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Invited Review

What is the value of proliferation markers in the normal and neoplastic cervix?

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Summary. Markers of cellular proliferation have been widely applied in cervical disease and include techniques which are applicable to routinely processed tissue including standard hematoxylin and eosin sections, and sections labelled with antibodies to Ki-67 and PCNA proteins. Flow cytometry and *in vivo* techniques including labelling with Bromodeoxyuridine (BrdU) and radiolabelled thymidine require more specialized facilities. Increases in the mitotic index and the Ki-67 and PCNA labelling indices, the incidence of aneuploidy together with increases in *in vivo* labelling with BrdU and radiolabelled thymidine have been demonstrated as the grade of cervical intraepithelial neoplasia (CIN) increases. With respect to invasive tumours increases in these parameters are associated with increased tumour size, stage and improved survival after radiotherapy.

At present the major practical application of these markers appears to be in distinguishing between postmenopausal atrophy and CIN lesions on histological sections and, in combination with the Papnet system, in identifying high grade dyskaryosis on blood stained cervical smears. Future development may permit the identification of those patients whose CIN lesion will progress, and who require treatment, to be distinguished from those whose lesions will stay static or regress and who can be followed up cytologically. This promises a more rational use of health care resources.

Most of the studies to date have been on small numbers of cases. Meta-analysis of existing studies and large, possibly multicentric, prospective studies are needed to elucidate the value of these markers.

Key words: Cervix, Cervix neoplasms, Cervical intraepithelial neoplasia, Cell division, Flow cytometry, Nuclear proteins, Nucleolus organizer region

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Introduction

A number of recent studies which assess the proliferative state in the human cervix have become available. Proliferation in the normal cervix, in cervical intraepithelial neoplasia and in invasive cervical carcinomas can be assessed by a range of techniques requiring technologies of varying sophistication and accessibility. These techniques can be considered in four broad groups:

1) methods which can be applied to routinely processed histological sections and cytological smears often requiring no additional technical input, for example, mitotic counts;

2) dynamic, biological techniques in which a marker compound, such as tritiated thymidine, is either injected locally into the cervix *in vivo* or in which fresh tissue is incubated with the marker *in vitro*;

3) techniques which use widely available immunoperoxidase and histochemical techniques and which can be easily performed using the facilities available in a typical diagnostic laboratory, for example, Ki-67 labelling index and AgNOR counts;

4) techniques requiring specialised equipment which is usually available only in research facilities and hospitals associated with university departments but which do not usually require special tissue handling, for example, flow cytometry.

Techniques based on routine preparations

In the normal cervix mitotic activity is usually confined to the basal and parabasal layers. With increasing histological grade of cervical intraepithelial neoplasia (CIN) the numbers and height of mitotic figures within the epithelium increases. Significant differences in the mitotic index in different grades of CIN have been described, perhaps reflecting variation in the speed of the cell cycle (Kobayashi et al., 1994). CIN lesions demonstrating aberrent mitoses are more likely to be associated with human papilloma virus type 16

(HPV 16) (Burger et al., 1997), a type of HPV particularly correlated with invasive and high grade intraepithelial lesions (Lorincz et al., 1992) and to exhibit DNA aneuploidy, a characteristic associated with the progression of low grade CIN to high grade lesions and of high grade CIN to invasive malignancy (Bibbo et al., 1989). Particular prognostic importance has been attributed to the presence of three group mitotic figures (Pieters et al., 1992). Morphological atypia and increasing mitotic rates are also associated with increased proliferation rates as assessed using the PCNA labelling index (Kobayashi et al., 1994; Steinbeck et al., 1995)

The availability of a mitosis specific antibody, which in combination with image analysis techniques can be used to study the frequency and distribution of mitotic figures in cervical epithelium will facilitate the study of these theoretical matters and assist in evaluating their practical applications (Hu et al., 1995).

Biological markers of proliferation

Autoradiography of tissue sections incubated in tritiated thymidine and the use of bromodeoxyuridine (BrdU) a pyrimidine analogue are sophisticated techniques. Special facilities, to accommodate living patients to whom radiolabelled thymidine or the oxygen sensitiser BrdU have been administered or to culture tissue biopsies in these compounds immediately after they are obtained from the patient, are required. These methods have not found a wide application since the need for such specialized facilities adds to their complexity and limits their routine use. Nevertheless they have contributed to our understanding of proliferation in the cervix.

Early studies using tritiated thymidine (Schellhas and Heath, 1969; Avarette et al., 1970) identified parabasal cells as the major proliferative component in the human cervix, the basal cells acting as a reserve cell layer. Increasing grades of CIN were associated with increasing numbers of labelled cells and with labelled cells being identified in the upper layers of cervical epithelium. In this early study increased labelling was identified in pregnant women without morphological evidence of CIN, the pattern of labelling resembling that seen in CIN I (Schellhas and Heath, 1969).

BrdU usually involves the incubation of a freshly obtained fragment of tissue *in vitro*. Increased proliferative activity has been identified in cervices showing squamous metaplasia and reserve cell hyperplasia with respect to samples of normal cervix (Fukuda et al., 1990). Progressive increases in the percentage of labelled cells and the layers of cervical epithelium containing labelled cells are identified with increasing histological grade of CIN (Fukuda et al., 1990; Kaneko and Izutsu, 1995).

In invasive cervical carcinomas Bolger et al. (1993) identified marked heterogeneity in the BrdU LI within tumours. Nevertheless the labelling index (LI) increased with advancing stage and increasing tumour size. Minagawa et al. (1993) suggested that the LI also correlated with the patient's age.

Special techniques available in routine diagnostic laboratories

Ki-67 is an antibody generated when mice were immunized with nuclear extract from the Hodgkin's disease derived L428 cell line (Gerdes et al., 1983, 1984). Ki-67 immunolabelling correlates with the cell cycle and consequently is a widely employed marker of cell proliferation (Sawhney and Hall, 1992). Ki-67 has been immunolocalised to the parabasal and basal layers in the normal (Al Saleh et al., 1995) and metaplastic (Bulten et al., 1996) ectocervix.

Variations in the Ki-67 labelling in the normal cervix in different phases of the menstrual cycle have been described in detail by Konishi et al. (1991). In their study they were unable to identify Ki-67 labelling in either the basal or reserve cell layers of the normal squamous epithelium during the follicular phase. There was some positivity in the parabasal layer. In the luteal phase the LI increased with focal positivity of basal and reserve cell epithelium and extensive parabasal cell positivity. The number of cycling cells also increased during pregnancy, reflecting earlier studies using tritiated thymidine (Schellhas and Heath, 1969). Ki-67 labelling was also identified in the parabasal, basal and intermediate layers of a series of condylomas studied by this group. In CIN lesions Ki-67 labelling in the basal and parabasal layers was again noted, with additionally, the identification of Ki-67 positive cells in the intermediate and superficial layers of the squamous epithelium, the percentage of positive cells and the height above the basement membrane at which they were located, increasing as the grade of CIN increased (Al Saleh et al., 1995; Konishi et al., 1991). Konishi et al. (1991) identified progesterone receptors in the majority (19 of 26) of cases of CIN. Condylomas were also found to be hormone-dependent, their growth increasing during pregnancy. They concluded that the growth of healthy and diseased cervical epithelium was regulated by sex hormones operating via progesterone and estrogen receptors.

Al Saleh et al. (1995) described a similar distribution of Ki-67 labelling in normal and dysplastic cervical epithelium but identified a higher Ki-67 labelling density in cervices in which HPV types 16/18 and 31/33/35 were identified in comparison with the labelling density associated with HPV types 6/11. The association between proliferation rate and particular HPV types has been confirmed by Tervahauta et al. (1994).

In Al Saleh et al. (1995) study the Ki-67 labelling density did not allow normal, metaplastic and low grade squamous intraepithelial lesions (SIL's) to be distinguished since there was considerable overlap between the groups. It was possible however to distinguish between the normal cervix and low grade

SIL's on the one hand and high grade SIL's on the other. Devictor et al. (1993) found a higher Ki-67 LI in cervices showing microinvasive and invasive cervical carcinomas than in those showing CIN III without invasion, a finding confirmed by others (Konishi et al., 1991).

In a small series of stage I and II invasive carcinomas Cole et al. (1992) identified no association between LI and tumour stage, lymph node status or short term survival rate. Nakano and Oka (1993) found that a high LI was associated with a better histological response and improved survival rate after radiotherapy. A low LI was associated with tumour recurrence and metastasis later. Oka et al. (1993) discovered that following radiotherapy (5.4-9.0 Gy) the growth fraction in patients with low LIs doubled. Unfortunately follow-up data was not available to indicate if this was transformed into an improved survival rate.

MIB-1, an antibody which detects the Ki-67 antigen on formalin fixed paraffin embedded tissue, allowing its use on routinely processed diagnostic and archival material, has shown similar results to those seen with conventional Ki-67 immunoreactivity on frozen section material (Bulten et al., 1996; McCluggage et al., 1996; Payne et al., 1996). The presence of a few scattered immunoreactive cells in the apparently maturing epithelium and at higher levels than the most superficially placed mitoses (Bulten et al., 1996; McCluggage et al., 1996) lends weight to the view that some degree of cellular abnormality involves the entire thickness of the squamous epithelium in CIN lesions. CIN is graded according to the third of the epithelium (lower, middle, upper) in which neoplastic basaloid cells appear. Grading the CIN lesion on the basis of the third of the epithelial thickness in which MIB-1 immunoreactive cells could be identified would result in the over-grading of some CIN lesions (Bulten et al., 1996). Bulten et al. (1996) developed a technique which used specialised image analysis equipment to quantify the Ki-67 LI; the number of Ki-67 positive cells per length of basement membrane; the maximum value of the relative distance of Ki-67 positive nuclei from the basement membrane and the 90th percentile of the relative distance of Ki-67 positive nuclei from the basement membrane. All four proliferation related parameters correlated statistically with the grade of CIN but the 90th percentile allowed precise classification of the CIN lesion. The need to use specialised image analysis equipment would however compromise the use of an otherwise simple antibody technique for use in routine diagnostic laboratories.

The combination of MIB1 with an image analysis system was also described by Boon et al. (1994) who used the Papnet system to detect labelled dyskaryotic cells in cervical cytology smears. Following microwave processing high grade SIL's (squamous intraepithelial lesions) were detectable but lower grade lesions were not. Although the system did not allow for the distinction between progressive and regressive lesions, Boon et al. (1994) concluded it provided a means of detecting intact fragments of high grade SIL's particularly on blood-stained smears. This is a very valuable innovation. A system which allowed these cases to be detected would improve the effectiveness of this cervical screening programme since Robertson and Woodend (1993) have shown that such cases are often overlooked by cytology screeners and are a major cause of false negative smears preceding a diagnosis of invasive cervical carcinoma.

In invasive carcinomas a lower cycling cell population has been demonstrated in adenocarcinomas than squamous cell carcinomas. The cycling population increases in squamous cell carcinomas after radiotherapy whilst that in adenocarcinomas remains static (Oka et al., 1996).

Proliferating cell nuclear antigen (PCNA) is an auxiliary protein for DNA polymerase delta and is an absolute requirement for DNA synthesis. It can therefore be used as a marker of cell proliferation in histological material (McCormick and Hall, 1992).

In the cervix the distribution of PCNA reflects that of Ki-67 with immunolocalisation to the basal and parabasal layers of the normal cervix (Al Nafussi et al., 1993; Mittal et al., 1993; Raju 1994; Steinbeck et al., 1995) and increasing number of PCNA immunopositive epithelial cells at progressively higher layers in the squamous epithelium as the grade of CIN increases (Al Nafussi et al., 1993; Mittal et al., 1993; Kobayashi et al., 1994; Raju, 1994; Kaneko and Izutsu, 1995; Steinbeck et al., 1995). The development of a condyloma is also associated with some cellular positivity in the upper cell layers. Kobayashi et al. (1994) compared the PCNA LI in the cells of the basaloid neoplastic layers in different grades of CIN. There was no evidence of a statistically significant difference in the lower grade lesions. A statistically significant difference was demonstrated in those CIN lesions in which the full thickness of the epithelium (carcinoma *in situ*) was replaced by neoplastic basaloid cells and those in which some superficial maturation (severe dysplasia) was identified. Raju (1994) identified a higher LI in foci of intracryptal extension of CIN with all the neoplastic cells reacting. These increases in LI reflect an alteration in biological behaviour since patients with intracryptal extension and full thickness dysplasia are at increased risk of recurrent or invasive disease (Tidbury et al., 1992).

As with the Ki-67 LI, Oka et al. (1996) identified a lower PCNA LI in adenocarcinomas than in squamous cell carcinomas. No increase in cycling cell population was demonstrated following radiotherapy. Both Raju (1994) and Steinbeck et al. (1995) found that proliferation was confined to the marginal neoplastic cells with weak or no labelling of keratinising epithelium. Lichtnegger (1995) who was unable to identify an association between PCNA LI and lymph node metastasis whilst Al Nafussi et al. (1993) found a high LI in squamous cell carcinomas irrespective of clinical outcome, with no association between the LI and either the tumour grade or survival.

In glandular lesions statistically significant differences in the Ki-67 and MIB-1 LIs between benign endocervical lesions and glandular intraepithelial neoplasia of the cervix have been identified. The differences between invasive adenocarcinoma and benign tuboendometrioid metaplasia was also found to be statistically significant (McCluggage et al., 1995; van Hoeven et al., 1997). Unfortunately the difference between the LI in invasive carcinomas and cervical intraepithelial glandular neoplasia, a frequent diagnostic dilemma especially in small focal or heavily inflamed lesions, was not statistically significant. Furthermore given the degree of overlap between the invasive and benign groups in one study, it is unlikely that these results will find a diagnostic application (McCluggage et al., 1995).

As with other proliferation markers, trends in the numbers and distribution of AgNOR's in association with increasing grades of CIN have been demonstrated (Rowlands, 1988; Thicket et al., 1989; Kobayashi et al., 1994; Kaneko and Izutsu, 1995). Although some authors have found statistically significant differences in AgNOR counts between normal and low grade as opposed to high grade CIN lesions (Rowlands, 1988; Kaneko and Izutsu, 1995) the diagnostic value of these findings is limited by the degree of overlap in the different grades of CIN (Thicket et al., 1988; Kobayashi et al., 1994; Kaneko and Izutsu, 1995).

In glandular lesions Allen and Gallimore (1992) identified statistically significant differences in the AgNOR counts in normal and microglandular hyperplasia compared with in-situ and invasive adenocarcinoma. AgNOR counts would not have allowed a distinction between in-situ and invasive glandular neoplasia.

AgNOR counts in invasive cervical carcinomas have shown statistically significant differences between adenocarcinoma and adenosquamous carcinoma (Miller et al., 1994) and between invasive and benign endocervical tissues (Allen and Gallimore, 1992). Statistically significant differences were also found in high grade as opposed to low grade tumours and in tumours with lymphovascular invasion. No association could be demonstrated between AgNOR counts and tumour size or stage (Miller et al., 1994). Thus AgNOR counts were a feature of histological type and differentiation rather than tumour extent.

Techniques requiring specialised equipment

Nuclear DNA content has been measured using flow cytometry (Thickett et al., 1989; Pieters et al., 1992; Minagawa et al., 1993; Kristensen et al., 1995; Zolzer et al., 1995) and static cytometry (Nasiell et al., 1979; Bibbo et al., 1989; Steinbeck et al., 1995) in both in-situ and invasive neoplasms. The proportion of aneuploid as opposed to polypoid lesions has been shown to increase with increasing grade of CIN (Steinbeck et al., 1995). A correlation between high grade CIN lesions and proliferative index (Thickett et al., 1989) has also been demonstrated.

In a large series of 211 cases, Bibbo et al. (1989) found that over 90% of aneuploid CIN II and III lesion either progressed to high grade disease or remained static. All aneuploid cases of CIN I progressed and all polyploid cases of CIN 1 regressed implying that this might provide a means of identifying those cases of CIN I which require follow up and treatment and those which might safely be discharged from follow up. All of the cases which progressed to invasive malignancy were aneuploid. Overall Bibbo found aneuploidy to be a better indicator of disease progression than the histological grade of CIN. Applying methods of DNA quantitation to cervical smears would therefore seem to offer a possible means of identifying those patients in whom colposcopy can be avoided. Unfortunately a follow up series of 22 patients did not identify a difference between the DNA distribution of 12 cases of moderate dyskaryosis which progressed and the 10 cases which did not (Nasiell et al., 1979).

In invasive squamous cell carcinoma of the cervix Minagawa et al. (1993) found that aneuploid tumours were more common in patients over 65 than in younger women. Kristensen et al. (1995) was unable to identify either DNA ploidy or S phase fraction as being of significance in predicting patient prognosis in a large study of 415 cases. Miller et al. (1994) found no correlation between either DNA index or S phase fraction and tumour stage, grade or histological type. Large tumours and those with lymphovascular involvement had a higher DNA index.

Practical applications

Can any of these findings be applied to diagnostic practice? Since Ki-67 and PCNA immunoreactivity are largely confined to the basal and parabasal layers of normal epithelium, with only occasional scattered positive cells in the intermediate and superficial layers in metaplastic epithelium, these techniques may permit the exclusion or confirmation of a CIN lesion in problematic cases. The technique would appear to be of particular value where the atrophic epithelium, encountered occasionally in hysterectomies from post-menopausal women, bears a resemblance to a CIN III lesion (McCluggage et al., 1996). If there is no evidence of Ki-67 labelling in the epithelial nuclei or if it is confined to the parabasal layer, the diagnosis of atrophy will be confirmed. This will avoid the need to process and examine additional blocks.

Unfortunately given the degree of overlap between different grades of CIN with AgNOR counts and, PCNA and Ki-67 labelling, it seems unlikely that these methods will provide an alternative to the morphological grading of CIN in routine practice. Initial results suggest that in the future these techniques or the assessment of nuclear DNA may allow those cases of CIN which will progress, and therefore require treatment, to be distinguished from

those which will regress or remain static requiring only follow up or even allowing the patient's discharge. To date most of these studies have been confined to small numbers of cases and further research in which statistically significant numbers of patients are followed up will be required before the role of proliferation markers in routine practice can be established. Meta-analysis of series individually consisting of small numbers of cases may also be of value in elucidating their value. In the meantime the second major application of proliferation markers would appear to be limited to detecting high grade squamous lesions in cervical smears which are obscured by blood (Boon et al., 1994).

With regards to invasive carcinomas, these techniques may allow the identification of those small localised lesions which have a high risk of progression and in which lymph node dissection to identify metastases would be of benefit. Alternatively larger carcinomas with a low risk of localised spread or lymph node metastases may be identified averting the need for systemic chemotherapy (Cole et al., 1992).

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References

Allen J.P. and Gallimore A.P. (1992). Nucleolar organizer regions in benign and malignant glandular lesions of the cervix. J. Pathol. 166, 153-156.

Al-Nafussi A.I., Klys H.S., Rebello G., Kelly C., Kerr G. and Cowie V. (1993). The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in the uterine cervix and cervical squamous neoplasia. Int. J. Gynecol. Cancer 3, 154-158.

Al-Saleh W., Delvenne P., Greimers R., Fridman V., Doyen J. and Boniver J. (1995). Assessment of Ki-67 antigen immunostaining in squamous intraepithelial lesions of the uterine cervix. Correlation with the histologic grade and human papillomavirus type. Am. J. Clin. Pathol. 104, 154-160.

Averette H.E., Weinstein G.D. and Frost P. (1970). Autoradiographic analysis of cell proliferation kinetics in human genital tissues. 1. Normal cervix and vagina. Am. J. Obstet. Gynecol. 108, 8-13.

Bibbo M., Dytch H.E., Alenghat E., Bartels P.H. and Wied G.L. (1989). DNA ploidy profiles as prognostic indicators in CIN lesions. Am. J. Clin. Pathol. 92, 261-265.

Bolger B.S., Cooke T.G., Symonds R.P., MacLean A.B. and Stanton P.D. (1993). Measurement of cell kinetics in cervical tumours using bromodeoxyuridine. Br. J. Cancer 68, 166-171.

Boon M.E., Kleinschmidt-Guy E.D. and Ouwerkerk-Noordam E. (1994). PAPNET for analysis of proliferating (MIB-1 positive) cell populations in cervical smears. Eur. J. Morphol. 32, 78-85.

Bulten J., Van Der Laak J.A.W.M., Gemmink J.H., Pahlplatz M.M.M., De Wilde P.C.M. and Hanselaar A.G.J.M. (1996). MIB-1, a promising marker for the classification of cervical intraepithelial neoplasia. J. Pathol. 178, 268-273.

Burger M.P.M, Van Leeuwen A.M., Hollema H., Quint W.G.V. and Pieters W.J.L.M. (1997). Human papillomavirus type influences the extent of chromosomal lag during mitosis in cervical intraepithelial neoplasia Grade III. Int. J. Gynecol. Pathol. 16, 10-14.

Cole D.J., Brown D.C., Crossley E., Alcock C.J. and Gatter K.C. (1992). Carcinoma of the cervix uteri: An assessment of the relationship of tumour proliferation to prognosis. Br. J. Cancer 65, 783-785.

Devictor B., Bonnier P., Piana L., Andrac L., Lavaut M.N., Allasia C. and Charpin C. (1993). C-myc protein and Ki-67 antigen immuno-detection in patients with uterine cervix neoplasia: correlation of microcytophotometric analysis and histological data. Gynecol. Oncol. 49, 284-290.

Fukuda K., Iwasaka T., Hachisuga T., Sugimori H., Tsugitomi H. and Mutoh F. (1990). Immunocytochemical detection of S-phase cells in normal and neoplastic cervical epithelium by anti-BrdU monoclonal antibody. Anal. Quant. Cytol. Histol. 12, 135-138.

Gerdes J., Scluvab U., Leinke H. and Stein H. (1983). Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int. J. Cancer 31, 13-20.

Gerdes J., Leinke H., Bausch H., Wacker H.H., Schwab U. and Stin H. (1984). Cell cycle analysis of a cell proliferation - associated human nuclear antigen defined by the monoclonal antibody KI-67. J. Immunol. 133, 1710-1715.

Hu W., Boyko Y., Mitchell M.F. and Hittelman W.N. (1995). Sequential dysregulation of proliferation during cervical carcinogenesis as measured by MPM-2 antibody staining. Proc. Am. Assoc. Cancer Res. 36, 588.

Kaneko S. and Izutsu T. (1995). Use of bromodeoxyuridine, proliferating cell nuclear antigen, and nucleolar organizer regions in the multi-parametric assessment of proliferating potency in cervical intra-epithelial neoplasia. J. Obstet. Gynaecol. 21, 133-144.

Kobayashi I., Matsuo K., Ishibashi Y., Kanda S. and Sakai H. (1994). The proliferative activity in dysplasia and carcinoma *in situ* of the uterine cervix analyzed by proliferating cell nuclear antigen immunostaining and silver-binding argyrophilic nucleolar organizer region staining. Hum. Pathol. 25, 198-202.

Konishi I., Fujii S., Nonogaki H., Nanbu Y., Iwai T. and Mori T. (1991). Immunohistochemical analysis of estrogen receptors, progesterone receptors, Ki-67 antigen, and human papillomavirus DNA in normal and neoplastic epithelium of the uterine cervix. Cancer 68, 1340-1350.

Kristensen G.B., Kaern J., Abeler V.M., Hagmar B., Trope C.G. and Pettersen E.O. (1995). No prognostic impact of flow-cytometric measured DNA ploidy and S-phase fraction in cancer of the uterine cervix: a prospective study of 465 patients. Gynecol. Oncol. 57, 79-85.

Lichtenegger W. (1995). The vaginal radical operation of cervical cancer. J. Obstet. Gynaecol. 21, 179-184.

Lorincz A.T., Reid R., Jenson A.B., Greenberg M.D., Lancaster W. and Kurman R.J. (1992). Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet. Gynecol. 79, 328-337.

McCluggage W.G., Maxwell P., McBride H.A., Hamilton P.W. and Bharucha H. (1995). Monoclonal antibodies Ki-67 and MIB-1 in the distinction of tuboendometrial metaplasia from endocervical adeno-carcinoma and adenocarcinoma *in situ* in formalin-fixed material. Int. J. Gynecol. Pathol. 14, 209-216.

McCluggage W.G., Buhidma M., Tang L., Maxwell P. and Bharucha H. (1996). Monoclonal antibody MIB-1 in the assessment of cervical squamous intraepithelial lesions. Int. J. Gynecol. Pathol. 15, 131-

136.

McCormick D. and Hall P.A. (1992). The complexities of proliferating cell nuclear antigen. Histopathology 21, 591-594.

Miller B., Flax S., Dockter M. and Photopulos G. (1994). Nucleolar organizer regions in adenocarcinoma of the uterine cervix. Cancer 74, 3142-3145.

Minagawa Y., Kigawa J., Kanamori Y., Hamochi H. and Terakawa N. (1993). Tumor cell kinetics in elderly patients with cervical cancer. Obstet. Gynecol. 81, 610 - 614.

Mitchell M.F., Hittleman W.K., Lotan R., Nishioka K., Tortolero-Luna G., Richards-Kortum R., Wharton J.T. and Hong W.K. (1995). Chemoprevention trials and surrogate end point biomarkers in the cervix. Cancer 76, 1956 -1977.

Mittal K.R., Demopoulos R.I. and Goswami S. (1993). Proliferating cell nuclear antigen (Cyclin) expression in normal and abnormal cervical squamous epithelia. Am. J. Surg. Pathol. 17, 117-122.

Nakano T. and Oka K. (1993). Differential values of Ki-67 index and mitotic index of proliferating cell population. An assessment of cell cycle and prognosis in radiation therapy for cervical cancer. Cancer 72, 2401-2408.

Nasiell K., Auer G., Nasiell M. and Zetterberg A. (1979). Retrospective DNA analyses in cervical dysplasia as related to neoplastic progression or regression. Anal. Quant. Cytol. 1, 103-106.

Oka K., Nakano T. and Hoshi T. (1993). Transient increases of growth fraction during fractionated radiation therapy for cervical carcinoma Ki-67 and PC10 immunostaining. Cancer 72, 2621-2627.

Oka K., Nakano T. and Hoshi T. (1996). Analysis of response to radiation therapy of patients with cervical adenocarcinoma compared with squamous cell carcinoma MIB-1 and PC10 labelling indices. Cancer 77, 2280-2285.

Payne S., Kernohan N.M. and Walker F. (1996). Proliferation in the normal cervix and in preinvasive cervical lesions. J. Clin. Pathol. 49, 667-671.

Pieters W.J.L.M., Koudstaal J., Ploem-Zaayer J.J., Janssens J. and Oosterhuis J.W. (1992). The three-group metaphase as a morphologic indicator of high-ploidy cells in cervical intraepithelial neoplasia. Anal. Quant. Cytol. Histol. 14, 227-232.

Raju G.C. (1994). Expression of the proliferating cell nuclear antigen in cervical neoplasia. Int. J. Gynecol. Pathol. 13, 337-341.

Robertson J.H. and Woodend B. (1993). Negative cytology preceding cervical cancer: causes and prevention. J. Clin. Pathol. 46, 700-702

Rowlands D.C. (1988). Nucleolar organising regions in cervical intraepithelial neoplasia. J. Clin. Pathol. 41, 1200-1202.

Sawhney N. and Hall P.A. (1992). Ki-67 structure, function and new antibodies. J. Pathol. 168, 161-162.

Schellhas H.F. and Heath G. (1969). Cell renewal in the human cervix uteri. A radioautographic study of DNA, RNA and protein synthesis. Am. J. Obstet. Gynecol. 104, 617-632.

Steinbeck R.G., Heselmeyer K.M., Moberger H.B. and Auer G.U. (1995). The relationship between proliferating cell nuclear antigen (PCNA), nuclear DNA content and mutant p53 during genesis of cervical carcinoma. Acta Oncol. 34, 171-175.

Tervahauta A.I., Syrjanen S.M., Mantyjarvi R. and Syrjanen K.J. (1994). Detection of p53 protein and Ki-67 proliferation antigen in human papillomavirus (HPV - positive and HPV-negative cervical lesions by immunohistochemical double-staining. Cytopathology 5, 282-293.

Thickett K.M., Griffin N.R., Griffiths A.P. and Wells M. (1989). A study of nucleolar organizer regions in cervical intraepithelial neoplasia and human papillomavirus infection. Int. J. Gynecol. Pathol. 8, 331-339.

Tidbury P., Singer A. and Jenkins D. (1992). CIN 3 the role of lesion size in invasion. B. J. Obstet. Gynaecol. 99, 583-586.

Van Hoeven K.H., Ramondetta L., Kovatich A.J., Bibbo M. and Dunton C.J. (1997). Quantitative image analysis of MIB-1 reactivity in inflammatory, hyperplastic and neoplastic endocervical lesions. Int. J. Gynecol. Pathol. 16, 15-21.

Zolzer F., Alberti W., Pelzer T., Lamberti G., Hulskamp F.H. and Streffer C. (1995). Changes in S-phase fraction and micronucleus frequency as prognostic factors in radiotherapy of cervical carcinoma. Radiother. Oncol. 36, 128-132.