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Original article ■

Karyometric Analysis of Squamous Metaplasia, Dysplasia and Squamous Cell Carcinoma of the Lung

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SUMMARY

The sequence of precursor lesions for squamous cell carcinoma may be hyperplasia-metaplasia-dysplasia-carcinoma in situ. The aim of this study was to perform a karyometric analysis of squamous metaplasia, moderate dysplasia and squamous cell lung carcinoma. Bronchoscopic biopsies of normal mucosa in chronic bronchitis patients (n=10), squamous metaplasia (n=10), moderate dysplasia (n=11), squamous cell lung carcinoma (n=48), and normal appearing mucosa surrounding carcinoma (n=11) were retrieved. Three nuclear variables were estimated using an image analysis system. The mean equivalent diameter, nuclear area and volume of equivalent sphere of squamous cell lung carcinoma were significantly larger than in moderate dysplasia, squamous metaplasia and normal bronchial mucosa. Also, the values of equivalent diameter, nuclear area and volume of equivalent sphere were significantly larger in normal appearing mucosa surrounding carcinoma compared to normal mucosa in chronic bronchitis patients. Karyometric analysis may be a helpful ancillary tool in distinguishing squamous cell lung carcinoma from dysplasia, and dysplasia from squamous metaplasia in bronchoscopic biopsy specimens.

Key words: **karyometry, normal bronchial mucosa, squamous metaplasia, dysplasia, squamous cell lung carcinoma**

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INTRODUCTION

The World Health Organization (WHO) published a tumor classification system that defines the three different preneoplastic lesions of the bronchial epithelium: (I) squamous dysplasia and carcinoma in situ (CIS), which may be precursors to squamous cell carcinoma; (II) atypical adenomatous hyperplasia which may be the progenitor lesion for adenocarcinoma, and (III) diffuse idiopathic pulmonary neuroendocrine cell hyperplasia, which may progress to carcinoid (1). It has been commonly accepted that the World Health Organization (WHO)/International Association for the Study of Lung Cancer (IASLC) morphology criteria is the gold standard. However, interobserver and intraobserver variation in histology reports exists among pathologists when using conventional histopathologic criteria (2, 3).

Nuclear morphometry is a method for quantitative measurement of histopathologic changes in the appearance of stained cell nuclei (4). Many studies have performed quantitative assessment of nuclear morphometry in various tumors, including pulmonary intraepithelial neoplasia and lung carcinoma (4-6).

The aim of this study was to estimate karyometric variables of squamous metaplasia, dysplasia and squamous cell carcinoma of the lung.

MATERIAL AND METHODS

Bronhoscopic biopsy samples of normal mucosa in patients with chronic bronchitis (n=10), squamous metaplasia (n=10), moderate dysplasia (n=11), squamous cell lung carcinoma (n=48), and normal appearing mucosa surrounding carcinoma (n=11) diagnosed at the Institute of Pathology, University of Niš, were retrieved from pulmonary pathology archives. All biopsies were reviewed by two pathologists. Normal mucosa was represented by pseudostratified ciliated columnar epithelium. Metaplasia, moderate dysplasia and squamous cell lung carcinoma were classified according to World Health Organization (WHO) criteria (1). After formaline fixation and paraffin embedding, serial histologic sections of 5μm thickness were routinely stained with hematoxylin end eosin.

Karyometric analysis was done using image analyzer LUCIA M 3.51 ab (Laboratory Imaging, Prague, Czeck Republic) at objective 40x (NA=0,65) and final magnification of 1,900:1 of FXA microscope (Nikon, Tokyo, Japan). The binary images were manually edited. In each case, a hundred nuclei were measured. Three nuclear variables were estimated: nuclear area, equivalent diameter and volume of equivalent sphere (7).

The nuclear area is the number of pixels.

The equivalent (E_q) diameter is the diameter of a circle having the same area as the corresponding object:

$$E_q\ diameter = \sqrt{4 \cdot area / \pi}$$

Volume E_q sphere is the volume of the ball:

$$Volume\ E_q\ sphere = \frac{\pi}{6} \cdot \mathrm{E_q\,diameter}^3$$

A statistical analysis was performed using Mann-Whitney test.

RESULTS

The values of the nuclear variables which were assessed are listed in Figures 1-3. The results are expressed as means ± standard deviation.

The mean nuclear equivalent diameter, area and volume of equivalent sphere of squamous cell lung carcinoma were significantly larger than in moderate dysplasia, squamous metaplasia and normal bronchial mucosa (Figures 1-3).

The values of nuclear size (equivalent diameter, nuclear area and volume of equivalent sphere) were found to be significantly larger in normal appearing mucosa surrounding carcinoma compared to normal mucosa in patients with chronic bronchitis. No significant differences were found between normal appearing mucosa surrounding carcinoma and squamous metaplasia.

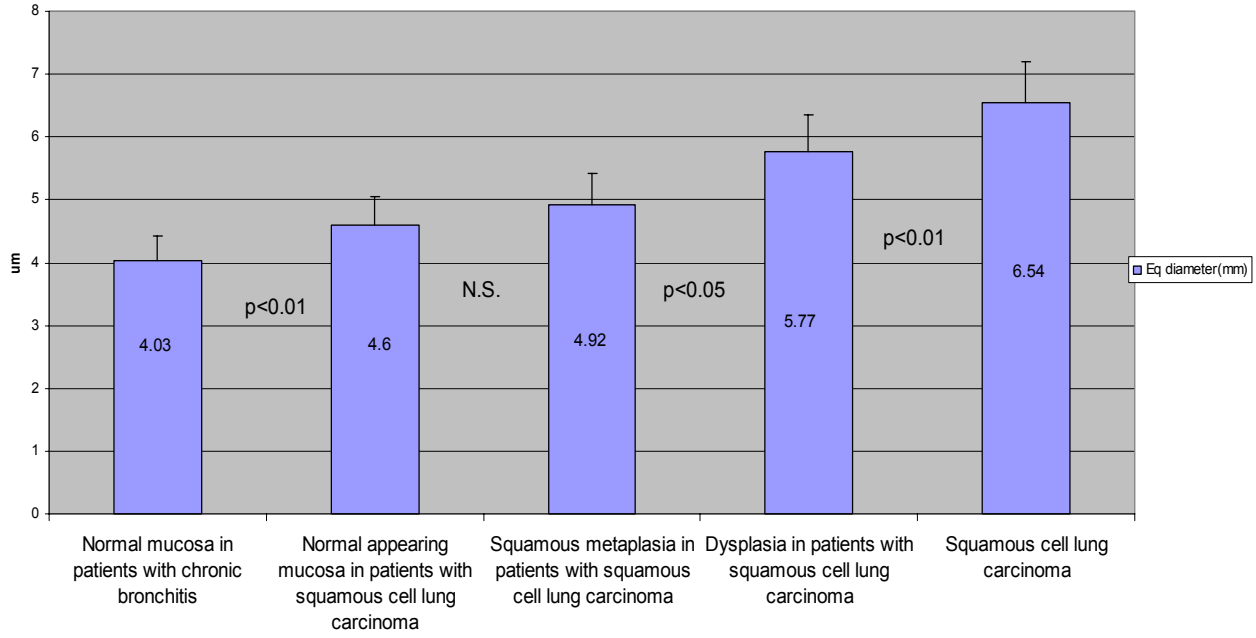

Figure 1. *Equivalent nuclear diameter (μm, mean ± standard deviation)*

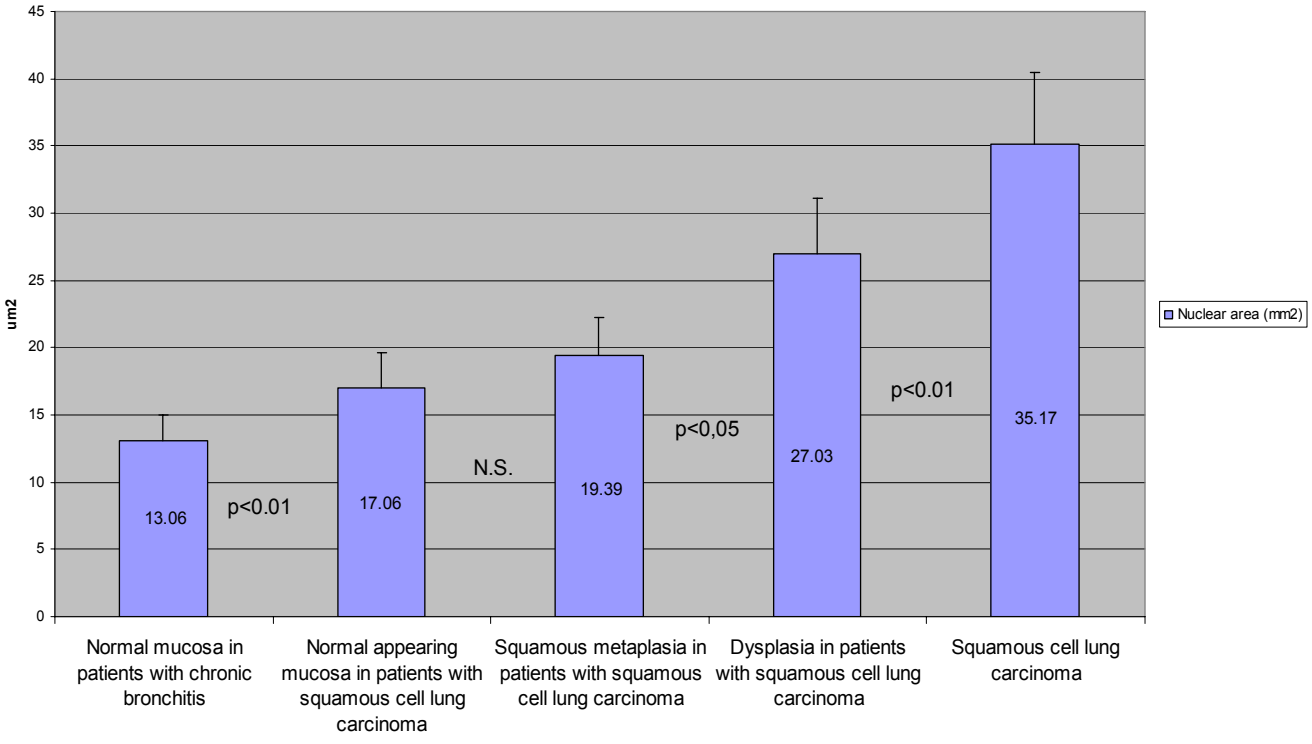

Figure 2. *Nuclear area (μm^2, mean ± standard deviation)*

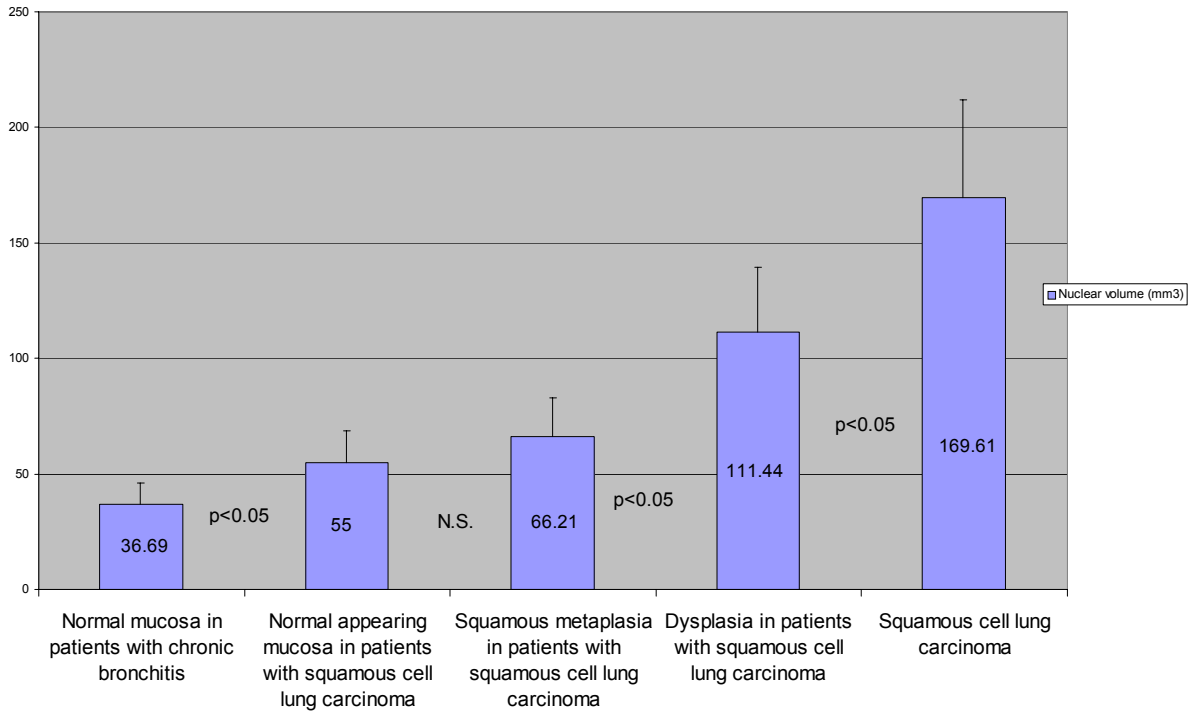

Figure 3. *Nuclear volume (μm^3, mean ± standard deviation)*

DISCUSSION

Lung cancer is not a result of a sudden transforming event in bronchial epithelium, but a multistep process characterized by accumulation of molecular genetic and epigenetic abnormalities, resulting in the formation of invasive tumor. This sequence has been defined for squamous cell carcinomas, although it is less understood for other cell types. It is accepted that squamous cell cancer develops in a gradual and stepwise fashion from normal epithelium, hyperplasia, squamous metaplasia, mild, moderate, severe dysplasia towards carcinoma in situ and microinvasive squamous cell carcinoma. Metaplasia exhibits stratified epithelium and cytoplasmic changes consistent with squamoid differentiation but lacking dysplastic changes. Moderate dysplasia is represented by mild increase in cell size, moderate cellular pleomorphism, and partial progression of maturation. Basilar zone is expanded with cellular crowding in lower two thirds of epithelium, vertically oriented nuclei in lower two thirds and mitoses in the lower third. Squamous cell lung carcinoma is associated with cytological malignant cells with squamous differentiation - keratinization and/or intercellular bridges (1).

The pathological and bronchoscopic diagnosis of preinvasive lesions remains difficult. Large biopsies are often difficult to perform; moreover, it is often difficult to repeat the bronchoscopy to improve the quality and size of biopsy specimens (8). Also, several problems occur with classification scheme. The classification assumes that the lesions progress in an orderly manner, from hyperplasia to metaplasia, and then to increasing degrees of dysplasia, but this may not be the case. There is also some overlap between categories, and in any particular case a range of grades may be seen. Therefore, the existence of a considerable observer variability in the histopathologic reporting of bronchial biopsy specimens is not surprising. However, even among experienced pathologists, full agreement of classifying lung malignancies may only reach a κ value of 0.73; the reproducibility for classifying preinvasive lesions showed intraobserver agreement of 0.71 and interobserver agreement was only 0.55 (2, 3). On the other hand, in a morphometric study by Guillard et al. (2005) (6), a nuclear morphometric index in the bronchial intraepithelial neoplasia is highly reproducible between individual technologists. The coefficient of correlation between the two histopathological technologists who made duplicate morphometric measurements for the 30 biopsies is 0.98 and the intrauser variability is similar.

The size, shape, and chromatin pattern of nuclei, which is operationally defined as quantitative nuclear morphometry of malignant cells, are known to differ from that observed in nonmalignant cell nuclei. Based on these differences, efforts have been made to use the quantitative assessment of nuclear morphometry to enhance diagnostic and prognostic efforts of pathologists. In addition, it has been observed that more subtle differences in nuclear morphometry are observed in histolo-

gically normal-appearing cells in areas that are peripheral to a malignant lesion (9). These differences are called malignancy associated changes (MAC) (10-12). Our findings are in agreement with these observations. In this study we identified significant differences with regard to nuclear size (diameter, nuclear area and volume of equivalent sphere) between normal mucosa in patients with chronic bronchitis and normal appearing mucosa in patients with squamous cell lung carcinoma.

The recent improvements in the resolution of captured images, and the algorithms that measure preneoplastic descriptors should increase our ability to detect and treat preneoplastic lesions. Lung cancer chemoprevention studies have used either metaplasia or a combination of metaplasia and dysplasia as primary intermediate end point biomarkers (13-15). Using nuclear morphometry to quantitate changes in dysplastic lesions and a morphometry index for each biopsy sample, Lam et al. (16) observed that the bronchial dysplasia is one of the best surrogates end point biomarkers currently available to assess the effects of new chemopreventive agents. Similarly, Guillaud et al. were found that in chemoprevention trials nuclear morphometry could supplement histopathology as a surrogate endpoint biomarker for bronchial intraepithelial neoplastic lesions (6). In the present study we also performed an image analysis of squamous metaplasia, dysplasia and squamous cell carcinoma of the lung. Nuclear equivalent diameter, area and volume of equivalent sphere were found to be significantly different between these lesions. Based on our morphometric measurements, we found increased nuclear features from metaplasia to squamous cell lung carcinoma. This finding supports the observation that preneoplastic lesions may be morphological phenotypes of the different steps in the progression from normal to malignant tissue (17). This progression pattern is in agreement with results of other authors (6). Guilard et al. (2005) show that morphometric index is significantly different between invasive carcinoma and carcinoma in situ, between carcinoma in situ and dyplasia, between moderate dysplasia and mild dysplasia and between mild dysplasia plus metaplasia and hyperplasia; however, there is no significant difference in the morphometric index between moderate and severe dysplasia or between mild dysplasia and metaplasia (6). Similarly, in our image analysis, nuclear area, equivalent diameter and volume of equivalent sphere of moderate dysplasia were significantly larger than in squamous metaplasia. These results support the view that bronchial hyperplasia and metaplasia are rather common reactive lesions, and that dysplasia and carcinoma in situ are true premalignant lesions with a high-risk of cancer development (8). In addition, Guillaud et al. (6) observed that the morphometric analysis correlated well with other biomarkers such as loss of heterozygosity (LOH) and cancer development. Nuclear variable that discriminate cells with LOH from those without LOH, in chromosomal regions that are thought to be important in the pathogenesis of lung cancer, is the size of the nucleus. The addition of nuclear morphometry or molecular analysis to histopathologic grading allows more accurate classification of preinvasive lesions and better identification of lesions that are biologically more aggressive (18).

CONCLUSION

Nuclear morphometry is a useful method for objective and reproducible distinction between metaplasia, dysplasia and squamous cell carcinoma of the lung on routine bronchoscopic biopsies, particularly in difficult cases.

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KARIOMETRIJSKA ANALIZA SKVAMOZNE METAPLAZIJE, DISPLAZIJE I PLANOCELULARNOG KARCINOMA PLUĆA

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Sažetak

Sekvence prekursornih lezija planocelularnog karcinoma mogu biti hiperplazija-metaplazija-displazija-karcinom in situ. Cilj rada bio je da se izvrši kariometrijska analiza skvamozne metaplazije, displazije umerenog gradusa i planocelularnog karcinoma pluća. Analizirane su bronhoskopske biopsije normalne mukoze kod bolesnika sa hroničnim bronhitisom (n=10), skvamoznom metaplazijom (n=10), umerenom displazijom (n=11), planocelularnim karcinomom (n=48) i "na izgled normalnom" mukozom u okolini karcinoma (n=11). Ispitivana su tri nuklearna parametra uz pomoć sistema za analizu prikaza. Prosečni ekvivalentni nuklearni dijametar, area i volumen ekvivalentne sfere planocelularnog karcinoma bili su signifikantno veći nego kod umerene displazije, skvamozne metaplazije i normalne bronhijalne mukoze. Vrednosti ekvivalentnog dijametra, areala i volumena ekvivalentne sfere jedara bili su signifikantno veći u "na izgled normalnoj" mukozi kod bolesnika sa planocelularnim karcinomom u odnosu na normalnu mukozu kod bolesnika sa hroničnim bronhitisom. Kariometrijska analiza može biti korisna pomoćna metoda u diferenciranju planocelularnog karcinoma od displazije i displazije od skvamozne metaplazije na bronhoskopskim biopsijskim uzorcima.

Ključne reči: **kariometrija, normalna bronhijalna mukoza, skvamozna metaplazija, displazija, planocelularni karcinom pluća**