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New Tools in Nail Disorders

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Tumors of the nail unit may be difficult to diagnose because of the screening effect of the nail plate. In longitudinal melanonychia, several new promising techniques assist with early diagnosis of melanoma (in vivo matrix dermoscopy and immunohistochemistry) as well as sparing as much of the healthy tissues as is possible (shave biopsy technique). Diagnosing nail disorders is in some instances difficult both for the clinician and the pathologist. New tools such as polymerase chain reaction have been proposed for onychomycosis, which accounts for more than half of nail conditions, will allow quick and accurate diagnosis. However, polymerase chain reaction analysis remains expensive and is not routinely used by clinicians. Scoring nail dystrophy by clinical observation remains very subjective; therefore, severity indexes have been proposed. Another emerging noninvasive technique is forensic analysis of nail clippings for detection of drug intake and abuse, as well as exposure to environmental pollution

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New Tools in Medical Imaging

Noninvasive assessment is of particular interest in nail disorders because it may facilitate differential diagnosis and delineate tumors.[1-3] Large studies imaging tumors of the nail apparatus with magnetic resonance imaging (MRI) demonstrated that this technique is mostly valuable for diagnosing vascular lesions and cysts filled with liquid, such as glomus tumors and ganglion cysts, and for solid lesions an accurate location is determined, thus helping the surgeon when planning its removal.[1,4] However, MRI requires specialized equipment, as the standard coils will not give proper images, that is not yet widely available.[4]

On the other hand, ultrasonography is widely available. Ultrasound imaging is based on the time delay that occurs when the ultrasound beams are reflected from different tissues. The clarity of the image depends on the structure of the tissue, its echogenicity, and several technical parameters. New generations of high-resolution ultrasound machines, reaching up to 15 MHz, can process great amounts of data (Fig. 1).

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New techniques have become available that allow the instantaneous integration of several overlapping ultrasound scans taken at different angles to produce a compound image with better information content. This technique, called real-time spatial compound imaging, reduces artifacts and provides a sharper Image.[5] The presence of blood flow also is studied with the use of color Doppler and power angiography, which allow for the detection of vessels and flow. Ultrasound can supply information on the anatomy and pathological processes in real time with the possibility of measuring and calculating parameters of thickness and volume of blood flow, with an accuracy of approximately 100 μm (depending on the device used).[6]

The nail apparatus contains tissues of various echogenicities, making it very suitable for ultrasound examination. Characteristic ultrasound pictures can be produced for psoriasis, glomus tumors, and myxoid cysts. Furthermore, ultrasound can be particularly useful when general edema and pain complicate the clinical examination. Imaging of the vasculature may be enhanced by the use of injected contrast media developed for ultrasonography. The use of contrast media may provide specific diagnoses, according to the washout curves of medium, and a better description of the vascular morphology of the lesion.[6] Further studies are still required.

Optical coherence tomography (OCT) works by emitting wave forms similar to ultrasound. The OCT probe sends infrared light (instead of acoustical waves), and its reflection is measured and the intensity is imaged as a function of position of the material reflecting the wave. The signal obtained from the scan is amplified, demodulated, and stored in a digital form. The image data are displayed by assigning color or gray scales to each reflection according to the measured signal strength. This technique provides cross-sectional, tomographic images of tissue *in situ* and in real time.[7] OCT imaging of the nail is easy to perform. Images clearly reflect the anatomical

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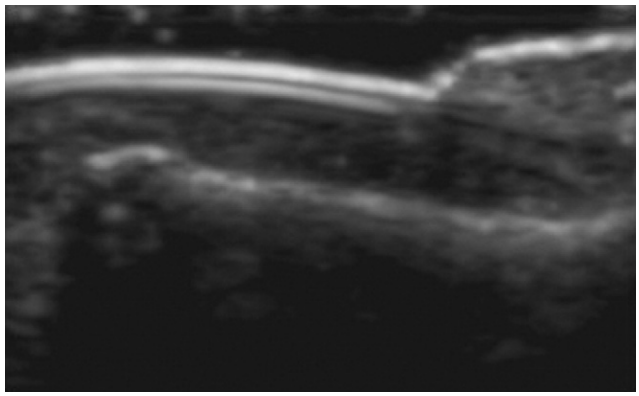

Figure 1 Real-time–HFUS of a normal nail apparatus. Note the bilamellar structure of the plate; the matrix that appears a bit darker than the bony phalanx (courtesy of G. Jemec, Denmark).

structure of the nail unit. These structures can also be imaged by high-frequency ultrasound (HFUS), but because OCT has greater resolution than HFUS, it has the ability to discriminate subtle changes not detected by ultrasound, thus providing more information about the nail unit. OCT has a resolution approximately 20 times greater that MRI, but a low penetrance depth; hence, OCT and MRI are not always mutually exclusive. Doppler OCT may add useful new information on nail vascular tumors.[7]

New Tools in Mycology Diagnosis

The clinical standard for diagnosing onychomycosis is direct examination (potassium chloride) combined with fungal culture.[8] For others,[9,10] histopathology with Periodic acid-Schiff staining combined with fungal culture is more sensitive. We strongly advise the combination of potassium chloride, histopathology and culture when sufficient infected material is available or if a nondermatophytes mold (NDM) is involved.

Chang[11] proposed a modified approach to the histologic diagnosis. As he usually favors plastic embedding because its use allows 2- to 2.5-μm sections as well as a better adherence to coated slides, he tried to find an alternative to this costly and time-consuming (2 weeks) method. Because distal and lateral onychomycosis is the most prevalent type of fungal infection, he postulated that examination of subungual hyperkeratosis alone may be equally effective in diagnosing onychomycosis as examination of the nail plate. Because subungual hyperkeratosis can be processed in the same manner as skin specimens, the time, the technical difficulty, as well as the costs are reduced. He decided to process the nail plate only if the subungual keratin was negative for hyphae, which accounted for 26% of their cases.

Using the method described by our master, Achten, in which nails are embedded in paraffin (without previous use of softening agents), cut into 15-μm thick sections with one-half stained with Periodic acid-Schiff and the other half with toluidine blue (allowing a better visualization of cellular details), it takes our technicians 8 hours to process entirely by hand 40 samples, which means that 16 person hours are necessary to process our 80 weekly samples. Although the most common diagnosis is onychomycosis, more than one-third of the samples are submitted to rule out onychomycosis, mainly in traumatized, psoriatic, black or white nails.

In onychomycotic nails, some physicians like to know the location of the fungus. As a result of the sampling technique, the Chang method lacks the ability to detect bipolar onychomycosis. The recommended way to obtain samples is to take scrapings of the upper plate in superficial white onychomycosis or subungual debris (by paring the proximal nail with a scalpel) in proximal subungual onychomycosis. Nail histopathology is also a valuable tool when a NDM grows in culture. Fungal elements might be found in subungual keratin but perforating hyphae, which are a clue to NDM invasion, will usually be found in the lower or upper nail plate (Fig. 2). For all these reasons, most nail experts usually use the older method of Achten.

The reliability and availability of mycologic evaluation of pathology specimens is limited, with well-known rates of false-negative results. Few laboratories have expertise in mycology, and erroneous results are frequent in laboratories.[12] Immunochemistry, flow cytometry, in vivo confocal microscopy, and scanning electron microscopy cannot be proposed as valid alternative methods because they are limited to even fewer laboratories, are complicated, and are even more costly.[8,12]

An essential step in understanding and treating onychomycosis is the identification of the fungus at the species level, as well as the assessment of its viability. Polymerase chain reaction (PCR)[13-17] is a genotypic method initially developed as an alternative to identify the species in false-negative cultures or in cultures in which the phenotypic characteristics were difficult to interpret. In recent years, it has been extended to detecting the fungus directly from the nail clippings, thus, allowing a diagnosis from small samples or in samples with little infection.[18-25] Also, PCR has allowed the discovery of different subtypes of *Trichophyton rubrum,*[14,26,27] which could be found simultaneously or at different times in the same patient. Moreover, this technique is time saving, as in most instances, the diagnosis is obtained in less than 48 hours compared with the classic 4 weeks needed for the conventional technique.[21]

There are several PCR methods[14] that require nonspecific primers usually when little is known about the target sequence (random amplification of polymorphic DNA and arbitrarily primed PCR). Others have targeted specific genes, for example, actin, chitin, and topoisomerase II. Most dermatophyte PCR studies have focused on the genes coding for ribosomal RNA (rRNA), including transcribed (internal transcribed spacers ITS1 and ITS2) and nontranscribed (nontranscribed spacer) DNA regions. The nontranscribed spacer region of the *Trichophyton rubrum* rRNA gene allows the study of it DNA subtypes.

Restriction enzyme digestion is used to cut the DNA at specific sites, allowing species-specific differentiation (restriction fragment length polymorphism).[17,18] The use of reverse transcriptase-PCR has the ability to follow gene expression and thus the viability of the fungus. Real-time PCR is a quantitative method in which the accu-

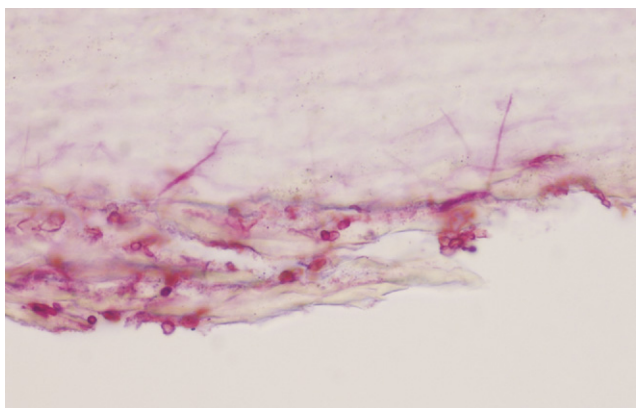

Figure 2 Onychomycosis due to nondermatophyte mold: fungal elements and perforating hyphae in the lower nail plate (×400).

mulation of PCR product is monitored in "real time," and the amount of initial target DNA is quantified using calibration curves (LightCycler PCR; Roche Molecular Biochemicals, Indianapolis, IN). Real-time reverse transcriptase-PCR is the most sensitive technique available for quantification and detection of viable fungi.[28]

It may be difficult to clinically evaluate the severity and burden of nail diseases, explaining the recent development of several indexes to quantitate the severity of disease. A valid and reliable measure that captures the severity of onychodystrophy is important both for clinical and research applications. Based on 39 questions, NailQol (ie, Nail Quality of Life) represents a new concise, valid, reliable, and responsive instrument for measuring the burden of skin disease for American patients with onychomycosis.[29]

A pilot evaluation suggests that Naildex (nail dystrophy severity index) may represent a new research method for quantifying the burden of nail disease. It has shown to be reliable and has both face and construct validity. It is relatively time-efficient and, as it does not involve imaging equipment, it is inexpensive. It takes into account all 10 finger-and/or toenails. The scores are calculated by a combination of percent of each nail infected, area of each nail and number of infected nails.[30] This pilot study was designed for patients with onychomycosis, but the authors propose that it could be used for patients with other diseases causing nail dystrophy, such as psoriasis, trauma and bacterial infections.

In the first part of an article reviewing antifungal therapy, Baran and colleagues[31] provide an onychomycosis severity index for assessing the response to treatment. The clinical characteristics of the nail infection, the age of the patient, the presence of predisposing factors, as well as the fungal type are considered. For each item, scores are given from 1 to 4. The highest score goes to diffuse melanonychia without paronychia, immunosuppression and *Scytalidium spp*.

New Tools in Forensics

Drugs and biological substances are stored in nails, where they can be detected and measured. This measurement will provide a history of drug intake and abuse, as well as toxin exposure and therefore represents a unique substrate for forensic purposes. The collection is easy and noninvasive, and only a small specimen is required for analysis. Because the nails grow very slowly, it makes them a useful tool for retrospective analysis. Fingernails are best for research of external contamination, but they reflect the exposure for the last 6 months. Although the great toenail is less exposed to external contamination, it gives information about the last year.[32] Several techniques have been shown to be useful in the detection of various substances, such as DNA,[33] pollutants (lead, nicotine) and metals in exposed workers (nickel, chromium) and poisons (arsenic, thallium). A new technique using laser ablation in combination with inductively coupled plasma sector field mass spectrometry succeeded in determining a homicide by thallium poisoning that took place 38 years ago.[34] Gas chromatography-mass spectrometry has been proved to be effective for the simultaneous qualification and quantification of amphetamine-type stimulants and cannabinoids in fingernails.[35]

Nephrogenic systemic fibrosis, a rare acquired disorder affecting renal insufficiency patients, seems be associated with the use of gadolinium-containing contrast agents for MRI. Gadolinium quantitative determination in fingernails by using inductively coupled plasma mass spectrometry showed, in specimens collected 6 months after the last gadolinium contrast agent (gadodiamide) injection, rates 1000 times higher than those observed among volunteers. Because the growth of fingernails is very slow (6 months), gadolinium quantitative determination in nails could be of major interest for diagnosing patients developing nephrogenic systemic fibrosis several weeks or months after the last gadolinium exposure.[36] In the future, more refined techniques will allow more specific and accurate diagnosis of drug intake and abuse, exposure to pollutants and may also help in the monitoring of some diseases from a simple nail clipping.

New Tools in Longitudinal Melanonychia

Melanonychia striata always represents a dilemma for the clinician and the pathologist. There remains some controversy about the way to address longitudinal melanonychia.[37] Therefore, several techniques in the surgical, medical, and pathological field have been proposed. All of them are aimed at diagnosing more accurately the cause of the pigmented band with limited postoperative dystrophy. For that, some authors proposed dermoscopy of the nail bed and matrix after nail removal. Nail avulsion associated with 2 lateral oblique incisions at the corner of the lateral nail fold-proximal nail fold allows exposure of the whole nail matrix and nail bed area. A digital tourniquet is placed to ensure a completely bloodless field. Dermatoscopic examination of the area may be performed by the use of a Dermlite® (3Gen LLC, San Juan Capistrano, CA) with polarized light with no direct tissue contact maintaining aseptic conditions. Alternatively, dermatoscopic examination can be performed with direct tissue contact by the use of a sterilized contact plate for inaccessible lesions and sterile saline solution 0.9% instead of immersion oil. Clear antiseptic gels are also an option. Only 12 cases were initially reported. The authors conclude that this novel technique does not replace dermatoscopic examination of the nail plate but collects complementary information on the lesion. Definitive diagnosis is always given by histopathology. They have shown some typical patterns of melanoma and melanocytic activation. The initial published series was a bit too small to reach a conclusion, but recently they presented a larger series, which is not published, of more than 70 cases that demonstrated very typical patterns highly correlated with histopathology. One point that is extremely attractive to the surgeon is that this technique makes it easier to locate pigmentation and allows perfect delineation of the surgical margins without avoiding omission of small pigment foci.[38,39]

To lessen postoperative dystrophy, a novel matrix biopsy technique, called the shave biopsy, was suggested by Haneke.[40,41] This procedure removes the matrix epithelium and a thin layer of the underlying dermis, allowing histologic examination of the whole pigmented lesion while limiting postoperative dystrophy. This new technique is very effective considering the discrete postoperative sequelae after removal of a large piece of matrix tissue. It allows the pathological examination of the whole lesion even for large bands (Fig. 3). It avoids partial or total ablation of the nail unit for benign lesions. However, this technique requires training of the surgeon

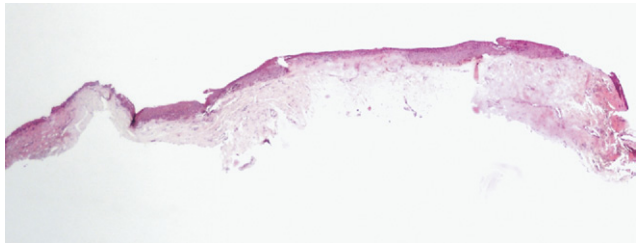

Figure 3 Shave biopsy. Note the thinness of the specimen (×20).

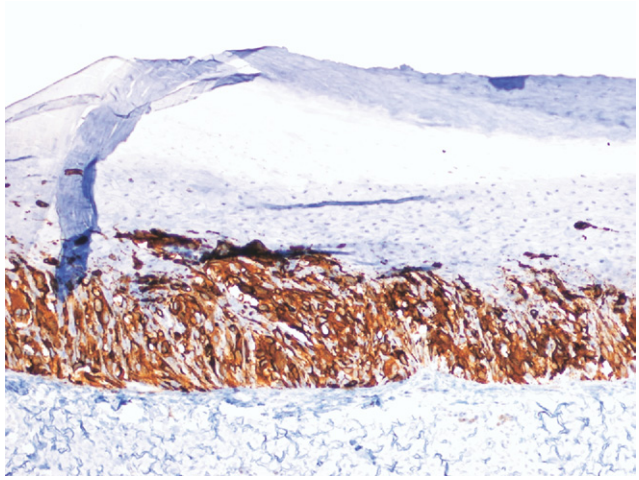

Figure 4 Mart-1 immunostaining in an in situ acral lentiginous melanoma (×100).

and excellent collaboration with the pathology laboratory to correctly orientate and read the thin specimen. Although this new technique is mentioned in several papers,[42-44] it still needs to be validated.

Pathology also tries to improve the ability to diagnose melanoma that might be in some instances be very challenging. Immunohistochemistry may help the pathologist and is being used more routinely. In melanoma, the sensitivity of the commonly used antibodies varies: 97-100% for the S-100 protein, 69-93% for HMB-45, and 75-92% for Mart-1.[45] In 20 invasive acral lentiginous melanomas (ALM), S-100 was reported to be more sensitive than HMB-45 and Mart-1.[46] Among 4 cases of S-100 negative melanomas, 2 were ALM.[47] Few data have been published about nail apparatus melanoma (NAM). In a case of *in situ* NAM, HMB-45 was proven to be the best method for detecting nail apparatus melanocytes.[48] Similarly, in 9 other cases of *in situ* ALM, including 4 NAM, all the lesions demonstrated strong positive staining with HMB-45 whereas S-100 protein was weakly positive or even negative in atypical melanocytes.[49] For a Belgian experienced team in nail pathology, immunochemistry is particularly useful for the diagnosis of early melanomas and for the determination of the lateral margins in ALM. When dealing with intraepithelial melanocytes, the sensitivity was better with HMB-45, than with Mart-1 (Fig. 4). S-100 protein was the least sensitive. However, in invasive NAM, S-100 protein was the most sensitive and was the only positive marker in case of desmoplastic melanoma and in areas with chondroid differentiation.[50]

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