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Atypical mitotic figures and the mitotic index in cervical intraepithelial neoplasia

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Abstract We surveyed cervical intraepithelial neoplasia (CIN) to quantify the proliferation rate and the presence of normal and atypical mitotic figures. In the cervical tissue specimens of 127 women with CIN, the area with the highest cell proliferation was identified and, at that site, the proliferation rate was assessed by calculating the mitotic index (MI). Lesions with an MI <2 were not considered further. In the area with the highest proliferation rate, 228 mitoses were classified into one of the following groups: normal mitotic figures (NMFs), lag-type mitoses (LTMs) comprising three group metaphases (3GMs), two group metaphases (2GMs) and other lag-type mitoses (OLTMs), multipolar mitoses (MPMs) comprising tripolar mitoses (3PMs) and quadripolar mitoses (4PMs), and other atypical mitotic figures (OAMFs). The median value of the MI increased significantly from 3 in CIN I through 4 in CIN II to 9 in CIN III ($P<0.001$). The occurrence of the different LTMs was mutually correlated. The frequency of LTMs increased significantly with increasing CIN grade ($P<0.001$), whereas the frequency of NMFs decreased significantly with increasing CIN grade ($P<0.001$). The frequency of OAMFs was not related to CIN grade ($P=0.94$). MPMs were present in low numbers in a minority of the lesions. Spearman's rank correlation coefficient (with 95% confidence limits) between the MI and the number of LTMs, OAMFs and NMFs was 0.66 (0.53; 0.75), –0.14 (–0.32; 0.05) and –0.51 (–0.63; –0.35), respectively. Increasing CIN grade is associated with increasing MI, increasing numbers of LTMs, and decreasing numbers of NMFs. MPMs are very rare events in CIN. The abundant presence of OAMFs seems to be independent of CIN grade and MI.

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Introduction

The presence of abnormal mitotic figures (AMFs) and an increased proliferation rate support a diagnosis of cervical intraepithelial neoplasia (CIN) in equivocal cases [1, 5, 15]. Although AMFs are considered to be characteristic features of CIN and are of diagnostic importance, little is known of the prevalence and interrelationships of specific AMFs in this histological entity. Therefore, we surveyed the mitotic figures and the proliferation rate of CIN lesions. More specifically, we investigated: the proliferation rate of CIN lesions, the spectrum of mitotic figures in CIN, and the relationship between the proliferation rate and the presence of particular atypical mitotic figures in CIN. The study was performed as a cross-sectional study on newly diagnosed patients with CIN.

Patients and methods

Patients were recruited from the colposcopy clinic of the Department of Gynaecology, University Hospital, Groningen. They were eligible for participation in the study if it was their first referral with an abnormal cervical cytology report indicating intraepithelial neoplasia; if they were subsequently diagnosed as having CIN grade I, II or III; if there were no abnormalities in the cylindrical epithelium; and if they were not pregnant. From 1 September 1988 to 1 May 1991, 148 consecutive patients were eligible for the study; 21 of these were excluded because of morphologically unsatisfactory material ($n=10$) or an insufficient amount of material ($n=11$), which left 127 patients.

Routine processing of the tissue specimens

If CIN was diagnosed in the biopsies, the whole transformation zone was excised about 6–10 weeks later by either loop electrosection or cold knife conization. With either treatment technique, the entire lesion was removed for morphological examination. The diathermy loop was used if the entire squamocolumnar junction could be seen and it did not extend up into the canal for more than

5 mm, measured from the anatomical os externum. Details of the technique have been described previously [4]. The tissue was fixed immediately in buffered formalin 8%, pH 7.42. After paraffin embedding, at least four 4-μm-thick sections were cut in an anterior-posterior direction and processed routinely for HE staining. Cold knife conization was performed when the neosquamocolumnar junction extended up into the endocervical canal for more than 5 mm, measured from the anatomical os externum. For histopathological analysis, the excised cone was incised at the 12 o'clock position, stretched, fixed in formalin and embedded in paraffin. A 4-μm section was cut at each hour position, and the 12 sections obtained were processed routinely for HE staining.

The histological diagnosis of CIN according to the WHO criteria [12] was made independently by both pathologists (H.H. and W.J.L.M.P.). If there was a discrepancy in the diagnosis, the specimens were re-examined by both the pathologists together and a consensus diagnosis was made. The pathologists were unaware of the mitosis count results.

Examination of mitoses

The microscopical examinations were performed with a Leitz dialux 20 EB microscope with a 40× npl fluotar Leitz objective (n.a. 0.70) and a 10× wide-field periplan ocular piece. The analysis of the mitotic figures was done by one person (A.M.v.L.).

For the purpose of this study, new sections 4 μm thick were made from all the tissue blocks, in order to achieve uniform quality of the study material. These sections were used to identify the area with the highest mitotic index (MI). This area could be localized in one of the biopsies or in the electrosectioned slice or in the cold knife cone. The MI was assessed by counting the number of mitoses per 1000 nuclei from basal to proximal through the epithelial layer by using a grid. We also assessed the MI on the basis of extended counting, and this estimate of the MI will be referred to as MIext. The MIext was assessed to analyse the correlation between an MI per 1000 nuclei and an MI per (approximately) 10,000 nuclei. For the assessment of MIext, 18 additional step sections were cut from the tissue block which contained the area with the highest MI. Every second section was taken for further analysis to ensure that none of the mitoses was considered twice. In nine step sections, the MI was assessed by counting the number of mitoses in an area of equal size to the area that contained 1000 nuclei in the first section. The MIext was defined as the mean value of the MI, as estimated in the first section and nine additional step sections.

Mitotic figures were defined as figures without a nuclear membrane, which indicated that the cell had passed the prophase, and in which clear hairy extensions of nuclear material were present. Pycnotic nuclei or nuclei with basophilic cytoplasm were not considered [16].

Mitoses were classified into normal and atypical mitoses. Normal mitotic figures (NMFs) were classified according to the definitions given by Rubio [13]. AMFs were defined as mitoses without the typical aspect of normal mitoses. We classified the AMFs into the following categories:

1. Lag-type mitoses (LTMs): figures with nonattached condensed chromatin in the area of the mitotic figure. These were subdivided into:
   a) Two group metaphases (2GMs): metaphases with nonattached condensed chromatin at one polar side;
   b) Three group metaphases (3GMs): metaphases with nonattached condensed chromatin at equidistant positions at the two polar sides (Fig. 1);
   c) Other lag-type mitoses (OLTMs): lag-type mitoses without the configuration of a 2GM or 3GM.
2. Multipolar mitoses (MPMs): metaphases with an abnormal configuration of the equatorial plate; the chromosomes were located along several radial axes. These figures were subdivided into:
   a) Tripolar mitoses (3PMs): metaphases with three radial axes;
   b) Quadripolar mitoses (4PMs): metaphases with four radial axes.
3. Other atypical mitotic figures (OAMFs): atypical mitoses with a morphological appearance which was not reconcilable with one of the abovementioned classes. The OAMFs included ring mitoses, asymmetrical mitoses, dispersed mitoses, etc.

The analysis of mitotic figures was performed on the first section from the area with the highest visual number of mitoses. On arbitrary grounds, we wanted to be 99% sure that any specific AMF was detected if its frequency among the mitoses in a lesion was actually 2% or more. Statistically, the number, n, of mitoses had to be so large that the chance $P(n)$ of not observing any specific AMF among n mitoses selected at random from a tissue specimen with a fraction p of that particular AMF was less than α. From the formula:

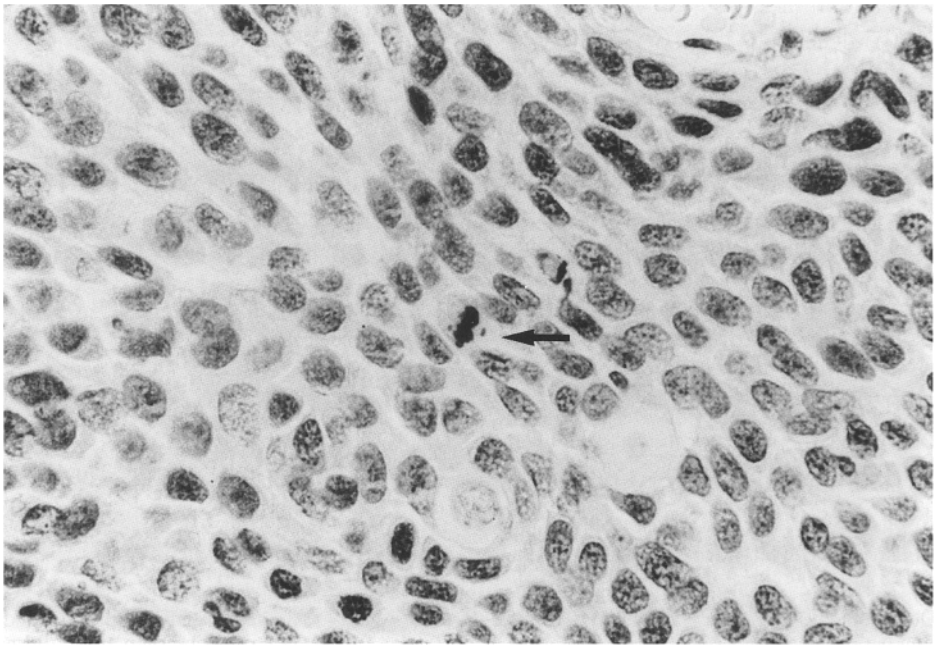

Fig. 1 Three-group metaphase (3GM). ×560

$P(n)=(1-p)^n \leq \alpha$

it followed that

$n \geq \ln(\alpha)/\ln(1-p)$.

For $\alpha=0.01$ and $p=0.02$, we calculated $n \geq 228$. If the selected area in the first section was too small to count 228 mitoses, we used additional odd-numbered step sections (which were made for the assessment of MIext). If the MI was 1 or less, the mitotic figures were not analysed in the particular lesion, because it was practically impossible to count 228 mitoses.

Statistical analysis

The Kruskal-Wallis one-way analysis of variance was used to examine whether there was a significant difference in the distribution of current age or the MI between the three groups of patients with CIN grade I, II and III. The Spearman's rank correlation coefficient was used to express the relationships between the MI and MIext, between the frequency of various mitotic figures, or between the frequency of mitotic figures and the MI. The statistical and graphical procedures were performed with the SYSTAT software package [18]. The 95% confidence interval of Spearman's rank correlation coefficient was obtained with the CIA (Confidence Interval Analysis) software package [9]. *P* values of less than or equal to 0.05 were considered significant.

Results

Patients

In the group of 127 patients, the histological diagnoses were CIN I (n=15), CIN II (n=28) and CIN III (n=84). The ages of the patients ranged from 20 to 66 years, with a mean age of 34.9 (SD 7.4) years. The mean ages of the patients with CIN I, CIN II and CIN III were 32.2 (SD 7.0) years, 35.0 (SD 7.5) years and 35.4 (SD 7.4) years, respectively. There was no difference in the age distribution between the three CIN grades (P=0.30; Kruskal-Wallis one-way analysis of variance).

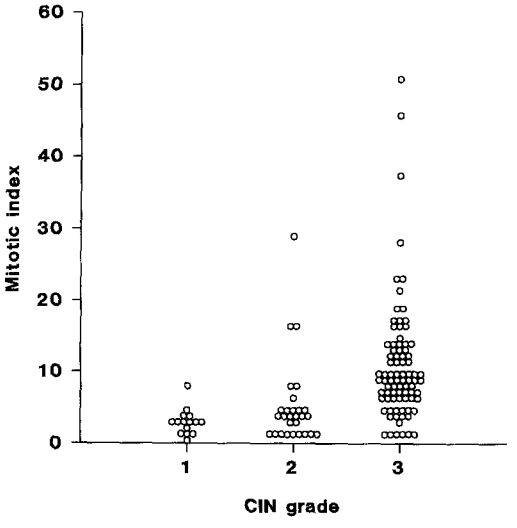

Fig. 2 Distribution of the mitotic index (MI) in relation to the grade of cervical intraepithelial neoplasia (CIN) (n=127)

The distribution of the MI and the MIext in CIN

The MI in the 127 lesions ranged from 0 to 51, with a median value of 8. The median values (and the range of the values) of the MI among the CIN I, CIN II and CIN III lesions were 3 (0–8), 4 (1–29) and 9 (1–51), respectively. The distribution of the MI per CIN grade is presented graphically in Fig. 2. There was a statistically significant difference in the MI between the three CIN grades (P<0.001, Kruskal-Wallis one-way analysis of variance).

The MIext ranged from 0.1 to 43.1 with a median value of 6.5. Spearman's rank correlation coefficient between the MI and the MIext was 0.92 (95% confidence limits 0.88 and 0.94). Of the 108 cases with an MI ≥2, the value of the MIext was <2 in 1 patient. However, of the 19 cases that had a MI <2, the MIext was ≥2 (i.e. 2.0, 2.3 and 2.7) in 3 patients. We conclude that the estimates of the MI and the MIext are very similar. We will use the MI in the further statistical analysis.

Table 1 Distribution of atypical and normal mitotic figures in the three CIN grades. Mitotic figures were counted among 228 mitoses in lesions which showed a mitotic index ≥2

	CIN I	CIN II	CIN III
Total number	11	19	78
3GM			
n	0	7	64
median		3	6
range		1–7	1–17
2GM			
n	10	14	77
median	3	12	22
range	1–4	1–34	2–61
OLTM			
n	10	19	78
median	3	10	21
range	1–7	2–42	2–69
3PM			
n	2	6	14
median	1.5	1	1
range	1–2	1–2	1–3
4PM			
n	0	1	7
median			1
range		1	1–2
OAMF			
n	11	19	78
median	112	109	104
range	73–163	61–160	41–196
NMF			
n	11	19	78
median	111	94	70
range	59–151	21–150	12–136

n=number of positive cases; median and range refer to the number of figures within the group of lesions which showed that particular figure

The AMF types in CIN

From the total group of 127 patients, 19 (15%) showed a MI <2. In this group of 19 patients, the histological diagnoses were CIN I (n=4), CIN II (n=9) and CIN III (n=6). These 19 patients were not included in the further analysis. We performed a quantitative analysis on the occurrence of 3GM, 2GM, OLTM, 3PM, 4PM, OAMF and NMF in all three CIN grades. The results are shown in Table 1.

The 3GM did not occur in CIN I lesions. Comparison with the group of CIN II lesions showed that the group of CIN III lesions had a higher percentage of 3GM-positive lesions, as well as a higher number of 3GMs per 228 mitotic figures in a lesion. The 3GM remains an infrequent finding, however: a total of 31 (44%) of the 71 3GM-positive lesions showed ≤4 3GMs per 228 mitoses, which means a frequency of less than 2%. The 2GM was found in nearly all of the lesions, including the CIN I lesions. Its frequency among the mitotic figures increased with increasing severity of the CIN lesion, from a median value of 3 2GMs per 228 mitoses in CIN I to 22 2GMs per 228 mitoses in CIN III. OLTMs were found in all of the lesions with the exception of one CIN I lesion, and the frequency also increased with increasing severity of the CIN lesion. The frequencies of OLTM and 2GM in CIN lesions were very similar.

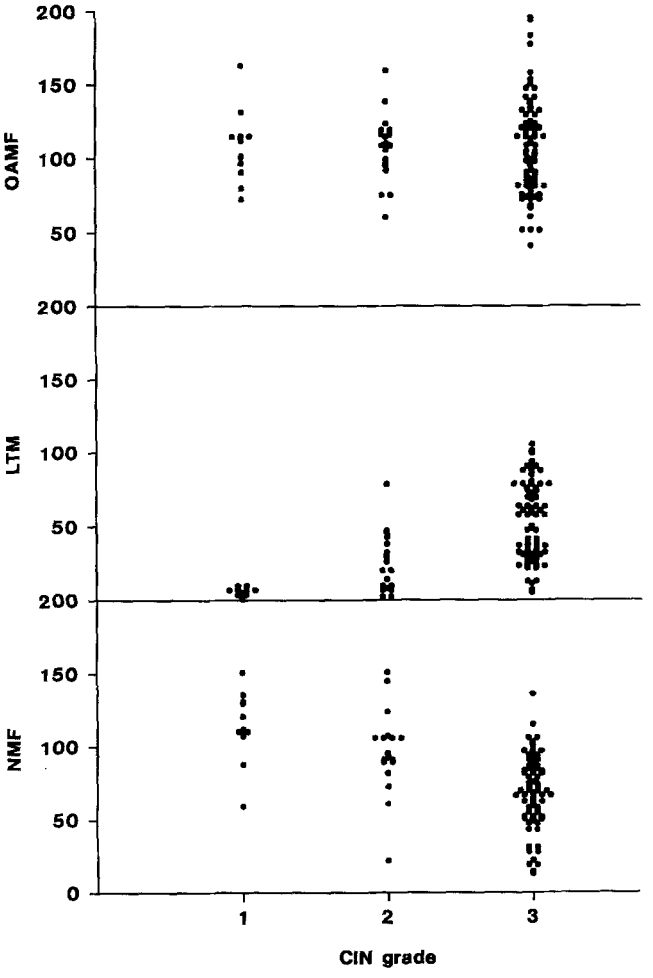

Fig. 3 Frequency of lag-type mitoses (LTM), other atypical mitotic figures (OAMF) and normal mitotic figures (NMF) among 228 mitoses in relation to the CIN grade (n=108; cases with MI<2 excluded)

We subsequently analysed the relationships between the specific LTMs. Spearman's rank correlation coefficient between the occurrence of 3GM and 2GM was 0.83, with a 95% confidence interval from 0.76 to 0.88. All the lesions that showed 3GM also showed 2GM. In 30 cases, 2GM was present without the simultaneous presence of 3GM. Spearman's rank correlation coefficient between the occurrence of 3GM and OLTM was 0.70, with a 95% confidence interval from 0.59 to 0.79. We conclude that the specific LTMs are strongly correlated with each other.

Only 24 (22%) of the 108 lesions displayed MPMs, i.e. 3PMs and/or 4PMs. In all these 24 cases, the total number of MPMs did not exceed 3 per 228 mitoses.

The group of OAMFs comprised the AMFs that could not clearly be delineated as a LTM or MPM. The median number of OAMFs per 228 mitoses ranged from 112 in CIN I lesions to 104 in CIN III lesions.

The median number of NMFs per 228 mitoses ranged from 111 in CIN I to 70 in CIN III.

Figure 3 shows the distribution of LTMs, OAMFs and NMFs per CIN grade graphically. The frequency of LTMs increased significantly with increasing CIN grade (P<0.001; Kruskal-Wallis one-way analysis of variance). The frequency of OAMFs was not related to CIN grade (P=0.94; Kruskal-Wallis one-way analysis of variance). The frequency of the NMFs decreased significantly with increasing CIN grade (P<0.001; Kruskal-Wallis one-way analysis of variance). Because of the small numbers, the frequency distribution of MPMs per CIN grade is not included in Fig. 3.

The relationship between the MI and AMF types in CIN

The 3GM type was found at MI values of 4 or higher. At MI values of 10 or higher, 3GM were absent in only 3 (7%) of the 43 cases. The presence of 3GM in relation to the MI is depicted in Fig. 4. The 2GM was found in a proportion of the lesions with MI values from 2 through 5, and this figure was always present at MI values of 6 or higher. As stated above, 2GM was present without the simultaneous occurrence of 3GM in 30 lesions, all of which had a comparatively low MI.

When we considered the LTMs as a group, Spearman's rank correlation coefficient between the number of LTMs and the MI was 0.66 (95% confidence limits 0.53 and 0.75). We conclude that the frequency of LTMs increases significantly with increasing CIN grade.

Spearman's rank correlation coefficient between the MI and the number of OAMFs was –0.14 (95% confidence limits –0.32 and 0.05), which means that these variables are not significantly associated. However, we did find a significant negative relationship between the

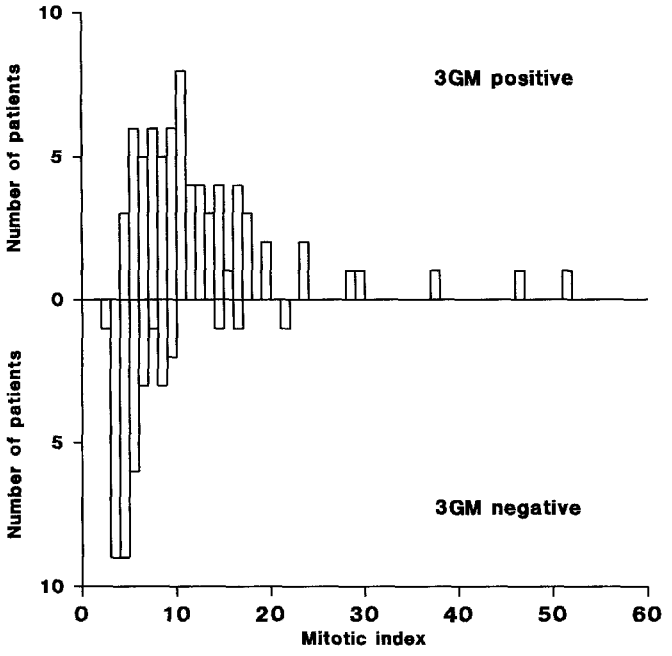

Fig. 4 Distribution of the MI in relation to the presence of three group metaphases (3GM) among 228 mitoses in CIN lesions (n=108; cases with MI<2 excluded)

MI and the presence of NMFs (Spearman's rank correlation coefficient between these variables was –0.51, with –0.63 and –0.35 as the 95% confidence limits). It can be argued that CIN grade confounds the association between the MI and the presence of AMFs, because the MI is also associated with CIN grade. We therefore performed the analysis within the group of CIN III lesions only. The correlation coefficients are somewhat lower, but the conclusions are essentially unchanged: Spearman's rank correlation coefficients (with 95% confidence limits) between the MI and the number of LTMs, OAMFs and NMFs in CIN III lesions were 0.44 (0.24; 0.60), –0.17 (-0.37; 0.06) and –0.24 (–0.44; –0.02), respectively. Because of the small numbers, the MPMs were not considered in this part of the analysis.

Discussion

In the literature, the proliferation rate of a lesion is commonly expressed as the number of mitoses per 10 high-power fields. This method has the disadvantage that the outcome depends on the cell size and the square area of the high-power field [7]. We preferred to assess the cell proliferation rate by counting the number of mitoses per 1000 nuclei (the MI) in the area with the highest number of mitoses visually. We demonstrated that this estimate did not differ substantially from the result obtained by counting the number of mitoses per 10000 nuclei in step sections from this area.

In this study, a standard number of mitoses was counted in the lesions from a large group of consecutively enrolled women. In each lesion, 228 mitoses were counted in sections cut and stained at one laboratory (Laboratory of Pathology, Winschoten); this condition produced slides of constant quality. To minimize intra-individual variation in the examination of mitoses, only the well-defined and clearly recognizable mitotic figures were considered. We adhered to the rules for mitosis counting, and any technically unsatisfactory specimens were excluded from the analysis [2].

We found two reports on mitosis counting in CIN [5, 15]. Both these reports refer to an increased proliferation with increasing CIN grade, which is in agreement with our findings. Very few reports have appeared on the quantitative analysis of different types of mitotic figures in cervical lesions. No meaningful comparison is possible between our results and those of others, because the selection of patients and counting procedures used were completely different. We demonstrated a strong association between the occurrence of LTMs and CIN grade. We found 3GM only in CIN II and CIN III lesions, which confirms the data of other investigators [6]. The 3GM is the best studied mitotic figure of the lag type and its presence seems to be characteristic of neoplasia. In chemically induced lesions of the uterine cervix in mice, 3GM was found in carcinoma in situ and invasive carcinoma, but was absent in dysplastic and inflammatory lesions [14]. In micro-invasive cervical carcinomas of humans, the intraepithelial part contained 3GM in 83–93% of the cases [6, 10]. In an image cytometry study, 3GM proved to be a better indicator of the presence of a large number of nonpolyploid high-ploidy cells in CIN than the traditional histological CIN classification [11]. As large numbers of these cells are related to aneuploidy [17], 3GM can be regarded as an indicator of aneuploidy, which is an established determinant of progressive CIN lesions [3, 8]. In a series of koilocytotic lesions, the presence of 3GM was also confined to the lesions that were aneuploid [19].

We demonstrated that MPMs are seldom found in CIN lesions. The OAMFs were present in large numbers in all of the CIN lesions, but their frequency was not associated with the CIN grade. The increase in the number of LTMs with increasing CIN grade seems to occur at the cost of the NMFs.

In the present study, we have described quantitatively the proliferation rate and the spectrum of AMFs in CIN. To study their possible diagnostic importance, particularly regarding the LTMs, we are planning a survey of the intraepithelial part of micro-invasive carcinomas and non-neoplastic cervical epithelial changes for the MI and the mitotic figures.

References

1. Anderson MC, Brown CL, Buckley CH, Fox H, Jenkins D, Lowe DG, Manners BTB, Melcher DH, Robertson AJ, Mells M (1991) Current views on cervical intraepithelial neoplasia. J Clin Pathol 44:969–978

2. Baak JPA (1990) Mitosis counting in tumors (editorial). Hum Pathol 21:683–685
3. Bibbo M, Dytch HE, Alenghat E, Bartels PH, Wied GL (1989) DNA ploidy profiles as prognostic indicators in CIN lesions. Am J Clin Pathol 92:261–265
4. Burger MPM, Hollema H (1993) The reliability of the histologic diagnosis in colposcopically directed biopsies. A plea for LETZ. Int J Gynecol Cancer 3:385–390
5. Chi CH, Rubio CA, Lagerlof B (1977) The frequency and distribution of mitotic figures in dysplasia and carcinoma in situ. Cancer 39:1218–1223
6. Claas ECJ, Quint WGV, Pieters WJLM, Burger MPM, Oosterhuis JW, Lindeman J (1992) Human papillomavirus and the three group metaphase as markers of an increased risk for the development of cervical carcinoma. Am J Pathol 140:497–502
7. Ellis PSJ, Whitehead R (1981) Mitosis counting. A need for reappraisal. Hum Pathol 12:3–4
8. Fu YS, Reagan JW, Richart RM (1981) Definition of precursors. Gynecol Oncol 12:S220–231
9. Gardner MJ, Gardner SB, Winter PD (1991) Confidence interval analysis (CIA) microcomputer program. British Medical Journal, London
10. Mourits MJE, Pieters WJLM, Hollema H, Burger MPM (1992) Three group metaphase as a morphological criterion of progressive cervical intraepithelial neoplasia. Am J Obstet Gynecol 167:591–595
11. Pieters WJLM, Koudstraal J, Ploem-Zaayer JJ, Janssens J, Oosterhuis JW (1992) The three group metaphase is a morphological indicator of high-ploidy cells in cervical intraepithelial neoplasia. Anal Quant Cytol Histol 14:227–232
12. Poulsen HE, Taylor CW, Sobin LH (1975) Histological typing of female genital tract tumours. World Health Organization, Geneva
13. Rubio CA (1991) Atypical mitosis in colorectal adenomas. Pathol Res Pract 187:508–513
14. Scarpelli DG, Von Haam E (1957) A study of mitosis in cervical epithelium during experimental inflammation and carcinogenesis. Cancer 17:880–885
15. Tanaka T (1986) Proliferative activity in dysplasia, carcinoma in situ and microinvasive carcinoma of the uterine cervix. Pathol Res Pract 181:531–539
16. Van Diest PJ, Baak JPA, Matze-Cok P, Wisse-Brekelmans ECM, Van Galen CM, Kurver PHJ, Bellot SM, Fijnheer J, Van Gorp LHM, Kwee WS, Los J, Peterse JL, Ruitenberg HM, Schapers RFM, Schipper MEI, Somsen JG, Willig AWPM, Ariens AT (1992) Reproducibility of mitosis counting in 2,469 breast cancer specimens. Results from the multicenter morphometric mammary carcinoma project. Hum Pathol 23: 603–607
17. Van Driel-Kulker AMJ (1986) Automated image analysis applied to the diagnosis of cervical cancer. Dissertation, University of Grenoble
18. Wilkinson (1990) SYSTAT: the system for statistics. SYSTAT, Evanston, Ill
19. Winkler B, Crum C, Fujii T, Ferenzy A, Boon M, Braun L, Lancaster WD, Richart RM (1984) Koilocytotic lesions of the cervix. The relationship of mitotic abnormalities to the presence of papillomavirus antigens and nuclear DNA content. Cancer 53:1081–1087