Leaders

Proliferation markers in tumours: interpretation and clinical value

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Proliferation is one of the most fundamental of biological processes because of its role in growth and in the maintenance of tissue homeostasis. In tumours especially, proliferation has traditionally received much attention. On the one hand, proliferation has been studied as an important cell biological mechanism in oncogenesis, often using rather laborious methods applicable only in experimental conditions, such as tritiated thymidine incorporation. On the other hand, assessment of proliferation has become popular in histopathology as a means of predicting the behaviour of tumours—that is, their likelihood of local recurrence, their metastatic potential, and the growth of metastases, and thereby the disease-free survival and survival to death. This is owing to the fact that different methods have become available to assess certain properties of cellular proliferation that are readily available in daily histopathological practice. This has led to vast numbers of studies on the value of these methods for the diagnosis of different kinds of tumours and clinical decision making. In this review we aim to provide an overview of methods currently available for assessment of proliferation, and to discuss critically their cell biological framework, their methodology, and some of the most important applications of these methods. First, however, a description of the cell cycle is necessary, to provide the context within which the different methods of assessing proliferation will be interpreted.

The cell cycle

Several phases of cellular proliferation can be recognised. From the resting (G_0) phase of highly variable duration, in which cells do not take part in the cell cycle, they eventually join the cycling population after suitable stimuli and enter the first gap (G_1) phase, which has a variable duration. In the G_1 phase, the cell prepares for the synthesis or S phase, in which DNA synthesis and doubling of the genome occur; this usually takes between seven and 12 hours. The S phase is followed by a period of apparent inactivity known as the second gap (G_2) phase (usually one to six hours), in which the cell prepares for further separation of chromatids during the mitotic (M) phase, which takes generally between one and two hours. After the M phase, each daughter cell may enter G_0 phase or move on to the G_1 phase of a

cell cycle. The interphase—which is comprised of the G_1 , S, and G_2 phases—forms the largest part of the cell cycle, but cells in these phases cannot be morphologically recognised. Cells in the mitotic phase can be identified because of the typical appearance of the chromosome sets during the different subphases of the M phase. This has been the basis for mitosis counting on light microscopy—the oldest way of assessing proliferation. Several other methods have become available to assess proliferation, based on other cell cycle fractions or on cell cycle phenomena besides mitosis. These include incorporation techniques, per cent S phase cell determination by DNA cytometry (%S-phase), immunohistochemistry of proliferation associated antigens, and nucleolar organiser regions. All these methods have their advantages and disadvantages from a cell biological or practical point of view, and these will be discussed later. Discussion of cell cycle regulation is beyond the scope of this review.

Proliferative activity and tumour growth

Growth is the overall increase in cell number, so is the net result of cell gain by proliferative activity and cell loss by apoptosis or necrosis. Proliferative activity results from cell cycling. The mechanisms responsible for proliferative activity (P) are the speed of the cell cycle, which is inversely proportional to the generation time (T) on the one hand, and to the proportion of cells committed to the cycle—the growth fraction (G)—on the other. The mathematical relation between proliferative activity, growth fraction, and generation time is $P = G/T$. A high proliferative activity can therefore be the result of either a high growth fraction or a short generation time, or both. Roughly, a proliferative activity of 0.01 cell/cell of the population/hour is obtained with a T of 100 hours and a growth fraction of 10%, as well as with a T of 500 hours and a growth fraction of 50%. Clearly neither of the techniques described below, defining growth fraction or generation time alone, can accurately describe proliferative activity.

The doubling time (Td) of a tumour in case of no cell loss is defined by $Td = T(\log 2/\log(G+1))$. A short doubling time therefore results from either a short cycle time or a high growth fraction, or both. Several methods are available to assess growth fraction as described

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below, but assessment of cell cycle time is much more complicated.

Markers of proliferation

INCORPORATION TECHNIQUES

Incorporation techniques are based on *in vivo* or *in vitro* incorporation of labelled nucleotide analogues, such as tritiated thymidine or bromodeoxyuridine (BrdU). Incorporated radioactive thymidine can be visualised by autoradiography, and incorporated BrdU can be revealed by immunohistochemistry. These incorporation methods are popular in biology and can be regarded as a gold standard marker of S phase cells. In a clinical setting, they are less useful. *In vivo* injection with radioactive thymidine is not popular, handling radioactive active material is cumbersome, and the autoradiographic development can take up to two weeks. It is also possible to incorporate tumour material *in vitro* with tritiated thymidine as for BrdU, but this does not take away the other disadvantages. By pulse labelling, an estimate can also be obtained on the number of cells entering S phase during a certain period of time, and thereby of cell cycle time.¹ It is not easy to count positively labelled cells reliably—as demonstrated by Meyer and McDivitt, who found coefficients of variation up to 55% on repeated counts.² In general, few studies have been devoted to optimising the counting of cells labelled with tritiated thymidine.

MITOTIC INDEX

Counting of mitotic figures is the oldest way of assessing proliferation. Ever since the introduction of microscopes made the recognition of mitotic figures possible, counting mitotic figures has been applied as a diagnostic tool, especially in tumour pathology. Even though many other ways of assessing proliferation have become available, the ease with which mitoses can be recognised without special equipment apart from a decent microscope and a well stained H&E slide has led to the increasing popularity of this way of counting of mitotic figures up to the present.

Strict morphological criteria should be applied for the recognition of mitotic figures.³⁻⁶ Mitoses can be defined as dark clots of chromosomes which can often be recognised by the presence of hairy extensions when focusing up and down, while the nuclear envelope is absent and the cytoplasm is basophilic rather than eosinophilic. These chromosomal clots can have the configuration of the metaphase, anaphase, or telophase.

Several approaches may be followed when counting mitotic figures. Most often, the number of mitoses is expressed as the total number in a defined number (for example, 10) high power fields (HPF). The exact area of the HPF must, however, be defined to be able to compare results from different studies, since the area of field of vision can vary considerably between different objectives. Unfortunately, most workers have failed to do so in the past, which has led to much criticism. Therefore, some use the number of mitotic figures per unit area (for example, 2 mm²). It may also be use-

ful to correct for the actual content of tumour cells within the slide by expressing the number of mitoses per a certain number of tumour cells (for example, 1000), which yields the mitotic rate, or to correct the number of mitotic figures for the area percentage of epithelium, which yields the mitoses per volume (MV) index.⁷ These methods are potentially time consuming, but stereological sampling approaches are quite useful to keep the extra time spent within acceptable limits.^{8,9}

The reproducibility of mitosis counting has been questioned. Indeed, when standardised methodology is not used quite variable results may be obtained on the same material by different observers,¹⁰ but after thorough training and following a strict protocol, excellent inter-reproducibility results have been obtained.⁵ Since counting of mitotic figures is not entirely objective, attempts have been made to automate the counting of mitotic figures. Kaman *et al* described a first setup of an image analysis system for automated recognition of mitotic figures in H&E stained sections.¹¹ Although this approach was partially successful, more recent approaches using Feulgen stained sections have been quite successful^{12,13} and may serve as a prescreening device. In one study, mitotic figures were counted by flow cytometry using a combination of DNA fluorescence and right angle light scatter.¹⁴ The sensitivity and specificity of this method may, however, not be optimal. In principle, when a good mitotic figure marker becomes available (for example, the AF-2 protein¹⁵), flow cytometry for counting mitotic figures may be feasible.

Obviously, mitoses comprise only part of the proliferating cells. In addition, the length of the mitotic phase may be highly variable (especially in DNA aneuploid tumours) and there are indications that in some tumours, such as Hodgkin's lymphomas, even metaphase arrests may occur, so that a mitotic count does not necessarily provide a good marker of proliferation in all situations.¹⁶ Indeed, the mitotic index only partially correlates with other proliferation markers such as the Ki67 labelling index^{15,17-19} and bromodeoxyuridine incorporation.²⁰ In spite of these objections, mitosis counting has proven to be useful for many diagnostic applications. Reproducibility of mitosis counting is good if a strict counting protocol is followed, as has been shown in a large interlaboratory reproducibility study.⁵ Proper fixation and staining as well as standardised section thickness are mandatory to obtain reliable counts, although some fixation delay is acceptable if the tissue is kept in the refrigerator.^{21,22}

DNA CYTOMETRIC %S-PHASE

In DNA cytometry, cell nuclei are stained with a stoichiometric DNA binding stain, and the amount of staining is measured. One popular approach is flow cytometry, whereby nuclei in a cell suspension are stained with a fluorescent dye, sucked into the flow cytometer where the fluorescence is excited, and measured by means of a photomultiplier system. Another approach that has become more popular in

recent years is by image (or static) cytometry, where an absorption stain (the Feulgen reaction) is applied to cells on a glass slide, and the optical density is measured by image analysis. The result of both approaches is a frequency histogram of DNA content, the DNA histogram. The DNA histogram is in fact a representation of the cell cycle, and the fractions of cells within the different cell cycle phases (G_0/G_1 , S and G_2/M phases) can be calculated from this histogram by computerised cell cycle analysis. The most widely used proliferation variable derived from DNA cytometry is the %S-phase cells, although in fact the S + G_2/M phase would represent the total proliferative fraction should the cells in G_1 that have passed the restriction point be neglected.

Traditionally, flow cytometry yields more reliable %S-phase calculations than static DNA histograms because of the higher resolution of the DNA histograms, which is related to the accuracy of measurement and number of events measured. However, increased computer power and more sophisticated software, allowing more events to be measured, have made %S-phase calculations from static DNA cytometric histograms a valuable alternative for those derived from flow cytometry.²³ %S-phase cells has been shown to correlate with other proliferation markers like Ki67 score^{15 18 24} and mitotic count,^{25 26} although not very strongly.

With regard to reliability of %S-phase assessments, several problems have been encountered in computerised cell cycle analysis. These are to a large extent caused by the different approach of the available programs and the different cell cycle fitting options within these programs, and partly to interobserver variations in interpretation. Nevertheless, some large studies have yielded important data as to the optimal fitting options,²⁷⁻²⁹ which may contribute to more consensus on cell cycle analysis. It seems that best results are obtained when using a semi-automated type of analysis.³⁰ Fixation delay does not seem to affect %S-phase measurements.^{21 22}

IMMUNOHISTOCHEMISTRY OF PROLIFERATION ASSOCIATED ANTIGENS

The proliferating cell nuclear antigen (PCNA) is a proliferation related protein that is upregulated in proliferating cells. Expression of PCNA correlates partly with other proliferation markers such as %S-phase fraction,³¹⁻³³ Ki67 staining,³⁴ and mitotic count.³² Not all studies, however, have confirmed the correlation with mitotic count,³⁵ and the PCNA labelling index could not be correlated with the Ki67 labelling index.³⁶ This is probably owing to the fact that PCNA is also involved in DNA repair. Since there is active ongoing DNA repair in many tumours, PCNA may also be upregulated in non-proliferating cells. Indeed, in some tumours, 100% of cells show positive staining. Therefore, after an initial period of popularity, PCNA is not considered to be a very reliable proliferation marker in tumours.

The Ki67 antigen, which is coded by a gene on chromosome 10, is expressed in the G_1 , S, and G_2 phases in cycling cells.^{37 38} The Ki67 score partly correlates with other proliferation markers like %S-phase cells,^{15 18 24 26} and mitotic count.^{17-19 36 39-41} Originally, the Ki67 antigen could only be studied in frozen sections. Recently, a series of Ki67 antibodies has been developed by the Kiel group that also works on paraffin embedded tumour material after antigen retrieval^{37 38}—the MIB antibodies. Of these, MIB-1 has been studied most widely and is regarded as a reliable marker of proliferating cells. MIB-1 assessments in paraffin sections are more reproducible than their counterparts in frozen sections.^{42 43} Adequate methodology is important to obtain reproducibility. To this end, a systematic random sampling procedure based on stereology has been proposed to obtain unbiased sampling of nuclei to be scored.^{44 45} Particularly when this is done with an automated scanning stage, quick and reproducible results are obtained.⁴⁶

DNA topoisomerase II catalyses the topologic isomerisation of DNA by passing one strand of DNA through a reversible break in a second DNA strand. The expression of DNA topoisomerase II increases rapidly at the transition of the S to G_2 phase and decreases rapidly at the end of mitoses. DNA topoisomerase II can be demonstrated by immunohistochemistry and can therefore be used as a marker of proliferating cells. There are as yet few applications of DNA topoisomerase II immunohistochemistry.

AgNORS

The nucleolus plays an essential role in the control of proliferation and protein synthesis. Nucleolar organiser regions (NORs) are segments of DNA closely associated with nucleoli which contain coding genes for ribosomal DNA. They therefore contribute strongly to the regulation of protein synthesis. NORs are argyrophilic and can therefore be visualised by a silver staining technique, what has led to the term AgNOR. AgNOR assessment correlates with rate of proliferation estimated by Ki67 score,^{19 47} %S-phase cells,⁴⁸⁻⁵⁰ and mitotic count.⁴⁸ More importantly, AgNOR assessments can probably be used to estimate cell cycle time, since the shorter the cell cycle, the more protein synthesis must take place for a subsequent mitoses per unit of time, so the more ribosomes must be generated and the higher the AgNOR score. AgNOR score has indeed been shown to correlate with population doubling time.⁵¹ There is, however, also protein synthesis activity in non-cycling cells, leading to a variable "baseline" AgNOR score. AgNORs as a measure of cell cycle time should therefore exclusively be assessed in proliferating cells, which can be done, for example, by double staining with Ki67.⁵² Standardised staining technique and measurement protocols have been prepared and are presently under evaluation to deal with silver staining variability and unified scoring of AgNORs (number *v* area, and so on), but at present these methods are difficult to apply in daily practice.

The problem of heterogeneity

Heterogeneity is one of the hallmarks of malignancy in general, and this also holds for proliferation. Heterogeneity for several proliferation markers has been well documented for several tumour types such as mitosis counting,⁵³ %S-phase,⁵⁴ and Ki67 labelling⁵⁵ in breast cancer, and for %S-phase⁵⁶ and Ki67 index⁵⁷ in gliomas, MIB-1 labelling index in squamous cell cancers of the head and neck⁵⁸ and gastrointestinal adenocarcinomas,^{59 60} and MIB1 labelling in lymphomas.⁶¹ In general, the highest proliferative part of the tumour will determine the clinical behaviour, so this must carefully be sought. With non-morphological methods such as DNA cytometric %S-phase determinations this is hardly possible, which explains the lack of agreement about the clinical value of this proliferation variable in different tumours such as breast cancer.²⁸ However, with morphological methods such as mitosis counting⁵³ it is possible to deal with heterogeneity by screening different tumour parts for the highest proliferative area. From a clinical point of view, morphological methods of assessing proliferation will therefore in general be preferable.

The frequently expressed requirement for a statistical representation of any index or score at the level of the malignant population should be considered as a false problem if the tumour is comprised of subpopulations with different kinetic behaviour. In this case proliferation has to be evaluated wherever it is high in the tumour, and there is no biological reason for averaging the indices observed with respect to regions where malignant cells are proliferating poorly or not at all. Sampling statistics might merely erase potentially meaningful signals like nests of mitoses or cycling cells. In this context, "cellular sociology" concepts—where proliferation indices are related to topography—will most probably change the conventional sampling rules.

Proliferation markers for diagnosis making and prognostication of tumours

BREAST

Breast cancer is probably the tumour in which the clinical implications of proliferation have been most studied. Several studies by different groups have been devoted to thymidine labelling, showing that high labelling indices are associated with poor prognosis; these have included large studies in node negative patients.^{62 63} As to mitosis counting, the number of mitotic figures assessed in cytological specimens has prognostic value in breast cancer as part of a cytological grading system.⁶⁴ Furthermore, it is also part of the prognostic histological grading system.^{65 66} Several studies have shown that the number of mitotic figures is the most important prognostic component of the Bloom–Richardson histological grade.^{67–69} In several retrospective and prospective studies, Baak *et al* showed that a highly systematised way of assessing the mitotic activity index (MAI) gave very useful prognostic information over and above that provided by tumour size and lymph node status,^{70–81} which was confirmed by others.⁸² The prognostic results from

a large prospective multicentre study by are eagerly awaited.⁸³ Several other groups from different countries have confirmed the prognostic value of mitosis counting in invasive breast cancer.^{48 49 67 78 82 84–89} The fact that the prognostic value of the MAI holds for premenopausal lymph node negative patients⁷³ has led to a clinical trial throughout Europe in which high risk (MAI ≥ 10), premenopausal, lymph node negative patients with invasive breast cancer are randomised over six cycles of CMF adjuvant chemotherapy or observation.

There have been many studies addressing the prognostic value of flow cytometrically assessed %S-phase cells in breast cancer (for an overview, see Bergers *et al*²⁸). In some there has been no prognostic value, but most studies using fresh/frozen material and sufficient numbers of patients show a significantly worse prognosis for high %S-phase values. Overall, one can therefore conclude that %S-phase cell count has prognostic value in invasive breast cancer, although the differences in survival are small. Intratumour heterogeneity is a major problem,^{34 90} as is the rather systematic correlation between increased %S phase and increased aneuploidy which indicates a potential bias.

The Ki67 labelling index was found to be prognostically relevant in several studies.^{39 41 91 92} More recent studies using MIB1 paraffin antibody have confirmed its prognostic value.^{93–98} One comparative prognostic study between Ki67 and MIB-1 showed that MIB-1 assessments on paraffin are at least as good as the Ki67 assessments on frozen sections.⁹⁹

Conflicting prognostic results for the PCNA index have been presented,^{100–102} so PCNA does not seem to be a relevant prognostic variable in breast cancer for technical reasons.

OVARY

Assessment of prognosis of borderline tumours of the ovary based only on morphological criteria is difficult, and proliferation markers are of value here. Sumithran *et al* showed that grading of borderline tumours by applying mitotic figure counts was of prognostic value.¹⁰³ Baak *et al* showed that the mitotic index (MI), assessed in the most epithelium-rich area of the tumour, exceeded the prognostic value of histological type, nuclear and histological grade, and even that of extent of disease.¹⁰⁴ Patients with high MI were at high risk, and the MI also has value additional to DNA ploidy, which is probably the strongest prognostic indicator in these tumours.¹⁰⁵ Others have described the importance of the presence of mitotic figures in peritoneal implants of borderline tumours.¹⁰⁶

The patients with poor prognosis among early stage cancers can be identified using a combination of mitotic index and volume percentage of epithelium (VPE).^{107–110} In advanced stage cancers, those cases with low MAI and low VPE seem to have a good chance of responding to cisplatin treatment, as shown by Baak *et al* and Rodenburg *et al*.^{111 112} Haapasalo *et al* confirmed the prognostic value of the

MAI/VPE combination for this group of patients, but showed that the M/V index had greater prognostic value in ovarian cancers.¹¹⁰ %S-phase has also been shown to be of prognostic value in combination with DNA ploidy and psammoma body content¹¹³.

BONE TUMOURS

In osteosarcoma, mitotic activity seems to be one of the most important histological features for grading.^{114–115} The lower the mitotic rate, the better the chance of long term survival, although overall prognosis is relatively poor.

In chondroid tumours, counting mitotic figures helps to distinguish chondroma from chondrosarcoma. In the study of Cuvelier and Roels,¹¹⁶ none of 23 chondromas had more than one mitosis per 200 nuclei, only one of six low grade chondrosarcomas had one mitosis per 200 nuclei, and in the other cases the mitotic rate ranged between 2 and 5. All high grade chondrosarcomas had higher mitotic rates, varying between 12 and 97. However, one chondroblastoma had a mitotic rate of 2 and one chondromyxoid fibroma a rate of 7. Mitotic rates therefore have to be used in combination with histopathological criteria. In chondrosarcomas, the presence of two or more mitoses per HPF ($\times 40$ objective) seemed to be the best indicator of metastases and poor prognosis in the study by Evans *et al.*¹¹⁷

In osteogenic fibrosarcoma, the mitotic frequency is the major histological feature in the grading system of the Netherlands Committee for Bone Tumours.¹¹⁸ Grade I tumours (< 1 mitosis/10 HPF) have a 65% five year survival and grade III tumours (≥ 10 mitoses/10 HPF) have only a 25% five year survival. Grade II has an intermediate prognosis.

In osteogenic giant cell tumours, mitotic activity is also used for grading. Grade I cases showing hardly any mitotic figures have a recurrence rate of about 10%, grade II cases (more mitotic figures but still less than one per HPF) have a recurrence rate of about 50%, grade III (at least one mitosis per HPF) have a recurrence rate of about 80%. Grade IV cases have all the features of sarcoma and are treated as such.

In aneurysmal bone cysts, mitotic activity can help to estimate the recurrence rate and to differentiate from telangiectatic osteosarcoma. Ruiter *et al* counted the number of mitosis per 50 HPF ($\times 750$, field diameter 275 μm^2), and the recurrence rate was markedly higher in cases with more than seven mitoses (13/17 *v* 2/28).¹¹⁹ In combination with the area of the largest nucleus, this mitotic index was helpful in discriminating aneurysmal bone cysts from telangiectatic osteosarcoma.¹²⁰

SOFT TISSUE TUMOURS

Assessment of malignancy in leiomyomatous tumours is a well recognised problem in which assessment of proliferation plays an important role. First, a discrimination has to be made between leiomyomatous tumours of the uterus and those in other sites. In extrauterine leiomyomatous tumours, a mitotic activity of five or more mitoses per 10 HPF is a strong

indicator of malignancy. In uterine leiomyomatous tumours, the situation is more complex. Classically,^{121–122} leiomyomatous tumours with 5–9 mitoses per 10 HPF were considered to be of “uncertain malignant potential,” and with 10 or more mitoses per 10 HPF to be malignant. However, there is a tendency to allow focally higher mitotic activity in younger women, especially in those using hormones.^{123–124} Indeed, in recent studies on borderline uterine leiomyomatous tumours in pre- and postmenopausal women, it was shown that presence of tumour necrosis and significant nuclear atypia are the most important indicators of malignancy,^{125–126} and that in the absence of significant nuclear atypia and tumour necrosis these tumours usually behave benignly, irrespective of the mitotic activity. When tumour necrosis or significant nuclear atypia is found, however, a high mitotic rate (> 10) indicates a clearly greater chance of relapse. There is no real consensus on the prognostic value of proliferation in true uterine leiomyosarcomas,^{126–128} and one study on uterine stromal nodules also did not find that this had any prognostic significance.¹²⁹

In gastrointestinal stromal tumours (GIST), MIB-1 labelling index and mitotic index were both very significantly correlated with patient survival in a study by Carrillo *et al.*¹³⁰ High proliferation is therefore an essential hallmark of high risk in these tumours, which is otherwise difficult to identify.

In soft tissue sarcomas, proliferation was found to be of prognostic value in several studies. High proliferation, as reflected by high mitotic index^{131–137} high Ki67 labelling index,^{137–140} and high %S-phase cells^{137–141–142} are indicators of poor prognosis in these malignancies. Several sarcoma grading systems that include a measure of proliferation have been proposed. Myhre Jensen *et al* combined mitotic index with cellularity, cellular anaplasia, and the presence of pyknotic or fragmented nuclei,¹³² and Coindre *et al* proposed a multivariate combination of mitotic index, cellular differentiation, and tumour necrosis.¹³⁵ Probably the most widely applied grading system for (locally adequately treated) sarcomas is that of the EORTC, including mitotic index only: patients with fewer than three mitoses per 2 mm² (grade I) do very well; patients with more than 25 mitoses per 2 mm² (grade III) do very poorly; and grade 2 patients with 4–25 mitoses per 2 mm² (grade II) have intermediate prognosis.^{133–134} Combining mitotic index with necrosis and tumour size provided the best results.

BRAIN TUMOURS

Mitoses play a role in the grading system of astrocytomas developed by Daumas-Duport *et al.* A point each is given for presence of nuclear atypia, mitoses, endothelial proliferation, and necrosis. This grading system is very reproducible and correlates well with prognosis.¹⁴³

In meningiomas, the number of mitoses counted in 10 fields at 400 $\times$ —grouped as 0, 1, 2–5, or 5 or more—correlates significantly with recurrence after subtotal resection.¹⁴⁴

LYMPHOMAS

Akerman *et al* counted mitotic figures in 101 non-Hodgkin's lymphomas.¹⁴⁵ Those patients with on average more than two mitoses per 400× field (counted in 10 fields) had a 37% two year survival, while cases with fewer than two mitoses an average had a 77% survival at that time. For follicle centre cell lymphomas, the prognostic value of their mitotic index exceeded that of growth pattern and predominant cell type. Griffin *et al* found comparable results.¹⁴⁶ Ki67 labelling index has also been shown to provide prognostic information in non-Hodgkin's lymphomas.^{147–150}

CERVIX

In normal cervix, proliferating cells are confined to the parabasal layer. This also holds for atrophic epithelium, koilocytic epithelium, and immature squamous metaplasia—conditions that may be difficult to distinguish from dysplastic epithelium. As has been shown in several studies,^{151–154} proliferation is increased in dysplastic epithelium, to an extent that correlates well with the grade of dysplasia. In addition, the higher the grade of dysplasia, the higher up in the epithelium are proliferating cells found (as demonstrated by MIB-1 immunohistochemistry). This topographical MIB-1 labelling index can therefore be useful to discriminate dysplastic epithelium from conditions mimicking dysplasia, as well as for grading dysplasia.

Conclusion

Several routinely applicable techniques have become available that reveal different properties of proliferation, all having their advantages and disadvantages. However, since cell cycle time is as yet very difficult to assess, they do not provide accurate information on rate of proliferation as such, but rather on the state of proliferation at the time of assessment.¹⁵⁵ Nevertheless, several of these methods have found their way into daily pathology practice, where they provide useful diagnostic and prognostic information in lesions and tumours from several sites. More methodological studies are necessary before reproducible protocols become available that provide similar information in different laboratories; the problem of heterogeneity must also be adequately dealt with, owing to the significant heterogeneity of most types of tumours. Although growth is obviously the net effect of proliferation and cell death, cell death is as yet difficult to quantify, although some attempts have been made.¹⁵⁶ Although some interesting results have been described, the lack of reliable data on the duration of cell death events hampers these attempts.

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