Prognostication and treatment decision-making in early breast cancer

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Prognostic value of mitotic counts in axillary node negative breast cancer patients with predominantly well-differentiated tumours.

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Journal of Surgical Oncology 2005; 31:128-133.

ABSTRACT

Background: In axillary node negative (ANN) breast cancer patients additional prognostic markers are needed to decide whether adjuvant systemic treatment might be useful.

Methods: In the present study the prognostic relevance of mitotic counts and Bloom-Richardson grade (BR-grade) was evaluated in 164 ANN breast cancer patients. No adjuvant systemic treatment was given to any of these patients. Mitotic counts were determined twice, in routine practice and in revision.

Results: A substantial reproducibility of mitotic counts was found, provided that the cut-off value chosen was high enough. After a median follow-up of 10 years, mitotic counts had no prognostic significance for survival at any cut-off value. A trend towards a significant worse survival was found for patients with Bloom-Richardson grade II or III in comparison with grade I.

Conclusions: Based on data in the literature a positive association between both mitotic counts and Bloom-Richardson grade and survival in axillary node negative breast cancer may exist, but the extent of this putative association and its clinical relevance can be argued, particularly in a group of patients with predominantly well-differentiated tumours.

INTRODUCTION

A number of guidelines for the adjuvant systemic treatment of axillary node negative (ANN) breast cancer have been published.[1-3] In these guidelines tumour size is used to decide whether adjuvant systemic treatment is indicated. However, in patients with tumours of intermediate size other prognostic factors are needed to define low or average/high risk subgroups. A number of markers have been suggested for this purpose. However, with the exception of histological grade, the clinical relevance of these markers specifically in ANN breast cancer is not established.

Proliferative capacity is important in the progression of cancer and mitotic counts (MC) represent tumour cell proliferation. MC are also an important component of all histological grading systems. In the present study we evaluated the reproducibility and prognostic relevance of MC and Bloom-Richardson grade (BR-grade) in 164 ANN breast cancer patients. No adjuvant systemic treatment has been administered to these patients. The objective was to determine whether either MC or BR-grade could be used to determine a subgroup of ANN breast cancer patients in whom adjuvant systemic treatment might result in a clinically relevant increase of survival.

PATIENTS AND METHODS

Patients

In 5 hospitals affiliated with the Comprehensive Cancer Centre Middle Netherlands (IKMN) consecutive patients with operable, stage I to III breast cancer, diagnosed between October 1989 and March 1993, were asked to

Table 5.1. Patient and treatment characteristics of eligible and non-eligible patients with negative axillary lymph nodes.

	Number of patients	
	Eligible (n=164)	**Non-eligible** (n=111)
Age median (range)	58	61
< 50 years	48	26
50 – 59 years	45	27
60 – 69 years	37	33
≥ 70 years	34	25
Primary treatment		
Modified radical mastectomy	55	37
Breast conserving therapy	108	68
Other	1	6
Histological type		
Ductal carcinoma	126	83
Lobular carcinoma	17	9
Mixed type	8	6
Other	13	13
Tumour size		
< 11 mm	31	36
11 – 30 mm	117	67
> 30 mm	15	8
Unknown	1	0

participate in our study. From 463 patients we obtained written informed consent. In the present study we specifically focused on ANN breast cancer patients (n=275). Not included were 38 (14%) patients who received adjuvant systemic therapy. Another 58 tumours were non-eligible because we were unable to acquire the exact routine MC from the pathology reports. Finally, specimens from 14 tumours could not be retrieved for revision and of 1 specimen fixation quality

was found not good enough to revise MC. So, eligible were 164 ANN breast cancer patients who received no adjuvant systemic therapy and in whom MC were performed both in routine practice and in revision. Patient- and treatment characteristics of the eligible patients and non-eligible ANN breast cancer patients were comparable and are shown in Table 5.1. The study was performed in a period when mammographic screening was systematically practiced in the IKMN district for patients between 50 and 70 years of age. Follow-up was assessed until December 2002. The median follow-up period was 10.2 years.

Mitotic counts

MC were determined routinely in three pathology departments. Data were obtained from the pathology reports. Routine MC were determined using microscopes with a 400x magnification, a 40x objective and a field area of 159 μm^2. Mitoses were counted in 10 consecutive high power fields. The MC were revised according to the criteria proposed by Baak and Clayton.[4-8] In most cases it was clear which slide was initially used for mitosis counting. In some cases we had to re-select a slide from the provided material. The quality of the provided sections varied, but was interpreted as good in the majority (91%) of cases. MC were revised using a microscope with a 400x magnification, a 40x objective and a field area of 310 μm^2. Mitoses were counted in 20 consecutive fields. Two observers (EF, FB) evaluated the sections simultaneously. In this study the MC were defined as the number of mitoses per 2 mm^2, instead of the number of mitoses per 10 high power fields. This was done in order to overcome the variety in field sizes of the various microscopes used.

Modified Bloom-Richardson grade

In all revised cases histological grade was evaluated using the modified Bloom Richardson grading system as proposed by Elston and Ellis.[9] In this grading

system three parameters: tubule formation, nuclear pleomorphism, and MC are determined. To each parameter a score of 1 to 3 is assigned. The final BR-grade is based on the summed score of these three parameters. For the MC Elston and Ellis used a field area of 274 μm^2. Up to 9 mitoses per 10 fields scored 1 point, 10-19 scored 2 points and more than 20 scored 3 points. This point system was recalculated from mitoses per 2.74 mm^2 (10 x 274 μm^2) to mitoses per 2 mm^2: Up to 7 mitoses per 2 mm^2 scored 1 point, 8 - 14 scored 2 points and more than 14 scored 3 points.

Statistics

Statistical analysis was carried out using the statistical package SPSS for Windows, release 10.0 (SPSS Inc.). Correlations between routine and revised MC were assessed using the nonparametric Spearman test. The agreement and the proportion of potential agreement beyond chance that was actually achieved (Kappa) between routine and revised MC were determined using cut-off values ranging from 4 to 18 mitoses / 2 mm^2. Association between MC and BR-grade was assessed using the Kruskal-Wallis test. Univariate and multivariate survival analyses were performed with the time-fixed Cox regression procedure. Survival endpoints of the study were disease free survival (DFS), distant metastasis free survival (DMFS) and overall survival (OS). For DFS time to failure was computed from the date of surgery until relapse or until the last day patient was known to be disease free. For DMFS time to failure was computed from the date of surgery until distant metastasis or until the last day patient was known to be free of distant metastasis. Patients who died during follow-up were censored at the date of death. Patients who developed contra-lateral breast cancer were censored at the date of diagnosis. OS was calculated from the date of surgery until death or until the date patient was last known to be alive.

RESULTS

Reproducibility

The mean and median MC measured routinely and after revision are listed per pathology department in table 5.2. Mean and median values were comparable between the 3 pathology departments and between routine and revised evaluation. In the revised evaluation significantly higher maximum MC were scored than in routine evaluation. In the revised specimens the BR-grade was determined as well (Table 5.2). Seventy-four tumours (45%) were histological well differentiated, 59 (36%) were of intermediate grade and 31 (19%) were poorly differentiated.

Table 5.2. Routine and revised mitotic counts and Bloom-Richardson grade according to pathology department.

	Pathology department		
	A	**B**	**C**
Number of patients	62	50	52
Routine mitotic counts			
Median (range)	7 (1-47)	6 (0-44)	8 (0-54)
Mean	11	10	12
Revised mitotic counts			
Median (range)	7 (0-92)	6 (0-85)	5 (0-91)
Mean	11	12	12
Bloom-Richardson grade			
I	45%	40%	50%
II	44%	34%	29%
III	11%	26%	21%

Routine and revised MC correlated well ($r = 0.76$, $p < 0.001$). The observed agreement between routine and revised MC varied between 0.76 and 0.90, kappa varied between 0.37 and 0.66, depending on the cut-off value used. Kappa was lower specifically when lower cut-off values were used. BR-grade and MC were strongly associated ($p<0.0001$). Median revised MC was 3 per 2 mm^2 in grade I tumours, 9 per 2 mm^2 in grade II tumours, and 22 per 2 mm^2 in grade III tumours.

Prognostic value

During follow-up 36 patients had recurrent disease (28 patients with distant metastases) and 37 patients died (23 deaths were caused by breast cancer). After 5 year DFS was 83% (DMFS 86%), OS was 90% (disease specific survival 94%). After 10 year DFS was 76% (DMFS 81%), OS was 77% (disease specific survival 85%).

The prognostic value of revised MC for DFS, DMFS and OS was analysed. Hazard ratios were determined using progressively higher cut-off values. Significance was not found for DFS, DMFS or for OS at any cut-off value. Comparable results were found when the analyses were performed on routine MC or were restricted to patients younger than 70 years of age, tumours 11 to 30 mm in diameter, or ductal carcinomas only (data not shown). As an example Figure 5.1 shows the overall survival curves according to revised MC using 13 mitoses / 2 mm^2 as cut-off value (Figure 5.1).

The risk for relapse (including loco-regional relapses) did not differ significantly between well, moderately and poorly differentiated tumours. The risk for distant metastasis was highest in patients with poorly differentiated tumours, but not significantly different from that of patients with well-differentiated tumours ($p=0.12$). Patients with moderately differentiated tumours had a significant higher

risk (p=0.04, RR 2.2) for death than patients with well-differentiated tumours (Figure 5.2).

In multivariate analysis including age, tumour size, BR-grade and MC, age was associated with OS (p=0.03) and BR-grade was associated with DSS (p=0.04)

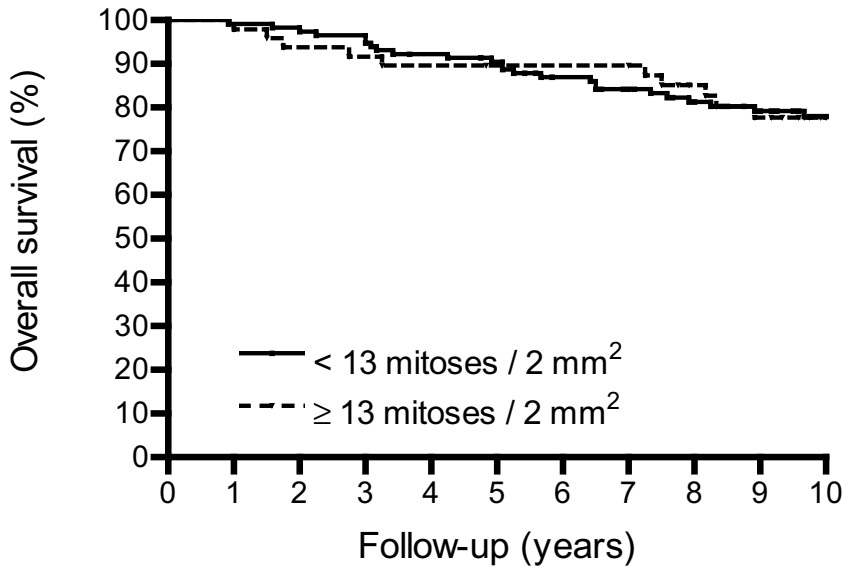
DISCUSSION

In published studies on MC in breast cancer the MC are usually expressed as number of mitoses per 10 high-power fields. But, these high-power fields are not uniformly defined. The area of the high-power fields used, if mentioned at all, varies from 0.102 mm^2 to 0.216 mm^2.[10,11] Consequently interpretation of results is difficult. To overcome this problem we have defined MC as the number of mitotic figures per 2 mm^2.

In the present study the median MC was 6 mitoses per 2 mm^2. In other reports the median MC (recalculated into mitoses per 2 mm^2) varied from 2.7 to 13.9 mitoses per 2 mm^2.[4,8,10-12] This variation can probably be explained by differences in patient characteristics: Tumours detected by screening have lower MC and MC in ANN patients are lower than those in node positive patients.[11,13] But, the observed wide variation in median values of MC also may suggest a low interobserver (or intergroup) reproducibility.

To assess the reproducibility of MC we have revised tumour samples from 164 patients. The MC were initially determined in routine practice at 3 separate pathology departments. The correlation coefficient found between routine and revised MC was 0.76. Bergers et al. found slightly better correlations.[14] The correlation coefficients found by van Diest et al. were much better with an overall r of 0.91.[7] But, in that study the counting areas were marked, which might explain

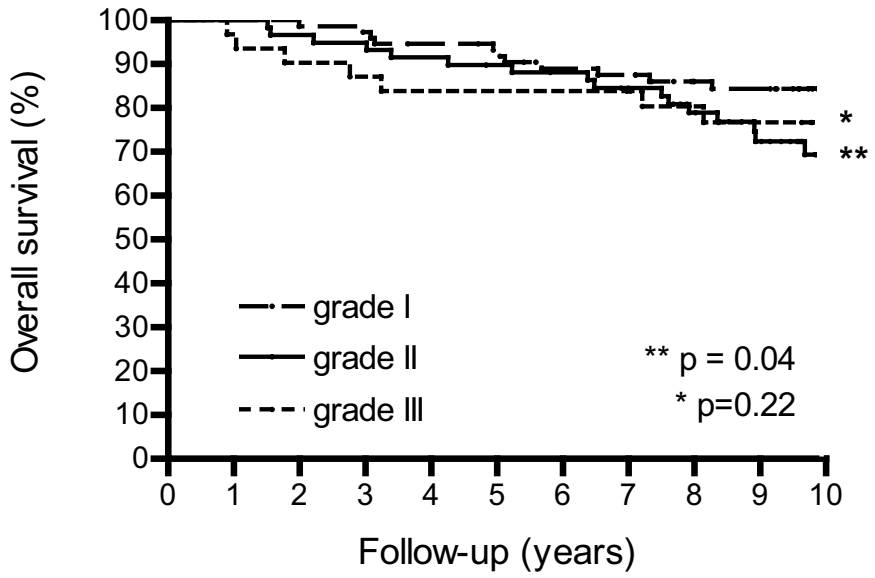
Figure 5.1. Overall survival according to mitotic counts using 13 mitoses / 2 mm^2 as cut-off value.



the higher correlation coefficients.[15] The reproducibility of MC is said to depend on the quality of the slides and on the pathologist's interpretation.[5] In our opinion the correlation coefficient of 0.76 is a good reflection of the reproducibility of MC obtainable in routine practice. The wide variation in median MC found among the investigational groups can probably be explained by a poor agreement between them in the recognition and/or interpretation of (abnormal) mitoses.[16]

For survival analyses the MC are often dichotomised, but the cut-off value used and proposed for this purpose varies. In dichotomised variables kappa is a measure of reproducibility. The reproducibility of the MC is smaller when the number of mitotic figures counted is smaller.[17] In the present study a substantial kappa (> 0.60) was reached when the cut-off value used was at least 6 mitoses per 2 mm^2. Reproducibility of MC and, as a consequence, its prognostic value

Figure 5.2. Overall survival according to Bloom-Richardson histological grade.



declined when lower cut-off values were used. Therefore, the cut-off value used must be sufficiently high to obtain reproducible and reliable analyses of the prognostic value of MC.

Mirza et al. have recently reviewed the published literature on prognostic factors in patients with ANN breast cancer, focusing principally on recent studies with large sample sizes and extended follow-up periods.[18] Four studies were identified that assessed the prognostic value of MC for decreased survival.[8,19-21] We have found three more studies.[22-24] In the present study no significant association between MC and survival was found, but the number of events (relapse and death) was relatively low. The strongest association between MC and DFS or OS in ANN breast cancer was reported by van Diest and Baak.[24] But the number of patients and events in that study was low. Clayton showed a positive association between MC and DSS in a study with sufficient events.[8] But, in that study the

median value for the MC was low, which might have had a negative influence on reproducibility.[17] In the largest study, performed on 1028 patients with T1N0 breast cancer, no significant association between MC and survival was found.[23] Page showed a significant association between MC and OS only when the analysis was restricted to the first 5 years of follow-up.[22] The association disappeared with longer follow-up time. In the study performed by Aaltomaa DFS and DSS were positively associated with MC, but DFS could not be predicted by MC in patients with tumours ≤2 cm in diameter.[19] Based on these studies we submit that a positive association between MC and survival in ANN breast cancer may exist, but that the extent of this putative association is a matter of debate. The extent probably depends on other tumour characteristics such as tumour size and histological grade.

In the present study a trend towards a significantly worse survival was found in patients with poorly or moderately differentiated tumours compared with patients with well-differentiated tumours. The number of well-differentiated tumours was relatively large (45%). In the study performed by van Diest only 12% of ANN tumours were well differentiated. In that study no significant association between BR-grade and OS was found, in contrast to a strong association between MC and OS.[24] In the studies performed by Aaltomaa, Clahsen, Clayton and Page the BR-grade was positively associated with DSS and OS respectively.[8,19,21,22] In the studies performed by Aaltomaa and Clayton the MC were slightly better in predicting DSS. In the studies performed by Clahsen and Page the BR-grade was slightly better.

In conclusion the determination of MC is an inexpensive, fast and reproducible way of assessing proliferation in routine practice. But, apparently, there is a poor agreement between the different investigational groups in the recognition and/or interpretation of (abnormal) mitoses. When cut-off values are used for survival analyses, they must be sufficiently high to obtain reproducible and reliable

analyses. Based on data in the literature it is likely that in patients with ANN breast cancer the MC are positively associated with survival, but the extent of this association can be a matter of debate. In the present study no significant association between MC and a number of relevant survival end-points was found. The favourable tumour characteristics and the associated low number of events can probably explain this. The prognostic value of the BR-grade is likely to be comparable to that of the MC. In the present study a trend towards a significant worse survival was found in patients with grade II or III tumours compared with patients with grade I tumours. In ANN breast cancer patients the prognostic value of the BR-grade may be superior to MC if the tumours are predominantly well differentiated, whereas MC may be superior to BR-grade if the tumours are predominantly poorly differentiated.

ACKNOWLEDGEMENTS

The authors wish to thank drs. M.E.I. Schipper, dr. H.M. Ruitenberg and dr. A. Hennipman for their help with this study. They thank the Comprehensive Cancer Center Middle Netherlands (IKMN) for their help with data collection, in particular drs. C.H.F. Gimbrere. They also thank the “Aart Huisman Stichting” and the “Stichting Medicina et Scientia” for their financial contributions.

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