

# *Aspergillus proliferans* Onychomycosis Forming Fungal Masses

## Onicomycosis por *Aspergillus proliferans* formando masas fúngicas

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### ABSTRACT

Onychomycosis are nail infections that can be caused by dermatophytes, yeasts or molds, representing the most frequent superficial mycosis worldwide. Dermatophytoma is a variety of onychomycosis characterized by forming fungal masses and it is caused by dermatophytes, particularly *Trichophyton rubrum*. However, other non-dermatophyte filamentous fungi, including *Aspergillus* sp., have been reported. This clinical variety has been associated to failure to conventional antifungal treatment.

The aim of this work is to present a clinical case of immunocompromised patient due to transplantation protocol, with diagnosis of onychomycosis caused by *Aspergillus proliferans* and propose the term onychofungoma to refer to onychomycosis caused by non-dermatophyte filamentous fungi forming fungal masses.

Several direct examinations of toenail scales, culture on Sabouraud dextrose agar with and without cycloheximide/chloramphenicol and molecular identification by PCR-sequencing using two molecular markers were performed.

The nail microscopic examination showed multiple clusters of light brown hyphae. In culture numerous colonies of a filamentous fungus, microscopically compatible with *Aspergillus* sp. developed. The molecular identification and the phenotypic study determined *Aspergillus proliferans* as the etiological agent of onychomycosis.

Onychomycosis whose microscopic study shows fungal masses must be timely attended to avoid patient life-threatening complications, especially when there are risk factors as immunosuppression in the present case. We propose the term onychofungoma (onycho = referent to nail; fungoma = fungal

### RESUMEN

Las onicomycosis son infecciones ungueales que pueden ser causadas por dermatofitos, levaduras u otros hongos filamentosos. Representan la micosis superficial más frecuente en el mundo. El dermatofitoma es una variedad de onicomycosis, se caracteriza porque forma masas fúngicas y es causado por dermatofitos, principalmente *Trichophyton rubrum*; sin embargo, otros hongos no dermatofitos, incluido *Aspergillus* sp., han sido reportados. Esta variedad clínica se ha asociado a falla en el tratamiento convencional.

El objetivo de este trabajo es presentar el caso de una paciente con inmunocompromiso debido a protocolo para trasplante renal, con diagnóstico de onicomycosis causada por *Aspergillus proliferans*, así como proponer el término "onicofungoma" para referirse a las onicomycosis causadas por hongos filamentosos no dermatofitos que forman masas fúngicas.

Se realizaron exámenes microscópicos de escamas de la uña del dedo grueso, cultivos en agar dextrosa Sabouraud con y sin antibióticos e identificación molecular por PCR-secuenciación utilizando dos marcadores moleculares.

El examen microscópico mostró múltiples conglomerados de hifas marrón. En el cultivo crecieron numerosas colonias de un hongo filamentosos, microscópicamente compatible con *Aspergillus* sp. La identificación molecular y el estudio fenotípico determinaron a *Aspergillus proliferans* como el agente etiológico de la onicomycosis.

La onicomycosis, cuyo estudio microscópico muestra masas fúngicas, debe ser atendida oportunamente para evitar complicaciones que pongan en peligro la vida del paciente, en particular cuando existen factores de riesgo como la inmunosupresión

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mass) to refer to onychomycosis cases showing fungal masses formed by non-dermatophytic filamentous fungi.

**KEYWORDS:** dermatophytoma, onychomycosis, *Aspergillus* sp., dermatophytosis, *Aspergillus proliferans*.

## Introduction

Onychomycoses are the most common superficial fungal infections around the world. The causal agents are known as dermatophytes, *Trichophyton rubrum* being the most frequent.<sup>1-4</sup> However, other non-dermatophyte filamentous fungi and yeasts are responsible for a significant number of onychomycosis cases.<sup>4-6</sup>

Dermatophytoma is a type of onychomycosis which was described by Roberts and Evans for the first time in 1998.<sup>7</sup> Clinically, and more frequently, it is characterized by a round or linear, white or yellowish-white area, mainly in the toenail, and the microscopic study of the affected area shows fungal masses formed by dermatophyte hyphae, from here comes the term dermatophytoma.<sup>7-9</sup> The causal agents mainly include *Trichophyton* species, but *Microsporium* and *Epidermophyton* have also been reported.<sup>10-12</sup>

Dermatophytoma has been reported in skin, clinically expressed as erythematous-squamous plaques closely similar to other dermatophytosis, which has been associated with different immunosuppression conditions. The main affected sites have been the face and the groin, caused by *T. rubrum* and *M. gypseum*.<sup>13-15</sup>

The frequency of nail dermatophytoma is not well known; however, Martínez and collaborators reported a 5.3% frequency of this variety in the cases studied in a period of 15 months.<sup>14,15</sup> This pathology is relevant because it has been associated with a poor treatment response.<sup>7,9,16,17</sup>

The aim of this work is to present the case of a patient in preparation for kidney transplant who present onychomycosis. The causal agent formed fungal masses in the nail plate and it was identified by PCR-sequencing using two molecular markers.

## Case report

A 38-year-old woman with a history of systemic arterial hypertension of approximately 10 years of evolution currently controlled. For five years she has presented chronic kidney disease of unknown cause and with hemodialysis treatment. Due to the severity of the kidney failure, the patient has been considered for transplant, so multiple check-up studies were required to rule out other pathol-

del caso que presentamos. Proponemos el término “onicofungoma” (onico = referente a la uña; fungoma = masa fúngica) para referirse a los casos de onicomicosis que muestran masas fúngicas por hongos filamentosos no dermatofitos.

**PALABRAS CLAVE:** dermatofitoma, onicomicosis, *Aspergillus* sp., dermatofitosis, *Aspergillus proliferans*.

ogies or sources of infection that could complicate the procedure. During the clinical inspection at the Dermatology Service, nail alterations were detected that, according to the questioning, started 12 months earlier in the nail of the left toenail, with pachyonychia and white-yellowish dyschromia. Months later, the condition spread to the rest of the nails. Scaling and interdigital itching were progressively added. She had previously received medical treatment with topical miconazole without improvement.

The physical examination at the time of consultation confirmed the condition of the nails, characterized by pachyonychia, melanonychia, yellowish-white longitudinal striae and onychogryphosis (figure 1). Keratoderma spread throughout the plants was also observed.

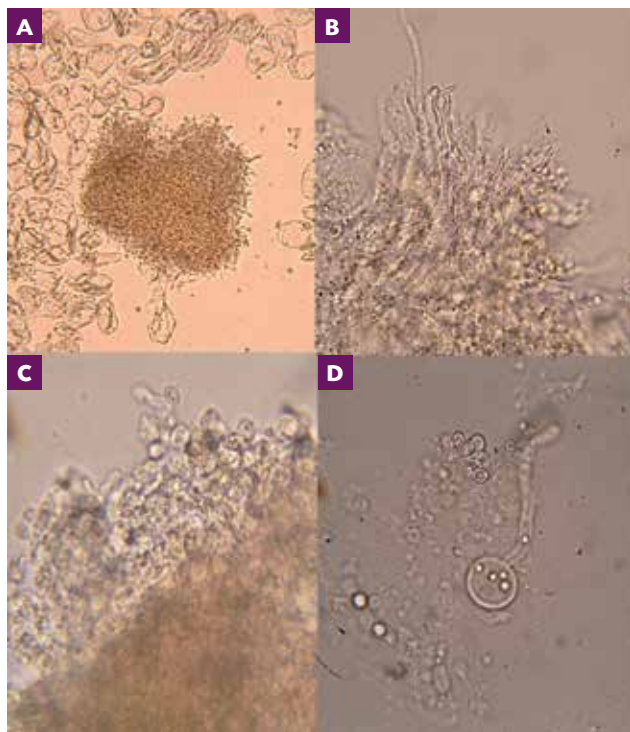
Because the right toenail showed severe deformity and dyschromia, uncommon in onychomycosis cases treated in our Service, and that the patient was under kidney transplant protocol, it was decided to excise that nail for a mycological study in order to rule out other pathologies. The sample was subjected to microscopic examination and culture by triplicate on SDA (BD, USA) with and without antibiotics (chloramphenicol and cycloheximide) and incubated at 28 °C for 21 days.



**Figure 1.** Clinical aspect of ten infected nails. Melanonychia is predominant. In insert longitudinal and whitish striae are more evident. Intertriginous peeling is also observed.

The microscopic examination of the nail (KOH 30%) showed multiple masses of brown hyphae (figure 2a), with fine filaments (figure 2b) and clusters of rounded cells (figure 2c) on its periphery. Among the scales, abundant hyaline filaments of irregular diameter, not compatible with dermatophytes, numerous yeast-like globose cells and other structures similar to germ tubes (figure 2d) were observed.

After three days of incubation, several whitish filamentous colonies (in total 28) appeared in both media (three with antibiotics and two without them), which over time they developed a yellow-orange pigment diffusible to the medium. The microscopic examination of different colonies with five days of growth, a hyaline, septate filamentous fungus was observed, with conidial apparatus corresponding to *Aspergillus* sp., with numerous yellow and equinulate conidia. On SDA without antibiotics the colonies were white and with microscopic morphology corresponding to *Aspergillus* sp. In addition, abundant colonies of bacteria developed in the cultures. A week later, we performed a second culture by duplicate on both SDA with and without antibiotics, with growth of four colonies of *Aspergillus* sp. only in one plate. A third culture was negative.



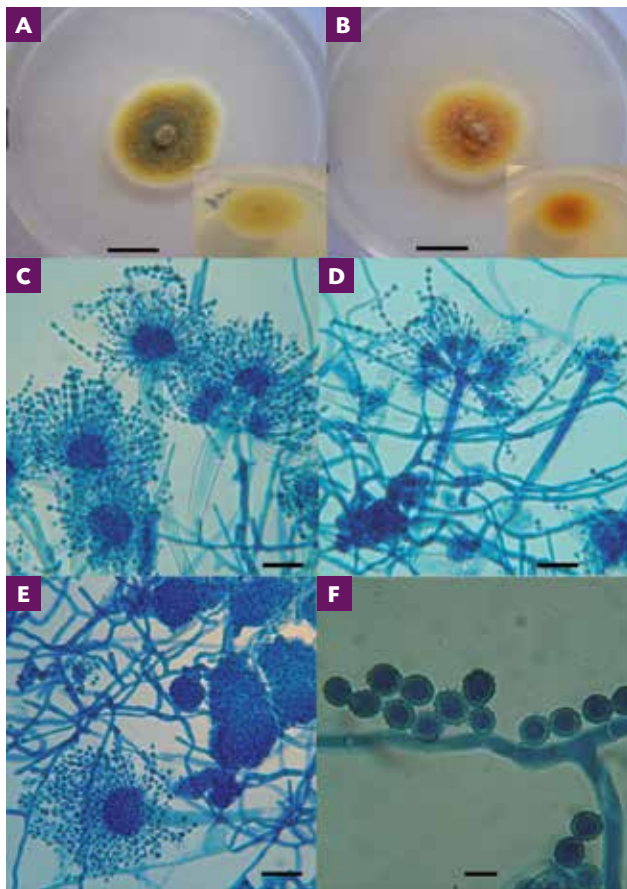
**Figure 2.** Direct microscopic examination of nail scrapings in a potassium hydroxide slide mount. A) Fungal mass. B) Fine or irregular filaments in periphery are observed. C) Other fungal masses showed rounded cells in periphery. D) Some germ tubes-like structures were also observed.

Considering that the microscopic examination of nail scales revealed fungal filaments not compatible with dermatophytes, the absence of growth of a dermatophyte during the three weeks of incubation and the higher number of colonies of *Aspergillus* sp. (a total of 32 colonies), this fungus was determined as the causal agent of onychomycosis with formation of fungal masses. Therefore PCR-sequencing tests, and a morphological study on potato dextrose agar (Dibiko, USA) were performed.

For the molecular identification, from a monosporic culture of *Aspergillus* sp., the total DNA extraction was performed using a commercial kit (GeneAll<sup>®</sup> Exgene<sup>™</sup> PLANT SV, Seoul, Korea), following the manufacturer's instructions. The quality of the genetic material was verified on a 0.8% agarose gel, stained with GelRed<sup>®</sup> Nucleic Acid Stain (Biotium, USA). With 50 ng of DNA, a polymerase chain reaction (PCR) was performed to amplify the ITS region and  $\beta$ -tubulin gene, with oligonucleotides and conditions recommended by Luo & Mitchell and Glass & Donaldson.<sup>18,19</sup> Amplicons were purified with a commercial kit (DNA Clean & Concentrator<sup>™</sup>-5, Zymo Research, USA), quantified and sent for sequencing. Sequence results were compared with the Genbank database (April 21, 2020). ITS sequence matched 100% identity with several species of the *A. glaucus* group (*A. ruber*, *A. medius*, *A. cibarius*, *A. niveoglaucus*, *A. neocarnoyi*, *A. glaucus* and *A. pseudoglaucus*; *Eurotium repens*, *E. berbariorum*, *E. niveoglaucum*, *E. medium*, *E. umbrosum* and *E. rubrum*), including several sequences corresponding to *Aspergillus proliferans* (MK2011244.1; KX696374, -76, -77; KC692212.1; HE615128.1).  $\beta$ -tubulin matched 100% identity with *A. pseudoglaucus* and 95.89% with *A. proliferans*. Based on the morphological characteristics described by Gómez de Membrillera,<sup>20</sup> and carefully compared with other recent descriptions that include all species of the *A. glaucus* group, *Aspergillus proliferans* was determined as the causal agent of the onychomycosis. The macroscopic and microscopic morphology of this species is shown in figure 3. In addition to the conidial apparatus characteristic of the genus, *A. proliferans* forms secondary aspergillar heads. This isolate did not form typical cleistothecia, as it was originally described for this species, but it formed abundant nodules considered as cleistothecial primordia.<sup>20,21</sup>

For the patient, a systemic treatment based on itraconazole 200 mg daily for six months was indicated, as well as topical treatment with ciclopirox olamine lacquer and 40% urea cream every 24 hours for six months. The patient showed improvement determined by healthy growth 3 mm from the proximal fold in all nails (figure 4). Currently the patient continues the protocol for kidney transplantation.





**Figure 3.** Macroscopic and microscopic morphology of *Aspergillus proliferans*. Culture on Sabouraud dextrose agar (A) and on potato dextrose agar (B) for seven days at 28 °C. Colonies are of slow growth, fluffy appearance and different color (white, yellow, orange, greenish or gray tones) depending on the medium. Inserts show the colony' reverse. Scale: 1 cm. C: Numerous conidial heads, uniseriate and radial phialides, spherical vesicles. Smooth and thin-walled conidiophores, 16 µm in diameter. D: Secondary conidial heads emerging from sterigma. E: Numerous nodulations, described as defective asci. F: Globose and echinulate conidia. Scales: C, D and E, 20 µm; F: 5 µm.

## Discussion

In 1998, Roberts and Evans reported the first case and used the term dermatophytoma for the first time. Since then, to our knowledge, there are approximately 222 reported cases of this pathology,<sup>7,8,10-12,14,22,23</sup> although there is probably a considerable underreporting. Extra-ungueal dermatophytoma cases associated to local or systemic immunocompromise have been reported.<sup>13</sup>

From a total of 1892 samples processed, Martínez and collaborators<sup>10</sup> reported a 5.3% frequency (100 cases) with a diagnosis of onychomycosis. Most of them (51%) corresponded to total dystrophic onychomycosis followed by a lateral distal subungual onychomycosis (47%). In the case here presented, clinically the patient mainly developed melanonychia, pachyonychia and onychogryphosis, in addition to the white-yellowish rounded areas, one of the two most reported manifestations.

Since the dermatophytoma is a type of onychomycosis, the main causal agent reported has also been *Trichophyton rubrum* followed by *M. gypseum*.<sup>10,12</sup> Unique cases have been caused by *T. mentagrophytes*, *T. interdigitale*, *M. canis* and *E. floccosum*.<sup>8,11,12,15</sup> However, one report involves non-dermatophyte filamentous fungi: *Scopulariopsis brevicaulis*, *Aspergillus* sp., *Fusarium* sp. and *Acremonium* sp.<sup>10</sup>

To determine that a non-dermatophyte fungus is the causal agent of onychomycosis, it is necessary to meet the following criteria: 1) observe fungal filaments on microscopic examination; 2) to obtain two independent isolates, at least one week apart; 3) inoculum count: growth in five of 20 nail fragments inoculated in the medium.<sup>24</sup> In this case report, evidence for criterion 1 is shown in figure 2; for criterion 2, three cultures from scales were made at



**Figure 4.** Nail clinical appearance after six months of combined treatment: oral itraconazole and topical cyclopirox-olamine and urea.

different times; the first and second cultures were positive for *Aspergillus* sp. Regarding criterion 3, from the first and second cultures, in a 10 cm plate, 32 colonies were obtained.

Several *Aspergillus* species have been reported as causing onychomycosis: *A. versicolor*, *A. terreus*, *A. flavus*, *A. niger*, *A. fumigatus* and *A. sydowii*,<sup>24</sup> including the *A. glaucus* group.<sup>25</sup> *Aspergillus proliferans* Smith is a species described since 1943,<sup>26</sup> and despite taxonomic changes this name continues to be valid. Phylogenetically it has been determined that the *Aspergillus* section contains six clades and *A. proliferans* is found, together with other species (*A. glaucus*, *A. niveoglaucus*, *A. brunneus* and *A. neocarnoyi*), within the clade *A. glaucus*.<sup>21</sup> The genus *Eurotium* (sexual phase of some *Aspergillus* species) has been transferred to the genus *Aspergillus*.<sup>26</sup>

The review by Gómez de Membrillera<sup>20</sup> indicates that *A. proliferans* does not produce perithecia and therefore does not produce ascospores but it forms secondary heads. Hubka and collaborators,<sup>26</sup> found that several of their isolates form ascoma, therefore they described the sexual phase of this species, but others did not.

The sequence of the ITS region of our isolate showed a 100% identity with the species of the clade *A. glaucus* and within the sequences corresponding to *A. proliferans* are those deposited by Hubka and collaborators<sup>21,26</sup> and by Mouhamadou and collaborators,<sup>28</sup> which is also coincident with the strain NRRL 1908, reported with defective development of ascoma.<sup>26</sup>

Among all the species of the clade *A. glaucus*, to our knowledge, *A. proliferans* is the only one in which the formation of secondary aspergillar heads has been described. This species also can form numerous ascoma primordia. Therefore, we define that the causative agent of the case of onychomycosis here presented corresponds to *A. proliferans*.

In the studies by Hubka and collaborators<sup>21,26</sup> included three clinical isolates of *A. proliferans*: two isolates from nails and one from skin infection, which indicates that this species has previously been found as an agent of onychomycosis and dermatomycosis.

Concerning to treatment, since the first report by Roberts and Evans,<sup>7</sup> they pointed out that dermatophytoma makes difficult that antifungal agents penetrate into the conglomerate of fungal elements. This observation was confirmed by Burkhart and collaborators<sup>9</sup> who established that the conglomerate represents the formation of fungal biofilms. The abundance of bacteria observed in the culture suggests that the association of these microorganisms could contribute to form a barrier that prevents the penetration of the antifungal drug, thus delaying or

canceling its therapeutic effect.<sup>9</sup> Oral antifungal treatment based on itraconazole or terbinafine is the same as that indicated for other types of onychomycosis. Some authors recommend partial or total nail avulsion (excision), in conjunction with topical and/or systemic treatment.<sup>11,16,27</sup>

The evaluation of an onychomycosis by dermatologist in patients with immunocompromise should be essential. Among the causes leading of death in transplant patients are opportunistic infections, as aspergillosis. Therefore, infections such as dermatophytoma must be timely and appropriately treated to avoid that a superficial lesion, apparently banal, constitutes a source of infection into the bloodstream.

Since cases of “dermatophytoma” caused by non-dermatophyte filamentous fungi, as is the present work, have been reported, we propose the term “onychofungoma” (onycho: nail; fungoma: fungal mass) to differentiate the onychomycosis in which fungal masses are formed by non-dermatophyte filamentous fungi, of those in which a dermatophyte is identified as the causative agent.

In this work we have shown a case of onychofungoma due to *Aspergillus proliferans*, based on morphological and molecular studies, and whose combined therapy (systemic with itraconazole, topical with cyclopyroxolamine/urea and surgical) resulted in a satisfactory recovery in six months.

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