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Original Article

The Usefulness of Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry in the Diagnosis of Onychomycosis in Patients with Nail Psoriasis

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Abstract

Background and objectives: Nail psoriasis is common in patients with plaque psoriasis and is associated with morbidity, including onychomycosis, which can complicate psoriasis treatments and be difficult to differentiate. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a fast and simple technique for identifying microorganisms through protein analysis. This study aimed to determine the sensitivity and specificity of MALDI-TOF for diagnosing onychomycosis in patients with nail psoriasis, by using conventional mycological and histological methods as the reference standard. ***Methods:*** A prospective study was conducted on 88 patients with clinically and histopathologically confirmed nail psoriasis. One hundred nail samples were obtained for direct examination, fungal culture, and mass spectrometry. None of the patients were receiving antifungal or systemic immunosuppressive therapy at the time of sampling. ***Results:*** Potassium hydroxide preparation and fungal culture were positive in 58 out of 100 nail samples from patients with psoriasis. MALDI-TOF identified onychomycosis in 68 out of 100 samples, distinguishing these cases from nail psoriasis without onychomycosis (32 out of 100). An excellent correlation (0.95) was found between MALDI-TOF and conventional onychomycosis diagnostic methods. The sensitivity and specificity of MALDI-TOF for diagnosing onychomycosis in patients with psoriatic nails were 95.4% and 97.5%, respectively. ***Conclusions:*** MALDI-TOF can be used to accurately differentiate cases of nail psoriasis without infection from those with onychomycosis.

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Keywords: MALDI-TOF; Mass spectrometry; Onychomycosis; Psoriasis; Nail disease; Differential diagnosis.

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Introduction

Dermatophyte onychomycosis is common in the general population, with a prevalence ranging from 6% to 26.9%. It is also frequently observed in patients with various inflammatory conditions (e.g., psoriasis, atopic dermatitis) and immunosuppressive diseases such as diabetes mellitus, transplantation, HIV, and malignancies.[1] Even in children, where onychomycosis was once considered rare, the incidence is now increasing. As the number of patients at risk for onychomycosis rises, both incidence and prevalence are expected to increase in the coming decades.[2] Fungal identification is essential for selecting the appropriate therapy.[3]

Standard diagnostic procedures include potassium hydroxide (KOH) preparation, fungal culture, and histologic examination of nail material using periodic acid-Schiff staining. However, especially in treated patients, both KOH preparation and fungal culture often yield false-negative results. The accuracy of results also depends on the knowledge of the examiner. The long incubation time, which can be up to three to four weeks, is the most notable drawback of fungal culture.[4] It is also important to differentiate nail dystrophies caused by onychomycosis from those due to non-infectious nail diseases such as psoriasis, dermatitis, or trauma.[5]

Psoriasis is a chronic, systemic, inflammatory disease of unclear etiology, primarily characterized by squamous plaques affecting the skin, including the nails.[6] Nail psoriasis is a common manifestation in patients with plaque psoriasis and can cause morbidity and cosmetic impairment.[7] Although psoriasis limited to the nail bed often mimics onychomycosis, making diagnosis challenging,[8] it affects 10% to 82% of patients with psoriasis.[9] In many cases, onychomycosis is superimposed on or mimics psoriasis.

Identification of the fungal pathogen is performed using conventional methods (biochemical and morphological tests) and molecular techniques (e.g., protein chain reaction test). Each test varies in sensitivity, availability, and cost (Table 1).[10] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is a soft ionization proteomic method that fragments ribosomal proteins to obtain a taxon-specific mass spectrum, known as a protein mass fingerprint, for each fungal taxon.[11]

This study was designed to evaluate the accuracy of MALDI-TOF for onychomycosis in psoriatic patients, using con-

Table 1. Summary of fungal identification methods in onychomycosis

Method	Availability	Cost	Time requirements	Personnel required	Database
Fungal culture	High	Low	Weeks	Experienced mycologist	Experience
Mass spectrometry	Medium	High	A few minutes to hours	Specialist	Library
Protein chain reaction	Medium	High	A few days	Specialist	Primer
Raman spectroscopy	Low	High	A few minutes to hours	Specialist	Library

ventional techniques, including direct 20% KOH examination and fungal culture as the reference standard.

Materials and methods

Patients

Nail samples were prospectively collected from patients clinically and histopathologically diagnosed with psoriatic disease affecting the nails from January 2021 to December 2023 in a tertiary-care hospital in Mexico. The study flow chart is detailed in Figure 1. Inclusion criteria were patients with histopathologically-confirmed psoriasis, aged 18 to 65 years, without systemic treatments or biologic therapy for psoriasis in the last six months. One hundred and thirty patients with plaque psoriasis were selected, of which four patients did not agree to participate in the study and 12 patients did not fulfill all inclusion criteria (out-of-range age, 7 patients; or receiving systemic or biologic psoriasis therapy, 5 patients). Twenty-six patients were excluded for erythroderma (10 patients), systemic comorbidities including diabetes or cancer (9 patients), or pregnancy or breastfeeding (7 patients). The study included a total of 88 patients.

Nail-bed biopsy was selected as the gold standard for diagnosing nail psoriasis, following the methodology of Barrera-Vigo *et al*.[12] Written informed consent was obtained from all patients, keeping their identity anonymous.

Inclusion criteria for all samples were as follows: 1) samples deemed usable by research team without age or gender restrictions; 2) nail psoriasis patient samples without severity restrictions. The exclusion criteria for all of the samples were as follows: 1) ambiguous time of sampling or missing information, 2) insufficient sample size due to testing failure, 3) samples that did not meet standard requirements for sampling, processing, and/or storing: 4) duplicate specimens; 5) specimens with incomplete or untraceable information; and 6) specimens that were otherwise deemed inappropriate by the investigators. Primary cultures were performed on Sabouraud's dextrose agar with chloramphenicol to inhibit bacterial growth. Two types of identification were carried out: 1) traditional mycology testing (KOH) and fungal culture, and 2) proteomic identification by MALDI-TOF.

Mycological procedures

All samples were examined microscopically in 20% KOH solution. Specimens were cultured on two plates and/or tubes: one containing Sabouraud-chloramphenicol-dextrose agar and the other containing Sabouraud-chloramphenicol-cycloheximide. Cultures were incubated at 22–27°C. Growth was assessed after 48 h and then once a week for one month. A specimen was considered positive if either microscopy or culture results were positive (excluding cases where direct

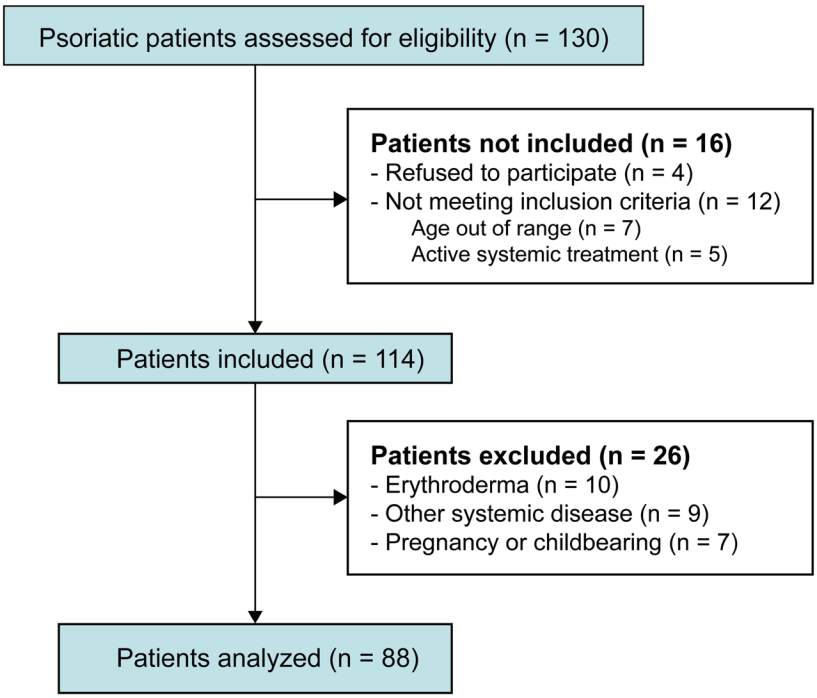

Fig. 1. Patient's enrollment for clinical and mycological examination.

Table 2. Demographic and clinical characteristics of the patients included

Characteristic	Patients n = 88
Age (years), mean ± SD	49.5 ± 17.45 (Range, 35–75 years)
Gender, Male/Female (%)	41/47 (47/53)
Duration of psoriasis (years) median (IQR)	9 (5–13)
Psoriatic arthritis (%)	23 (26.13)
Psoriasis severity, PASI median (IQR)	7 (3.6–13.2)
Nail psoriasis severity, NaPSI (IQR)	4 (2–4)
Comorbidities (%)	46 (52.2)
Systemic arterial hypertension	28 (31.8)
Diabetes mellitus	17 (19.3)
Depression/Anxiety	22 (25)
Hypotiroidism	10 (11.3)
Dyslipidemia	18 (20.4)
Chronic venous insufficiency	13 (14.7)
Heart disease (angina, myocardial infarction, coronary artery disease)	8 (9)

IQR, interquartile range; NaPSI, Nail Psoriasis Severity Index; PASI, Psoriasis Area and Severity Index; SD, standard deviation.

examination showed negative yeast growth). Mycological diagnosis was confirmed by the presence of fungal elements (pseudohyphae, hyphae, and/or spindle-shaped spores) on direct examination at least twice, in association with significant fungal growth, and in the absence of dermatophyte isolation, to exclude non-dermatophyte filamentous fungi and yeasts other than *Candida albicans*.

Cultures with no growth were considered negative and discarded after four weeks of incubation. Positive samples for yeast colonies were identified phenotypically by germination and urease tests, then maintained in brain-heart broth supplemented with 15% glycerol and stored at −20°C until MALDI-TOF mass spectrometry (MS) analysis. Filamentous colonies were identified based on macro- and microscopic characteristics and maintained by normal subculturing.

MS

The MALDI-TOF Vitek-MS® instrument was used according to the manufacturer's instructions. Samples were treated with 0.5 µL of 25% formic acid to disrupt the cell wall and 1.0 µL of a 3.1% v/v solution of alpha-cyano-4-hydroxycinnamic acid for protein crystallization. Identifications with high agreement (>98%) were considered accurate. Data for each species were merged into consensus spectra or superspectra, containing only mass signals present in at least 80% of individual mass spectra. The protein profiles obtained from reference spectra were compared using Biotyper software. For clinical validation, Biotyper was used to identify spectra with corresponding log scores using either the in-house library (5,945 spectra) or the Bruker commercial fungal database (4,111 spectra).

Data analysis

Data were recorded using Microsoft Excel 2013 and transferred to SPSS version 24 (for Windows) for processing. The significance level for statistical calculations was set at 5% ($P < 0.05$), using the Chi-square or Fisher test. Differences between treatment groups were analyzed using the Student's two-tailed independent sample t-test. All analyses and calculations were performed using SPSS version 24 (for Windows).

Results

A total of 100 nail samples from 88 patients with histopathologic evidence of psoriatic nail disease were examined. Patient characteristics are displayed in Table 2. Direct KOH examination was performed, revealing fungal species in 58 samples (58%), of which 46% were dermatophytes.

Fungal culture of nail specimens was positive in 54 samples (54%), allowing for the identification of the etiologic agents. Table 3 shows the isolation sites of the species included in the study and patient characteristics.

MALDI-TOF analysis identified 68 fungal species, including 53 dermatophytes, 12 yeasts, and three non-dermatophytes (Fig. 1). The most common dermatophyte species was *Trichophyton rubrum* (44%). The most common yeast species was *Candida albicans* (7%), and the most common non-dermatophyte species was *Neoscytalidium dimidiatum* (2%).

Each isolate produced a mass spectrum with 60 to 120 signals ranging from 2,000 to 200,000 Da. The extraction and analysis procedures previously established for bacteria were similarly effective and reproducible for dermatophytes, yeasts, and non-dermatophyte fungi.

A kappa coefficient of 0.76 was obtained between conventional identification techniques and MALDI-TOF, indicating excellent concordance according to Landis and Koch.[13]

The sensitivity and specificity of MALDI-TOF for dermatophytes were 95.4% (95% confidence interval (CI): 88.5–97.5%) and 99% (95% CI: 93.33%), respectively. The sensitivity and specificity for yeasts and non-dermatophytes were not determined due to their low prevalence.

Discussion

The identification of superficial mycoses, regardless of the cause, is typically performed using conventional methods.[11] The proteomic technique for identifying the causative spe-

Table 3. Identification of fungal species isolated by conventional and MALDI-TOF techniques

Fungal species	Conventional (n = 58)	MALDI-TOF (n =68)	*P*-value
Dermatophyte			
T. rubrum	38 (65)	44 (65)	NS
T. tonsurans	8 (14)	9 (13)	NS
Yeast			
Candida albicans	5 (9)	7 (10)	NS
Candida parapsilosis	3 (5)	5 (7)	NS
Candida sp	1 (2)	0 (0)	NS
Non-dermatophytes molds			
Neoscytalidium (previously *Scytalidium*) *dimidiatum*	0 (0)	2 (3)	NS
Fusarium oxysporum	0 (0)	1 (2)	NS
Not identified	3 (5)	0 (0)	NS

MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; NS, not significant.

cies in superficial mycoses is used in less than 20% of laboratories in Latin America and the Caribbean.[14] Its disadvantages are the high cost and time-consuming nature.[11] MALDI-TOF is a useful and rapid technique for identifying various microorganisms.[15] Few authors have discussed its advantages and disadvantages compared to other methods, but it is considered more sensitive than automated biochemical methods.[15] Other studies indicate that MALDI-TOF results are equivalent to molecular biological techniques up to 95%.[16]

The high frequency of onychomycoses in psoriatic patients observed in our study (68% of cases) aligns with previous studies that reported a prevalence of up to 63.1%.[17] In our study, out of 100 samples from 88 patients with nail psoriasis, 58% were positive, increasing to 68% with proteomic analysis. These results are similar to those reported in the literature.[11,18] Most studies have reported the association of onychomycosis with nail psoriasis in 4% to 60% of cases.[19] These variations appear to be due to the methodological design of the study and the geographic region.

Psoriasis is an inflammatory disease that, due to its chronicity, may affect natural protective mechanisms in the nail apparatus, predisposing patients to more frequent onychomycosis than the general population.[20] Additionally, psoriasis onychopathy may create a moist environment that predisposes to fungal proliferation.[21,22] Other theories attempting to explain the association include defects in keratopoiesis with elevated glycoproteins and inhibitory peptides that could increase the risk of fungal superinfection,[21] or even the immunomodulatory and immunosuppressive treatments used for the treatment of psoriasis, both local and systemic, including biologic treatments such as tumor necrosis factor-alpha inhibitors, including adalimumab and infliximab.[22]

Similar to previous studies,[18,22–34] dermatophytes like *T. rubrum* were the most common fungi associated with onychomycoses in patients with the psoriatic disease (Fig. 2). Regarding yeasts, we found a lower frequency than reported by Chularojanamontri *et al*.,[20] who noted a prevalence of *Candida* onychomycosis of 33.3% in 150 cases of nail psoriasis, with a higher frequency in patients treated with methotrexate (half of the *Candida* onychomycosis cases). No association between the current treatment of psoriasis and the etiologic agent was found in our study. A higher frequency of *Candida* species in patients with nail psoriasis has been reported in some previous studies.[35–38]

As for non-dermatophyte fungi, *N. dimidiatum* and *Fusarium oxysporum* were the cause of onychomycosis in two and one case, respectively, which is interesting because these fungi are rare causes of onychomycosis in patients with nail psoriasis. These fungi were not detected by culture but only by proteomic analysis. In a previous study by Chadeganipour *et al*.[39] in 289 patients with psoriasis, fungal infections were detected in 46 cases, of which four cases were onychomycoses caused by non-dermatophytes (two cases of *Aspergillus* sp. and two cases of *Fusarium* sp.), but the species could only be identified by conventional methods (direct examination and mycology culture). Non-dermatophyte fungi are more common in patients with nail psoriasis, according to one report.[26]

MALDI-TOF MS could be a useful tool for the routine and rapid identification of fungi in clinical mycology laboratories, especially since reference methods based on molecular sequencing are not currently available in our developing countries (Table 1). It remains important to differentiate dermatophytes from non-dermatophyte species, which do not respond to antidermatophyte therapy and cause dermatophytosis-like infections, as well as to distinguish species in concomitant infections. According to our study and previous reports, MALDI-TOF MS can discriminate between onychomycosis and non-fungal nail diseases, whereas KOH preparation and fungal culture are often used to confirm or rule out onychomycosis.[40]

MALDI-TOF MS does not require any living or non-living fungal material to confirm or exclude onychomycosis. The novelty of this technique lies in detecting solid biochemical features related to mycological infections or non-infectious diseases, represented by proteolytic degradation products of native nail proteins.[41]

The main limitation is that MALDI-TOF identification is only possible if the species is included in the reference spectra library.[42]

Neoscytalidium dimidiatum is the most common causative agent of onychomycosis by non-dermatophyte fungi and has a high rate of resistance to antifungal treatments.[43] We found no reports of the association of *N. dimidiatum* in patients with psoriasis; more interestingly, it was not identified by conventional means, only by proteomic analysis.

The reliability of this tool, especially for identifying derma-

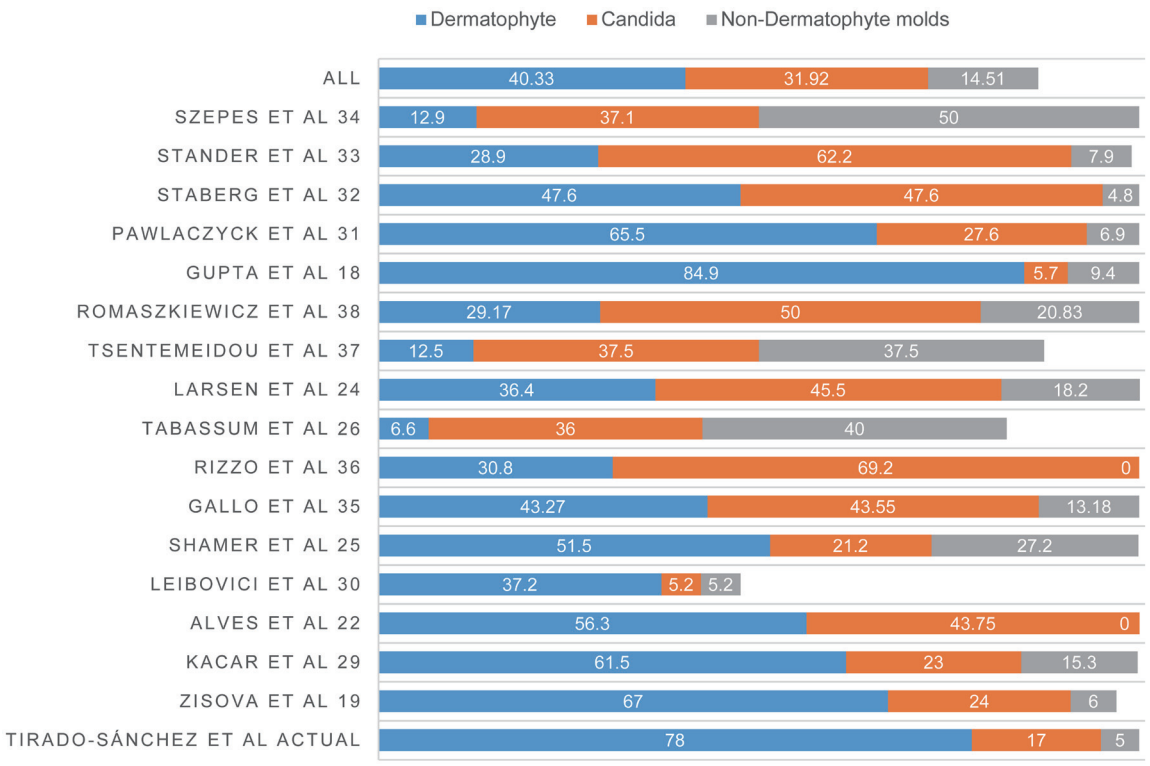

Fig. 2. Comparative chart of the pathogens found in psoriatic patients with onychomycosis based on literature review.

tophytes, has been demonstrated in several previous studies. However, limitations have been noted in differentiating species within complexes such as the *T. mentagrophytes* complex or the *T. rubrum* complex. Similar restrictions have been found in genera revealing cryptic species within morphologically recognized "morph species" such as *Aspergillus* and *Fusarium*. This may explain our low species-level identification rate for these fungi, as well as the use of commercially available databases. However, recent studies using complementary in-house databases have achieved over 90% correct identification at the species level.[44,45]

In our study, MALDI-TOF showed a good correlation with conventional diagnostic methods, with sensitivities and specificities of 95.4% and 99%, respectively. This is consistent with other studies.[11,46–50] Although our study was performed only in patients with nail psoriasis and not in a healthy population, it is relevant because the underlying chronic inflammatory pathology may affect the sensitivity and specificity of proteomic analysis. The major limitation of this study was the inability to perform follow-up analyses of patient outcomes after the intervention. An additional limitation includes the missing information on antifungal pretreatment, since may significantly decreases the sensitivity of fungal culture but to a lesser extent with MALDI-TOF. Considerably more work is needed to explore the utility of MALDI-TOF MS in patients with psoriasis with biologic therapy and other systemic treatments.

Conclusions

MALDI-TOF offers speed and, with upgrades to the base series, increased sensitivity and specificity. Its compatibility with most commercially available kits makes it a valuable addition to clinical practice. The introduction and increased availability of MALDI-TOF may expand the diagnostic landscape, allowing for the identification of rare and difficult-to-identify species, especially non-dermatophyte species with high resistance to antifungal therapy, thereby facilitating new management options for complex cases.

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Conflict of interest

The authors declare no conflict of interest in this study.

Author contributions

Study concept and design, drafting of the manuscript (ATS, SB), acquisition of data (ATS, AB, JA), analysis and interpretation of data (AB, JA), critical revision of the manuscript for important intellectual content (ATS, AB), administrative, technical, or material support (JA, SB), and study supervision (AB). All authors have made significant contributions to this study and have approved the final manuscript.

Ethical statement

This study was carried out in accordance with the Institutional Review Board of the Hospital General de México, which approved the protocol with the number DI/21/109/03/58. All

subjects gave written informed consent in accordance with the Declaration of Helsinki.

Data sharing statement

All data used to support the findings of this study are included in the article.

References

[1] Gupta AK, Stec N, Summerbell RC, Shear NH, Piguet V, Tosti A, *et al*. Onychomycosis: a review. J Eur Acad Dermatol Venereol 2020;34(9):1972–1990. doi:10.1111/jdv.16394, PMID:32239567.

[2] Chanyachailert P, Leeyaphan C, Bunyaratavej S. Cutaneous Fungal Infections Caused by Dermatophytes and Non-Dermatophytes: An Updated Comprehensive Review of Epidemiology, Clinical Presentations, and Diagnostic Testing. J Fungi (Basel) 2023;9(6):669. doi:10.3390/jof9060669, PMID:37367605.

[3] Yousefian F, Smythe C, Han H, Elewski BE, Nestor M. Treatment Options for Onychomycosis: Efficacy, Side Effects, Adherence, Financial Considerations, and Ethics. J Clin Aesthet Dermatol 2024;17(3):24–33. PMID:38495549.

[4] Lipner SR, Scher RK. Onychomycosis: Clinical overview and diagnosis. J Am Acad Dermatol 2019;80(4):835–851. doi:10.1016/j.jaad.2018.03.062, PMID:29959961.

[5] Frazier WT, Santiago-Delgado ZM, Stupka KC 2nd. Onychomycosis: Rapid Evidence Review. Am Fam Physician 2021;104(4):359–367. PMID: 34652111.

[6] Hwang JK, Lipner SR. Treatment of Nail Psoriasis. Dermatol Clin 2024;42(3):387–398. doi:10.1016/j.det.2024.02.004, PMID:38796270.

[7] Canal-García E, Bosch-Amate X, Belinchón I, Puig L. Nail Psoriasis. Actas Dermosifiliogr 2022;113(5):481–490. doi:10.1016/j.ad.2022.01.006, PMID:35697407.

[8] Bozdemir NY, Yuksel EI, Toraman ZA, Cicek D, Demir B, Gunbey F. Factors affecting onychomycosis in patients with psoriasis. Dermatol Ther 2022;35(7):e15513. doi:10.1111/dth.15513, PMID:35426221.

[9] Battista T, Scalvenzi M, Martora F, Potestio L, Megna M. Nail Psoriasis: An Updated Review of Currently Available Systemic Treatments. Clin Cosmet Investig Dermatol 2023;16:1899–1932. doi:10.2147/CCID.S417679, PMID:37519941.

[10] Noguchi H, Matsumoto T, Mimura Y, Kubo M, Higuchi S, Kashiwada-Nakamura K, *et al*. Dermatophyte antigen kit: A useful diagnostic tool for onychomycosis. J Dermatol 2023;50(12):1614–1618. doi:10.1111/1346-8138.16924, PMID:37605440.

[11] Bonifaz A, Montelongo-Martínez F, Araiza J, González GM, Treviño-Rangel R, Flores-Garduño A, *et al*. [Evaluation of MALDI-TOF MS for the identification of opportunistic pathogenic yeasts of clinical samples]. Rev Chilena Infectol 2019;36(6):790–793. doi:10.4067/S0716-10182019000600790, PMID:33660761.

[12] Barrera-Vigo MV, Tejera-Vaquerizo A, Mendiola-Fernández M, Cid J, Cabrade Luna B, Herrera-Ceballos E. [Diagnostic utility of nail biopsy: a study of 15 cases]. Actas Dermosifiliogr 2008;99(8):621–627. PMID:19080893.

[13] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(1):159–174. PMID:843571.

[14] Falci DR, Pasqualotto AC. Clinical mycology in Latin America and the Caribbean: A snapshot of diagnostic and therapeutic capabilities. Mycoses 2019;62(4):368–373. doi:10.1111/myc.12890, PMID:30614600.

[15] Chalupová J, Raus M, Sedlářová M, Sebela M. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnol Adv 2014;32(1):230–241. doi:10.1016/j.biotechadv.2013.11.002, PMID:24211254.

[16] Westblade LF, Jennemann R, Branda JA, Bythrow M, Ferraro MJ, Garner OB, *et al*. Multicenter study evaluating the Vitek MS system for identification of medically important yeasts. J Clin Microbiol 2013;51(7):2267–2272. doi:10.1128/JCM.00680-13, PMID:23658267.

[17] Klaassen KM, Dulak MG, van de Kerkhof PC, Pasch MC. The prevalence of onychomycosis in psoriatic patients: a systematic review. J Eur Acad Dermatol Venereol 2014;28(5):533–541. doi:10.1111/jdv.12239, PMID:24033871.

[18] Gupta AK, Lynde CW, Jain HC, Sibbald RG, Elewski BE, Daniel CR 3rd, *et al*. A higher prevalence of onychomycosis in psoriatics compared with non-psoriatics: a multicentre study. Br J Dermatol 1997;136(5):786–789. PMID:9205520.

[19] Zisova L, Valtchev V, Sotiriou E, Gospodinov D, Mateev G. Onychomycosis in patients with psoriasis—a multicentre study. Mycoses 2012;55(2):143–147. doi:10.1111/j.1439-0507.2011.02053.x, PMID:21771106.

[20] Chularojanamontri L, Pattanaprichakul P, Leeyaphan C, Suphatsathienkul P, Wongdama S, Bunyaratavej S. Overall Prevalence and Prevalence Compared among Psoriasis Treatments of Onychomycosis in Patients with Nail Psoriasis and Fungal Involvement. Biomed Res Int 2021;2021:9113418. doi:10.1155/2021/9113418, PMID:34938812.

[21] Rigopoulos D, Papanagiotou V, Daniel R 3rd, Piraccini BM. Onychomycosis in patients with nail psoriasis: a point to point discussion. Mycoses 2017;60(1):6–10. doi:10.1111/myc.12542, PMID:27523738.

[22] Alves NCPOP, Moreira TA, Malvino LDS, Rodrigues JJ, Ranza R, de Araújo LB, *et al*. Onychomycosis in Psoriatic Patients with Nail Disorders: Aetiological Agents and Immunosuppressive Therapy. Dermatol Res Pract 2020;2020:7209518. doi:10.1155/2020/7209518, PMID:32411192.

[23] Jha B, Sharma M, Gc S, Sapkota J. Onychomycosis among Clinically Suspected Cases Attending the Dermatology Out-patient Department of a Tertiary Care Centre: A Descriptive Cross-sectional Study. JNMA J Nepal Med Assoc 2021;59(237):450–453. doi:10.31729/jnma.6277, PMID:34508426; PMCID: PMC8673452.

[24] Larsen GK, Haedersdal M, Svejgaard EL. The prevalence of onychomycosis in patients with psoriasis and other skin diseases. Acta Derm Venereol 2003;83(3):206–209. doi:10.1080/00015550310007229, PMID:12816157.

[25] Shemer A, Trau H, Davidovici B, Grunwald MH, Amichai B. Onychomycosis in psoriatic patients - rationalization of systemic treatment. Mycoses 2010;53(4):340–343. doi:10.1111/j.1439-0507.2009.01726.x, PMID:19496936.

[26] Tabassum S, Rahman A, Awan S, Jabeen K, Farooqi J, Ahmed B, *et al*. Factors associated with onychomycosis in nail psoriasis: a multicenter study in Pakistan. Int J Dermatol 2019;58(6):672–678. doi:10.1111/ijd.14364, PMID:30615189.

[27] Natarajan SB, Baalann KP. Psoriasis with onychomycosis in a diabetic patient. Pan Afr Med J 2021;40:72. doi:10.11604/pamj.2021.40.72.26025, PMID:34804340.

[28] Méndez-Tovar LJ, Arévalo-López A, Domínguez-Aguilar S, Manzano-Gayosso P, Hernández-Hernández F, López Martínez R, *et al*. [Onychomycosis frequency in psoriatic patients in a tertiary care hospital]. Rev Med Inst Mex Seguro Soc 2015;53(3):374–379. PMID:25984624.

[29] Kaçar N, Ergin S, Ergin C, Erdogan BS, Kaleli I. The prevalence, aetiological agents and therapy of onychomycosis in patients with psoriasis: a prospective controlled trial. Clin Exp Dermatol 2007;32(1):1–5. doi:10.1111/j.1365-2230.2006.02215.x, PMID:16824053.

[30] Leibovici V, Hershko K, Ingber A, Westerman M, Leviatan-Strauss N, Hochberg M. Increased prevalence of onychomycosis among psoriatic patients in Israel. Acta Derm Venereol 2008;88(1):31–33. doi:10.2340/00015555-0323, PMID:18176747.

[31] Pawlaczyk M, Rokowska A, Chmielewska I, Janicka D, Rzepecka B, Mazurek M, *et al*. Does onychomycosis more frequently affect patients suffering from psoriasis? Mikologia Lekarska 2007;14(1):52–55.

[32] Staberg B, Gammeltoft M, Onsberg P. Onychomycosis in patients with psoriasis. Acta Derm Venereol 1983;63(5):436–438. PMID:6197846.

[33] Ständer H, Ständer M, Nolting S. [Incidence of fungal involvement in nail psoriasis]. Hautarzt 2001;52(5):418–422. doi:10.1007/s001050051335, PMID:11405160.

[34] Szepes E. [Mycotic infections of psoriatic nails]. Mykosen 1986;29(2):82–84. PMID:2938001.

[35] Gallo L, Cinelli E, Fabbrocini G, Vastarella M. A 15-year retrospective study on the prevalence of onychomycosis in psoriatic vs non-psoriatic patients: A new European shift from dermatophytes towards yeast. Mycoses 2019;62(8):659–664. doi:10.1111/myc.12925, PMID:31062415.

[36] Rizzo D, Alaimo R, Tilotta G, Dinotta F, Bongiorno MR. Incidence of onychomycosis among psoriatic patients with nail involvement: a descriptive study. Mycoses 2013;56(4):498–499. doi:10.1111/myc.12042, PMID:23368934.

[37] Tsentemeidou A, Vyzantiadis TA, Kyriakou A, Sotiriadis D, Patsatsi A. Prevalence of onychomycosis among patients with nail psoriasis who are not receiving immunosuppressive agents: Results of a pilot study. Mycoses 2017;60(12):830–835. doi:10.1111/myc.12681, PMID:28877373.

[38] Romaszkiewicz A, Bykowska B, Zabłotna M, Sobjanek M, Sławińska M, Nowicki RJ. The prevalence and etiological factors of onychomycosis in psoriatic patients. Postepy Dermatol Alergol 2018;35(3):309–313. doi:10.5114/pdia.2017.68299, PMID:30008651.

[39] Chadeganipour M, Shadzi S, Mohammadi R. Fungal Infections among Psoriatic Patients: Etiologic Agents, Comorbidities, and Vulnerable Population. Autoimmune Dis 2021;2021:1174748. doi:10.1155/2021/1174748, PMID:34567800.

[40] Gupta AK, Hall DC, Cooper EA, Ghannoum MA. Diagnosing Onychomycosis: What's New? J Fungi (Basel) 2022;8(5):464. doi:10.3390/jof8050464, PMID:35628720.

[41] Pföhler C, Hollemeyer K, Heinzle E, Altmeyer W, Graeber S, Müller CS, *et al*. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a new tool in diagnostic investigation of nail disorders? Exp Dermatol 2009;18(10):880–882. doi:10.1111/j.1600-0625.2008.00838.x, PMID:19320735.

[42] Ranque S, Normand AC, Cassagne C, Murat JB, Bourgeois N, Dalle F, *et al*. MALDI-TOF mass spectrometry identification of filamentous fungi in the clinical laboratory. Mycoses 2014;57(3):135–140. doi:10.1111/myc.12115, PMID:23895114.

[43] Leeyaphan C, Chai-Adisaksopha C, Tovanabutra N, Phinyo P, Bunyaratavej S. Prognostic factors for mycological cure in patients with onychomycosis caused by Neoscytalidium dimidiatum: A retrospective cohort study. Mycoses 2023;66(6):497–504. doi:10.1111/myc.13575, PMID:36740753.

[44] Wigmann EF, Behr J, Vogel RF, Niessen L. MALDI-TOF MS fingerprinting for identification and differentiation of species within the Fusarium fujikuroi species complex. Appl Microbiol Biotechnol 2019;103(13):5323–5337. doi:10.1007/s00253-019-09794-z, PMID:31037383.

[45] Triest D, Stubbe D, De Cremer K, Piérard D, Normand AC, Piarroux R, *et al*. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of molds of the Fusarium genus. J Clin Microbiol 2015;53(2):465–476. doi:10.1128/JCM.02213-14, PMID:25411180.

[46] Criseo G, Scordino F, Romeo O. Current methods for identifying clinically important cryptic Candida species. J Microbiol Methods 2015;111:50–56. doi:10.1016/j.mimet.2015.02.004, PMID:25659326.

[47] Jamal WY, Ahmad S, Khan ZU, Rotimi VO. Comparative evaluation of two matrix-assisted laser desorption/ionization time-of-flight mass spectrom-

etry (MALDI-TOF MS) systems for the identification of clinically significant yeasts. Int J Infect Dis 2014;26:167–170. doi:10.1016/j.ijid.2014.05.031, PMID:25080355.
[48] Porte L, García P, Braun S, Ulloa MT, Lafourcade M, Montaña A, *et al*. Head-to-head comparison of Microflex LT and Vitek MS systems for routine identification of microorganisms by MALDI-TOF mass spectrometry in Chile. PLoS One 2017;12(5):e0177929. doi:10.1371/journal.pone.0177929, PMID: 28542393.
[49] Kassim A, Pflüger V, Premji Z, Daubenberger C, Revathi G. Comparison of biomarker based Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and conventional methods in the identification of clinically relevant bacteria and yeast. BMC Microbiol 2017;17(1):128. doi:10.1186/s12866-017-1037-z, PMID:28545528.
[50] Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, *et al*. Identification and typing of the emerging pathogen Candida auris by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses 2016;59(8):535–538. doi:10.1111/myc.12519, PMID:27292939.