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BRIEF REPORT

Rhino-orbito-cerebral mucormycosis, a retrospective study of 7 cases

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Abstract
Mucormycosis is an opportunistic fungal infection caused by fungi of the Mucorales order. It has a low incidence and is a potentially lethal infection that generally affects patients who are immunocompromised due to systemic disease. We report 7 cases of rhinocerebral mucormycosis in an 8-year retrospective study (2000-2008) in haematological patients. Early diagnosis is essential, and there must therefore be a high level of clinical suspicion in patients with predisposing factors. Certain diagnosis requires fungal cultures or biopsies of the affected areas that prove an invasion of the tissues by the characteristic hyphae. The key to treatment is early and aggressive surgical treatment, together with high intravenous doses of amphotericin B. Despite this, prognosis is poor and mortality is about 70%-80%.

PALABRAS CLAVE
Inmunodeprimidos;
Diabetes mellitus;
Sinusitis fúngica;
Mucormicosis rinocerebral;
Zigomicetos;
Rhizopus oryzae

Mucormicosis rinoorbitocerebral, un estudio retrospectivo de 7 casos

Resumen
La mucormicosis es una infección oportunista, producida por los hongos del orden mucorales. Tiene una baja incidencia. Es potencialmente letal y afecta a pacientes inmunocomprometidos. Presentamos 7 casos de mucormicosis rinocerebral en un estudio retrospectivo de 8 años (2000-2008) en pacientes hematológicos.
Es preciso realizar un diagnóstico precoz, para lo cual se ha de mantener un alto índice de sospecha clínica en pacientes con factores predisponentes. El diagnóstico de certeza requiere la realización de cultivos o biopsias de las zonas afectadas que demuestren la invasión de los teji-

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dos por las hifas características. La clave del tratamiento es el desbridamiento quirúrgico precoz y agresivo, junto con altas dosis de amfotericina B intravenosa. A pesar de este tratamiento, el pronóstico es desfavorable y la mortalidad es de un 70-80%

Introduction

Rhinocerebral mucormycosis (RCM) is a disease of low incidence that is potentially fatal. We conducted this retrospective study to confirm that there has been a progressive increase of the disease in recent years, and to describe the causes of this upsurge in incidence. In addition, it is important to know the disease and keep it in mind to facilitate early diagnosis and appropriate treatment.

RCM is an opportunistic mycological infection caused by fungi of the Mucorales group, of which the most important family is *Mucoraceae* and, of these, the genera most frequently involved are *Rhizopus*, *Absidia, Mucor* and *Rhizomucor*.[1,2] The entry point is the upper aerodigestive tract. Even with medical and surgical treatment, depending on the series, mortality is around 70%-80%.[3] Predisposing factors would be decompensated diabetes mellitus, major burns, leukaemia and lymphoma and other haematological diseases, transplants, kidney failure, severe dehydration, sepsis and cortico-therapy or continued anticancer treatment.[4,5] In our series, all patients were immunocompromised patients with haematological pathologies and many had been subjected to bone marrow suppression.

There have been several types of mucormycosis described: pulmonary, gastrointestinal, mucocutaneous, disseminated and rhinocerebral. The latter is the most frequent.[6]

It typically begins in the nasal cavity, paranasal sinuses or palate (Figure 1), from which it may extend to the orbit and brain; the most frequent early clinical manifestations are facial pain, proptosis and ophthalmoplegia.[7] The rapid, fatal course of the illness is explained by the invasion at the tissue level of blood vessels by hyphae that cause thrombosis and massive necrosis and quickly reach the central nervous system. Sometimes, there is a fulminant course capable of ending the patient's life in less than 24 hours.[8,9]

Material and methods

A retrospective study was conducted from 2000 to 2008 in our hospital. We found 7 patients with clinical diagnosis of RCM. All were patients with haematological diseases, and 3 of them were diabetic (Table).

One patient was excluded due to an intraoperative false positive. The patient was diagnosed with acute leukaemia with a high grade of malignancy, presenting a unilateral nasal obstruction and a crusty blackish rhinitis in the immediate post-transplant. He was intervened urgently and rapid biopsies were taken. At a first instance, the biopsies could not rule out the presence of hyphae and mucormycosis. It was decided to perform a micro-debridement and resection of inflammatory tissue with necrotic appearance, which corresponded to the turbinates and part of the septum. After culture and the corresponding microbiological analysis, the disease was ruled out.

The fungal culture was carried out using Agar-Sabouraud media at 30 ºC. Histological samples were tested under white fluorescent lighting (calcofluor, according to the technique of Becton-Dickinson, Le-Pont-de-Claix, France) adding 20% potassium hydroxide. They were incubated at 37, 45 and 56 ºC and subjected to both macroscopic and microscopic study with haematoxylin-eosin staining and silver-methanamide staining. The typical fungal structures were elongated, branched at variable angles and with an irregular diameter.

Treatment with intravenous liposomal amphotericin B (2 mg/kg/day) was always introduced upon suspicion of RCM. A rigid trans-nasal endoscopy was performed in search of blackish necrotic plaques. Once the rapid biopsy and urgent microbiological culture (search for non-septate hyphae and spores) were carried out, a computed tomography (CT) of the paranasal sinuses and the facial mass was performed urgently, to seek signs of destruction or disruption of soft tissue, or occupation of sinuses by soft tissue. Only in one case was cranial magnetic resonance performed, since the endoscopy showed no evidence of necrosis, and there were doubts about the CT.

The surgical treatment received ranged from micro-debridement and maxillary antrostomy, anterior and

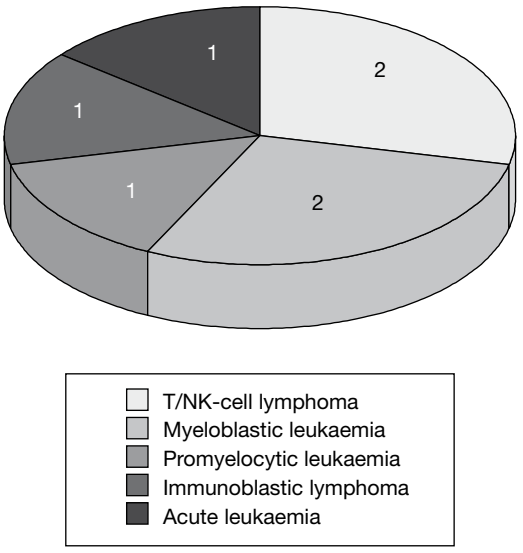

Figure 1 Distribution of haematological patients in our sample: 2 patients with T/NK-cell lymphoma, 2 with myeloblastic leukaemia, 1 with promyelocytic leukaemia, 1 with immunoblastic lymphoma, and 1 with acute leukaemia.

Table Patients and clinical parameters

Patient	Age/ Gender	Month	Surgical Treatment	Favouring factors	Symptoms	Prognosis
1	54 M	May	SNES (U, MA, Et)	AB, MH, corticotherapy, nasal T/NK-cell lymphoma	Fever. Unilateral NRF. Algia over right maxillary sinus	Good. Recovery from medullar aplasia
2	59 M	September	SNES: resection of inferior and middle turbinates, U, MA, Et, Sp	AB. Post-transplant MAe (acute leukaemia). Not treated with amphotericin B previously. Type II DM	Fever. Hypoesthesia and paresthesias in left hemiface. Unilateral NRF	Bad. Dies after 72 h. No recovery from aplasia. There is no progression of mucormycosis
3	38 F	September	Open surgery. Maxilloethmoidectomy and resection of septum. Orbital exenteration	MAe. Immunoblastic lymphoma. Corticotherapy	Fever. NRF, epistaxis. Intense periorbital pain. Ptosis. Unilateral anisocoria. Periorbital oedema	Bad. No recovery from aplasia. Dies 24 h after surgery
4	32 M	October	SNES: resection of part of the septum U, MA, Et	MH. Dialysis with deferoxamine. Acute leukaemia	Pain only under maxillary palpation. Acute unilateral NRF	Good. Continues with hypoplasia for 11 more days
5	49 F	August	SNES: resection of inferior turbinate, U, MA, Et, Sp	MAe. Advanced nasal T/NK-cell lymphoma. Type I DM	Fever. Epistaxis and expulsion of black scab. Periorbital pain	Bad. Survival of 22 days after surgery. Progression of basal disease
6	68 M	June	SNES: resection of septum, MA, Et, Sp	Moderate MH. Myeloblastic leukaemia. Corticotherapy. AB. Type I DM	Hemifacial oedema, nasal NRF. Fever, mucopurulent and bloody rhinorrhea	Bad. Dies after 4 days. Worsening of general condition, progression of disease
7	51 F	September	SNES: MA, Et, Sp. Resection of inferior turbinate	MAe. Promyelocytic leukaemia. Corticotherapy. AB. Not treated with amphotericin B	NRF. Epistaxis. Right hemifacial intense pain. Fever	Bad. Dies after one week. No recovery from aplasia

AB: Broad-spectrum antibiotic therapy in the last week (imipenem, amikacin and vancomycin); DM: diabetes mellitus; Et: ethmoidectomy; F: female; M: male; MA: maxillary antrostomy; MAe: medullar aplasia. Normally in immediate post-transplant of bone marrow or after post-radiotherapy aplasia; MH: medullar hypoplasia; NRF: nasal respiratory failure; SNES: sinonasal endoscopic surgery; Sp: sphenoidectomy; U: unciformectomy.

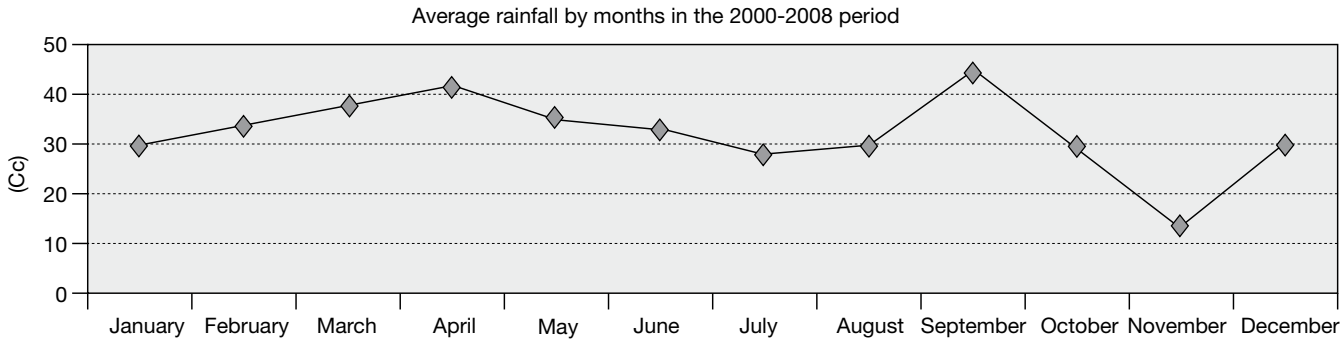

Figure 2 Average rainfall in Salamanca during the study period. Source: National Institute of Meteorology.

posterior ethmoidectomy and sphenoidectomy through endoscopic sinonasal surgery through to maxillectomy, maxilloethmoidectomy, orbital exenteration and partial resection of the soft palate. It was essential, as far as possible, to correct the metabolic condition of the patient or the underlying disease. Adjuvant antifungal therapy consisted of liposomal amphotericin B in doses of 2-4 mg/kg/day.

Results

We obtained 7 cases with clinical and microbiological diagnostic of RCM in the last 8 years at our hospital. All patients were haematological and 3 (42.86%) were diabetic. This is explained because in our environment we have a renowned haematology service, which carries out a large number of bone marrow transplants. If we analyze the sample of patients, we find disparity in the basal haematological disease: 2 patients (28.5%) had nasal T/NK-cell lymphoma as their basal disease, and one had immunoblastic lymphoma (14.29%), 2 had acute leukaemia immediately after transplantation (28.5%), one had promyelocytic leukaemia (14.29%), and other myeloblastic leukaemia (14.29%) (Figure 1).

The mortality of the series was 71.5%. The two patients who survived 3 months after surgery presented nasal T/NK-cell lymphoma (Case no. 1) and acute leukaemia (Case no. 4). In Case no. 1, the surgery was very early, 14 h after the patient showed nasal symptoms of nasal respiratory failure and pain above the maxillary region. The patient died from progression of the disease itself within 14 months. Case number 4 corresponds to a patient who sill had medullar hypoplasia 11 days after surgery. Like the previous case, the patient underwent surgery on the same day the symptoms began, due to suspicious clinical signs and observation of blackish nasal plates upon endoscopic examination.

Discussion

RCM is a rare opportunistic infection that affects immunocompromised patients. When it attacks immunocompetent patients, it is found in cases of extensive trauma or burns, drug addiction or prior major surgery.[10] Mucorales species are ubiquitous: they live in soil and water and are common inhabitants of decaying organic matter. In recent years, we have recorded an increase in cases, with an incidence of about one patient affected by RCM per year in our hospital. This might be attributed to an increased number of immunocompromised patients and also to increased awareness of this entity.

The entry point of Mucorales is the upper aerodigestive tract (nasal mucosa or digestive tract). The invasive form has high affinity for the arteries and grows along the internal elastic lamina, eventually penetrating the endothelium and cause thrombosis, stroke and extensive tissue necrosis.

In our series, 85.72% of the cases (6/7) occurred in the warmer and wetter months (May-September) (see Figure 2). These types of fungi require a high degree of humidity only at the time of inoculation for infection to occur, and a constant temperature around 24 °C is sufficient to perpetuate them. Herrero described that most of the colonies of the Mucorales can only be cultured from March to October, and that there is an autumn peak for zygomycetes, with a higher concentration of *Rhizomucor* at that time in our environment.[11] In our series, as in others reviewed, the most commonly implicated agent was *Rhizopus oriyzae*. This is found in non-sterile hospital adhesives, depressants, needles, catheters or wooden rods in microbiology laboratories[12] or even in hospital air.[13] Air conditioning favours the spread, infection and colonisation. In addition to inhalation, we are in contact with these species via the digestive tract or through direct inoculation. Mucorales are phagocytosed and are not pathogenic. However, if the defence mechanisms are impaired, they can become aggressive and produce acute invasive fungal rhinosinusitis, which may extend towards the orbit and into the central nervous system by the apex or the lamina cribrosa. There are two predisposing factors for the disease: metabolic acidosis and a deficit in the function of neutrophils and monocytes. In this sense, in those cases suffering from diabetic ketoacidosis, the fungal infection is favoured, since the Mucorales have a system of acetone-reductase that facilitates their growth in media rich in glucose with acid pH.[14] The pathogenesis of this disease is arterial invasion by the fungi themselves, causing thrombosis and tissue infarction.[15] This fact, coupled with a rapid progression and possible brain damage, is what determines its extreme gravity. The underlying illness, the time between the onset of the disease and the

establishment of treatment, and the occurrence of cerebral ischemic events play a role in worse survival rates. The favouring causes in our cases would be, in the first place, the aplasia that our haematological patients are subjected to, either due to their own illness or from being in recovery from bone marrow transplantation. This situation was more acute in the case of the 3 patients with diabetes (although none presented ketoacidosis at the time of diagnosis). Moreover, many of them (75%) were being treated with broad spectrum antibiotics, thus causing a destruction of bacterial flora and favouring colonisation by these opportunistic pathogens. Other favouring conditions in our series would be concomitant corticosteroid therapy (40%) or treatment with cytostatics (30%), the lymphoproliferative process itself or the patient who required dialysis and was being treated with deferoxamine. Deferoxamine saturates the iron-transferrin binding and favours an increase in the serum concentrations of this factor, thus enhancing fungal infection.[16]

The prognosis depends on the extent of infection, underlying diseases and the establishment of an early treatment.[17] Liposomal amphotericin B is the medical treatment of choice, coupled with an adequate surgical debridement of devitalised areas and the correction of the underlying metabolic and immune factors. Before the introduction of amphotericin B, survival was at 0-6%[18] Kidney function must also be controlled in order to prevent blood urea nitrogen and creatinine exceeding 50 and 3 mg/dl, respectively. If this happens, the dose should be decreased until kidney function recovers.

The disease progression and invasion of maxilla, orbits and brain occur frequently in just a few hours. Without aggressive surgical treatment, mortality reaches 100% in a short time. Adams describes an overall mortality of 67% But if analysed according to the clinical form, mortality reaches 100% in the gastrointestinal and disseminated form, 67% in the rhinocerebral and 16% in the cutaneous.[19] Our mortality was similar (71.5%). The worst prognostic factors involved in our series were immunosuppressive therapy (86%), bone marrow aplasia in patients in the immediate post-transplant period or bone marrow hypoplasia from the basal disease, orbital involvement (18%) and diabetes mellitus (43 %).

Although clinical signs are very suggestive, the definitive diagnosis requires cultures or biopsies of affected areas that show tissue invasion by the characteristic hyphae. In one patient in our series, the infection was not confirmed in the postoperative microbiological analysis. Indeed, the patient presented the non-specific clinical signs of nasal respiratory failure, pain and generalised nasal oedema with scabs adhered to the septum and turbinates. The imaging test showed only a pansinusitis and the rapid intrasurgical biopsy could not rule out the presence of hyphae. Therefore, a surgical micro-debridement of all the crusted and oedematous area was carried out. Such false positives are rare, but the aggressiveness of the disease and its high mortality without early treatment justify surgical intervention in cases of diagnostic doubt.

ACT is recommended as part of follow up, one month after the end of amphotericin treatment, and reviews by the ENT every 3 months for a period of not less than a year.[19]

Conclusion

Fungi are ubiquitous, but more likely to cause infection in warm and wet seasons. There are other factors involved in the pathogenicity of these fungi. Perhaps these organisms have a seasonal variation in their power to spread or pathogenic characteristics not yet well known. The entry point is the upper aerodigestive tract. The most frequently implicated agent is *R. oriyzae.*

Although clinical signs are very suggestive, the definitive diagnosis requires the performance of cultures or biopsies of affected areas showing tissue invasion by the characteristic hyphae. This devastating disease requires early diagnosis and treatment. Even with medical and surgical treatment, it may reach 70%80%mortality.

Conflict of interests

The authors declare no conflict of interests.

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