Evaluation of diagnostic utility of step sections in dermatopathology: A prospective study of 200 consecutive punch biopsies

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ABSTRACT

Background: Obtaining deeper sections or step sections is a common practice for small skin biopsies. Much of the available literature highlights the importance of step sections in neoplastic diseases of skin. However, the routine dermatopathology practice in developing countries shows a predominant burden of nonneoplastic diseases, and the utility of step sections in this context has not been much reported. Objective: The study was aimed to evaluate the utility of prospective step sections in routine dermatopathology practice. Materials and Methods: The present study comprising 200 consecutive skin biopsies was carried out in a prospective manner. Three slides were prepared in each case: Slide 0 was prepared from the ribbon of tissue obtained from untrimmed block, step sections 1 and 2 were obtained at 50 µm and 100 µm depth, respectively. The diagnosis was rendered on slide 0 and subsequently reviewed after examining step section 1 and 2. Results: Of the 200 cases, additional findings on step sections were found in 18 cases (9%) which led to change in diagnosis in 10 (5%) cases. Step section 1 led to correct diagnosis in 6 cases (3%). Step section 2 led to correct diagnosis in 10 cases (5%); however, this was statistically not significant (P ≥ 0.065) when comparing to step section 1. Additional findings which led to diagnosis was most commonly found in the cases of borderline tuberculoid leprosy (5 out of 10 cases) followed by bullous disorders. Conclusion: We therefore believe that step sections improve the diagnostic accuracy in skin biopsies and they are, especially useful in suspected cases of Hansen’s disease. There is no statistical advantage of step section 2 versus step section 1; although, step section 2 had shown to include all the additional findings which led to a change in diagnosis.

Key words: Leprosy, prospective, skin biopsy, step sections

INTRODUCTION

Skin biopsy is indicated in all suspected dermatoses and to clarify a diagnosis when a number of cutaneous entities are under consideration.[1] Incomplete sectioning through the tissue block is a known cause of false-negative diagnosis in dermatopathology.[2,3] Deeper sectioning of small biopsy specimens such as needle core and endoscopic biopsies is commonly used to enhance diagnostic sensitivity and accuracy. Deeper sections in biopsies can be random or in form of step sections. Step sections are taken at a fixed distance, usually 50 µm from each other and subsequently labeled as step one, two, three, etc. The utility of step sections has been evaluated in prostatic, rectal, oral, colonic, esophageal, trephine, and sentinel lymph node biopsies.[4-9]
In some laboratories, step sections or “deeper levels” are prepared before receipt of the slides by the pathologist (prospective step sections), and in some laboratories, they are requested after reviewing the initial slide.\(^2\)\(^,\)\(^10\) The use of prospective step sections may improve diagnostic accuracy and reduce the time for diagnosis.

The present study was carried out with the aim to evaluate the diagnostic utility of prospective step sections and to compare the diagnostic utility of step section 1 in comparison to step section 2.

**MATERIALS AND METHODS**

The present study was conducted on consecutive 200 punch skin biopsies received in the Department of Pathology from the Dermatology department irrespective of the provisional diagnosis and type of biopsy. The study excluded excisions. The punch biopsies received were of 3.5 mm, that is, 3500 \(\mu\)m in thickness. In case of patch, plaque, and macular lesions, the punch was taken from the center of lesion. In vesiculobullous/papular disorders, early intact vesicles/bulla/papule with scanty normal margin was preferred. The punch biopsies were submitted intact. All the skin biopsies comprising the study were received in formalin and had both dermis and epidermis on microscopic examination.

The biopsies underwent routine tissue processing and paraffin embedding. Sections of 3 \(\mu\)m thickness were prepared from the paraffin blocks. For each specimen, 3 prospective sections, each consisting of 1 ribbon of tissue containing 4–6 sections, were obtained at 50 \(\mu\)m intervals from the paraffin block. The initial slide was prepared from the ribbon of tissue obtained from untrimmed block of tissue and was labeled as slide 0; the second slide obtained after 50 \(\mu\)m interval was labeled as step section 1 and the third slide obtained after 50 \(\mu\)m interval was labeled as step section 2. Sections were stained with hematoxylin and eosin. Special histochemical (periodic acid-Schiff [PAS], Masson’s Trichrome, Elastic Van Gieson, Lepra, acid-fast bacteria [AFB]) and immunohistochemical (IHC) stains were done wherever required. Sections for special stains and IHC were taken after sections for slide 0 had been taken if the orders had been placed at the time of grossing or after SS2 if the orders had been placed subsequently.

A diagnosis was rendered using slide 0 only, and subsequently, step section 1 and step section 2 were reviewed. The utility of step sections were classified into:
- No additional information
- Additional findings which contributed to the diagnosis rendered earlier on slide 0
- Additional findings which led to diagnosis or changed the diagnosis.

Statistical analysis was performed using Chi-square test and simple proportion test.

**RESULTS**

These 200 biopsies were received over approximately 1 year 3 months. Out of a total of 7114 surgical pathology specimens received over this period, the burden of skin punch biopsy was 2.8% (200/7114). The patients’ age ranged from 1 to 80 years with a mean of 37.9 ± 18.19 years. Maximum number of cases were in the third decade of life. Of 200 cases, 119 were males and 81 were females with a ratio of 1.4:1. The most common clinical finding was a patch or a plaque (43 cases each = 21.5%), followed by macular or ulcerated lesion (35 cases each = 17.5%), followed by papular type of lesion (18 = 9%). The most common provisional clinical diagnosis was Hansen’s disease followed by cutaneous vasculitis. Out of 200 cases, 191 (95.5%) cases were reported as nonneoplastic/benign and 9 (4.5%) as malignant [Tables 1 and 2].

The special stains (Lepra, AFB, PAS, Masson’s Trichrome, Elastic Van Gieson) were required in 76 out of 200 cases (38%). Some cases required only one special stain, but in some cases, more than one special stain was required for diagnostic workup. In cases suspected of Hansen’s, an order for lepra stain was put at the time of grossing. Once the special stain had been carried out it was not repeated, only in the cases where the section had floated or stain had not worked properly a repeat stain was requested.

On step section 1 and 2 combined together, additional findings were observed in 18 cases (9%). Thus, in 9% of cases, step sections were helpful. The point estimate is 9%, and the preferred (Wilson score) 95% confidence limits are 5.76%–13.78%.

In 10 cases, additional findings led to diagnosis or changed the diagnosis, whereas in 8 cases, the diagnosis was not changed but only contributed to the diagnosis already made on slide 0.
Additional findings which led to diagnosis or changed the diagnosis (10 cases)

In 5 cases, granulomas not present in initial slide 0 were seen in step sections 1 and/or 2 which led to a diagnosis of borderline tuberculoid (BT) leprosy [Figure 1]. There was one case each of pemphigus vulgaris [Figure 2] and bullous pemphigoid [Figure 3] in which bulla and its contents were not present on slide 0 but were present in step sections 1 and 2, and that helped in making the diagnosis. In one case of pustular psoriasis, step sections showed the presence of pustules, besides features of psoriasis leading to diagnosis. In a case of lichen planus pigmentosus (LPP), band-like infiltrate and pigment incontinence not present on the initial slide was seen on step sections. The step sections changed diagnosis from chronic nonspecific dermatitis to LPP. In yet another case of chronic folliculitis, follicular destruction not seen on initial section was present on step section leading to diagnosis. In these 10 cases, had there been no step sections the diagnosis would not have been made or diagnosis would have been wrong.

Additional findings which contributed to the diagnosis rendered on slide 0 (8 cases)

Three cases were of BT leprosy, 2 showed the presence of nerve destruction which was not present in slide 0 and in one case granulomas became more in number on step sections 1 and/or 2 [Figure 4]. In a case of spongiotic dermatitis, step sections showed an additional finding of granulomas not present in initial slide 0.
of vesicle formation which was not seen on slide 0. In one case of suspected morphea, presence of adequate adnexa on serial sections ruled out the possibility of morphea. In one suspected case of dermoid cyst, only keratinous debris was seen on initial section, but the presence of adnexal structures in cyst wall in step sections helped to contribute to the diagnosis but did not change the diagnosis. In one case, each of lupus vulgaris and lupus miliaris disseminated fascel, granulomas, and caseation necrosis became more prominent, respectively, on step sections.

In the present study, additional findings were found in 10 cases of granulomatous inflammation, followed by 4 cases of bullous disorders, 3 cases of follicular disorders, and one case of LPP. The number of additional findings were statistically significant ($P < 0.001$). Step section 1 detected additional findings in 9 (4.5%) cases, whereas step section 2 detected additional findings in 16 (8%) cases out of 200 cases. On comparing step section 1 with 2, in 7 (3.5%) cases, additional findings were only seen on step section 2; however, this was statistically not significant ($P \geq 0.05$) when comparing to step section 1. The diagnosis was changed in 10 out of these 18 cases. This was statistically significant ($P < 0.002$) compared to slide 0. Step sections 1 and 2 detected change in diagnosis in 6 (3%) and 10 (5%) cases, respectively. There was a significant overlap of six cases. On comparing step section 1 with 2, in 4 (2%) cases, change in diagnosis was seen only with step section 2; however, this was statistically not significant ($P \geq 0.065$) when compared to step section 1 [Table 3].

Out of 200 cases, 9 cases were reported as malignant and remaining 191 were labeled as nonneoplastic or benign. In malignant cases, changes or additional findings were not observed in step sections. The statistical advantage of step sections in benign over malignant conditions was statistically insignificant $P = 0.066$ ($P \geq 0.05$).

### DISCUSSION

Skin biopsy material should always be examined carefully and thoroughly as incomplete sectioning through the tissue block is a known cause of false diagnosis. In the present study, additional findings were seen in 18 (9%) cases on step section 1 and 2 combined together which led to improved diagnostic accuracy in 10 (5%) cases. In a study by Bruecks et al., prospective step sections improved diagnostic accuracy in 7% of small skin biopsies and step sections were found to be helpful in 30% of cases. The study design in this study was similar to ours, that is, the inclusion of all skin biopsy cases irrespective of their clinical diagnosis. However, in a study by Maingi and Helm et al., where step sections were requested after reviewing the initial slide, the deeper section requirement has been reported to be 7%. This led to additional diagnostic information in 37.3% of the cases.

The majority of cases (95%) in the present study were nonneoplastic as excisional biopsies were excluded, which is the preferred technique for the evaluation of neoplastic lesions. The additional step sections in case of malignant disorders only confirmed the diagnosis rendered on slide 0 and did not yield any additional information. As the number of malignant cases was only 9, the applicability of step sections for malignant disorders cannot be concluded. However, various authors have found the usefulness of step sections in malignancies. In cases where malignancy is strongly suspected or an in situ lesion is seen up to 10 step sections have also been taken.

In a study done on cases showing actinic keratosis in the initial level; step sections showed additional findings in 33% of cases. These additional findings were malignant lesions such as squamous cell carcinoma...
(in situ and invasive type); basal cell carcinoma. However, additional findings in benign lesion such as seborrhoeic keratosis and porokeratosis were also seen. There was one case each of actinic keratosis and Bowen’s disease in our study which did not show any significant finding on step sections. In another study performed on cases diagnosed as melanoma on initial level; step sections were found to be prognostically important, as variables such as tumor thickness and tumor depth were found to be greater on step sections leading to change in management in some cases.\[13\]

In this study, additional findings which led to diagnosis were most commonly found in BT leprosy (5 out of 10 cases) followed by bullous disorder. Step sections may help in showing the granuloma, may increase the number of granulomas, suggesting their distribution or condition of nerves. Furthermore, step sections are helpful in diagnosing leprosy reactions. Hence, in a patient with a presumptive clinical diagnosis of leprosy, we suggest prospective step sections in addition to lepra stain should be taken. Step sections should also be done in bullous disorders literature reveals that one of the bullous disorder that maximally benefits should also be done in bullous disorders literature reveals that one of the bullous disorder that maximally benefits in arriving at a correct diagnosis by step sections is herpes simplex virus infection.\[15\] In a study from Nepal also it was suggested that a 3 mm punch with step sections may yield additional diagnostic information in granulomatous disorders and inflammatory/infective dermatitis.\[16\] The other nonneoplastic diseases in which step sections have been found to be useful are scabies, folliculitis, granuloma annulare, viral wart, solar lentigo, etc.\[2,10\]

On comparing step section 1 and 2, step section 2 detected additional findings in 16 (8%) whereas step section 1 detected additional findings in 9 (4.5%). The difference of additional diagnosis on step sections was 7 cases (3.5%) which was statistically not significant (P ≥ 0.05). This difference as reported by Bruecks et al. was 2%.

The prospective cross-sectional studies have been used to evaluate turn around time.\[16\] Prospective sections help in reducing the turn around time, but they incur a slight increase in the cost due to material cost (storage space, slides, and stains) and labor cost. However, the technicians find it more convenient to cut prospective sections because retrieval of the block, bringing it to the right temperature and then cutting it makes it more laborious and time-consuming. The retrospective sections increase the turnaround time by 1–2 days, but they are cost-effective.\[2,16\] The comparison of our study with other studies available in literature is mentioned in Table 4.

**CONCLUSION**

To conclude, step sections improve the diagnostic accuracy in skin biopsies especially in Hansen’s disease.

**Table 4:** The present study is reviewed along with the studies available in literature

| Headings | Maingi and Helm[14] | Carag et al.[12] | Dyson et al.[13] | Bruecks et al.[2] | Kattel et al.[14] | Present study |
|----------|---------------------|----------------|----------------|----------------|----------------|--------------|
| **Type of step sections** | Retrospective | Retrospective | Retrospective | Prospective | Prospective and retrospective (cross-sectional study) | Prospective |
| **n** | 110 out of 1445 Neoplasms of skin (81) + inflammatory diseases (29); not mentioned 3 (25-50 µ interval) | 69 Actinic keratosis on initial slide. shave, punch, and elliptical specimens 10 (50 µ interval) | 100 Melanomas cases. Shave (56%), punch (28%), and fusiform excision (16%) 6 (50-100 µ interval for smaller biopsies; 300-500 µ for excisions) | 500 All consecutive small skin biopsies (skin curettings, shave, punch) | 2 (50 µ interval) | 2 (50 µ interval) |
| **Type of lesion; type of biopsy** | | | | | | |
| **Number of step sections in addition to initial level or slide 0** | Not done | Not done | Not done | Not done | 20-28 h versus 36-50 h | Not done |
| **TAT calculation (prospective versus retrospective)** | | | | | 2.56 days versus 4.64 days | |
| **Additional findings on SSs** | 41 cases (37.3%) | - | 43% showed increase in tumor thickness. depth increased in 10% of cases | 117+34 cases (30%) | - | 18 cases (9%) |
| **Improved diagnostic accuracy with SSs** | 26 cases (23.6%) | 23 cases (33%) | - | 34 cases (7%) | Increase in sensitivity from 90% to 93.8% | 10 cases (5%) |

TAT: Turnaround time
Jerath, et al.: Value of step sections in dermatopathology

The authors strongly advocate the use of prospective step sections in punch biopsies; at least one step section should be taken (keeping the cost issues in mind), preferably at 100 μ, which was shown to include all the additional findings that led to the diagnosis.

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Conflicts of interest
There are no conflicts of interest.

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