

Practical Tips for More Productive Skin Biopsies

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INTRODUCTION

Clinical dermatology in common with family practice is one the last bastions of clinical medicine. Under most circumstances, high standard practice can be accomplished with the assistance of relative simple tools such as a magnifying glass, facilities for Wood's lights examination etc, and 'high tech' - though undoubtedly now a fashion - is unnecessary for the most of the time. However, even among the best trained dermatologists, clinical assessment is not always sufficient, and support for a clinical diagnosis from ancillary studies is necessary for a definitive diagnosis. By virtue of the easy accessibility of this organ, skin biopsy for histological examination of the disease process remains a most important diagnostic procedure that permits the dermatologist to arrive at specific diagnoses. Unfortunately, this collaboration between the dermatologist and the pathologist is not always a fruitful event, to the frustration of both parties, as well as that of the patient. There are, however, few discussions in the literature that address specifically to this failing process.¹

This paper attempts to enlist the reasons that account for the failure of the skin biopsy consultation process and discusses some of the fine points that are of practical interest and importance to the practising dermatologists and pathologists. There is a frank discussion of the practical factors that affect the results, including those that are rarely discussed in print. Many of the statements are a matter of opinion and, for sure, will be the author's bias.

WHY DOES THE DERMATOPATHOLOGY ASSESSMENT PROCESS OF SKIN BIOPSY FAIL ?

From the review of the results of a continuing clinicopathologic audit of biopsies received from one of our senior dermatologists, as well as that of the skin biopsy sent in from our in-house clinicians over a period of 4 years, a listing of the most common factors that account for the failure of a satisfactory dermatopathological evaluation for skin biopsies can be concluded. These includes faulty selection of lesion for biopsy, application of the inappropriate biopsy technique, mishandling of biopsy samples, nonspecific histological features in these lesions biopsied, difficulties arisen from the lives (natural history) of the lesions for that disease process, atypical clinical and histological manifestations of lesions, failure on the part of the clinicians to provide pertinent and essential clinical data, failure on the part of the pathologists in terms of their diagnostic ability, as well as failure to exercise the powerful tool of clinicopathologic correlation properly. Some of these factors are recognised by other workers in the field.²

SELECTING THE RIGHT LESIONS FOR BIOPSY

The site of biopsy is absolutely critical. One should aim at obtaining a lesion that is, or at least thought to be, the most characteristic and representative of all lesions. The dictum is to select the "ESTABLISHED" or "fully developed" lesion, and avoid the tempered old lesions (especially blistering lesions). It should however be noted that lesions that are too young generally show nonspecific histology. Likewise, there is nothing to be learned from biopsy of a lesion that is resolving either. One should also avoid the traumatised and complicated lesions. At times, it may be more worthwhile to spend a few minutes closely examining the patient, particularly those with widespread eruptions, to find the best lesion

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for biopsy. In other instances, the opinion of an experienced dermatopathologist colleague will also be of great value.

In general, it is recommended to biopsy lesions at the borders, particularly those that evolve in a centrifugal manner. For example, biopsy of the edge of a leg ulcer, including the deep part of the lesion, may reveal a carcinoma or a deep fungal infection, whereas biopsy of the ulcer centre show only inflammation. With a bullous or pustular lesion, it is best to find the smallest lesion that can be removed in total, as the presence of the roof is often necessary for the diagnosis.

An exception to the general rule of selecting a fully developed lesion for biopsy is vesiculobullous lesions in which an early intact lesion not older than 24-48 hours is preferred, as re-epithelialisation in subepidermal vesiculations rapidly sets in and will result in erroneous interpretation of an intraepidermal vesicular dermatitis. In cases where immunofluorescent study is of diagnostic importance, selection of the biopsy site is also critical for best results. Perilesional biopsy is recommended for bullous pemphigoid, pemphigus, and perhaps dermatitis herpetiformis, while normal skin may be better for selected cases of systemic lupus erythematosus and dermatitis herpetiformis.

SELECTING THE BIOPSY TECHNIQUE

Productive tissue procurement depends not only on selecting the right lesion for biopsy but also on employment of the appropriate biopsy procedure. Inappropriate technique renders biopsy uninformative. For diagnostic purpose, the dermatologists may choose among shave, punch, incisional as well as excisional biopsy techniques. Each dermatologist obviously has his own preference, but the manner in which the biopsy is performed should depend on the *anticipated* depth of the disease process, understanding that scarring may be a consideration.³ Thus, superficial disease limited to the epidermis, such as viral warts and seborrhoeic keratosis, can be easily biopsied by shave technique, with acceptable cosmetic results. Shave biopsy should, however, not be used for inflammatory dermatoses nor suspected melanocytic lesions because these specimens generally yield little dermal tissue for assessment. Deep processes such as lupus erythematosus or panniculitis is best approached with scalpel incisional biopsy,

including the deep dermis and adequate amount of the subcutaneous fat.

In fact, for inflammatory dermatoses, whenever possible, the biopsy specimen should include the subcutaneous fat because in many such disease processes, characteristic histologic features are found in the lower dermis and subcutis.³ Furthermore, it should be noted that it is generally not possible to obtain adequate amount of subcutaneous tissue by the punch technique, elliptical incisional biopsy that includes subcutaneous fat of at least 6 mm, or a few lobules deep (a lobules measures about 1 mm in diameter), is the recommended method for the study of subcutaneous pathology.⁴ The use of punch procedure for this purpose is to be discouraged.⁴ Likewise, biopsies for alopecia should always include a generous amount of subcutaneous fat, as this is where the normal hair follicle bulbs reside, preferably taken by a scalpel, and at least 5mm across.

Elliptical excisions are also the recommended method for the removal of neoplasms (benign or malignant), as well as subtle connective tissue abnormalities, such as atrophoderma, anetoderma and connective tissue nevi, to include both lesional and adjacent normal skin so that the transition at the two zones can be scrutinised. This assessment is simply not achievable with a solitary punch biopsy.

Vesicles and bullae should also be excised for examination with a scalpel and *not* a punch, which so often deroof the blister, and the roof material that housed a wealth of information are lost during processing.

In the case of pigmented lesions that is clinically suspected of being malignant, an incisional biopsy that extend beneath the *deepest* part of the lesion and included as much of the lesion as possible should be obtained, even if complete excision cannot be achieved. The important and yet difficult differentiation between benign and malignant melanocytic proliferations depends not on scrutinising the centre of the lesion and cytology, but rather on crucial assessment of the growth pattern at the periphery and deep part of the lesion. Similar attitude applies to the proper evaluation of cutaneous lymphoid infiltrate as well as well-differentiated malignant epidermal proliferations such as verrucous squamous cell carcinoma. Biopsy using the punch technique giving only the superficial and central part of the proliferation is non-diagnostic in

almost all cases. Non-committal terms such as pagetoid melanocytic hyperplasia and verrucous epidermal proliferation are the consequence of such practice.

Regarding the punch technique, the 4mm knife is the standard. At times, the 6mm or 8mm are useful for removal of larger lesions and serves as a substitute for the elliptical biopsy. In all events, if the punch technique is used, use it correctly - always include the subcutaneous fat, and **by all means avoid squeezing** the specimen with forceps which is seen in almost all such biopsies currently.

Curette biopsy yields tiny tissue fragments, that are liable to loss during processing and precludes tissue orientation, has therapeutic indications but *not* diagnostic ones.

It should also be reminded that considerations must be given to regional variations in the thickness of the various layers of the skin. This is particularly a problem regarding biopsies taken from the palm and sole that so often yield only a thick layer of stratum corneum with practically no dermal tissue for assessment.

As a practical guide, it is worth to exercise the following manoeuvre before proceeding to biopsy - ALWAYS visualise in one's mind the predicted pathological process based on the clinical scrutiny: are the changes going to reside within the epidermis alone/ the superficial dermis/ the deep dermis or the subcutaneous fat? One will be better led to select the most appropriate method of biopsy that permit adequate histopathologic evaluation, should this simple mental exercise is performed.

HANDLING OF THE BIOPSY SPECIMEN

The primary objective is to ensure the specimen reach the laboratory in a state suitable for the relevant diagnostic technique. In most instances, the specimens are sent for paraffin histologic diagnosis and should be fixed in 10% buffered formalin. Immunofluorescent (IMF), immunohistochemical (IMHC) studies and much less commonly enzyme histochemical study require fresh unfixed tissue for the preparation of frozen sections. There are now special transport solution, the Michel's transport solution, for transporting biopsies intended for IMF at times when the specimen cannot

be delivered to the laboratory promptly, which allow preservation of tissue-bound immunoglobulin for one to two days.⁵ Specimens should not be left dried, nor should fresh unfixed biopsy be immersed for prolonged period in normal saline, as most un-informed local hospital internists practise, when autolysis and overhydration will produce ostensible sclerodermatoid changes.⁶ Likewise, the biopsy specimens may be refrigerated or kept on ice for transport, but it should not be frozen in uncontrolled manner over-night or over the weekend in the dermatology clinic at the conclusion of the clinic session, only to be delivered to the laboratory later, in which event irreparable artefact and ice crystal damage will ensue.

Clinicians are also discouraged to prospect the specimen too much before it is examined by the pathologists. Very often, the clinician divides or transects the biopsy in order to cater tissue for different laboratories for varying studies, including histological, microbiological or even forensic examinations. When this is improperly performed, it often damages the lesion or confounds subsequent orientation, or the diagnostic lesion is simply lost to the microbiologist only to be minced for culture.⁷

LACK OF PERTINENT CLINICAL INFORMATION

Failure to provide pertinent clinical data often leads to unhelpful histological report - the so-called GARBAGE IN, GARBAGE OUT phenomenon. While most dermatologists give very relevant clinical summary and differential diagnoses, some players do not offer a single word for the history or diagnosis on the request form. There is nothing as exasperating for the pathologists as to receive a specimen for which not even the anatomical location is given on the request. In fact it has been advised that it could be medicolegally dangerous to attempt a morphologic interpretation in the absence of all these basic information.⁷

The basic items like *name, age, sex* should be given. In ideal situations, the *lesional morphology, distribution, duration* and *differential diagnoses* are most welcomed as they are essential for correlative interpretation by the committed pathologists. The **site of biopsy** is equally essential for meaningful interpretation. It is more rewarding to write **'scalp**

biopsy for histology' rather than **'skin biopsy for histology'**.

NON-SPECIFIC HISTOLOGIC FEATURES IN THE BIOPSY

Perhaps in no other field of medicine does one encounter such diverse disease processes as in dermatology. However, the skin has only limited ways to respond to insults of divergent nature. As a result, it is not surprising to find that *few inflammatory dermatologic lesions have pathognomonic histological features*. Clinical distinctive lesions may have similar histologic appearance. Thus both viral exanthem and Schamberg's lesions present as non-specific perivascular lymphocytic infiltrate, pityriasis rosea and erythema annulare centrifugum as spongiotic dermatitis, erythema multiforme and graft-versus-host disease as vacuolar type interface dermatitis, psoriasis and Reiter's disease as psoriasiform dermatitis, bullous pemphigoid and herpes gestationis as subepidermal blister with eosinophils, Henoch-Schonlein purpura and microscopic polyangiitis as leucocytoclastic vasculitis, erythema nodosum and subacute migratory panniculitis as septal panniculitis and scleroderma and morphea as fibrosing dermatitis showing collagen homogenisation, and there are endless examples. It is therefore, in all these instances, that the histologic findings should be understood in the light of the *full clinical data* in order to better serve the patients.

CONCEPT OF LIVES OF SKIN LESIONS

Most traditional and time-honoured textbooks of dermatology and dermatopathology picture the histology of cutaneous diseases at but a moment in their course, as if these are static phenomenon rather than dynamic processes. Many pathologists were pre-occupied with the warped view of the histopathology of cutaneous diseases as snap-shot pictures, when in fact the histology of all skin lesions varies at different stages of their live history of evolution and devolution. Classic descriptions stipulate only the established lesions, and therefore, for most surgical pathologists, the greatest chance for a diagnosis is rendered only with biopsies taken of an established lesion. Lesions biopsied

at other time of their evolution are simply not recognised and defied interpretation by most pathologists.

Pathologists, and dermatologists alike, should conceive the pathologic processes in time lapse, as indeed these processes play themselves out in actuality. Thus, psoriasis should not be recognised morphologically as simply and only a psoriasiform dermatitis, but also recognised at other stages of the development of the lesion as spongiotic dermatitis, spongioform pustular dermatitis, and a subcorneal or intracorneal pustular dermatitis, depending on what stage in the life history of the psoriatic lesion was interrupted by the biopsy procedure.⁸ Lesions have lives, just as human being have, and that lesions look different at different stages in their lives. It should be understood that the durations of some lesions are short, while others are long, but every life will be better understood when viewed over its entire time rather than at one point only. Understanding this, many of the non-diagnostics and non-matchings become diagnostically specific.

ATYPICAL LESIONS

Classic lesions present no problem to all. Atypical features in the history or clinical appearance of the lesion with consequent uncertainty in the diagnosis is frequently the indication for biopsy and frequently be the problematic case histologically. This is true because lesions that are atypical clinically will almost certainly be atypical histologically, as the clinical gross appearance is the summation and consequence of the pathologic changes. In this circumstances, it is important for the pathologist to have the full clinical data, and also the *differential diagnosis*. The pathologist may not be able to give a single definitive answer but still may contribute by *ruling out one or more of the differential diagnoses*. Ultimately, the disease will declare itself with further biopsy material.

THE 'WRONG' PATHOLOGIST

No matter how perfect is the clinical assessment, lesion selection and biopsy technique, the information critical for generating the correct histologic diagnosis will not be observed and extracted unless the pathology

is interpreted by an experienced and committed dermatopathologist.

Neoplastic conditions of the skin may at times be properly interpreted by the general surgical pathologists, but the ability to scrutinise the myriad of inflammatory dermatoses, for sure, requires special training, deliberation and experience.

Some general pathologists argue that it is only necessary to send the more complicated specimens to the dermatopathologists. However, it must be realised that it is often the "uncomplicated" or "simple" cases that may seem misleadingly simple by the unwary, when in fact they are something else. The author had witnessed on many occasions that lesions of lichen amyloidosis been mistaken as wart, and perhaps even more distressing was that a superficial basal cell carcinoma being misread as *non-specific dermatitis* in a university setting.

The *uninterested pathologists* is no less problematic than the inexperienced and ignorant. They may fail to submit the correct part of the biopsy for processing with consequent inability to come to a helpful diagnosis. There are often times when only the normal peripheral part of a skin biopsy taken from an obvious blistering lesion is submitted for processing, and only finally realise that the case is a life threatening erythema multiforme major after his dermatopathology colleague submits the rest of the specimen for examination.

Equally distressing to the dermatologists are that some pathologists never attempt to render a specific diagnosis despite the presence of all diagnostic features in the biopsy specimen, or they fail to couch their diagnosis in the language of clinical dermatology. Often, they just issue a descriptive diagnosis, such as inflammation in subcutaneous fat, or they rely entirely on the clinicians' input, and their reports are replete with terms such as "consistent with" and "cannot rule out", even when the implied diagnosis is absolutely out of question. Further frustration and confusion are caused by failure of the pathologists to communicate with the dermatologists using a language that is understandable to their clinical colleagues. Terms such as dermatofibroma are certainly better understood than fibrous histiocytoma, wart better than squamous papilloma, seborrhoeic keratosis better than

basosquamous papilloma, and malignant melanoma better than pagetoid melanocytic proliferation.

CLINICAL CORRELATION IN DERMATOPATHOLOGY

Lastly, a few words must be made on this important aspect of the skin biopsy-histologic diagnostic process, in order that the dermatologists are properly oriented as to what they should be expecting from the laboratory. As alluded to previously, the skin reacts in only a limited number of ways, so that any given pathologic change often has several possible causes. While in some cases, an astute microscopist may render a correct diagnosis in a difficult case without knowledge of the clinical features by careful attention to subtle clues, more often, correlation of the microscopic findings with the clinical features will aid in making the correct diagnosis or limit the differential diagnosis to few possibilities. The following are some scenarios and examples when clinicopathological correlation is of greatest diagnostic assistance.

1. Conditions that shows a same general reactive pattern, such as seen in the various spongiotic dermatitides of allergic contact dermatitis, dyshidrotic dermatitis, nummular dermatitis, id reactions as well as dermatophytosis; leucocytoclastic vasculitis as seen in the various settings including Henoch-Schonlein purpura, microscopic polyangiitis, hypocomplementaemia and various drug and chemical hypersensitivity.
2. Conditions that show a un-common characteristic histologic abnormality but distinctive and heterogeneous clinical picture, such as epidermolytic hyperkeratosis in bullous congenital ichthyosiform erythroderma and some cases of palmoplantar keratoderma, focal acantholytic dyskeratosis in Darier's disease as well as Grover's disease.
3. Instances in which the histologic changes are subtle such as those seen in the various form of ichthyosis, as well as conditions with abnormality in the elastic tissue like atrophoderma and scleredema.
4. Instances when the purported lesion may not have been actually biopsied. The author has the experience of almost reporting a superficial biopsy as a

dermatitis when the biopsy is intended for an angiolipoma in the subcutis.

5. Technical pitfalls, when ostensible scleroderma is interpreted for biopsy which has been overhydrated by prolong soaking in the water bath.⁶ Correlating to the clinical feature immediately discloses this pitfall and thus avoids an erroneous interpretation.

With the above discussion, which is by no means comprehensive and without bias, I wish that it will improve the diagnostic value of the skin biopsy. Below is a summary of the suggestions for effective skin biopsy diagnosis sublimed upon the above discussion:

TIPS FOR AN EFFECTIVE AND SATISFACTORY DERMATOPATHOLOGY CONSULTATION

1. *Select a fresh and established lesion.*
2. *Select the most appropriate biopsy technique (mentally anticipate the histopathology).*
3. *Use SCALPEL technique for panniculitis, alopecia, and suspected malignant tumors (particularly melanocytic lesions).*
4. *Avoid crushing the tissue during the biopsy procedure.*
5. *Place tissue in proper fixative.*
6. *Make sure that the patient's name, sex, age are indicated in the request form.*
7. *Include a legible summary of relevant medical history, site of biopsy, and a differential diagnoses.*
8. *Discuss the results with your pathologist.*

CONCLUSION

Skin biopsy for histology, although a rather unsophisticated and primitive investigative technique, provides a wealth of information that is accurate in predicting the biologic behaviour of the majority of skin disorders. But in order to do so, it must be obtained at the appropriate time in the evolution of the eruption, from the appropriate site, and in a proper manner, and must be interpreted by someone skilled in dermatopathology. Often, dialogue between the dermatologist and a committed pathologist with correlation of the microscopic findings and clinical features will be helpful and perhaps necessary in order to arriving at a correct diagnosis to the benefit of the patient.

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