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Nail diseases are generally held to be challenging in diagnosis and treatment and many physicians are very reluctant to take a biopsy. Thus, non-invasive diagnostic methods are often performed that are time-consuming, expensive and often do not allow a conclusive diagnosis to be made. However, a definitive correct diagnosis is needed before embarking on a long-term therapy that is potentially hazardous.

## **PREREQUISITES OF A NAIL BIOPSY**

A histopathological diagnosis cannot be better than the biopsy, which is crucial in onychology. This requires in-depth knowledge of nail anatomy, at least some basics of nail pathology and how nail changes develop, skills in atraumatic surgery, and how to manage potential complications. Alterations of the nail plate surface derive from the apical matrix, those of the nail plate substance from the proximal to the distal matrix, and nail bed changes remain under the nail. Pigment that is localized in the middle layer of the nail originates in the middle matrix, whereas a melanocyte lesion in the nail bed cannot give pigment to the nail plate. [1]

### **PATIENT PREPARATION**

Except for nail plate only biopsies, anaesthesia and sterile conditions are necessary. The patient has to be aware that there might be some pain after the anesthesia waned off. Smoking is forbidden prior to any nail surgery and sports activities should be stopped until at least a week after biopsy. [2] The extremity is elevated for 24-48 hours, and the patients should bring a large open shoe in case of a toenail biopsy. [3]

### **ANAESTHESIA**

After disinfection, local anaesthesia is applied. Some prefer a so-called distal fan block, which is in fact just a local infiltration anaesthesia, others perform a proximal finger block the advantage of which is not to interfere with cytological details of the biopsied soft tissue. [4]

The local anaesthetic agent of our choice is ropivacaine 1% as it works as fast as lidocaine and as long as bupivacaine, i.e. usually longer than 12 hours. Lidocaine, mepivacaine or another agent may be used but their duration of action is much shorter. Although adrenaline in a fixed concentration of 1:200,000 does not cause complications we do not use it as we prefer a tourniquet.

## **BIOPSY TECHNIQUES**

*Nail clippings* give a lot of information about fungal infection, potential nail psoriasis, causes of leukonychia and nail pigmentation, particularly in longitudinal melanonychia.

The nail is cleansed with a simple alcohol swab and as much of the nail plate plus subungual keratin as possible is clipped off. This material may be fixed in formalin or submitted dry to the lab. *Cytologic smears* may allow herpes simplex/herpes zoster and pemphigus to be diagnosed. The blister roof may be examined for the degree of necrosis to differentiate epidermal necrolysis from other blistering conditions.

Shave biopsies are adequate for superficial lesions of the paronychial skin, e.g. for pigment spots or vesicles in case of a suspected viral vesicle. The specimen is transferred to filter paper, stretched out and fixed in formalin as usual. Punch biopsies may be performed from any part of the nail unit. Their diameter should not exceed 4 mm for nail bed and 3 mm for matrix biopsies. Whether or not the overlying nail plate is avulsed depends on the clinical diagnosis as well as the thickness and hardness of the plate. To soften the nail the digit may be immersed into warm water for 10 minutes prior to punching through the nail. However, as the consistency of the nail and the underlying soft tissue are very different the plate usually shears off the nail bed or matrix and often remains in the punch from where it has to be taken out with the help of an injection needle #30, the tip of which had been bent 100° to form a tiny hook. The punch is usually run down to the bone from where it is dissected with pointed iris scissors. The punch hole is left for second intention healing or a piece of collagen foam may be pressed in to stop the bleeding. If the soft tissue and the plate are separated they should be submitted in different fixation jars.

Punching through the proximal nail fold, underlying nail and matrix down to the bone is very rarely indicated and retrieving the tissue cylinder without the specimen falling into several pieces is difficult.

*Fusiform biopsies* may be taken from any nail region. The orientation of their long axis is important as it should always be in transverse direction in the matrix and longitudinally in the nailbed. A narrow spindle is sufficient as it will be sectioned longitudinally in the lab, which has to be indicated for the technician by the surgeon. A fusiform matrix biopsy's distal incision line should be parallel to the border of the lunula. It is sutured with 6-o absorble stitches, e.g. Vicryl rapid<sup>®</sup>.

Dermaticum Freiburg, Germany; Dept Dermatol, Inselspital, Univ Bern, Switzerland; Centro Dermatol Epidermis, Inst CUF, Porto, Portugal; Kliniek voor Huidziekten, Univ Hosp, Gent, Belgium The biopsy specimen is oriented on filter paper before immersion into the fixative.

The lateral longitudinal nail biopsy is the most versatile technique for all nail disorders. It comprises the proximal nail fold, matrix, nail bed, nail plate and hyponychium. It often allows to histologically follow the course of a nail disorder for a period of several months. After softening the nail with a warm bath, the first incision starts at the distal crease of the distal interphalangeal joint and is carried distally and on the bone with a straight line through the proximal nail, nail plate, matrix, nailbed to the digit tip. The second incision starts 3mm lateral from the first and runs parallel to it through the lateral sulcus till the hyponychium. The two incisions are joined proximally in a slightly slanted manner to be sure that no proximal-lateral matrix remnant will be left. The whole narrow long tissue block is dissected from the bone using pointed sharp scissors. The narrow defect is sutured with 5-0 stitches. The tissue specimen is fixed as usual.

The superficial tangential biopsy is ideal for the diagnosis of superficial matrix and nailbed lesions, particularly for the diagnosis of longitudinal melanonychias. [5] The proximal nail fold is incised on both sides and separated from the underlying nail to allow it to be reclined. The nail is cut transversely beyond the brown band at about 5 mm distal from the suspected distal margin of the lesion, detached from the matrix and elevated in a trap-door manner to expose the melanocyte spot in the matrix. A shallow incision is carried around the lesion about 1 mm deep with an adequate safety margin, usually 3-5 mm. A #15 scalpel is laid on the neighbouring matrix and gently pressed down. With sawing motions, the lesion is tangentially removed. The specimen is about 0.8 mm thick so that the scalpel blade is seen shining through the tissue specimen. This is transferred to filter paper and stretched out to give a perfectly flat specimen. Fixation is as usual.[6]

## **PROCESSING OF TISSUE SPECIMENS**

The aim of any nail biopsy is its histopathological examination. Nails are feared for their resilience to sectioning and staining. There are many prescriptions how to treat nail biopsies to facilitate sectioning in the lab and indeed, many labs have their own recipes. Pure nail clippings may be softened overnight in 10% aqueous urea solution before formalin fixation and routine embedding. We have found the immersion of compound nail specimens in cedar wood oil for 3 (to 5) days after fixation to sufficiently soften the nail plate to allow good sections of the plate and soft tissue. Embedding, sectioning and staining are then as usual. This technique also permits immunohistochemical reactions to be performed. Hematoxylin & eosin as well as PAS are routinely performed.

## **READING NAIL SLIDES**

Histological sections of nail biopsies are often difficult to read. They require experience in dermato- and onychopathology. However, reporting follows general rules in dermatopathology although some criteria are different: Where parakeratosis develops in epidermis this is usually orthokeratosis in the nail apparatus, spongiosis may be very obvious in the nail where it is usually lacking in the skin, e.g. ungual lichen planus or psoriasis, inspissation of serum is frequently a sign of repeated minor trauma and must not be mistaken for an eczema or fungal elements. [7]

# CONCLUSION

Histopathology is the gold standard in the diagnosis of nail diseases. It requires an optimal nail biopsy in terms of technique and localization, which in turn, is the prerequisite for a reliable diagnosis.

#### REFERENCES

- 1. Haneke E. Anatomy of the nail unit and the nail biopsy. Semin Cutan Med Surg 2015;34:95-100.
- 2. Richert B, Di Chiacchio N, Haneke E. Nail Surgery. New York-London: Informa healthcare, 2011.
- 3. Haneke E. Anatomy, biology, physiology and basic pathology of the nail organ. Hautarzt 2014;65:282-90.
- 4. Haneke E. Nail surgery. Clin Dermatol 2013;31:516-25.

The complete bibliography is, from three weeks after publication in this magazine, available at www.nvdv.nl.

# **Abstract**

Histopathology is the gold standard for almost all nail diagnoses. It may require additional examinations like direct microscopy and culture in case of suspected onychomycoses, X-ray for traumatic nail changes, ultrasound and magnetic resonance imaging for special lesions like particular tumors. For the histopathological examination, the biopsy has to be taken from the correct site, contain the necessary portions of the nail apparatus to allow a diagnosis to be made, to be large enough to be processable in the lab, and be stained with relevant routine and immunohistochemical reactions. The surgeon has to be familiar with the particular anatomy of the nail unit and the way how and where nail changes develop. Nail plate biopsies, which are simple nail clippings, give a high yield of positive results in onychomycoses and often also permit the differential diagnosis of nail psoriasis as well as to rule out some other nail conditions. Nail bed lesions may be diagnosed with a punch or fusiform biopsy, the latter being performed in a longitudinal arrangement. Matrix lesions may be punched with a maximum diameter of 3 mm, biopsied with a narrow transverse fusiform or a superficial tangential biopsy. The optimal biopsy is the lateral longitudinal nail biopsy as it takes a narrow piece of all nail unit components without leaving obvious sequelae.

#### **Keywords**

nail biopsies – onychopathology – nail surgery – nail diagnoses

# CORRESPONDENCE

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