



Concordance between direct microscopy and fungical culture for the diagnostic of feet's onychomycosis*

Concordância entre o exame micológico direto e a cultura para fungos no diagnóstico das onicomicoses dos pés

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Abstract: Prospective study compared the agreement between the direct microscopy and fungical culture from subungueal samples of the patients with clinical suspicion of feet's onychomycosis. The agreement occurred in 56.1% of the exams with dermatophytes, in 52.4% by others fungi and in 90.4% of the negative cases, 0,54 according to the Kappa`s test. In 39.3% of the onychomycosis caused by dermatophytes and 31.8% by nondermatophytes, these were identified only for direct microscopy. The direct microscopic showed more sensibility compared with the culture, being superior in 19.5% of the total sample and maintaining agreement with the culture in 71.5% of the sample.

Keywords: Culture; Dermatomyces; Microscopy; Onychomycosis

Resumo: Estudo prospectivo avaliando a concordância entre os resultados do exame micológico direto e da cultura para fungos de material coletado de pacientes com suspeita clínica de onicomicose dos pés. Ocorreu concordância em 56,1% dos exames com dermatófitos, em 52,4% dos exames com outros fungos e em 90,4% dos exames negativos (0,54 de acordo com o teste *kappa*). Em 39,3% das onicomicoses por dermatófitos e em 31,8% das por não dermatófitos, os agentes etiológicos foram identificados somente pelo exame direto. O exame direto demonstrou maior sensibilidade, comparado ao cultural, sendo superior em 19,5% da amostra total e mantendo concordância com a cultura em 71,5% da amostra.

Palavras-chave: Cultura; Dermatomicoses; Microscopia; Onicomicose

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Articles on the literature have proposed different tests as the golden standard for the diagnosis of onychomycosis. However, none of them was methodologically designed to detect false-negative and false-positive results.¹⁷ This can mask a more detailed assessment of the potential use of mycological culture as golden standard test for the diagnosis of onychomycosis.^{8,9} The objective of this prospective study is to determine the concordance between the direct microscopic examination (DME) and the culture for fungi on the diagnosis of onychomycosis of the feet.

The protocol for the prospective study was approved by the ethics committee of the Complexo Hospitalar Santa Casa de Porto Alegre-Brasil and a verbal consent was obtained from each participant. All the patients admitted to the outpatients' service of the Dermatology Service during a 12 month period (year 2005) with the clinical diagnosis of onychomycosis were included on the study. Samples from all patients were analysed according to the following procedures: 1) subungual curettage was used to obtain as much subungual material as possible; 2) DME preparations were done by placing the samples in glass plates with KOH20%; 3) specimens were lightly heated and subsequently microscopically analysed for the presence of fungal elements; 4) mycological cultures were obtained with Sabouraud (dextrose agar and mycosel); 5) the cultures were stored in incubators at 25° C for 5 weeks and were periodically analysed for fungal growth; 6) specimens from each positive culture were determined by microscopic and macroscopic examination; 7) the technical assistant proceeded with the collection and the preparation of the specimens for the DME and the fungal culture, while the reading of the results was performed by a experienced mycologist; 8) both the collector and the mycologist were blind to the objectives and hypothesis of the study; 9) the results from DME and fungal culture were classified according to the following category: dermatophytes, (arthrospored hyaline hyphae), non-dermatophyte fungi (septated hyaline hyphae)

and yeasts (yeasts cells and pseudohyphae). For the purpose of statistical analyses the cultures were re-categorized into the following groups: dermatophytes, non-dermatophytes and negative. The data was double entered into Epi Info 6.04 with automatic evaluation for consistency. Afterwards, it was transferred to Stata version 9, where the analyses were done.

During the study period 890 DME were performed with the respective cultures. There was concordance in 170 of the dermatophytes (56, 1% of these cases), in 89 by other fungi (52, 4% of these cases) and 377 of the negative cases (90, 4 % of these cases), making up a kappa of 0, 54 which is considered moderate (Table 1 – in blue). The p-value was <0, 0001 and the confidence interval was (95%CI) =0, 54 (0,498 - 0,607). On the other hand, the DME was able to diagnose 119 cases of dermatophytes and 54 cases of other fungi, which were negative in the culture (Table 1 – in green). The culture diagnosed only 9 cases of infection by dermatophytes and 31 by other fungi when the DME was negative (Table 1 – in red). There was also a qualitative discordance in 14 cases of dermatophyte at the DME, but with the culture other fungi were identified, and in 27 cases where the DME had detected other fungi but dermatophytes grew on culture (Table 1 in black).

According to the findings presented here, the DME detected a higher number of positive cases compared to the fungal culture. In 119 cases (representing 13, 4 % of the total sample and 39, 3 % of those caused by dermatophytes) the dermatophytes were identified only by the DME, as well as in 54 cases of non-dermatophyte (representing 6, 1 % of the total sample and 31, 8 % of the cases caused by these fungi), where the cultures were also negative. In accordance with these results *Staats et al* also demonstrated that in cases of infection by dermatophytes the DME detects more cases of onychomycosis of the feet than the culture for fungi.¹⁰ The authors believe that these findings are a result of the difficulty on cultivating dermatophytes in laboratory. A disadvantage of this method is

TABLE 1: Comparison of the results of the direct microscopic examination and the fungal culture from the patients of the dermatology department of the Santa Casa de Porto Alegre

		Examination of the Culture			
		Dermatophytes (%)	Other fungi (%)	Negative (%)	Total
Direct Exam	Dermatophytes (%)	170 (56.1)	14 (4.6)	119 (39.3)	303 (100)
	Other fungi (%)	27 (15.9)	89 (52.4)	54 (31.7)	170 (100)
	Negative (%)	9 (2.2)	31 (7.4)	377 (90.4)	417 (100)
Total		206 (23.14)	134 (15.1)	550 (61.8)	890 (100)

that the recognition of fungal elements by microscopy requires considerable experience and expertise.

Our results suggest that the mycological culture was not capable of effectively improving the number of cases of onychomycosis caused by dermatophytes diagnosed, with only 9 cases (1,01% of the total sample) with negative DME when the culture was positive. In cases caused by non-dermatophytes, in 31 exams (3, 48% of the total sample) the agents were identified only by culture. These findings constitute a reasonable argument against the use in large scale of direct microscopic examination for unguis infections of the feet by dermatophytes, which are the most usual causative agents.

On the sample studied, the costs involved with the DME reached 8, 9 dollars per exam while the costs involved with culture exams reached 22, 25 dollars per culture. When the DME is negative and the suspicion of onychomycosis is high, probably a second DME is more useful than the culture in order to confirm the diagnosis, especially due to its costs. One of the main limitations of the present study was the absence of a gold standard test to detect the false-positive and false-negative results. Therefore, prospective studies must be conducted in order to determine the natural history of the onychomycosis and the proportion of these results (false-positive and false-negative) in each diagnostic test. □

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