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Longitudinal study of malignancy associated changes in progressive cervical dysplasia

Margareta Fležar[1], Jaka Lavrenčak[1], Mario Žganec[2], and Marija Us – Krašovec[1]

[1]*Institute of Oncology,* [2]*Faculty of Electrical Engineering, Ljubljana, Slovenia*

Eight of 29 patients with progressive CIN were followed for 2 to 10 years. Their consecutive Pap smears were destained and stained according to Feulgen thionin method. Cyto-Savant™ high resolution image cytometer (Oncometrics Technol. Corp., Vancouver, Canada) was used for image acquisition and analysis. Average values of nuclear texture features and their probability distributions for consecutive Pap smears from each patient were calculated. Three out of 5 discriminant MAC, highDNAamount, highDNAcomp and highDNAarea, were found to increase as a function of time in 5 out of 8 patients. A preliminary analysis which was performed on non-standardized archival material demonstrated a monotonous increase of discrete texture features as a function of time in patients with progressive CIN.

Key words: *cervix dysplasia; image cytometry; cell nucleus*

Introduction

The goal of an efficient screening of uterine cervical smears is the eradication of invasive cancer of the cervical squamous epithelium. This could be achieved by an early detection and subsequent treatment of precancerous lesions. However, the debatable issue remains the treatment of moderate dysplasias (CIN 2). The follow up of the patients with moderate dysplasias that were not treated showed that some of these lesions regressed to mild dysplastic lesions or even regressed back to normal squameous epithelium, while some progressed to severe dysplasia, carcinoma in situ and invasive carcinoma. At the time of diagnosis of moderate dysplasia one cannot predict whether the lesion will progress and whether the treatment of the lesion should be administered. Additional diagnostic tools would therefore be helpful to establish the biological potential of moderate dysplasias.

Some authors claim that the analysis of cells and – or nuclei by means of image cytometers can yield data about the progression or regression of the lesions.[1,2,3] Their hypothesis is based on measurements of chromatin structure and organization (nuclear texture features). Moderate dysplastic lesions that will progress to cancer differ from the ones that will regress mainly in nuclear texture features. The combination of nuclear texture features discriminating

Correspondence to: Margareta Fležar, MD, MSc, Institute of Oncology, Dept. Cytopathology, Zaloška 2, 1105 Ljubljana. Tel: +386 61 323 063 ext. 4946; Fax:+386 61 1314 180; E-mail: mflezar@onko-i.si

between the two lesions that have a different biological potential is described as MAC (malignancy associated changes) or in some other studies as kariometric factors.[1,2,4]

In our previous study we found significant differences between progressive and regressive moderate dysplasias (CIN 2) by image cytometric analysis in five nuclear texture features which could be explained as MAC.[5] In the present study our aim was to determine the time of onset of MAC which would be characteristic for progressive moderate dysplasias. The occurance of MAC was studied on normal intermediate cells present in the cervical smears obtained by the routine screening program.

Materials and methods

From the files of 3 different laboratories, 29 patients with progressive and 21 patients with regressive moderate dysplasias (CIN 2) were found. However, only 8 patients (ages 22 to 42 years) were followed up with at least three consecutive cervical smears before the diagnosis of progression to CIN 3 (severe dysplasia or carcinoma in situ) was established (Table 1). Their follow-up ranged from 2 to 10 years.

The cervical smears that were originally Papanicolaou stained were destained in acid alcohol and nuclear DNA was stained stechiometrically according to modified Feulgen method by thionin. Image cytometric analysis was performed by an automated high resolution image cytometer Cyto-Savant™ (Oncometrics Technol. Corp., Vancouver, Canada).[6]

Table 1. Patient's diagnosis, age at the time of diagnosis and the length of follow-up in years

Patient	Diagnosis	Age at diagnosis (years)	Follow-up (years)
1	CIN 3	23	2
2	CIN 3	33	6
3	CIN 3	29	9
4	CIN 3	29	7
5	CIN 3	23	6
6	CIN 3	42	10
7	CIN 3	25	6
8	CIN 3	22	3

From the several hundred nuclei acquired automatically, we selected an average of 76 ranging from 15 to 177, well preserved nuclei of normal (non-diagnostic) intermediate squamous cells per cervical smear. Over 100 nuclear features and most importantly nuclear texture features were calculated for each nucleus scanned, detailed description and formulas of nuclear features are described elsewhere.[7] Probability distributions were calculated for each nuclear texture feature, however, longitudinal observation of MAC appearance was performed only for the nuclear texture features found to have discriminative power between progressive and regressive moderate dysplasias in our previous study (Table 2). These features belong to the group of discrete nuclear texture features and reflect subtle differences in condensation between the neighbouring chromatin particles.

Results

The longitudinal observation of discriminating nuclear texture features showed a monotonous increase of values of 3 out of 5 nuclear texture features as a function of time in progressive moderate dysplasias in 5 patients.

The values of "highDNAcomp" increased through the time, that is from the first smear, diagnosed as moderate dysplasia, to the last one diagnosed as severe dysplasia or carcinoma (Figure 1). In five patients (patients 2, 5, 6, 7, 8) we observed a slight decrease, no increase, or slight increase of value between the first and the second smear. However, a substantial increase was found between their second and the third (last) smear. In two patients (patients 3, 4) the values of "highD-

Table 2. Nuclear texture features, which discriminated between progressive and regressive dysplasias

Discriminative nuclear texture features	
HighDNAarea:	Fraction of the total nuclear area occupied by high condensed chromatin
HighDNAamount:	Ratio of IOD* of high condensed chromatin (IOD^*_{high}) to IOD*
HighDNAcomp**:	Shape of high condensed chromatin

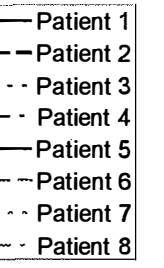
* Integrated Optical Density ** highDNAcompactness

NAcomp" remained basically unchanged throughout the whole period, even though there was a slight increase between their first and the second smear. One patient (patient 1) showed exactly a reverse pattern of nuclear feature changes, that is an overall decrease in the value of this feature throughout the period.

A very similar pattern in changes of values was found for "highDNAamount" (Figure 2). Again, five patients (patients 2, 5, 6, 7, 8) showed slight or no increase or even some decrease between the first and the second smear, and significant increase between their second and the last smear. The same two patients (patients 3, 4) presented with relatively unchanged values of this feature throughout the period. One patient (patient 1), the same as above, showed significant decrease in "hiDNAamount" values from her first to the last smear.

In addition, the longitudinal observation of "highDNAarea" showed a similar pattern of changes to the other two features in the same five patients (patients 2, 5, 6, 7, 8) (Figure 3). For this feature the values for five patients were practically unchanged between the first and the second smear. Afterwards, the values increased substantially. Two patients (patients 3, 4) had the constant values of this feature through the time, and, again, in one patient (patient 1) the values decreased from her first to the last smear.

Other features that were discriminative in the previous study did not show a convincing pattern of increase in the majority of patients.



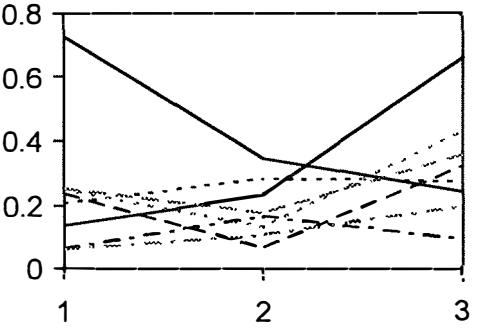


Figure 1. The dynamics of "highDNAcomp" in consecutive smears of patients with progressive dysplasias.

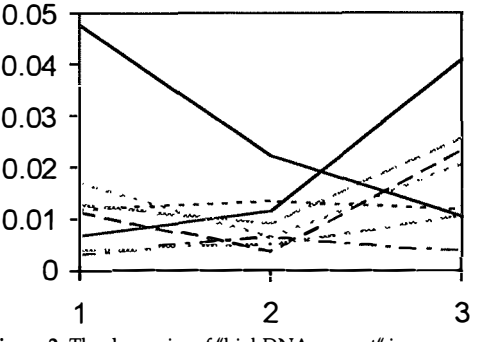


Figure 2. The dynamics of "highDNAamount" in consecutive smears of patients with progressive dysplasias.

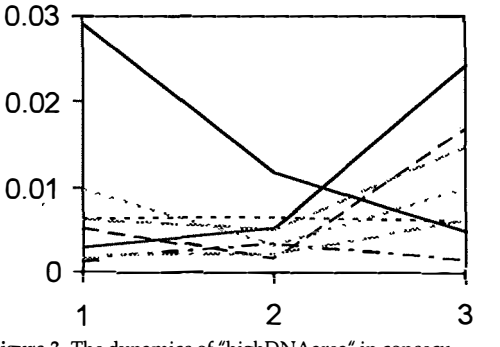


Figure 3. The dynamics of "highDNAarea" in consecutive smears of patients with progressive dysplasias.

Discussion

Population screening of cervical smears for an early detection of preneoplastic lesions of uterine cervix is becoming an important issue in health care programs in different countries all over the world. Some countries have already established efficient screening programs that cover high percentage of women, while others are still in the process of introducing a widespread screening. A successful screening program detects more women with early preneoplastic changes, such as mild and moderate dysplasias of cervical epithelium.[8] By light microscopy it is not possible to assess the biologic behaviour of cervical dysplasias. To avoid the overtreatment of women harbourning dysplasias with nonprogresive course, they should possibly be defined as such by an additional diagnostic analysis.

Image cytometry was used to measure the chromatin structure and organization in cervical neoplastic lesions objectively. Initially, the nuclei of diagnostic cells in dysplastic lesions were analyzed and cytometric differences between the nuclei of different grades of dysplasias were reported.[9,10] These studies were mostly performed as a part of projects that would introduce automated image cytometric screening of cervical smears, and nuclear texture features were used to correct classification of the cells into normal and dysplastic ones.

Besides, the subvisual chromatin changes were also studied in normal intermediate cells, which were also present in the cervical smears together with dysplastic cells.[11, 12, 13, 14, 15] Subtle chromatin changes were first detected by means of light microscopy examination in normal cells of patients with a malignant disease and were described as malignancy associated changes (MAC).[16] With the development of high resolution image cytometers the MACs were objectively measured and they were found not only in connection with different malignant tumors, but also in normal cells of dysplastic lesions, hence also in dysplasias of cervical epithelium.[1,2,11,12,13,14,15]

Subsequently, the idea of MAC was introduced in the studies of progression or regression of cervical dysplastic lesions. Some authors claim that chromatin structure of progressive dysplastic lesion differs from the structure of regressive lesions.[1,2,3] In our previous study we were also able to discriminate between the two types of lesions by using certain nuclear texture features.[5] However, the dynamics of subvisual chromatin structure changes as a function of time has not been studied yet. In our small group of patients, followed-up for 2 to 10 years, the increase of discriminative nuclear texture features in subsequent smears was found in some patients in normal intermediate cells. Since the discriminative texture features from the previous study reflect the condensed portion of chromatin particles in the nucleus, the results of this study suggest a condensation of chromatin with the progression of the lesion. A larger data set with more patients involved in the study should be made to confirm our observations. Also, a larger and more consistent increase of the feature values could be expected, if there were no variations in the initial preparation (the effect of fixation and original staining), which were found to cause differences in nuclear texture features.[17] Besides, nuclear texture features are also influenced by the age of the patients, vaginal inflammations and hormonal status.[13,18]

We expect that more extensive image cytometric studies on different grades of cervical dysplasias will define which patients would progress to more severe lesions and which lesions will regress. If several subsequent smears of the same patient were obtained through a more efficient screening program, we could retrospectively learn more precisely, when the exact progression begins. Accord-

ingly, most appropriate treatment would be planned and most of all the aggressive overtreatment of patients, still in childbearing age, would be avoided.

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