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

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] Given the rarity of IM, these results have not been evaluated systematically in a clinical setting. Therefore, the value of combining a lipid formulation of AmB (Lip-AmB) with posaconazole for the treatment of IM remains a subject of controversy and 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. Of these patients, 31 were adults and all were affected by hematologic 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 less than $0.5x10^9$/L. Within one month prior to diagnosis, 12 patients had received steroids for the treatment of graft-versus-host disease after allo-SCT (n=7) or for the treatment of the underlying disease (n=5). Only 3 patients (9%) were affected by diabetes mellitus that was unrelated to steroid administration.

The diagnosis of IM was proven in 20 cases (63%) and probable in 12 cases (38%). In approximately one-third of cases (n=11), 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 had 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: L-AmB (n=12), lipid complex AmB (n=2), AmB (n=1). In the remaining 5 cases, posaconazole (n=2), voriconazole (n=1) and caspofungin (n=2), 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

Table 1. Clinical characteristics and risk factors of 32 patients who developed invasive mucormycosis.

	N	%
Gender		
M	18	56
F	14	44
Underlying disease		
AML	20	64
ALL	3	9
Multiple myeloma	3	9
Lymphoma	3	9
SAA	3	9
Phase of hematologic disease		
Induction AML / ALL	13	41
Relapse/salvage	4	12
Consolidation AML	2	6
allo-SCT	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 <0.5x10⁹)		
Yes	22	69
No	10	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	5	16
L-AmB	27	84
L-AmB dose		
3 mg/kg	10	37
5 mg/kg	14	48
>5 mg/kg	3	15
Surgery		
Yes	13	41
No	19	59
Recovery from neutropenia*		
Yes	16	6
No	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-SCT	4	12

*AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; SAA: severe aplastic anemia; allo-SCT: allogeneic hematopoietic stem cell transplantation; CyA: cyclosporine A; HC: hematologic condition; L-AmB: liposomal amphotericin B; IM: invasive mucormycosis; POS: posaconazole. ^1 CNS 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 immunosuppressants. #1 L-AmB, 2 voriconazole.*

Table 2. Univariate analysis of factors influencing treatment success.

	All	Favorable	Death due to IM	P
	Cases	23	9	
Gender				0.9
M	18	13	5	
F	14	10	4	
Age (years)				0.2
<50	12	10	2	
>50	20	13	7	
Underlying HC				0.1
AML	20	16	4	
other	12	7	5	
Allo-SCT				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
3 mg/kg	10	6	4	
=/> 5 mg/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	

*AML: acute myeloid leukemia; HC: hematologic condition; SAA: severe aplastic anemia; allo-SCT: allogeneic hematopoietic stem cell transplantation; L-AmB: liposomal amphotericin B; POS: posaconazole. *Out of 22 patients neutropenic at the onset of IM. §These data coinciding with immunosuppressive therapy.*

to only 3 patients (9%). Among the 29 patients (91%) receiving Lip-AmB+POS as second- or third-line treatment, 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%). Median duration of combined treatment was 32 days (3-157 days). In 3 cases (9%), deferasirox was added to Lip-AmB+POS.

None of the patients had to stop antifungal treatment because of drug-related toxicity. A comparison of patients who received L-AmB at 3 mg/kg with those who received L-AmB at 5 mg/kg or higher found that none of them showed relevant nephrotoxicity.

After a median follow up of three 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 hematologic disease; a clinical improvement of IM was observed in 5 of these cases. Maintenance treatment with oral posaconazole was administered in all 18 responsive cases (56%) for a median of 74 days (range 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 hematologic malignancy 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, no parameters were identified as being significant. 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 favorable clinical response was achieved (>70% if stable disease was included into the definition). This rate compares favorably 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 favorable response; all 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 was 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 the limited sample size and a tendency to perform surgery only on severely ill patients.

Finally, the influence of deferasirox could not be assessed in our analysis. While previous *in vitro* studies as well as animal studies suggested a synergistic effect of deferasirox in combination with L-AmB,[12] a recent interventional trial examining this failed to confirm such an association.[13] In our series, deferasirox was added only in 3 cases, all with a favorable outcome. Nevertheless, this should only be considered a subjective observation.

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 neu-

tropenia. Interestingly, in 6 (19%) of these cases, an allo-SCT could be performed.

Our case series may have been subject to selection bias, as only those patients who survived long enough to receive a combination therapy were included. However, since interventional trials on this question are unlikely to be performed any time soon, data from registries remain the only available source of structured information on the treatment of mucormycosis.

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.

Livio Pagano,[1] Oliver A. Cornely,[2,3] Alessandro Busca,[4] Morena Caira,[1] Simone Cesaro,[5] Cristiana Gasbarrino,[6] Corrado Girmenia,[7] Werner J. Heinz,[8] Raoul Herbrecht,[9] Cornelia Lass-Flörl,[10] Annamaria Nosari,[11] Leonardo Potenza,[12] Zdenek Racil,[13,14] Volker Rickerts,[15] Donald C. Sheppard,[16] Arne Simon,[17] Andrew J. Ullmann,[8] Caterina Giovanna Valentini,[1] Jörg Janne Vehreschild,[2] Anna Candoni,[18] and Maria J.G.T. Vehreschild[2]

[1]Istituto di Ematologia, Università Cattolica S. Cuore, Roma, Italy; [2]1st Dept. Internal Medicine, University Hospital Cologne, Cologne, Germany; [3]Clinical Trials Centre Cologne, Center for Integrated Oncology CIO KölnBonn, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), German Centre for Infection Research, University of Cologne, Cologne, Germany; [4]Divisione di Ematologia, San Giovanni Battista Hospital, Torino, Italy; [5]Oncoematologia Pediatrica, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; [6]Divisone di Ematologia, Università Cattolica S. Cuore, Campobasso, Italy; [7]Dipartimento di Ematologia, Azienda Policlinico Umberto I, Roma, Italy; [8]University of Würzburg Medical Center, Department of Internal Medicine II, Würzburg, Germany; [9]Department of Hematology and Oncology University Hospital of Strasbourg, Hautepierre Hospital, Strasbourg, France; [10]Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria; [11]Divisione di Ematologia e Centro Trapianti Midollo, Niguarda Ca' Granda Hospital, Milano, Italy; [12]Dept. Oncology and Hematology, Modena e Reggio Emilia University, Azienda Ospedaliera Policlinico, Modena, Italy; [13]Dept. of Internal Medicine, Hematology and Oncology, Masaryk University and University Hospital Brno, Brno, Czech Republic; [14]CEITEC - Central European Institute of Technology, Masaryk University Brno, Brno, Czech Republic; [15]Dept. Infectious Diseases, Robert Koch Institute, Berlin, Germany; [16]Departments of Medicine and Microbiology and Immunology, McGill University, Montreal, Canada; [17]Dept. Pediatric Oncology and Hematology, Saarland University Hospital, Homburg, Saarland, Germany, and [18]Clinica di Ematologia, Università di Udine, Italy

Funding: Fungiscope is supported by unrestricted grants from Astellas Pharma, Gilead Sciences, MSD/Merck and Pfizer Pharma GmbH. This work was also supported by CELL – The CzEch Leukemia Study Group for Life. This study was partially supported by grants from Italian Ministry for University and Scientific Research (Fondi Ateneo Linea D-1-2011-2012).

Acknowledgments: 8 patients were already published elsewhere2, 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.

Correspondence: Livio Pagano. E-mail: lpagano@rm.unicatt.it
doi:10.3324/haematol.2012.083063

Key words: posaconazole, lipid formulations of amphotericin B, mucormycosis, combination therapy, invasive fungal infections, hematologic malignancies.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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