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Original Articles

Risk factors for invasive aspergillosis in acute myeloid leukemia patients prophylactically treated with posaconazole

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Invasive aspergillosis (IA) is an important cause of morbidity and mortality in neutropenic patients with hematological malignancies. To investigate the immediate and mid-term benefits of posaconazole prophylaxis in AML patients undergoing first induction chemotherapy and to study the infection risk factors, we prospectively studied the IA incidence in these patients at our hospital between years 2007 and 2008; then we compared them to a matched control group without prophylaxis. There were 55 and 66 patients in each group respectively. At day 32 post-induction, two probable cases (3.6%) were scored in the prophylaxis group compared to 8 cases (12.1%) in the control group (4 possible and 4 probable). At day 100, it reached 7.27% and 15.5% respectively. Kaplan-Meier analysis at day 100 showed lower mortality rate in the prophylaxis group compared to the control group [3.64% (n = 2, none due to IA) and 10.61% (n = 7, four due to IA) respectively, P = 0.002]. Multivariate analysis showed age and lack of response to induction as independent infection risk factors. Posaconazole prophylaxis resulted in lower incidence of IA and significantly improved survival. Patient's age and response to induction treatment are two independent infection risk factors, and need more attention during future clinical trials linked to antifungal prophylaxis.

Keywords AML induction therapy, aspergillosis, posaconazole, prophylaxis, infection risk factors

Introduction

Invasive aspergillosis (IA) is a threatening opportunistic infection in immunocompromised patients including those with hematological malignancies [1]. At least 10% of patients with acute myeloid leukemia (AML) develop IA during the chemotherapy-induced period of aplasia, of which nearly a half dies despite early antifungal (AF) therapy [2,3].

Patient outcome improvement due to the empiric use of various AF drugs has not been further demonstrated [1,4]. Despite its feasibility and superior efficacy, the preemptive strategy requires patient surveillance *via* consensually approved IA-predictive tests to be efficiently implemented [5,6]. Hence, systematic prophylaxis has become a challenging alternative, with new available AF drugs. Among them posaconazole, a triazole drug with a spectrum of activity which encompasses that of other drugs, except

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amphotericin B [7]. Two large studies have shown significant reductions in the incidence and invasive fungal infection (IFI)-related mortality by using posaconazole as prophylactic treatment compared to other azole drugs in patients undergoing HSCT or with AML and myelodysplastic syndrome (MDS) [8,9]. Meanwhile, little is known regarding the effect of prophylaxis beyond the induction period in long-term neutropenic patients with AML. Moreover, no clinical, AF-based trial has yet studied which factors commonly assigned to AML prognosis may influence patient outcome in this setting. Therefore, we analyzed AML patients who underwent posaconazole prophylaxis during the induction phase in order (i) to investigate the occurrence of IA and patient outcome at short and mid-term periods of follow-up; and (ii) to stratify the underlying conditions and risk factors commonly ascribed to AML, hypothesizing these may also influence the incidence of IA. We compared the observed results to a matched control group without prophylaxis to point out the importance of IA prophylaxis within the same centre, under the same environmental and treatment settings.

Material and methods

Study design

Between November 2007 and November 2008, AML patients hospitalized in our hospital, undergoing first induction chemotherapy were enrolled to receive posaconazole prophylaxis against IA; a written informed consent was signed by each eligible patient before enrollment. These patients were matched to a control group, AML patients cared in the same department of the same hospital between November 2006 and November 2007 and who did not receive any AF prophylaxis during the induction period. Matching criteria consisted of sex, age, FAB classification, cytogenetics and molecular markers. No change in strategies of AML treatment was made in our service throughout the 24-month study. This observational study was carried out according to the institutional guidelines and was approved by the local institutional review board.

Posaconazole administration

In the experimental group, posaconazole was administered in an oral suspension 200 mg three times daily, from the first day of induction chemotherapy, until recovery of neutropenia (absolute neutrophil count $\leq$500/µl for at least 7 consecutive days) or leukemia complete remission, until occurrence of an IFI, whichever came first. Patients in the control group were susceptible to receive any AF therapy other than posaconazole in case of IA.

Patient monitoring

Each patient had a daily clinical examination. Presence of *Aspergillus* galactomannan antigen was tested on sera sampled twice-a-week by using the double-sandwich ELISA Platelia *Aspergillus*® (Bio-Rad, Marnes-La-Coquette, France); an optimal density index $\geq$0.5 was considered to be positive [10,11]. Total body CT-scan was repeated in case of positive antigenemia result and persistence of fever. Proven, probable and possible IA was defined according to the 2008 revised EORTC criteria [6].

Data documentation and end-points

The primary end-point was to identify which AML prognosis factors may have an impact on the occurrence of IA upon the induction phase and after day 100, depending on antifungal prophylaxis. These variables were qualitative: sex, FAB classification, cytogenetic groups, prognostic groups (including cytogenetic features and molecular markers), effect of laminar air-flow room, chemotherapeutic protocol intensity, response to induction; and quantitative: age $>$ 55.

The secondary end-points included incidence of IA at day 32 and at day 100 after induction chemotherapy, as well as patient outcome *via* study of survival over time until 24 months. The cumulative incidences of IA were calculated with respect to the time to proven, probable or possible IA (from day 1 of hospitalization).

Statistical analyses

The impact of posaconazole prophylaxis and AML prognostic factors was assessed using the Exact Fisher test (unilateral). Qualitative and quantitative comparisons between patients with and without IA were assessed using the Monte-Carlo χ^2 test and the Kruskal-Wallis test, respectively. Cumulative incidences of IA were analyzed by using the Gray tests and the Fine and Gray competitive risk model, respectively [12]. We used the Kaplan-Meier method to measure time to death from any cause, time to death related to IA, time to IA, and survival without IA. The Log-rank test and the Cox regression model were applied for the assessment of survival benefit between the two cohorts [13]. All statistical tests were performed using the R software version 2.9.2.

Results

Studied groups

Among the 121 patients studied (59 males and 62 females), 55 patients were enrolled in the experimental posaconazole group and 66 matched patients consisted of the control group, and were followed-up with a median duration of

Table 1 Characteristics of posaconazole and control cohorts upon matching.

	Condition	Control group ($n = 66$)	Posaconazole group ($n = 55$)	P value
Age, median (years) [range]		53 [19–77]	53 [18–73]	NS
Sex	Male	36	23	NS
	Female	30	32	
LAF room	+	52	46	NS
	–	14	9	
AML type	*de novo*	52	39	NS
	Secondary	14	16	
FAB group	0	3	0	NS
	1	11	8	
	2	14	12	
	3	11	7	
	4	2	5	
	5	15	8	
	6	1	0	
	7	3	0	
	ND	6	14	
Cytogenetic group	Favorable	8	10	NS
	Intermediate	20	23	
	Unfavorable	36	22	
	ND	2	0	
Molecular biology	Normal	15	13	NS
	Abnormal	44	35	
	ND	7	7	
Prognostic group	Good	13	16	NS
	Poor	44	31	
	ND	9	8	
Chemotherapy intensity	Low	2	5	$P < 0.01$
	Standard	50	28	
	High	14	22	
Response to induction	CR	54	46	NS
	Failure	10	9	
	Death	2	0	
Median duration of aplasia (days) [range]		28 [9–52]	29 [7–91]	NS
Median duration of hospitalization (days) [range]		37 [22–57]	38 [25–101]	NS

IA, invasive aspergillosis; LAF, laminar air-flow; AML, acute myelogenous leukemia; CR, complete response; ND, not done; NS, not significant.

6 months (1.1–14.4) and 14.9 months (0.7–26.7), respectively. Characteristics of the two groups at baseline are shown in Table 1.

In the posaconazole group, the median duration of prophylaxis was 27 days (8–94) and the median time to recovery from neutropenia was 29 days (7–91). Among these 55 patients, 35 (64%) were discharged as a result of recovery from aplasia or end of hospitalization, three patients (5%) had discontinued prophylaxis because of hepatic toxicity grade II which was related to posaconazole, while four patients had discontinued due to other reasons not related to posaconazole [renal failure ($n = 1$) and transfer to intensive care unit ($n = 3$)], none developed IA later. For the remaining 13 patients (23%), posaconazole prophylaxis was switched to either pre-emptive or curative AF treatment with (an)other antifungal drug(s) because of probable invasive candidiasis ($n = 5$), probable IA ($n = 3$), or suspected IFI ($n = 5$). The five IFI were suspected by imaging techniques and were rejected later after negative confirmation by antigenemia tests and thus were not taken into account with the infected patients in the analysis. In the control group, all patients with probable or possible IA were treated by voriconazole.

Incidence of IA

During the induction period, reports of IA doubled between day 15 and day 32 in both cohorts (4.13% *vs* 8.2%). At day 32, two probable cases (3.6%) were scored in the posaconazole group compared to the 8 cases (12.1%) in the control group, which consisted of four possible IA and four probable IA cases (OR: 0.3 [95% CI, 0–1.2], $P = 0.085$) (Fig. 1a). The median time to IA discovery in the total population was 15 days (4–32). At day 100 post-induction treatment, the cumulative

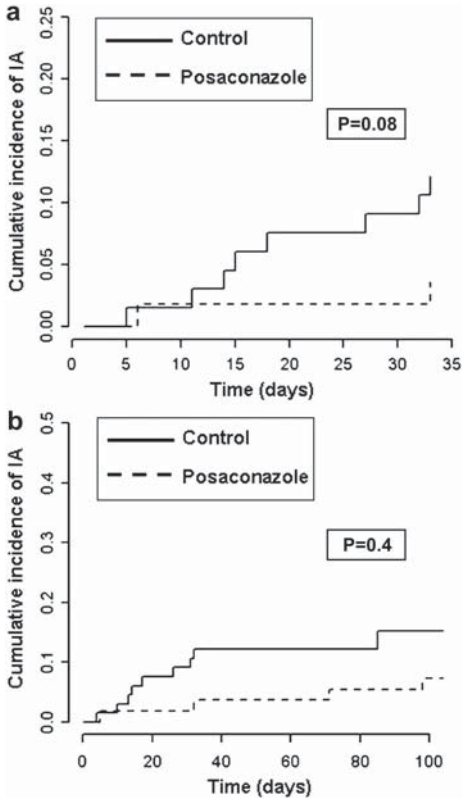

Fig. 1 Cumulative incidence of invasive aspergillosis (IA) during the induction period (a), and at day 100 post-induction chemotherapy (b).

incidence of IA reached 7.3% in the experimental group and 15.5% in the control group (OR: 0.4 [95% CI, 0.18–2], $P = 0.4$) (Fig. 1b). No proven IA cases were reported in the whole population.

Prognosis factors according to the presence of IA

Comparison of patient characteristics at induction, according to the presence or absence of IA (probable or possible) showed that infected patients were significantly older (i.e., > 55 years; $P = 0.03$), while treatment response failure ($P = 0.09$) might have the potential to lead to IA occurrence (Table 2).

To investigate the potentially protecting factors against the occurrence of IA, we performed a series of statistical analyses applied to AML prognosis factors.

At the end of the induction period, univariate analysis showed that cytogenetics significantly impacted on IA incidence (OR: 4.66 [95% CI, 1.075-Inf], $P = 0.04$). There was only a trend for response to chemotherapeutic induction (OR: 3.63 [95% CI, 0.875–Inf], $P = 0.07$), or for posaconazole prophylaxis (OR: 0.28 [95% CI, 0–1.97], $P = 0.085$) to impact on it (Table 3). Multivariate analysis revealed the negative impact of age over 55 (HR = 1.05 [1.01–1.10]; $P = 0.015$) and failure or partial response to treatment response (HR = 4.31 [1.18–15.67]; $P = 0.027$). In contrast, it merely supported bad cytogenetics (HR: 0.894 [97.5% CI, 0.793–1.010]; $P = .067$) and invalidated the lack of posaconazole prophylaxis (HR = 0.64 [0.14–2.93]; $P = 0.57$) as risk factors for IA. In addition, when considering probable IA (not possible IA), the same significant variables were retrieved ($P = 0.013$ and $P < 0.001$, respectively), and a potential link was detected with cytogenetic prognosis ($P = 0.057$), similarly to univariate analysis.

Beyond the induction period or beyond day 100, none of the variables studied by multivariate analysis significantly emerged as an independent factor impacting on IA occurrence. Instead, only a trend to increase IA was detected with severe cytogenetics (SHR: 0.829 [97.5% CI, 0.68 2–1.01]; $P = 0.058$).

Table 2 Characteristics of the cohorts according to the presence or absence of invasive aspergillosis (IA).

	Condition	No IA	Possible IA	Probable IA	*P* value
Age, median (years) [range]		54.6 [18.4–72.9]	59.8 [52.2–58.3]	62.82 [47.9–77.1]	$P = 0.03$
Sex	Male	53	3	3	$P = 0.61$
	Female	58	1	3	
LAF room	+	90	3	4	$P = 0.65$
	–	19	1	2	
AML type	*de novo*	82	4	5	$P = 0.55$
	Secondary	29	0	1	
FAB group	0	2	0	1	$P = 0.38$
	1	15	2	2	
	2	25	1	0	
	3	17	1	0	
	4	7	0	0	
	5	22	0	1	
	6	1	0	0	
	7	3	0	0	
	ND	18	0	1	
Cytogenetic group	Favorable	17	1	0	$P = 0.32$
	Intermediate	42	0	1	
	Unfavorable	50	3	5	
Molecular biology	Normal	75	1	2	$P = 0.59$
	Abnormal	25	1	2	
Prognostic group	Good	28	1	0	$P = 0.38$
	Poor	70	1	4	
Chemotherapy intensity	Low	6	0	1	$P = 0.68$
	Standard	70	4	4	
	High	35	0	1	
Response to induction	CR	94	2	4	$P = 0.09$
	Failure	16	2	1	
	Death	1	0	1	
Median duration of aplasia (days) [range]		28 [7–91]	33 [25–52]	28 [21–42]	$P = 0.4$
Median duration of hospitalization (days) [range]		37 [22–101]	49 [40–55]	37 [29–55]	$P = 0.09$

IA, invasive aspergillosis; LAF, laminar air-flow; CR, complete response; ND, not done.

Table 3 Studied risk factors for invasive aspergillosis (IA) at induction (univariate analysis).

Risk factor	Condition	IA%	Odd-ratio (95% CI)	*P* value
Age (years)	≤55	4.9	OR = 2.53 [0.66–Inf]	$P = 0.15$
	>55	11.67		
LAF room	+	7.2	OR = 2.01 [0.41–Inf]	$P = 0.27$
	–	13.6		
AML type	*De novo*	9.9	OR = 0.31 [0–1.97]	$P = 0.24$
	Secondary	3.3		
Posaconazole prophylaxis	+	3.6	OR = 0.28 [0–1.197]	$P = 0.085$
	–	12.1		
Cytogenetic group	Favorable/intermediate	3.3	OR = 4.66 [1.075–Inf]	$P = 0.04$
	Unfavorable	13.8		
Prognostic group	Good	10.3	OR = 1.99 [0.27–Inf]	$P = 0.46$
	Poor	16		
Response to induction	CR	6	OR = 3.63 [0.875–Inf]	$P = 0.07$
	failure	19		
Duration of aplasia	≤28 days	6.55	OR = 1.57 [0.43–Inf]	$P = 0.36$
	>28 days	10		

IA, invasive aspergillosis; LAF, laminar air-flow; AML, acute myelogenous leukemia; CR, complete response.

Outcomes

At day 100, nine deaths were recorded – two in the posaconazole group (3.64%) and seven in the control group (10.61%). The corresponding mortality probability curve was significantly lower in the posaconazole group compared to the control group (SHR: 0.103 [95% CI: 0.0238–0.445]; $P = 0.0023$) (Fig. 2a). All seven control patients died within 2.2 months after the initiation of induction, of whom four were diagnosed with IA. Deaths were due to IA despite complete response to induction chemotherapy ($n = 1$), failure of induction chemotherapy ($n = 5$, including one with IA), and cardiovascular-related toxicity ($n = 1$). The two posaconazole patients died from induction failure and in the absence of IA or other IFI (Fig. 2b).

Given the last follow-up end-point during the observational period (24 months), the median overall survival (OS) was 13.3 months in the posaconazole group and 23.6 months in the control group. The equivalence of the corresponding OS probability curves ($P = 1.0$) evidenced the lack of impact of posaconazole prophylaxis on long-term OS. By contrast, corresponding curves according to IA illustrate the significantly reduced survival of patients with IA (median of 7.5 months; $P = 0.02$) (Fig. 3).

Outcome prognostic factors

Within the IA subset, survival was significantly influenced by age ($P = 0.011$), cytogenetics ($P = 0.041$), and response to treatment ($P = 0.035$). A moderate relation was detected with the chemotherapy protocol ($P = 0.067$) but not with posaconazole prophylaxis ($P = 0.89$).

Data from multivariate analysis also revealed age (HR = 1.054 [1.017–1.09]; $P = 0.0042$), unfavourable cytogenetics (HR = 2.524 [1.72–5.44]; $P = 0.0018$), and failure to induction (HR = 7.73 [3.58–16.7]; $P < 0.001$) as the independent negative factors for long-term survival.

Discussion

In this monocentric study, we documented the potential for some AML prognosis factors to impact on IA incidence depending on prophylaxis with posaconazole. This goal was implemented with respect to the recently approved benefit of posaconazole as IA prophylaxis in some categories of hematological patients [8,9]. In our study, median duration of prophylaxis coincided with that of recovery from neutropenia; this feature and the daily administration were similar to those previously reported [8]. However we did not reach a significant level of reduction of incidence of IA using prophylaxis ($P = 0.085$). In one other similar study very recently published, restricted to a single German centre, the IA incidence rates in the same patient category under prophylaxis were 2.6% with posaconazole and 13.4% with local polyene [16]. These results are very close to ours; nevertheless these led to a significant decrease of IA cases under posaconazole. In contrast to the German situation, we did not record any case of proven IA. Moreover, given the annual incidence of IA below 10% in our service before posaconazole systematic prophylaxis and the shorter length of the trial with respect to that of the German study, we could not ascertain a sufficient number-to-treat (NTT) patients to provide a significant decrease of IA cases in relation to prophylactic posaconazole. Finally, because death is considered as a necessary short and mid-term outcome, a NNT is ethically not affordable, beyond model-based reasons [14,15].

Beyond this, our goal was to address whether factors ascribed to AML prognosis may behave also as potential

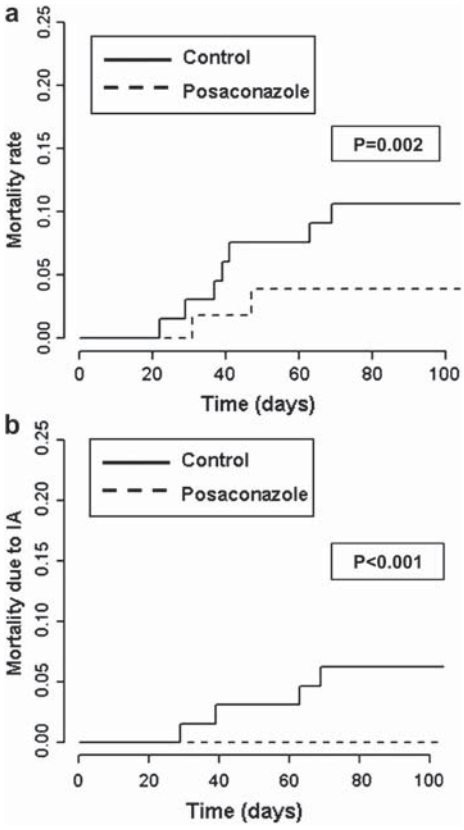

Fig. 2 Cumulative incidence of mortality over the 100-day period according to the presence or absence of posaconazole prophylaxis, as overall mortality (a) and mortality attributable to invasive aspergillosis (b).

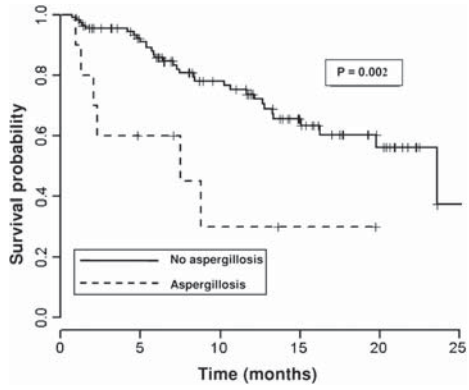

Fig. 3 Overall survival curves according to the presence or absence of invasive aspergillosis.

infection factors for patients, as a means to detect more accurately who will be more susceptible to infection even in the presence of antifungal prophylaxis. Indeed, we stress local epidemiology as a meaningful but still neglected problematic in neutropenic, hematological patients. Many previous clinical trials did not consider the differences in the environmental settings between different centers – whether national or multinational studies [17–20]. Likewise, the heterogeneity in chemotherapy regimens has not been integrated into these trials whereas this may have a substantial impact on response to disease, thereby on IFI incidence. In this respect, monocentric studies appear appropriate to highlight this issue. The only monocentric trial documenting the efficiency of posaconazole prophylaxis did not address this issue [16]. In that, our study is the first report providing evidence for a time-related variation of such factors onto IA incidence and survival in AML neutropenic patients.

We found that age (over 55) and treatment response (partial or failure) were independently associated as measured upon the induction period and at day 100. These two variables also decreased significantly short-term OS. Model-based prognostic groups on factors significantly associated with short-term aspergillosis-free-survival and OS have been recently released, that concerned various leukemia conditions with mainly allogeneic 7 transplant recipients [17–20]. Further, larger analyses would be warranted to explore clinical and biological variables in the context of AF prophylaxis with respect to types and conditions of AML. Our statistical-based results did not show LAF implementation to be more protective against IA compared to standard rooms. Although most patients were cared in LAF rooms, IA was diagnosed as four probable cases and three possible cases. Nevertheless, efficient, controlled ventilation systems are recognized protective measures against airborne aspergillosis in high-risk patients [21]. However, one main problem lies in the fact that airway contamination before the induction period is not achievable, nor may be the abortion of any pathogenic process of already inhaled conidia, or even waterborne infection [22]. Conversely, these data raise the question of the efficacy of posaconazole prophylaxis in patients already harboring *Aspergillus* elements before hospitalization, keeping in mind that AML-associated characteristics may worsen prognosis by 'favoring' fungal pathogenicity.

Similarly to others, our results indicate that posaconazole prophylaxis may contribute to keep low short-term mortality rates [8,9,16]. However, a significantly lower mortality rate was achieved within the posaconazole group at day 100, which contrasts with the negative data obtained in the German monocentric study [16]. Again, this may be explained by the different AML settings, including the chemotherapeutic regimen (as documented in the former study), as these may vary depending on the type of AML – and thus the intensity of chemotherapy – and the availability of protocols, also potentially dependent on the centers and/or country. In our case, the therapeutic regimens statistically differed between the two cohorts because of improving protocols including dosage and availability. Nevertheless, the IA cases were diagnosed in patients who received different chemotherapeutic regimens; time of recovery from neutropenia was identical between both groups. Another explanation of the differences observed

between the German study and ours is that the former study assigned topical polyene prophylaxis as the control group [16], whereas our control group was exempt from any antifungal prophylaxis. In our case, the two posaconazole patients who died did not suffer from any IFI, whereas four of the seven control patients who died were diagnosed with IA.

We addressed the potential survival benefit of long-term posaconazole prophylaxis after chemotherapy initiation, an end-point not yet documented in previous studies. Whereas univariate analysis contorted its benefit, the integration of AML variables into multivariate analysis revealed the higher strength or weight of some AML prognosis factors onto long-term survival, from 9 months after starting chemotherapy. However the loss of long-term benefit from posaconazole may be taken with caution given the shortened follow-up period of the posaconazole cohort compared to the control cohort. Whether prolonged prophylaxis would improve long-term survival is therefore one of the issues future clinical trials should integrate into their goals.

Overall, our study confirms that posaconazole prophylaxis at induction chemotherapy in AML patients can lower the incidence of IA and improve survival at day 100 which recommends its use in all AML patients during induction. In addition, our results revealed that patient's age, response to induction treatment, and potentially cytogenetic prognosis, are independent infection risk factors that may require more attention especially in trials analyzing prophylaxis efficacy without concomitant analysis of patient's and disease characteristics. Cytogenetic prognosis may need supplementary validation since it can be a confounding factor with age and response to induction chemotherapy. Nevertheless, a careful selection of AML risk factors would help to address the optimal conditions for delivering AF prophylaxis over time especially beyond the induction period.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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