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RESEARCH ARTICLE

Evaluation of Onychomycosis in Nail Psoriasis: Comparison of Diagnostic Methods

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ABSTRACT

Background: Epidemiological studies indicate that onychomycosis may affect up to 79% of psoriatic patients. Onychomycosis in psoriatic patients is more commonly caused by yeasts comparing with non-psoriatic. There are different methods for diagnosing onychomycosis that could be used separated or together.

Objectives: To compare the histopathological findings (nail *clipping*) with Direct Mycological Examination (DME) and mycological culture on patients with psoriasis and nail psoriasis.

Methods: Eighty-three finger nails of 12 patients with the diagnosis of nail psoriasis were analyzed. None were under topical therapy or the nails. Samples for *clipping*, Direct Mycological Examination and mycological culture were collected. The proportions were analyzed using the chi-square test. The comparison of Direct Mycological Examination, mycological culture and *clipping* results were analyzed through sensitivity, specificity, positive and negative predictive value. Spss® 22 software was used for the analysis.

Results: Direct Mycological Examination x mycological culture: Direct Mycological Examination and mycological culture show agreement regarding their negative test. Of the 51 negative mycological culture exams, 5 were positive on Direct Mycological Examination and of the 51 negatives on Direct Mycological Examination, 5 were positive on mycological culture ($p<0,0000$). Therefore, Direct Mycological Examination and mycological culture are reliable and complementary methods on diagnosis of onychomycosis. *Clipping* x mycological culture: in the 51 negative mycological culture tests, were found 30 positive clipping tests (61.2%). Of the 34 negatives clipping, 13 were considered positive on mycological culture (38,2%) ($p=0,96$). *Clipping* x Direct Mycological Examination + mycological culture: Of the 56 negative results Direct Mycological Examination +mycological culture, 32 were positive on *clipping* (57,1%). Of the 34 negatives *clipping*, 10 were considered positive on Direct Mycological Examination + mycological culture (37%) (p: 0,613). This means that nail *clipping* "rescues" negative results from mycological culture. In Direct Mycological Examination there was a predominance of blastoconidia. No septae hyphae were detected. In mycological culture, the most prevalente fungi was Candida. There was no dermatophyte growth.

Conclusion: Nail *clipping* was positive more than half of the negative samples for mycological culture, and can be considered a great diagnostic aid, complementary to Direct Mycological Examination and mycological culture. These results are consistent with some previous reports, Candida was the fungus with higher frequency on the psoriatic nails, however, the role of these fungi is controversial (contamination x colonization x infection).

Keywords: psoriasis, onychomycosis, nail *clipping*, direct mycological exam, mycological culture

Introduction

Patients with nail psoriasis may have nail changes that are similar to onychomycosis. Some authors state that psoriasis is an important risk factor for onychomycosis since morphological changes in nails with psoriasis may facilitate fungal colonization.[1]

Epidemiological studies so far have yielded ambiguous results; some authors have shown that onychomycosis can affect up to 79% of patients with psoriasis, while others have found a lower incidence (15%).[2] From a clinical perspective, the association between these two diseases holds particular significance, as it influences the clinical management of the patient. The presence of untreated or unidentified fungi in the nail bed may increase the severity of nail psoriasis (Köbner phenomenon) and may be the cause of treatment failure.[2]

Onychomycosis affects 4 to 18% of the population.[1] Onychomycosis is mainly caused by anthropophilic dermatophyte fungi of the genus *Trichophyton*, especially *T. rubrum*, followed by *T. interdigitale*. Non-dermatophyte fungi exhibit a low prevalence, with *Fusarium* spp., *Aspergillus* spp., and *Scopulariopsis brevicaulis* being the most commonly isolated species, typically found in the toe regions.[2,3] Fungi belonging to the *Candida* spp. genus, particularly Candida *albicans* and *Candida parapsilosis*, demonstrate heightened prevalence among immunocompromised and diabetic patients.[1]

The most commonly used methods for the diagnosis of onychomycosis are the Direct Mycological Examination (DME) and mycological culture from nail scrapings.

Nail *clipping* consists in the histopathological analysis of fragments cut from the distal nail plate. Although literature has featured studies on this procedure since 1948, it remains underutilized in the clinical practices of dermatologists.[5] It is a swift, practical, and painless examination that does not induce nail dystrophy.[6] Nail *clipping* is recommended for assessing nails exhibiting subungual keratosis and distal onycholysis-common clinical findings in both psoriasis and onychomycosis. This aids in confirming the presence of fungal agents, but not the species, as only the mycologic culture can confirm the specific etiological agent.[6,7] They are also useful for monitoring antifungal treatment, as both DME and cultures can yield false negative results.[8]

In the histopathological analysis of nail *clippings*, the presence of uniform septate hyphae invading the nail plate suggests infection by dermatophytes. Twisting hyphae with thicker walls represent non-dermatophytic fungi, while conidia on the ventral surface of the lamina, especially when accompanied by buds and pseudo-hyphae, suggest Candida infection.[9]

Studies demonstrate that nail *clipping* exhibits a higher diagnostic sensitivity compared to the Direct Mycological Exam (DME) and mycological culture.[8]

Hence, it is evident that a combination of these three tests is necessary for establishing a diagnosis, with culture maintaining its status as the "gold standard" and the sole method capable of identifying the etiological agent.[8] This shortens the patient's journey by avoiding multiple negative tests and prolonged antifungal treatments. Correct diagnosis is important due to prolonged and expensive treatment, with possible adverse events, such as elevated liver enzymes and drug interactions.

This study also aims to emphasize the role of nail *clipping* as a diagnostic tool that is often neglected by dermatologists.

Methods

This descriptive and observational cross-sectional study utilized non-probabilistic sampling with outpatients diagnosed with nail psoriasis at the Clementino Fraga Filho University Hospital (HUCFF/UFRJ), Rio de Janeiro-Brazil. The evaluation period spanned from October 2017 to October 2018, encompassing patients aged 18 or older with a confirmed diagnosis of psoriasis and nail alterations consistent with nail psoriasis.
Exclusion criteria involved patients with decompensated systemic diseases, those with conditions involving ischemic or perfusion alterations in extremities, post-traumatic nail alterations, and other inflammatory conditions affecting the nails. All participants provided informed consent by signing the informed consent form.

A questionnaire assessing the progression of psoriatic disease, current medications (including immunosuppressive therapy), prior fungal infections, past nail alterations, and the number of affected nails was administered. A total of 83 nails from the digits of 12 patients with nail psoriasis were analyzed. Among these, 5 received only topical skin treatment (moisturizer, coaltar, and derivatives), while 7 underwent systemic treatment (methotrexate, phototherapy, secukinumab, etanercept). None of the patients were undergoing topical treatment specifically for the nails.

All samples were submitted to direct mycological examination (DME), mycological culture and nail *clipping*. Specimen collection and laboratory procedures: Nails were cleaned with gauze soaked in 70% ethanol. Using appropriate collecting instruments, the affected areas were scraped off, discarding the first portions. Deeper scraping was then made, placed between two wide glass slides, wrapped by paper (all materials sterile) and sent for laboratory analysis.

Direct microscopy examination was made with KOH 20% added to Parker Ink (Quink) (1:1 ratio), searching for the presence of fungal elements. Specimens were then inoculated onto Sabouraud-Dextrose agar 2% (BD-DIFCO™) and Mycosel agar (BD-DIFCO™), incubated under aerobic conditions for 30 days at 25°C and examined daily. Filamentous fungi were identified initially by macro and micromorphological characteristics of colonies and then by slide cultures on Potato-Dextrose–agar (BD-DIFCO™)[10]. Yeast isolates recovered from primary culture were streaked on ChromAgar Candida (BD-DIFCO™) to check for purity, and then subcultured on Sabouraud-Dextrose agar 2%. Additional tests included germ tube production, slide cultures on Corn-meal-tween 80 agar and carbohydrates fermentation and assimilation[11].

Following the collection of the DME and mycological culture, nail *clippings* were obtained by cutting the distal

portion of the nail plate with a minimum length of 5 millimeters and a width of 2 millimeters. The nail plate fragments were fixed in 10% formalin, processed, and embedded in paraffin. Histological sections of 4 micrometers were obtained and stained with Hematoxylin and Eosin as well as Periodic Acid-Schiff (PAS). The PAS staining aimed to describe the presence of hyphae or round forms[7].

The Spearman correlation model was employed for correlation analysis. Proportions were assessed using the Chi-square test. The comparison of DME, mycological culture, and nail clipping analyses was conducted through sensitivity, specificity, positive predictive values, and negative predictive values. The analyses were performed using the SPSS® 22 software.

Results

The results obtained from nail clipping, DME, and mycological culture were compared. Sensitivity and specificity tests were conducted for culture, and the findings were correlated with the DME (Table 1).

Table 1:

DME * mycological culture Cross tabulation					
			Mycological culture		Total
			,00	1,00	
DME	,00	Score	46	5	51
		% within DME	90,2%	9,8%	100,0%
		% within mycological culture	90,2%	15,6%	61,4%
	1,00	Score	5	27	32
		% within DME	15,6%	84,4%	100,0%
		% within mycological culture	9,8%	84,4%	38,6%
Total		Score	51	32	83
		% within DME	61,4%	38,6%	100,0%
		% within mycological culture	100,0%	100,0%	100,0%

The DME and mycological culture exhibited concordance regarding negative results. Out of 51 negative culture tests, 5 were positive in the DME, and of the 51 negative DME results, 5 were positive in culture ($p < 0.0000$). Therefore, DME and culture are reliable and complementary methods in the diagnosis of onychomycosis.

Sensitivity, specificity, and positive and negative predictive values were assessed in the comparison between nail clipping and mycological culture (Table 2).

Table 2:

Nail *clipping* * mycological culture cross tabulation					
			Nail *clipping*		Total
			,00	1,00	
Mycological culture	,00	Score	21	30	51
		% within nail *clipping*	61,8%	61,2%	61,4%
		% within mycological culture	41,2%	58,8%	100,0%
	1,00	Score	13	19	32
		% within nail *clipping*	38,2%	38,8%	38,6%
		% within mycological culture	40,6%	59,4%	100,0%
Total		Score	34	49	83
		% within nail *clipping*	100,0%	100,0%	100,0%
		% within mycological culture	41,0%	59,0%	100,0%

In the 51 negative culture tests, 30 were positive in nail clipping (61.2%). Out of the 34 negative nail clipping results, 13 were positive in culture (38.2%) (p=0.96).

Sensitivity, specificity, and positive and negative predictive values were assessed in the comparison between nail clipping and the combined DME and culture. The results from the DME and mycological culture were combined, considered positive only when both results were positive (Table 3).

Out of the 56 negative results in DME+ mycological culture, 32 were positive in nail clipping (57.1%). Among the 34 negatives in clipping, 10 were considered positive in DME+ mycological culture (37%) (p=0.613).

The predominant findings in the DME were blastoconidia, with no detection of septate hyphae. In mycological culture, Candida was the most frequently identified agent, and there was no growth of dermatophytes.

Table 3:

DME and mycological culture * nail *clipping* Cross tabulation					
			Nail *clipping*		Total
			,00	1,00	
DME and mycological culture	,00	Score	24	32	56
		% within DME and mycological culture	42,9%	57,1%	100,0%
		% within nail *clipping*	70,6%	65,3%	67,5%
	1,00	Contagem	10	17	27
		% within DME and mycological culture	37,0%	63,0%	100,0%
		% within nail *clipping*	29,4%	34,7%	32,5%
Total		Score	34	49	83
		% within DME and mycological culture	41,0%	59,0%	100,0%
		% within nail *clipping*	100,0%	100,0%	100,0%

Discussion

There are few studies mentioning microscopic alterations in nail clippings from nails without suspected onychomycosis.[18,19,20] The majority of studies focused on nail clippings in the assessment of onychomycosis. [15,21,22,23,24,25].

Nail *clipping* serves as a valuable tool, in conjunction with the DME and mycological culture, in the differential diagnosis of nail alterations. It plays a significant role in distinguishing between fungal colonization and infection, revealing characteristics relevant to nail dystrophies. Nail *clipping* is useful in monitoring and diagnosing patients undergoing antifungal treatment, as well as in cases where multiple direct exams and cultures have yielded negative results despite persistent clinical suspicion. Some authors suggest that all patients with psoriasis and subungual keratosis or onycholysis should be investigated for the presence of fungi, particularly before initiating topical corticosteroid treatment.[26,27,28] Thus, if the presence of fungal infection is confirmed, clinical management with antifungal drugs can prevent "Koebnerization" of the nails, as well as spare the individual from unnecessary complications.[29]

A diagnostic algorithm is available for clinical suspicion of onychomycosis. (table 4)

Table 4:

DIAGNOSTIC ALGORITHM ON CLINICAL SUSPICION OF ONYCHOMYCOSIS:

DME + mycological culture-> negative

↓

DME + mycological culture -> negative

↓

nail *clipping*

A study conducted by Velasquez-Agudelo et al. compared, through meta-analysis, nail clipping, DME, and mycological culture in individuals with clinical suspicion of onychomycosis. Nail clipping with Periodic Acid-Schiff (PAS) staining was the most sensitive test (84%), while culture had a sensitivity of 56%, and DME showed sensitivity ranging from 44% [14] to 100% [15]. When test combinations were made, sensitivity values were 57% for clipping + DME[13] and 98.3% for clipping + mycological culture [16].

In our study, nail clipping, in the analysis of fungi, proved to be an excellent complementary test when both the Direct Mycological Exam (DME) and mycological culture returned negative results. It revealed 61% of positive results that culture did not detect and 57% that were not detected by DME + mycological culture. Dermatophytic fungi were not found in DME and mycological culture, aligning with literature which identifies *Candida* spp. as the primary etiological agent of onychomycosis in patients with psoriasis.[12]

Culture is the gold standard method for diagnosing onychomycosis, and the histopathological analysis of nail clippings does not replace it.[13] Inadequate collection, insufficient samples, and the inability to identify the etiological fungus are limiting factors of nail *clipping*.[7,30] However, the association with other methods increases its diagnostic sensitivity.[9]

Nail *clipping* with PAS staining is useful to confirm the presence of fungi in the nail plate and its invasion by visualization of fungal structures.[17]

Although mycological culture was found to be the least sensitive test in Velasquez-Agudelo *et al.*[8], this test should not be ignored in the diagnosis of onychomycosis because it is the only one of the three tests that can detect the etiological agent with greater accuracy and provides highly useful information to decide on the most appropriate antifungal therapy.

Therefore, the results indicate the necessity of a combination of the three tests to establish a diagnosis, given their complementarity in identifying which patients were affected, the causative agent, and the degree of invasion.

It is worth mentioning that fungal invasion in the nail occurs, in most cases, through the distal bed, in the opposite direction to the nail's growth, making the PAS-stained nail clipping temporally reliable compared to the presented clinical symptoms.

The coexistence of psoriasis and onychomycosis has been reported in the literature, with some studies suggesting a higher prevalence of onychomycosis in patients with psoriasis (18% versus 9% in a control group), as indicated by a systematic review by Klaassen et al.[12] and Stander et al. found yeast in 23.9% of patients with nail psoriasis, 6.1% in patients with psoriasis without nail changes, and in 6.9% of the controls.[31]

This study successfully compared the results of the DME, mycological culture, and nail clipping in the same patient and on the same nails. This approach distinguishes it from other studies in the current literature, which primarily involve meta-analyses comparing different studies.

Conclusion

Nail clipping was positive in more than half of the samples that tested negative for mycological culture, establishing itself as an auxiliary tool alongside the DME and mycological culture in the differential diagnosis of nail alterations in patients with psoriasis. Accurate diagnosis of nail dystrophy prevents ineffective, prolonged, and costly treatments, along with potential adverse events and drug interactions.

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