

Assessment of Consistency in Assignment of Severe (Grade 3) Squamous Metaplasia to Human Bulbar Conjunctiva Impression Cytology Cell Samples

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ABSTRACT Nucleus-to-cytoplasm (N:C) characteristics of published images of human bulbar conjunctival cells designated as showing severe (grade 3) squamous metaplasia were retrospectively assessed. From publications over a 40-year period, measurements were made from images of cell and nucleus size (based on areas and dimensions) and four different calculations were made for nucleocytoplasmic (N:C) ratios. From 54 published images, the mean nucleus-to-cytoplasm area ratio (NU/CYT AREA ratio) was 0.145 ± 0.077 (range 0.052 to 0.346), compared to two different reference set values of 0.069 ± 0.017 and 0.080 ± 0.021 . Similarly, a nucleus-to-cytoplasm length ratio (as LNLONG) was 0.308 ± 0.080 (range 0.191 to 0.475) compared to reference values of 0.226 ± 0.032 and 0.236 ± 0.034 . Similar differences in reference values were obtained using two other N:C ratio calculations. A wide range of values was found for morphometric N:C indices from published images, especially those without a scale bar, indicating a high incidence of inconsistent grading assignments. Overall, only about 30% of the published images showed morphological features consistent with severe

squamous metaplasia (i.e., with the rest neither enlarged nor showing substantially changed N:C ratios) with no substantial pictorial evidence indicating that cells from the human bulbar conjunctiva have pyknotic nuclei. Current evidence indicates that grade 3 squamous metaplasia cells should be substantially enlarged.

KEY WORDS bulbar conjunctiva, impression cytology, nucleus-to-cytoplasm ratio, N:C ratio, squamous metaplasia

I. INTRODUCTION

Along with the corneal epithelium, the superficial epithelial cell layer of the bulbar conjunctiva constitutes an integral part of the exposed ocular surface.¹ In general terms, any abnormality in lacrimal secretion and/or properties of the tear film will likely lead to alterations in the bulbar conjunctiva and the development of symptoms.^{2,3} As the condition worsens and the tear film undergoes repeated cycles of excessive evaporation, dry eye syndrome or dry eye disease (DED) can develop.⁴ The etiology of the disease is multifaceted, and can be associated directly with lacrimal deficiency due to lacrimal gland disease or indirectly as a result of contact lens wear or ocular surgery.⁴ This repeated desiccation and/or inflammation is expected to result in an alteration of the bulbar conjunctival epithelial cells,⁵⁻⁸ widely referred to as squamous metaplasia. It is characterized by cell enlargement and a notable increase in the nucleocytoplasmic (N:C) ratio of the cells, i.e., the relative area occupied by the nucleus gets proportionately smaller compared to the overall size of the cell. Concurrent changes in the conjunctival goblet cells are generally expected as well.⁵⁻⁸

Conjunctival impression cytology (CIC) has become widely adopted as a method to allow for sampling of cells of the bulbar (or palpebral) conjunctiva without surgically taking a biopsy of the same cells⁷⁻¹⁰ or scraping cells from the conjunctival surface with a spatula.^{11,12} Cells obtained by CIC can be fixed and stained and provide a coronal (or *en face*) perspective of the cells.⁷ From the stained

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OUTLINE

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CIC specimen, the size of the cells and their nucleus staining characteristics can be assessed by light microscopy. The cells can be graded as normal or abnormal, or as one of several stages between normal and severe (substantial) squamous metaplasia, according to cell size and the N:C ratio.^{5,6,13-18}

Various investigators have published descriptions of their grading systems,¹³⁻¹⁸ especially for assignment of the N:C ratio, but it is largely unknown how consistently these grading schemes have been utilized and interpreted. This issue is important, as, for example, differences could be reported between the outcomes of CIC studies simply because the same criteria were not being applied in grading schemes. Therefore, some type of assessment of the application of these grading schemes, specifically to evaluate the consistency of the assignment of cell grades, is needed. One way to address this application of the grading schemes and a way to validate their use would be to undertake masked comparisons of grading assignments.^{18,19} A slightly different approach would be to objectively assess the conjunctival cells on images that have been subjectively assigned different grades of squamous metaplasia in a single-center study,¹⁸ or assigned the same grade (of normal) in multicenter studies.⁵

A substantial number of objective assessments have been reported on squamous metaplasia, with a range of different calculations for assessing cell size and N:C ratio,^{20,21,23,24} thus facilitating objective comparisons. This type of approach to validating CIC image grading could be applied to any published examples of cells considered to show the features of severe squamous metaplasia, now with the specific objective of determining the comparability of the grade assignments. This is possible because numerous reports have included images of cells collected by CIC, often with specific commentary on the extent of cell changes (as a grade squamous metaplasia) and/or particular features of the cells the authors considered to indicate squamous metaplasia.

With the publication of CIC images purported to illustrate squamous metaplasia, it is reasonable to consider that such images were included as representative examples. If the utilization and interpretation of subjective grading schemes for CIC samples have been consistent, then the nucleocytoplasmic characteristics, as assessed objectively, should at least be similar. The present studies were undertaken to assess if this has been the case.

II. MORPHOLOGICAL FEATURES OF CONJUNCTIVAL SURFACE CELLS

In considering the changes to conjunctival cells associated with squamous metaplasia, these cells must be compared with normal cells. With different collection devices (filters) being used to obtain impression cytology specimens, as well as differences in fixation and staining, some differences in the appearance of cell images are to be expected. Notwithstanding, numerous grading schemes have been proposed, two of which are summarized in [Table 1](#).

Overall, any definition of severe squamous metaplasia of the conjunctival epithelial surface cells is likely derived from the most widely used grading scheme provided by Nelson.^{14,15} This scheme has 4 grades or stages for the cells. A number of other grading schemes have been developed that appear to show a rather high degree of similarity to Nelson's.⁶ Another slightly different 4-grade scheme, developed for superior palpebral conjunctiva, is that of Saini and colleagues,¹⁶ while a 6-point scheme was proposed by Tseng.¹³ All schemes include statements on cell size, cell shape, and N:C ratio. Assessment is presumably made by a simple visual inspection of a color image of cells and their nuclei.

In the application of grading schemes, no clear guidelines exist on the type of image upon which the grading should be made or how the relative sizes of the nuclei and the epithelial cells should be compared. The use of medium power fields (200 X final magnification) has been recommended as a suitable balance for allowing visibility of adequate cell detail while providing adequate numbers of cells for subjective and objective (morphometric) assessments,²³⁻²⁵ especially as CIC samples can be very heterogeneous.^{6,23-27} For subjective assignments, the N:C ratios could be based on comparisons of dimensions (e.g., length of nuclei with respect to the length of the cells) or areas (i.e., the areas of the nuclei compared to the overall area of the cells). Since area-based assessments would be expected to result in numerical values for N:C ratios that are very different from dimension-based assessments,^{21,23,24} the importance of defining which is to be used should be self-evident, but this has not been done in published reports.

Regardless of the actual magnification used, the first morphological features noted for the non-goblet cells of the conjunctiva are their overall size and shape. As summarized in [Table 1](#), terms like "small" and "large" are used, along with descriptions of shape, e.g., with "small and round" denoting normal, and "larger" and "polygonal" describing developing squamous metaplasia. This overall assessment, however, likely depends on how densely packed the cells in the sample actually are. For example, cells taken from the exposed nasal bulbar conjunctiva of essentially asymptomatic non-contact lens wearing and nominally ocular surface disease-free individuals may appear approximately round in shape ([Figure 1A](#)).²⁸

In [Figure 1A](#), the visual impression of 'roundness' is probably partly driven by the similarly round appearance

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