Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007

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Abstract

Zygomycosis is an important emerging fungal infection, associated with high morbidity and mortality. The Working Group on Zygomycosis of the European Confederation of Medical Mycology (ECMM) prospectively collected cases of proven and probable zygomycosis in 13 European countries occurring between 2005 and 2007. Cases were recorded by a standardized case report form, entered into an electronic database and analysed descriptively and by logistic regression analysis. During the study period, 230 cases fulfilled pre-set criteria for eligibility. The median age of the patients was 50 years (range, 1 month to 87 years); 60% were men. Underlying conditions included haematological malignancies (44%), trauma (15%), haematopoietic stem cell transplantation (9%) and diabetes mellitus (9%). The most common manifestations of zygomycosis were pulmonary (30%), rhinocerebral (27%), soft tissue (26%) and disseminated disease (15%). Diagnosis was made by both histology and culture in 108 cases (44%). Among 172 cases with cultures, *Rhizopus* spp. (34%), *Mucor* spp. (19%) and *Lichtheimia* (*formerly Absidia*) spp. (19%) were most commonly identified. Thirty-nine per cent of patients received amphotericin B formulations, 7% posaconazole and 21% received both agents; 15% of patients received no antifungal therapy. Total mortality in the entire cohort was 47%. On multivariate analysis, factors associated with survival were trauma as an underlying condition (p 0.019), treatment with amphotericin B (p 0.006) and surgery (p <0.001); factors associated with death were higher age (p 0.005) and the administration of caspofungin prior to diagnosis (p 0.011). In conclusion, zygomycosis remains a highly lethal disease. Administration of amphotericin B and surgery, where feasible, significantly improve survival.

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Introduction

Although often described as a rare fungal infection, zygomycosis (mucormycosis) appears to be increasing in frequency [1]. It mainly affects immunocompromized patients, such as those with haematological malignancies, recipients of haematopoietic stem cell (HSCT) [2] or solid organ transplants (SOT) [3], patients with diabetes mellitus and ketoacidosis [4,5], and infants with prematurity [6]. Zygomycosis may also affect immunocompetent patients with trauma or burns, or patients with elevated serum levels of iron under treatment with deferroxamine. The increased frequency of this infection is attributed to the rising prevalence of diabetes mellitus, as well as to the increased use of immunosuppressive treatments. The introduction of newer antifungals, in particular voriconazole, has also been suggested to play a role in the increased incidence of zygomycosis [7]. Due to the relative rarity of the disease it is difficult to perform stringent epidemiological studies to estimate its exact incidence. Most of the available data stem from case series and pertain to haematological patients [8] or to patients who have undergone transplantation [9]. An active population-based surveillance in the Bay Area of San Francisco, USA, was published in 1998 [10].

While mortality approached 100% in the older literature depending on the patients' underlying disease and the type of zygomycosis [11], it has been reduced since amphotericin B has become available [12]. Since then, however, it has remained essentially unchanged. Nevertheless, with the availability of the less toxic lipid formulations, survival rates of up to 85% have recently been reported in select patient populations [13,14].

Taking into consideration the challenges related to epidemiology, treatment and outcome of zygomycosis, a working group on zygomycosis was formed by the European Confederation of Medical Mycology (ECMM). The aim of the group was to analyse the clinical characteristics, microbiology, treatment practices and outcome of zygomycosis in Europe through a voluntary case registry. We present here the results of the first 3 years (2005–2007) of this effort.

Patients and Methods

Study design

In each participating European country, a national coordinator was appointed, who prospectively collected zygomycosis cases, recorded by the treating physicians in standardized case report forms (CRFs), which were then sent either by e-mail or fax to the general study coordinator. The national coordinators were all experts in the field of zygomycosis and in most cases were appointed by the respective national Mycology Societies. The participating hospitals were selected by the national coordinator on the basis of their capacity to document all episodes of zygomycosis occurring during the study period.

Mucorales isolates were initially identified at the participating institutions by the routine methods used in each laboratory. Molecular identification was then performed either at a national centre (Institut Pasteur in Paris, Medical University in Innsbruck, etc.) or the isolates were sent to a central laboratory in Spain (National Centre for Mycology, Madrid) for sequencing. Furthermore, coordinators were informed that they could send paraffin-embedded tissue for PCR identification to a central laboratory in Germany [15].

In order to be included as a case in the registry, sufficient information regarding diagnosis, predisposing factors and clinical presentation had to be provided. The CRFs were reviewed by the principal investigators (GP and AS) and queries were sent to the national coordinators in order to complete missing data. After completing the database, a data review committee examined all the data. The study was approved by the Ethics Committee of the University of Athens 'Laikon General Hospital', in Athens, Greece, the institution of the principal investigators. In addition, approval was also obtained from local ethics committees of all collaborating countries according to local regulations.

Definitions

For the classification of each case as proven or probable, the revised definitions of invasive fungal disease of the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) were used [16], with the following modification: if the diagnosis was made by histology and there was PCR testing on tissue positive for zygomycetes (from the central laboratory described above), the case was classified as proven zygomycosis, even if there were no cultures available. In addition, diabetes mellitus was also included in the host criteria.

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Diagnosis by histology was made if large, non-septate hyphae were reported. The sites of infection were classified as recently described by Roden *et al.* [4]. Mortality was assessed as all-cause mortality during the course of zygomycosis.

Statistical analysis

Statistical analyses were conducted using SPSS v.16. Differences between the qualitative variables in two or more groups were analysed by a chi-square test. A two-sided p value of <0.05 was considered significant. For the estimation of predictors of outcome the following method was used. Univariate analysis was performed using logistic regression for each variable separately. Variables entered into univariate analysis included age, sex, underlying diseases, treatment with corticosteroids, immunosuppressives or antifungals prior to the diagnosis of zygomycosis, sites of infection and types of treatment. The variables for which a statistically significant relationship was shown (p <0.05) were used to construct a new multivariate model using the logistic regression approach. The multivariate results presented are those from the most parsimonious model, including only the selected covariates.

TABLE 1. Underlying conditions predisposing to zygomycosis and mortality[a]

Underlying condition	Number of patients (%)	Number of patients who died (%)[b]
Haematological malignancies[c]	102 (44)	49/95 (52)
Acute myeloid leukaemia	49/102 (48)	
Acute lymphoblastic leukaemia	22/102 (22)	
Non-Hodgkin lymphoma	11/102 (11)	
Myelodysplastic syndrome	6/102 (6)	
Other	12/102 (12)	
Haematopoietic stem cell transplantation[d]	21 (9)	13/17 (76)
Other malignancies	11 (5)	5/9 (56)
Solid organ transplantation	10 (4)	5/10 (50)
Diabetes mellitus	39 (17)	18/33 (55)
Trauma	39 (17)	13/32 (41)
Burn	7 (3)	2/6 (33)
HIV/AIDS	4 (2)	0/3 (0)
Aplastic anaemia	4 (2)	1/4 (25)
Other[e]	9 (4)	4/9 (44)

[a]Nineteen patients (8%) had more than one underlying disease, so the total is more than 100%.
[b]The number of patients is smaller in this column than the number in the left column because some data regarding outcome were missing.
[c]The patients who underwent transplantation are not included in this group.
[d]Haematopoietic stem cell transplantations (n = 21) were allogeneic, except for two cases, for whom the treating physicians stated that the patients had undergone 'bone marrow transplantation' without further clarification.
[e]One patient had neutropenia due to treatment for psoriatic arthritis, one was receiving deferroxamine, and the other seven were receiving corticosteroids for various reasons (two for chronic obstructive pulmonary disease, two for Wegener's granulomatosis, one for panarteritis nodosa, one for Goodpasture's syndrome and one for rheumatoid arthritis)

Results

During the period between January 2005 and December 2007, 15 countries submitted 237 cases to the study, of which 230 were eligible for inclusion: Italy (60 cases), Greece (36), Germany (35), Switzerland (22), France (21), Belgium (16), Austria (12), Spain (9), Russia (6), Norway (5), Finland (2), Turkey (2), Czech Republic (2), Netherlands (1), UK (1). Seven cases were excluded because the data provided were not sufficient to classify them either as proven or as probable zygomycosis. The median age of the patients was 50 years, ranging from 1 month to 87 years; 60% of the patients were of male gender. Seventeen patients were younger than 14 years and three were ≤1 year old.

Underlying conditions

The patients' underlying conditions are listed in Table 1. In the month prior to diagnosis of zygomycosis, 106 (46%) patients received corticosteroids and 101 (44%) received other immunosuppressive medications (cyclosporine, mycophenolate-mofetil, tacrolimus, everolimus, methotrexate, cyclophosphamide, daunorubicin, vincristin, cytarabin, chlorambucil, fludarabine or novantrone). One hundred and ten patients (48%) received antifungals in the same period: fluconazole (19%), itraconazole (3%) and posaconazole (1%) as prophylaxis, and voriconazole (19%), caspofungin (13%) and amphotericin B (13%) as empirical treatment.

Eight patients with haematological malignancies also had diabetes mellitus, secondary to steroid use in most cases, while in one case, the patient had acute lymphoblastic leukaemia and AIDS.

Diabetes was the sole predisposing factor in 21 (9%) patients. In another 18 (8%), diabetes was combined with other underlying conditions, such as malignancy or trauma. Of the 39 cases with trauma, 18 had surgical trauma and 4 had various underlying diseases (non-Hodgkin lymphoma, Ewing sarcoma, solid organ transplantation and diabetes). The remaining 16 patients were immunocompetent and trauma was the sole predisposing factor for zygomycosis. In another two patients no predisposing factor was found. Thus, in total, there were 18 (8%) patients that were considered immunocompetent.

The majority of children in the <14 year age group had an underlying haematological malignancy (n = 8, 47%), while the rest had trauma (2), solid organ transplantation (2), allogeneic bone marrow transplantation (1), osteosarcoma (1), type 1 diabetes mellitus (1), aplastic anaemia (1) and Pearson's syndrome (1). Two of the three infants had undergone solid organ transplantation and one had Pearson's syndrome.

Sites of infection

The types of infection by site are shown in Table 2. The distribution of reported sites in children was similar. Statistical

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TABLE 2. Sites of infection and mortality among 230 patients. The outcome was evaluated in 200 patients, of whom 94 (47%) died

Type of infection by site	Number of patients (%)	Number of patients who died (%)[a]
Sinus		
Overall	62/230 (27)	28/58 (48)
Sinusitis	27/62	9/26 (35)
Sino-orbital	9/62	5/8 (63)
Rhinocerebral	26/62	14/24 (58)
Pulmonary		
Overall	68/230 (30)	33/59 (56)
Localized[b]	65/68 (96)	30/56 (54)
Deep extension	3/68 (4)	3/3 (100)
Cutaneous		
Overall	59/230 (26)	13/47 (28)
Localized[c]	36/59	8/30 (27)
Deep extension	23/59	5/17 (29)
Cerebral, isolated[d]	5/230 (2)	1/5 (20)
Disseminated[e]	34/230 (15)	18/31 (58)
Lung	31/34	15/28 (54)
Sinus	11/34	6/10 (60)
Soft tissue	7/34	3/7 (43)
CNS[f]	18/34	13/17 (76)
Liver	6/34	3/5 (60)
Kidney	5/34	2/4 (50)
Other organ	8/34	7/8 (88)
Heart	1/230	1/1 (100)
Liver	1/230	0/1 (0)

[a]The total number of cases for each site is smaller than the one in the second column, because data about outcome were missing in some cases and were not included.
[b]Lung infection was characterized as localized when it was only in the lung tissue and with deep extension when it extended to adjacent tissues, such as the pleura, the heart, etc.
[c]Cutaneous infection was characterized as localized when it did not extend to underlying tissues (muscle or bone), in which case it was characterized as having deep extension.
[d]The total number of cases with involvement of the brain, including isolated cerebral, rhinocerebral and disseminated disease, was 49/230 (21%).
[e]Various combinations of involved organs were reported. The most common combination was sino-pulmonary zygomycosis (10/34, 29%).
[f]Five of the 18 cases of CNS involvement in disseminated disease were rhinocerebral zygomycosis with dissemination.
[g]Sino-orbital refers to cases involving the sinus and orbit, without extension to the brain.

analysis (comparison of frequencies of sites of infection for every underlying disease) showed a significant relationship between underlying disease and site of infection (Fig. 1). Haematological malignancy correlated with pulmonary disease: of 68 patients with pulmonary zygomycosis, 35 had a haematological malignancy (51.47%). *Vice versa*, diabetes correlated with rhinocerebral disease: of the 21 patients with rhinocerebral disease, 11 had diabetes (52.38%; $p < 0.001$).

Diagnosis

In 10 (4%) cases the diagnosis of zygomycosis was made post-mortem, and in a further six cases it was diagnosed ante-mortem and confirmed at autopsy. The various methods of diagnosis are presented in Table 3. Results of cultures were available in 172 patients (74%; Table 4). These results were confirmed by molecular sequencing methods in only 40% of cases. In the 58 patients with no culture results, either no material was sent for culture, or cultures were performed but were negative. Diagnosis was made by both histology and culture in 108 cases (44%). These 108 cases were classified as proven according to the modified EORTC/MSG criteria. Another four cases were classified as proven because the diagnosis was made by histology and PCR. The remaining 118 cases were classified as probable zygomycosis. The 35 patients who were diagnosed as having probable zygomycosis based only on positive cultures, had underlying malignancies or diabetes mellitus and a clinical picture of pulmonary or rhinocerebral disease.

Treatment and outcome

Various approaches to treatment were used, including antifungal chemotherapy and surgery (Table 5). Of the 230 patients included in the study, data regarding antifungal medication were available for 225 patients. Thirty-three patients received no antifungal medication, either because the diagnosis was made at post-mortem (in 10), or during the last 24 h prior to death (in 14), or because only surgical treatment was performed (in 9). Zygomycosis was successfully cured by the sole use of surgery in two patients who were burn victims with cutaneous disease, in two patients who had maxillary sinusitis but were otherwise immunocompetent and in one patient who had a brain abscess.

Of the 192 patients who received antifungal medication, 167 (87%) received amphotericin B as first line treatment. The formulation used most frequently was liposomal amphotericin B (130/167 patients, 78%). In 10 (6%) cases the formulation of amphotericin B used was unspecified, in 21 (13%) it was amphotericin B deoxycholate and in 7 (4%) it was amphotericin B lipid complex. Amphotericin B was given either alone or in combination with other antifungal agents (Table 6). In some cases where administration of amphotericin B was followed by posaconazole, there were reports of lengths of treatment of as long as 603 days. In the group of patients who received liposomal amphotericin B as the only antifungal medication and were cured, the median duration of treatment was 55 days (range, 14–169 days) and the median daily dose was 5 mg/kg (range, 3–10 mg/kg).

Total mortality was 47%. Mortality in the children's group was 27%. Mortality rates were variable depending on the underlying diseases (Table 1), on the site of infection (Table 2), and on treatment (Tables 5,6). Table 7 shows the risk factors affecting mortality found to be significant on univariate and multivariate analysis. As shown on multivariate analysis, age was an independent predictor of death. Increase of age by 1 year augments the risk of death by 3%. The same statistical model showed that patients who developed zygomycosis as a result of trauma had an 86% smaller probability of death compared with patients with haematological

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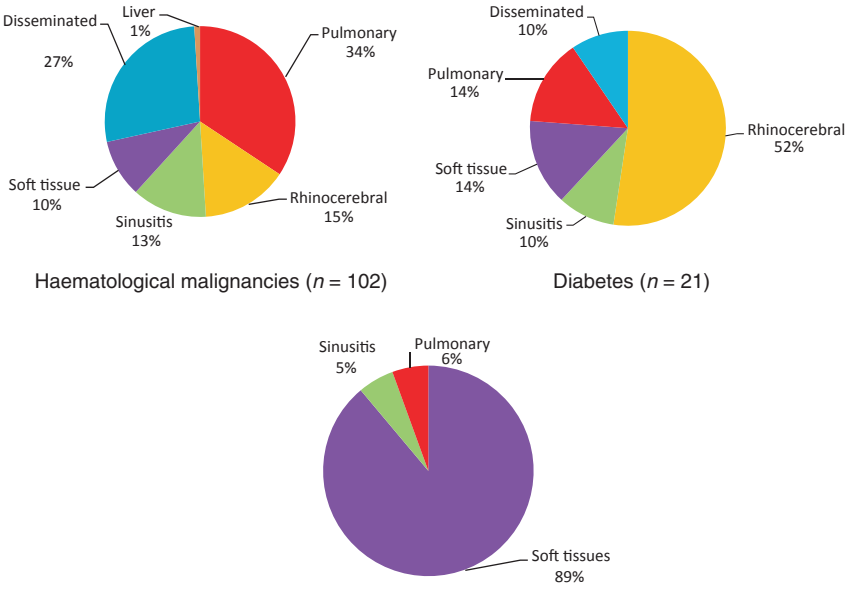

FIG. 1. Sites of infections in relation to underlying disease. Infection localized to sinuses is termed 'sinusitis', while if it extends to the orbit or the brain it is termed "rhinocerebral". Disseminated infection is defined as infection involving at least two non-contiguous sites.

TABLE 3. Laboratory methods used for the diagnosis of zygomycosis

Diagnostic methods	Number of patients (%)
Histology + culture + direct examination	69 (30)
Histology + culture	39 (17)
Histology + PCR[15]	4 (2)
Only histology	24 (10)
Only culture	35 (15)
Culture + direct examination	29 (13)
Histology + direct examination	16 (7)
Only direct examination	14 (6)

malignancies. Amphotericin B, either alone or with posaconazole or with posaconazole and other antifungals (in combination or sequentially), significantly decreased the risk of death. Finally, surgical treatment decreased the risk of death by 79%. Regarding administration of antifungals prior to the diagnosis of zygomycosis, voriconazole and caspofungin were found to be risk factors on univariate analysis, but only caspofungin remained as an independent risk factor on multivariate analysis.

Discussion

Thus far, this is the largest and geographically most diverse study on the contemporary epidemiology of zygomycosis in Europe. While no conclusions regarding incidence rates are feasible due to the absence of a denominator, several important observations can be made that are helpful to better understand the current evolution of the disease. The most common underlying diseases were haematological malignancies (n = 102, 44%), while diabetes was found in 39 patients (17%). This is in contrast to the extensive literature compilation by Roden *et al.* [4], who reported that malignancies constituted 17% of all cases, whereas diabetes was the underlying condition in 36% of 929 cases. This could be explained by the fact that the number of immunocompromized patients has dramatically increased in recent decades. It may also be partly due to the predominance of haematology centres participating in the study. In the analysis of 41 cases by Ruping *et al.* [17], malignancies were the predisposing factor in 63% of cases and diabetes in 17%, but in that study also the majority of contributing investigators were haematologists. It is not surprising that incidence rates are different in each study, because there has been no defined denominator in any of them, including the present one. Trauma was found to be a predisposing factor in 17% of cases. Of note, eight of the 18 cases (44%) with surgical trauma were submitted from the same centre and constituted an outbreak of zygomycosis in a Greek hospital [18], thus significantly influencing the percentage.

The most common sites of infection were the lungs, the sinuses and the soft tissues. Pulmonary zygomycosis was most common in patients with haematological malignancies,

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TABLE 4. Species of isolated zygomycetes. In another five cases, the fungus did not grow in cultures but was diagnosed by tissue PCR[a]

Isolated fungi	Number (%)
Rhizopus species	58 (34)
Not speciated	25
Rhizopus arrhizus (*Rhizopus oryzae*)	24
Rhizopus microsporus	7
Rhizopus microsporus var. rhizopodiformis	1
Rhizopus pusillus	1
Mucor species	33 (19)
Not speciated	31
Mucor circinelloides	2
Rhizomucor pusillus	5
Rhizomucor variabilis	4
Lichtheimia species[b]	32 (19)
Not speciated	5
Lichtheimia corymbifera	27
Cunninghamella species	8 (5)
Not speciated	3
Cunninghamella bertholletiae	5
Apophysomyces elegans	2 (1)
Saksenae species	1
Zygomycetes not specified	19 (11)
Total	172 (100)

[a]Two cases of *Lichtheimia corymbifera* from brain tissue, one of *Rhizopus oryzae* from sinus biopsy, one case of *Rhizopus pusillus* from lung tissue and one of *Rhizomucor* from bronchial aspirate.
[b]Older names *Absidia* and *Mycocladus*.

TABLE 5. Patients who received medical or surgical treatment

Treatment	Number of patients (%)[a]	Number of patients who died (%)[b]
Medical and surgical	90 (40)	20/84 (24)
Only medical	102 (46)	49/85 (58)
Only surgical	9 (4)	4/9 (44)
None	24 (11)	21/22 (95)
Total number of medical treatments	192 (85)	69/169 (41)
Total number of surgical treatments	99 (44)	24/93 (26)

[a]Data regarding treatment were missing in five cases. Percentages were calculated using 225 as the denominator.
[b]Data regarding outcome were missing in 30 cases, so mortality was calculated based on available data.

TABLE 6. Antifungal drugs administered as treatment for zygomycosis

Medication	Number (%)	Number of patients who died (%)[a]
Amphotericin B alone[b]	90 (39)	32/82 (39)
d-AmB	12/90 (13)	6/12 (50)
L-AmB	68/90 (76)	20/62 (32)
ABLC	4/90 (4)	2/4 (50)
Unspecified ampho B	6/90 (7)	4/4 (100)
Amphotericin B + posaconazole[b]	48 (21)	13/43 (30)
Amphotericin B + Posa + other[c]	13 (6)	2/11 (18)
Amphotericn B + other[c]	16 (7)	11/16 (69)
Posaconazole alone	17 (7)	6/10 (60)
Other[d]	8 (3)	3/6 (50)
None	33 (14)	26/31 (84)
Unknown	5 (2)	1/1 (100)
Total	230	94/200 (47)

[a]Data regarding outcome were missing in 30 cases, so mortality was calculated based on available data.
[b]Posaconazole was administered either in combination with (18 cases, 38%) or sequentially following amphotericin B treatment (28/48 cases, 58%). In two cases it was unclear whether they were given in combination or sequentially. The formulation of amphotericin B used was liposomal amphotericin B in 43 cases (90%).
[c]Caspofungin, itraconazole, voriconazole, fluconazole.
[d]Fluconazole, itraconazole, voriconazole, caspofungin. The three patients who survived had all undergone surgery.

and rhinocerebral disease in patients with diabetes (Fig. 1). These associations have been reported in other studies [4,17,19] and various pathogenetic mechanisms have been proposed [4]. However, the exact pathogenesis leading to the association between diabetes and rhinocerebral disease is not clear [20].

The diagnosis of zygomycosis is difficult, despite the availability of modern methods. As a result, 4% of all cases were diagnosed post-mortem (by histology and culture), while another 6% were diagnosed during the last 24 h before death. Of note, in older series, rates of post-mortem diagnosis were much higher. In a series published by Pagano *et al.* [21], ante-mortem diagnosis of zygomycosis was made in 54% of cases, while in another series of 185 cases of disseminated zygomycosis published by Ingram *et al.* [22], > 90% were diagnosed at post-mortem examination. Methods for diagnosis of zygomycosis varied from centre to centre (i.e. molecular typing was not performed on all isolates). The responsible pathogen was isolated in cultures in 172/230 (75%) cases. Similar rates have been reported by Roden *et al.* [4]: a positive culture result was obtained in 50% of cases, but there was a clear increase in culture positivity over time, with 71% of all cases since 2000 diagnosed on the basis of culture results. In another five cases the diagnosis was made by tissue PCR. The use of PCR is an emerging method aiding in the diagnosis of zygomycosis and, although it has several limitations, can be an important diagnostic tool if no other method is available, provided that the proper techniques are selected [15,23]. The most commonly isolated pathogens in the present study were *Rhizopus* spp., followed by *Mucor* spp. and *Lichtheimia corymbifera* (formerly *Absidia corymbifera*). Of note, *Lichtheimia* species reported in the present study constituted 19% of all zygomycetes isolated, while in the study by Roden *et al.* [4], the respective percentage was only 5%. This may be due either to an actual rise of these species, to improved identification of the fungus in recent years, to geographic differences, or to bias in reporting.

Treatment of zygomycosis was medical and/or surgical. Liposomal amphotericin B was the antifungal used in 68% (130/192) of patients who received medical antifungal treatment, while amphotericin B lipid complex was given to 4% (7/192) of patients. In some countries amphotericin B deoxycholate is still widely used, despite its toxicity. In this study conventional amphotericin B was administered to 11% (21/

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TABLE 7. Factors related to outcome on univariate analysis

	Univariate analysis				Multivariate analysis			
Outcome	**Odds ratio**	**p**	**95% confidence interval**		**Odds ratio**	**p**	**95% confidence interval**	
Age	1.02	0.002	1.00921	1.040164	1.03	0.005	1.008854	1.051944
Soft tissue	0.30	0.02	0.1170924	0.8145019	0.13	0.019	0.0265983	0.7209237
Trauma	0.09	0.003	0.0215813	0.4525246	–	–	–	–
Corticosteroids	2.15	0.008	1.223825	3.790619	–	–	–	–
Prior antifungal	2.08	0.011	1.182969	3.681554	–	–	–	–
Prior voriconazole	2.60	0.011	1.241533	5.452466	–	–	–	–
Prior caspofungin	3.69	0.009	1.388624	9.809659	5.70	0.011	1.492133	21.82155
Prior azole	2.04	0.014	1.153278	3.624237	–	–	–	–
Medical treatment	0.18	0.001	0.0731678	0.488193	–	–	–	–
Treatment with amphotericin B alone	0.18	0.001	0.0661397	0.50083	0.14	0.006	0.036421	0.5659631
Treatment with amphotericin B and posaconazole[a]	0.14	0.001	0.0503203	0.4342687	0.09	0.003	0.0215417	0.442235
Treatment with amphotericin B and posaconazole and other antifungal[b]	0.04	0.008	0.0043601	0.4335625	0.05	0.029	0.0046377	0.7538557
Surgical treatment	0.16	<0.001	0.086244	0.303416	0.21	<0.001	0.0913973	0.4832143

Variables entered into univariate analysis included age, sex, underlying diseases, treatment with corticosteroids prior to the diagnosis of zygomycosis, treatment with immunosuppressives prior to the diagnosis of zygomycosis, treatment with antifungals prior to the diagnosis of zygomycosis, sites of infection and types of treatment. Only the statistically significant are shown ($p < 0.05$).
[a]Posaconazole was given either in combination with or sequentially following amphotericin B administration.
[b]Posaconazole and other drugs were given either in combination with or after amphotericin B administration.

192) of patients who were treated medically. No comparison could be made between groups taking the various formulations of amphotericin B, because the numbers were not large enough and there were several confounding factors such as different underlying diseases and sites of infection. Multiple regression analysis showed that treatment with amphotericin B in general, whether alone or in combination, significantly improved outcome. The other factors favouring a better outcome were surgical treatment and trauma as an underlying condition.

Administration of voriconazole before the diagnosis of zygomycosis was found to be a risk factor for zygomycosis on univariate but not on multivariate analysis. There have been several reports suggesting that voriconazole use may play a role in the increased occurrence of zygomycosis [7,24]. It has also been postulated that exposure to voriconazole may lead to increased virulence of zygomycetes organisms [25]. Previous treatment with caspofungin was shown to be related to an adverse outcome on multivariate analysis. However, the confidence interval was very large, thus weakening the relevance of this observation. A possible explanation for the relatively poor outcome of patients receiving this antifungal prior to the diagnosis of zygomycosis is that caspofungin was given empirically for a presumed fungal infection, thus delaying the initiation of a drug with potent *in vitro* activity against zygomycetes (amphotericin B).

A significant number of patients developed breakthrough zygomycosis while receiving prophylactic therapy with an azole (40 received fluconazole, 7 received itraconazole and 2 received posaconazole). *In vitro* studies have shown that fluconazole has no activity against the Mucorales. Itraconazole may be active against some species [26–28], while posaconazole demonstrates good *in vitro* activity against several zygomycetes [28,29], whereas *in vivo* activity in animal models is species dependent [30] Breakthrough zygomycosis while on prophylaxis with these drugs has been reported in other studies [16,31,32]. These observations may signify resistance of some species to these antifungals; another factor that must be taken into account is that both itraconazole and posaconazole have problematic pharmacokinetics and even if the administered dose is correct, the serum levels may be suboptimal and therefore ineffective.

Total mortality in the present study was 47%, which is higher than that reported in two recent series [17,21]. Comparing the various clinical presentations, patients with disseminated disease had the highest mortality, followed by patients with pulmonary disease. The best outcome was seen in patients who were immunocompetent, of whom only one of 18 died (6%). The underlying disease leading to the worst prognosis was haematopoietic cell transplantation (mortality 76%), while patients with haematological malignancies and diabetes mellitus had similar outcomes (mortality 52% and 55% respectively). However, in patients who had only diabetes as an underlying disease, without any additional conditions such as malignancies or trauma (Table 1), mortality was 44% (8/18).

In conclusion, this large European study found that zygomycosis continues to be a disease with a dismal prognosis in about half of the cases. Clinicians treating patients with diabetes, haematological malignancies or trauma, or patients who are immunosuppressed for any reason, should have a high index of suspicion for the disease and make every effort

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to obtain tissue for histology, culture and, if possible, PCR. A combination of liposomal amphotericin B with surgery offers the best chance of recovery.

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Transparency Declaration

Potential conflicts of interest

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Appendix

Members of the ECMMM Working Group on Zygomycosis

The following investigators (listed by country) also participated in this study. Austria: A. Mayr (Innsbruck Medical University, Innsbruck). Belgium: M. Aoun (Institut Jules Bordet, Brussels). Germany: K. Tintelnot (Robert Koch Institute, Berlin), V. Rickerts (University Hospital, Frankfurt am Main), F. von Loewenich (University Hospital of Freiburg). Greece: A. Antoniadou (University of Athens, Athens). Italy: C. G. Valentini (Institute of Hematology, Catholic University, Rome), B. Posteraro (Institute of Microbiology, Catholic University, Rome), C. Girmenia (Department of Hematology, Policlinico Umberto I, University of Sapienza, Rome), C. Ossi (San Raffaele Scientific Institute, Milan), A. Pan (Institute of Tropical and Infectious Diseases, Spedali Civili, University of Brescia, Brescia). UK: B. Jones (Royal Infirmary, Glasgow).

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