Prognostic value of proliferation in invasive breast cancer: a review

P J van Diest, E van der Wall, J P A Baak

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.

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

Abbreviations: BrdU, bromodeoxyuridine; MAI, mitotic activity index; MoM, minichromosome maintenance; PCNA, proliferating cell nuclear antigen
through a defined process in which several phases can be recognized. 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. Cell cycle time is difficult to assess, but preliminary studies have assessed argyrophilic nuclear organisators in Ki67 positive cells, with promising results. The growth fraction can be more easily assessed by immunohistochemistry of proliferation associated antigens, such as Ki67, Ki-S1 topoisomerase IIα, proliferating cell nuclear antigen (PCNA), geminin, or minichromosome maintenance (McM) proteins, or by DNA flow cytometric or image cytometric (two dimensional or three dimensional) 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. 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. 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.

**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, and patients with a high thymidine labelling index benefit from adjuvant chemotherapy. 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, and Weidner et al confirmed the good correlation between BrdU and mitotic index. Goodson et al found BrdU to be slightly superior to Ki67. 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. However, in view of the high intratumour heterogeneity of the S phase fraction, this feature cannot be used for individual patients.

**Proliferation associated antigens**

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

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

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. Indeed, cyclin A labelling appeared to have prognostic value in invasive breast cancer. Using frozen sections, the Ki67 labelling index was prognostically relevant in several studies in invasive breast cancer. 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, including tissue from lymph node negative patients, and there was a good correlation between Ki67 and MIB-1 staining. In predominantly in situ cancers, even the Ki67 labelling index of the in situ parts seems to have prognostic value. A pronounced decrease in the Ki67/MIB-1 labelling index is associated with a good response to preoperative treatment. However, not all studies on Ki67/MIB1 reached significance. Few studies have dealt with the methodological issues, such as sampling strategies, intratumour heterogeneity, and reproducibility, most studies are retrospective, and thresholds vary.

Because of its conflicting results, PCNA immunohistochemistry does not provide a prognostically relevant assessment of proliferation in breast cancer. 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, but there are well known problems with reproducibility of grading because of the lack of strict protocols. In different studies from our group, we have shown that a highly standardised way of assessing the mitotic activity index (MAI; counting at ×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 and two prospective studies. Several other groups from different countries have confirmed the prognostic value of mitosis counting in primary invasive breast cancer, including prospective studies. Elkhuhuzen 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. The threshold in the different studies varies...
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

<table>
<thead>
<tr>
<th>First author</th>
<th>Ref</th>
<th>P/R</th>
<th>N</th>
<th>Subgroup</th>
<th>Overall survival</th>
<th>Significance</th>
<th>Independent value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaltomaa</td>
<td>121</td>
<td>R</td>
<td>293</td>
<td>LN–</td>
<td>0.005</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>121 R 224 LN+</td>
<td>0.004</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>122 R 106 All</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119 R 281 LN–</td>
<td>0.0115</td>
<td>0.0007</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>118 R 688 All</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 R 611 All</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boak</td>
<td>104</td>
<td>R</td>
<td>271</td>
<td>Ductal</td>
<td>–</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>105 R 82 Ductal</td>
<td>0.0254</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>159 P 576 LN–</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barbareschi</td>
<td>124</td>
<td>R</td>
<td>178</td>
<td>LN–</td>
<td>0.03</td>
<td>–</td>
<td>No</td>
</tr>
<tr>
<td>Biesterfeld</td>
<td>125</td>
<td>R</td>
<td>104</td>
<td>All</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>152 R 108 LN+</td>
<td>0.0093</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bos</td>
<td>107</td>
<td>R</td>
<td>153</td>
<td>All</td>
<td>0.046</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>Chen</td>
<td>155</td>
<td>R</td>
<td>255</td>
<td>LN–</td>
<td>NS</td>
<td>NS</td>
<td>No</td>
</tr>
<tr>
<td>Clausen</td>
<td>82</td>
<td>R</td>
<td>441</td>
<td>LN–</td>
<td>&lt;0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clayton</td>
<td>127</td>
<td>R</td>
<td>378</td>
<td>LN–</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>126 R 399 LN+</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collan</td>
<td>108</td>
<td>R</td>
<td>120</td>
<td>All</td>
<td>–</td>
<td>0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>Colpaert</td>
<td>128</td>
<td>R</td>
<td>104</td>
<td>LN–</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>No</td>
</tr>
<tr>
<td>Jorg</td>
<td>13</td>
<td>R</td>
<td>112</td>
<td>All</td>
<td>–</td>
<td>0.0009</td>
<td>Yes</td>
</tr>
<tr>
<td>Ekelinen</td>
<td>129</td>
<td>R</td>
<td>216</td>
<td>All</td>
<td>0.01</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Groenendijk</td>
<td>4</td>
<td>R</td>
<td>387</td>
<td>All</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ikppart</td>
<td>130</td>
<td>R</td>
<td>300</td>
<td>All</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>Jannink</td>
<td>112</td>
<td>R</td>
<td>186</td>
<td>All</td>
<td>–</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>113 R 189 All</td>
<td>–</td>
<td>&lt;0.001</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joensuu</td>
<td>131</td>
<td>R</td>
<td>311</td>
<td>All</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>–</td>
</tr>
<tr>
<td>Kato</td>
<td>156</td>
<td>R</td>
<td>70</td>
<td>LN–</td>
<td>NS</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>132 R 422 All</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keshgegian</td>
<td>133</td>
<td>R</td>
<td>126</td>
<td>All</td>
<td>0.0003</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kronarizaf</td>
<td>135</td>
<td>R</td>
<td>364</td>
<td>All</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>134 R 202 All</td>
<td>–</td>
<td>0.0001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ladekarl</td>
<td>138</td>
<td>R</td>
<td>71</td>
<td>Ductal</td>
<td>0.1</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>137 R 98 LN–</td>
<td>0.0005</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laroye</td>
<td>157</td>
<td>R</td>
<td>76</td>
<td>All</td>
<td>–</td>
<td>NS</td>
<td>Yes</td>
</tr>
<tr>
<td>Le Doussal</td>
<td>95</td>
<td>R</td>
<td>1262</td>
<td>Ductal</td>
<td>&lt;0.0001</td>
<td>0.002</td>
<td>Yes</td>
</tr>
<tr>
<td>Linden</td>
<td>160</td>
<td>P</td>
<td>195</td>
<td>All</td>
<td>0.001</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>114 R 156 All</td>
<td>0.001</td>
<td>0.005</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipponen</td>
<td>139</td>
<td>R</td>
<td>111</td>
<td>All</td>
<td>0.001</td>
<td>0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>141 R 363 All</td>
<td>0.004</td>
<td>0.001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140 R 202 All</td>
<td>0.012</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liu</td>
<td>142</td>
<td>R</td>
<td>87</td>
<td>All</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>Mandard</td>
<td>144</td>
<td>R</td>
<td>281</td>
<td>LN–, prem.</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>Manders</td>
<td>145</td>
<td>R</td>
<td>137</td>
<td>All</td>
<td>0.0070</td>
<td>0.0017</td>
<td>Yes</td>
</tr>
<tr>
<td>Page</td>
<td>146</td>
<td>R</td>
<td>311</td>
<td>LN–</td>
<td>NS</td>
<td>0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>Pietilainen</td>
<td>78</td>
<td>R</td>
<td>191</td>
<td>All</td>
<td>–</td>
<td>0.0025</td>
<td>Yes</td>
</tr>
<tr>
<td>Russo</td>
<td>90</td>
<td>R</td>
<td>646</td>
<td>All</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Simpson</td>
<td>147</td>
<td>R</td>
<td>560</td>
<td>LN+</td>
<td>0.004</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Theissig</td>
<td>148</td>
<td>R</td>
<td>92</td>
<td>All</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>Thor</td>
<td>46</td>
<td>R</td>
<td>486</td>
<td>All</td>
<td>0.0056</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Toikkanen</td>
<td>149</td>
<td>R</td>
<td>217</td>
<td>Lobular</td>
<td>–</td>
<td>0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>Tosi</td>
<td>150</td>
<td>R</td>
<td>350</td>
<td>All</td>
<td>0.025</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Uyterlinde</td>
<td>115</td>
<td>R</td>
<td>63</td>
<td>Ductal</td>
<td>–</td>
<td>0.008</td>
<td>Yes</td>
</tr>
<tr>
<td>116 R 225 Ductal</td>
<td>&lt;0.001</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>117 R 295 Ductal</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Diet</td>
<td>111</td>
<td>R</td>
<td>211</td>
<td>&lt;55</td>
<td>–</td>
<td>&lt;0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>110 R 20 LN–</td>
<td>0.004</td>
<td>–</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 R 148 All</td>
<td>0.0001</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Younes</td>
<td>151</td>
<td>R</td>
<td>300</td>
<td>Ductal</td>
<td>–</td>
<td>0.0032</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

www.jclinpath.com
MAI has been shown to be reproducible in multicentre studies involving routine laboratories, and (b) the prognostic value of the MAI holds for premenopausal lymph node negative patients, which was confirmed in a nationwide prospective study in the Netherlands. An additional, the College of American Pathologists’ consensus statement 1999 mentions mitotic figure counting as a category I prognostic factor for breast cancer; and the mitotic count has also been recognised by the EIA as an “essential prognostic factor”.

“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. Ideally, mitosis should be counted before chemotherapy, but even after chemotherapy, the mitotic index has prognostic value. 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. 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. 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.12

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.

REFERENCES


Proliferation in breast cancer

49 Bergers E, Diei PJ van, Baak JPA. Prognostic implications of different cell cycle analysis models of flow cytometric DNA histograms of 1,301 breast cancer patients: results from the multicenter study on the flow cytometric mammographic carcinoma project. Int J Cancer 1997;74:260–9.


www.jclinpath.com


Proliferation in breast cancer


