

## Nail Biopsy: A User's Manual

### Abstract

Nail biopsy is a procedure not routinely resorted to; but when indicated, it is often the only clue left for diagnosis. At such times, it pays to be conversant with it. It is an investigation that not only provides etiologic, diagnostic, and prognostic information but also aids in understanding the pathogenesis of nail diseases. It can be of therapeutic value, especially with respect to nail tumors. This article compiles the procedural techniques for nail biopsy of various types and attempts to summarize the evidence available in the literature. The objective of nail biopsy is to clinch a precise diagnosis of nail pathology with a simple and safe surgical procedure, avoiding pain or permanent nail damage. Patient selection is of utmost importance, wherein, the patient does not have typical skin lesions, yields inadequate information on routine nail investigations, and has no peripheral vascular compromise. The patient needs to be explained about the risks associated, the expected functional handicap, the time required for regrowth, a possibility of permanent nail dystrophy, and a possibility of not achieving a diagnosis even after the biopsy. Techniques and types of various nail biopsies are being discussed in this article. The specimen could be collected as an excision biopsy, punch biopsy, shave biopsy, or longitudinal biopsy. The trick lies in choosing the appropriate area for biopsy. Various biopsy types discussed in this article include nail plate biopsy (easiest and least scarring); nail bed biopsy (elliptical excision or punch); nail matrix biopsy (elliptical excision, punch excision ( $\leq 3$  mm) or tangential/shave excision); and nail fold biopsy. Complications reported along with means to minimize them are also discussed.

**Keywords:** *Histopathology, nail bed biopsy, nail matrix biopsy, nail plate biopsy, processing and softening*

### Introduction

Most dermatologists believe nail biopsy to be a complex procedure, with a significant risk of scarring; however, there are many scenarios in which it could be the only diagnostic clue available.<sup>[1,2]</sup> These include patients with isolated nail dystrophy and no cutaneous clues towards diagnosis.<sup>[1]</sup> Nail as a unit has very limited morphologic patterns of reaction; hence, nail features of various dermatoses tend to overlap. For example, distal onycholysis with subungual hyperkeratosis is a common feature for both onychomycosis and nail psoriasis. Thus, histopathological diagnosis remains the gold standard. Though our knowledge in the field of dermoscopy of nail (onychoscopia) is fast advancing, it still remains in its infancy due to a lack of controlled studies establishing the sensitivity or specificity of onychoscopic diagnostic criteria.<sup>[3,4]</sup> This further underscores the importance of nail unit histopathology, which is the gold standard against which onychoscopia is evaluated.

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Contrary to popular belief, a nail biopsy seldom leads to scarring.<sup>[1,2,5]</sup> It is most useful in isolated nail manifestations as it gives a definitive approach to management rather than a conjectural one.<sup>[5]</sup> Among individual conditions, biopsy is especially useful in the diagnosis of onychomycosis,<sup>[6]</sup> longitudinal melanonychia,<sup>[7]</sup> isolated nail psoriasis or lichen planus, and suspected malignant melanoma. It has therapeutic benefit in nail bed tumors, especially the glomus tumor.<sup>[8]</sup>

Nail biopsy is done with an objective to arrive at a precise diagnosis of nail unit pathology with a simple and safe surgical procedure, simultaneously avoiding pain or permanent dystrophy. Towards this end, a proper attention to the biopsy technique and the site chosen for biopsy is imperative. If biopsy is properly done, scarring is entirely avoidable. This article aims to summarize the essential nuances of nail biopsy procedure, serving as a user manual for those needing to do it. It answers the when, where, and how of nail biopsy.

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For the purpose of this review an extensive search of English language scholarly articles was carried out on PubMed using the key words “nail biopsy,” “nail histopathology,” and “nail histology.” Full texts of articles identified were downloaded and evaluated. A methodical analysis of nail biopsy related information was done.

### When to do a Nail Biopsy?

Of prime importance is selecting the appropriate patient. An ideal patient is the one with no typical skin lesions from which a diagnosis could be more easily achieved.<sup>[5]</sup> Quite often, routine nail investigations such as direct microscopy, fungal culture, and imaging techniques may fail to confirm the suspected diagnosis.<sup>[6]</sup> At the same time, the patient should not have any contraindications to nail surgery. One should take special care for diabetes with neurological changes, peripheral vascular disease, arterial insufficiency, etc. which can compromise digital vascularity and hence healing post-surgery.<sup>[2]</sup> Like any other dermatosurgical procedure, an informed written consent needs to be taken. The patient needs to be explained about the procedure, the need for dressing post-biopsy, and the expected functional handicap (be it the fingernail or the toenail). The patient also needs to be aware that even a biopsy may not be able to confirm the diagnosis in all the cases.<sup>[5]</sup> A pre and post-biopsy photographic record is maintained.

### How to do a Nail Biopsy?

For preoperative preparation and techniques of nail unit anesthesia, readers can refer to standard dermatosurgery texts.<sup>[9,10]</sup> A brief mention is being made here regarding the various techniques of anesthesia applicable to the nail unit.<sup>[10]</sup> Effective local anesthesia is essential as the nail unit is a very sensitive structure with a large number of free nerve endings. Most of the apprehension about nail surgery is attributable to the pain associated with this procedure, and effective anesthesia can go a long way in allaying these fears. Prior to administering anesthesia, one should check for a history of allergy to lidocaine, bupivacaine, or parabens; the same can be confirmed with an intradermal injection first. One should always assess any history of cardiac disease including heart block.

#### Procedure of local anesthesia

Like any other injection, the administration of local anesthesia can rarely precipitate a vasovagal attack; hence, it is best to have the patient in a reclining position during the administration of anesthesia. Various techniques of nail unit anesthesia are described below.

#### Proximal digital block

This is the workhorse technique most useful for majority of nail unit surgeries. With the patient's hand in a pronated position, the anesthetic agent is administered at

the base of the digit on both sides (lateral aspect of the proximal phalanx) [Figure 1a and b]. The needle is inserted at an angle of 45-degree to the finger and the plunger is withdrawn before injecting to ensure that a major lateral digital vessel has not been entered. Approximately 1.5 to 2 mL of the anesthetic is deposited to numb the lateral digital nerves. The same procedure is repeated on the opposite side of the digit to complete the block. A complete block usually takes 10–15 minutes as this is a field block.

#### Distal digital block (wing block)

This technique produces a more localized (lateralized) area of anesthesia; however, it is useful as only a small amount of anesthetic is to be injected and the effect is immediate. The injection site is at a point approximately 1 cm proximal and lateral to the junction of the proximal nail fold and the lateral nail fold [Figure 2]. The needle is inserted at a 45-degree angle, directed distally, and down up to the bone. This is followed by a slow injection of approximately 0.5 mL of anesthetic, which blanches both nail folds and anesthetizes the lateral half of the nail unit. For complete anesthesia, the procedure needs to be repeated on the opposite side as well, with additional infiltration of the proximal nail fold required at times.

The preferred technique of anesthesia for nail biopsy is the proximal digital block as it anesthetizes the entire nail unit compared to the distal block (wing block), which anesthetizes limited parts of the nail unit.<sup>[9,10]</sup> Other blocks which have been found useful in the nail unit include matricial block (for localized matrix surgery), transthecal digital block (requires only a single injection on the palmar aspect; however, can be used for the middle three fingers only), and hyponychial block (for more distal surgery).

The next essential step after achieving adequate nail unit anesthesia is exsanguination of the digit and application



Figure 1: (a and b) Proximal digital block being administered in the digit to be operated. Note the anesthetic agent being administered at the base of the digit on both sides



**Figure 2: Administration of local anesthesia for distal digital block. The site of injection is 1 cm proximal and lateral to the junction of the proximal and lateral nail folds. A very limited amount of anesthetic agent can be infiltrated at this site**

of tourniquet.<sup>[9-12]</sup> This helps in achieving a bloodless operative field, which is essential for an adequate biopsy and can be easily achieved with a gauze strip tourniquet [Figure 3a-e],<sup>[13]</sup> in addition to conventional methods such as the use of Penrose drain, Foley's catheter, or surgical glove (with the tip of the finger to be operated being cut off and rolled back over the base of the digit).

To minimize potential postoperative scarring, a practical knowledge of nail unit anatomy is necessary.<sup>[12]</sup> Useful tips include respecting the boundaries of the ventral and proximal nail matrices, distal nail matrix, and extensor tendon insertion [Figure 4].

Nail surgery requires the use of certain specialized instruments, which makes the task precise and easy [Figure 5].<sup>[9-12]</sup> These include the nail spatula (used to separate the nail plate from the nail bed) and the English nail splitter (a heavy duty instrument to cut even thick nail plates and at the same time preventing damage to the underlying nail bed) [Figure 5a]. Other than these two basic instruments, one requires sharp biopsy punches (3 mm for matrix and up to 4 mm for nail bed) and fine curved Castroviejo's scissors and Jeweller forceps (for retrieving the biopsy specimens) [Figure 5b]. Nail biopsies (except tangential excisions) need to be taken down to the level of periosteum as there is no subcutaneous tissue in the nail unit.

### Where to Take a Nail Biopsy From?

This is the most vital question to be answered before taking the biopsy. The nail unit is composed of 6 components which form, ensheath, and protect the structure. These include the germinative portion (nail matrix); product



**Figure 3: (a-e) Exsanguination and tourniquet using a sterile gauze strip. A long sterile piece of gauze is wound across the digit from the distal to the proximal end. This enables exsanguination up to the base. Thereafter, the strip is opened from the distal end to expose the area to be operated upon. The last 2-3 folds of the gauze are not released to form a tourniquet at the base, which is tied tightly**

portion (nail plate); ensheathing portion (cuticle); supporting system (nail bed mesenchyme and phalanx); anchoring portion (ligaments); and framing portion (nail folds). Because of this complex organizational structure, the appendage behaves differently than the rest of the skin. It needs to be understood that the changes seen in the nail plate (on its surface or within it) are in fact a result of the pathology localized to the matrix. Thus, for nail pitting or onychorrhexis, the biopsy needs to be taken from the matrix rather than the nail plate itself. Similarly, for a longitudinal pigmented band, the diagnostic biopsy is taken from the point of origin of the band within the matrix (visible only after retracting the proximal nail fold).

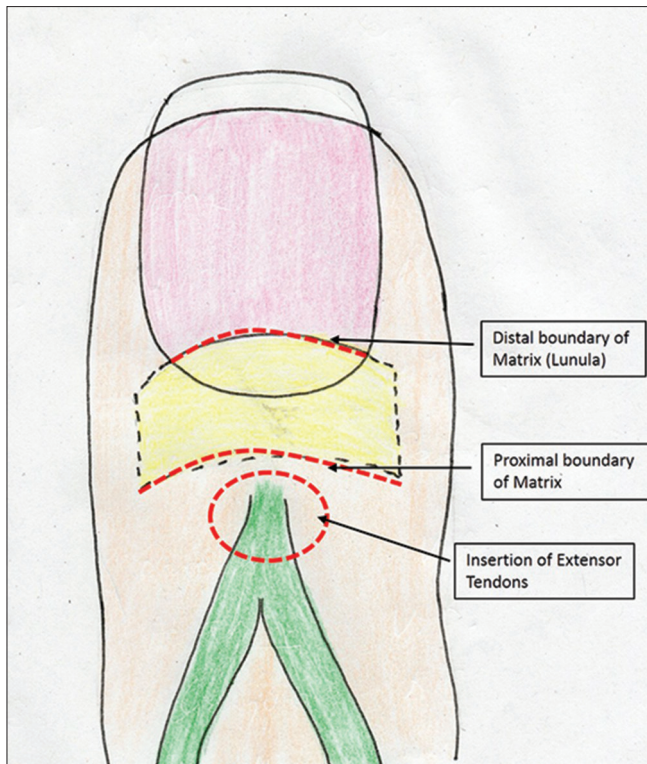
As is well known, the changes in the nail plate occur as a result of the pathology of the nail matrix; hence, the histopathological features of disorders such as melanonychia, erythronychia, and pitting are best represented in a nail matrix biopsy. However, for features such as onycholysis, subungual hyperkeratosis, and salmon patch one would need to take a nail bed biopsy.<sup>[5]</sup> Dermatoscopic examination of nail may help to locate the lesion or pathology to precisely mark the site of biopsy. The ideal sites for taking nail biopsy, as determined by the visualized nail feature, are summarized in Table 1.

### Techniques of Nail Biopsy

Just like a skin biopsy, the nail biopsy could be taken with a scalpel (excision) or with a punch. In addition, three special types of biopsy are described in the nail unit [Figure 6].

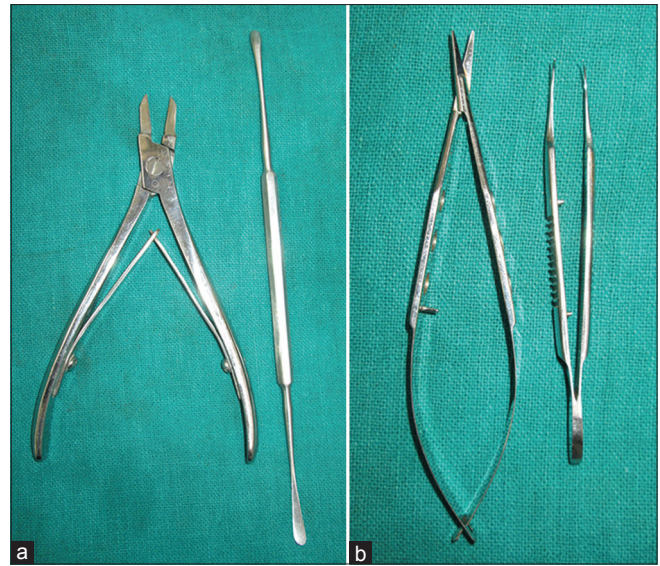
Excision biopsy is generally applicable to tumorous growths arising from the nail bed or matrix, most





**Figure 4:** Dorsal view of the nail showing showing the “no-go areas ” (marked in red-dotted line) while performing a nail biopsy

commonly the glomus tumor. This requires partial or complete nail plate removal for exposing the nail bed or matrix, localization of the growth, and its subsequent excision from the surrounding tissue. Punch biopsy is generally a diagnostic biopsy to retrieve a cylinder of tissue from the suspected area of pathology.<sup>[14,15]</sup> In contrast to these, a nail plate biopsy involves taking a specimen (>3–4 mm) of the nail plate only. This is attempted for onycholysed nail plate which can be clipped easily with a nail splitter.<sup>[6]</sup> Expectedly, this biopsy gives only limited information; however, this is sufficient when the sole purpose is to rule out onychomycosis in direct microscopy negative patients. A shave biopsy, better known as tangential excision biopsy, is recommended for the nail matrix origin of longitudinal melanonychia.<sup>[7,16]</sup> This helps minimize scarring, as explained subsequently. A longitudinal nail biopsy is a specialized type of biopsy of the nail unit which is more of academic interest and not performed routinely now. It is a single biopsy which samples all the parts of the nail unit including the matrix, bed, fold, and plate.<sup>[17]</sup> This gives a wealth of histopathologic information; however, it has certain constraints. Only the changes confined to the lateral part of the nail unit can be sampled adequately, and the potential for scarring and acquired malalignment is present.<sup>[17]</sup> Nevertheless, it is a useful technique for larger lesions placed asymmetrically in the nail unit as a representative area of each part of the nail unit is sampled. The biopsy being potentially scarring its utility in day to day practice is low.



**Figure 5:** (a and b) Specialized instruments used for nail surgery. (a) The nail spatula and nail splitter. (b) Castroviejo's scissors and jeweler's nontoothed forceps, both with a curved tip

## Types of Nail Biopsy

### Nail plate biopsy

As explained above, this is the easiest and the least scarring type of nail biopsy.<sup>[18]</sup> It is generally reserved for cases with an onycholysed nail plate; though an attached nail plate can also be separated after administering anesthesia. The nail plate is clipped and sent for histopathological examination, including PAS staining.<sup>[6,19]</sup>

### Indications

These include cases with distal onycholysis varying from suspected onychomycosis<sup>[6]</sup> (distal and lateral subungual onychomycosis); subungual warts (causing distal onycholysis); or nail psoriasis. It can also help distinguish melanin pigmentation from hemosiderin.<sup>[20]</sup> Its utility in systemic disease has also been reported as distal nail clippings can give a wealth of histopathological information in conditions such as gout or show up other crystals.<sup>[18,21]</sup>

### Procedure

Digital anesthesia may not be necessary, especially in cases with distal onycholysis, even though the procedure may be somewhat uncomfortable for the patient. However, if proximal sampling is required (e.g., proximal subungual onychomycosis) or nail plate partially needs to be separated from nail bed, anesthesia is needed.

The procedure is as simple as clipping of onycholysed nail plate (should be more than 3–4 mm in size) with a nail splitter [Figure 7a-d]. The nail is cut taking along as much of subungual hyperkeratosis as possible [Figure 7c]. For sampling of proximal subungual onychomycosis, a 3–4 mm punch needs to be driven into the proximal part of the nail plate after giving anesthesia.

**Table 1: Site for nail biopsy guided by nail manifestation**

Presenting feature	Common probable etiologies	Site of pathologic involvement	Where to biopsy from?
Nail pitting	Psoriasis, psoriatic arthritis Lichen planus Alopecia areata Atopic and contact dermatitis <i>Others causes:</i> Sarcoidosis, pemphigus vulgaris, reactive arthritis, incontinentia pigmenti, etc.	Pits are a result of nail matrix pathology. <i>Very superficial</i> pits suggest pathology in the dorsal nail matrix (ventral aspect of proximal nail fold) <i>Deeper pits</i> suggest more extensive involvement of the ventral (germinative nail matrix)	Nail matrix biopsy (NMB) targeting the proximal matrix proximal to the nail plate area showing pits
Onychorrhexis	Lichen planus Ageing nails Nail psoriasis <i>Other causes:</i> hypothyroidism, anemia, nail paint removers, etc.	This also signifies nail matrix pathology. The severity commonly correlates with the degree of effect on matrix	NMB from proximal matrix proximal to the ridges
True Leukonychia	Psoriasis Lichen planus Onychomycosis Idiopathic (total or subtotal)	The histopathologic correlate of leukonychia is persistence of parakeratotic cells and retained nuclei within the otherwise transparent nail plate This persistence of parakeratosis is again a sign of proximal matrix pathology	NMB to be taken from the matrix source in the intermediate or distal matrix NMB (from leukonychia nail plate) will show only parakeratosis Unlikely to reveal pathology. Nail plate biopsy can help rule out fungal etiology only (endonyx), if that is suspected
Erythematous lunula	Alopecia areata Nail psoriasis Lichen planus Drug induced Idiopathic <i>Other causes:</i> rheumatoid arthritis, systemic lupus erythematosus, hepatic cirrhosis, carbon monoxide poisoning	Distal matrix	NMB (distal matrix without retracting the PNF if the size of the lunula permits) can reveal the etiology However, it should be kept small ( $\leq 3$ mm) and should not cross the lunula margin to avoid scarring and distortion of shape of lunula
Longitudinal Melanonychia	Melanocyte hyperplasia (may involve multiple nails) Lentigine Melanocytic nevus Malignant melanoma	At the origin of longitudinal melanonychia, which may lie in the proximal matrix, less commonly in the distal matrix	NMB after retracting the proximal nail fold and avulsing the proximal part of the nail plate. To fully expose the point of origin of the band. Thereafter, a tangential (shave excision) can be done to retrieve a specimen at least 1 mm in thickness
Oil drop sign or Salmon Patch	Nail psoriasis	Nail bed	Nail bed biopsy (NBB) without avulsing the nail plate
Distal onycholysis with Subungual hyperkeratosis	Psoriasis Nail lichen planus Onychomycosis Subungual wart <i>Other causes:</i> Subungual tumors such as exostosis, acquired digital fibrokeratoma, subungual acral fibromyxoma, etc.	Nail bed and hyponychium	NBB without nail plate avulsion for suspected inflammatory dermatoses Nail plate biopsy (NPB) if the purpose is only to rule out onychomycosis or wart. Even with this biopsy, try to include as much subungual debris as possible. NBB after avulsing nail plate for suspected subungual tumors

Contd...



Table 1: Contd...

Presenting feature	Common probable etiologies	Site of pathologic involvement	Where to biopsy from?
Splinter hemorrhages	Nail psoriasis Trichinosis Traumatic hemorrhages Subacute bacterial Infective endocarditis, <i>Other causes:</i> Systemic lupus erythematosus, scleroderma, rheumatoid arthritis, vasculitis, Raynaud's disease	Nail bed	NBB without nail plate avulsion
Subungual Hematoma	Nail bed injuries caused by blunt/sharp trauma Idiopathic Can mask underlying melanoma	Nail bed	NPB can be taken to rule out the presence of blood. Nail plate overlying the discolored area is taken.
Total dystrophic nail (crumbling and destruction)	Nail psoriasis Total dystrophic onychomycosis Nail lichen planus	Nail matrix and nail bed	NBB (Punch biopsy)
Painful lesion of the nail bed or nail matrix	Glomus tumor Other tumors of the nail unit (less commonly) Nail bed pustules/abscess	Nail bed or nail matrix mesenchyme (dermis). Diagnostic as well as therapeutic biopsy	Partial or complete nail avulsion (depending on the extent and localization of the lesion) followed by fusiform excision (longitudinal in nail bed and horizontal for nail matrix)

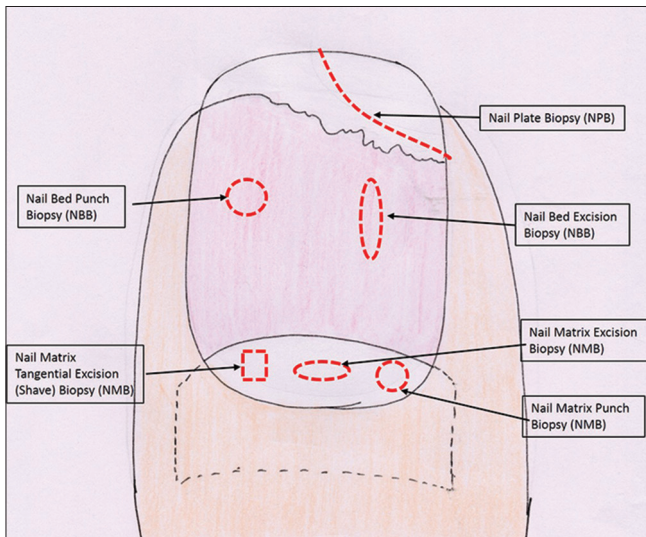


Figure 6: Commonly done nail biopsies. Dotted lines show marks of excision

**Limitations**

It samples a limited part of the nail unit only, hence, gives only limited histopathological data; e.g., it can rule out the presence of fungal elements within the nail plate structure but tells us precious little regarding other possible diagnoses.

**Complications**

Hardly any complications are expected with this minor procedure. Potential risk of scarring lies when the nail plate is being biopsied proximally, where the underlying matrix may be damaged, but even that is minimal.



Figure 7: (a-d) Nail plate biopsy for a case with distal onycholysis due to suspected wart. No anesthesia has been administered. The distal onycholysed part of the nail plate is being cut with a nail splitter taking care to include as much subungual debris as possible

**Nail bed biopsy**

This is probably the most common type of biopsy performed on the nail unit. If the site is chosen carefully, it can yield a wealth of confirmatory histopathological information.

### Indications

Nail bed biopsy can be used to distinguish between conditions with similar clinical patterns, e.g., psoriasis and onychomycosis.<sup>[22]</sup> It is also recommended for cases with discoloration of nail bed, e.g., salmon patch, proximal subungual onychomycosis, etc. Any painful nail bed lesion or any tumorous growth of nail bed will also need a nail bed biopsy to confirm the diagnosis.

### Types

The nail bed biopsy can be either an excision biopsy or a punch biopsy. An excision biopsy will necessitate prior avulsion of nail plate (partial or total) with the subsequent excision being longitudinally oriented to minimize scarring.<sup>[5]</sup> Size should be kept small as defects of nail bed larger than 3–4 mm often result in secondary damage and permanent onycholysis.<sup>[10]</sup> A punch biopsy offers the advantage of not necessitating a prior avulsion of plate. This is important as the epithelium of the nail bed is tightly adherent to the ventral aspect of the nail plate and gets easily removed while avulsing the plate,<sup>[10]</sup> compromising the histopathologic interpretation of biopsy specimen.

### Procedure

1. Without nail plate avulsion [Figure 8a-d]: This technique is important for evaluating nail plate epithelium, the dermoepidermal junction, or interface changes histopathologically; hence, almost all diagnostic biopsies for suspected inflammatory disorders affecting the nail should be done with this technique. However, it can be difficult to drive the punch through the nail plate
  - To facilitate nail plate penetration with a punch, the digit can be soaked in warm water for few



**Figure 8:** (a-d) Nail bed biopsy without nail plate avulsion using a 3–4 mm punch. The punch is driven straight down to the periosteum. The resulting tissue cylinder is lifted with a curved Castroviejo's scissor to ensure intact delivery. The resultant defect can be seen in (d)

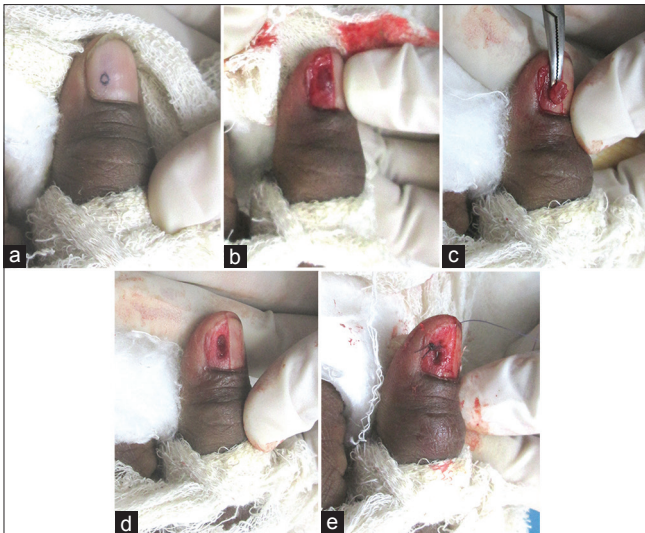
minutes or the nail plate can be thinned out by grinding

- After preparation for nail surgery and localization of the site for biopsy [Table 1], a 3–4 mm punch is pushed through the plate down to the bone [Figure 8a]
  - While withdrawing the punch, one should be careful to minimize rotation to prevent detachment of the nail plate from the specimen
  - Harvesting of the specimen is done by fine curved Castroviejo's scissors and Jeweller's forceps, if needed (avoid toothed forceps) [Figure 8b and c]
  - In the event of separation of the plate while withdrawing the punch (this may happen if the nail plate is too thick), the specimen needs to be delivered from the metallic cylinder of the punch with the help of a needle. The remaining nail bed tissue (left *in situ* in the nail) can be retrieved separately from the punched-out cylinder and sent for histopathological processing. In general, for disorders associated with nail thinning (e.g., nail lichen planus), such a separation of nail plate is unlikely to happen (author's personal experience)
2. With nail plate avulsion [Figure 9a-d; Figure 10a-e]: This is attempted for disorders confined to the nail bed dermis, where the nail plate and nail bed epithelium are not essential for confirming diagnosis. For this biopsy, several techniques can be used
    - The extent and type of nail plate avulsion is determined based on the area to be biopsied. Total nail avulsion [Figure 9a and b] is preferably avoided unless it is absolutely necessary; the advantages of partial nail avulsion [Figure 10a and b] being lesser invasiveness, better postoperative recovery, lesser risk of long-term scarring, and damage to nail bed. After avulsion, the punch biopsy can be taken simply like a skin biopsy from the chosen site [Figure 9d]. The punch (3 mm or maximum 3–4 mm) needs to be driven down to the level of the bone. A fine tipped curved Castroviejo's scissors is inserted at the side of the cylinder to deliver the specimen. The use of forceps should be minimized. Hemostasis is secured and dressing done
    - Double-punch technique can be used for fairly localized lesions.<sup>[15]</sup> For this, a larger 6-mm punch



**Figure 9:** (a-d) Nail bed biopsy with total nail plate avulsion (punch biopsy). This is to be resorted to only when the prior location of the lesion cannot be approximated (in this case a glomus tumor)





**Figure 10: (a-e) Nail bed biopsy with partial nail plate avulsion (excision biopsy). Note that the fusiform excision is oriented in a longitudinal manner in the nail bed**

is used to make a hole in the nail plate only and the disk is removed securely. Then, a smaller 3-mm punch is used to retrieve a specimen from the nail bed, which is separated from the periosteum with a curved fine Castroviejo's scissors. The plate defect is kept larger to enable the manoeuvrability of the fine scissors. Following this, the punched-out nail plate disk can be replaced to cover the nail bed defect

- Elliptical biopsy of the nail bed is performed after nail avulsion (preferably partial). The ellipse should be oriented longitudinally (parallel to the nail bed ridges) and kept narrow (approximately 2 mm) [Figure 10c]. The specimen should be detached from the bone with a fine, curved Castroviejo's scissors, avoiding the use of forceps [Figure 10d]. This is followed by primary closure with 4-0 or 5-0 absorbable sutures [Figure 10e]. Re-approximation is generally enough as opposed to a full closure. At times, this may need undermining of the lateral edges with a sharp blade.

After taking a biopsy, the avulsed nail plate is preferably placed back after trimming the edges, as far as possible. This improves healing and minimizes postoperative discomfort to the patient.

### Limitations

Nail bed biopsy can be a technically demanding procedure, and inexpertly performed biopsies can risk obtaining an inadequate specimen or damaging the fragile sample obtained, thus compromising histopathological information. There may be the risk of causing nail dystrophy due to injury to the distal matrix, if one is not careful.<sup>[23,24]</sup> Separation of the nail plate from the nail bed while

retrieving the specimen is a constant threat in this type of biopsy. If this happens, the histopathological interpretation becomes somewhat more difficult.

### Postoperative outcomes

Postoperative pain is proportional to the extent of nail plate removal. It is generally very little for a punch biopsy. Healing is fast without scarring or nail dystrophy. Nail bed defects larger than 3-4 mm have a potential to lead to permanent onycholysis, although the nail bed regenerating capabilities are high.

### Nail matrix biopsy

This type of biopsy is reserved only for lesions arising from the nail matrix.<sup>[14,15]</sup> It is technically the most difficult type of nail biopsy, especially because of the heightened risk of scarring. Being the germinative part of the nail unit, any inadvertent and excessive damage to the matrix is likely to result in permanent scarring. However, at times, biopsying the matrix is an absolute must and careful planning can help avoid any undesirable complications. As far as possible, matrix biopsies should be confined to the distal matrix rather than the proximal matrix, as this helps reduce the risk of scarring.

### Indications

This is required for histopathological diagnosis of lesions arising from the nail matrix, commonly the longitudinal pigmented bands or inflammatory nail dystrophies such as nail lichen planus or nail psoriasis [Table 1]. In addition, there are numerous tumors of nail matrix origin, such as the onychomatricoma, onychopapilloma, or glomus tumor, where this biopsy can be diagnostic as well as therapeutic.

### Types

This can be taken as a punch biopsy (size to be kept small, up to 3mm); as an excision biopsy (the excision to be oriented horizontally compared to the longitudinal orientation in the nail bed); or a tangential (shave) biopsy.<sup>[16]</sup>

### Procedure

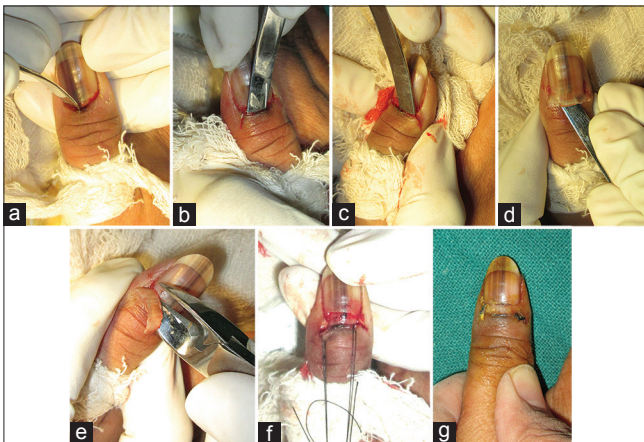
Various techniques are described for nail matrix biopsy [Figure 11a-g]

- After administering digital block, exsanguination, and tourniquet, the proximal nail fold is cut through on both the sides at the junction with the lateral nail folds [Figure 11b and c]. This is a full thickness division after separating the proximal nail fold from the dorsal surface of the nail plate. The cut is carried backwards and outwards up to at least half-way through to the distal interphalangeal joint. This separation helps retract the proximal nail fold and expose the underlying matrix
- The proximal half or one-third of the nail plate can be avulsed through proximal approach; only if required [Figure 11d and e]. Similar to the nail bed biopsy, we need to determine whether the specimen needs



to have the nail plate attached for histopathological examination or whether the nail plate should be avulsed. NMB being done for inflammatory dermatoses necessitates that the nail plate should remain attached; whereas for longitudinal pigmented band or matricial tumors, the overlying nail plate is better avulsed to expose the point of origin of the band

- The retracted proximal nail fold is held back with the help of skin hooks or stay sutures [Figure 11f]. The use of artery forceps is not particularly encouraged as it can cause significant crush injury to the retracted fold and lead to subsequent necrosis
- A punch biopsy is taken with a punch 3 mm in size, driven down to the bone, and the specimen cylinder is delivered with curved, fine scissors [Figure 12]. The chances of separation of the thin nail plate in this area are minimal to absent. At times, it may not be necessary to cut and retract the proximal nail fold. It may serve to just temporarily hold back with a skin hook or nail spatula [Figure 13]
- For longitudinal pigmented bands, the proximal nail plate is separated, and the origin of the band is visualized [Figure 11]. The origin is then scored with a sharp blade and a tangential excision (shave excision) is done.<sup>[7,25]</sup> The retrieved specimen should be at least 1 mm thick to permit evaluation of any potential malignancy
- Whenever an elliptical excision is planned in the matrix area, it should be oriented horizontally as it allows primary closure with the least risk of scarring [Figure 14]
- For even bigger matricial lesions such as the glomus tumors, the matrix epithelium underlying the avulsed



**Figure 11:** (a-g) Nail matrix biopsy for a case with longitudinal pigmented band with spread of pigment in the proximal nail fold (Hutchinson's sign). (a) Separation of the proximal nail fold from the nail plate. (b) Lateral release incisions to enable retraction of the proximal nail fold. (c) Lateral release incision on the other side. (d) Proximal partial avulsion of the nail plate with a nail spatula. (e) Separation of the proximally avulsed nail plate with nail splitter. (f) Exposed origin of the pigmented band which is then tangentially excised. (g) The proximal nail fold is then allowed to fall back and sutured in place. The avulsed nail plate can be replaced temporarily after trimming its margins

nail plate needs to be adequately separated to expose the extent of the tumor and separate it from the surrounding tissue [Figure 15].<sup>[26]</sup> After tumor excision, a primary closure of the matrix defect needs to be attempted after undermining the edges, if required<sup>[26]</sup>

- Post-surgery, the retracted proximal nail fold should be allowed to fall back and sutured in place. The avulsed nail plate is preferably trimmed from the edges and secured over the exposed nail bed or matrix. This makes the postoperative period less symptomatic and prevents possible adhesions between the nail bed and fold.

### Modifications

Several modifications of this procedure have been reported in the literature.

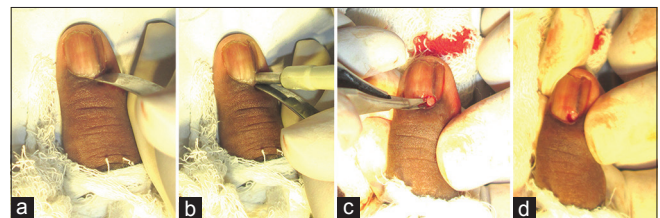
- Trap door/"pop the bonnet" technique:<sup>[27]</sup> The nail plate is not detached from the proximal nail fold. It is just avulsed with a distal approach and lifted like a car bonnet after separating from the nail bed. After an adequate sample has been taken, the plate is placed back on the bed and secured in place. This could be done with a suture also, if required
- "Submarine hatch" technique:<sup>[28]</sup> This technique is better known as a "nail bed biopsy," but even distal nail matrix can be biopsied with this technique, ensuring minimal damage. This is reserved for a lesion located in the proximal portion of the nail unit. The proximal nail fold is separated from the nail plate and lifted with the spatula. The biopsy punch is then driven through the chosen area taking care to avoid the lunula margin. The retracted nail fold is then allowed to fall back on the plate and held in place with the cyanoacrylate glue.

### Limitations

Nail matrix biopsy is a technically demanding procedure which needs to be learnt carefully. The small size of the retrieved specimens may compromise histopathological interpretation also at times. For larger excisions (e.g., nail matrix tumors), postoperative primary closure may not be possible.

### Postoperative outcomes

The immediate postoperative period may not be very symptomatic; however, an eye needs to be kept on the long-term outcomes post matrix surgery. The risk of



**Figure 12:** (a-d) Nail matrix biopsy taken with a punch. The nail matrix is being exposed without dividing the nail fold. The nail fold is just retracted with the help of nail spatula



**Figure 13:** Proximal nail fold can also be retracted with a skin hook to obtain a punch biopsy

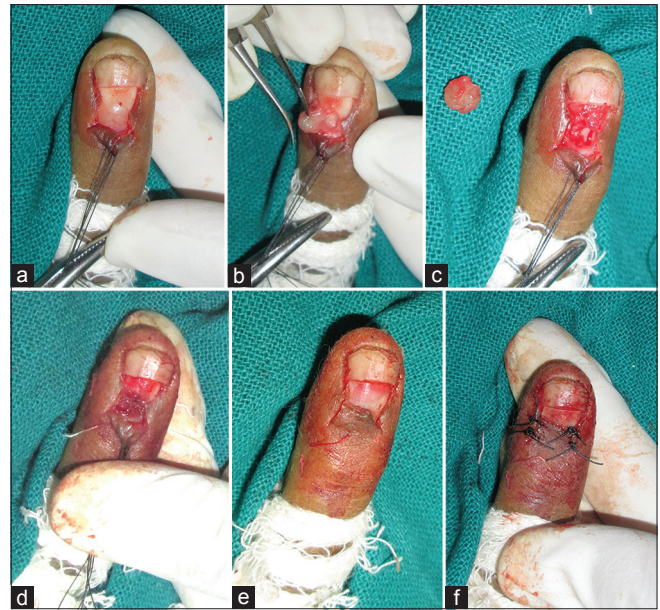
scarring and postoperative split nail deformity is always present, if the matrix area is not handled properly.

### *Nail fold biopsy*

Nail fold biopsy can be done from the proximal nail fold or lateral nail fold, and is indicated for paronychia dermatoses, inflammation, or nail fold tumors (benign or malignant). It can be shave biopsy, elliptical excision, punch, or *en bloc* excision (for proximal nail fold). Prior to any excision over the nail folds, it is wise to insert a nail spatula underneath the concerned fold to prevent any inadvertent damage to the underlying nail bed or matrix.<sup>[1]</sup>

### *Postoperative care*

Postoperative instructions to the patient are similar to those for any other type of nail surgery.<sup>[9,10]</sup> For dressing of the operated digit, a greasy antiseptic should be used as a bottom layer and a bulky, adsorbent dressing as the top layer to ensure maximum patient comfort. Patients should be specifically instructed to avoid keeping the operated digit in a dependent position for the initial 48 hours. This helps minimize the risk of postoperative edema in the digit which can be dangerous as it turns the dressing into a tourniquet. The patient should also be instructed to loosen the dressing and report back in case of severe, throbbing pain in the digit or change in color. The patient is advised to avoid soaking of dressing to prevent infection. The first dressing change is recommended after 48 hours.



**Figure 14:** (a-f) Fusiform excision in the proximal matrix area oriented horizontally

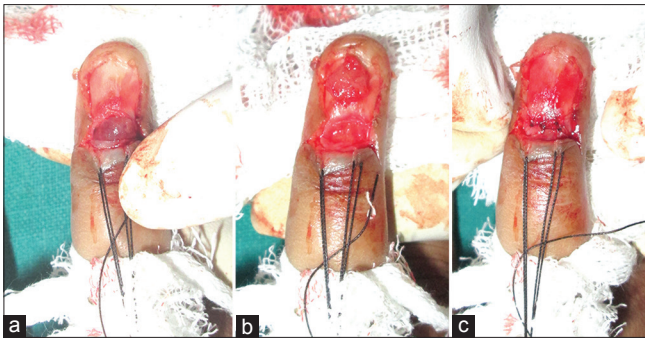
This digit may again have to be soaked in warm, sterile saline to enable painless removal of the adherent dressing. Subsequent dressings can be kept less bulky. The patients generally require analgesic support for the first 3–4 days. Stitches over the proximal nail fold can be removed after 7 days. A routine postoperative use of antibiotics is not recommended. However, there can be a significant risk of secondary infection.<sup>[5]</sup> For a tropical climate like ours, and especially in patients engaging in manual work, it is better to prescribe oral anti-staphylococcal antibiotics (author's personal experience).

### *What to expect?*

The expectation alignment from a nail biopsy procedure is essential for the clinician as well as the patient. One can broadly remain assured that most nail biopsy procedures are not scarring. We only expect a temporary disfigurement of the nail plate which will improve over subsequent months with the onward growth of the nail plate [Figure 16]. Permanent scarring can result with damage to the germinative matrix or with the more radical procedures like the longitudinal nail biopsy, which are not routinely resorted to.

Nail biopsy is important for the wealth of histopathological information it provides, especially needed for dermatoses confined to the nail unit. For onychomycosis, it provides definitive proof of fungal etiology by demonstrating nail plate invasion by fungus;<sup>[6,17]</sup> apart from being the most sensitive diagnostic technique for this condition.<sup>[29]</sup> Similarly, for suspicious longitudinal pigmented or red bands, nail biopsy is the only way to rule out subungual melanoma.<sup>[14,16]</sup> The same stands true for other tumors of matrix origin.





**Figure 15: (a-c) A large matricial excision biopsy. Note that the defect created by the large glomus tumor which has been excised collapses back and can be successfully closed with 6-0 absorbable suture. The suturing is done in a horizontal fashion as opposed to longitudinal closure in nail bed**

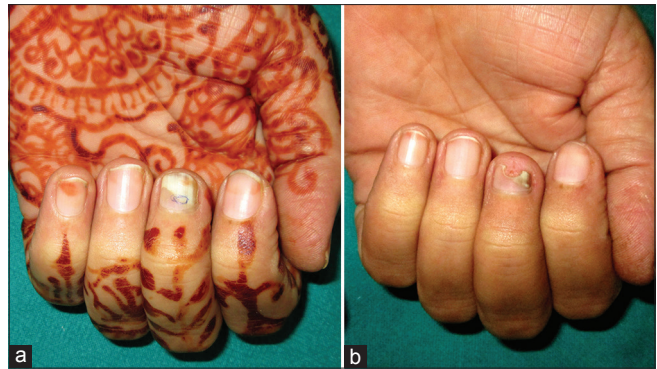
At the same time, one should anticipate certain inherent pitfalls. If the site for biopsy within the nail unit is not carefully chosen, then the diagnostic histopathologic information may not be forthcoming. The procedure may itself become complicated, especially with thickened or dystrophic nails, leading to a compromised histopathologic specimen. Interpretation of nail biopsies requires a trained dermatopathologist aware of the variations in the histology of the nail unit compared to skin. In the absence of well-defined histopathologic diagnostic criteria for various nail diseases, the interpretation may become all the more subjective.<sup>[22]</sup>

The patient also needs to be explained about the functional handicap which nail surgery entails (author's personal experience). Whether it is the toenail or the fingernail, the patient should be ready for a disruption of normal activities (such as typing, texting, driving, etc.) till the biopsy site heals. When a toenail is operated upon, the need for appropriate footwear to accommodate the bulky dressing needs to be reinforced to the patient beforehand. The time-period expected for regrowth of the operated nail should be reasonably specified. The patient also needs to be aligned regarding the possibility of permanent nail dystrophy despite well-conducted surgery as well as the possibility of not achieving a diagnosis even after undergoing a nail biopsy.

#### *Processing and interpreting nail biopsies*

When a biopsy specimen is sent to the histopathology lab, including a nail diagram showing the exact site of biopsy is very useful.<sup>[2,24]</sup> Standardized templates have been devised in the form of nail maps or cassettes.<sup>[30,31]</sup> In addition, inking the nail plate surface to suggest the right orientation of the specimen can greatly facilitate subsequent embedding and cutting of specimens.<sup>[31]</sup> This helps maximize histopathologic information from correctly oriented specimens. It can also help evaluation of margins for neoplastic disorders.<sup>[30]</sup>

The nail biopsy first needs to be fixed in 10% neutral buffered formalin for 24 hours. This ensures fixing of the



**Figure 16: Nail Bed Biopsy done to differentiate between Nail psoriasis vs onychomycosis. The chosen biopsy site is marked with a pen (16A). Regrowth of normal nail after 4 weeks with a healed nail bed (16B). Note that there is no residual scarring.**

histopathological details. Then, the pathologist grossly assesses thickness of the biopsy before subjecting it to further processing.<sup>[32]</sup> Processing of nail biopsy specimens in the laboratory also requires special skill as the specimen has both soft tissue and hard tissue (nail plate) together.<sup>[30]</sup> The nail plate structure does not lend itself to easy cutting due to hard keratins; hence, some degree of softening of the specimen is always required. Thinner nail plate specimens, e.g., those from children may not require any softening.<sup>[30]</sup>

Various softeners have been devised in literature. The most common three softening agents used in laboratories are Mollifex Gurr, potassium hydroxide 10% solution, and potassium thioglycollate 10%.<sup>[30]</sup> It is advised to avoid decalcification solutions as softening agents as they alter the morphology. Softening not only increases the ease of section cutting but also improves the section quality by reducing tissue shattering. Cutuly *et al.* and Tahmisian *et al.* demonstrated that paraffin blocks can be stored in water to decrease brittleness.<sup>[33,34]</sup> Baker introduced the use of a mixture of nine volumes of 60% ethanol and one volume of glycerol (Baker's fluid) and found it to be better than water alone.<sup>[35]</sup> Carlquist recommended the use of ethylenediamine as a softening agent but it could not be extended for human tissues due to its hazardous nature.<sup>[36]</sup> It was subsequently discovered that household chemicals including detergents, fabric conditioners, and hair removal creams could also be used as softening agents.<sup>[37]</sup> Diegenbach *et al.* indicated the use of commercial fabric softeners for easy sectioning.<sup>[38]</sup> Phenol-based softeners gained popularity; however, due to significant safety risks, they should not be considered for widespread use. Orchard *et al.* conducted a study on normal human nail clippings to evaluate a series of keratin softening reagents showing that fabric conditioner, and hair removal creams proved to be effective keratin softeners.<sup>[39]</sup> Other agents proposed in the literature include cedar wood oil, and chitin softening solution (mercuric chloride, chromic acid, acetic acid, and 95% alcohol).<sup>[5]</sup>

After softening and cutting, the sections are mounted on poly-L-lysine coated slides, heat dried, and stained with

hematoxylin and eosin following standard protocols. It is always helpful to include a fungal stain when processing nail specimens (periodic acid Schiff stain or Gomori methenamine silver stain) as onychomycosis is commonly encountered in nail specimens and may not be distinguished easily on routine stains.<sup>[30]</sup>

### Complications

The commonly encountered complications are bleeding and secondary infection. Nail is a very vascular structure and techniques to minimize bleeding can help improve diagnostic outcomes. A prophylactic use of antibiotics with more invasive procedures such as nail bed or matrix biopsy is preferred, especially in tropical climates like ours. Few biopsies may result in scarring of nail bed leading to subsequent onycholysis. Rarer complications such as a reduction in nail width, malalignment of the axis of re-growing nail, or growth of lateral nail spicules can be expected only with more radical procedures like the longitudinal nail biopsy.<sup>[17]</sup>

### Conclusions

Nail biopsy is a not so commonly resorted to procedure. Nevertheless, dermatologists need to be attuned with the biopsy procedure and subsequent optimum processing to derive maximum histopathological information. A properly done nail biopsy can go a long way in minimizing complications and optimizing treatment outcomes in nail disorders.

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### Conflicts of interest

There are no conflicts of interest.

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