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# New Diagnostic Applications in Sporotrichosis

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## 1. Introduction

Sporotrichosis is a subcutaneous mycosis that is globally distributed. Areas of high endemicity are Latin America, South Africa, India and Japan (Lopez-Romero et al., 2011; Queiroz-Telles et al., 2011). This infection is caused by the dimorphic fungus, previously described as the single species *Sporothrix schenckii* (Coura, 2005), that is now named the *S. schenckii* species complex (Marimon et al., 2006, 2007, 2008), which is associated with plants and soil (Ramos e Silva et al., 2007). In humans, sporotrichosis has been regarded as a job-related disease that usually occurs in the form of isolated cases or small outbreaks and affects only people exposed to plants or soil rich in organic matter and occasionally laboratory technicians (Copper et al., 1992; Hajjeh et al., 1997).

The fungus *S. schenckii* was isolated for the first time in 1896 by Benjamin Schenck from a 36-year-old male patient who presented with lesions on his right hand and arm. This isolate was classified into the genus *Sporotrichum* (Schenck, 1898). Hekton and Perkins described the second sporotrichosis case in 1900. These investigators isolated the fungus and created the current denomination, *Sporothrix schenckii* (Hekton & Perkins, 1900, as cited in Coura, 2005).

In the early 1900s, sporotrichosis was a common disease in France, and many features of the fungus and the less-common clinical manifestations were described. At the same time, potassium iodide began to be used to treat sporotrichosis (Kwon-Chung & Bennett, 1992) and is a satisfactory therapy for treatment of sporotrichosis, until now.

The first reported case of natural animal infection was described in 1907 by Lutz and Splendore in rats from Brazil (Lutz & Splendore, 1907, as cited in Coura, 2005). In 1908, in Brazil, Splendore described the asteroid bodies around *Sporothrix* yeast cells, which are used to diagnose sporotrichosis during histological examinations (Kwon-Chung & Bennett, 1992).

Classically, subcutaneous mycosis is usually associated with puncture injuries in farmers, florists, leisure gardeners, landscapers and greenhouse workers. However, in the zoonotic sporotrichosis endemic area of Rio de Janeiro, Brazil, transmission of the disease has been occurring broadly in the populace through the scratches or bites of *S. schenckii* infected cats (Schubach et al., 2008). Less commonly, other form of acquisition of the infection is inhalation of fungus conidia from soil (Rohatgi, 1980) and from infected animals, being cats the most involved. Sporotrichosis is primarily a cutaneous disease. After inoculation, a

papule develops at the site of inoculation and generally ulcerates, forms into nodules and then develops proximal to the primary lesion followed by lymphatic distribution and often ulceration. This is the lymphocutaneous form and is followed in frequency by the fixed form, which is characterised by a localised lesion without lymphatic involvement (Schubach et al., 2008). Disseminated forms are unusual and frequently occur in immunosuppressed hosts (Schechtman, 2010). Disseminated cutaneous sporotrichosis is primarily characterised by multiple lesions scattered over several parts of the body or the spread of haematogenous lesions. Osteoarticular (Campos-Macías et al., 2006) and pulmonary lesions (Losman & Cavanaugh, 2004; Callens et al., 2005) are the most common extracutaneous forms. All forms are rare in normal hosts. Mucosal involvement is sporadic, and the conjunctiva is the most commonly affected mucosa (Schubach, et al., 2005). Meningitis is one of the worst complications of *S. schenckii* infection, and several recently reported cases describe AIDS as an underlying risk factor (Galhardo et al., 2010).

Pathogenic fungi of the genus *Sporothrix* are dimorphic in the environment or when cultured in the laboratory at 25-30°C. In the filamentous form, they present as hyaline, septate, branched hyphae with single-celled conidia of two types: hyaline and brown (dematiaceous). Hyaline conidia are small, ovoid and usually occur in the apical portion of conidiophores. Dematiaceous conidia are large and ovoid with a thick cell wall and are present along the entire length of the hyphae (Siegler et al., 1990). Macroscopically, filamentous *S. schenckii* colonies are initially white and gradually become brown to dark black as the fungus produces the conidia, which are unable to form chains. In contrast, the parasitic phase and the yeast phase of *S. schenckii* that develops when the fungus is cultured in appropriate culture media at 35°-37°C, resembles cigar-shaped oval yeast cells, which may have one or more buds (Ramos e Silva et al., 2007). Macroscopically, colonies of *S. schenckii* grown at 37°C have a yellowish-beige colour and a creamy texture.

*Sporothrix schenckii* belongs to the Fungi Kingdom. After the substantial fungal taxonomy revision by Guarro and co-workers, this fungus was placed in the *Ascomycota* Division, *Pyrenomycetes* Class, *Ophiostomatales* Order, *Ophiostomataceae* Family (Guarro et al., 1999). Recently, it was suggested that *S. schenckii* should not be considered the only taxon, which causes sporotrichosis in human and animals because four new species have been identified (Marimon et al., 2006, 2007, 2008).

Mycoses can be challenging to diagnose, and accurate interpretation of laboratory data is important to ensure appropriate treatment. Although the clinical manifestations of sporotrichosis are well described, the diagnosis of this mycosis cannot be based on clinical information alone because the symptoms of sporotrichosis overlap with those of other diseases. Also, a definitive diagnosis requires isolation of *Sporothrix* spp. on specific culture media or visualisation of the yeast form during direct examination of clinical specimens using specific fungal staining techniques. However, these procedures are time consuming, usually taking a minimum of 15 days, and lack sensitivity. Furthermore, *Sporothrix* spp. structures can be confused with those of other fungal pathogens when visualised microscopically. Given these difficulties, other techniques have been developed to supplement culture and microscopic examination. These laboratory tests have a rapid turnaround time and reasonable specificity and sensitivity.

In this chapter, some of the well-established clinical and laboratorial diagnoses will be discussed and current conventional diagnostic tools will be reviewed. Additionally, we will outline the development of novel diagnostic methods and discuss their relative merits and disadvantages.

## 2. Diagnosis

Sporotrichosis is classically diagnosed by correlation of clinical, epidemiological and laboratorial data. Erythematous, ulcerated, or verrucous lesions that appear at the site of a skin injury that are caused by trauma with material from vegetal origin or by a scratch or bite from an animal, such as a cat, with subsequent nodular lymphangitic spread, are strong clinical and epidemiological indicators of mycotic infection (Coura, 2005). To confirm the diagnosis, laboratorial examinations must be performed. Typical laboratory analyses involved in sporotrichosis diagnosis include microscopic examination and culture of clinical specimens, such as skin biopsies or pus, from the patient's lesions. Although, in cases of disseminated or extracutaneous sporotrichosis other specimens such as sputum, urine, blood, synovial and cerebrospinal fluids can be analysed depending on the affected organs or systems (Kwon-Chung & Bennet, 1992).

Currently, there are additional diagnostic tools available for the diagnosis of sporotrichosis. For instance, serological techniques involving antibody detection against *S. schenckii* antigens have been developed using different enzyme linked immunosorbent assays (ELISA) formats and antigenic preparations (Almeida-Paes et al., 2007a; Bernardes-Engemman et al., 2005). Molecular methods to detect *S. schenckii* DNA in clinical specimens, including tissue fragments, are also being studied in several laboratories to facilitate quick diagnosis of infection (Hu et al., 2003; Kano et al., 2003). The next sections will focus on these diagnostic applications for diagnosis sporotrichosis.

### 2.1 Clinical types and differential diagnosis

#### 2.1.1 Clinical types

Sporotrichosis affects both sexes and can occur at any age. In most cases, sporotrichosis is a benign infection that is restricted to the skin, subcutaneous tissue and adjacent lymph vessels (Kwon-Chung & Bennett, 1992; Rippon, 1988).

The disease can be divided into four clinical forms (Sampaio et al., 1954, as cited in Ramos-e-Silva et al., 2007):

The **lymphocutaneous** form is the most common presentation and accounts for up to 75% of cases and is the easiest sporotrichosis to diagnose. The lesions are usually located on the upper extremities and are characterised by the appearance of a primary lesion at the site of inoculation after two or three weeks. This lesion can be ulcerated with an infiltrated base or a papular, nodular, nodular-ulcerative, ulcerative-gummy or vegetative plaque. From the initial injury, it forms a chain of painless nodules along the path of the lymph vessels, which may soften and ulcerate with little exudates that can be classified as sporotrichoid (Figure 1). Usually, the regional lymph nodes are not involved, and no skin changes are observed between the nodes. Erythema can be present, but the pain is usually mild. A secondary infection may occur and is associated with increased erythema and suppuration.

The **fixed cutaneous** form is the second most common and accounts for approximately 20% of cases. The lesion remains confined to the inoculation site and the lymph vessels are not involved. The lesions are ulcers (Figure 2), verrucous, or infiltrated plaques. Small satellite lesions are common and are frequently observed in children. The upper and low extremities are the most common sites of lesions, and children present with a high index of lesions on the face. Unlike the lymphocutaneous form, patients with the fixed cutaneous form do not present systemic symptoms. However, if left untreated, the lesions may evolve into a chronic course, and a spontaneous involution of the lesion can occur.



Fig. 1. Lymphocutaneous sporotrichosis.



Fig. 2. Fixed cutaneous sporotrichosis.



The **disseminated cutaneous form** presents with nodular, gummy, or ulcerated disseminated lesions (Figure 3). Following inoculation of the skin, there is a haematogenous dissemination, which initially presents as softened subcutaneous lesions that can ulcerate after weeks or months. Although quite rare, it has been reported in AIDS patients and in patients submitted to a long course of steroid therapy as the first manifestation of this syndrome. Disseminated cutaneous sporotrichosis is more frequently observed (up to 16% of cases) during endemic zoonotic sporotrichosis in non-immunosuppressed patients infected by cat zoonotic transmission of the fungus (Barros et al., 2004; Freitas et al., 2010), which is attributed to multiple inoculations.



Fig. 3. Disseminated cutaneous sporotrichosis.

The **extracutaneous form** appears in less than 5% of cases and is difficult to diagnose. It arises after haematogenous spread of the fungus, conidial inhalation, contact with a skin lesion or direct inoculation of a mucosal site. Any organ or tissue can be affected by sporotrichosis, and the symptoms are specific to the organ involved and are then followed by fever and general commitment in some cases. Involvement can be unifocal or multifocal. Immunosuppression, caused by conditions such as diabetes, alcoholism, malignancy,

steroid therapy, chronic obstructive pulmonary disease (COPD) and AIDS, is common in the pulmonary and nervous forms (Kwon-Chung & Bennett, 1992; Rippon, 1988). Some points of emphasis are:

- Bones and joints are the most commonly affected sites after the skin. The most commonly affected bones are the tibia, small bones of hands, radius, ulna, skull and face. Additionally, the joints of the hands, elbow, wrist, knee and ankle are also commonly affected. Most patients have infection of a single joint, which presents as a solitary granuloma. Destructive arthritis (Figure 4) or tenosynovitis may occur.



Fig. 4. Bilateral destructive lesions on the knees and on the left wrist in a patient with osteoarticular sporotrichosis.

- Pulmonary infection is frequently caused by inhalation of conidia and is not caused by dissemination of the fungus. Clinically, pulmonary sporotrichosis manifests as a chronic pulmonary disease with cavitation or hilar massive lymphadenopathy, the later has a certain frequency of spontaneous resolution.
- Ocular infection can result from an exogenous infection or haematogenous dissemination. It may manifest as conjunctivitis with characteristic visible granulomas, episcleritis, dacryocystitis, corneal ulceration, uveitis, nodular iritis, retrobulbar lesion, panophthalmitis, ulceration or ectropia and can lead to total blindness in rare cases. Cases of isolated granulomatous conjunctivitis have been reported in Brazil without cutaneous disease and are related to cat zoonotic transmission (Barros et al., 2004).
- The mucosa in the mouth, pharynx, larynx and nose can be infected by both direct and haematogenous routes. Exanthema, ulceration, suppuration and vegetation are clinical manifestations.
- Infection of the nervous system presents as brain abscesses or chronic meningitis, which is indolent and is associated with hypoglycorrhachia, hyperproteinorrhachia and low mononuclear cell counts. A common and potentially serious complication in these cases is hydrocephalus.

The first-choice for treatment of the cutaneous forms is oral itraconazole. Oral terbinafine is an alternative medication. Potassium iodide, despite being a classical and inexpensive drug, is difficult to tolerate and does not have sufficient scientific background to be recommended for sporotrichosis. Treatment with venous amphotericin B is reserved for severe patients and is also an option for the disseminated forms involving internal organs. Immunosuppressed patients often need higher doses of itraconazole and longer courses of treatment (Kauffman et al., 2007).

### 2.1.2 Differential diagnosis

Lymphocutaneous sporotrichosis is fairly common and can be confidently diagnosed. However pyoderma, atypical *Mycobacterium* and *Nocardia* infection as well as leishmaniasis must also be considered (Coura, 2005).

The clinical symptoms of the fixed form can be similar to pyoderma, paracoccidioidomycosis, chromoblastomycosis, cutaneous tuberculosis, atypical mycobacteria, tertiary syphilis, leishmaniasis, and even skin cancer.

Lesions of the disseminated cutaneous form can be confused with other deep mycosis, such as paracoccidioidomycosis or histoplasmosis, or with atypical mycobacteria and non-infectious granulomatous diseases.

The disseminated extracutaneous forms are diagnosed differently according to the affected organ. Other fungal conjunctivitis, bacterial osteomyelitis, pulmonary fungal infections, tuberculosis and sarcoidosis should also be considered.

### 2.2 Conventional diagnosis

Depending on which clinical specimen is sent to the mycology laboratory for diagnosis, some procedures must be performed before microscopic and culture analyses. Skin biopsies should be sent to the laboratory in a sterile physiological saline solution. Water and formaldehyde are not suitable as transporter because they interfere with the microbiological tests. In the laboratory, skin/mucosa fragments have to be triturated using surgical scissors (Figure 5). Grinding the clinical specimen with a mortar and pestle should be avoided because it can destroy fungal structures present on the material. Pus from ulcerated lesions does not require special treatment and can be analysed directly after sample collection (Molinaro et al., 2010).



Fig. 5. Skin fragment being triturated for mycological examination.

Direct microscopic examination of the specimens is typically performed on specimens in 10% potassium hydroxide or 4% sodium hydroxide to detect parasitic cigar shaped-budding yeast-like cells using a light microscope with a magnification of 400X to 1000X. These fungal cells are small (2-6 $\mu$ m in diameter), rare and hard to detect during direct examination of the



specimens obtained from human patients (Kwon-Chung & Bennett, 1992) or from domestic animals, such as dogs (Schubach et al., 2006). On the other hand, when this test is performed on skin biopsies collected from cats infected with *S. schenckii* yeast cells are easily observable (Figure 6A) because cats have a high fungal burden on their lesions (Schubach et al., 2004). Although yeast-like cells can be observed during microscopic examination in a few cases, they do not provide a definitive diagnosis of sporotrichosis. *Histoplasma capsulatum* var. *capsulatum* and *Candida glabrata*, two other pathogenic fungi that may cause skin infections, can also appear as small, round or oval yeast cells similar to *S. schenckii* (Larone, 2002).

To identify the fungus, fluorescent methods using calcofluor white dye or fluorescent-antibody staining can be performed. However, these techniques are expensive and are not available in most laboratories, especially in underdeveloped countries. Staining methods, including the Gram and Giemsa preparations, also aid in the microscopic identification of clinical specimens from patients with sporotrichosis. When the Gram stain is performed on the clinical specimens, the yeast cells are positively stained and occasionally surrounded by giant or polymorphonuclear cells (Figure 6B). Although both fluorescent and staining methodologies facilitate the identification of fungal cells when compared to the clarification by hydroxide solutions, both procedures lack sensitivity (Lacaz et al., 2002).

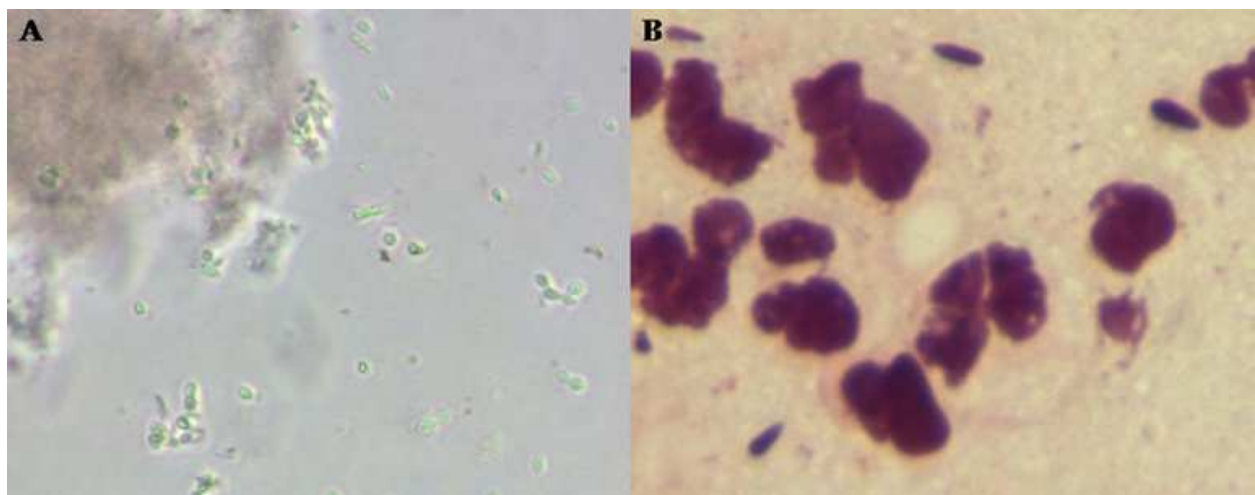


Fig. 6. Direct examination of clinical specimens from patients with sporotrichosis. (A) Skin biopsy of a cat with sporotrichosis showing several yeast-like budding cells. Magnification 400X. (B) Gram stain of pus from a human patient with sporotrichosis showing cigar shaped yeast-like cells. Magnification 1000X.

Histopathological examination using a tissue fragment in formaldehyde is important for the diagnosis of sporotrichosis. Although *S. schenckii* yeast-like cells may be seen in skin biopsies with the routinely used hematoxylin and eosin (H&E) stains, other stains such as Gomori methenamine silver (GMS) or periodic acid-Schiff (PAS) can be used to confirm identification of fungal elements (Figure 7A). Parasitic cells of *S. schenckii*, which can be found within phagocytic cells or in the extracellular space, are difficult to see due to the paucity of these cells in lesions from human patients (Larone, 2002; Quintella et al., 2011).

The reaction of the tissue is another important characteristic that should be evaluated during histopathologic examination of skin biopsies during diagnosis of sporotrichosis. *S. schenckii* usually causes a mixed suppurative and granulomatous inflammatory response in the dermis and adjacent subcutaneous tissue (Figure 7B). Cases of disseminated disease also present a

mixed inflammatory reaction. Fibrosis and micro abscess are frequently observed on cutaneous infections. Moreover, hyperkeratosis, parakeratosis and pseudoepitheliomatous hyperplasia may also occur (Larone, 2002). In addition to intact polymorphonuclear cells, the granuloma formed during sporotrichosis may also contain cellular debris, caseous material, lymphocytes, plasmocytes, giant and epithelioid cells, fibroblasts and yeasts (Quintella et al., 2011). Some authors also report that foreign material of vegetal origin related to the traumatic inoculation of the agent may be found in rare cases (Orellana et al., 2009).

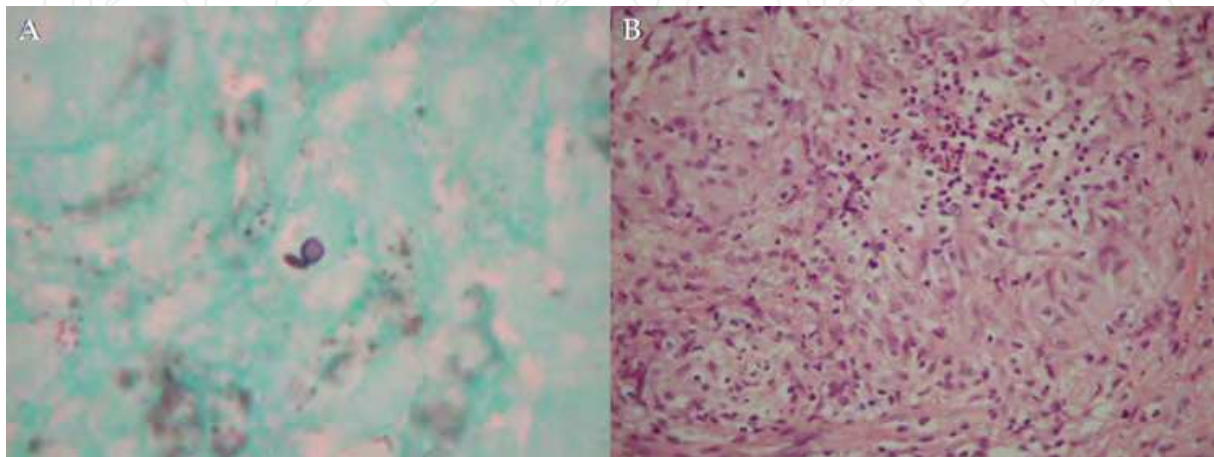


Fig. 7. Histological examination of skin biopsies from patients with sporotrichosis. (A) Gomori methenamine silver stain presenting a yeast-like cell with a cigar-shaped bud, suggestive of *S. schenckii*. Magnification 400X. (B) Hematoxylin and eosin stain presenting a suppurative and granulomatous reaction with neutrophils. Magnification 400X. (Courtesy of Leonardo P. Quintella.)

Some histopathologic changes on analysed tissues may be also related to the observation of the etiologic agent on tissue sections. The presence or predominance of epithelioid granulomas, caseous/fibrinoid necrosis and fibrosis as well as the occurrence of foreign body granulomas with predominance of lymphocytes is related to a lack of fungal observation. Suppurative granulomas, neutrophils and liquefaction are more common when *S. schenckii* yeast-like cells are encountered (Quintella et al., 2011).

Several authors observed the Splendore-Hoeppli reaction on histopathologic tissue sections from sporotrichosis patients. This reaction indicates a localised immunologic response to antigens of several infectious organisms, such as fungi, bacteria and parasites (Hussein, 2008), and appears as a radiating homogenous, refractile, eosinophilic, club-like material surrounding a central eosinophilic focus (Larone, 2002). Splendore first observed this structure in 1908 on tissue sections from patients with sporotrichosis and afterwards by Hoeppli in 1932 around schistosome larvae (Kwon-Chung & Bennett, 1992). Positive identification of this structure in tissue sections from patients with sporotrichosis ranges from 20% to 66% (Gezuele & Rosa, 2005); although other authors reported that this structure was not observed in several analysed samples (Quintella et al., 2011).

These histopathologic findings help to differentiate sporotrichosis from other diseases. For instance, during histoplasmosis infection, the neutrophilic inflammatory response is a mixed of suppurative and granulomatous reactions, and in sporotrichosis are not observed. Suppurative tissue reactions are observed in candidiasis, especially when it is caused by *C. glabrata*, where the absence of hyphae and pseudohyphae elements can mimic

sporotrichosis; however, granulomas are not usually formed (Larone, 2002). On dogs with sporotrichosis, lesions present well-formed granulomas with marked neutrophil infiltration. Frequently, lymphocytes and macrophages are absent on peripheral infiltrate, which facilitates the differentiation between sporotrichosis and leishmaniasis (Miranda et al., 2010). Definitive diagnosis of sporotrichosis is based on the isolation and identification of its etiological agent in culture (Kwon-Chung & Bennett, 1992). Isolation of *S. schenckii* can be easily attained after spreading the clinical specimens on Sabouraud Dextrose Agar supplemented with 400mg/L chloramphenicol to avoid bacterial contamination and on culture media containing cycloheximide, such as Mycosel or Mycobiotic agar. Cycloheximide inhibits the growth of several anemophilous fungi that can contaminate cultures from clinical specimens obtained from non-sterile sites; however, this drug does not inhibit the mycelial form of *S. schenckii*, which grows well on this culture medium (Molinari et al., 2010).

Traditional identification of *S. Schenckii* is based on the macro and micromorphologies of the mycelial and yeast forms (Figure 8). These analyses, however, do not differentiate the newly described species in the *S. schenckii* complex (Marimon et al., 2007). To differentiate the species within this complex, other tests such as carbohydrate assimilation (especially sucrose and raffinose), growth rates at 30°C and 37°C and the production of dematiaceous conidia are necessary. Moreover, molecular methods, which will be discussed below, are also important for the differentiation of these new species of *Sporothrix*.

Although *S. schenckii* can grow around body temperature, this is not the optimal temperature for this fungus growth. Therefore, to enhance the chance of fungal isolation, cultures must be incubated at 25 to 30°C after removing the fungus from stress condition and allowing it to grow, even if the patient fungal burden is low. After five to ten days of incubation at 25°C, filamentous hyaline colonies often start to develop. Only a few strains require extended incubation for growth. These colonies, after some time, may develop a dark colour, which is usually located in the centre or sectors of the colony (Kwon-Chung & Bennett, 1992). If there is no growth of *S. schenckii* after four weeks of incubation, cultures can be considered negative.

To identify a fungal isolate as *S. schenckii*, one needs to determinate if it can undergo *in vitro* dimorphism. Thus, it is necessary to make subcultures of the fungus at 35 to 37°C on enriched media such as brain heart infusion (BHI) agar, blood or chocolate agar, for five to seven days. Colonies with a creamy texture and with a yellow to tan colour will grow. Microscopically, they are composed of round to oval hyaline yeast-like cells that often have cigar-shaped narrow base buds (Larone, 2002; McGinnis, 1980).

Because environmental fungi of the genus *Sporothrix* can also form yeast-like cells when grown on the appropriate medium at temperatures around 35°C, a micromorphologic study of the filamentous form of the isolated fungus is necessary to correctly identify *S. schenckii* because environmental *Sporothrix* strains are not able to produce the virulence-related dematiaceous conidia of *S. schenckii* (Dixon et al., 1991). Slide culture preparations using potato dextrose agar (PDA) or corn meal agar incubated at 30°C are ideal to study *S. schenckii* conidiogenesis (Marimon et al., 2007). For this test, *S. schenckii* strains present hyaline hyphae usually less than 3 µm in diameter with regular septation and branching produced in strands. Hyaline conidia are produced in a flower-like arrangement at the tip of the sympodial conidiophores that arises at right angles from the sides of the hyphae. Dematiaceous conidia are produced mainly along the hyphae on extremely short denticles (Dixon et al., 1991; Kwon-Chung & Bennett, 1992; Larone, 2002).



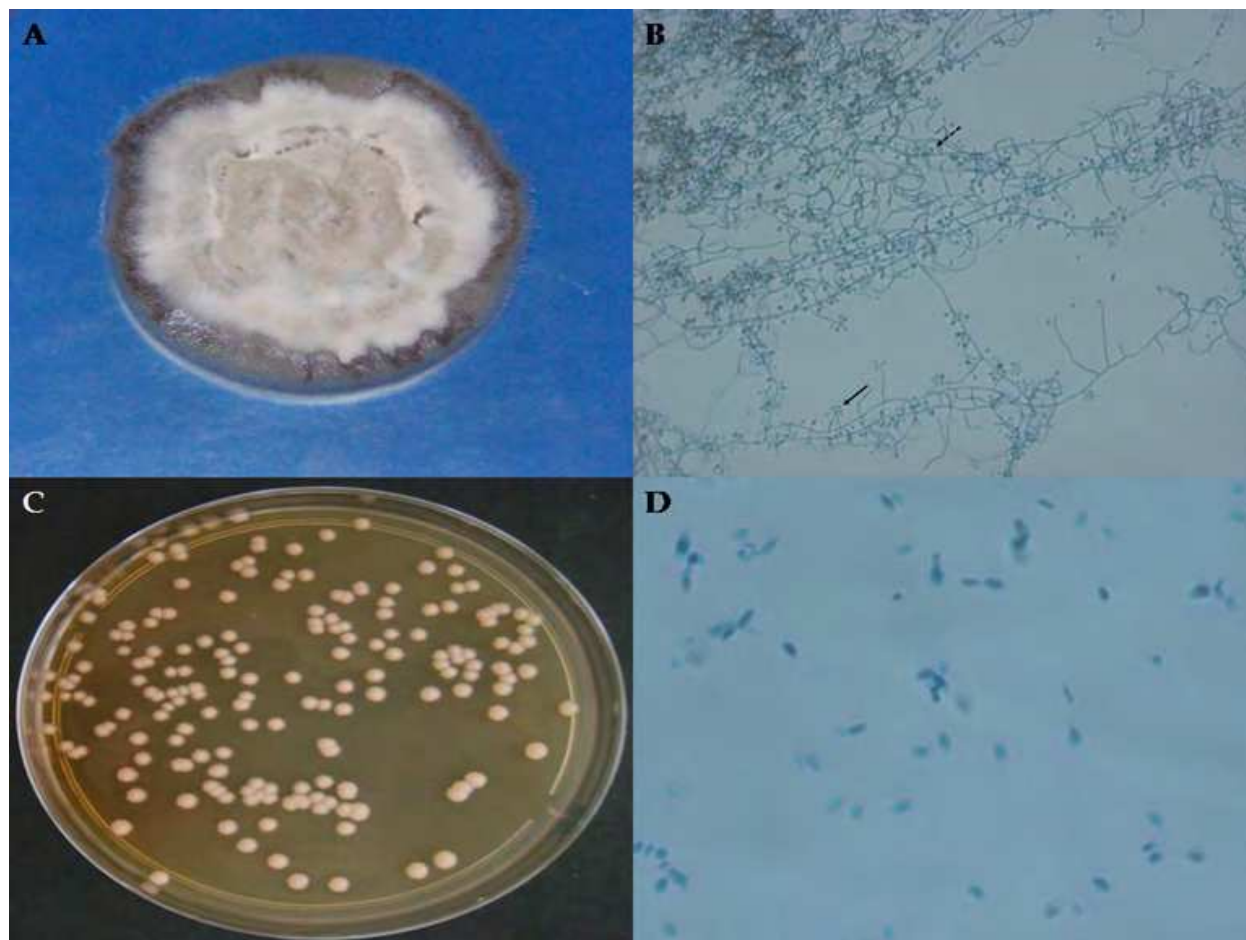


Fig. 8. *S. schenckii* identification: (A) Mycelial colony on Potato Dextrose Agar (PDA) after 21 days of incubation at 25°C. (B) Slide culture of the mycelial form on PDA, incubated at 30°C for 15 days and stained with lactophenol cotton blue. The continuous arrow indicates hyaline conidia on a sympodial conidiophore. The dashed arrow indicates dematiaceous conidia on a short denticle arising from the hypha. Magnification 400X. (C) Several white, smooth yeast colonies on a Brain Heart Infusion (BHI) agar plate after 10 days of incubation at 36°C. (D) Lactophenol cotton blue stain of oval yeast-like cells with cigar-shaped buds after growth on BHI agar. Magnification 400X.

### 2.3 New diagnostic approaches

Positive cultures provide the strongest evidence for sporotrichosis, but culture diagnosis has significant limitations. In particular, in some manifestations of the disease, such as *S. schenckii*-induced arthritis, the collection of material for culture is difficult (Morris-Jones, 2002.). Also, sporotrichosis may be mistaken for other infections, such as tuberculosis, leishmaniasis, paracoccidioidomycosis, gummatous syphilis, and chromoblastomycosis (Rippon, 1988; Sharma et al., 2005). Non-culture methods have been developed to improve the rate and speed of diagnosis. These tests include antibody detection as well as newer molecular techniques that have been developed to improve the diagnosis and identification of *Sporothrix* spp. in clinical specimens, as well for taxonomic purposes. The results from the described tests can provide a presumptive diagnosis of sporotrichosis and require clinical correlation for the correct evaluation and determination of the final diagnosis.

### 2.3.1 Serology

Several methodologies have been described for the immunological diagnosis of sporotrichosis based on antibody detection in sera from infected patients. Precipitation and agglutination techniques were first used (Albornoz et al, 1984; Blumer et al., 1973; Casserone et al., 1983; Karlin & Nielsen, 1970). Double immunodiffusion for sporotrichosis usually does not cross-react with sera from patients with chromoblastomycosis or leishmaniasis, which are infectious diseases with similar clinical manifestations. Immunoelectrophoresis has also been used, and in all positive cases, an anodic arc, called an S arc, is observed (Albornoz et al., 1984). Both methodologies that use an antigenic complex from fungal culture filtrate are highly sensitive. Tube and latex agglutination both have high sensitivity and specificity and have been used for sporotrichosis serodiagnosis since the 1970s (Blummer et al., 1973; Casserone, et al., 1983; Karlin & Nielsen, 1970). However, these tests lack sensitivity in cases of cutaneous sporotrichosis (Albornoz et.al., 1984; Rippon, 1988) and do not permit the determination of the immunoglobulin isotype involved.

Immunoenzymatic assays are increasingly used for the serodiagnosis of this mycosis. The first immunoblot assay used for serodiagnosis of sporotrichosis dates back to 1989, when exoantigen preparations from a *S. schenckii* yeast form presented 100% sensitivity and 95% specificity for the detection of antibodies (Scott & Muchmore, 1989). Later, another immunoassay (ELISA) was developed, using the concanavalin A binding peptide-rhamnomannan from the *S. schenckii* yeast cell wall, and antibodies were detected in 35 serum samples from patients with culture proven sporotrichosis, resulting in 100% sensitivity. However, the specificity was lower than previous tests because the sera from patients with cutaneous leishmaniasis cross-reacted (Loureiro Y Penha & Lopes Bezerra, 2000). The same group reported an ELISA test using the same antigenic preparation against sera from 92 patients with different clinical forms of sporotrichosis in Rio de Janeiro and reported 90% sensitivity, 80% specificity, and a global efficiency of 86% (Bernardes-Engemann, et al. 2005). Other studies showed that the use of different strains during the preparation of the antigen might result in different sensitivity and specificity, despite the purification of the antigen involved in this methodology. This difference is due to the O-glycan residues linked to the molecules (Bernardes-Engemann, et al. 2009).

The development of an enzyme immunoassay using exoantigens produced by the mycelia phase of a *S. schenckii* strain isolated in the zoonotic sporotrichosis endemic area of Rio de Janeiro was also reported (Almeida-Paes et al., 2007a). This antigen was described by Mendoza and collaborators (2002) and showed no cross-reactivity with the antigen and serum samples from patients with coccidioidomycosis, histoplasmosis and paracoccidioidomycosis (Mendoza et al., 2002). The same antigen was used previously in immunodiffusion and immunoelectrophoresis techniques without cross-reactivity with sera from patients with leishmaniasis or chromoblastomycosis (Albornoz et.al., 1984). This test had a sensitivity of 97% and specificity of 89% when performed on 90 sera from patients with different clinical forms of sporotrichosis, 72 sera from patients with other infectious diseases and 76 healthy controls (Almeida-Paes et al., 2007a). The overall efficiency of this test for diagnosis and follow-up of human sporotrichosis is increased when IgG, IgM, and IgA isotypes are also measured providing the most accurate results (Almeida-Paes et al., 2007b).

When the immunoassays probed with different antigenic preparations are compared, the crude exoantigens (Almeida-Paes et al., 2007a) gave values of sensitivity and specificity a little higher than those using the concanavalin A binding fraction of the *S. schenckii* yeast cell wall (Bernardes-Engemann, et al. 2005). A similar observation was found when using this



purified antigen and crude exoantigens for the serodiagnosis of feline sporotrichosis. Purified antigens showed 90% sensitivity and 96% specificity, whereas crude exoantigens presented 96% sensitivity and 98% specificity (Fernandes et al., 2011).

### 2.3.2 Molecular or DNA-based

The *Sporothrix* genus is traditionally identified by phenotypic characteristics, such as macro- and micromorphology and sugar assimilation, but *Sporothrix* spp. colonies grow slowly, and differentiation from other fungi that have similar colony or microscopic morphology is challenging. To confirm the diagnosis, conversion to the yeast phase should be performed, which takes at least 2 to 3 weeks. Methods to identify fungal isolates, such as species-specific DNA, can decrease this time-consuming step while maintaining or improving the specificity, accuracy and sensitivity.

Until now, few molecular methods have been applied to the diagnosis of sporotrichosis to detect *S. schenckii* DNA from clinical specimens, and to identify *Sporothrix* spp. in culture. Diagnosis using the Polymerase Chain Reaction (PCR) is based on the amplification of fungal gene sequences and is a powerful tool for identifying mycoses. One of the pioneering DNA-based methodologies used for the diagnosis of fungal infections was reported by Sandhu and collaborators who developed 21 specific nucleic acid probes targeting the large subunit rRNA gene from several fungi, including *S. schenckii*. The results show a high level of specificity (Sandhu et al., 1995).

In past years, several molecular taxonomic studies using different methodologies, such as restriction fragment length polymorphism (RFLP) from different gene targets, random amplified polymorphic DNA (RAPD), DNA sequencing of internal transcriber spacer (ITS) regions of the ribosomal RNA (rRNA), PCR targeting the DNA topoisomerase II gene, amplified fragment length polymorphism (AFLP), and M13 PCR fingerprinting have demonstrated that *Sporothrix schenckii* isolates have different genetic characteristics, which suggests that they do not belong to the same species (Ishizaki et al., 2000; de Beer et al., 2003; Gutierrez-Galhardo et al., 2008; Kanbe et al., 2005; Mesa-Arango et al., 2002; Neyra et al., 2005; Reis et al., 2009; Watanabe et al., 2004; Zhang et al., 2006). In addition, Marimon and collaborators (2007) supported these findings by suggesting that, according to a combination of phenotypic and genetic features, *S. schenckii* should not be considered a single taxon that causes sporotrichosis in human and animals but should instead be considered a species complex that is comprised of at least four species: *S. brasiliensis*, *S. globosa*, *S. luriei*, and *S. schenckii* (Marimon et al., 2006, 2007, 2008). *S. globosa* is distributed worldwide (Madrid et al., 2009; Oliveira et al., 2010), whereas *S. mexicana* is restricted to Mexico and *S. brasiliensis* to Brazil. Also, *S. brasiliensis*, *S. globosa* and *S. luriei* are related as etiological agents of sporotrichosis (Marimon et al., 2007, 2008). Additionally, other phylogenetic analysis based on the rDNA and the  $\alpha$ -tubulin regions from *S. albicans*, *S. pallida* and *S. nivea* revealed a significant similarity. Therefore, it has been proposed that all the three species were called *S. pallida* when they were first described (de Meyer et al., 2008). An identification key for the *Sporothrix* complex has now been proposed (Marimon et al., 2007) that includes analysis of conidial morphology, auxonogram analysis using raffinose and sucrose, genotyping via PCR amplification, and sequencing of the calmodulin gene. Based on this last analysis, Romeo and collaborators (2011), who were studying the molecular phylogeny and epidemiology of a *S. schenckii* species complex isolated in Italy, demonstrated that 26 environmental strains co-clustered with *S. albicans*, and two clinical isolates grouped with *S. schenckii stricto sensu* (Romeo et al., 2011).

PCR diagnosis based on the amplification of the fungal gene sequences is a powerful tool for identifying invasive mycoses. The first description of PCR for the diagnosis of sporotrichosis was reported by Kano and colleagues (2001). Specific oligonucleotide primers based on the chitin synthase 1 gene were designed, and with this primer pair, PCR was able to detect a 10 pg genomic DNA fragment of *S. schenckii*. A nested PCR assay for the detection of *S. schenckii* was evaluated in clinical samples using the 18S rRNA gene sequence as the target. However, nested PCR could detect *S. schenckii* DNA in tissue samples from infected animals or from clinical specimens from patients with sporotrichosis confirmed by culture or histochemical staining. The test showed high sensitivity and specificity, indicating that the assay could provide rapid diagnosis with sufficient accuracy to be clinically useful for patients with sporotrichosis (Hu et al., 2003). More recently, the same assay was used to detect *S. schenckii* DNA from 38 strains (including all 24 mitochondrial DNA (mtDNA) types) collected from different areas of the world, in the tissues of eight mice infected with the ATCC10268 strain of the fungus, and in skin biopsies of nine patients with sporotrichosis. In addition, the same procedures were used with two strains of *Ceratomyces minor* and isolates of 10 species of other pathogenic fungi. The authors demonstrated that nested PCR could identify *S. schenckii* from all of the mtDNA types and in isolates recovered from different areas of the world, corroborating the data obtained by Hu and collaborators (2003) that the nested PCR assay is highly sensitive and specific and is a rapid method for diagnosis of sporotrichosis under contamination free conditions (Xu et al., 2010).

### 3. Conclusions

Sporotrichosis is a chronic infection caused by the dimorphic fungus now named the *S. schenckii* species complex. Lymphocutaneous infection is the most common presentation. The diagnosis of sporotrichosis is classically attained by correlation of clinical, epidemiological and laboratorial data. Considerable advances have been made in non-culture-based diagnosis of sporotrichosis with the development of a scarcity of methods for the detection of antibodies, antigens, and nucleic acids. The methods described for the diagnosis of sporotrichosis each have their strengths and weaknesses and require critical analysis by microbiologists and clinicians. However, not all tests described are universally available, which complicates the capacity to diagnose and treat individuals with sporotrichosis. Also, the immunological status of the patient and manifestation of the disease influences the efficacy of the diagnostic test. Continuing efforts to improve or develop diagnostic tests will facilitate our diagnostic aptitude. However, such assays will require validation in populations from diverse regions of the world prior to general applications in routine diagnosis.

Results obtained from a panel of serological diagnostic test play an important role in the diagnosis of sporotrichosis. Nevertheless, nucleotide probes, specific for the *Sporothrix* species complex, and DNA amplification procedures, such as PCR, allow more rapid and precise diagnosis, which can lead to earlier treatment. However, the gold standard in diagnosis continues to be culture and the correlation of molecular data and phenotypic characteristics.

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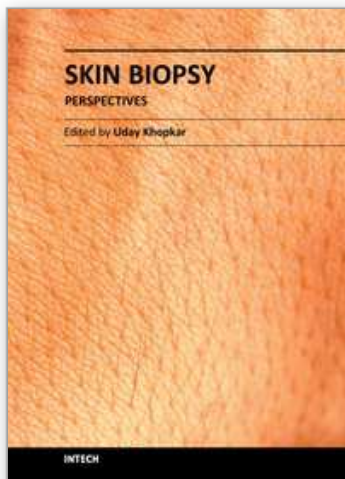
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### **Skin Biopsy - Perspectives**

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Skin Biopsy - Perspectives is a comprehensive compilation of articles that relate to the technique and applications of skin biopsy in diagnosing skin diseases. While there have been numerous treatises to date on the interpretation or description of skin biopsy findings in various skin diseases, books dedicated entirely to perfecting the technique of skin biopsy have been few and far between. This book is an attempt to bridge this gap. Though the emphasis of this book is on use of this technique in skin diseases in humans, a few articles on skin biopsy in animals have been included to acquaint the reader to the interrelationship of various scientific disciplines. All aspects of the procedure of skin biopsy have been adequately dealt with so as to improve biopsy outcomes for patients, which is the ultimate goal of this work.

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