Evaluation of haematoxylin subtypes for the optimal microscopic interpretation of cutaneous malignancies

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Introduction

Non-melanoma keratinocyte carcinomas are on the rise with around 156,000 cases diagnosed annually in the United Kingdom1. Basal cell carcinoma (BCC) is the most predominant form that is encountered2. In recent years Mohs micrographic surgery has gained popularity for the treatment of BCC due to the rapid result turnaround, improved surgical results and preservation of healthy tissue3. The Mohs technique employs mainly H&E-stained frozen sections for surgical margin assessment of cutaneous excisions, utilising microscopic evaluation of the complete, circumferential, peripheral and deep margins. This study aimed to determine which mordant based haematoxylin (Ehrlich’s, Cole’s, Mayer’s, Gill’s I, II, III, Weigert’s, Harris or Carazzi’s) produced the optimal morphological clarity of staining for the identification of cellular morphology of cutaneous BCC.

Method-assessment criteria

Upon completion of the staining of all 100 cases with each haematoxylin subtype, the slides were independently evaluated by two assessors. The scoring criteria were based on a modified UKNEQAS CPT Mohs scheme assessment criteria4. Each assessor allocated scores between 1 to 5 based on the scoring criteria4. The assessment focused mainly on the quality of the haematoxylin staining highlighted in Table 2. The results assigned by each observer for the specificity and sensitivity of each slide were then combined to generate an overall score for each slide out of 10. These results were then added together and divided by 100 to calculate the mean and then a sensitivity and specificity score was generated as a percentage for each dye. These sensitivity and specificity scores were critically evaluated to determine if a particular haematoxylin preparation provided a better pathological assessment of BCC tumours.

Results

All 900 slides stained as expected with each haematoxylin dye subtype demonstrating nuclear staining at different degrees of intensity (Figures 2-11). Staining was limited to the maximum capacity that was possible on the linistainer of 2 minutes 30 seconds. The specificity and sensitivity results for each haematoxylin subtype based on the criteria that were set out in Table 2 are shown in Table 2. Figure 3 shows the sensitivity and specificity scores of all the haematoxylin subtype dyes graphically.

Discussion and Conclusion

The diagnosis and classification of most neoplastic disorders rely on the information gathered from the evaluation of H&E stained sections, with the interpretation of haematoxylin stained nuclear detail playing a key role in determining morphological characteristics. This study identified Carazzi’s haematoxylin as the most optimal staining dye for the identification of BCC tumours for use as part of the Mohs procedure.

The use of Carazzi’s haematoxylin as part of any frozen section procedure, including Mohs, has not been widely assessed. However, this study has highlighted the vastly improved and clear visualisation of nuclear and chromatin detail of Carazzi’s haematoxylin when used as part of the H&E staining process. This was reflected in the higher sensitivity and specificity scores that Carazzi’s obtained overall in this study. Nationally in the UK, there is no standardised staining protocol for use in the MMS procedure. This study helps towards quantifiably determining an optimal H&E staining protocol that can be used as part of this procedure.

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References