SUPPLEMENTARY APPENDIX

CONTENTS

SUPPLEMENTAL METHODS

INCLUDED PATIENTS

We investigated 198 unselected consecutive patients with newly diagnosed AML, which correspond approximately to 75% of patients with newly diagnosed AML registered at MDACC during the years of study. Forty-six patients (23%) were excluded for the following reasons: 15 patients were not followed in our institution, 13 patients had prior or subsequent stem cell transplantation during the study period and 18 patients did not receive any PAP at baseline (14 patients) or during the study (4 patients). Consequently, our study population consisted of 152 patients who had received > 2 days of PAP and were evaluable for type and duration of PAP drug use as well as for a diagnosis of IFI. Algorithm S1 shows the study population and case-control groups.

DEFINITIONS

Primary antifungal prophyalxis was defined as any systemic antifungal therapy given as prophylaxis for more than two consecutive days to a patient without current or previous clinical, microbiological and/or radiological evidence of either documented or presumed IFI. Documented and possible IFIs were determined in accordance with the EORTC revised definitions (2008) (8). Patients with possible IFIs or those with clinical and microbiological evidence of IFI and non-characteristic CT scan abnormalities, according to EORTC definitions (2008), were categorized as having presumed IFIs. Overall IFIs included patients with documented and presumed IFIs. EAT was defined as the administration of an antifungal drug(s) in a patient with persistent febrile neutropenia and/or clinical findings possibly related to an IFI in which microbiologic and radiologic findings were negative for IFI. Galactomannan results for diagnosis of "probable aspergillosis" included one or more positive serum samples with an index ≥ 0.5 within a 2-week timeframe of the clinical and radiological evidence of IFI. The date of IFI onset was considered as the date of the first clinical manifestations attributable to IFI or the date of the clinical/radiologic/microbiologic diagnosis.

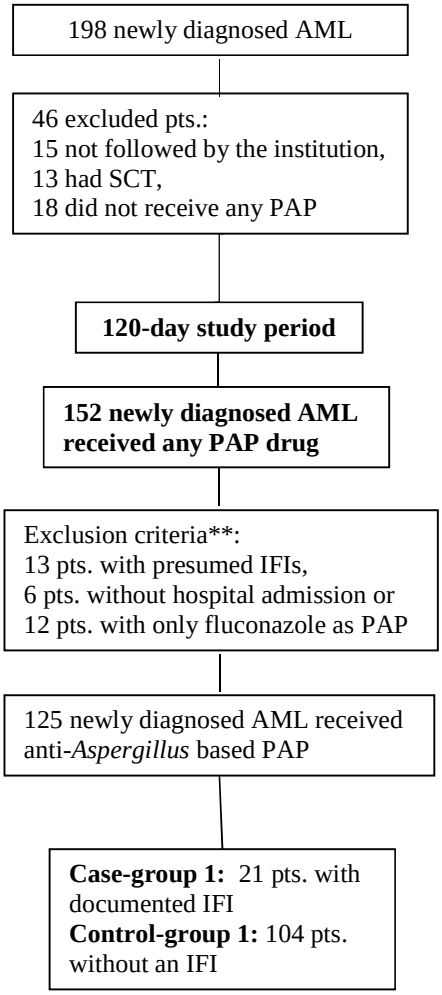
DATA COLLECTION

We performed a pilot study to make uniform the data collection. Demographic, clinical and laboratory data were collected from the time of registration to the hospital until diagnosis of an IFI, loss of the patient to follow up, death or completion of 120 days after first remission-induction chemotherapy, whichever came first. Data concerning antifungal use were collected from the institution's pharmacy database and matched with the information in patients' electronic medical records. We collected data about the type, dosage and duration of antifungal

drugs used as PAP in each prophylaxis period (PAP drug used without interruption) in each patient during the study period. We also collected information regarding clinical, epidemiological, laboratorial or complementary parameters (Table 1). The diagnosis of IFI was revised by 3 infectious diseases specialists (M.Z.R.G., V.M. and D.P.K.).

PAP AND DIAGNOSTIC WORKUP OF IFIS IN THE STUDY HOSPITAL

There was no explicit protocol for PAP usage in AML patients during the study. The diagnostic workup of IFIs in the hospital during the study period was standardized, patients were closely followed and exams and procedures were performed when indicated. The diagnostic workup did not change during the study period, including procedures in the clinical microbiology laboratory. Serum galactomannan tests were the only biomarker for IFI routinely used as part of diagnostic workup during the years of study (9).



Supplemental Figure S1.

Table S1: Epidemiology, clinical features and outcome of 21 AML patients with documented IFIs during 120-day study period

n.	Age/ Gender	PAP drug[a]	Duration of PAP drug use[a] (days)	Time to IFI[b]	Diagnosis of Documented IFI[c]	Agents of IFI	Positive GM; total n., n. of consecutives, mean level (range)[d]	Clinical forms	Death
1	21/F	Voriconazole (PO)	3	33	Probable	IA	4, 3 consecutives, 0.76 (0.53 – 1.3)	Pulmonary	No
2[e,f]	55/F	Caspofungin	46	56	Definite	*Aspergillus niger* (bronchial washing); *Paecilomyces* sp. and hyphae invanding tissue (lung tissue); rare septated hyphae (BAL)	1, 3.51	Pulmonary/ Osteomyelitis (rib)	No
3[e]	57/F	Posaconazole	17	23	Probable	IA	1, 0.83	Pulmonary	No
4	70/F	Posaconazole	26	32	Definite	Mold sterile hyphae (skin biopsy)	1, 0.76	Disseminated	No

Table S1. (Continued)

n.	Age/ Gender	PAP drug[a]	Duration of PAP drug use[a] (days)	Time to IFI[b]	Diagnosis of Documented IFI[c]	Agents of IFI	Positive GM; total n., n. of consecutives, mean level (range)[d]	Clinical forms	Death
5[e]	72/M	Micafungin	11	16	Definite	*Coccidioides* sp. (positive IGM immunodiffusion test),	-	Pulmonary	No
6[e]	61/F	Fluconazole (PO)	64	104	Probable	IA	6, 2 consecutives, 0.90 (0.57 – 1.41)	Pulmonary	No
7[e]	63/F	Posaconazole	82	111	Probable	*Fusarium* sp. (sputum)	3, non-consecutives, 0.61 (0.53 – 0.72)	Disseminated	No
8[f]	65/M	Anidulafungin	28	60	Probable	IA	1, 0.56	Pulmonary	No
9[e,f]	62/F	Micafungin	5	15	Definite	*Candida krusei* (blood)[g] and *Candida parapsilosis* (blood and urine)[g]	-	Disseminated	No
10[e]	82/M	Fluconazole (PO)	1[h]	29	Probable	IA	2, non-consecutives, 0.60 (0.58 – 0.63)	Pulmonary	No
11	64/F	Anidulafungin	24[i]	29	Definite	*Candida glabrata* (blood)[g1]; few yeast (BAL)	-	Disseminated	No
12	61/M	Caspofungin	6	7	Probable	IA	5, 3 consecutives, 0.72 (0.55 – 0.97)	Pulmonary	No
13[e]	53/F	Caspofungin	4	17	Probable	IA	1, 0.80	Pulmonary	No
14[e]	72/M	Micafungin	9	11	Definite	Hyphae invanding tissue (nasal lesion)	1, 1.26	Sino-pulmonary	No

15[e]	67/F	Posaconazole and Micafungin	18	21	Probable	IA, previous *Aspergillus fumigatus* (sputum) colonization	1, 0.64	Pulmonary	Yes[i]
16[e,i]	40/F	Anidulafungin	9	13	Definite	*Geotrichum capitatus*, a.k.a. *Blastoschizomyces capitatus* (blood)	-	Disseminated	No

Table S1. (Continued)

n.	Age/ Gender	PAP drug[a]	Duration of PAP drug use[a] (days)	Time to IFI[b]	Diagnosis of Documented IFI[c]	Agents of IFI	Positive GM; total n., n. of consecutives, mean level (range)[d]	Clinical forms	Death
17	69/F	Caspofungin	9	8	Probable	IA	4, 4 consecutives, 0.73 (0.56 – 0.81)	Pulmonary	No
18[e,i]	63/M	Caspofungin	13	17	Definite	*Fusarium* sp. (lymph nodes)	1, 0.86	Disseminated	Yes[i]
19[e]	77/M	Voriconazole (PO)	19	21	Probable	IA	1, 1.20	Pulmonary	Yes[i]
20[e,f]	70/F	Caspofungin	10	84	Probable	IA	4, 2 consecutives, 0.70 (0.52 – 1.07)	Pulmonary	No
21[e,f]	54/F	Anidulafungin	20	20	Definite	*Candida glabrata* (2 blood and 1 bone marrow cultures)[g2]; yeast like forms invading tissue (skin lesion); yeast < 10.000CFU/ml (urine)	-	Disseminated	Yes

Note: AML, acute myeloid leukemia; BAL, bronchoalveolar lavage; CFU, *colony-forming unit;* GM, serum galactomannan tests; FRIC, first remission-induction chemotherapy; IA, "invasive aspergillosis"; IFI, invasive fungal infection; IGM, *immunoglobulin M;* PAP, primary antifungal therapy; PO, per os; [a] PAP drug associated to D-IFI; [b] time to IFI after FRIC (days); [c] definite or probable IFI according to EORTC/MSG criteria; [d] among our probable IFI cases, GM levels were low and did not differentiate from those with definite *Fusarium* or *Paecylomyces* infections with *Aspergillus niger* colonization; [e] case with absolute neutrophil counts below 0.10 at time of D-IFI started; [f] therapy-related AML; [g] MIC of caspofungin was 0.5 mcg/ml for both *C. krusei* (intermediate) and *C. parapsilosis* (susceptible); [g1] 0.12 mcg/ml for *C. glabrata* (susceptible); [g2] 8 mcg/ml for *C. glabrata* (non-susceptible). Micafungin and anidulafungin were not tested; [h] patient was taking caspofungin for 26 days during hospital admission and then was discharged with oral fluconazole; [i] initial period of use as empirical antifungal therapy; [j] death related to IFI; [l] received 3 days of fluconazole prophylaxis before the start of echinocandin PAP.