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# A morphometric study of nucleolar organiser regions in cervical intraepithelial neoplasia

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*The study sought a correlation between the number of AgNOR granules and the degree of cervical intraepithelial neoplasia (CIN). Thirty-five sections (5 normal, 10 CIN1, 10 CIN2 and 10 CIN3) were subjected to retrospective analysis. The percentage of cells with 1, 2, 3, 4 and more AgNORs was calculated and the number of granules per 100 cells was counted. The number of cells containing single granules decreases. However, the number increases with CIN level when the cells contain 4 and more AgNORs. The number of granules per 100 cells also increases with the degree of CIN. It can be thus concluded that the number of cells with 4 and more AgNOR granules can serve as a CIN differentiation exponent.*

**Key words: cervical intraepithelial neoplasia, argyrophilic nucleolar organiser regions**

## INTRODUCTION

Three grades of dysplasia are defined in the uterine cervix, namely cervical intraepithelial neoplasia (CIN) grades 1–3, which represent progressive degrees of neoplasia. Subjective grading systems used in classical histopathology are often unable to predict correctly the biological behaviour of these lesions. Therefore, a simple laboratory method based on the evaluation of biopsy material that would predict the behaviour of CIN would, be of significant clinical value. Silver staining of nucleolar organiser region-associated proteins (AgNORs) has become a widely used method in pathology, mainly for assessing the prognosis of malignant tumours. AgNORs are loops of ribosomal DNA, which transcribe to ribosomal RNA as the first step of protein synthesis [4]. They are partly located within the fibrillar centres of nucleoli and can be visualised as small black dots, single or in clusters, within the nucleus. An increase in the number of AgNORs is associated with increased tumour aggressiveness, as the mean number of AgNORs per nucleus is higher in malignant than in benign tissues, higher in high grade than in low grade malignancies, and higher in tumours with a poor prognosis compared with those with a good prognosis [2, 3, 7, 8]. In the present study the role of AgNOR measurements as a prognostic marker in CIN was evaluated.

## MATERIAL AND METHODS

A total of 35 cervical biopsy specimens were taken in retrospect from the case files of the Department of Clinical Pathomorphology. Five were normal on histological examination, and there were 10 of each of the three grades of CIN. Two 4 $\mu$m sections were cut from the pretreatment formalin-fixed, paraffin-embedded block. One section was stained with H&E to verify the histopathological

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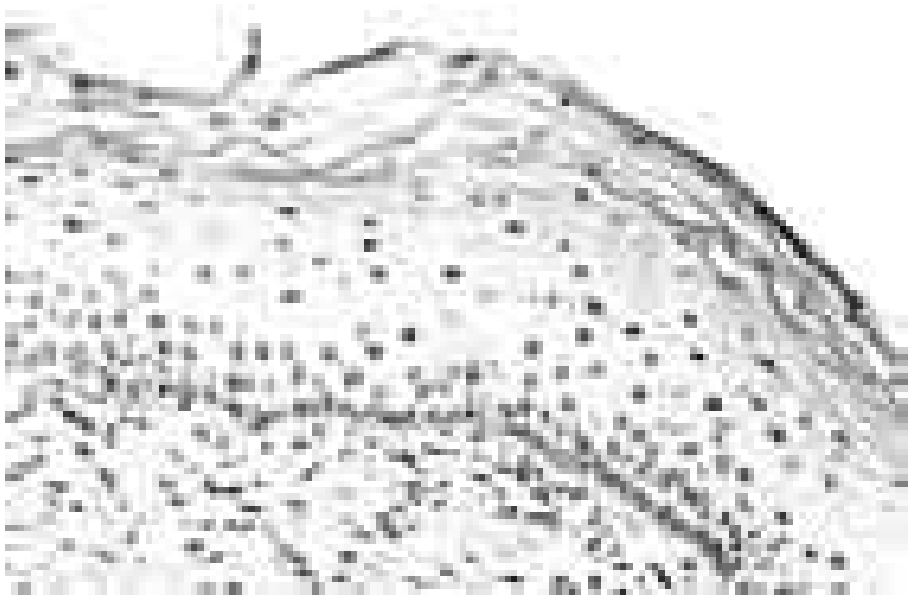

**Figure 1.** Nucleolar organizer regions stained by argyrophilic technique in normal cervical squamous epithelium. Silver-binding black dots are evident in the nuclei of epithelial cells. Mag. 250 ×.

diagnosis; the other was stained by the one-step silver colloid technique according to Ploton et al. [4]. The quantitative analysis of the AgNOR expression was performed by means of digital image analysis, using the DP-SOFT PC software. The images were generated by a BX41 microscope (Olympus Optical, Poland) connected to a Camedia C3030Z camera (Olympus Optical, Poland) and fed into the computer through a DP-DISPLAY 3.0 (Olympus Optical, Poland) for off-line processing. A total of 100 cells/case were analysed with the aid of a 1000x magnification and an oil-immersion lens. The numbers of nuclei with 1, 2, 3, 4, and > 4 AgNORs within all the squares in 5 fields were counted and their percentages were calculated. The study was approved by the Medical University of Białystok Ethical Committee (protocol no. R-I-003/229/2003). The data were stored and analysed by means of CSS Statistica statistical software (Statsoft Inc., Poland). The two-tailed Student's t-test was used for comparison of group means. A P value of 0.001 was taken as significant.

## RESULTS

After incubation with $AgNO_3$ solution, proteins associated with nucleolar organiser regions were seen as tiny granules grouped mainly within the nucleoli and sometimes in the nuclear caryoplasm. The normal squamous epithelium showed single granules mainly in the cell nuclei of the reproductive layer (Fig. 1). In early lesions (CIN1) AgNOR granules were found in the cells of the basement and parabasement layer (Fig. 2). In CIN3 numerous AgNOR granules were observed throughout the epithelium (Fig. 3). Mean AgNOR counts per cell and the percentage of cells with 1, 2, 3, 4 and more granules are presented in Table 1.

## DISCUSSION

The results of our study are in accordance with previous studies and confirm that AgNOR counts allow for discrimination between normal cervical epithelium and CIN, and between CIN and squamous cell carcinoma (SCC) [1–3, 5]. Mean AgNOR counts and the percentage of cells with more than 4 AgNORs were significantly higher in malignancy than in benign epithelium, thus confirming previous reports by Sakai et al. [6]. Our analysis has demonstrated that the number of cells containing single granules decreases, increasing with CIN degree when the cells contain 4 and > 4 AgNORs. The number of granules per 100 cells also increases with CIN. It can thus be concluded that the number of cells containing 4 and more AgNOR granules can serve as a CIN differentiation exponent. The presence of a large number of nucleolar organiser regions that express an increase in the cellular proliferative activity can be treated as an additional index of neoplastic transformation risk in correlation with routine histopathological evaluation.

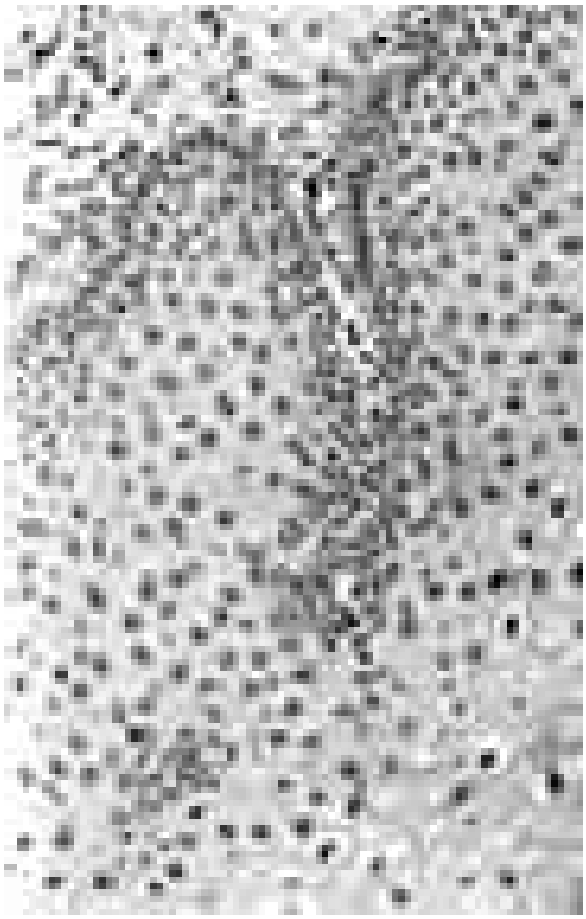

**Figure 2.** Nucleolar organizer regions stained by argyrophilic technique in cervical intraepithelial neoplasia (CIN1). Mag. 250 ×.

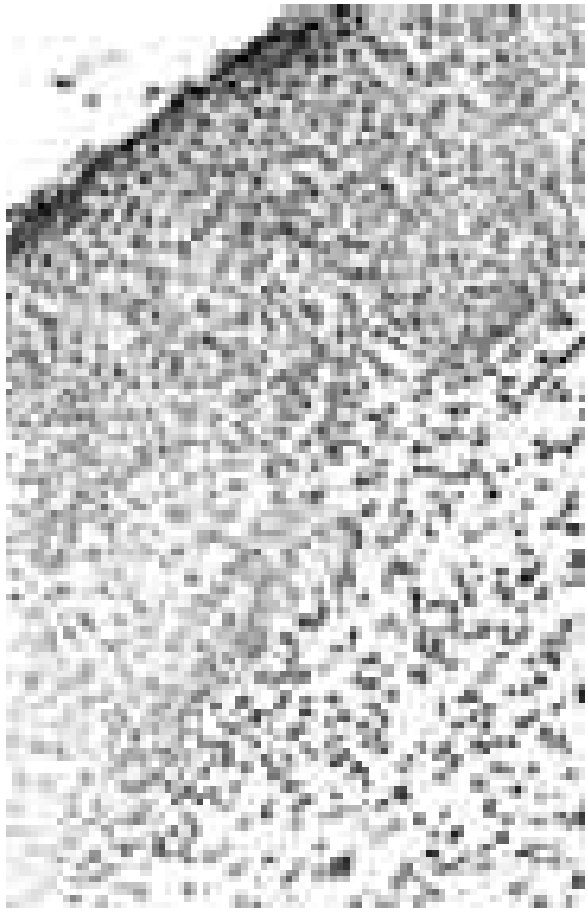

**Figure 3.** Nucleolar organizer regions stained by argyrophilic technique in cervical intraepithelial neoplasia (CIN3). Mag. 250 ×.

**Table 1.** Mean AgNOR counts and percentages of nuclei with 1, 2, 3, 4 and more AgNORs in cervical intraepithelial neoplasia

| | Mean AgNOR[a] | p | 1 AgNOR[b] | p | 2 AgNORs[b] | p | 3 AgNORs[b] | p | 4 and > AgNORs[b] | p |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal (n = 5) | 1.3 ± 0.6 (0.6–1.9) | | 65 ± 7 (57–73) | > 0.001 vs. CIN 1, CIN 2 and CIN 3 | 38 ± 8 (29–47) | | 11 ± 6 (5–16) | | 12 ± 5 (6–18) | |
| CIN 1 (n = 10) | 1.4 ± 0.2 (1.1–1.7) | NS | 54 ± 6 (47–61) | > 0.05 vs. CIN 2 and CIN 3 | 41 ± 4 (36–46) | NS | 14 ± 8 (6–22) | NS vs. normal | 14 ± 5 (8–19) | NS vs. normal |
| CIN 2 (n = 10) | 1.5 ± 0.2 (1.2–1.7) | NS | 49 ± 6 (42–55) | > 0.03 vs. CIN 3 | 38 ± 7 (31–45) | NS | 16 ± 5 (13–24) | NS vs. normal and CIN 1 | 19 ± 7 (12–25) | > 0.001 vs. normal and CIN 1 |
| CIN 3 (n = 10) | 2.3 ± 0.6 (1.6–2.9) | > 0.001 vs. normal CIN 1 and CIN 2 | 45 ± 7 (38–51) | | 36 ± 6 (29–42) | NS | 24 ± 8 (16–32) | > 0.001 vs. normal CIN 1 and CIN 2 | 32 ± 6 (25–39) | > 0.001 vs. normal CIN 1 and CIN 2 |

[a]mean ± standard deviation/cell, [b]mean ± standard deviation (%)

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