Onychomycosis Due to *Aspergillus* Species

Ricardo Negroni

Abstract Fungal infections of the nail plate accounts for 50% of nail diseases. The prevalence of onychomycosis varies between 2 and 8% of the general population, being more frequent amongst adults aged 60 years or more. Several fungi have been implicated, including dermatophytes, yeasts and non-dermatophyte moulds. Non-dermatophyte filamentous fungi are the most heterogeneous groups of organisms which may cause onychomycosis. These are environmental fungi which may behave as contaminants or colonisers of a previously damaged nail or pathogens. Several *Aspergillus* species have been isolated from nails, including *A. fumigatus, A. flavus, A. terreus, A. sclerotiorum*, and *A. nidulans.* These are not common conditions – only ∼2% of onychomycosis cases are caused by *Aspergillus* species. Diabetes, peripheral vascular disease, orthopaedic trauma and advanced age are the most important underlying conditions, tough in half of the cases no risk factor is evident. *Aspergillus* onychomycosis may present with distinct clinical forms. In the majority of the patients lesions resemble those produced by dermatophytes – proximal subungueal onychomycosis with painful paronychia without pus is the most characteristic clinical manifestation. The results of treatments are less predictable than in dermatophyte nail infections. Itraconazole and terbinafine by oral route are effective against *Aspergillus* onychomycosis. Additionaly, nail antifungal lacquers with ciclopirox or amorolfine, as well as partial nail avulsion with 40% urea ointment usually improve the clinical response of systemic treatment.

Keywords *Aspergillus* spp · Non-dermatophyte fungal nail infection · Onychomycosis · Superficial mycoses

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Contents

1 Introduction

Onychomycosis refers to a fungal infection that affects the nail plate. These may be caused by three different groups of fungi: dermatophytes (named *Tinea Unguium*); yeast-like fungi and non-dermatophyte filamentous fungi [1–5]. Onychomycosis are responsible for 50% of total nail diseases and its prevalence in general population varies between 2 and 8%, according to different authors. The frequency of onychomycosis is lower in children and higher in persons above 60 years of age (> 25%), affecting both sexes [1, 3, 4, 6–9]. Nail fungal infection are usually asymptomatic conditions that have an important impact on the quality of life of affected patients, interfering in interpersonal relationships and the work environment, particularly when fingernails are affected.

There are several local and general predisposing factors which may increase the prevalence of onychomycosis [10, 11]. These are summarized in Table 1.

Dermatophytes are responsible for > 90% of onychomycosis cases located in toenails. The most prevalent species in this group is *Trichophyton rubrum,* followed by *T. mentagrophytes, T. tonsurans*, *Epidermophyton floccosum* and *T. schoenleinii*.

Table 1 Predisposing factors for *Aspergillus* onychomycosis

General predisposing factors
Age: prevalence is greater in elder people (slow nail growth)
Peripheral vascular diseases
Genetics: *T. rubrum* infection shows a familiar pattern of autosomal dominant inheritance
Diabetes mellitus
Hypothyroidism and other endocrine diseases
Cell-mediated immunity impairment, HIV-infection, steroids, anti-blastic therapy
Local factors
Orthopaedic trauma
Trauma caused by sports or non-proper foot wears (e.g., football, rugby, tennis, jogging, female's foot wears)
Hyperhydrosis
Poor hygiene
For fingernail onychomycosis: chronic contact with water, solvents, detergents and chemical products

These are all anthropophilic species and the presentation of onychomycosis caused by dermatophytes is influenced by genetic predisposing factors [3, 4, 7, 10, 12, 13].

Yeast-like fungi frequently attack fingernails – in this location, yeasts are more prevalent than dermatophytes as agents of onychomycosis, which is often associated with onycholysis and paronychia [1, 3, 12].

Non-dermatophytic filamentous fungi are responsible for 2–20% of the nail fungal infections. These are the most heterogeneous group of fungi which may cause onychomycosis, and include different environmental fungal species which live in the soil or on rotting vegetation. Some of these fungi are often found as primary pathogens of the nails, including *Scopulariopsis brevicaulis, Scytalidium hyalinum, Scytalidium dimidiatum,* and *Onychocola canadensis*; but a great variety of other fungi may also cause onychomycosis or are isolated from nail clinical samples, such as *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp., *Chaetomium globosum,* and species of *Alternaria* and *Curvularia* [12–22].

The interpretation of a positive nail culture for non-dermatophyte moulds is always difficult. The recovery of some of these fungi from a nail sample culture does not represent unequivocal evidence linking the aetiological agent to the nail disorder. For instance, fungi may contaminate the nail surface or behave as a saprobe, transiently colonising the nail without causing invasion. Alternatively, fungi might persistent colonise a nail that was previously damaged by trauma or other causes. Mixed infections in which a dermatophyte and a non-dermatophyte mould are associated have also been described [2, 6, 7, 16, 17, 23, 24]. Onychomycosis caused by non-dermatophyte moulds is often observed in adults of both sexes and is more prevalent in elder persons, suffering orthopaedics or/and peripheral vascular alterations [5, 10, 11, 21, 25, 26].

2 Clinical Forms

Clinical characteristics of onychomycosis correlate with the infection's route of entry. The most prevalent clinical form is distal or lateral subungueal onychomycosis (DLSO), in which the hyphae invade the hyponichium and/or the nail bed from the distal part and then spread proximally causing thickening of the horny layer. Onycholysis is often observed and the nail becomes yellow-brown and opaque. This type of onychomycosis is commonly observed in toenails and they may be caused by both dermatophytes and moulds [1, 3, 7, 12, 23].

White superficial onychomycosis (WSO) is observed in fingernails and toenails. The infection begins at the superficial layer of the nail plate, invading progressively deeper layers, appears as small white patches on the nail dorsal surface. It is often produced by *T. mentagrophytes* var. *interdigitale*, but some moulds such as *Aspergillus terreus, Fusarium oxysporum* and *Acremonium* spp. have also been implicated [1, 3, 4, 12]. Black superficial onychomycosis is a very rare form of nail infection; some cases due to *T. rubrum* and *Scytalidium dimidiatum* have been published [1].

Proximal subungueal onychomycosis (PSO) is produced by hyphae invasion of the proximal nail fold and spreads distally under the nail plate. Clinically it appears as a white patch from the cuticle to 2–6 mm distally. Its most frequent aetiological agent is *T. rubrum*. PSO may affect toenails and less commonly fingernails; the majority of the patients suffer from cell-mediated defects in immunity. Rarely this type of onychomycosis is associated with proximal nail destruction and chronic paronychia without pus. This type of lesions is often caused by *Fusarium* spp. and less frequently by *Aspergillus* species [4, 6, 25–29].

"Endonyx" onychomycosis (EO) is produced by the distal penetration of the fungi to the nail plate without nail bed invasion. This type of onychomycosis causes white patches without subungueal hyperkeratosis or onycholysis. This has been related to *T. violaceum* or *T. soudanensis*. Non-dermatophyte mycelial fungi have not been isolated from this clinical form [1, 7].

Total dystrophy (TDO) is final status of any type of fungal nail infection. In these cases, the nail bed appears hyperkeratotic and no normal nail structure is detected. The nail plate looks thickened, opaque and brown-yellow in colour. The primary total dystrophy is observed in patients suffering from chronic mucocutaneous candidosis [1, 30].

Paronychia of the fingernails is usually produced by yeasts, mostly affecting adult women who work handling carbohydrates-containing foods, water, detergents or chemical active substances. It is also detected in patients suffering from hyperhydrosis, maceration and acrocyanosis. Initially the cuticle is damaged followed by a chronic inflammation of the proximal nail fold near to the matrix, which compromise leading to a nail plate dystrophy as irregular transverse grooves. *Candida* spp. and bacteria are frequently isolated from paronychia [1, 2, 7, 12].

3 Mycological Diagnosis

The diagnosis of onychomycosis is based on the finding of fungal elements such as hyphae, pseudohyphae and budding yeast in direct microscopic examination with KOH and the isolation of fungi in cultures. Histopathological study of nail clippings using Periodic Acid-Schiff (PAS) staining is a very important step in the diagnosis, especially in onychomycosis due to non-dermatophyte moulds. In a study carried out in Italy, histopathology presented positive results in more than 82% of the cases [31].

A proper specimen should be obtained by a professional with experience in the field. It is very important to advise patients in advance not to use any powder, cream or nail lacquer as well as systemic or topical antifungal drugs. In order to prevent dust contamination, patients should wear socks and closed shoes. Nail samples should be taken from the nail bed or from ventral nail plate in DLSO or by scraping the nail dorsum in cases of WSO, EO and PSO. These nail specimens are usually collected in small Petri dishes. Before microscopic examination the sample is divided into small fragments [6, 12, 1–3].

For microscopic examination one half of the clinical sample is put on a glass slide with a drop of 20–40% KOH, then a cover ship is applied and the preparation is gently heated in a Bunsen flame. The slide is observed at 200× or 400× using a light microscope, searching for hyphae, pseudohyphae, yeast cells or spores.

Other type of solutions may be used for clearing of the keratin and allowing the visualization of fungal elements include 30% KOH plus 10% glycerine, to avoid dessecation; 20% KOH in 60% water plus 40% dimethyl sulphoxide; 30% KOH with a drop of chlorazol black E or a fluorochrome named calcofluor white, which requires a fluorescence microscope (Fig. 2) [1, 3, 5, 7, 9, 12].

Cultures are the second step in the mycological examination. It is very important to inoculate the nail sample into different culture media such as Sabouraud dextrose agar with 0.05% chloramphenicol; Borelliś lactrimel with the same antibiotic con-

Fig. 1 Onychomycosis of the big toenail due to *Aspergillus terreus* following orthopaedic trauma in a 56-year-old woman

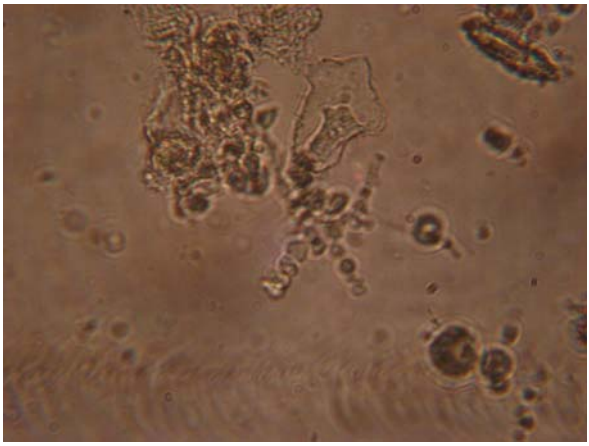

Fig. 2 Direct microscopic examination with 40% KOH of a nail sample shown in Fig. 1 revealing hyaline septated hyphae of a non-dermatophyte mould, 400×

Fig. 3 Nail culture from Fig. 1 showing several colonies of *Aspergillus terreus*

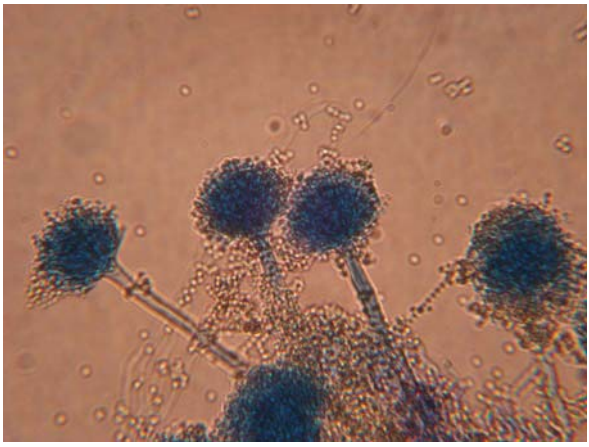

Fig. 4 Microscopic examination with lactophenol cotton blue, 400× (same as Figs. 1–3)

centration; and Sabouraud dextrose agar with chloramphenicol and cycloheximide (Actidione^{R}). The latter inhibits growth of bacteria, some yeast and most of the non-dermatophyte moulds. The nail sample is seeded with sterile needle in several points on the medium surface and the specimen is gently pushed into de medium. Cultures are incubated for 4 weeks at 26–28°C (Fig. 3 and 4) [1, 3, 12].

A molecular biology kit for the diagnosis of dermatophyte infections has been presented recently, but it is still not widely used in clinical laboratory [32].

4 Special Characteristics of Onychomycosis Due to *Aspergillus* Species

Several *Aspergillus* species have been isolated from nails, including *A. fumigatus, A. flavus, A. versicolor, A. niger, A. terreus, A. sclerotiorum, A. nidulans* and *Emericella quadrilineata* (which is the teleomorphic state of *A. tetrazonus*) [15, 17, 22, 25, 26, 29, 31, 33, 34].

The prevalence of onychomycosis due to *Aspergillus* spp. seems to be variable in different parts of the world. Apparently it is more often observed in Mexico, Europe, Pakistan and India, and less common in South America (where *Fusarium* spp. and *Acremonium* spp. are the non-dermatophyte moulds most frequently involved). In Italy *Aspergillus* spp. were responsible for 2.6% of all onychomycosis and in Pakistan, *Aspergillus* species were isolated from 2% of cases [4, 10, 12–15, 17, 19, 20, 26–28, 31, 35, 36].

In Mexico, Bonifaz et al. described that 37.1% (29/78) of onychomycosis cases due to non-dermatophyte moulds were caused by *Aspergillus* spp. The following *Aspergillus* species were identified: *A. niger* ($n = 13$), *A. terreus* ($n = 8$), *A. fumigatus* ($n = 7$), and *A. flavus* ($n = 1$). Predisposing factors for *Aspergillus* onychomycosis include peripheral vascular diseases, diabetes, orthopaedic trauma, and advanced age – however, none of these are found in 50% of the cases [11, 15].

From the clinical point of view *Aspergillus* onychomycosis may produce different clinical forms: onycholysis, DLSO, WSO or PSO with painful paronychia without pus (Fig. 5,6 and 7). The latter clinical form is the most characteristic of this type of nail infection, but it is also observed in onychomycosis due to *Fusarium* spp. [16, 25, 26, 29, 31]. Other types of nail invasion are rarely detected.

5 Treatment

Onychomycosis is one of the most difficult fungal infections to treat, due to the slow growth of the nails, the hardness of the nail plate and the location of the infectious process between the nail bed and plate. Although systemic antifungal drugs as terbinafine and itraconazole are effective in the treatment of onychomycosis caused by *Aspergillus* spp., fungi are difficult to eradicate and these drugs should always be associated with nail avulsion using a 40% urea ointment and antifungal nail lacquers containing amorolfine or ciclopirox olamine [1, 31, 3–5].

Itraconazole is a first generation triazole active against dermatophytes, yeasts and non-dermatophytes moulds including *Aspergillus* species. It is usually administered

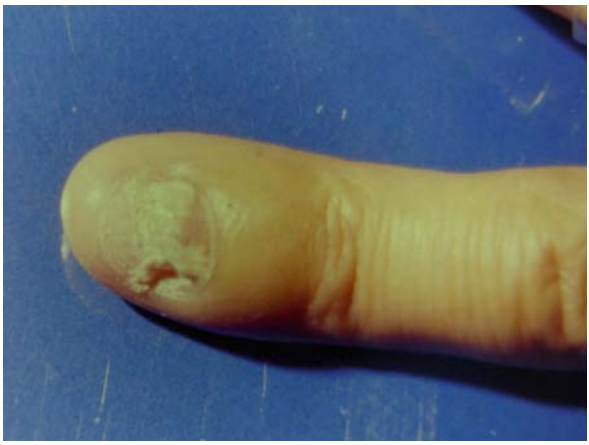

Fig. 5 Total dystrophic onychomycosis with chronic paronychia due to *Aspergillus flavus*. This was located in the little finger of the right hand of a 65-year-old woman suffering from chronic bacterial endocarditis who was submitted to a long-term treatment with antibacterial antibiotics

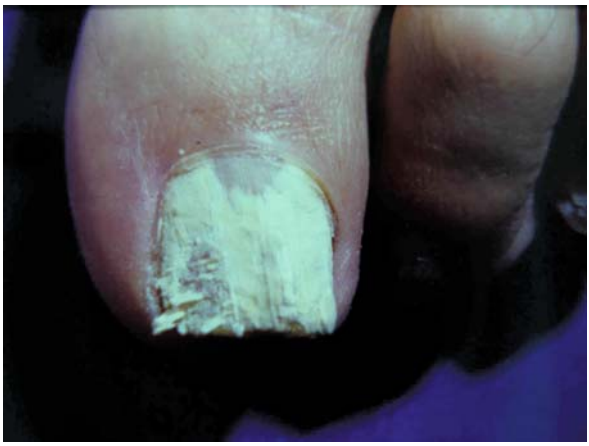

Fig. 6 White total dystrophy of the big toenail due to *Aspergillus terreus* in a 71-year-old man with peripheral vascular insufficiency

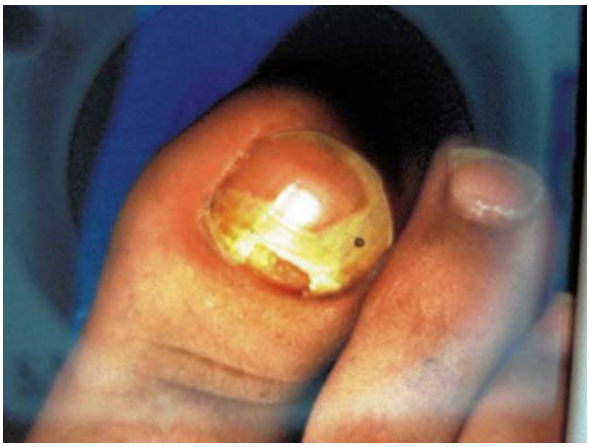

Fig. 7 Proximal subungueal onychomycosis in a 46-year-old woman following orthopaedic trauma. There was onychomadesis (spontaneous separation of the nail plate from the matrix area) and chronic paronychia but no signs of pus. The infection was caused by *Aspergillus nidulans* and located in the big toenail

in capsules, either as a continuous treatment (200 mg once daily for 3–4 months) or as a pulse therapy (200 mg 12 hourly for 1 week each month, over 3–4 months for toenail and shorted period for fingernails). Both regimens seem to be equally effective [37, 38]. Monitoring itraconazole blood levels has been recommended to ensure proper drug absorption – this is discussed in more detail elsewhere in this book. A pharmacokinetic comparison study [37] showed that intermittent therapy with itraconazole resulted in higher maximum itraconazole plasma concentrations but lower total drug exposure, and hence lower itraconazole nail tip concentrations, than continuous therapy. However, the intermittent schedule was not associated with a lower cure rate, suggesting that itraconazole nail concentrations remained within the therapeutic range. Headache and gastrointestinal tract upset are the most frequent side effects of itraconazole. Asymptomatic rise of hepatic enzymes is observed in less than 3% of patients. The drug should not be given to patients with evidence of

ventricular dysfunction and a wide range of drug interactions should be considered when itraconazole is indicated in patients who are receiving other treatments [1, 39, 40].

Terbinafine is the first orally active allylamine, an antifungal drug especially active against dermatophytes and moulds. The drug is fungicidal and remains at therapeutic levels in keratinized tissues, but with a short plasma half-life of 36 h. For the treatment of onychomycosis terbinafine is given at 250 mg daily for 3–4 months for toenails and during 2 months for fingernails. Although several studies have been performed with pulsed therapy it is not clear if it is as effective as continuous treatment. Gastrointestinal alteration and rash are the most common side effects in patients treated with terbinafine. Interactions with rifampin, cimetidine, cyclosporine, tricyclic antidepressants and other psychotropic drugs should be taken in consideration when terbinafine is indicated [1, 3, 39, 40]. More detail on terbinafine are given in the chapter by Drs. Cuenca-Estrella and Rodriguez-Tudela elsewhere in this book.

Nail lacquers should always be applied in those patients with WSO, nail partial avulsion with 40% urea ointment is indicated in those cases with deep onycholysis, in LSO or when a remarkably nail plate thickening is detected.

Combination therapy using topical and systemic treatment has shown to be more effective than monotherapy with orally active drugs. The association of itraconazole or terbinafine by oral route with nail lacquers containing 8% ciclopirox olamine or 5% amorolfine has been successfully used.

Oral sequential therapy using two pulses of itraconazole 200 mg every 12 hours for 1 week per month for 2 months followed by one pulse of terbinafine 250 mg every 12 hours for 1 week and if it is necessary a second pulse of terbinafine 3–4 months later proved to be more efficient than monotherapy with itraconazole or terbinafine [1, 31, 40].

The results oftreatment in *Aspergillus* onychomycosis are less predictable than in *Tinea Unguium* due to the lack of a wide clinical experience. According to Italian authors itraconazole and terbinafine are very effective in the treatment of this fungal infection and the pulsed regimen is more economical and less demanding [26, 31].

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