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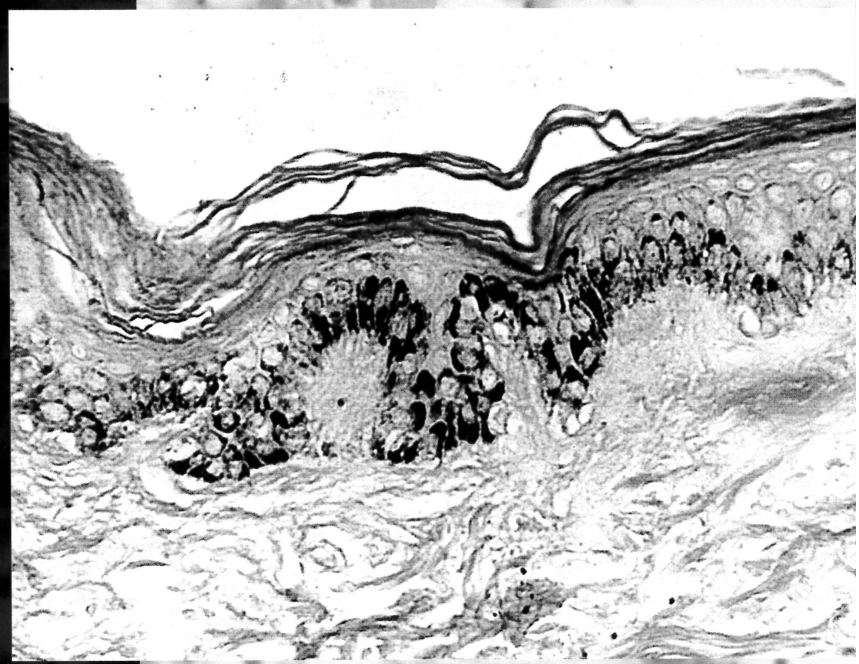

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CONTENTS

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Cover Photograph
Masson-Fontana melanin staining of biology samples from the dorsal aspects of the hand, from a hyperpigmented macule.

Book reviews

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Clinical and Laboratory Investigations

Onychomycosis: the development of a clinical diagnostic aid for toenail disease. Part I. Establishing discriminating historical and clinical features

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Summary

Background The ideal method for diagnosing onychomycosis is unclear. Mycological investigation is currently the method of choice, although there is a false-negative culture rate of at least 30%.

Objectives To establish a clinical diagnostic aid which may be used alongside laboratory-based mycological tests and in epidemiological studies.

Methods Patients with nail disease ($n = 209$) were enrolled in the study. The examining clinician completed a questionnaire containing four historical questions and 21 questions related to the clinical findings. All patients had samples taken for mycological analysis. The gold standard for the diagnosis of onychomycosis was a positive result on both direct microscopy and culture of nail samples. Following exclusions, questionnaire responses from 169 patients were analysed using Stata. Multiple logistic regression with forward stepwise selection of variables was performed.

Results Both microscopy and culture results were positive in 32% of cases and negative in 42%. Dermatophytes formed the majority of isolates. Four parameters were found to be significantly related to positive mycology results: a history of tinea pedis in the last year, scaling on one or both soles, white crumbly patches on the nail surface, and an abnormal colour of the nail plate.

Conclusions Our results have shown one historical feature and three clinical features to be strongly associated with onychomycosis. The questionnaire has been revised to include only these stems and is being tested further with the aim of achieving a binary definition.

Keywords: diagnostic aid, onychomycosis

The incidence of onychomycosis is increasing[1–3] and the development of newer, more effective antifungal agents has led to a renewed interest in this condition, both in the medical and the public domains. Despite the advances in antifungal treatments, the optimal method for diagnosing onychomycosis in routine practice remains unclear. Most mycologists and dermatologists agree that mycological investigation is the method of choice. However, even in the best laboratories there exists a false-negative culture rate of approximately 30%.[4,5] Additionally, the sensitivity of direct microscopy is dependent upon many factors, including the skill of the operator and the quality and quantity of nail samples obtained. Various other procedures have been employed to improve the accuracy of diagnosis, such as the histological examination of periodic acid–Schiff-stained nail clippings[6,7] and *in vivo* confocal microscopy,[8] but these are not widely available, nor in general use. We hope to develop a clinical diagnostic algorithm to be used as an adjunct to mycological investigation. It may also be helpful in epidemiological studies where large-scale mycology sampling may not be feasible.

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Materials and methods

A questionnaire was designed which contained four historical questions largely concerned with eliciting a history of tinea pedis or a family history of nail problems. Twenty-one further questions were related to the clinical examination and included those known to be associated with onychomycosis such as nail bed thickening and onycholysis. Ethics Committee approval was obtained. The questionnaire was piloted and refined and was subsequently applied to patients with abnormal nails, regardless of what was felt to be the underlying cause, attending the dermatology and chiropody clinics at Guy's and St Thomas' Hospitals Trust. Four other large teaching hospitals in the U.K. and the Chelsea School of Podiatry in London contributed patients to this study (43 and 58 patients, respectively). The only exclusion criterion employed was that subjects should be over 10 years of age.

All questionnaires were completed by the observing clinician. Full-thickness nail clippings and subungual debris, when present, were collected from one representative nail. Skin signs of local dermatophyte infection were also sought and skin scrapings taken for mycological analysis.

Laboratory methods

All samples were processed in a single laboratory. Direct microscopy was carried out using wet preparations with 30% potassium hydroxide solution. Additional calcofluor white staining was performed and the specimens were examined under ultraviolet radiation. Nail samples were cultured on modified Sabouraud's agar, both with and without cycloheximide. Incubation at 26 °C was maintained for at least 2–3 weeks under controlled humidity.

As the objective was to determine signs of nail disease that were significantly associated with onychomycosis, the 'gold standard' for the diagnosis of onychomycosis in this study was taken as positive results on both direct microscopy including calcofluor-treated nails, and culture of nail samples.

Statistical methods

Questionnaire responses were collated and entered into a database (Microsoft Excel). Responses were recorded in binary format except for one question concerning the number of abnormal nails, which was recorded numerically. Details were taken of the age and sex of each patient.

Data analysis was performed with the aid of the Stata statistical software package. Crude sensitivities and specificities for each question were determined and χ^2 tests performed. The relative value of each question was calculated by adding the sensitivity and specificity of each and subtracting 100 (Youden's J statistic).

The number of variables in the regression analysis was limited by entering those with a P-value $< 0{\cdot}1$ along with questions that were felt to be clinically relevant (onycholysis, nail bed thickening and abnormal colour of the nail plate). Multiple logistic regression with forward stepwise selection of variables was performed, using mycology results as the dependent variable and questionnaire responses, age and sex as the independent variables.

Results

A total of 209 questionnaires and corresponding nail samples was received. There were only 14 fingernails sampled and due to the lower prevalence of fingernail onychomycosis in comparison with toenail involvement, we decided to exclude the corresponding questionnaires from further analysis. A further 26 questionnaires were excluded for the following reasons: samples taken from both fingernails and toenails ($n = 3$); missing data for site of nail samples ($n = 7$); growth of nondermatophyte moulds and contaminants ($n = 4$); and nails that were negative on direct examination but culture positive ($n = 12$). In all, 169 questionnaires were available for data analysis. Demographic information was obtained for 164 subjects: 57% were male and 43% female. The age range was 10–95 years (mean 54·9; median 55·5).

Mycology results

Of the nails sampled, 32% had positive results on both direct examination and culture for fungi, 42% had entirely negative results, and 20% were positive on direct microscopy but culture negative. Almost all isolates were dermatophytes (92%), comprising *Trichophyton rubrum* (44 cases) and *T. mentagrophytes* var. *interdigitale* (11 cases). One case was positive on culture for *Scytalidium dimidiatum*. There were more males than females with onychomycosis, 36% compared with 19%.

Signs suggestive of local dermatophyte infection were identified in 65 individuals and skin samples were taken from the foot, and, in one case, the body for mycological testing. Half of these had positive results on direct microscopy for fungal elements although only one-fifth were culture positive. Of the skin samples which were positive both on direct examination and on culture, 19% were associated with onychomycosis. The same organism was cultured in all cases.

Number of abnormal nails

One question concerned the total number of abnormal fingernails and toenails. The fifth toenails were excluded, as these are often abnormal due to pressure from footwear, giving a maximum total of 18. The mean number of abnormal nails was 5·1 (median 3, range 1–18).

Data analysis

Three separate data analyses were performed. In the first analysis, questionnaire responses relating to nails with positive mycology results were combined with those relating to nails with entirely negative results, representing 133 individuals. In the second, questionnaire responses relating to nails that were positive on direct microscopy but were culture negative were analysed along with the mycology-negative nails, making a total of 113. For the final analysis, the two previous datasets were combined, giving a total of 169 patients.

Initial analysis of the data to determine the sensitivities and specificities of the questionnaire stems revealed the same results for the first and third sets of data. Results for the third dataset are shown in Table 1.

Analysis of the second set of data corresponding to questionnaires from direct microscopy-positive/culture-negative nails and those with entirely negative results showed fewer statistically significant questionnaire stems. The seven that had significant *P*-values were also found in the other two analyses. However, the following questions failed to reach significance: dry scaly skin on the soles/palms, scaling on one/both soles, peeling/maceration/vesicles in the toe webs, abnormal toenails, swelling of the nail folds, white crumbly areas on the nail surface, pitting and oil spots.

Table 1. Sensitivity and specificity of questionnaire stems (questionnaires relating to direct microscopy positive/culture positive, direct microscopy positive/culture negative and direct microscopy negative/culture negative nails; $n = 169$)

Question	Sensitivity (%)	Specificity (%)	χ^2	*P*-value	Relative value
History					
Tinea pedis in the last year?	**50·0**	**80·5**	**21·0**	**0·000**	**30·5**
Household contacts with tinea pedis?	13·0	92·2	1·2	0·270	5·2
Close family with nail problems?	13·0	84·4	0·2	0·637	− 2·6
Dry, scaly skin on soles/palms?	**59·8**	**64·9**	**10·3**	**0·001**	**24·7**
Examination					
Scaling on one/both soles?	**68·5**	**54·5**	**9·1**	**0·003**	**23·0**
Peeling/maceration/vesicles on toe webs?	**67·4**	**55·8**	**9·2**	**0·002**	**23·2**
Scaling on one/both palms?	10·9	83·1	1·3	0·256	− 6·0
Abnormal toenails?	**100·0**	**6·6**	**6·2**	**0·013**	**6·6**
Abnormal fingernails?	**20·7**	**52·6**	**8·1**	**0·004**	**− 26·7**
Nails on both hands affected?	**7·6**	**68·8**	**15·5**	**0·000**	**− 23·6**
Swelling of nail folds?	**8·7**	**80·5**	**4·2**	**0·042**	**− 10·8**
Erythema of nail folds?	15·2	80·5	0·5	0·464	− 4·3
Pain on pressing nail folds?	4·3	90·9	1·6	0·213	− 4·8
Discharge on pressing?	0·0	100·0	–	–	0·0
Thickened nail plate?	51·6	51·3	0·2	0·703	2·9
Onycholysis?	**69·6**	**35·1**	**0·4**	**0·522**	**4·7**
Lateral onycholysis?	**61·5**	**40·8**	**0·2**	**0·632**	**2·3**
Thickened nail bed?	**84·4**	**10·7**	**0·9**	**0·358**	**− 4·9**
Abnormal colour of nail plate?	**92·4**	**18·2**	**4·3**	**0·038**	**10·6**
White crumbly areas on nail surface?	**27·2**	**88·3**	**6·3**	**0·012**	**15·5**
Partial/complete loss of nail plate?	15·2	79·2	0·9	0·346	− 5·6
Pitting?	**7·6**	**78·9**	**6·4**	**0·012**	**− 13·5**
Oil spots?	**1·1**	**86·8**	**9·9**	**0·002**	**− 12·1**
Koilonychia?	1·1	97·4	0·6	0·458	− 1·5

Questions in bold type have significant *P*-values or were felt to be clinically significant.

Symptom/sign	Odds ratio	Standard error	*P*-value	95% confidence interval
Tinea pedis in the last year?	3·17	1·35	0·007	1·38–7·32
Scaling on one/both soles?	2·35	0·92	0·028	1·10–5·04
White crumbly areas on nail surface?	4·53	2·53	0·017	1·52–13·51
Age	0·97	0·01	0·020	0·95–1·00
Abnormal colour of nail plate?	3·96	2·62	0·038	1·08–14·51

Table 2. Results of multiple logistic regression

Multiple logistic regression

Three separate sets of data were analysed, as detailed above. The third, and largest, set of data confirmed the findings seen in the other two analyses. The parameters shown in Table 2 were found to be discriminating for onychomycosis.

Discussion

Five subtypes of onychomycosis are recognized:[9] distal lateral onychomycosis, proximal subungual onychomycosis, superficial onychomycosis, total dystrophic onychomycosis, and endonyx onychomycosis. These subtypes cause a large number of possible changes in the nail apparatus. The purpose of this study was to determine the most reliable predictor for the diagnosis of onychomycosis. The resulting clinical diagnostic aid would be used alongside mycological analysis and as such it cannot be expected to identify all patients with onychomycosis. A particular area where its use will be limited is in subjects with other causes of nail dystrophy. Psoriasis in the nails can mimic onychomycosis, and indeed the two conditions may coexist.[10]

Interestingly, only two clinical signs in the nail apparatus were found to be significantly associated with onychomycosis: white crumbly areas on the nail surface, and an abnormal colour of the nail plate. A history of tinea pedis, or signs of this on the soles, and increasing age, were also significant. Tinea pedis is a known risk factor for toenail onychomycosis.[11] Although peeling/maceration/vesicles in the toe webs had a significant *P*-value in the initial analysis (0·002) and the third highest relative value (23·2), it did not maintain significance following multiple logistic regression analysis.

Further evaluation of the data showed that increasing age was linearly associated with onychomycosis; there was no apparent cut-off above which onychomycosis became more likely. However, the low odds ratio suggests that age has a poor predictive value for onychomycosis. Intraclass correlation studies failed to show any association between the number of abnormal toenails observed and the likelihood of onychomycosis.

It is interesting that onycholysis, a commonly reported sign in onychomycosis, did not reach statistical significance as a predictive sign. Although often traumatic, onycholysis was present in 73% of the nails which were found to have positive mycology results. In spite of its unremarkable *P*-value (0·522) in univariate analysis, it was included in the regression analysis along with lateral onycholysis (*P*-value 0·632) and nail bed thickening (*P*-value 0·358), both of which were also felt to be important clinically. None of these three signs was discriminating for onychomycosis.

An abnormal colour, or discoloration, of the nail is frequently seen in onychomycosis. No additional information was collected in this study regarding any specific colour changes observed. However, it is possible that some of the clinicians noted the opacity of the nail plate secondary to separation from the nail plate as 'an abnormal colour of the nail plate' rather than onycholysis *per se*. This effect, however, is likely to be small, as 80% of the questionnaires were completed by one clinician (C.L.F.).

White crumbly areas on the nail surface are generally associated with superficial white onychomycosis. This pattern of nail infection is most commonly associated with *T. mentagrophytes* var. *interdigitale*. In this study, only 11 nail samples grew this organism and in only 23% of the corresponding questionnaires was there a positive response to this question. This suggests that perhaps more severe cases of onychomycosis were picked up in this study, including those with a total dystrophic pattern of nail disease. Certainly more than half of the patients were recruited from dermatology clinics, with the remainder coming from chiropody clinics.

We tried to control, in part, for the false-negative culture rate in the laboratory by including in the data analysis questionnaires relating to nails that were direct microscopy positive but culture negative ($n = 77$). Some of these patients may also have received antifungal therapy before inclusion in this study. No data were collected to investigate this

further, although it would be prudent to exclude from further studies individuals who had recently received systemic antifungal therapy. The false-negative rate in the laboratory was additionally reduced by taking skin scrapings from the soles or toe webs whenever possible.

The questionnaire has been refined to include only the discriminating questions and is being validated by applying it to patients over 10 years of age presenting with abnormal toenails to primary care. Following further statistical evaluation, it may be possible to achieve a reduction to a binary definition. The sensitivity and specificity of this clinical aid can thereby be determined.

In summary, mycology should remain the investigation of choice in suspected onychomycosis. If negative, the clinical algorithm that we are developing may be useful in identifying patients with a high likelihood of fungal disease, who would benefit from repeat mycological studies.

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