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Combined antifungal approach for the treatment of invasive mucormycosis in patients with hematological diseases: a report from the SEIFEM and FUNGISCOPE registries

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Dear Sirs,

Invasive mucormycosis (IM) in patients with acute leukemia and allogeneic stem cell transplant (allo-SCT) recipients treated with antifungal monotherapy is associated with high mortality rates of 44-49% [1-3]. Among the available antifungals, amphotericin B (AmB) formulations and posaconazole demonstrate the most promising *in vitro* activities against Mucorales [4,5], and their combination displays synergistic *in vitro* activity [6,7]. However, pre-clinical studies in neutropenic and diabetic ketoacidotic mice with IM reported no improvement in survival under a combination of posaconazole and liposomal amphotericin B (L-AmB), compared to L-AmB monotherapy [8,9]. To date, these results have not been evaluated systematically in a clinical setting, given the rarity of IM. Therefore, the value of combining a lipid formulation of AmB (Lip-AmB) with posaconazole for the treatment of IM remains a matter under discussion.

Thirty-two patients with proven/probable IM treated with a combination of Lip-AmB and posaconazole (Lip-AmB+POS) between 2007 and 2012 were identified in two large registries: SEIFEM (Sorveglianza Epidemiologica Infezioni Fungine Emopatie Maligne) and Fungiscope – A Registry for Emerging Fungal Infections.

Clinical characteristics of these patients are summarized in table 1. All patients, but one, were adults and were affected by hematological malignancies except 3 cases presenting with severe aplastic anemia. Most IM occurring in AML patients were documented during the first induction treatment for the underlying disease. At diagnosis, 22 patients (69%) had a neutrophil count of $<0.5x10^9$/l. Within one month prior to diagnosis 12 patients had received steroids: in 7 cases, all allo-HSCT for the treatment of graft versus host disease, in the remaining 5 cases for the treatment of the underlying disease. Only 3 patients (9%) were affected by diabetes mellitus, unrelated to steroid administration.

The diagnosis of IM was proven in 20 cases (63%) and probable in 12 cases (38%). In about one third of cases (11 cases), the infection was localized in the lower respiratory tract, while a disseminated infection (≥ 2 non-contiguous sites) was detected in another 35% of

cases (n=11). Overall, 21 patients (66%) had received antifungal prophylaxis before the onset of IM for a median duration of 35 days (range 2-109). Only 3 cases received prophylaxis with agents with anti-Mucorales activity. Among the 22 patients (69%) who were neutropenic at the onset of IM, 16 (73%) recovered from neutropenia. Thirteen patients (41%) underwent surgical excision of infected tissue. In the majority of patients (29 cases, 91%) Lip-AmB+POS was initiated due to lack of response to antifungal monotherapy. In 20 patients (63%) only one line of monotherapy had been administered for a median time of 18 days (range 13-64) before initiation of Lip-AmB+POS. In 75% of these cases (n=15), an AmB formulation had been administered: 12 L-AmB, 2 lipid complex AmB, 1 AmB. In the remaining 5 cases, posaconazole (2 cases), voriconazole (1 case) and caspofungin (2 case), had been given. In 9 cases (28%) two different lines of treatment had been administered prior to Lip-AmB+POS.

Lip-AmB+POS was administered as first-line treatment to only 3 patients (9%). Among the 29 patients (91%) receiving Lip-AmB+POS as second or third line therapy, 27 (93%) received posaconazole as an addition to an ongoing treatment with Lip-AmB. In 28 patients (88%), posaconazole was administered at 800 mg/d, in 2 patients (6%) at a lower dosage (400 mg/d and 600 mg/d) and in 2 patients (6%) at a higher dosage (1600 mg/d and 3200 mg/d). Lipid complex AmB was chosen for combination with posaconazole in 5 patients (16%), L-AmB in 27 patients (84%). The standard dosage of L-AmB (3 mg/kg) was used in 10 cases (32%) and a higher dosage (5 mg/kg or more) in 17 cases (53%). The median duration of combined treatment was 32 days (3-157 days). In 3 cases (9%), deferasirox was added to Lip-AmB+POS.

In any patients the antifungal treatment was stopped for drug-related toxicities. Comparing patients that received L-AmB at 3 mg/kg and those who received L-AmB at 5 mg/kg or higher, none of them showed relevant nephrotoxicity.

After a median follow-up of 3 months, clinical improvement of IM was observed in 18 patients (56%): 11 (34%) complete and 7 (22%) partial responses. Stable disease was demonstrated in 5 patients (16%). Nine patients (28%) did not respond to treatment and died

of progressive IM. Of the 3 patients (9%) receiving Lip-AmB+POS as front-line therapy, only 2 experienced a complete response, while the third died of IM.

At day 90 after the diagnosis of IM, 19 patients (59%) had died, 9 due to progression of IM and 10 due to progression of the underlying hematological disease, even though a clinical improvement of IM was observed in 5 cases. Maintenance treatment with oral posaconazole had been administered in all 18 responsive cases (56%) for a median of 74 days (10-175 days), without relapse of IM. Thirteen patients (41%) were still alive at least 12 months after diagnosis of IM and displayed no signs of active infection; 11 of these patients (85%) were able to continue treatment of the underlying hematological malignacy and 4 (12%) underwent an allo-SCT without relapse of IM after a time ranging between 9 to 16 months. In a univariate analysis, allo-SCT and steroid administration were negatively associated with treatment success. Recovery from neutropenia was identified as a potentially protective factor (table 2). Due to the low number of cases at multivariate analysis we did not identified any significant parameter

In the vast majority of our cases, Lip-AmB was used as front-line treatment, and posaconazole was added when no satisfactory response was observed. Hence, Lip-AmB+POS was prescribed as a salvage approach. In 56% of our cases a favourable clinical response was achieved (>70% if stable disease was included into the definition). This rate compares favourably with recent case series, in which response rates ranged from 32% to 59% [1-3], and with the response rates reported from a compassionate use trial that evaluated posaconazole as salvage therapy. In the latter trial, 6 of 13 patients (46%) receiving Lip-AmB+POS displayed a favourable response; all of which were partial responses [10].

Clearly, many factors besides the choice of antifungal agents may have contributed to patient outcome. We were not able to evaluate the impact of different Lip-AmB and posaconazole dosages on patient outcome due to the limited number of cases and the lack of regular therapeutic drug monitoring. Another important factor we could not adequately control for is the impact of surgical debridement on patient outcome. In contrast with previous analyses [3,11], surgical removal of infected tissue was not identified as a protective factor. This

may, however, be explained by limited sample size and a tendency to perform surgery on severely ill patients, only.

Finally, the influence of deferasirox could not be assessed in our analysis. While previous studies *in vitro* as well as animal studies suggested a synergistic effect of deferasirox in combination with L-AmB [12], a recent interventional trial on this issue failed to confirm such an association [13]. In our series, deferasirox was added in 3 cases only, all with a favourable outcome. Nevertheless, this observation should be considered no more than suggestive.

In patients responding to therapy, maintenance treatment with posaconazole was frequently administered for prolonged periods of time. It permitted 11 patients (34%) to continue treatment for the underlying malignancy, and prevented relapse of IM during subsequent periods of neutropenia. Of note, in 6 (19%) of these cases, an allo-SCT could be performed.

The risk that our series may suffer from a selection bias due to the inclusion of only those patients that survived long enough to receive a combination therapy it is possible, however we wanted to analyze the role of a combination therapy in those patients who have performed using 2 large registries, in which were collected both patients have a good outcome than those who have a bad course.

In conclusion our analysis suggests that a combined antifungal treatment with Lip-AmB+POS may be considered in patients with very aggressive forms of IM.

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Eight patients were already published elsewhere[2, 3], however, the assessment of Lip-AmB+POS was not the focus of these analyses.

Trasparency declaration

LP has received honoraria from Gilead Sciences, Schering-Plough, Astellas Pharma, Merck, and Pfizer Pharmaceuticals, he has been speaker for Gilead Sciences, Schering-Plough, Merck, Pfizer Pharmaceuticals, Astellas Pharma. OAC is supported by the German Federal Ministry of Research and Education (BMBF grant 01KN1106), has received research grants from 3M, Actelion, Astellas, Basilea, Bayer, Biocryst, Celgene, Cubist, F2G, Genzyme, Gilead, GSK, Merck/Schering, Miltenyi, Optimer, Pfizer, Quintiles, and Viropharma, is a consultant to 3M, Astellas, Basilea, Cubist, F2G, Gilead, GSK, Merck/Schering, Optimer, and Pfizer, and received lecture honoraria from Astellas, Gilead, Merck/Schering, and Pfizer. AB has received honoraria from Gilead Sciences, Schering-Plough and Merck; he has been speaker for Gilead Sciences, Schering-Plough, Merck, Pfizer Pharmaceuticals, Astellas Pharma, Cephalon and Novartis. MC has received honoraria from Gilead Sciences, Merck, Pfizer Pharmaceuticals, Schering-Plough. SC was a member of Advisory Board for Pfizer and Gilead Sciences and received fees for lectures by Merck-Sharp Dohme. CG has received honoraria from Gilead Sciences, Schering-Plough, Astellas Pharma, Merck, and Pfizer Pharmaceuticals; he has been speaker for Gilead Sciences, Schering-Plough, Merck, Pfizer Pharmaceuticals. WJH. has received research support from Astellas, Basilea, Gilead, MSD／Merck and Pfizer, and compensation as a member of the scientific advisory board to MSD／Merck, and Pfizer, and has served as speaker for Gilead, MSD／Merck and Pfizer. CL-F has received grant support in the past 5 years from Astellas Pharma, Gilead Sciences, Pfizer, Schering Plough and Merck Sharp and Dohme. She has been an advisor/consultant to Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough. AN has received honoraria from Gilead Sciences, Schering-Plough, Merck, Pfizer and Cephalon. Le.Po. has received honoraria from Merck. MC has received honoraria from Gilead Sciences, Schering-Plough, Merck. ZR has served at the speakers' bureau of Pfizer and Astellas Pharma, and has been a consultant to Astellas Pharma. VR has received research grants from Gilead Sciences and Pfizer and received lecture honoraria from Gilead Sciences, Pfizer, Merck/Schering. DS has been as speaker for and received research funding from Merck. Speaker for Astellas. AS has received research grants from Gilead and Pfizer. AU has received research grants from MSD (Schering-Plough), and is/was an advisor or received lecture honorarium from Astellas, Aicuris, Basilea, Gilead, MSD, and Pfizer. JJV has received research grants from or has been a speaker for Astellas, Merck, Pfizer, and Schering-Plough. AC has received honoraria from Gilead Sciences, Schering-Plough, Merck, and Pfizer Pharmaceuticals. MJGTV has served on the speakers' bureau of Schering-Plough/Essex, Pfizer, MSD and Gilead Sciences. She has received a research grant from 3M. RH, Cr.Ga, VCG : none to declare.

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246 **Table 1. Clinical characteristics and risk factors of 32 patients who developed IM**

	n	%
Gender ❖ M ❖ F	 18 14	 56 44
Underlying disease		
❖ AML	20	64
❖ ALL	3	9
❖ Multiple Myeloma	3	9
❖ Lymphoma	3	9
❖ SAA	3	9
Phase of hematological disease		
❖ Induction AML/ALL	13	41
❖ Relapse/ Salvage	4	12
❖ Consolidation AML	2	6
❖ allo-BMT	8	25
❖ Supportive/No Treatment	5	16
Immunosuppressive therapy before diagnosis of IM		
❖ Steroids	12 §	37
❖ Immunosuppressors (CyA and others)	7 §	
Diabetes mellitus	3	9
Neutropenia at onset IM (ANC <0.5x10^9)		
❖ Yes ❖ No	22 10	69 31
Prophylaxis	21	66
❖ None	11	34
❖ Itraconazole	4	13
❖ Posaconazole	2	6
❖ Fluconazole	12	38
❖ Other#	3	9
Species	**n**	**%**
❖ *Lichtheimia corymbifera*	6	19
❖ *Cunninghamella bertholletiae*	1	3
❖ *Mucor* spp.	10	31
❖ *Rhizomucor* spp.	6	19
❖ *Rhizopus* spp.	9	28
Site of infection		
❖ Lung only	11	35
❖ Rhinocerebral only	5	15
❖ Skin only	2	6
❖ Other	3 ^	9

❖ Multiple	11	35
Lines of therapy prior Lip-AmB+POS		
❖ 0	3	9
❖ 1	20	63
❖ 2	9	28
Lipid formulation in Lip-AmB+POS		
❖ Lipid complex AmB ❖ L-AmB	5 27	16 84
L-AmB dose		
❖ 3 mg/kg	10	37
❖ 5 mg/kg	14	48
❖ >5 mg/kg	3	15
Surgery		
❖ Yes ❖ No	13 19	41 59
Recovery from neutropenia *		
❖ Yes ❖ No	16 6	73 27
Treatment response		
❖ Favorable (CR +PR)	18	56
❖ Stable	5	16
❖ Deterioration/Failure	9	28
Continuation of therapy with POS	18	56
Outcome at 90 days after diagnosis of IM		
❖ Death due to HM	10	31
❖ Death due to IM	9	28
Subsequent allo-HSCT	4	12

Legend: AML: Acute myeloid leukemia; ALL: Acute Lymphoid leukemia; SAA: severe aplastic anemia; allo-HSCT: allogeneic hemopoietic stem cell transplantation; CyA: cyclosporine A; HC: hematological condition; L-AmB: liposomal amphotericin B; IM: invasive mucormycosis; POS: posaconazole

^ 1CNS only, 1 liver and small bowel, 1 soft tissue, * out of 22 patients neutropenic at the onset of IM

§ 7 patients received both steroids and other immunusuppressants

1 L-AmB, 2 Voriconazole

Table 2. Univariate analysis of factors influencing treatment success

	all	favourable	death due to IM	p-value
	Cases	23	9	
Sex				0.9
❖ M	18	13	5	
❖ F	14	10	4	
Age (yrs)				0.2
❖ <50	12	10	2	
❖ >50	20	13	7	
Underlying HC				0.1
❖ AML	20	16	4	
❖ other	12	7	5	
allo HSCT				**0.01**
❖ yes	8	3	5	
❖ other	24	20	4	
Steroids §				**0.03**
❖ yes	12	6	6	
❖ no	20	17	3	
Neutropenia at onset of IM				0.2
❖ yes	22	17	5	
❖ no	10	6	4	
Prophylaxis				0.3
❖ yes	21	14	7	
❖ no	11	9	2	
Lip-AmB+POS				0.5
❖ L-AmB	27	19	8	
❖ Lipid complex AmB	5	4	1	
L-Amb dose				0.6
❖ 3mg/kg	10	6	4	
❖ =/> 5mg/kg	17	13	4	
Site of Infection				0.1
❖ Lung	11	6	5	
❖ Multiple	11	8	3	
❖ Other	10	9	1	
Surgery				0.1
❖ yes	13	11	2	
❖ no	19	12	7	
Recovery from neutropenia **				0.06
❖ yes	16	14	2	
❖ no	6	3	3	

Legend: AML: Acute myeloid leukemia; HC: heamatological condition; SAA: severe aplastic anemia; allo-HSCT: allogeneic hemopoietic stem cell transplantation; L-AmB: liposomal amphotericin B; POS: posaconazole.

* out of 22 patients neutropenic at the onset of IM

§ this data coinciding with immunosuppressive therapy