

REVIEW

Prognostic value of proliferation in invasive breast cancer: a review

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Breast cancer is the leading cause of death among solid tumours in women, and its incidence is increasing in the West. Adjuvant chemotherapy and hormonal treatment improve survival but have potentially serious side effects, and are costly. Because adjuvant treatment should be given to high risk patients only, and traditional prognostic factors (lymph node status, tumour size) are insufficiently accurate, better predictors of high risk and treatment response are needed. Invasive breast cancer metastasises haematogenously very early on, so many breast cancer prognosticators are directly or indirectly related to proliferation. Although studies evaluating the role of individual proliferation regulating genes have greatly increased our knowledge of this complex process, the functional end result—cells dividing—has remained the most important prognostic factor. This article reviews the prognostic value of different proliferation assays in invasive breast cancer, and concludes that increased proliferation correlates strongly with poor prognosis, irrespective of the methodology used. Mitosis counting provides the most reproducible and independent prognostic value, and Ki67/MIB1 labelling and cyclin A index are promising alternatives that need methodological fine tuning.

woman over the age of 50 years,³ but preferentially detect slowly growing and more well differentiated tumours that inherently have a better prognosis and miss fast growing aggressive tumours, which often present as interval cancers.^{4 5} Therefore, the overall effect is still a matter of debate.⁶

“Adjuvant treatment should only be given to high risk patients, which requires good prognostic factors to indicate high risk and additional factors to predict response to treatment”

Adjuvant chemotherapy and hormonal treatment have been shown to improve survival in patients with breast cancer but have potentially serious side effects, and are costly. Therefore, adjuvant treatment should only be given to high risk patients, which requires good prognostic factors to indicate high risk and additional factors to predict response to treatment (for example, steroid receptors⁷ and HER-2/neu⁸). Traditional prognostic factors such as lymph node status and tumour size are not accurate enough. Therefore, to improve the indication for adjuvant treatment, additional predictive and prognostic factors are required. A multitude of prognostic factors have been identified for breast cancer. Many of them are directly (for example, cell cycle regulators⁹) or indirectly (for example, growth factors^{10–12} or angiogenesis¹³) related to proliferation. This is no surprise, because invasive breast cancer can conceptually be regarded as a disease that metastasises haematogenously in a very early phase. Hence, prognosis does not depend on the mere presence of distant metastases but on whether they grow or not.

Although many studies evaluating the role of individual genes regulating these processes have tremendously increased our knowledge of the complex process of proliferation, the functional end results of this process—a cell dividing—has remained the most important prognostic factor so far. Here we review the prognostic value of different proliferation assays in invasive breast cancer.

Assessment of proliferation

Different methods based on the concepts of the cell cycle have become available to assess the rate of proliferation, and have recently been reviewed extensively.^{14–19} Cellular proliferation takes place

Breast cancer is the leading cause of death among solid tumours in women, and the incidence is still increasing in the Western world, especially among younger woman. In the Netherlands, there are over 10 000 new cases of breast cancer every year in a population of about 16 million people. At this moment, the disease will affect 12% of all women and about 30–40% of patients will die from metastatic disease despite radical surgery. Although there are several recognised risk factors, such as early menarche, late menopause, nulliparity, and positive family history, there are at present no realistic options for primary prevention in patients not known to have a germline mutation in one of the hereditary breast cancer related genes, such as BRCA1 and BRCA2,¹ PTEN (Cowden's disease),² and p53 (Li Fraumeni syndrome).

Improvements in survival can therefore be expected from early detection and adjuvant treatment. Mammographic screening strategies result in the early diagnosis of breast cancer and a 25–30% decrease in breast cancer mortality in

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Abbreviations: BrdU, bromodeoxyuridine; MAI, mitotic activity index; McM, minichromosome maintenance; PCNA, proliferating cell nuclear antigen

through a defined process in which several phases can be recognised. From the resting (G0) phase they join the active cycling population after appropriate stimuli and enter the first gap (G1) phase. Both phases have a highly variable duration. In G1, the cell prepares for the synthesis (S) phase, in which DNA synthesis and doubling of the genome take place. The S phase is followed by a period of apparent inactivity known as the second gap (G2) phase, in which the cell prepares for further separation of chromatids during the mitotic (M) phase. After the M phase, each daughter cell may enter G0 phase or move on to the G1 phase to repeat the cell cycle. The interphase, which comprises the G1, S, and G2 phases, forms the largest part of the cell cycle, but cells in these phases cannot be morphologically recognised. However, cells in the mitotic phase can easily 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 light microscopical counting of mitotic figures, the oldest form of assessing proliferation.

However, the duration of the mitotic phase can vary, especially in aneuploid tumours, so the number of mitoses is not linearly correlated with the rate of proliferation. Therefore, cell biologists in particular have explored other methods. An optimal assessment of the proliferation rate of a tumour includes measurements of the growth fraction, in addition to measurements of the cell cycle time.¹⁴ Cell cycle time is difficult to assess, but preliminary studies have assessed argyrophilic nuclear organiser regions in Ki67 positive cells, with promising results.²⁰ The growth fraction can be more easily assessed by immunohistochemistry of proliferation associated antigens, such as Ki67, Ki-S1 topoisomerase II α , proliferating cell nuclear antigen (PCNA), geminin,²¹ or minichromosome maintenance (McM) proteins,²² or by DNA flow cytometric²³ or image cytometric (two dimensional²⁴ or three dimensional²⁵) assessment of the S phase fraction. Incorporation techniques (for example, with bromodeoxyuridine (BrdU) and tritiated thymidine) theoretically provide the gold standard of cellular proliferation. All these methods have their good and bad points from a cell biological or practical point of view.¹⁴ However, incorporation techniques are impractical because fresh material is needed, patients need to be injected intravenously, and/or radioactivity is involved, making them unattractive in daily practice. The percentage S phase is hampered by pronounced intratumour heterogeneity.²⁶ Therefore, mitosis counting and the Ki67 index are the most practical methods. Mitosis counting has been best studied from a methodological point of view and larger retrospective and prospective studies have been used (see below).

Prognostic value of proliferation in breast cancer

The different methods to assess proliferation have all been tested for prognostic value in invasive breast cancer. Most studies have been performed on sporadic patients, and a few on BRCA1/2 related cases, which in general show higher proliferation, compatible with their worse prognosis.^{27 28}

Incorporation techniques

A high thymidine labelling index has been shown to be associated with poor prognosis in lymph node positive and negative patients with breast cancer,²⁹⁻⁴⁴ and patients with a high thymidine labelling index benefit from adjuvant chemotherapy.⁴⁵ For BrdU, only few clinical studies have been published. Thor *et al* compared BrdU with the mitotic index and Ki67 index, and found comparable prognostic value for these three techniques,⁴⁶ and Weidner *et al* confirmed the good correlation between BrdU and mitotic index.⁴⁷ Goodson *et al* found BrdU to be slightly superior to Ki67.⁴⁸ However, as stated above, incorporation methods are impractical for routine use, which hampers their worldwide

application, despite the very good prognostic value of the thymidine labelling index.

Flow cytometric S phase

With regard to the flow cytometric S phase fraction, most studies that used fresh/frozen material and a sufficient number of patients found a relation between high S phase fraction and an unfavourable prognosis.⁴⁹⁻⁵² However, in view of the high intratumour heterogeneity of the S phase fraction, this feature cannot be used for individual patients.²⁶

Proliferation associated antigens

The monoclonal antibody Ki-S1 is thought to recognise a cell cycle associated antigen, related to the mitotic count,⁵³ but only a few clinical studies have been reported using this antibody, and most have revealed no prognostic value.⁵³⁻⁵⁵

Topoisomerase II α is a recently established marker of proliferating cells.⁵⁶ In one study, topoisomerase II α and Ki67 scores closely paralleled one another, indicating that the topoisomerase II α labelling index reflects the proliferative activity of tumour cells.^{57 58} Topoisomerase II α provided independent prognostic value in two studies.^{57 58}

Cyclin A is expressed in the late S, G2, and M phases of the cell cycle, and is therefore one of the most useful markers of proliferating cells.⁵⁹ Indeed, cyclin A labelling appeared to have prognostic value in invasive breast cancer.^{60 61}

Ki67 labelling correlates with the S phase fraction⁶² and mitotic index.^{62 63} Using frozen sections, the Ki67 labelling index was prognostically relevant in several studies in invasive breast cancer.⁶⁴⁻⁶⁶ The MIB1 antibody, which is reactive against Ki67 and can be used on paraffin wax embedded tissues, confirmed the prognostic value of Ki67 on archival material,^{30 48 51 58 64 67-81} including tissue from lymph node negative patients,^{82 83} and there was a good correlation between Ki67 and MIB-1 staining.⁸² In predominantly in situ cancers, even the Ki67 labelling index of the in situ parts seems to have prognostic value.⁷¹ A pronounced decrease in the Ki67/MIB-1 labelling index is associated with a good response to preoperative treatment.⁸⁴⁻⁸⁶ However, not all studies on Ki67/MIB1 reached significance.⁵² Few studies have dealt with the methodological issues, such as sampling strategies, intratumour heterogeneity, and reproducibility, most studies are retrospective, and thresholds vary.⁸⁷

Because of its conflicting results, PCNA immunohistochemistry does not provide a prognostically relevant assessment of proliferation in breast cancer.⁸⁸⁻⁹³ For geminin and McM proteins, no prognostic results have been described to date.

Mitosis counting

Several studies have shown that the mitotic count is the most important constituent of histological grade,^{94 95} but there are well known problems with reproducibility of grading because of the lack of strict protocols.⁹⁶⁻¹⁰² In different studies from our group, we have shown that a highly standardised way of assessing the mitotic activity index (MAI; counting at $\times 400$, magnification in an area of 1.6 mm², in the highest proliferative invasive area in the periphery of the tumour) provided a very strong prognostic factor, with additional prognostic value to tumour size and lymph node status in several retrospective studies^{13 103-117} and two prospective studies.^{101 102} Several other groups from different countries have confirmed the prognostic value of mitosis counting in primary invasive breast cancer,^{4 46 51 78 82 90 95 118-151} including prospective studies.¹⁵² Elkhuizen *et al* found that patients who had undergone breast conserving treatment and had a recurrence after an interval of more than two years, but who had a high mitotic count, had an equally poor prognosis as those patients with local recurrence detected after a short interval.¹⁵³ The threshold in the different studies varies

Table 1 Overview of different studies on the prognostic value of mitotic activity in invasive breast cancer

| First author | Ref | P/R | N | Subgroup | Overall survival | Significance | Independent value |
|--------------|-----|-----|------|------------|------------------|--------------|-------------------|
| Aaltomaa | 121 | R | 293 | LN- | 0.005 | - | Yes |
| | 121 | R | 224 | LN+ | 0.004 | - | Yes |
| | 122 | R | 106 | All | <0.001 | <0.001 | Yes |
| | 119 | R | 281 | LN- | 0.0115 | 0.0007 | Yes |
| | 118 | R | 688 | All | <0.0001 | <0.0001 | Yes |
| | 120 | R | 611 | All | <0.001 | <0.001 | Yes |
| Baak | 104 | R | 271 | Ductal | - | <0.001 | Yes |
| | 105 | R | 82 | Ductal | - | 0.0254 | Yes |
| | 159 | P | 576 | LN-, <55 | <0.0001 | <0.0001 | Yes |
| Barbareschi | 124 | R | 178 | LN- | 0.03 | - | No |
| Biesterfeld | 125 | R | 104 | All | - | <0.0001 | Yes |
| | 152 | R | 108 | LN+ | - | 0.0093 | Yes |
| Bos | 107 | R | 153 | All | 0.046 | 0.017 | Yes |
| Chen | 155 | R | 255 | LN- | NS | NS | No |
| Clahsen | 82 | R | 441 | LN- | <0.01 | - | - |
| Clayton | 127 | R | 378 | LN- | - | <0.0001 | Yes |
| | 126 | R | 399 | LN+ | - | <0.0001 | Yes |
| Collan | 108 | R | 120 | All | - | 0.001 | Yes |
| Colpaert | 128 | R | 104 | LN- | <0.0001 | - | No |
| Jong | 13 | R | 112 | All | - | 0.0009 | Yes |
| Eskelinen | 129 | R | 216 | All | 0.01 | - | Yes |
| Groenendijk | 4 | R | 387 | All | <0.0001 | - | - |
| Ikpat | 130 | R | 300 | All | - | <0.0001 | Yes |
| Jannink | 112 | R | 186 | All | - | <0.001 | Yes |
| | 113 | R | 189 | All | - | <0.001 | Yes |
| Joensuu | 131 | R | 311 | All | - | <0.0001 | - |
| Kato | 156 | R | 70 | LN- | - | NS | No |
| | 132 | R | 422 | All | <0.0001 | <0.0001 | Yes |
| Keshgegian | 133 | R | 126 | All | 0.0003 | - | - |
| Kronqvist | 135 | R | 364 | All | <0.0001 | - | Yes |
| Ladekarl | 134 | R | 202 | All | - | 0.0001 | Yes |
| | 138 | R | 71 | Ductal | - | 0.1 | Yes |
| | 137 | R | 98 | LN- | - | 0.0005 | Yes |
| Laroye | 157 | R | 76 | All | - | NS | Yes |
| Le Doussal | 95 | R | 1262 | Ductal | <0.0001 | 0.002 | Yes |
| Linden | 160 | P | 195 | All | 0.001 | - | Yes |
| | 114 | R | 156 | All | 0.001 | 0.005 | Yes |
| Lipponen | 139 | R | 111 | All | 0.001 | 0.001 | Yes |
| | 141 | R | 363 | All | 0.004 | 0.001 | Yes |
| | 140 | R | 202 | All | 0.012 | - | Yes |
| Liu | 142 | R | 791 | All | <0.0001 | <0.0001 | Yes |
| Mandard | 144 | R | 281 | LN-, prem. | <0.001 | <0.001 | Yes |
| Manders | 145 | R | 137 | All | 0.0070 | 0.0017 | Yes |
| Page | 146 | R | 311 | LN- | NS | 0.01 | Yes |
| Pietilainen | 78 | R | 191 | All | - | 0.0025 | Yes |
| Russo | 90 | R | 646 | All | <0.0001 | - | Yes |
| Simpson | 147 | R | 560 | LN+ | 0.004 | - | Yes |
| Theissig | 148 | R | 92 | All | - | <0.0001 | Yes |
| Thor | 46 | R | 486 | All | 0.0056 | - | Yes |
| Toikkanen | 149 | R | 217 | Lobular | - | 0.0001 | Yes |
| Tosi | 150 | R | 350 | All | 0.025 | - | Yes |
| Uyterlinde | 115 | R | 63 | Ductal | - | 0.008 | Yes |
| | 116 | R | 225 | Ductal | - | <0.0001 | Yes |
| | 117 | R | 295 | Ductal | - | <0.0001 | Yes |
| Van Diest | 111 | R | 211 | <55 | - | <0.0001 | Yes |
| | 110 | R | 20 | LN+ | - | 0.004 | - |
| | 9 | R | 148 | All | - | 0.0001 | Yes |
| Younes | 151 | R | 300 | Ductal | - | 0.0032 | Yes |

Not all these studies used independent patient groups.
LN, lymph node; NS, not significant; P, prospective, R, retrospective.

slightly, but there seems to be a consensus threshold at about 10–12 mitosis/2 mm², as in histological grading. Mitosis counting in lymph node metastases also provides some prognostic value.¹⁵⁴

“We have shown that a highly standardised way of assessing the mitotic activity index provided a very strong prognostic factor, with additional prognostic value to tumour size and lymph node status”

Table 1 provides an overview of the different studies on the MAI in breast cancer. The total number of patients investigated is difficult to estimate because not all of the

studies shown used independent patient groups, but the table makes it clear that the MAI has been studied in thousands of patients with usually strong independent prognostic value. Only a few smaller studies failed to reveal prognostic value.^{155–157} In several studies, mitotic count has been shown to have additional prognostic value to tumour size and lymph node status, a combination denoted the multivariate prognostic index.^{104 108 111 120 150 158} Not many data have been published on other subgroups, such as oestrogen receptor positive and negative patients, but in general it can be stated that mitosis counting has independent prognostic value, even from the oestrogen receptor status.

Nevertheless, for practical reasons, it seems that the MAI by itself is preferred for clinical practice. The fact that (a) the

MAI has been shown to be reproducible in multicentre studies involving routine laboratories,¹⁶¹ and (b) the prognostic value of the MAI holds for premenopausal lymph node negative patients,^{111–117} which was confirmed in a nationwide prospective study in the Netherlands,^{160–162–163} has led to acceptance of the MAI by the Netherlands Society of Clinical Oncology as a high risk (MAI $\geq 10/1.59 \text{ mm}^2$) indicator for lymph node negative patients with invasive breast cancer necessitating adjuvant chemotherapy. According to this consensus,¹⁶⁴ lymph node negative patients with breast cancer who have tumours between 1 and 3 cm and a MAI lower than $10/1.6 \text{ mm}^2$ receive no adjuvant treatment, whereas patients with a MAI $\geq 10/1.6 \text{ mm}^2$ receive adjuvant chemotherapy and/or endocrine treatment, depending on their steroid receptor status. In addition, the College of American Pathologists' consensus statement 1999 mentions mitotic figure counting as a category I prognostic factor for breast cancer,¹⁶⁵ and the mitotic count has also been recognised by the UICC as an "essential prognostic factor".¹⁶⁶

"A prospective comparison between the prognostic and predictive value of mitosis counting and microarray expression analysis would be of great interest"

MAI is not seriously affected by fixation delay, although fixation delay does lead to worse morphology, which makes counting more difficult. Therefore, it is advisable to avoid fixation delay when possible, and to keep specimens in the refrigerator until fixation.¹⁶⁷ Ideally, mitosis should be counted before chemotherapy, but even after chemotherapy, the mitotic index has prognostic value.^{168–170} Mitoses should preferably be counted on excision biopsies or mastectomies to avoid sampling error, but even measurements on core biopsies seem to have some value. Several studies have shown that mitosis counting on biopsies can reach the highest score in the histological grading system, but the mitotic index as such is often underestimated in core biopsies.^{171–173} Currently, this issue is even more important, because neoadjuvant chemotherapy is planned based on the prognostic factors assessed on core biopsies.

The advantage of a section based morphological method to assess proliferation such as mitosis counting is that intratumour heterogeneity (for example, central and peripheral tumour parts) is relatively easy to deal with.¹⁷⁴ The MAI has been criticised for not correcting for cellularity, but correction for volume percentage epithelium or cellularity does not lead to a relevant increase in prognostic value, although it does

dramatically increase the time required for a proper assessment.^{112–113}

CONCLUSION

Proliferation plays an important role in the clinical behaviour of invasive breast cancer. Increased proliferation correlates strongly with poor prognosis, irrespective of the methodology used. However, of the different methods to assess proliferation, mitosis counting has been shown most convincingly to provide reproducible and independent prognostic value in invasive breast cancer. Therefore, the MAI is already used in clinical practice in several countries as a single prognostic marker, and is the most well established component of the histological grade. Ki67/MIB1 labelling and the cyclin A index are promising alternatives, which need further methodological fine tuning. In general, however, little attention has yet been paid to the value of these proliferation markers in predicting response to treatment. A prospective comparison between the prognostic and predictive value of mitosis counting and microarray expression analysis would be of great interest.

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REFERENCES

- Marcus JN, Watson P, Page DL, *et al.* Hereditary breast cancer: pathobiology, prognosis, and BRCA1 and BRCA2 gene linkage. *Cancer* 1996;**77**:697–709.
- Eng C, Stratton M, Ponder B, *et al.* Familial cancer syndromes. *Lancet* 1994;**343**:709–13.
- Kerlikowske K, Grady D, Rubin SM, *et al.* Efficacy of screening mammography: a meta-analysis. *JAMA* 1995;**273**:149–54.
- Groenendijk RP, Bult P, Tevarie L, *et al.* Screen-detected breast cancers have a lower mitotic activity index. *Br J Cancer* 2000;**82**:381–4.
- Cowan WK, Angus B, Gray JC, *et al.* A study of interval breast cancer within the NHS breast screening programme. *J Clin Pathol* 2000;**53**:140–6.
- Olsen O, Gotsche PC. Cochrane review on screening for breast cancer with mammography. *Lancet* 2001;**358**:1340–2.
- Collett K, Harveit F, Skjæerven R, *et al.* Prognostic role of oestrogen and progesterone receptors in patients with breast cancer: relation to age and lymph node status. *J Clin Pathol* 1996;**49**:920–5.
- Ellis IO, Dowsett M, Bartlett J, *et al.* Recommendations for HER2 testing in the UK. *J Clin Pathol* 2000;**53**:890–2.
- Van Diest PJ, Michalides RJ, Jannink I, *et al.* Cyclin D1 expression in invasive breast cancer: correlations and prognostic value. *Am J Pathol* 1997;**150**:705–11.
- De Jong JS, Van Diest PJ, Valk Pvan der, *et al.* Expression of growth factors, their receptors and growth inhibiting factors in invasive breast cancer I. An inventory in search of autocrine and paracrine loops. *J Pathol* 1998;**184**:44–52.
- De Jong JS, Van Diest PJ, Valk Pvan der, *et al.* Expression of growth factors, their receptors and growth inhibiting factors in invasive breast cancer II. Correlations with proliferation and angiogenesis. *J Pathol* 1998;**184**:53–7.
- De Jong JS, Van Diest PJ, Michalides RJAM, *et al.* Concerted overexpression of the genes encoding p21 and cyclin D1 is associated with growth inhibition and differentiation in various carcinomas. *Mol Pathol* 1999;**52**:78–83.
- De Jong JS, Van Diest PJ, Baak JPA. Hot spot microvessel density and the mitotic activity index are strong additional prognostic indicators in invasive breast cancer. *Histopathology* 2000;**36**:306–12.
- Van Diest PJ, Brugal G, Baak JP. Proliferation markers in tumours: interpretation and clinical value. *J Clin Pathol* 1998;**51**:716–24.
- Dowsett M, Archer C, Assersohn L, *et al.* Clinical studies of apoptosis and proliferation in breast cancer. *Endocr Relat Cancer* 1999;**6**:25–8.
- Landberg G, Roos G. The cell cycle in breast cancer. *APMIS* 1997;**105**:575–89.
- Daidone MG, Silvestrini R. Prognostic and predictive role of proliferation indices in adjuvant therapy of breast cancer. *J Natl Cancer Inst Monogr* 2001;**30**:27–35.
- Quinn CM, Wright NA. The clinical assessment of proliferation and growth in human tumours: evaluation of methods and applications as prognostic variables. *J Pathol* 1990;**160**:93–102.
- Sherbet GV, Patil D. Genetic abnormalities of cell proliferation, invasion and metastasis, with special reference to gynaecological cancers. *Anticancer Res* 2003;**23**:1357–71.

Take home messages

- Because adjuvant treatment for invasive breast cancer should be given to high risk patients only, and traditional prognostic factors (lymph node status, tumour size) are insufficiently accurate, better predictors of high risk and treatment response are needed
- Most of the multitude of breast cancer prognosticators are directly or indirectly related to proliferation and increased proliferation correlates strongly with poor prognosis, irrespective of the methodology used
- Mitosis counting has the most reproducible and independent prognostic value
- Ki67/MIB1 labelling and the cyclin A index are promising alternatives, although they require further methodological fine tuning

- 20 **Biesterfeld S**, Farokhzad F, Kluppel D, *et al.* Improvement of breast cancer prognostication using cell kinetic-based silver-stainable nucleolar organizer region quantification of the MIB-1 positive tumor cell compartment. *Virchows Arch* 2001;**438**:478–84.
- 21 **Wohlschlegel JA**, Kutok JL, Weng AP, *et al.* Expression of geminin as a marker of cell proliferation in normal tissues and malignancies. *Am J Pathol* 2002;**161**:267–73.
- 22 **Endl E**, Kausch I, Baack M, *et al.* The expression of Ki-67, MCM3, and p27 defines distinct subsets of proliferating, resting, and differentiated cells. *J Pathol* 2001;**195**:457–62.
- 23 **Bergers E**, Van Diest PJ, Baak JPA. Cell cycle analysis of 1664 flow cytometric DNA histograms of fresh breast cancer material. Correlations between flow cytometric, clinical and pathological variables. *Cancer* 1996;**77**:2258–66.
- 24 **Montironi R**, Diamanti L, Santinelli A, *et al.* Computer-aided S-phase fraction determination in DNA static cytometry in breast cancer. A preliminary methodologic study on cytologic material. *Anal Quant Cytol Histol* 1992;**14**:379–85.
- 25 **Tekola P**, Baak JPA, Ginkel HAHM van, *et al.* Three-dimensional confocal laser scanning DNA ploidy cytometry in thick histological sections. *J Pathol* 1996;**180**:214–22.
- 26 **Bergers E**, Van Diest PJ, Baak JPA. Tumour heterogeneity of DNA cell cycle variables in breast cancer measured by flow cytometry. *J Clin Pathol* 1996;**49**:931–7.
- 27 **Noguchi S**, Kasugai T, Miki Y, *et al.* Clinicopathologic analysis of BRCA1- or BRCA2-associated hereditary breast carcinoma in Japanese women. *Cancer* 1999;**85**:2200–5.
- 28 **Lakhani SR**, Jacquemier J, Sloane JP, *et al.* Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *J Natl Cancer Inst* 1998;**90**:1138–45.
- 29 **Cooke TG**, Stanton PD, Winstanley J, *et al.* Long-term prognostic significance of thymidine labelling index in primary breast cancer. *Eur J Cancer* 1992;**28**:424–6.
- 30 **Courdi A**, Hery M, Dahan E, *et al.* Factors affecting relapse in node-negative breast cancer. A multivariate analysis including the labeling index. *Eur J Cancer Clin Oncol* 1989;**25**:351–6.
- 31 **Daidone MG**, Silvestrini R, Valentini B, *et al.* Proliferative activity of primary breast cancer and of synchronous lymph node metastases evaluated by [³H]-thymidine labelling index. *Cell Tissue Kinet* 1990;**23**:401–8.
- 32 **Meyer JS**, McDivitt RW. Reliability and stability of the thymidine labeling index of breast carcinoma. *Lab Invest* 1986;**54**:160–4.
- 33 **Meyer JS**, Province M. Proliferative index of breast carcinoma by thymidine labeling: prognostic power independent of stage, estrogen and progesterone receptors. *Breast Cancer Res Treat* 1988;**12**:191–204.
- 34 **Meyer JS**, Friedman E, McCrate MM, *et al.* Prediction of early course of breast carcinoma by thymidine labeling. *Cancer* 1983;**51**:1879–86.
- 35 **Silvestrini R**, Daidone MG, Di Fronzo G, *et al.* Prognostic implication of labeling index versus estrogen receptors and tumor size in node-negative breast cancer. *Breast Cancer Res Treat* 1986;**7**:161–9.
- 36 **Silvestrini R**, Daidone MG, Valagussa P, *et al.* 3H-thymidine-labeling index as a prognostic indicator in node-positive breast cancer. *J Clin Oncol* 1990;**8**:1321–6.
- 37 **Silvestrini R**, Daidone MG, Mastore M, *et al.* Cell kinetics as a predictive factor in node-positive breast cancer treated with adjuvant hormone therapy. *J Clin Oncol* 1993;**11**:1150–5.
- 38 **Silvestrini R**, Daidone MG, Luisi A, *et al.* Biologic and clinicopathologic factors as indicators of specific relapse types in node-negative breast cancer. *J Clin Oncol* 1995;**13**:697–704.
- 39 **Silvestrini R**, Daidone MG, Luisi A, *et al.* Cell proliferation in 3,800 node-negative breast cancers: consistency over time of biological information provided by 3H-thymidine labeling index. *Int J Cancer* 1997;**74**:122–7.
- 40 **Silvestrini R**, Luisi A, Zambetti M, *et al.* Cell proliferation and outcome following doxorubicin plus CMF regimens in node-positive breast cancer. *Int J Cancer* 2000;**87**:405–11.
- 41 **Tubiana M**, Pejovic MH, Chavaudra N, *et al.* The long-term prognostic significance of the thymidine labelling index in breast cancer. *Int J Cancer* 1989;**33**:441–5.
- 42 **Tubiana M**, Pejovic MH, Koscielny S, *et al.* Growth rate, kinetics of tumor cell proliferation, and long-term outcome in human breast cancer. *Int J Cancer* 1989;**44**:17–22.
- 43 **Volpi A**, De Paola F, Nanni O, *et al.* Prognostic significance of biologic markers in node-negative breast cancer patients: a prospective study. *Breast Cancer Res Treat* 2000;**63**:181–92.
- 44 **Nio Y**, Tamura K, Kan N, *et al.* In vitro DNA synthesis in freshly separated human breast cancer cells assessed by tritiated thymidine incorporation assay: relationship to the long-term outcome of patients. *Br J Surg* 1999;**86**:1463–9.
- 45 **Paradiso A**, Schittulli F, Cellamare G, *et al.* Randomized clinical trial of adjuvant fluorouracil, epirubicin, and cyclophosphamide chemotherapy for patients with fast-proliferating, node negative breast cancer. *J Clin Oncol* 2001;**19**:3929–37.
- 46 **Thor AD**, Liu S, Moore DH 2nd, *et al.* Comparison of mitotic index, in vitro bromodeoxyuridine labeling, and MIB-1 assays to quantitate proliferation in breast cancer. *J Clin Oncol* 1999;**17**:470–7.
- 47 **Weidner N**, Moore DH 2nd, Ljung BM, *et al.* Correlation of bromodeoxyuridine (BRDU) labeling of breast carcinoma cells with mitotic figure content and tumor grade. *Am J Surg Pathol* 1993;**17**:987–94.
- 48 **Goodson WH 3rd**, Moore DH 2nd, Ljung BM, *et al.* The prognostic value of proliferation indices: a study with in vivo bromodeoxyuridine and Ki-67. *Breast Cancer Res Treat* 2000;**59**:113–23.
- 49 **Bergers E**, Diest PJvan, Baak JPA. Prognostic implications of different cell cycle analysis models of flow cytometric DNA histograms of 1301 breast cancer patients: results from the multicenter morphometric mammary carcinoma project. *Int J Cancer* 1997;**74**:260–9.
- 50 **Joensuu H**, Toikkanen S, Klemi PJ. DNA index and S-phase fraction and their combination as prognostic factors in operable ductal breast carcinoma. *Cancer* 1990;**66**:331–40.
- 51 **Mirza AN**, Mirza NQ, Vlastos G, *et al.* Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. *Ann Surg* 2002;**235**:10–26.
- 52 **Pinto AE**, Andre S, Pereira T, *et al.* Prognostic comparative study of S-phase fraction and Ki-67 index in breast carcinoma. *J Clin Pathol* 2001;**54**:543–9.
- 53 **Morris ES**, Elston CW, Bell JA, *et al.* An evaluation of the cell cycle-associated monoclonal antibody Ki-S1 as a prognostic factor in primary invasive adenocarcinoma of the breast. *J Pathol* 1995;**176**:55–62.
- 54 **Bevilacqua P**, Verderio P, Barbareschi M, *et al.* Lack of prognostic significance of the monoclonal antibody Ki-S1, a novel marker of proliferative activity, in node-negative breast carcinoma. *Breast Cancer Res Treat* 1996;**37**:123–33.
- 55 **Sampson SA**, Kreipe H, Gillett CE, *et al.* KiS1—a novel monoclonal antibody which recognizes proliferating cells: evaluation of its relationship to prognosis in mammary carcinoma. *J Pathol* 1992;**168**:179–85.
- 56 **Lynch BJ**, Guinee DG Jr, Holden JA. Human DNA topoisomerase II-alpha: a new marker of cell proliferation in invasive breast cancer. *Hum Pathol* 1997;**28**:1180–8.
- 57 **Rudolph P**, Olsson H, Bonatz G, *et al.* Correlation between p53, c-erbB-2, and topoisomerase II alpha expression, DNA ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas: prognostic implications. *J Pathol* 1999;**187**:207–16.
- 58 **Rudolph P**, MacGrogan G, Bonichon F, *et al.* Prognostic significance of Ki-67 and topoisomerase II alpha expression in infiltrating ductal carcinoma of the breast. A multivariate analysis of 863 cases. *Breast Cancer Res Treat* 1999;**55**:61–71.
- 59 **Bukholm IR**, Bukholm G, Holm R, *et al.* Association between histology grade, expression of HsMCM2, and cyclin A in human invasive breast carcinomas. *J Clin Pathol* 2003;**56**:368–73.
- 60 **Michalides R**, van Tinteren H, Balkenende A, *et al.* Cyclin A is a prognostic indicator in early stage breast cancer with and without tamoxifen treatment. *Br J Cancer* 2002;**86**:402–8.
- 61 **Bukholm IR**, Bukholm G, Nesland JM. Over-expression of cyclin A is highly associated with early relapse and reduced survival in patients with primary breast carcinomas. *Int J Cancer* 2001;**93**:283–7.
- 62 **Spyratos F**, Ferrero-Pous M, Trassard M, *et al.* Correlation between MIB-1 and other proliferation markers: clinical implications of the MIB-1 cutoff value. *Cancer* 2002;**94**:2151–9.
- 63 **Weidner N**, Moore DH 2nd, Vartanian R. Correlation of Ki-67 antigen expression with mitotic figure index and tumor grade in breast carcinomas using the novel "paraffin"-reactive MIB1 antibody. *Hum Pathol* 1994;**25**:337–42.
- 64 **Sahin AA**, Ro J, Ro JY, *et al.* Ki-67 immunostaining in node-negative stage I/II breast carcinoma. Significant correlation with prognosis. *Cancer* 1991;**68**:549–57.
- 65 **Veronese SM**, Gambacorta M, Gottardi O, *et al.* Proliferation index as a prognostic marker in breast cancer. *Cancer* 1993;**71**:3926–31.
- 66 **Veronese SM**, Maisano C, Scibilia J. Comparative prognostic value of Ki-67 and MIB-1 proliferation markers in breast cancer. *Anticancer Res* 1995;**15**:2717–22.
- 67 **Beck T**, Weller EE, Weikel W, *et al.* Usefulness of immunohistochemical staining for p53 in the prognosis of breast carcinomas: correlations with established prognosis parameters and with the proliferation marker, MIB-1. *Gynecol Oncol* 1995;**57**:96–104.
- 68 **Depowski PL**, Brien TP, Sheehan CE, *et al.* Prognostic significance of p34cdc2 cyclin-dependent kinase and MIB1 overexpression, and HER-2/neu gene amplification detected by fluorescence in situ hybridization in breast cancer. *Am J Clin Pathol* 1999;**112**:459–69.
- 69 **Dettmar P**, Harbeck N, Thomssen C, *et al.* Prognostic impact of proliferation-associated factors MIB1 and S-phase in node-negative breast cancer. *Br J Cancer* 1997;**75**:1525–33.
- 70 **Harbeck N**, Dettmar P, Thomssen C, *et al.* Prognostic impact of tumor biological factors on survival in node-negative breast cancer. *Anticancer Res* 1998;**18**:2187–97.
- 71 **Imamura H**, Haga S, Shimizu T, *et al.* Prognostic significance of MIB1-determined proliferative activities in intraductal components and invasive foci associated with invasive ductal breast carcinoma. *Br J Cancer* 1999;**79**:172–8.
- 72 **Jager JJ**, Jansen RL, Arends JW, *et al.* Anti-apoptotic phenotype is associated with decreased locoregional recurrence rate in breast cancer. *Anticancer Res* 2000;**20**:1269–75.
- 73 **Jensen V**, Ladekar M, Holm-Nielsen P, *et al.* The prognostic value of oncogenic antigen 519 (OA-519) expression and proliferative activity detected by antibody MIB-1 in node negative breast cancer. *J Pathol* 1999;**176**:343–52.
- 74 **Kenny FS**, Willsher PC, Gee JM, *et al.* Change in expression of ER, bcl-2 and MIB1 on primary tamoxifen and relation to response in ER positive breast cancer. *Breast Cancer Res Treat* 2001;**65**:135–44.
- 75 **Lau R**, Grimson R, Sansome C, *et al.* Low levels of cell cycle inhibitor p27^{kip1} combined with high levels of Ki-67 predict shortened disease-free survival in T1 and T2 invasive breast carcinomas. *Int J Oncol* 2001;**18**:17–23.
- 76 **Locker AP**, Birrell K, Bell JA, *et al.* Ki67 immunoreactivity in breast carcinoma: relationships to prognostic variables and short term survival. *Eur J Surg Oncol* 1992;**18**:224–9.

- 77 Nakagomi H, Miyake T, Hada M, *et al*. Prognostic and therapeutic implications of the MIB-1 labeling index in breast cancer. *Breast Cancer* 1998;**5**:255-9.
- 78 Pietiläinen T, Lipponen P, Aaltomaa S, *et al*. The important prognostic value of Ki-67 expression as determined by image analysis in breast cancer. *J Cancer Res Clin Oncol* 1996;**122**:687-92.
- 79 Pinder SE, Wenczyk P, Sibbering DM, *et al*. Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. *Br J Cancer* 1995;**71**:146-9.
- 80 Thor AD, Moore DH II, Edgerton SM, *et al*. Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. *J Natl Cancer Inst* 1992;**84**:845-55.
- 81 Trihita H, Murray S, Price K, *et al*. Ki-67 expression in breast carcinoma: its association with grading systems, clinical parameters, and other prognostic factors—a surrogate marker? *Cancer* 2003;**97**:1321-31.
- 82 Clahsen PC, van de Velde CJ, Duval C, *et al*. The utility of mitotic index, oestrogen receptor and Ki-67 measurements in the creation of novel prognostic indices for node-negative breast cancer. *Eur J Surg Oncol* 1999;**25**:356-63.
- 83 Lee AK, Loda M, Mackarem G, *et al*. Lymph node negative invasive breast carcinoma 1 centimeter or less in size (T1a,bNOMO): clinicopathologic features and outcome. *Cancer* 1997;**79**:761-71.
- 84 Billgren AM, Rutqvist LE, Tani E, *et al*. Proliferating fraction during neoadjuvant chemotherapy of primary breast cancer in relation to objective local response and relapse-free survival. *Acta Oncol* 1999;**38**:597-601.
- 85 Chang J, Powles TJ, Allred DC, *et al*. Prediction of clinical outcome from primary tamoxifen by expression of biological markers in breast cancer patients. *Clin Cancer Res* 2000;**6**:616-21.
- 86 Nole F, Minchella I, Colleoni M, *et al*. Primary chemotherapy in operable breast cancer with favorable prognostic factors: a pilot study evaluating the efficacy of a regimen with a low subjective toxic burden containing vinorelbine, 5-fluorouracil and folinic acid (FLN). *Ann Oncol* 1999;**10**:993-6.
- 87 Brown DC, Gatter KC. Ki67 protein: the immaculate deception? *Histopathology* 2002;**40**:2-11.
- 88 Aaltomaa S, Lipponen P, Papinaho S, *et al*. Proliferating-cell nuclear antigen (PC10) immunolabelling and other proliferation indices as prognostic factors in breast cancer. *J Cancer Res Clin Oncol* 1993;**119**:288-94.
- 89 Haerslev T, Jacobsen GK. Proliferating cell nuclear antigen in breast carcinomas. An immunohistochemical study with correlation to histopathological features and prognostic factors. *Virchows Arch* 1994;**424**:39-46.
- 90 Russo A, Bazan V, Morello V, *et al*. Vimentin expression, proliferating cell nuclear antigen and flow cytometric factors. Prognostic role in breast cancer. *Anal Quant Cytol Histol* 1994;**16**:365-74.
- 91 Schoenborn I, Minguillon C, Moehner M, *et al*. PCNA as a potential prognostic marker in breast cancer. *Breast* 1994;**3**:97-102.
- 92 Tahar SR, Neuberg DS, Dieffenbach A, *et al*. Prediction of early relapse and shortened survival in patients with breast cancer by proliferating cell nuclear antigen score. *Cancer* 1993;**71**:3552-9.
- 93 Thomas M, Noguchi M, Kitagawa H, *et al*. Poor prognostic value of proliferating cell nuclear antigen labelling index in breast carcinoma. *J Clin Pathol* 1993;**46**:525-8.
- 94 Genestie C, Zafrani B, Asselain B, *et al*. Comparison of the prognostic value of Scarff-Bloom-Richardson and Nottingham histological grades in a series of 825 cases of breast cancer: major importance of the mitotic count as a component of both grading systems. *Anticancer Res* 1998;**18**:571-6.
- 95 Le Doussal V, Tubiana-Hulin M, Friedman S, *et al*. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. *Cancer* 1989;**64**:1914-21.
- 96 Boiesen P, Bendahl PO, Anagnostaki L, *et al*. Histologic grading in breast cancer—reproducibility between seven pathologic departments. South Sweden breast cancer group. *Acta Oncol* 2000;**39**:41-5.
- 97 Dalton LW, Page DL, Dupont WD. Histologic grading of breast carcinoma. A reproducibility study. *Cancer* 1994;**73**:2765-70.
- 98 Delides GS, Garas G, Georgouli G, *et al*. Intralaboratory variations in the grading of breast carcinoma. *Arch Pathol Lab Med* 1982;**106**:126-8.
- 99 Frierson HF Jr, Wolber RA, Berean KW, *et al*. Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating ductal carcinoma. *Am J Clin Pathol* 1995;**103**:195-8.
- 100 Harvey JM, de Klerk NH, Sterrett GF. Histological grading in breast cancer: interobserver agreement, and relation to other prognostic factors including ploidy. *Pathology* 1992;**24**:63-8.
- 101 Theissig F, Kunze KD, Haroske G, *et al*. Histological grading of breast cancer. Interobserver reproducibility and prognostic significance. *Pathol Res Pract* 1990;**186**:732-6.
- 102 Tsuda H, Akiyama F, Kurosumi M, *et al*. Establishment of histological criteria for high-risk node-negative breast carcinoma for a multi-institutional randomized clinical trial of adjuvant therapy. Japan national surgical adjuvant study of breast cancer (NSAS-BC) pathology section. *Jpn J Clin Oncol* 1998;**28**:486-91.
- 103 Baak JPA, Kurver PHJ, Snoo-Nieuwlaet AJE, *et al*. Prognostic indicators in breast cancer—morphometric methods. *Histopathology* 1982;**6**:327-39.
- 104 Baak JPA, Dop Hvan, Kurver PHJ, *et al*. The value of morphometry to classic prognosticators in breast cancer. *Cancer* 1985;**56**:374-82.
- 105 Baak JPA, Chin D, Diest PJvan, *et al*. Comparative long term prognostic value of quantitative Her2/Neu protein expression, DNA ploidy, morphometric and clinical features in paraffin-embedded invasive breast cancer. *Lab Invest* 1991;**64**:215-22.
- 106 Baak JPA, Wisse-Brekelmans ECM, Kurver PHJ, *et al*. Regional differences in breast cancer survival are correlated with differences in differentiation and rate of proliferation. *Hum Pathol* 1992;**23**:989-92.
- 107 Bos R, Van Der Groep P, Greijer AE, *et al*. Levels of hypoxia-inducible factor-1 alpha independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer* 2003;**97**:1573-81.
- 108 Collan Y, Kumpusalo L, Pesonen E, *et al*. Prediction of survival in breast cancer: evaluation of different multivariate models. *Anticancer Res* 1998;**18**:647-50.
- 109 De Jong JS, Van Diest PJ, Baak JPA. Number of apoptotic cells as a prognostic marker in invasive breast cancer. *Br J Cancer* 2000;**82**:368-73.
- 110 Van Diest PJ, Baak JPA, Matze-Cok P, *et al*. Prediction of response to adjuvant chemotherapy in premenopausal lymph node positive breast cancer patients with morphometry, DNA flow cytometry and HER-2/neu oncoprotein expression: preliminary results. *Pathol Res Pract* 1992;**188**:344-49.
- 111 Van Diest PJ, Baak JPA. The morphometric multivariate prognostic index (MPI) is the strongest prognosticator in premenopausal lymph node negative and lymph node positive breast cancer patients. *Hum Pathol* 1991;**22**:326-30.
- 112 Jannink I, Van Diest PJ, Baak JPA. Comparison of the prognostic value of mitotic activity index (MAI), random MAI (rMAI), M/V-index, and random M/V-index (rM/V-index) in breast cancer patients. *Hum Pathol* 1995;**26**:1086-92.
- 113 Jannink I, Van Diest PJ, Baak JPA. Comparison of the prognostic value of mitotic frequency and mitotic activity index in breast cancer. *Breast* 1996;**5**:31-6.
- 114 Linden JCVan der, Lindeman J, Baak JPA, *et al*. The multivariate prognostic index and nuclear DNA content are independent prognostic factors in primary breast cancer patients. *Cytometry* 1989;**10**:56-61.
- 115 Uyerlinde AM, Schipper NW, Baak JPA, *et al*. Limited prognostic value of cellular DNA content to classical and morphometrical parameters in invasive ductal breast cancer. *Am J Clin Pathol* 1988;**89**:301-7.
- 116 Uyerlinde AM, Baak JPA, Schipper NW, *et al*. Further evaluation of morphometric and flow cytometric features in breast cancer patients with long term follow up. *Int J Cancer* 1990;**45**:1-7.
- 117 Uyerlinde AM, Baak JPA, Schipper NW, *et al*. Prognostic value of morphometry and DNA flow cytometry features of invasive breast cancers detected by population screening: comparison with control group of hospital patients. *Int J Cancer* 1991;**48**:173-81.
- 118 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Prognostic factors after 5 years follow-up in female breast cancer. *Oncology* 1992;**49**:93-8.
- 119 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Prognostic factors in axillary lymph node-negative (pN-) breast carcinomas. *Eur J Cancer* 1991;**27**:1555-9.
- 120 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Predictive value of a morphometric prognostic index in female breast cancer. *Oncology* 1993;**50**:57-62.
- 121 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Prognostic scores combining clinical, histological and morphometric variables in assessment of the disease outcome in female breast cancer. *Int J Cancer* 1991;**49**:886-92.
- 122 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Nuclear morphometry and mitotic indexes as prognostic factors in breast cancer. *Eur J Surg* 1991;**157**:319-24.
- 123 Aaltomaa S, Lipponen P, Eskelinen M, *et al*. Mitotic indexes as prognostic predictors in female breast cancer. *J Cancer Res Clin Oncol* 1992;**118**:75-81.
- 124 Barbareschi M, Caffo O, Veronese S, *et al*. Bcl-2 and p53 expression in node-negative breast carcinoma: a study with long-term follow-up. *Hum Pathol* 1996;**27**:1149-55.
- 125 Biesterfeld S, Noll I, Noll E, *et al*. Mitotic frequency as a prognostic factor in breast cancer. *Hum Pathol* 1995;**26**:47-52.
- 126 Clayton F, Hopkins CL. Pathologic correlates of prognosis in lymph node-positive breast carcinomas. *Cancer* 1993;**71**:1780-90.
- 127 Clayton F. Pathologic correlates of survival in 378 lymph node-negative infiltrating ductal breast carcinomas. Mitotic count is the best single predictor. *Cancer* 1991;**68**:1309-17.
- 128 Colpaert C, Vermeulen P, Jeuris W, *et al*. Early distant relapse in "node-negative" breast cancer patients is not predicted by occult axillary lymph node metastases, but by the features of the primary tumour. *J Pathol* 2001;**193**:442-9.
- 129 Eskelinen M, Lipponen P, Papinaho S, *et al*. DNA flow cytometry, nuclear morphometry, mitotic indices and steroid receptors as independent prognostic factors in female breast cancer. *Int J Cancer* 1992;**51**:555-61.
- 130 Ikpat OF, Kuopio T, Collan Y. Proliferation in African breast cancer: biology and prognostication in Nigerian breast cancer material. *Mod Pathol* 2002;**15**:783-9.
- 131 Joensuu H, Toikkanen S. Identification of subgroups with favorable prognosis in breast cancer. *Acta Oncol* 1992;**31**:293-301.
- 132 Kato T, Kameoka S, Kimura T, *et al*. p53, mitosis, apoptosis and necrosis as prognostic indicators of long-term survival in breast cancer. *Anticancer Res* 2002;**22**:1105-12.
- 133 Keshgegian AA, Cnaan A. Proliferation markers in breast carcinoma. Mitotic figure count, S-phase fraction, proliferating cell nuclear antigen, Ki-67 and MIB-1. *Am J Clin Pathol* 1995;**104**:42-9.
- 134 Kronqvist P, Kuopio T, Collan Y. Quantitative thresholds for mitotic counts in histologic grading: confirmation in nonfrozen samples of invasive ductal breast cancer. *Ann Diagn Pathol* 2000;**4**:65-70.
- 135 Kronqvist P, Kuopio T, Collan Y. Morphometric grading in breast cancer: thresholds for mitotic counts. *Hum Pathol* 1998;**29**:1462-8.

- 136 **Kronqvist P**, Kuopio T, Jalava P, *et al*. Morphometrical malignancy grading is a valuable prognostic factor in invasive ductal breast cancer. *Br J Cancer* 2002;**87**:1275–80.
- 137 **Ladecarl M**, Jensen V. Quantitative histopathology in lymph node-negative breast cancer. Prognostic significance of mitotic counts. *Virchows Arch* 1995;**427**:265–70.
- 138 **Ladecarl M**. Quantitative histopathology in ductal carcinoma of the breast. Prognostic value of mean nuclear size and mitotic counts. *Cancer* 1995;**75**:2114–22.
- 139 **Lipponen P**, Collan Y, Eskelinen MJ. Volume corrected mitotic index (M/V index), mitotic activity index (MAI), and histological grading in breast cancer. *Int J Surg* 1991;**76**:245–9.
- 140 **Lipponen P**, Aaltomaa S, Kosma VM, *et al*. Apoptosis in breast cancer as related to histopathological characteristics and prognosis. *Eur J Cancer* 1994;**30A**:2068–73.
- 141 **Lipponen P**, Papinaho S, Eskelinen M, *et al*. DNA ploidy, S-phase fraction and mitotic indices as prognostic predictors of female breast cancer. *Anticancer Res* 1992;**12**:1533–8.
- 142 **Liu S**, Edgerton SM, Moore DH 2nd, *et al*. Measures of cell turnover (proliferation and apoptosis) and their association with survival in breast cancer. *Clin Cancer Res* 2001;**7**:1716–23.
- 143 **Lynch J**, Pattekar R, Barnes DM, *et al*. Mitotic counts provide additional prognostic information in grade II mammary carcinoma. *J Pathol* 2002;**196**:275–9.
- 144 **Mandard AM**, Denoux Y, Herlin P, *et al*. Prognostic value of DNA cytometry in 281 premenopausal patients with lymph node negative breast carcinoma randomized in a control trial: multivariate analysis with Ki-67 index, mitotic count, and microvessel density. *Cancer* 2000;**89**:1748–57.
- 145 **Manders P**, Bult P, Sweep CG, *et al*. The prognostic value of the mitotic activity index in patients with primary breast cancer who were not treated with adjuvant systemic therapy. *Breast Cancer Res Treat* 2003;**77**:77–84.
- 146 **Page DL**, Gray R, Allred DC, *et al*. Prediction of node-negative breast cancer outcome by histologic grading and S-phase analysis by flow cytometry: an Eastern cooperative oncology group study (2192). *Am J Clin Oncol* 2001;**24**:10–18.
- 147 **Simpson JF**, Gray R, Dressler LG, *et al*. Prognostic value of histologic grade and proliferative activity in axillary node-positive breast cancer: results from the Eastern cooperative oncology group companion study, EST 4189. *J Clin Oncol* 2000;**18**:2059–69.
- 148 **Theissig F**, Baak JP, Schuurmans L, *et al*. 'Blind' multicenter evaluation of the prognostic value of DNA image cytometric and morphometric features in invasive breast cancer. *Anal Cell Pathol* 1996;**10**:85–99.
- 149 **Toikkanen S**, Pylkkanen L, Joensuu H. Invasive lobular carcinoma of the breast has better short- and long-term survival than invasive ductal carcinoma. *Br J Cancer* 1997;**76**:1234–40.
- 150 **Tosi P**, Luzi P, Sforza V, *et al*. Correlation between morphometrical parameters and disease free-survival in ductal breast cancer treated only by surgery. *Appl Pathol* 1986;**4**:33–42.
- 151 **Younes M**, Lane M, Miller CC, *et al*. Stratified multivariate analysis of prognostic markers in breast cancer: a preliminary report. *Anticancer Res* 1997;**17**:1383–90.
- 152 **Biesterfeld S**, Reitmaier M. Re-evaluation of prognostic mitotic figure counting in breast cancer: results of a prospective clinical follow-up study. *Anticancer Res* 2001;**21**:589–94.
- 153 **Elkhuizen PH**, Hermans J, Leer JW, *et al*. Isolated late local recurrences with high mitotic count and early local recurrences following breast-conserving therapy are associated with increased risk on distant metastasis. *Int J Radiat Oncol Biol Phys* 2001;**50**:387–96.
- 154 **van Diest PJ**, Matze-Cok E, Baak JP. Prognostic value of proliferative activity in lymph node metastases of patients with breast cancer. *J Clin Pathol* 1991;**44**:416–18.
- 155 **Chen SC**, Chao TC, Hwang TL, *et al*. Prognostic factors in node-negative breast cancer patients: the experience in Taiwan. *Changgen Yi Xue Za Zhi* 1998;**21**:363–70.
- 156 **Kato T**, Kimura T, Miyakawa R, *et al*. Clinicopathologic features associated with long-term survival in node-negative breast cancer patients. *Surg Today* 1996;**26**:105–14.
- 157 **Laroye GJ**, Minkin S. The impact of mitotic index on predicting outcome in breast carcinoma: a comparison of different counting methods in patients with different lymph node status. *Mod Pathol* 1991;**4**:456–60.
- 158 **Carbone A**, Serra FG, Rinelli A, *et al*. Morphometric prognostic index in breast cancer. *Anal Quant Cytol Histol* 1999;**21**:250–4.
- 159 **Baak JPA**, Van Diest PJ, Peterse JL, *et al*. Selection of lymph node negative unfavorable premenopausal breast cancer patients for adjuvant systemic therapy can best be done by the mitotic activity index (MAI). *J Pathol* 1999;**189**(suppl):4a.
- 160 **Linden JCVan der**, Baak JPA, Lindeman J, *et al*. Prospective evaluation of the prognostic value of morphometry in primary breast cancer patients. *J Clin Pathol* 1987;**40**:302–6.
- 161 **Van Diest PJ**, Baak JPA, Matze-Cok P, *et al*. Reproducibility of mitosis counting in 2469 breast cancer specimens: results from the multicenter morphometric mammary carcinoma project. *Hum Pathol* 1992;**23**:603–7.
- 162 **Baak JPA**, Kurver PHJ, Diest PJ van, *et al*. Data processing and analysis in the multicenter morphometric mammary carcinoma project (MMMCP). *Pathol Res Pract* 1989;**185**:657–63.
- 163 **Baak JPA**, Diest PJ van, Ariens Ath, *et al*. The multicenter morphometric mammary carcinoma project (MMMCP). A nationwide prospective study on reproducibility and prognostic power of routine quantitative assessments in the Netherlands. *Pathol Res Pract* 1989;**185**:664–70.
- 164 **Bontenbal M**, Nortier JW, Beex LV, *et al*. Adjuvant systemic therapy for patients with resectable breast cancer: guideline from the Dutch national breast cancer platform and the Dutch Society for Medical Oncology. *Ned Tijdschr Geneeskde* 2000;**144**:980–4.
- 165 **Fitzgibbons PL**, Page DL, Weaver D, *et al*. Prognostic factors in breast cancer. College of American Pathologists' consensus statement 1999. *Arch Pathol Lab Med* 2000;**124**:966–78.
- 166 **Gospodarowicz MK**, Henson DE, Hutter RVP, *et al*. *UICC prognostic factors in cancer*, 2nd ed. New York: Wiley-Liss, 2001.
- 167 **Bergers E**, Jannink I, Diest PJ van, *et al*. Influence of fixation delay on mitotic activity and flow cytometric %S-phase. *Hum Pathol* 1997;**28**:95–100.
- 168 **Akashi-Tanaka S**, Tsuda H, *et al*. Prognostic value of histopathological therapeutic effects and mitotic index in locally advanced breast cancers after neoadjuvant chemotherapy. *Jpn J Clin Oncol* 1996;**26**:201–6.
- 169 **Honkoop AH**, Pinedo HM, De Jong JS, *et al*. Effects of chemotherapy on pathologic and biologic characteristics of locally advanced breast cancer. *Am J Clin Pathol* 1997;**107**:211–18.
- 170 **Somlo G**, Simpson JF, Frankel P, *et al*. Predictors of long-term outcome following high-dose chemotherapy in high-risk primary breast cancer. *Br J Cancer* 2002;**87**:281–8.
- 171 **Di Loreto C**, Puglisi F, Rimondi G, *et al*. Large core biopsy for diagnostic and prognostic evaluation of invasive breast carcinomas. *Eur J Cancer* 1996;**32A**:1693–700.
- 172 **Sharifi S**, Peterson MK, Baum JK, *et al*. Assessment of pathologic prognostic factors in breast core needle biopsies. *Mod Pathol* 1999;**12**:941–5.
- 173 **Harris GC**, Denley HE, Pinder SE, *et al*. Correlation of histologic prognostic factors in core biopsies and therapeutic excisions of invasive breast carcinoma. *Am J Surg Pathol* 2003;**27**:11–15.
- 174 **Jannink I**, Risberg B, Diest PJ van, *et al*. Heterogeneity of mitoses counting in breast cancer. *Histopathology* 1996;**29**:421–8.