Stepping on diagnosis with step sections- An institutional experience

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Abstract

Introduction: A thorough morphological assessment plays a central role in evaluation of skin biopsies. Overlapping microscopic features pose additional diagnostic challenges in dermatopathological practice. More over skin biopsies are of small size and diagnostic findings may not be present in the initial section. Hence, step sectioning may have added value as in adjunct in contemplating the final diagnosis.

Objective: The study was aimed to evaluate the diagnostic utility of step sectioning in dermatopathology.

Materials and Methods: A total of 478 skin biopsies were received over a period of 2 years from January 2015 to December 2016 at the Department of Pathology, JJMMC. Two prospective step sections (A2, A3) were cut at an interval of 50µm from the original section (A1). The findings were evaluated by two pathologists independently. The results were analysed and evaluated statistically.

Results: Majority of cases (n= 453, 94.7%) were diagnostic. Out of these, many cases (n= 400, 83.6%) showed no additional findings on step sections. In a few cases (n= 32, 6.7%), step section provided additional findings which contributed to the diagnosis. There were some cases (n= 14, 3%) in which findings were noted only on step section leading to the diagnosis. Some cases (n=7, 1.46%) were such that the step sections changed the diagnosis made on original section.

Conclusion: Step sectioning enhances the diagnostic accuracy of morphological assessment in ermatopathology practice.

Keywords: Prospective, Step sections, Skin biopsy, Lichen planus.

Introduction

Histopathological examination of biopsies is considered gold-standard for diagnosis of skin lesions, either neoplastic or inflammatory. There are many causes for sampling error, during evaluation one of them being incomplete sectioning of tissue blocks. Thus, assessment of only superficial section can miss the representative portion of the tissue block leading to false-negative diagnosis.

Examination of multiple levels of the tissue block has been cited as a means of avoiding such diagnostic error.[1] In this context multiple levels or deep sections which are taken at specific depth are called step sections. There are studies in which the utility of step sections has been evaluated in biopsies from various organs including skin.[2-9]

Interestingly small skin biopsies have a cosmetic advantage and are thus preferred in day-to-day practice, but for dermatopathological diagnosis they pose difficulty because most of the skin biopsy specimens often exhibit nonspecific and overlapping microscopic features. There are not many studies done on the utility of step sections in dermatopathological practice and also, currently no standard protocols are available for its routine use.[10] To overcome this difficulty, we have adopted a practice of obtaining sections at three levels with a specific depth of interval.

The study aimed to examine the use of such prospective step sections of small skin biopsies and its effect on dermatopathological diagnosis.

Materials and Methods

The present study included consecutive 478 skin punch biopsies, received over a period of 2 years- from January 2015 to December 2016 at the Department of Pathology, JJMMC, Davangere, Karnataka.

All skin punch biopsies and incisional scalpel biopsies received at Pathology Department during study period, irrespective of provisional diagnosis and excision biopsies of skin were excluded.

Routinely, punch biopsies measuring 2 mm to 4 mm in thickness are performed in our hospital. For cosmetic purpose, biopsies with 2mm thickness were preferred for face and other exposed areas of body. For lesions like plaque, and macular lesions, the biopsies were taken from the centre of lesion. In cases of vesiculo-bullous/ papular disorders, intact vesicle/ bulla/ papule with scanty normal margin was taken. Punch biopsies measuring 6mm or greater in diameter were divided at 2 mm interval. Elliptical specimens less than 6 mm long were bisected longitudinally and submitted for histopathological examination except for vesiculo-bullous lesions.

All the skin biopsies were fixed in formalin and underwent routine tissue section processing, tissue embedding in paraffin. This was followed by prospective sections at 50 microns with each section measuring 4-5 microns in thickness. Each ribbon of tissue consisted of 8-10 sections of skin bits. The initial slide was prepared from the ribbon obtained from untrimmed block of tissue and was labelled as slide A1; the second slide obtained after 50 µm interval was labelled as A2 and the third slide obtained after 50 µm

interval from A2, was labelled as A3. Routinely A1 and A2 were asked for examination and sections were stained with haematoxylin and eosin.

Initially A1 was reviewed by two pathologists independently in the light of clinical findings and morphological features were noted down and subsequently A2 section were reviewed by both pathologists independently to look for additional findings. This was followed by discussion by both the pathologists to arrive at a final diagnosis.

The cases in which the findings were non-diagnostic on A1 and A2, a subsequent step section- A3 was asked. Special histochemical stains like- Masson's trichrome, Van Gieson, Lepra stain for acid-fast bacteria, Periodic acid-Schiff and others were done wherever required on A3 step section.

The utility of step sections were classified into:

1. Category 1-No additional information
2. Category 2-Additional findings which contributed to the diagnosis
3. Category 3-Additional findings which led to diagnosis
4. Category 4-Findings on step section which changed the diagnosis.

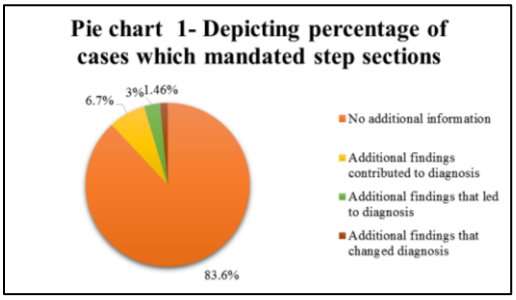
Descriptive statistic analysis was applied and results were depicted as pie charts and tables.

Results

Total of 478 biopsies were received over a period of two years. Maximum skin biopsies were from the patients aged between 30-40 years with male to female ratio of 1.3:1. Among 478 biopsies, 453 were diagnostic and 35 were non diagnostic.

Out of 453 diagnostic cases, 400 cases showed no additional information on step sections. However in 32 cases, step section was useful in getting additional findings which contributed to the diagnosis rendered on A1. There were 14 cases in which A1 had no diagnostic finding and on step sectioning, additional findings led to the diagnosis. 7 cases were such that the step sections changed the diagnosis made on initial section as depicted in pie chart 1.

Chart 1:



The cases encountered in these categories are shown in Table 1, 2 and 3.

Category 2

There were 32 cases in this category which showed additional findings which contributed to the diagnosis rendered on slide A1

Table 1: Showing distribution of lesions in category 2

Squamous cell carcinoma	1
Psoriasis	**7**
Lichen planus	6
PLC, Pleva	4
Discoid lupus erythromatosis	2
Verruca	3
Seborrhic Keratosis	2
Morphoea/ Scleroderma	2
Leukocytoclastic vasculitis	2
Fungal infection	2
Solar lentigo	1
Total	**32**

There were 7 cases of psoriasis, in which slide A1 showed psoriform hyperplasia, suprapapillary thinning and parakeratosis. Thus, giving a clue towards diagnosis. On further step sectioning, slide A2 showed Munro's microabscesses, spongiform pustules, features which were contributing to the definitive diagnosis. (Fig 1a & 1b)

6 cases of lichen planus showed features like presence of compact orthokeratosis, wedge shaped hypergranulosis, degeneration of basal layer of epidermis and the showed lichenoid infiltrate at the dermoepidermal junction on slide A1. Step section A2 and A3 showed civatte bodies, Max-Joseph spaces and saw toothed elongation of rete ridges, features which contributed to diagnosis.

There was one case with full thickness dysplastic changes of epithelium, the diagnosis of squamous cell carcinoma was confirmed for this case as the slides A2 and A3 showed of subepithelial stromal invasion.

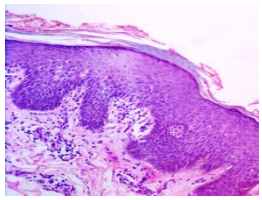


Fig 1a: Slide A1

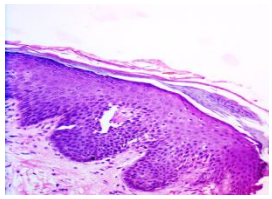


Fig 1b- Slide A2

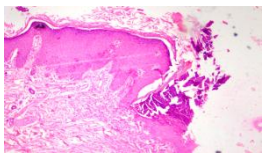


Fig 2a: Slide A1

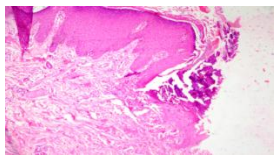


Fig 2b: Slide A2

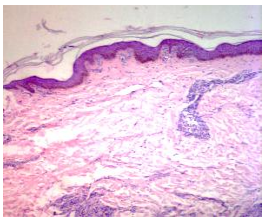
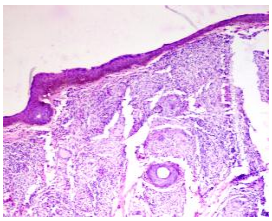



Fig 3a: Slide A1 **Fig 3b: Slide A2**

Figures showing findings on step sections – Fig 1: Psoriasis, Fig 2: Perforating lesion, Fig 3: Hansen's disease

Category 3

There were 14 cases included in this category which showed additional findings which led to the diagnosis.

There were 4 cases of perforating lesions which had dilated follicular infundibulum filled with keratotic plug containing few neutrophils, follicular epithelium was surrounded by neutrophilic infiltrate on slide A1, features which were nonspecific for diagnosis. Step section A2 led to the diagnosis as it showed perforation of epithelium through which inflammatory cells, degenerated material was extruded in the dilated infundibulum. (Fig 2a and 2b))

Hansen's disease was diagnosed on slide A2 as it showed lymphohistiocytic aggregates and multinucleated giant cells which were absent in slide A1, with A1 which showed only perivascular lymphocytic infiltrate. (Fig 3a and 3b)

Table 2: Showing distribution of lesions in category 3

Perforating Lesions	4
Hansen's Disease	3
Syringoma	1
Pytriasis Rubra pilaris	1
Granuloma Annulare	1
Pemphigus Vulgaris	1
Dermatits Herpetiforms	1
Tubercular Verrucosa Cutis	2
Total	**14**

Initial section A1 of syringoma showed solid nests of epithelial cells with tail like extension, with occasional lumen formations. On step sections A2 and A3, majority of the solid nest, showed lumen with amorphous debris, helping to arrive at definitive diagnosis. (Fig 4a & 4b))

There was one case of Pytriasis rubra pilaris with A1 showing Orthokeratosis, hypergranulosis, and broadened rete ridges. A2 and A3 showed shoulder parakeratosis leading to diagnosis. (Fig 5a & 5b)

In case of granuloma annulare initial had no specific findings and step sections showed palisaded granulomas with central degenerated material surrounded by histiocytes.

Similarly, diagnosis of pemphigus vulgaris was made in one of the cases when step section showed suprabasal bulla with characteristic row of tombstones appearance.

Category 4

There were 7 cases included in this category which showed additional findings which changed the diagnosis

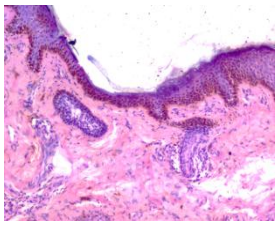
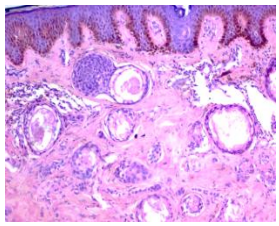



Fig 4a: Slide A1 **Fig 4b: Slide A2**

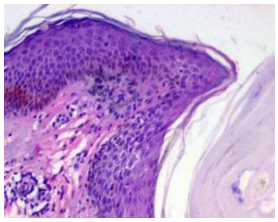
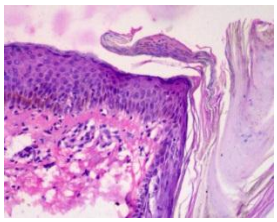



Fig 5a: Slide A1 **Fig 5b: Slide A2**

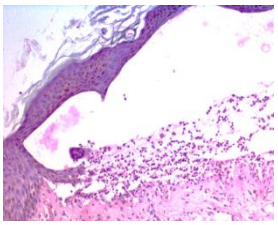
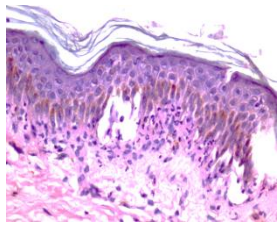



Fig 6a: Slide A1 **Fig 6b: Slide A3**

Figures showing findings on step sections– Fig 4: Syringoma; Fig 5: Pytriasis Rubra Pilaris; Fig 6= 6a-Bullous pemphigus, 6b: Dermatitis herpetiformis

On initial slide (A1) a case was diagnosed as Bullous pemphigoid due to presence of inflammatory infiltrate comprised of neutrophils and lymphocytes within the bullous cavity. But on further step section, (A3) there were seen papillary neutrophilic microabscesses and karyorrhexis in dermis, changing the diagnosis to Dermatitis herpetiformis. (Fig 6a & 6b)

Two cases of Actinic keratosis showed epidermal cell atypia with disorderely maturation, hyperkeratosis, parakeratosis and dermal chronic inflammatory inflitrate. Further step sectioning in both the cases showed invasive squamous cells into the dermis.

Table 3: Showing distribution of lesions in category 4

Initial Diagnosis	Changed Diagnosis	No of cases
Bullous Pemphigoid	Dermatitis Herpetiformis	1
Actinic keratosis	Squamous cell carcinoma	2
Seborrheic Keratosis	Basal cell carcinoma	1
Subacute spongiotic dermatitis	Pityriasis Lichenoides Et Varioliformis Acuta	1
Seborrheic Dermatitis	Psoriasis	1
Follicular keratosis	Discoid Lupus Erythematosus	1
Total		7

Discussion

Step sections are defined as sections from ribbon of paraffin tissue block obtained at specific microtoming depth followed by mounting these sections on multiple slides and labelling with respect to initial section.

The utility of step section have been studied in past and these studies suggest that deeper levels provide a more accurate diagnosis in about one-third of skin biopsy specimens.[10-12] The study by Werner et al.[13] best reflects what in reality occurs in a dermatopathology service – 63% of the step sectioned cases could be signed out without ordering them, with no change in diagnosis. On the other hand, if no step sectioning were performed, 37% of the patients would not benefit maximally from the diagnostic power of skin biopsy, which could be crucial as in case of malignant lesions.

Deeper sections are named according to methodology, namely-Serial sectioning, which is defined as obtaining a continuous ribbon of sections from a paraffin block & placing all the sections on multiple slides when compared to step sections which are a form of sampling in which sections are collected at specified depths in the block.[14] In step sectioning, sections are discarded in between the sections that are mounted on the glass slide. Step sections are preferred to serial sections as the intervening unstained sections are available for special stains if needed.[2]

There are no fixed protocols for taking step sections. Our study makes an attempt to standardize the method of taking step sections for the skin biopsies in the view of their diagnostic utility.

The present study the sizes of the skin biopsy specimen were 2 mm, 3 mm and 4 mm, with 3 mm being most common. It was found by one of the study groups that 3 mm is the smallest size likely to give sufficient tissue for consistently accurate histological diagnosis and less likely to cause significant scarring.[15] Bahram et al.[16] have suggested a punch biopsy for diagnosing inflammatory dermatoses and recommend 3 mm to 4 mm size as adequate. 2mm biopsies face problems with orientation of specimen while processing the slides by technicians and have frequent problems related to crushing artefacts[16, 17] but few studies have shown that 2 mm biopsy is sufficient for diagnosing wide dermatological conditions.[18]

According to Khopkar et al.,[19] choice of site for biopsy and proper technique of a punch biopsy has a profound effect on the diagnostic information that can be retrieved from punch biopsies.[18]

The depth of paraffin tissue block which can yield the best possible representative or diagnostic step section was studied by piloting. We subjected 5 skin punch biopsies for step sectioning at 10 µm depth and labelled as A1, A2, A3 so on. We found that there were no significant changes in findings between A1 and A2. On other hand additional findings started appearing from A5-A6 slides corresponding to depth of 50 µm-60 µm. 5 of the skin punch biopsies were also subjected to depth of 100 µm. Such depth of step sectioning lead to tissue exhaustion from tissue block. Likewise, for small biopsies of skin, taking step sections at 50 µm has shown to be diagnostic by many study groups. Similarly, Dyson et al.[20] had advocated in their study step section at 50 µm for small specimens and 100 µm for large specimens. Thus, depth of 50 µm for step sectioning was finalised for the present study.

Studies by Carag HR et al.[12] and Maingi CP et al.[11] where deeper sections revealed a diagnosis in 37.3 % of the cases, had concluded that step sections were particularly helpful in case of skin cancers. Likewise, 30 % and 9 % cases showed additional findings on step section in the study conducted by Bruecks et al.[3] and Jerath et al.[4] The present study showed additional findings in 11.1 % of cases on step sectioning.

Among all the cases where step section showed additional findings, inflammatory lesions were most common followed by neoplastic lesions. Among all 5 neoplastic lesions, there was 1 case of benign lesion, syringoma and 4 cases of malignant lesions- 3 cases of squamous cell carcinoma and 2 cases of basal cell carcinoma. Our study had less number of malignant lesions as the excisional biopsies were excluded which is preferred method for malignant lesions.

The study by Carag et al.[12] studied step sections in cases showing actinic keratosis on initial sections found malignant lesions in 20% of cases after evaluation of deeper levels.[13] Thus, the step sections improved the diagnostic accuracy & also discovered hidden malignancies without compromising on economy & turnaround time.

Among all the inflammatory cases, majority of the cases were inflammatory dermatosis followed by granulomatous lesions among all the diagnostic cases.

Our study showed similar findings to Bruecks et al.[3] who examined the utility of prospective step sections in improving diagnostic accuracy and turn-around time for small skin biopsies.

There are other literature focusing on scientific utility of step sections. Along with skin, there are studies showing step sectioning being used to improve the diagnostic accuracy of several specimen like prostatic, rectal, oral, colonic, esophageal, trephine, endometrial and sentinel lymph node biopsies.[2,4-6, 8,17, 21,22] Study pattern and outcome were different for different tissues esophageal biopsy specimens were subjected to step sections for detection of intestinal metaplasia by chikara et al.[6] Nash et al[21] performed step section on colorectal polyps with no pathologic diagnosis (NPD) or those in which only lymphoid aggregates (LAs) showed additional findings including neoplastic features. Step section preparation of transbronchial lung biopsy significance in the diagnosis of diffuse lung disease showed that among 112 patients with nondiagnostic transbronchial lung biopsy findings, the step sectioning resulted in specific findings in 7 cases.[22]

However, focusing on utility of step sectioning, our study on skin biopsy showed that there are increased supply costs for prospective sections which include staining materials, glass slides and increased storage volume. But more important was, step sectioning helped us in obtaining in additional findings in significant proportion of cases (11.1%), warranting the use of step sectioning on routine basis.

Table 4: Shows comparison of different studies done of step sectioning

	Carag et al[12]	Bruecks et al[3]	Kattel et al[15]	Jerath et al[4]	Present study
Type of step section	Retrospective	Retrospective	Retrospective & Prospective	Prospective	Prospective
No. of cases	69	500	100	200	478
Type of lesion	Actinic keratosis	All skin biopsies	All skin biopsies	All skin biopsies	All skin biopsies
Type of biopsy	Shave, punch Biopsies	Shave, punch Biopsies	Punch biopsies	Punch Biopsies	Punch biopsies
Initial+ step sections	10	2	2	2	3
Interval between step sections	50 µm	50 µm	50 µm	50 µm	50 µm
Additional finding on Step sections	_	117+34 cases (30%)	_	18 cases (9%)	11.1%

Conclusion

Histopathological examination of skin biopsies is peculiar owing to the fact that the findings are overlapping across the lesions and pathognomonic findings may be present at only certain sections of biopsy. Hence, sectioning of the skin biopsies at multiple levels will help to pick up these additional findings and formulate the final diagnosis.

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