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***Chronic Invasive Rhinosinusitis by Conidiobolus***

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# Chronic Invasive Rhinosinusitis by

## *Conidiobolus*

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## Abstract

Chronic invasive fungal rhinosinusitis is a rare and potentially aggressive infection, characterized by nasal obstruction due to the presence of fungal hyphae infiltrating the mucosa, submucosa, bone, or blood vessels of the paranasal sinuses, facial pain and hyposmia. *Conidiobolus* is a very rare cause of chronic invasive fungal rhinosinusitis. This fungus predominates in tropical forests and usually is not present in Europe.

We aim to present the first case of chronic invasive fungal rhinosinusitis due to *Conidiobolus* diagnosed in a Portuguese patient.

We present a Caucasian 65 years old male patient with progressive nasal obstruction, bilateral frontal headache and a hyposmia with 8 months of evolution. He was diagnosed a chronic invasive rhinosinusitis associated with hypertrophied inferior turbinates and ulcerative nasal mucositis. The identification of *Conidiobolus* was performed in samples from surgical excision biopsies, by macroscopic observation of the colony, microscopic observation of the mycelium and molecular biology techniques. The patient was treated using liposomal B amphotericin and followed up for 3 years without interurrences.

Chronic invasive fungal rhinosinusitis by *conidiobolus* is not restricted to tropical forests anymore. European physicians must be aware of this possibility. Diagnosis is only possible by strong suspicious and conjugated efforts between surgeons and pathologists.

Key Words: invasive fungal sinusitis; Entomophthoromycosis; *Conidiobolus Coronatus*

## Introduction

Chronic invasive fungal rhinosinusitis (CIFR) is characterised by nasal obstruction or nasal discharge due to the presence of fungal hyphae within the mucosa, submucosa, bone, or blood vessels of the paranasal sinuses, facial pain and/or hyposmia.<sup>1,2</sup> It is potentially fatal<sup>3,4</sup> and its diagnosis and treatment are hard to achieve.

*Aspergillus*, *Rhizopus* and *Mucor* fungi are the most frequent aetiologies.<sup>3,5,6</sup>

A entomophthoromycosis is a zygomycosis that affects the subcutaneous tissues and can be classified as basidiobolomycosis or conidiobolomycosis<sup>7-13</sup>. In most cases, conidiobolomycosis is caused by *Conidiobolus Coronatus*, a filamentous fungus that predominates in African, Central and South American and Southeast Asian tropical forests. Usually, it is not present in Europe.<sup>7-10</sup>

This fungus affects more frequently the higher respiratory tract. Inhaled conidia penetrate the nasal mucosa and invade cellular layers, reaching blood vessels and disseminating to the rest of the nasal tissue, paranasal sinuses and other subcutaneous tissues of the face.<sup>7-9</sup>

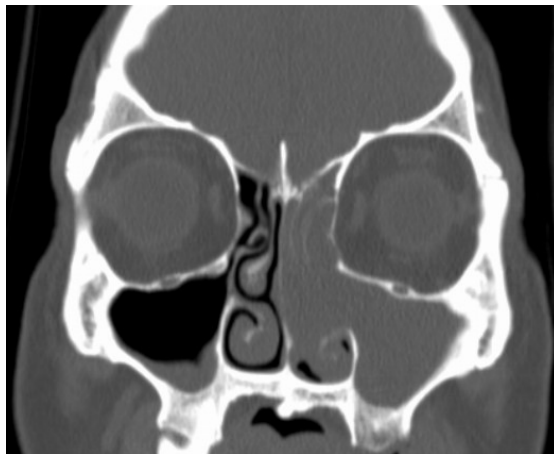
The diagnosis is based on the association of clinical and imagiological presentation, histopathological exam and mycological techniques of lesions. To identify the fungi, a macroscopic observation of the colony, microscopic observation of the mycelium and molecular biology techniques are necessary.<sup>9-11</sup>

The treatment relies on surgical excision of the tissues and correction of anatomical damage due to mucosal infection, and antifungal agents, such as amphotericin B or itraconazole.<sup>12-13</sup>

To the best of our knowledge, we present the first described case of a Portuguese patient with a CIFR by *Conidiobolus Coronatus*.

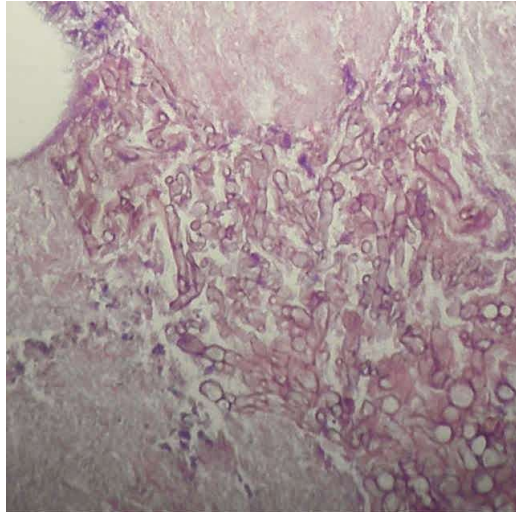
## Case Report

A sixty-five-year-old Caucasian male, living in the centre of Portugal without recent trips abroad, farmer, immunocompetent, presented with a progressive intractable nasal obstruction for 8 months, facial pain and hyposmia. Nasal endoscopy revealed nasal crusting and rhinorrhoea. Examination showed no focal neurologic complaints or other findings. Computed tomography (CT) showed a mass and bone erosion of the ethmoidal cells, maxillary sinus and nasal cavity. An initial diagnosis of mucocele was done and prompt surgery was proposed due to the erosive aspect.



*Figure 1 – Coronal CT shows a large left maxillary-ethmoidal-frontal mass*

Endoscopic functional sinus surgery with tissue debridement and tumoral resection was performed. Histopathology revealed fungal hyphae in the mucosa and submucosa, necrosis of tissue, and calcification, indicating the existence of a filamentous fungal infection.



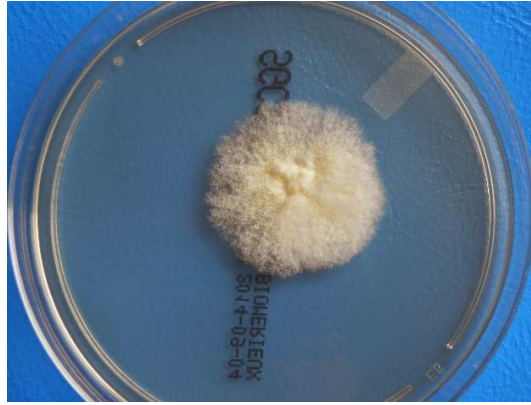
**Figure 2** – Tissue section stained with haematoxylin evidencing a mass of hyphae; Magnification 200x

The sample was initially cultured in chocolate-agar plates and a subcultured using Sabouraud/chloramphenicol-agar plates, incubated at 30°C. Four days later a colony with a macroscopic aspect remembering wax was observed.



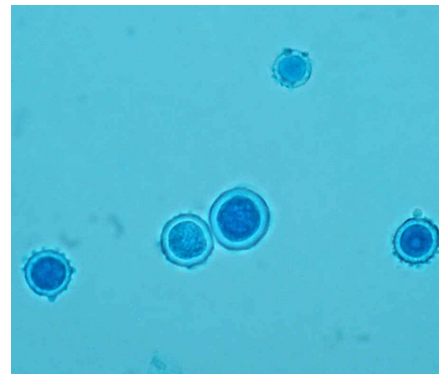
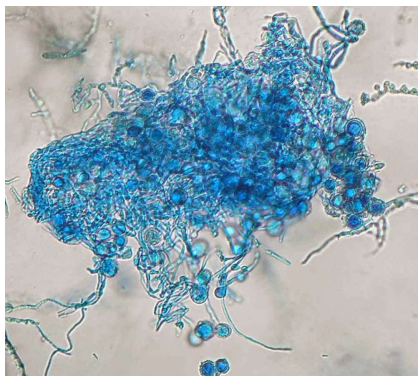
**Figure 3**- Culture of the sample in chocolate-agar plate, incubated at 37°C Typical wax-like and cream-colored colony

Afterwards, the periphery of the colony presented new small colonies contributing to a characteristic radial distribution. A new colony was cultured and four days later became radially folded and covered by a fine, powdery, white surface mycelium. The colony color became tan to brown.



**Figure 4** – A subculture of the colony in Sabouraud/chloramphenicol-agar plate, four days after sowing: the colony became radially folded and covered by a fine, powdery, white surface mycelium.

Microscopically, simple conidiophores forming solitary, single celled terminal spherical conidia with a prominent papilla (10-25  $\mu\text{m}$  diameter) were observed. Conidia may also produce hair-like appendages, called *villae*. These laboratory features and epidemiological data, supported the identification as *Conidiobolus*.



**Figure 5a and 5b**- At the microscopic level it was possible to observe hyphae presenting typical simple conidiophores forming solitary, terminal spherical conidia, 10 to 25  $\mu\text{m}$  in diameter, single-celled with a prominent papilla. Conidia may also produce hair-like appendages, called *villae*

A segment of the ITS and D1/D2 rDNA regions was amplified and the product sequenced by DNA Sanger method. The molecular analysis confirmed the identification of *Conidiobolus Coronatus*.

Post operatively, intravenous amphotericin B was initiated for 10 weeks. No significant morbidities were observed during follow up, and 3 years after discharge, the patient remained asymptomatic.

## Discussion

Rhinosinusitis is defined as an inflammation of the nose and paranasal sinuses mucosa. The criteria defined by the European Position Paper on Rhinosinusitis and Nasal Polyps are: nasal obstruction and/or nasal discharge, associated with facial pain and/or hyposmia<sup>1</sup>. Rhinosinusitis can be bacterial, viral or fungal. It is chronic if present for more than 12 weeks.  
1,2,3,6

Fungal rhinosinusitis can be classified as non-invasive, allergic or invasive. The invasion is defined by the progression of the disease and the ability to penetrate through vessels, spreading into nearby tissues, such as the central facial bones, dural venous sinuses or the eye.  
3-6

Several theories have been presented to explain the development of chronic rhinosinusitis (CRS) but there is still no international consensus. However, it is widely accepted that the basic mechanism relies on an unbalanced interaction between host defences and environmental factors at the site of the inflammation. In fungal CRS, nasal cavity invasion stimulates the development of an inflammatory process with subsequent eosinophilic degranulation. These two processes induce tissue damage to the nasal cavity and the development of CRS symptomatology.<sup>1,2</sup>

A entomophthoromycosis is caused by fungi of the entomophthorales order, where are included the *Conidiobolus* genus which is constituted by the species *Coronatus*, *Lamprauges* and *Incongruus*.<sup>7,9,11-13</sup> *Conidiobolus Coronatus* was first described in 1897, as a filamentous saprophytic commensal fungus present in soil and dry vegetations, and a pathogen in the digestive tract of several species of insects and reptiles. It has a higher prevalence in tropical forests of Africa, South America and Asia.<sup>7-9</sup>



The fungus from this genus can enter the human body through the nasal cavity by inhalation of conidia that penetrate the mucosa through small traumatic breaches and the action of some enzymes.<sup>7,8-9,11-13</sup> Usually, the patients are immunocompetent. Although some literature points to an especially higher risk for rural outdoor workers, there are no proven risk factors. Small reports describe a higher prevalence in males (8:1 versus females) between 20 and 60 years of age.<sup>9,13</sup>

Clinically, the proliferation of the fungus originates an inflammatory process, induces nasal obstruction, inflammation of the paranasal sinuses, and hyposmia. In addition, the proliferation of the fungus will originate a hyphal mass that together with the inflammatory process will induce tissue invasion that may extend to other subcutaneous tissues of the face.<sup>7-14</sup> Later, subcutaneous nodules may develop worsening nasal obstruction and inducing facial deformities.<sup>12</sup>

Diagnosis is difficult. It's crucial to correlate clinical findings, facial CT-scan and the histopathological and mycological examination.<sup>7-14</sup>

Fungal cultures are difficult and frequently false negative. The histopathological study, upon the staining of the tissues sections with haematoxylin, PAS or Gomory-Grocott, allow the visualization of scarcely septate hyphae surrounded by eosinophils, which indicates the existence of a filamentous fungus.<sup>9,12,13</sup>

The mycological analysis, based on the macroscopic visualization of the colonies and the microscopic visualization of the hyphae and conidiophores confirm the existence of a filamentous fungus, identifies the genus and molecular biology techniques allow species identification.<sup>8,9,13</sup>

The biggest difficulty in the diagnosis of this pathology relies on the late suspicion of its agent due to the lack of a pathognomonic or highly suggestive clinical presentation. Pre-

operative suggestion of fungal sinusitis (e.g. calcifications in the CT) may induce the surgeon to collect adequate quantities of biopsy specimens of diseased mucosa and bone adjacent areas. It also helps the pathologist to the prompt use of special stains and cultures for the detection of fungus.<sup>15</sup> In most cases, the existence of conidiobolomycosis was only investigated when a high deformity level of the face was visible. The later the diagnosis, the greater the mass of hyphae and the inflammatory process which increases the level of invasion and reduces the chances of an easy remission.<sup>12</sup>

Beyond the difficulties associated to the diagnosis, there is no consensus regarding the treatment, dosage or duration. Previous studies suggest the use of potassium iodide, itraconazole, and amphotericin B, combined with surgical removal of the lesions, if necessary. Moreover, there is no information on what to do in relapsing patients and the follow-up scheme used.<sup>7-14</sup>

Most published papers present cases discovered in high prevalence areas<sup>-14</sup>. Only four cases have been reported in Europe. Last June the first European case of *Conidiobolus sp.* in a patient who never travelled to tropical areas was published, making this the first reported case of a Portuguese patient.<sup>11,12,14</sup>

The literature on entomophthoromycosis by *Conidiobolus* is scarce and most aspects remain unclear. Our patient remains without nasal obstruction, regaining the sense of smell after 3 years of follow up. Being a farmer, makes him more exposed to the soil and microorganisms, but by itself does not explain the appearance of this infection for the first time in Portugal. The patient has not travelled recently to any high prevalence areas. Our case report, together with the report from Falces-Romero et al. (2017) suggest a change in geographic distribution of *Conidiobolus* which could be associated with environmental conditions.

A high index of suspicion for invasive fungal disease may lead to earlier diagnosis and probably to a better treatment with fewer morbidities and mortality. More studies are needed to fully understand the pathogeny of entomophthoromycosis and CRFS itself, as well as a broad study on antifungal drugs, making it possible to define the most adequate drug, dosage and duration of treatment, raising the percentage of cases on full remission and preventing complications and relapses.

## **Conclusion**

The authors present the first case of a CIFR by *Conidiobolus Coronatus* diagnosed in a Portuguese patient and emphasised diagnosis and treatment difficulties.

*Conidiobolus Coronatus* is a rare CIFR aetiology. Since there is not a pathognomonic presentation and the geographic distribution of this fungus is altered, it is important to collect adequate samples for mycological analysis to achieve an early and correct diagnosis, leading to a better outcome.

Fully pathogenic and epidemiologic comprehension of *Conidiobolus* infections should be future goals for further investigation, as well as defining a consensus on the treatment.

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## References

1. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* 2012; 3:1–298
2. Lam K, Schleimer R, Kern RC. The Etiology and Pathogenesis of Chronic Rhinosinusitis: a Review of Current Hypotheses. *Curr Allergy Asthma Rep.* 2015;15(7).
3. Jose Gavito-Higuera TTU, Carola Birgit Mullins TTU, Luis Ramos-Duran TTU, Hugo Sandoval TTU, Nassim Akle TTU, Ramon Figueroa MC of G. Sinusal Fungal Infections and Complications: A Pictorial Review. *Journal of Clinical Imaging Science;* 2016. p. 9.
4. Deshazo RD. Syndromes of invasive fungal sinusitis. *Med Mycol.* 2009;47 Suppl 1(May 2008):S309–14.
5. Chakrabarti A, Denning DW, Ferguson BJ, Ponikau J, Buzina W, Kita H, et al. Fungal Rhinosinusitis : A Categorization and Definitional Schema Addressing Current Controversies. 2009;(September):1809–18.
6. Brook I. Microbiology of sinusitis. *Proc Am Thorac Soc.* 2011;8(1):90–100.
7. Chowdhary A, Randhawa HS, Khan ZU, Ahmad S, Khanna G, Gupta R, et al. Rhinoentomophthoromycosis due to *Conidiobolus coronatus*. A case report and an overview of the disease in India. *Med Mycol* [Internet]. 2010;48(6):870–9. Available from: <http://mmy.oxfordjournals.org/content/48/6/870.full>
8. Gugnani HC. ENTOMOPHTHOROMYCOSIS DUE TO CONIDIOBOLUS. *Eur J Epidemiol* [Internet]. 1992 [cited 2017 May 31];392(3):391–6. Available from: <http://download.springer.com/static/pdf/608/art%253A10.1007%252FBF00158574.pdf?originUrl=http%253A%252F%252Flink.springer.com%252Farticle%252F10.1007%252FBF00158574&token2=exp=1496238293~acl=%252Fstatic%252Fpdf%252F608%252Fart%2525253A10.1007%2525252FBF00158574.pdf%253ForiginUrl%253>
9. Tadano T, Paim NP, Hueb M, Fontes CJ. [Entomophthoramycosis (zygomycosis) caused by *Conidiobolus coronatus* in Mato Grosso (Brazil): case report]. *Rev Soc Bras Med Trop* [Internet]. 2005;38(2):188–90. Available from: [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=15821798](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15821798)
10. Nayak DR, Pillai S, Rao L. Rhinofacial Zygomycosis Caused By *Conidiobolus Coronatus*. *Indian J Otolaryngol Head Neck Surg.* 2004;56(3):225–7.
11. Fischer N, Ruef C, Ebnöther C, Bächli EB. Rhinofacial *conidiobolus coronatus* infection presenting with nasal enlargement. *Infection.* 2008;36(6):594–6.
12. Bento DP, Tavares R, Martins MD, Faria N, Maduro AP, Araujo C, et al. Atypical presentation of entomophthoromycosis caused by *Conidiobolus coronatus*. *Med Mycol.* 2010;48(December):1099–104.

13. Moncada DC, Montes M, Molina V, Velásquez JB, Gómez CI. Infección orofacial por *Conidiobolus coronatus*. *Biomedica* [Internet]. 2016;36(supl.1):15–22. Available from: [http://www.scielo.org.co/scielo.php?script=sci\\_arttext&amp%5Cnpid=S0120-41572016000500003](http://www.scielo.org.co/scielo.php?script=sci_arttext&amp%5Cnpid=S0120-41572016000500003)
14. Falces-Romero I, Alastruey-Izquierdo A, García-Rodríguez J, First isolation of *Conidiobolus* sp. in a respiratory sample of a patient in Europe, *Clinical Microbiology and Infection* 2017, doi: 10.1016/j.cmi.2017.06.015.
15. Aribandi M, McCoy VA, Bazan C. Imaging features of invasive and noninvasive fungal sinusitis: a review. *Radiographics* [Internet]. 2007;27(5):1283–96. Available from: <papers3://publication/doi/10.1148/rg.275065189>