Allergic fungal rhinosinusitis due to *Curvularia lunata*

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SUMMARY

We report a case of *Curvularia lunata* infection in an immunocompetent male with an initial diagnosis of suspected left side allergic fungal rhinosinusitis (AFRS), treated surgically. He had a relapse of nasal polyposis and underwent a surgical revision under local anaesthesia with endoscopic nasal polypectomy. The histological examination of the surgical specimen showed an inflammatory polyp of the paranasal sinuses, with eosinophil and lymphocyte infiltration, but without evidence of fungi. However, *Curvularia* spp fungus grew in cultures of nasal sinus drainage and bioptical specimens. The fungus was identified by DNA sequencing as C. lunata. The patient was then treated with itraconazole (200 mg BID for 4 weeks), mometasone furoate nasal spray (100 mcg BID for 6 months) and normal saline nasal irrigations. At the last follow-up endoscopic evaluation after 19 month from treatment, the patient was symptomless and free from disease. No polyp recurrence nor seromucous discharges were noticed. This first case of *C. lunata*-associated AFRS reported in Italy, highlights the difficulty of this diagnosis and the usefulness of molecular identification of the fungal species involved.

KEY WORDS: Curvularia lunata, Rhinosinusitis, Nasal polyposis, Itraconazole.

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INTRODUCTION

Curvularia is a dematiaceous fungus present in the environment worldwide, mainly in tropical and subtropical regions (Revankar, 2007). The genus *Curvularia* comprises several species. Of these, mostly three ubiquitous species are known to cause several types of infection in both immunocompetent and immunocompromised hosts: *C. lunata*, *C. pallescens* and *C. geniculate*, *C. lunata* being the most commonly reported in human infections (Brandt, 2003). In immunocompromised subjects, these include superficial and deep mucocutaneous infections, brain abscesses, endophthalmitis and disseminated infection (Revankar, 2007). In immunocompetent patients, *C. lunata* is most commonly associated with allergic fungal sinusitis (Shubert, 2009), although the involvement of *C. inaequalis* has also been reported recently (Posteraro, 2010; Cruz, 2013).

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CASE REPORT

A 16-year-old immunocompetent male was admitted to the ORL Clinic of San Matteo Hospital Foundation, Pavia, Italy, in November 2006 with a diagnosis of suspect left side allergic fungal rhinosinusitis (AFRS). The suspicion was based on the presence of three out of the five Bent and Kuhn diagnostic criteria (Bent, 1994):

1) type I hypersensitivity,
2) nasal polyposis,

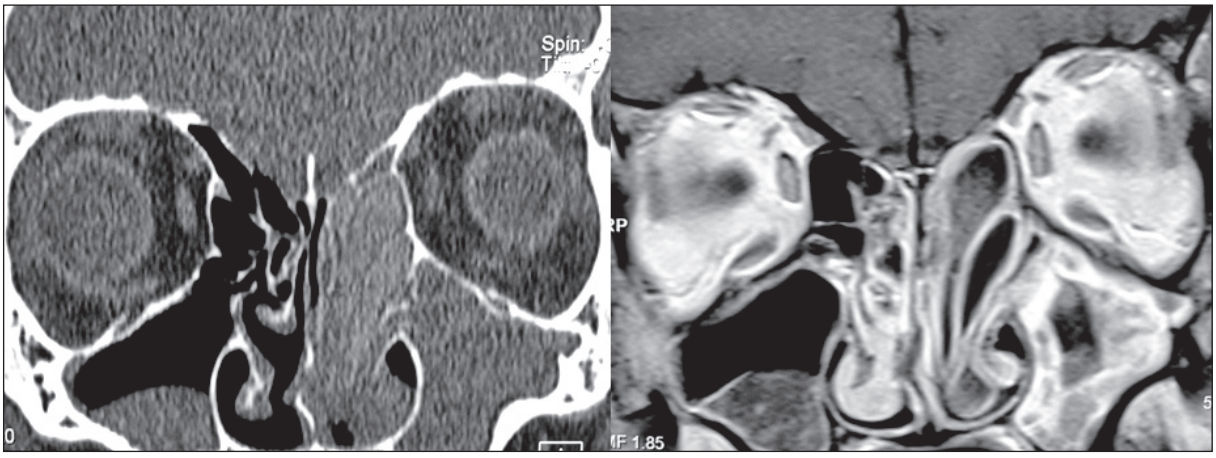

FIGURE 1 - *Computed tomography and magnetic resonance (coronal view) performed in 2006 showing thickening of the left side nasal mucosa, hyperdensity of mucin within left ethmoid and maxillary sinuses and polyps within left ethmoid and maxillary sinuses.*

3) characteristic findings on CT scan (Figure 1). He had a medical history of bronchial asthma since childhood, and atopy (prick tests were positive for pollen, *Dermatophagoides pteronyssinus*, *D. farinae*, *Parietaria*, and *Alternaria*). He underwent left side functional endoscopic sinus surgery (FESS): uncinectomy, ethmoidectomy, middle meatal antrostomy, and opening of the frontal recess.

In July 2008 he had a relapse of nasal polyposis and underwent surgical revision under local anaesthesia with endoscopic nasal polypectomy using a microdebrider. In April 2009, a second relapse of nasal polyposis was observed. A medical therapy with oral steroids followed by topical steroid administration was started. Subsequently, in October 2009, due to a further relapse of nasal polyposis, he underwent a left side FESS surgical revision under general anesthesia (endoscopic nasal polypectomy, revision of ethmoidectomy, middle meatal antrostomy and wide opening of the frontal recess) with removal of allergic mucin. The patient was discharged three days after surgery, with broad spectrum antibiotic therapy, tapering oral steroid therapy and normal saline nasal irrigations.

The histological examination of the surgical specimen showed an inflammatory polyp of the

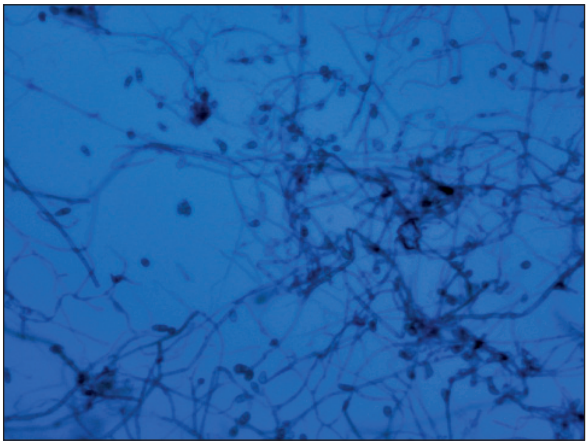

FIGURE 2 - *Curvularia lunata microscopic image (25X) showing dark septated hyphae, conidophores and multiseptated conidia.*

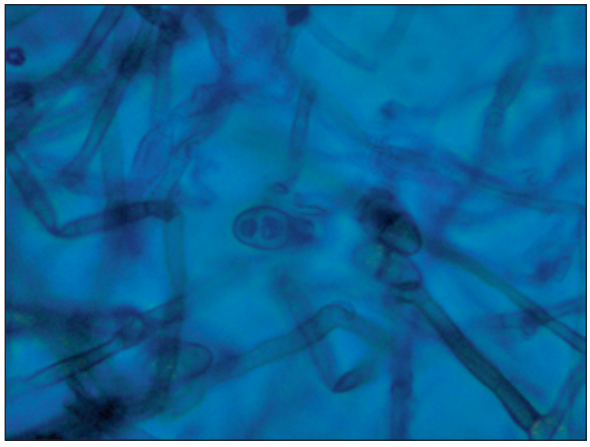

FIGURE 3 - *Curvularia lunata microscopic image (40X). Detail of a septated conidium with enlarged central cell and thick cell walls.*

paranasal sinuses, with eosinophil and lymphocyte infiltration, but without evidence of fungi. However, *Curvularia spp* fungus grew in cultures of nasal sinus drainage obtained before surgery in 2008 and again in 2009 from bioptical specimens. On both occasions the fungus was subsequently identified at the species level as *C. lunata* by molecular characterization, as described below. The patient was then treated with itraconazole (200 mg BID for 4 weeks), mometasone furoate nasal spray (100 mcg BID for 6 months) and normal saline nasal irrigations.

At the last follow-up endoscopic evaluation (May 2011), the patient was symptomless and free from disease. No polyp recurrence or seromucous discharges were noticed.

Microbiology

Macroscopic and microscopic examinations of fungal colonies were performed. Brown to blackish colonies with a moderate brown to black *verso* grew in 5 days in Sabouraud Dextrose Agar. On microscopic examination using lactophenol cotton blue, hyphae were dark and septated, with geniculated and elongated conidiophores; conidia were cylindrical or slightly curved, with one of the central cells larger and darker than the others (width 8-15 µm, length 20-35 µm) (Figures 2 and 3).

Molecular characterization was performed on isolates. Genomic DNA was extracted using the Ultra Clean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) and amplified using the universal fungal-specific primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) for internal transcribed spacer regions of fungal ribosomal DNA (White, 1990). PCR was performed in a 2700 thermal cycler (Applera, Foster City, CA, USA) set to the following conditions: denaturation at 94°C for 5 min; 40 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Amplicons were purified using a Microcon Centrifugal Filter Device (Millipore Corporation, Bedford, MA, USA) and sequenced using Big Dye terminators (Applera) in a 310 ABI PRISM® sequencer (Applera). Nucleotide sequences were analyzed using Finch TV software Version 1.4.0. and the GenBank BLAST database. The strain was identified as *C. lunata* (100% identity).

COMMENTS

Allergic fungal rhinosinusitis (AFRS) is a relatively rare form of chronic rhinosinusitis associated with uninvasive fungal infection affecting the immunocompetent host (Shubert, 2009). This pathology has been recently reviewed by Glass and colleagues (Glass, 2011). Clinically, patients with AFRS present with atopy, chronic sinusitis/rhinitis poorly responsive to standard therapy and with recurrent nasal polyposis, a high eosinophil count and elevated IgE levels (Taxy, 2006).

The diagnosis of AFRS is primarily histopathologic, being characterized by the presence of allergic mucin, with fungal hyphae on fungal staining, and/or surgically obtained sinus cultures positive for fungi (Das, 2009) and absence of mucosal fungal invasion, including commonly associated features of tissue invasion like mucosal necrosis, granuloma formation, or giant cells (Granville, 2004). Fungal allergens elicit an IgE-mediated and possibly type III hypersensitivity mucosal inflammation in the absence of invasion in an atopic host (Luong, 2005). The resulting inflammation leads to the obstruction of sinus ostia, which may be exacerbated by anatomic factors such as septal deviation or turbinate hypertrophy, resulting in stasis within the sinuses. This, in turn, creates an ideal environment for fungus proliferation, thus increasing the antigenic exposure (Luong, 2005). The local inflammatory response is responsible for inducing polyposis and allergic mucin (Das, 2009). AFRS needs to be differentiated from eosinophilic mucinous rhinosinusitis (EMRS), in which allergic mucin resembles AFRS, but no fungus is demonstrated on histopathology or culture (Shubert, 2009).

The diagnostic criteria for AFRS include the Bent and Kuhn criteria (Bent, 1994) and the Schubert and Goetz criteria (Schubert, 1998). The Bent and Kuhn criteria are more commonly applied in the clinical setting (Glass, 2011) and include:

1) type I hypersensitivity,

2) nasal polyposis;
3) characteristic findings on CT scan;
4) presence of fungi on direct microscopy or culture;
5) allergic mucin containing fungal elements without tissue invasion.

The Schubert and Goetz criteria (Schubert, 1998) include:

1) characteristic allergic mucin seen histopathologically and/or grossly;
2) positive fungal stain for hyphae within the allergic mucin, but not in the mucosa, or positive surgical sinus fungal culture in an otherwise characteristic patient;
3) sinus mucosa demonstrating eosinophilic-lymphocytic inflammation without evidence for tissue necrosis, granulomas, or fungal invasion;
4) exclusion of other fungal diseases. In our case AFRS could be confirmed as all criteria were met according to both systems.

The diagnosis of AFRS can be difficult since in roughly 30% of cases no fungi are demonstrated either on routine examination or using special stains, such as PAS or Grocott stain, and approximately 40% of cases are misdiagnosed or wrongly classified during the initial evaluation (Das, 2009; Luong 2005). There might be a discrepancy between histological and culture results, with fungal hyphae found on histology but negative culture, which can be explained by entrapment of fungal hyphae in mucus, thus preventing contact with culture media (Luong 2005), however, in our case, the opposite scenario was found. Moreover, it is important to emphasize that a differential diagnosis with EMRS without fungal involvement is required to avoid diagnostic pitfalls (Shubert, 2009).

Fungal rhinosinusitis is best treated with the association of surgical removal of mucin and postoperative medical treatment with steroids and itraconazole (Khun, 2000; Luong, 2004; Glass, 2011), and after such interventions our patient had no recurrence up to the last follow-up, 1 year and 7 months later. FESS is the standard of care for AFRS, followed by medical therapy. Surgery aims to restore ventilation of the paranasal sinuses and the natural drainage paths. Moreover, surgery aims at the complete removal of mucin and debris in order to eliminate the fungus antigenic stimulus. Postoperative endoscopic follow-up is pivotal in the clinical diagnosis of AFRS recurrence. The efficacy of systemic and topic corticosteroid therapy has been well documented. Benefits include increased cure rates (and milder disease in case of recurrence), longer time to revision surgery, and reduced systemic IgE levels.

Preoperative start of therapy is generally used to improve the intervention (exposure, blood loss), with a taper period after surgery. Antifungal therapy has been introduced due to high rates of recurrence following surgical therapy alone. Systemic treatment with antifungal drugs can significantly reduce the rate of recurrence of AFRS after FESS. Several investigators have evaluated the use of intranasal antifungal preparations with mixed results, and this approach needs further evaluation (Khalil, 2011). Finally, Mabry and colleagues (Mabry, 2000) showed that immunotherapy may also be effective in the treatment of AFRS, with elimination of nasal crusting and mucin deposits.

In conclusion, the case presented here highlights the difficulty of AFRS diagnosis, since the patient underwent several surgical procedures before a diagnosis could be made. In AFRS dematiaceous fungi (such as *Bipolaris* or *Curvularia*) are generally isolated from cultures of surgically drained mucin (deShazo, 1995). *Aspergillus* species, such as *A. fumigatus*, *A. niger* or *A. flavus* are isolated (Revankar, 2007). In this case, the *Curvularia* genus was isolated by culture of mucin and subsequent species identification by DNA sequencing. To our knowledge, this is the first case of *C. lunata*-associated AFRS reported in Italy. Molecular identification can help the diagnosis of AFRS and may be useful for differentiating dermatiaceous from other filamentous fungi in tissues, involved in the development of this pathology.

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