

# The importance of mycological investigations in diagnostics of nail changes

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## Abstract

The aim of the study was an analysis of correlation between mycological examination results and clinical features of changed toenails in patients that visited mycological laboratory due to suspected onychomycosis.

**Material and methods:** Samples of changed toenails were collected from 579 patients. From all that cases a precise patient's history was taken paying a special attention on previous antimycotic treatment. In the clinical examination features of toenail changes were estimated.

**Results:** Onychomycosis was confirmed by the mycological examination in 23.3% of patients and exclude in 56.3% of individuals. In 20.4% of all cases the fungi growth was not obtained despite of positive results of direct microscopic examination. Among the cultured fungi species the most frequently observed were strains of *Trichophyton rubrum* – 46.6%. 46% of all patients were previously treated with antifungals but the therapy was not efficacious in 23% of them.

**Conclusions:** In all the cases of toenail changes it is important to take a precise patient's history, because it has an essential influence on the results of diagnostic examinations.

Diagnosing onychomycosis one cannot rely only on the clinical examination because in over 50% of patients with typical for onychomycosis toenail changes the mycological examination do not confirm fungal infection.

**Key words:** clinical nails disorders, onychomycosis, diagnostics difficulties.

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## Introduction

Onychomycosis occurs especially in the elderly. It is connected with extremely slow growth of the nail plates as well as with impaired blood supply to the limbs found more frequently in the elderly [1].

Moreover, different non-mycotic nail lesions are more frequently observed in the elderly. Those lesions may be found in the course of other diseases as well as may be due to different traumas from which the nails are very likely to suffer quite often [2].

Pathological lesions in the nails caused by a mycotic infection may very often resemble the clinical picture of other nail diseases.

For that reason it is vital to confirm a diagnosis of onychomycosis correctly with adequately performed mycological diagnostics before the introduction of antimycotic treatment [3-8].

Contemporary mycological diagnostics includes methods such as:

- nail observation through a magnifying glass,
- direct microscopic examination,
- cultures using standard Sabouraud dextrose agar and Sabouraud medium with the addition of chloramphenicol and actidion,
- microcultures,
- detection and differentiation media: DTM, urease medium and chlamydo-spore agar,
- carbohydrate and nitric zymograms and auxanograms
- the estimation of drug sensitivity of cultured fungi,
- the examination of enzymatic properties of fungi,
- histopathological examinations.

The very simple, valuable, but not appreciated test allowing to diagnose dermatophyte onychomycosis is nail observation through a magnifying glass in order to find so called "nets". The symptoms of nets, although present in some groups of patients, are of significant diagnostic importance as they are pathognomic for dermatophyte onychomycosis [9]. Consequently, the diagnosis of that disease can be made in those cases where mycological cultures are negative. However, the fungal species responsible

for the infection cannot be determined in such a situation, although it is known that when the symptoms of onychomycosis are present the only causative factor for the observed clinical lesions in the nail plates may be dermatophyte.

## The aim of the research

The aim of the research was to perform a deepened analysis of the participation of mycological infections in the development of pathological lesions in the toenail plates present in patients who had reported at the mycological laboratory with suspected onychomycosis.

## Material and methods

579 patients who had visited the microbiological laboratory either worried by the abnormal appearance of their toenails or with suspected onychomycosis having a referral note from a general practitioner, participated in the research.

The material for mycological examination collected from the nail plates in the case of suspected onychomycosis was every time following collection observed under the microscope in the form of preparations prepared with the reagent containing dimethyl sulfoxide (DMSO) and KOH, and it was cultured on solid Sabouraud media without antibiotics but with actidion and chloramphenicol being selective media. The material for mycological examination in patients with the infection of the nailfolds was collected in a direct way with a sterile loop or a sterile thread saturated with Sabouraud medium was placed under the nailfold for the period of 24 h and then it was placed in a typical way on solid Sabouraud medium.

When fungal growth was present subsequent strain determination was based on the use of additional media such as DTM (Dermatophyte Test Medium), Christensen's medium or chlamyospore agar as well as on developing microcultures which in the case of dermatophytes and moulds most frequently encountered in nails allow to determine their species basing on the micromorphological features. Species determination of fungi from *Candida* genus consisted of developing cultures with Nickerson-Mańkowski medium in order to distinguish *Candida albicans* from other fungi of *Candida* genus marked as *Candida sp.*, which only in some cases were subsequently differentiated using API-20 AUX<sup>®</sup> test allowing a detailed determination of species belonging to yeast-like fungi in a simple way.

## Results

Toenail onychomycosis was confirmed by mycological examination in 23.3% of examined patients as positive results of mycological culture were achieved. In 56.3% of cases negative results of direct mycological examination and culture were achieved despite of twice repeated, consequently, toenail onychomycosis was excluded.

In 20.4% of all examined patients no fungi were cultured despite the detection of fungal strands in direct mycological examination.

Figure 1. A proportional participation of patients in the study with previous antifungal treatment

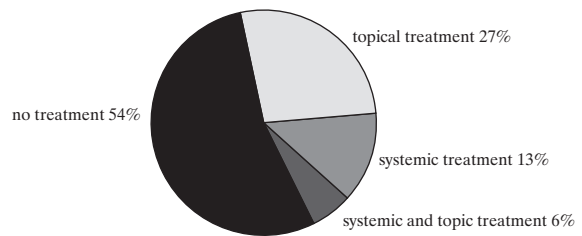
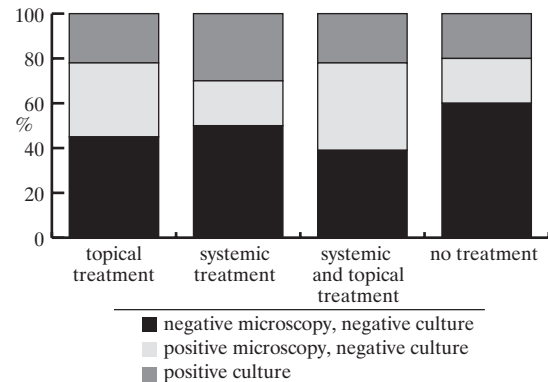


Figure 2. Analysis of influence of previous treatment on results of mycological examinations



Among fungal species which were cultured from the obtained material the following strains were the most frequent: *T. rubrum* – 46.6% (63 cultures), then *T. mentagrophytes*, both in granular and interdigital modification – 21.5% (29 cultures) and *T. tonsurans* – 5.2% (7 cultures), *C. albicans* – 10.4% (14 cultures), *C. species* – 4.4% (6 cultures) and *Scopulariopsis brevicaulis* – 11.9% (16 cultures).

All patients who underwent mycological examination were asked if they had earlier been administered antimycotic therapy (Fig. 1).

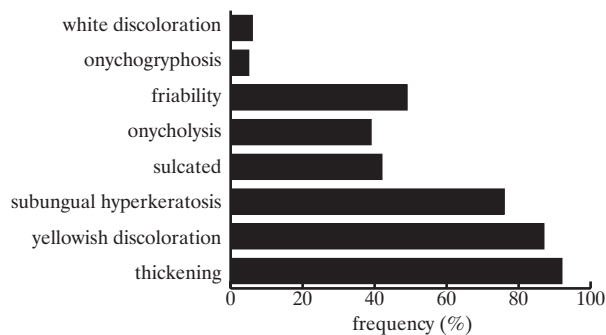
The patients who admitted having been given antimycotic medicines in the past were asked if mycological diagnostics had been performed before the introduction of that therapy.

It was found that as much as 47% of examined patients had previously been treated with antimycotic therapy, however, some of them had surely been treated ineffectively as they showed positive results of mycological cultures (Fig. 2). In subject examination of the toenails in all patients the following were observed: appearance properties of nail changes (Fig. 3) and the extent to which they were connected with mycotic and non-mycotic aetiology responsible for their pathological appearance (Fig. 4).

## Discussion

While analysing the results of mycological examinations it was determined that more than 50% patients who had visited the dermatologist due to toenail lesions did not have onychomycosis. Therefore some appearance properties of abnormal nails such as yellowish discoloration, thickening, subungual keratosis, onycholysis, whitish discoloration, sulcated and friability which

Figure 3. The analysis of different clinical symptoms occurring in changed toenails

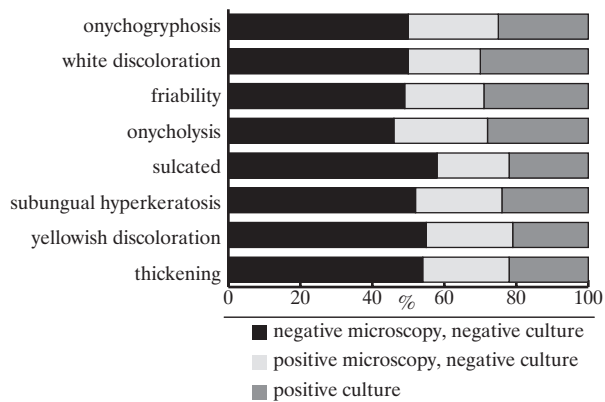


are most frequently connected with onychomycosis in about 50% of cases were related to disturbances of toenail appearance due to non-mycotic aetiology (Fig. 4).

What is especially worth emphasizing is that 47% of examined patients had previously been treated with antimycotic therapy and in some of them the treatment had surely been ineffective as they still have positive results of mycological cultures (Fig. 2). In the cases where lesions of the nail plates are maintained and currently mycological examinations do not confirm onychomycosis it is impossible to determine in a reliable way if pathogenic fungi had been present before previous treatment. It is due to the fact that fungi frequently colonise the nail plates which had been changed by other pathogenic agents, therefore antimycotic treatment eliminates them from the nails but it hardly changes the clinical picture of the nails [10]. Moreover, it should be remembered that in the patients who had previously been treated negative results of mycological examinations may be the result of the fact that the viability of the fungus weakened due to previous treatment. Such fungi are not able to grow on artificial media although viable fungal strands may be found in deep, hardly accessible nail layers or in keratotic material covering the nail bed under the nail plate. In some of these patients positive results of direct microscopic examinations are found in the material collected from the nails, however, no fungal culture can be obtained.

Other problem connected with frequently seen non-mycotic aetiology of clinical changes in the nails is the interpretation of mycological results in the cases where fungal culture is present. If a dermatophyte is cultured from the nail lesions, there are usually no doubts that it is a pathogenic agent responsible for the nail lesions. However, when yeast-like fungi and moulds are obtained it is not always possible to accept unanimously that these fungi are pathogenic agents responsible for the nail lesions. Whereas when a great number of *Candida albicans* colonies are obtained from the nail material it can generally be accepted that this fungus is a pathogenic agent, however, in other species from *Candida* genus such a statement requires additional confirmation by several cultures of the same fungus from the material collected from the same nail [11]. Moreover, the cultures of moulds obtained from the nail material should also be treated with great caution as they may quite often secondarily grow in the nail plates changed in the course of different diseases. In our geographical conditions only *Scopulariopsis brevicaulis* is responsible for specific clinical changes in the toenails [12]. In the case when the culture of other moulds

Figure 4. A proportional participation of different clinical symptoms in changed toenails in patients with and without onychomycosis



is obtained the diagnosis of onychomycosis needs also to be confirmed with subsequent mycological examinations.

## Conclusions

1. In all cases of the nail changes it is extremely important to perform a detailed insight into patient's history regarding the course of the disease and possible trials of treatment as it may have a significant influence on the results of diagnostic examinations.

2. When a diagnosis of onychomycosis is recognized it cannot exclusively be based on clinical examination as in more than 50% patients with the lesions of the nail plates specific for onychomycosis mycological examination does not confirm mycosis.

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