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Diagnostic Accuracy of Dermatoscopy Versus Microbiological Culture and Polymerase Chain Reaction in the Diagnosis of Onychomycosis: A Cross-Sectional Study

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ABSTRACT

Background: Several clinical signs in dermatoscopy are very characteristic of onychomycosis and can be a quick complement for the diagnosis of onychomycosis.

Objectives: The aim of this study was to evaluate the diagnostic accuracy of dermatoscopy compared to microbiological culture and polymerase chain reaction (PCR), as well as the clinical signs associated with onychomycosis.

Methods: The clinical signs of 125 patients were assessed cross-sectionally using dermatoscopy, and a positive or negative result was assigned. A sample was then taken for PCR and microbiological culture.

Results: Of the 125 patients, 69.6% (87/125) had positive results when both laboratory tests were combined. When they were not combined, the prevalence was lower at 48% (60/125) with PCR and at 43.2% (54/125) with culture. Furthermore, 76.8% (96/125) were classified as positive with dermatoscopy with a sensitivity of 1, a specificity of 0.76, positive predictive value of 0.91 and negative predictive value of 1 (with 95% confidence intervals). Of the 96 dermatoscopy-positive samples, 36 were negative with PCR ($p < 0.001$), 42 were negative with culture ($p < 0.001$) and nine were negative when both tests were combined ($p < 0.001$). Clinical signs that were significantly associated with the presence of onychomycosis were subungual hyperkeratosis (dermatoscopy: $p = 0.004$, odds ratio (OR) = 2.438; PCR + microbiological culture: $p = 0.004$, OR = 3.221), subungual detritus ($p = 0.033$, OR = 3.01, only with dermatoscopy) and dermatophytoma (dermatoscopy: $p = 0.049$, OR = 3.02; PCR + microbiological culture: $p = 0.022$, OR = 2.40).

Conclusions: The results suggest that dermatoscopy is a good tool for the diagnosis of onychomycosis but should be used as a complementary test or for screening patients to be sampled for laboratory testing. The combination of the three tests can lead to a reduction of false-positive and false-negative clinical and laboratory results. This allows for early diagnosis and specific treatment based on test results.

1 | Introduction

Onychomycosis is a fungal nail infection that affects approximately 5.5% of the world's population and accounts for half of all nail lesions [1]. It predominantly affects the toenails, and the main clinical signs are thickening, hyperkeratosis or subungual detritus, discolouration, onycholysis, dermatophytoma and longitudinal striae, among others [1–3]. Advanced age, immunodeficiency, peripheral vascular disease and diabetes increase the prevalence of onychomycosis and the occurrence of dystrophic or thickened nails [2, 4, 5]. It is a major health problem due to its high prevalence and the subungual and periungual lesions that it can cause, which are a serious problem in patients with diabetes [2, 4].

Onychomycosis can be caused by dermatophytes (which account for 90% of cases), moulds and yeasts. In addition, some microorganisms may also be present and cause mixed infections [1, 6]. Before treating a patient, it is necessary to determine the causative microorganism using valid laboratory tests such as microbiological culture, KOH tests or polymerase chain reaction (PCR) [7]. However, these tests have advantages and disadvantages. Microbiological culture identifies the causative pathogen and confirms its viability but has a waiting time of 2–3 weeks and a high false-negative rate, while KOH has immediate results but does not confirm pathogen viability and is dependent on the examiner. Furthermore, PCR has a short waiting time of 24–48 h and high sensitivity but does not confirm pathogen viability and is expensive [7, 8]. However, different professionals do not consider laboratory tests necessary for diagnosis and are simply guided by clinical diagnosis using tools such as dermatoscopy [9].

Dermatoscopy is a noninvasive, fast, inexpensive and reproducible diagnostic tool [10]. In some countries, it is indicated to prescribe antifungal treatment with clinical or dermatoscopic diagnosis, leaving laboratory tests for treatment failures or in case of doubt [11, 12]. In other countries, only 21.4% of podiatrists use laboratory tests despite the fact that 86.3% consider them necessary [13].

Many clinical signs in dermatoscopy are very characteristic of onychomycosis, so it can be a quick complement for diagnosing onychomycosis until the results of laboratory tests are available [14, 15]. A severe appearance, leukonychia and black discolouration are statistically significantly associated with distal lateral onychomycosis according to Kaynak et al. [16] Jesús-Silva et al. [17] described the presence of longitudinal striae, linear borders and irregular borders in patients with onychomycosis. Kayarkatte et al. [14] observed statistically significant associations with herringbone patterns, subungual hyperkeratosis, irregular distal nail ends and severe nail appearance.

The combination of microbiological culture and PCR has been described as one of the most useful and effective tools in the diagnosis of onychomycosis. However, it can sometimes pose financial problems in resource-poor settings [7, 18]. Previous studies have described dermatoscopic signs that can guide the diagnosis of ONM, but to date, no study has compared diagnosis by dermatoscopy versus more than one laboratory test [14, 16, 17]. Therefore, the aim of this study was to evaluate the diagnostic accuracy of dermatoscopy compared to microbiological culture and PCR, as well as the clinical signs associated with onychomycosis.

2 | Materials and Methods

2.1 | Participants and Samples

This study was conducted in accordance with the Declaration of Helsinki and current national legislation governing research and the involvement of patients [19]. A descriptive cross-sectional study was conducted with 125 patients presenting with symptoms of onychomycosis and selected consecutively. These patients were enrolled between October 2023 and March 2024 in a specialised clinic focusing on the treatment of onychomycosis. They were assessed using an Illuco IDS-100 dermatoscope (Gyeonggi-do, Republic of Korea) by positioning the dermatoscopy over the affected nail and diagnosed as positive or negative, and then samples were collected for microbiological culture and PCR. A positive dermatoscopy diagnosis was considered when any of the following dermatoscopic signs were observed: Onychogryphosis, chromonychia, subungual hyperkeratosis, subungual detritus, onycholysis, longitudinal striae, fragile nails, dermatophytoma.

Dermatoscopy assessment, the recording of identified clinical signs and sample collection were always performed by the same professional.

The inclusion criteria for the study were a presumptive diagnosis of onychomycosis and age over 18 years. Patients were excluded if they were previously diagnosed with onychomycosis, were undiagnosed but treated with topical or oral medication within the last month, had psoriasis or subungual tumours, and were receiving immunosuppressive treatment. The sample selection required sufficiently large portions of the nail to allow for both diagnostic procedures.

The samples were examined through microbiological culture in Sabourad dextrose agar for 1–3 weeks. PCR was performed based on the protocols of previous studies [20]. After 1–3 weeks, the laboratory provided the results. The PCR results are reported as 'dermatophyte positive' or 'dermatophyte negative' and the microbiological culture results are reported 'positive' or 'negative' along with the isolated pathogen. Prior to participation in the study, the patients signed an informed consent form. All cultures were analysed in an external microbiology laboratory.

2.2 | Statistical Analysis

Statistical analyses were performed with SPSS for MacOS version 25.0 (SPSS Inc.). The samples were described using tables and graphs with qualitative variables presented as frequencies and percentages and quantitative variables presented as means and standard deviations. The chi-squared test was used to investigate possible associations between qualitative variables.

The sensitivity, specificity, positive predictive value and negative predictive value of dermatoscopy for the diagnosis of onychomycosis were determined by comparison with the combined results of microbiological culture and PCR. Epidat v.3.1 (Galicia, Spain) was used for the analysis. The minimum sample size for the analysis was estimated as 121 samples with a statistical power of 0.80 and an alpha level of 0.05 using an online sample-size calculator (GRANMO version 8.0, *Institut Municipal d'Investigació Mèdica*).

3 | Results

3.1 | Patient Factors

Data from all 125 patients were included in the study. The mean age of the study participants was 67.02 ± 12.43 (range: 18–96) years. The sample included 87 males (69.6%) and 38 females (30.4%). Samples were taken in the last quarter of the year 2023 and the first quarter of the year 2024. Other demographic data can be found in Table 1.

3.2 | Onychomycosis

Among all patients, the hallux was the most affected nail plate with clinical signs of infection (44.8%), followed by both hallux nails (24%), a hallux and lesser toes (20.8%), the lesser toes only (7.2%) and all nail plates (3.2%). Table 2 shows the clinical signs observed using dermatoscopy (Figure 1). A statistically significant association was found between positive results and subungual hyperkeratosis (dermatoscopy: $p = 0.004$, odds ratio (OR) = 2.438; PCR + microbiological culture: $p = 0.\ 004$, OR = 3.221), subungual detritus ($p = 0.033$, OR = 3.01, only with dermatoscopy) and dermatophytoma (dermatoscopy: $p = 0.049$, OR = 3.02; PCR + microbiological culture: $p = 0.022$, OR = 2.40). Table 3 shows the types of onychomycosis detected.

3.3 | Comparison of Tests

Of the samples collected, 76.8% (96/125) were considered positive after dermatoscopic assessment. However, only 69.6% (87/125) of tests were positive when PCR and culture were combined. The prevalence was higher when PCR was performed at 48% (60/125) compared to the culture result of 43.2% (54/125). Of the 96 dermatoscopy-positive samples, 36 were negative according to PCR ($p < 0.001$), 42 were negative according to culture ($p < 0.001$) and nine were negative when both tests were combined ($p < 0.001$) (Tables 4–6).

Of the 87 participants diagnosed with onychomycosis, 54 were identified by microbiological culture, and mixed infection was the most detected (27.77%). The identified pathogens in Table 7 include the detected pathogens, but not all can be accepted as the primary or causative pathogens of onychomycosis. Table 8 shows the diagnostic accuracy with 95% confidence intervals of dermatoscopy using the laboratory tests as the gold standard.

4 | Discussion

This study examined the diagnosis of onychomycosis based on clinical signs. Three different methods were used: dermatoscopy, microbiological culture and PCR. More positive results were diagnosed with PCR than with culture, which is explained by PCR's higher sensitivity to dermatophytes, which account for about 90% of fungal infections [1, 6]. Microbiological culture is three to four times more likely to give false-negative results than PCR [7, 21]. Navarro-Pérez et al. [7] concluded that the prevalence of onychomycosis is higher when PCR testing is used compared to microbiological culture, that the combination of both techniques improves the detection rate of onychomycosis, and that it may help to reduce false-negative results.

Of the 96 positive results with dermatoscopy, nine were diagnosed as negative using PCR + culture, while the 87 positive results with the PCR + culture combination were all positive with dermatoscopy. Thus, a sensitivity of 1, a specificity of 0.76, a positive predictive value of 0.91 and a negative predictive value of 1 were obtained. This suggests that dermatoscopy has a high capacity to detect this disease. However, the false-positive results can be a problem. Acquired resistance to terbinafine in the treatment of dermatophyte fungi has been reported recently due to either clinical reasons from the patient or microbiological reasons related to previous exposure to drugs from empirical prescriptions without diagnostic confirmation by laboratory testing [8, 22–25]. If we had prescribed terbinafine based on dermatoscopic diagnosis, 45.97% (40/87) of patients diagnosed with onychomycosis by laboratory tests would have received an antifungal that was ineffective against the pathogens that infected their nails (15 cases of mixed species, 14 cases of *Candida* sp., 4 cases of *Penicillium* sp., 3 cases of *Cladosporium* sp., 2 cases of *Aspergillus niger*, 1 case of *Curvularia* sp. and 1 case of *Aspergillus flavus*). Therefore, it is important to confirm the

TABLE 1 | Patient demographics. 0.8% had an underweight BMI, 33.6% were normal, 40.8% were overweight and 24.8% were obese.

	Medium	Standard deviation	Minimum	Maximum
Age	67.02	12.43	18	96
Weight (kilograms)	79.30	14.89	50	126
Height (centimetres)	170.65	9.58	145	198
IMC (%)	27.12	3.85	17.99	39.06

TABLE 2 | Clinical signs identified by dermatoscopy and the association with positive dermatoscopy and laboratory test results.

Clinical signs	Frequency (125 patients included)	Percentage (%)	*p* value dermatoscopy	*p* value PCR + culture
Onychogryphosis	90	72	0.067	0.146
Chromonychia	93	74.4	0.211	0.571
Subungual hyperkeratosis	88	70.4	**0.004*** **(OR = 2.438)**	**0.004*** **(OR = 3.221)**
Subungual detritus	42	33.6	**0.033*** **(OR 3.01)**	0.121
Onycholysis	31	24.8	0.925	0.275
Longitudinal striae	17	13.6	0.560	0.299
Fragile nails	14	11.2	0.402	0.439
Dermatophytoma	11	8.8	**0.049*** **(OR = 3.02)**	**0.022*** **(OR = 2.40)**

Note: Bold values and * indicate statistically significant association.

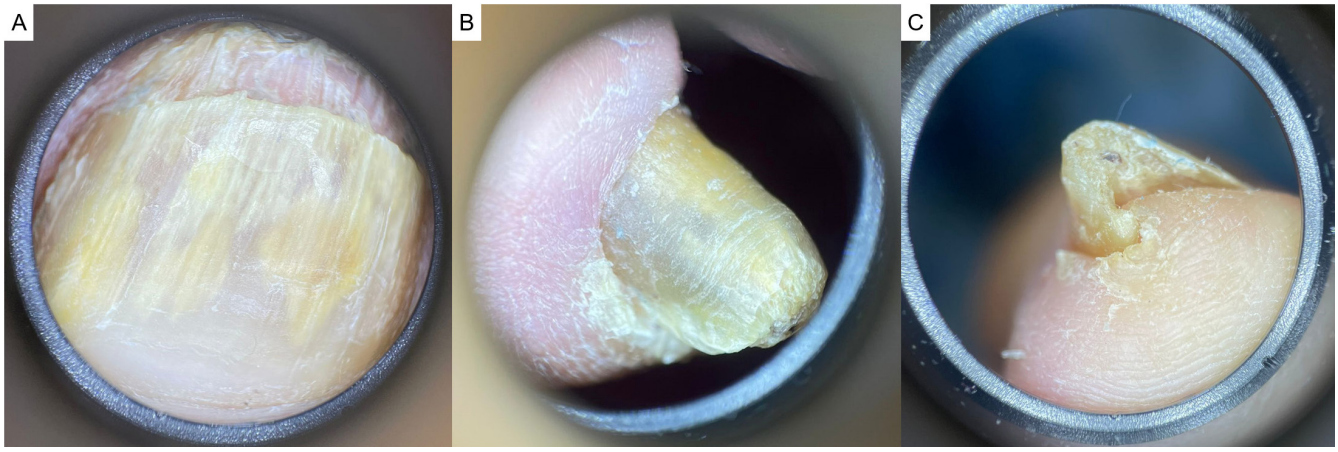

FIGURE 1 | Example of signs assessed using dermatoscopy. (A) Dermatophytoma and longitudinal striae pattern/spiked pattern. (B) Chromonychia. (C) Subungual hyperkeratosis.

TABLE 3 | Type of ONM based on the combined results of culture and PCR.

Type of ONM	Frequency	Percentage (%)
Total dystrophy	49	39.2
Distal lateral	18	14.4
Superficial	11	8.8
Distal	9	7.2
Total	87	100

TABLE 4 | Cross-table between dermatoscopy and PCR results. Detection rates were statistically significantly different between dermatoscopy and PCR ($p < 0.001$).

		PCR results		
		Positive	**Negative**	**Total**
Dermatoscopy	Positive	60	36	96
	Negative	0	29	29
Total		60	65	125

diagnosis with complementary laboratory tests in order to prescribe the appropriate antifungal drug [7, 8]. In addition, certain characteristics of onychomycosis may closely resemble those of other conditions, such as psoriasis vulgaris, lichen planus, trauma and malignant neoplasms. These contribute to about 40–50% of all nail abnormalities and require different management approaches than onychomycosis [26, 27].

The present study has demonstrated dermatoscopy to be a great tool for the diagnosis of onychomycosis as all positive results from the laboratory tests were positive with dermatoscopy. However, false-positive results and failure to detect the pathogen causing the infection are a major limitation in prescribing the correct antifungal therapy as the efficacy of oral and topical therapies may differ depending on the fungal

TABLE 5 | Cross-table between dermatoscopy and culture results. Detection rates were significantly different between dermatoscopy and culture ($p < 0.001$).

		Microbiological culture		
		Positive	**Negative**	**Total**
Dermatoscopy	Positive	54	42	96
	Negative	0	29	29
Total		54	71	125

TABLE 6 | Cross-table between dermatoscopy results and combined PCR and culture results. Detection rates were significantly different between dermatoscopy and PCR + culture ($p < 0.001$).

		PCR + Microbiological culture		
		Positive	**Negative**	**Total**
Dermatoscopy	Positive	87	9	96
	Negative	0	29	29
Total		87	38	125

TABLE 7 | Microorganisms detected by microbiological culture.

Pathogen	Frequency	Percentage
*Mixed**	15	27.77
Candida sp.	14	25.92
Trichophyton rubrum	11	20.37
Penicillium sp.	4	7.40
Cladosporium sp.	3	5.55
Trichophyton mentagrophytes	2	3.70
Aspergillus niger	2	3.70
Curvularia sp.	1	1.85
Trichophyton violacium	1	1.85
Aspergillus flavus	1	1.85
Total	54	100

Note: Cases where the dermatophyte pathogen was not specified were identified by PCR but not by microbiological culture.
**Mixed infections: Candida sp.* + dermatophyte (10/15); *Candida sp.* + *Trichophyton rubrum* (1/15); *Candida sp.* + *Fusarium sp.* (1/15); *Candida albicans* + *Trichophyton rubrum* (1/15); *Candida sp.* + *Fusarium sp.* (1/15); *Aspergillus sp.* + dermatophyte (1/15).

species involved and the severity of the classification [1, 3, 28]. Therefore, dermatoscopy is a good tool that can help in deciding whether to perform a diagnostic laboratory test, especially if subungual hyperkeratosis ($p = 0.004$), subungual detritus ($p = 0.033$) or dermatophytoma ($p = 0.049$) is observed. But it should not be used for diagnosis and treatment selection without complementary laboratory tests such as microbiological culture, PCR, direct microscopy (KOH) or histological techniques [7, 12, 17, 29].

This study has several strengths. It had a large sample size, included patients whose diagnostic suspicion was negative after dermatoscopic assessment, and included several age groups with data collected over 6 months. However, several limitations have to be taken into account. The study did not collect information on patients' comorbidities, and the sample was predominantly male (69.6%), which could influence the results.

The authors believe that these results are relevant in clinical practice as the use of dermatoscopy allows the establishment of a clinical suspicion of ONM and helps to determine when diagnostic confirmation by laboratory tests should be performed, which facilitates early diagnosis and management of the disease.

5 | Conclusions

Our results suggest that dermatoscopy is a good tool for the diagnosis of onychomycosis. However, it should be used as a complement to laboratory tests in order to choose the appropriate treatment according to the pathogen detected or as a screening test to determine whether to sample patients for laboratory tests. Moreover, the combination of the three tests can lead to a reduction of false-positive and false-negative clinical and laboratory results. The use of the dermatoscopy guides the healthcare professional to an early diagnosis with the help of laboratory tests which allows, depending on the pathogen detected, to establish a specific treatment. This could reduce the current problem of antifungal resistance.

Furthermore, future studies should consider dermatoscopic assessment by different practitioners to assess the interobserver reliability of this test and include patients who do not have dermatoscopic signs to eliminate biases in the specificity of the test.

TABLE 8 | Diagnostic accuracy of dermatoscopy with 95% confidence interval.

	Dermatoscopy				
Gold standard	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Positive likelihood ratio**
Culture	1	0.41	0.56	1	1.69 (1.39–2.05)
PCR	1	0.45	0.63	1	1.81 (1.45–2.25)
PCR + Culture	1	0.76	0.91	1	4.22 (2.39–7.47)

Abbreviations: NPV, Negative predictive value; PPV, Positive predictive value.

Author Contributions

David Navarro-Pérez: conceptualization, investigation, writing – original draft, methodology, writing – review and editing, data curation, formal analysis, project administration, visualization. **Aroa Tardáguila-García:** conceptualization, methodology, software, writing – original draft, validation, formal analysis, investigation. **Sara García-Oreja:** data curation, writing – review and editing. **Diego León-Herce:** software, investigation, visualization. **Francisco Javier Álvaro-Afonso:** visualization, supervision, project administration. **José Luis Lázaro-Martínez:** project administration, supervision, resources, validation, writing – review and editing, funding acquisition.

Ethics Statement

The protocol was approved by the medical ethics committee of the *Instituto de Investigación Sanitaria del Hospital Clínico San Carlos* (IdISSC) (protocol number 23/610-E). All patients voluntarily signed consent statements for the use of their images and publication of their case details. The participants in this study have given written informed consent for the publication of their case details.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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