Chapter 10
Direct Microscopy and Culture: What You Need to Know

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Key Features

KOH, chlorazol black, calcofluor white, and culture are the current mycological studies to confirm onychomycosis.

Direct examination in dermatophytic onychomycosis shows septate filaments, arthrospores, or dermatophytoma.

In yeast infections pseudohyphae and blastospores are usually observed.

Onychomycoses are nail infections caused by dermatophytes, yeasts, and non-dermatophytic molds (NDM). Direct examination with KOH, chlorazol black, calcofluor white, and culture are the current mycological studies to confirm the clinical diagnosis [1–4].

Direct examination is performed from the sample obtained by nail scraping with a scalpel blade, a curette, or a vertical perforation of the nail plate [4]. The most convenient site to obtain it, in distal subungual onychomycosis, is from the nail bed or from the ventral area, as well as the proximal part of the affected nail; in the white superficial forms, one scratches the friable part of the dorsal aspect of the nail; in the proximal forms, one must perform a small hole in the proximal part of the nail to expose the affected site of the nail and perform the scraping. In cases of candidal paronychia, one can take small fragments from the cuticle.

This examination is performed with sodium or potassium hydroxide (KOH or NaOH) 10–40% on the nail scraping and slightly heating the sample with a Bunsen lighter, or we can avoid the heating if the KOH is mixed at equal proportions with dimethyl sulfoxide (DMSO). Nowadays, chlorazol black is suitable because it stains just the fungal chitin and is easily observed with a dark or blue color, and artifacts are not confused with fungi structures (Fig. 10.1). If a fluorescence microscope is

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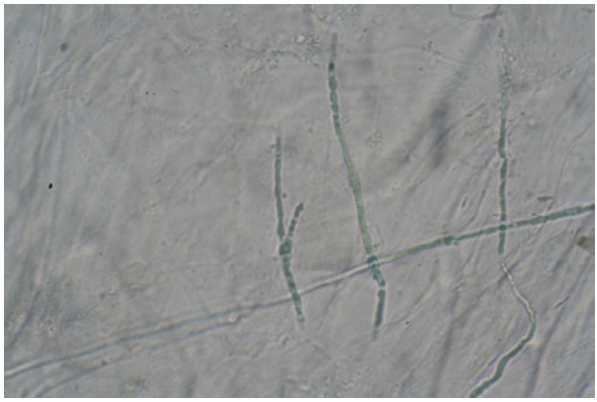

Fig. 10.1 Direct examination in nail clipping (chlorazol black 40×)

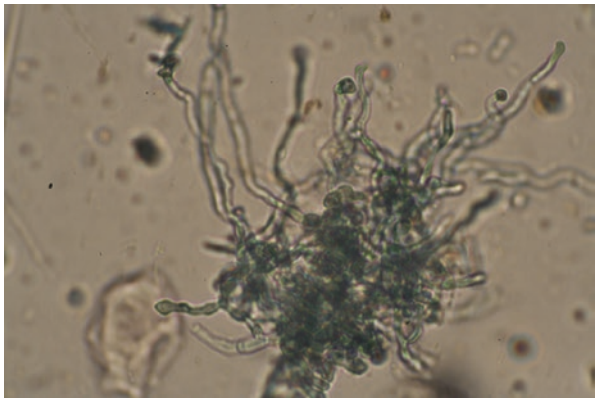

Fig. 10.2 Dermatophytoma (chlorazol black 40x)

available, we can perform the fluorochrome, calcofluor white stain; this is a desirable method, because it allows to visualize even few elements [4, 5].

Long or septate filaments and arthrospores are observed under the microscope. In dermatophytic infection, a dermatophytoma can be found, this is a large accumulation of filaments and spores, and these fungal elements can explain in some cases the lack of response to treatment (Fig. 10.2).

In *Candida* onychomycosis, one can visualize hyphae, pseudohyphae, and blastospores. In dematiaceous fungi we can find dark hyphae, but some non-dermatophytic molds may give similar images to dermatophytes, but in some of them, special characteristics are observed as, in *Aspergillus,* aspergillar heads; in *Scopulariopsis*, huge lemon-shaped spore; and, in *Scytalidium*, narrow and tortuous hyphae [4].

False negatives are due to inexperience in obtaining or reading the sample (20%). Microscopic exam does not allow us to distinguish the viable and nonviable forms [4].

Culture is performed in Sabouraud dextrose agar (SDA) with antibiotics such as chloramphenicol and cycloheximide (Actidione®) if a primary pathogenic fungus is suspected, but the use of culture mediums is convenient without Acti-dione® if

Fig. 10.3 *Candida* spp. culture in SDA

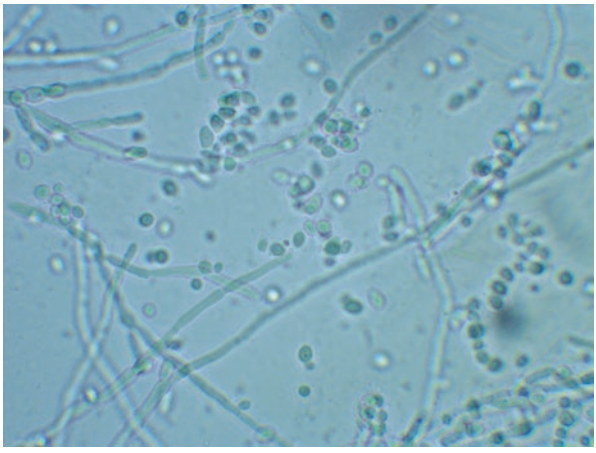

Fig. 10.4 *Candida* spp. blastospores, hyphae, and pseudohyphae

one suspects a non-dermatophytic mold or a yeast (Figs. 10.3 and 10.4). It is convenient to put in the tube or in the Petri dish small fragments of the nail or to pulverize the sample [4].

Usually, it is difficult to isolate the causal agent given the poor sample and the low viability (30–60%); this percentage of false negatives increases after having received a recent treatment or when the scraping is not taken in the limit between the healthy and diseased nail.

Cultures must be incubated at room temperature (24–28° C), and the colonies can be observed from 1 week to 1 month, but NDM and yeasts can grow in less than 1 week; culture is considered negative after 3–6 weeks. If negative, it could be convenient to repeat the complete mycological study [1–4], especially if we have also a negative KOH.

Isolated fungi require a proper interpretation of the mycological samples, because not all the yeasts and non-dermatophytic molds are necessarily pathogens, and should be isolated in several occasions or should grow in abundance in the cultures.

Fig. 10.5 *Trichophyton rubrum.* Culture in SDA

If a specialized personnel is not available, DTM (dermatophyte test medium) is recommended; this is a medium with antibacterial and red phenol as indicators, so bacteria will be inhibited, and if a dermatophyte grows, there is a color virage from yellow to red.

The fungi more frequently isolated among dermatophytes are mainly *Trichophyton rubrum* (Fig. 10.5) and *T. mentagrophytes* var. *interdigitale* and very rare are *T. mentagrophytes* var. *mentagrophytes*, *T. tonsurans*, *Epidermophyton floccosum*, *Microsporum canis, M. gypseum*, *T. soudanense*, *T. violaceum*, *T. erinacei*, and *T. equinum*. Among the yeasts especially *C. albicans* and *C. parapsilosis,* some cases related with *Candida ciferri* and in immunocompromised hosts *Candida glabrata.* Among NDM: *Scopulariopsis brevicaulis* (Fig. 10.6), *Fusarium* sp. and *Aspergillus* sp., *Acremonium* sp., *Paecilomyces* sp.*, Onychocola canadensis* Also *Neoscytalidium* sp., *Alternaria alternata, Chaetomium globosum* and another black fungi. It has been informed of lethal dissemination of the infection by *Fusarium* from an onychomycosis in a neutropenic patient [4].

Biopsy is the gold standard, but usually it can be performed when a case of onychomycosis is highly suspicious and the mycological exams are negative or in uncommon cases. A 4 mm punch biopsy can be performed or a longitudinal, and lateral 2 mm biopsy is preferred that includes all the length of the nail, including matrix, proximal fold, nail bed, and hyponychium [5–7].

In onychomycosis, microabscess with leukocytes in the horny layer can be found, spongiosis, leukocytes exostosis, and lymphocytes, sometimes spongiform pustules and the presence of fungi elements in variable quantities.

In lateral and distal subungual onychomycosis, the filaments are visualized in the hyponychium or in the nail bed, and they take a longitudinal distribution; also, round spores can be observed and correspond to dermatophyte arthrospores

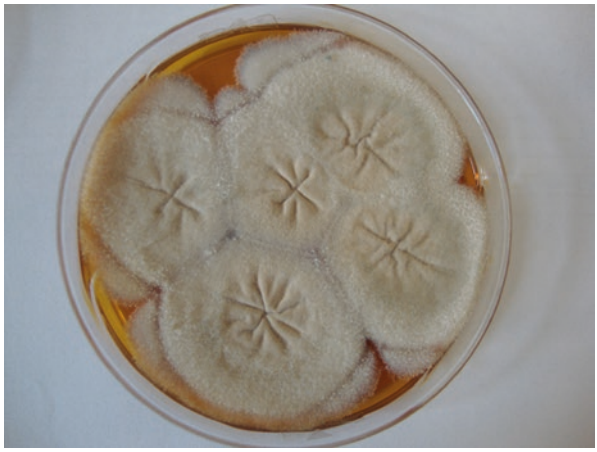

Fig. 10.6 *Scopulariopsis brevicaulis*. Culture in SDA

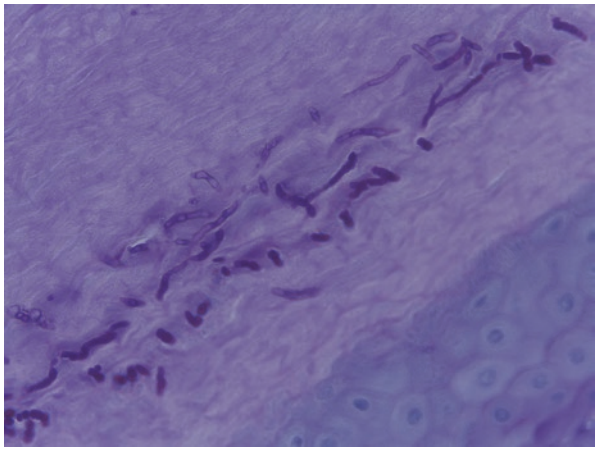

Fig. 10.7 Hyphae and spores (PAS 40×)

(Fig. 10.7); papillomatosis can be present in the nail bed as well as neutrophil microabscesses in the horny layer, spongiosis, and exocytosis; the inflammatory reaction can be mild. In leukonychia cases, the fungi behave as saprophytes with "tortuous" hyphae, arthrospores in chains, short hypha, and even the so-called perforation organs; there is not an inflammatory reaction in the nail bed [4]. In the PWSO a thick lamina is observed with abundant fungal elements and a mild inflammatory reaction. In the total dystrophic onychomycosis, the nail plate is irregular or can lose all its structure. When *Candida* is the causal agent, one can visualize spores and germinated tubes and lymphocyte exocytosis with cerebriform nuclei [4].

In some cases of nail psoriasis, we can find more pronounced parakeratosis, subungual abscesses in the horny layer, spongiform pustules, and parakeratosis

foci. Histopathology of the nail allows to differentiate onychomycosis from other inflammatory entities such as lichen planus, alopecia areata, and eczema. Some saprophytic fungi or secondary colonization can mainly affect previously damaged nails [4–7].

The biopsy can be an important diagnostic tool, detecting the fungus using H&E and PAS stains. The histological test with PAS stain is fast, simple, and reliable in evaluating onychodystrophies, and the onychomycosis of the nail plate shows a low sensitivity (62%) but a high specificity (100%); Gomori-Grocott or Gridley [6–8] can be performed and also the KONCPA technique (KOH treated nail clipping stained with PAS) or fluorescence techniques [9].

We can use antibodies, immunohistochemistry, and flow cytometry as other techniques to identify pathogenic fungi in the nail.

In another section of this book, we mention molecular techniques such as PCR to increase sensitivity and specificity of the diagnostic tools. It has been found that the gene fragments that codify for 18S-rARN are amplified in the infected nails, but not in the healthy ones, to be able to recognize the spices and also the RFLP (restriction fragment length polymorphism) patterns, using Haell endonucleases [10–12].

Summary for the Clinician

Direct examination with KOH, chlorazol black, calcofluor white, and culture are the current mycological studies to confirm clinical diagnosis. It is performed after nail scraping with a scalpel blade or a curette. Chlorazol black is suitable because it stains just the chitin and fungal elements that are easily observed with a dark or blue color.

Causal agents are difficult to isolate, especially dermatophytes (30–60%). Yeasts and NDM must be isolated in a huge quantity.

Biopsy is the gold standard, but it is usually performed when a case of onychomycosis is highly suspicious and the mycological exams are negative.

Molecular tools are also available.

Clinical Pearls

Nail scraping and examination with KOH, chlorazol black, and calcofluor white usually confirm clinical diagnosis of onychomycosis.

Dermatophytes are causal agents which are difficult to isolate. Also yeasts and NDM can be etiological agents.

Biopsy is usually performed when one suspects onychomycosis and mycological exams are negative.

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