# DNA cytometry as a first-line method for diagnosis of cervical precancer with respect to clinical behaviour

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**Summary**

*Background:* The objective of this study was to estimate DNA image cytometry (DNA-ICM) as a first-line diagnostic method for diagnosis of cervical precancer with respect to its clinical behaviour. *Methods:* 30 consecutive patients with Papanicolaou smears that yielded diagnoses of LSIL or HSIL and showed single cell or stem line aneuploidy were included in a prospective cohort study. Slides were classified according to the Bethesda system. DNA-ICM was performed according to the consensus reports of the European Society of Analytical Cellular Pathology. *Results:* 24 (80%) patients with DNA aneuploid cervical epithelial cell abnormalities had cervical intraepithelial neoplasia (CIN) (CIN I: n = 5; CIN II: n = 6; CIN III n = 13). Six (20%) patients showed no evidence of CIN in subsequent biopsies. During follow-up of three years none of the six patients with negative histology developed cervical precancer or cancer. All 24 (100%) lesions confirmed as CIN by histology showed DNA aneuploidy in cytology. *Conclusions:* DNA-ICM should be used as an objective first-line diagnostic tool for predicting cervical precancer. Yet, due to immune response, DNA aneuploid cervical cell abnormalities do not seem to be enough to predict the definitive clinical outcome in each patient.

*Key words:* Cervix; Cancer; Natural history; DNA cytometry.

## Introduction

None of the established diagnostic methods (cytology, HPV testing, molecular markers, colposcopy, and even guided biopsy) can be relied on for an accurate diagnosis of cervical intraepithelial neoplasia (CIN). The positive predictive value (PPV) of these methods is low [1,2]. Colposcopy can evaluate only lesions limited to the ectocervix. The diagnostic accuracy of colposcopically guided biopsies depends entirely on the sites from which they are taken. Biopsy cannot always detect glandular involvement or invasion. Endocervical curettage shows only extension of CIN into the cervical canal.

DNA aneuploidy, as identified by DNA image cytometry (DNA-ICM), represents the quantitative cytometric equivalent of chromosomal aneuploidy and has been widely accepted as an objective marker of malignant cell transformation [3-7]. However, CIN represents a heterogeneous group of lesions, particularly with respect to their clinical behaviour. CIN in individual women can undergo any of four possible options: 1: regression; 2: persistence, 3: progression, and 4: recurrence.

The objective of this prospective cohort study was to estimate DNA-ICM as a first-line diagnostic method for diagnosis of cervical precancer with respect to its clinical behaviour.

## Material and Methods

### *Patients*

Between February and June 2006, Pap smears from 59 women yielded diagnoses of low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL) at the Cytological Laboratory of the Department of Obstetrics and Gynecology, Medical University of Graz, Austria. Cytological samples were obtained consecutively from routine input at this institution. DNA-ICM was performed in all Pap smears. The median patient age was 34 years (range, 16-78 years).

### *Sample processing and assessment*

Samples from the uterine cervix were obtained using a spatula and/or cervix brush. Colposcopy generally was not performed. The specimens were fixed in alcohol, subjected to Pap staining, and screened by medical technical assistants. The 2001 Bethesda system was used for cytological classification [6].

After morphologic investigation, the smears underwent destaining and restaining according to the method described by Feulgen [8]. Measurements of nuclear DNA content were performed as previously described using a computer-based image analysis system consisting of a Zeiss Axioplan 2 microscope (Zeiss, Jena, Germany) with a 40× objective (numeric aperture, 0.75; Köhler illumination) and a charge-coupled device black-and-white video camera with 572 lines of resolution (VariCam CCIR; PCO Computer Optics, Kehlheim, Germany).

The software package used in the current study was the AutoCyte QUIC-DNA-Workstation (AutoCyte Inc., Burlington, NC), which provides shading and glare correction. The latter was performed at a rate of 2.2%. In each case, at least 30 intermediate squamous cells with normal appearance were measured as internal reference cells. Using squamous cells as an internal reference, latent human papillomavirus (HPV) infection must be considered as a potential cause of a slightly changed peridiploid DNA content [9, 10]. Since a clonal change would be unlikely in cells with normal appearance, latent viral infection should increase the coefficient of variation of reference cells rather than shifting the respective DNA histogram peak. The former potential confounder was limited in the current study, because the coefficient of variation for reference cells was always ≤ 5%. At least 200 epithelial cells with abnormal (i.e., hyperchromatic), enlarged or polymorphic nuclei were

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measured, starting with encircled areas. To increase the detection rate of 9*c*-exceeding events (9cEEs), all Feulgen-stained smears were checked during measurement. All technical instruments and all software used in the study met the standard requirements of the consensus reports of the European Society for Analytical Cellular Pathology (ESACP) [9, 11-13].

Two parameters were assessed for diagnostic interpretation [3-6]. DNA stem line is the G0/G1 cell phase fraction for a proliferating cell population (with a first peak and a second doubling peak or with nuclei in the doubling region). DNA stem line ploidy was defined as the modal value of a DNA stem line in *c* units (*c* = DNA content). DNA stem line aneuploidy was assumed if the modal value of a stem line was < 1.80*c* or > 2.20*c* and < 3.60*c* or > 4.40*c*. Rare DNA events included the 9cEEs, which were defined as the number of cells with a DNA content > 9*c*. Single cell aneuploidy was diagnosed when at least one cell per slide had DNA content > 9*c* (9cEE > 1) [14].

*Follow-up*

The reference standard was histologic examination. The following procedure was agreed upon with the gynaecologists sending samples to the cytological laboratory: Depending on the location of the transformation zone, each patient should have one or more biopsies and/or endocervical curettages within six months after cytological diagnosis. Histological diagnoses were classified according to the CIN system [15]. For patients whose histology showed no evidence of CIN, we considered cytological follow-up at time intervals of six months. Final data retrieval of follow-up cytology was carried out in August 2009.

## Results

In 40 of the 59 patients with LSIL or HSIL, histology was performed within six months after cytological examination. Nineteen patients were lost to follow up due to different reasons. In 24 (60%) of 40 patients histology showed CIN. None of the patients had invasive cancer.

In the collective of 40 patients with subsequent histology, DNA-ICM showed DNA aneuploidy in 30 (75%) of the patients (stem line n = 12, single cell n = 12) whereas ten patients (25%) had normal DNA histograms.

Twenty-four (80%) of the 30 patients with DNA aneuploidy had CIN (CIN I: n = 5; CIN II: n = 6; CIN III: n = 13) and six (20%) patients were negative for CIN (Table 1). During follow-up of three years, none of the six patients with negative histology developed cervical precancer or cancer.

Table 1. — *DNA-ICM characteristics of six women with DNA aneuploid cervical epithelial cell abnormalities showing regressive behaviour during follow-up of three years*.

| Patients | 9c exceeding events | DNA stem line ploidy |
|---|---|---|
| 1 | 12 | no |
| 2 | 8 | no |
| 3 | 5 | 2.52 (12 cells) |
| 4 | 3 | 2.25 (37 cells) |
| 5 | 12 | 4.68 (35 cells) |
| 6 | 1 | no |

## Discussion

The International Consensus Conference on the Fight Against Cervical Cancer (International Academy of Cytology Task Force 8) recommended DNA-ICM as a useful adjunctive method for identifying cervical intraepithelial lesions which require further clinical management [16]. Studies that applied a retrospective or prospective design reported high PPVs (84-100%) for the development of in situ or invasive carcinoma from mild to moderate cervical dysplasias with proven DNA aneuploidy [3-5, 7, 14, 17]. Compared to DNA-ICM, HPV testing and surrogate molecular markers of HPV infection (p16INK4a) may also help identify cases that are associated with underlying CIN; however, the PPVs of these diagnostic methods are reported to be much lower [1].

In the current study, all 24 (100%) lesions confirmed as CIN by subsequent histology had preceding DNA aneuploid smears. This confirms the high value of DNA-ICM for identification of CIN in cervical cytology. In contrast, six (20%) patients with DNA aneuploid cell abnormalities were negative in subsequent histology and during follow-up of three years. This is interpreted as morphological regression, showing that CIN has a substantial variability in prognosis. Four of six patients with regressive morphological follow-up revealed DNA stem line aneuploidy and two had single cell DNA aneuploidy.

DNA stem line aneuploidy reflects the clonal expansion of cells with distinct chromosomal aneuploidy. Abnormal stem lines have also been reported in most invasive cervical carcinomas and have exhibited some degree of correlation with tumour grade and histologic subtype [18-20]. In addition to stem line abnormality, rare events may indicate DNA aneuploidy. These events are likely to be attributable to nonproliferating abnormal cells with different chromosomal aneuploidies and abnormally high numbers of chromosomes [10]. Therefore, rare events may also serve as markers of malignant cell transformation, even if they are not relevant to tumour growth [7].

From the clinical viewpoint, immune surveillance plays a critical role in spontaneous regression, persistence or progression for CIN. Lesions are cleared as a result of a successful cell-mediated immune response directed against early human papilloma virus (HPV) proteins [21]. The HPV type and other partly unknown factors play a role in this. The most comprehensive review of the literature on progression, regression, and persistence rates for CIN comes from a compilation of all studies on the natural history of CIN dated from 1952-1992 [22]. In this review, regression rates for CIN 1 were 60%; for CIN 2 they were 40% and for CIN 3 they were 33%.

Our DNA cytometric finding of a 20% regression rate in patients with DNA aneuploid cell abnormalities is well comparable with the findings reported by Ostor [22] and may reflect a special feature of squamous intraepithelial lesions with HPV protein expression. In squamous intraepithelial lesions without HPV protein expression (i.e., oral cavity), a closer relationship between DNA aneuploidy and clinical outcome was reported [23].

In summary, DNA-ICM should be used as an objective first-line diagnostic tool for predicting cervical precancer. Yet, due to immune response, DNA aneuploid cervical cell abnormalities do not seem to be sufficient to predict the definitive clinical outcome in each case. Longer follow-up is necessary to confirm these findings.

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