Chapter 12
Onychomycosis: Role of Histopathology

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Key Features
- Histopathological examination of nail clippings is the most sensitive technique for onychomycosis diagnosis.
- Special stains like periodic acid-Schiff (PAS) and Grocott methenamine silver (GMS) are required to highlight the presence of fungal hyphae.
- The infecting agent can be suspected depending on its exposed architecture; nevertheless, the precise identification is not possible.

Onychomycosis is the term used to describe fungal infection of one or more nail units, caused primarily by dermatophytes, yeasts, or non-dermatophyte molds (NDM). It represents the most frequent onychopathy in adults, accounting for up to 50 % of all nail disorders and affecting approximately 5–13 % of the general population, proportionately increasing with age [1, 2]. Clinical presentation can be variable and may mimic other nail diseases so diagnostic tests need to be conducted to confirm the presence of fungi [3].

Traditionally, onychomycosis is diagnosed by two standard methods, direct examination with KOH-Chlorazol black® (Delasco, Council Bluffs) and fungal culture; nevertheless, inconsistent low sensitivities of these techniques have been reported, resulting in potentially delayed diagnosis, which may lead to total nail dystrophy, with low rates of recovery despite treatment [2, 4]. Thus, histopathological examination can be useful when the latter are negative and can be helpful in

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establishing the extent of the fungal invasion, suggesting an infective agent or ruling out the infection by proposing an alternative diagnosis [2, 5].

The infected nail is clinically and histopathologically different from the healthy nail. Electron microscopy has been useful in characterizing the microscopic nail plate architecture in normal and affected nails. Healthy nails have a smooth surface made of parallel layers of flat and keratinized cells called onychocytes, resulting in a compact barrier; they have an average reported thickness of 0.49 mm and a density of 1.34 g/cm^3. On the other side, onychomycotic nails present a fragmented surface with cell separation and lifting, indicating its rigidity has changed; also, they tend to be thicker (1.29 mm) and have a lower density (1.29 g/cm^3) than non-affected nails, traducing a more porous plate [5, 6].

Dermatophytes are known to have a variety of proteases and lipases that hydrolyze keratin, collagen, and elastin, which alter the nail matrix, disrupt cell interactions, and help them invade the nail plate, so explaining the reported microscopic changes [7, 8].

The most frequent type of nail biopsy for diagnosing onychomycosis is the nail clipping dyed with special fungal stains, which is now considered the gold standard because of its high sensitivity. This is the preferred diagnostic method because it is a non-painful procedure for the patient and is fast and simple to perform at a low cost.

Clippings are fragments cut from the distal portion of the nail plate, which according to reports should be at least 4 mm in length to improve the diagnostic performance; these samples are then processed and stained for histopathological assessment [9, 10].

With hematoxylin and eosin (HE) stain, the study frequently reveals dystrophy of the nail plate with dissociated keratin layers (Fig. 12.1), as well as the presence of parakeratosis and plasma globules, which according to several studies are statistically and significantly more common in slides where fungi are found than in those where they are not present. Neutrophils and bacteria are variably described; nevertheless, their finding is not statistically different with the presence or absence of fungi. All these microscopic findings are more frequent and easily found on the ventral part of the nail plate, which is in close proximity to the nail bed. In a white superficial onychomycosis, fungal elements are commonly found in the dorsal surface of the nail plate [10, 11].

The fungal hyphae on HE routinely stained sections are difficult to visualize; therefore, special fungi stains are needed to highlight its presence; these classically include periodic acid-Schiff (PAS) and Grocott methenamine silver (GMS). PAS technique works by exposing tissue to periodic acid, which oxidizes hydroxyl groups of cell wall polysaccharides into dialdehydes; the latter react with Schiff reagent, forming a magenta compound. The background is a faint pink, while fungi cell membranes stain a magenta-red color. In GMS stain, chromic acid is used to oxidize the hydroxyl groups forming aldehydes, which then react with the silver nitrate reducing it to metallic silver, making them visible. The slide has a light green background, while the hyphae are stained dark brown to black [1].

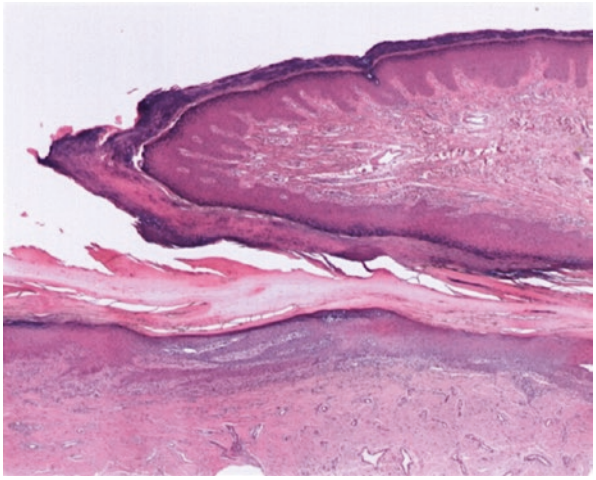

Fig. 12.1 Longitudinal nail biopsy of an onychomycotic nail. The nail plate of the matrix and proximal nail bed is dystrophic showing an irregular, fragmented, and lifted surface with mounds of parakeratosis

For a while, GMS was thought to be superior to PAS staining for finding fungal structures in biopsies; however, after several conducted studies, they concluded the deeper levels were increasing the detection of the fungi, rather than the stain used. Although no significant differences were reported between these two, PAS was found to be a more cost-effective stain [1, 2, 12].

Although the fungi structures are well visualized with the special stains described, histological examination does not allow the precise identification of the infecting agent; however, it can suggest the implicated pathogen by analyzing their morphology.

Usually, a dermatophytic infection is suspected when regular, septate hyphae running parallel to the nail surface are observed (Fig. 12.2).

Yeasts are suspected when small round spores, some even budding, pseudohyphae, and short filaments are found. Spores without pseudohyphae can be contaminants (Fig. 12.3).

NDM can display truncated spores with vertically thin arising perforating hyphae.

Although the former are the main causative onychomycosis agents, other ND fungi have been reported to cause infection like *Aspergillus* spp., *Alternaria* spp., *Scopulariopsis brevicaulis*, *Emericella quadrilineata*, and other microorganisms like *Prototheca* spp., and also Medlar bodies have been found in a case of melanonychia [13–17].

An uncommon and unique clinical presentation of onychomycosis caused by dermatophytes, also known as dermatophytoma, typically presents as a yellow longitudinal band or yellow or white patch. Histologically a dense mass of hyphae is found [10].

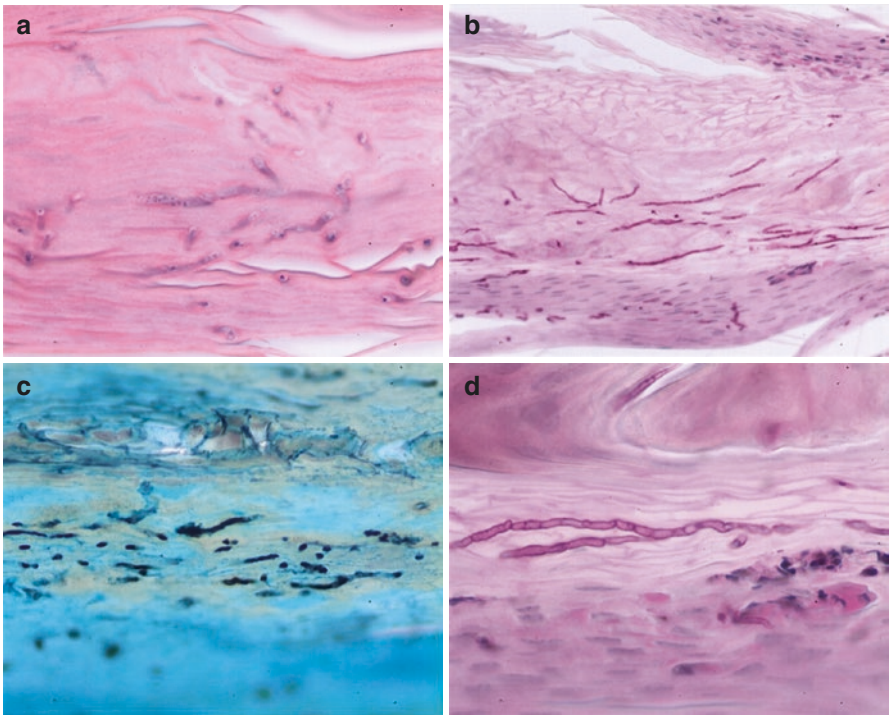

Fig. 12.2 Histological examination of dermatophytic onychomycosis. (**a**) H&E stain showing translucent hyphae spreading between corneocytes. (**b**) PAS stain highlighting hyphae in *red-magenta* color. These are regular and parallel-oriented to the nail surface. (**c**) Hyphae dyed in *black* with Grocott methenamine silver stain. (**d**) PAS stain showing regular, branching, septate hyphae

Punch samples of nail plate and nail bed or longitudinal nail biopsies are rarely performed, except when other inflammatory diseases are clinically suspected. When these are taken, the histological picture displays subungual hyperkeratosis with mounds of parakeratosis or foci of neutrophils, psoriasiform dermatitis with hyperplasia of the nail bed epithelium, and variable spongiosis with neutrophil exocytosis. In the absence of onychomycosis, another nail disorder can be recalled like psoriasis, lichen planus, or even a hematoma. Psoriasis can be difficult to differentiate from onychomycosis clinically and histopathologically [5].

KOH has a sensitivity between 53 and 76 % and mycological culture between 35 and 53 %; both tend to be sample dependent; nevertheless, the latter can identify the fungal species and sensitivities to antifungals but requires long incubation periods to yield a diagnosis. PAS-stained sections of nail clippings have the highest reported sensitivity, varying between 75 and 92 % according to different studies, and therefore are considered the gold standard diagnostic technique. It is the least likely to be affected by sampling methods and is also considered the most sensitive to monitor residual infection after adequate antifungal treatment. Combining these techniques has established decreased chances of false negatives; PAS staining combined with KOH reported sensitivities between 89 and 99.4 %, while PAS with culture gave

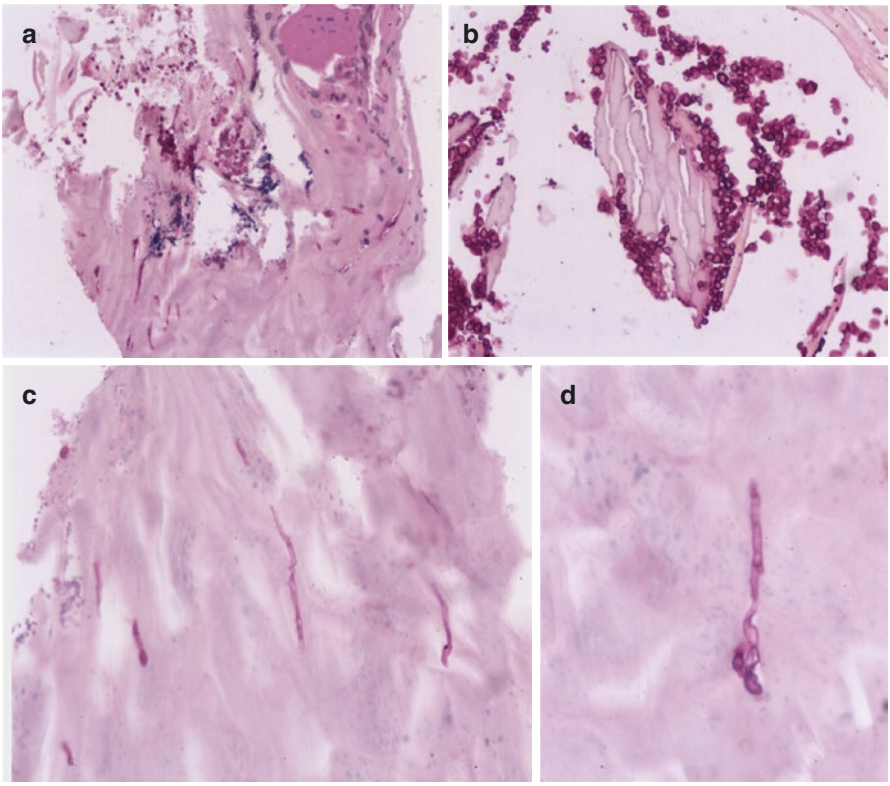

Fig. 12.3 *Candida* onychomycosis on a histological section stained with H&E. (**a**) Dystrophic nail plate with parakeratosis, plasma, bacterial colonies, and *red-magenta* dyed spores. (**b**) Multiple small round spores admixed with keratin. (**c**, **d**) Short and thin filaments and pseudohyphae, vertically oriented to the nail plate, are also found

between 93 and 96 %; however, PAS alone was better than the combination of KOH direct microscopy and culture with a sensitivity of 88.8 %. Hence, combination of PAS-stained nail clippings with either KOH or culture can give higher values of sensitivity and negative predictive values [4, 11, 18].

Although histopathology is a relatively fast diagnostic test, the nail needs to be fixed, dehydrated, embedded in paraffin, and sectioned before being stained [3]. A nail biopsy can be technically harder to process than a routine cutaneous specimen because of the hard keratin in the nail plate, so pretreatment with softening agents is needed to obtain high-quality sections. Useful softener agents described include potassium hydroxide (KOH), 4 % phenol, 5 % trichloroacetic acid in 10 % formalin, cider oil, chitin softening agent with mercuric chloride containing solutions, and more recently sodium hydroxide (NaOH), which has reported an improved ease of sectioning and adherence to slides, although fainter PAS staining and damage to melanin and hemosiderin pigments have been reported [10, 19].

More novel techniques have been described for detecting dermatophytes, for example, fluorescence microscopy, from the basis that some pathogenic fungi

fluoresce under ultraviolet light, as in Wood's lamp test. This method uses hematoxylin- and eosin-stained sections under fluorescence microscopy, where the fungus shows a clear bright fluorescent ring at the periphery, without investing time in special stains. Other available tests are flow cytometry, immunohistochemistry, phase-contrast hard X-ray microscopy, optical coherence tomography, and DNA-based rapid diagnostic techniques like PCR; nevertheless, these are limited and infeasible for daily use because of high costs, complexity, and poor availability [18, 20].

Summary for the Clinician

Histological examination of nail clippings is a simple and relatively fast technique for diagnosing onychomycosis. Sections typically need PAS or GMS stains to highlight the presence of hyphae. PAS nail clipping has the highest reported sensitivity and, therefore, is considered the gold standard; nevertheless, combination with KOH or mycological culture is recommended to enhance diagnostic yield.

Histological examination does not allow the precise identification of the fungal agent; however, it can suggest the implicated pathogen depending on its morphology. Dermatophytes display regular septate hyphae parallel to nail surface; yeasts show round spores, pseudohyphae, and short filaments; and non-dermatophyte molds present with truncated spores with thin and vertical perforating hyphae.

Clinical Pearls

- PAS staining of nail clippings is the gold standard for diagnosing onychomycosis. This is usually performed when KOH or mycological culture yields negative results and clinical suspicion is high.
- Morphology of fungi can suggest the etiological pathogen.
- When a longitudinal nail biopsy is performed, it displays subungual hyperkeratosis with mounds of parakeratosis or foci of neutrophils, psoriasiform hyperplasia of the nail bed epithelium, and variable spongiosis with neutrophil exocytosis.
- Nail biopsies allow other nail disorders to be excluded. Psoriasis is the principal differential diagnosis.
- Although it is relatively simple and fast technique, nail biopsy can be technically harder to process.
- Combination of diagnostic tests is recommended to improve diagnostic performance.

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