Prognostic Value of Mitotic Counts in Breast Cancer of Saudi Arabian Patients

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Abstract. *Background: Quantitative methods in combination with other objective prognostic criteria can improve the evaluation of a cancer patient's prognosis, and possibly predict response to therapy. One of the important prognostic and predictive markers is the mitotic count, which has proven valuable in many aspects. In this study, the prognostic value of the mitotic count was assessed in breast cancer (BC) patients in Saudi Arabia. Patients and Methods: The study comprised a series of 87 patients diagnosed and treated for breast cancer at the Departments of Surgery and Oncology, King Abdul-Aziz University Hospital, between 2000 and 2008. Mitotic counts were carried out using a standard laboratory microscope (objective, ×40; field diameter, 420 μm). The number of mitotic figures in 10 consecutive high-power fields (hpf) from the most cellular area of the sample gave the mitotic activity index (MAI, mitotic figures/10 hpf). The standardized mitotic index (SMI) recorded the mitotic count as the number of mitotic figures by area of the neoplastic tissue in the microscopic field, thus the number of mitoses in 10 consecutive fields was corrected for the volume fraction and field size (mitotic figures/mm^2). Results: The means of MAI and SMI of the tumors in the entire series of 87 patients were 15 mitotic figures/10hpf (range 4-45) and 4 mitotic figures/mm^2 (range 1-9), respectively. The mitotic counts were higher in advanced stages than in early cancer ($p<0.04$). The mitotic counts were significantly larger in patients with high-grade tumor ($p<0.004$) and in cases with tumor metastasis ($p<0.004$). The mitotic counts were also significantly larger in the recurrent cases than in non-recurrent ones ($p<0.02$). Conclusion: The quantitatively measurable mitotic counts of cancer cell nuclei are of significant prognostic value in invasive ductal carcinoma of the breast in Saudi Arabia and the mean cut-off values of MAI and SMI can be applied as objective (quantitative) criteria to distinguish breast cancer patients into groups with favorable and less favorable prognosis.*

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According to the Saudi Cancer Registry Report (2005) (1), breast cancer (BC) is the most common cancer among women, accounting for 22.4% of all newly diagnosed carcinomas, with an age-specific incidence rate of 15.4/100,000. The median age at diagnosis is 47 years (range 18-96 years). BC has been intensely studied worldwide, but many aspects still remain unclear, including some intriguing special features of BC encountered in different global regions. The possibility that these geographic differences may have a genetic basis is one favored hypothesis. The variation in the distribution of different BC genetic marker haplotypes with a clear difference in distribution between Western Central Africa and Northern Africa and similarly between Asia and Europe has substantiated this suggestion (2).

Approximately, 20%-30% of the patients with lymph node-negative (LN^-) BC die of recurrent disease. A relative survival improvement of 15%-20% over the next decade might be expected from improvements in adjuvant systemic therapy (3). Accurate and reliable prognostic markers are needed to help identify the high-risk patients. The BC prognosis can be evaluated by combining different clinico-pathological features such as tumor size, stage, grade and LN status (4). Also the histological grading system provides high prognostic potential (5, 6), but it suffers from being subjective and still leaves a substantial group of patients with an unclear prognosis (7). In this

respect, accurate quantitative measurements would be expected to be more reproducible than the subjective methods of tumor grading (8).

Many recent studies have shown that proliferation markers exceed the prognostic value of classical predictors (9-12). A variety of methods have become available to assess the rate of proliferation based on the cell cycle (13, 14). The growth fraction can be evaluated using immunohistochemistry for different proliferation-associated antigens, such as Ki-67 (15), topoisomerase IIα (16), proliferating cell nuclear antigen (PCNA) (17) and geminin (14), or by analysis of the S-phase fraction using DNA flow cytometry or DNA static image cytometry (18). However, the S-phase fraction method is hampered by pronounced intra-tumor heterogeneity (19). Therefore, mitosis counting and the Ki-67 index are the most practical methods. However, in a Finnish material it became quite clear that the mitotic count was even much better prognosticator than quantified Ki-67 staining (quantified as the fraction of positively staining nuclei) (20). Out of these two methods, mitosis counting has been best studied from a methodological point of view, based on larger retrospective and prospective studies (21). Mitotic counting has been reported to be a powerful, practical, easily assessable, inexpensive and highly reproducible prognosticator (9, 21-24). Furthermore, several studies have indicated that the mitotic count is the most important constituent of the histological grade (25, 26), but well-known problems with reproducibility of the grading exist due to the lack of strict protocols (27, 28). Aaltomaa *et al*. (29) have suggested that all types of BC could be graded using the same principles when mitotic indices are determined, based on the observed minor differences only in the proliferative activity between ductal carcinomas and all special BC forms (29).

Among a wide range of quantitative histopathology approaches for unbiased assessment of potential prognostic factors, nuclear morphometry (30-32) and mitotic counting (33), have been shown to be able to distinguish between benign and malignant lesions. With others, we have suggested that the mitotic count in combination with other objective prognostic criteria could improve the evaluation of prognosis in BC and possibly predict response to therapy, independently of the geographic peculiarities of this disease.

As part of our efforts to introduce a mitotic count grading system specifically suitable for BC in Saudi women, the prognostic value of mitotic count was assessed in BC patients in Saudi Arabia, with special reference to similar data reported from other countries.

Patients and Methods

The material for this study was derived from a cohort of 201 consecutive women diagnosed with BC at the Department of Pathology, King Abdul-Aziz University, Jeddah, Saudi Arabia between 2000 and 2008. Patients were excluded from this study on the basis of the following criteria: histopathological diagnosis was not invasive ductal carcinoma (IDC); patient history, medical files or BC specimens were not found. The remaining cohort of 87 women with IDC was eligible for the counting of mitotic figures.

The pertinent clinicopathological features (age, menopausal status, stage, grade, and LNN status) and follow-up and survival data were collected from the patient files and are summarized in Table I. The mean age at the time of diagnosis was 47.5 years (range: 19-81 years).

Treatment and follow-up. Almost all the patients were subjected to surgery, *i.e*., lumpectomy, radical or modified radical mastectomy with axillary clearance. Postoperative early adjuvant systemic therapy in the form of chemotherapy, radiotherapy and hormonal therapy was given to 72%, 56% and 38% of the patients, respectively. After treatment, the patients were seen at 6-12 month intervals until death or the end of follow-up (FU) in mid August, 2009. Some patients were lost to FU. The mean FU time for the whole series was 47 months (range: 4-118 month). During FU, the patients were subjected to repeated clinical examination and bone isotope scan, chest and abdominal-pelvic CAT scan were performed whenever needed. In most instances, the cause of death was obvious on clinical grounds alone. Autopsy was not performed in any case.

During the FU period, 15 (17%) patients developed recurrence and 12 (13%) patients developed distant metastasis in different organs. Disease-free survival (DFS) and disease-specific survival (DSS) were calculated as the time from diagnosis to the appearance of recurrent disease (or date last seen disease-free), and time from diagnosis to death (due to disease) or to the date last seen alive, respectively. In calculating the DSS, the patients who died of other or unknown causes were censored.

Counting mitotic figures. All the tissue samples has been obtained from the primary tumor at the time of diagnosis. The samples were fixed in buffered formalin and embedded in paraffin. Sections were cut at 5 μm and stained with H&E. Mitotic figures were characterized by an absent nuclear membrane with clear, hairy extensions of nuclear material (condensed chromosomes) either clumped (beginning metaphase), in a plane (metaphase/anaphase) or in separate chromosomal aggregates (anaphase/telophase). The cytoplasm of the mitotic cells was often larger during mitosis than in the resting cells. Special attention was paid to distinguishing between apoptotic bodies and mitotic figures (34). The recognition of at least one chromosome, usually appearing as a small protuberance at the outline of the chromosome clump was required for inclusion in the mitotic count. The absence of nuclear membrane was also an important feature, but did not alone constitute mitosis.

Sampling rules. The mitotic figures were counted in the most cellular area at the periphery of the tumor from 10 consecutive high- power fields (hpf) (35). Necrotic and inflammatory areas were avoided. If several areas met these criteria, the area with the highest number of mitotic figures, assessed subjectively, was chosen. Two parallel clearly separate chromosome clumps were counted as one mitotic figure. Mitotic counting was carried out using a standard laboratory microscope (objective, ×40; field diameter, 440 μm). Two methods were used record mitoses in the

Table I. *Clinicopathological characteristics of the patients.*

Characteristic	No. of patients (%)
Age (years)	
<50	50 (57%)
>50	37 (43%)
Menopausal status	
Premenopausal	48 (55%)
Postmenopausal	39 (45%)
Localization	
Right	43 (49%)
Left	44 (51%)
Neurovascular invasion	
No	25 (29%)
Yes	37 (42%)
Unknown	25 (29%)
Lymph node	
N0	24 (28%)
N1	33 (38%)
Nx	30 (34%)
Metastasis	
M0	38 (44%)
M1	12 (13%)
Mx	37 (43%)
Grade	
G1	17 (20%)
G2	48 (55%)
G3	21 (24%)
Gx	1 (1%)
Stage	
1	16 (18%)
2	44 (51%)
3	4 (5%)
4	12 (14%)
Unknown x	11 (12%)
Recurrence during follow-up	
Yes	15 (17%)
No	56 (65%)
Unknown	16 (18%)
Response to treatment	
CR	45 (51%)
PR	7 (8%)
PD	11 (13%)
Unknown	24 (28%)
Status at the end of follow-up	
Alive	66 (76%)
Died of disease	8 (9%)
Unknown	13 (15%)

CR: complete response, PR: partial response, PD: progressive disease.

cancer cells the mitotic activity index (MAI) and the standardized mitotic index (SMI). The number of mitotic figures in the 10 consecutive hpf gave the mitotic activity index (MAI). From the counted areas, SMI (also called M/Vv index) was calculated as the number of mitotic figures by area of the neoplastic tissue in the microscopic field. Thus the number of mitoses in 10 consecutive fields was corrected for the volume fraction and field size (37).

$$SMI=k\ (\sum MI)/(\sum Vv)$$

Table II. *Clinicopathological features and associated mitotic activity (MAI) and standardized mitotic (SMI) indices.*

Clinicopathological features	Mean mitotic count *p*-value	
	MAI	SMI
Age	0.840	0.40
Menopausal status	0.790	0.84
Site (L, R)	0.770	0.02
Margins	0.760	0.54
Invasion	0.870	0.85
Lymph node	0.690	0.47
Metastasis	0.004	0.01
Grade	0.004	0.01
Stage	0.040	0.05
Response to treatment	0.050	0.01
Recurrence	0.020	0.01
DFS	0.300	0.30
DSS	0.300	0.40

L: Left, R: right, DFS: disease-free survival, DSS: disease-specific survival.

Where $k=100/r^2$, r=the radius of the field and MI=number of mitotic figures in the studied fields. Vv is the volume fraction of malignant epithelium in the field.

Statistical analysis. Statistical analyses were performed using the SPSS® (SPSS, Inc., Chicago state, USA) and STATA (Stata Corp., town, TX, USA) software packages (PASW Statistics for Windows, version 18.0.1 and STATA/SE 11.0). Student *t*-tests and ANOVA were used to test differences between the groups. Bivariate correlations between the mitotic counts and DFS and DSS were evaluated using Pearson's correlation test. For univariate survival analysis, Kaplan-Meier curves were plotted and differences between the strata (MAI and SMI cut-offs) were analyzed using the log-rank test. In addition, multivariate analysis was performed using Cox's regression model with known prognostic predictors (age, family history, site, tumor grade, LNN involvement, response to treatment, stage) were entered in stepwise backward approach, to evaluate the independent prognostic value of MAI and SMI. In all the analyses, *p*-values below 0.05 were regarded as significant.

Results

Clinicopathological features. The correlation of the mitotic counts (MAI and SMI) with the different clinicopathological features is shown in Table II. MAI and SMI means were 15 mitotic figures/10 hpf, (range 4-45), and 4 mitotic figures/mm^2 (range 1-9), respectively, in the whole series of 87 samples. The MAI and SMI means were used as the cut-off in further calculations to correlate the mitotic counts with the clinical parameters and disease outcome.

Higher values of SMI were seen in the left breast tumors than the right side tumors ($p<0.02$), while MAI did not show this trend ($p<0.77$). Significant associations were observed between mitotic count and histological grade.

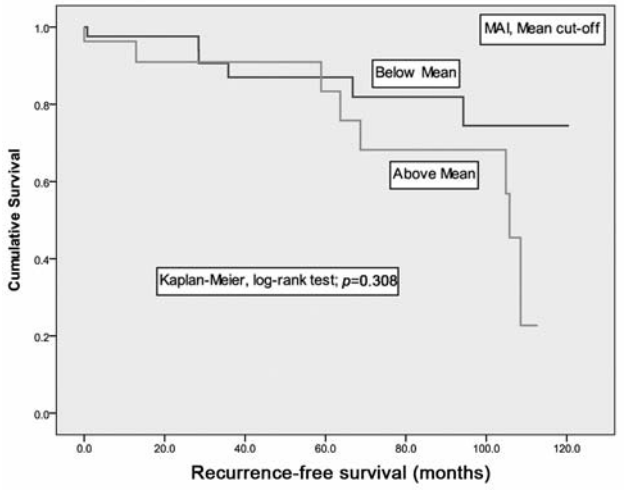

Figure 1. *MAI as predictor of disease-free survival (DFS).*

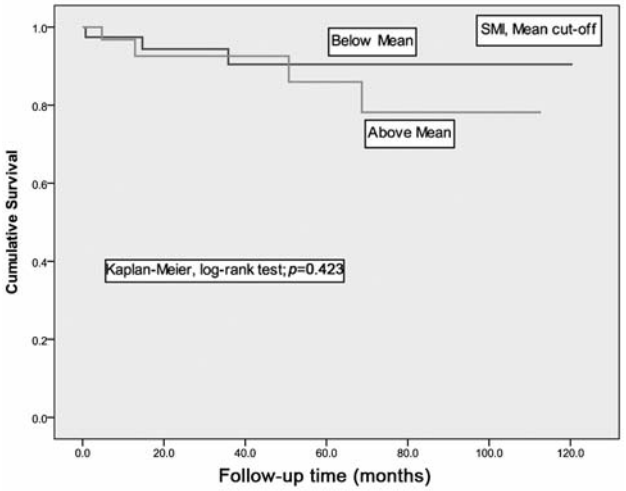

Figure 2. *SMI as predictor of disease-specific survival (DSS).*

High-grade tumors showed higher mitotic counts (19 mitotic figures/10 hpf) for MAI and for SMI (5.1 mitotic figures/mm^2) as compared with low-grade tumors (12 mitotic figures/10 hpf and 3.8 mitotic figures/mm^2, respectively), (p<0.0004, MAI; p<0.01, SMI, respectively). Similarly, mitotic counts were significantly higher in the tumors that subsequently recurred (19 mitotic figures/10 hpf for MAI and 5 mitotic figures/mm^2 for SMI) when compared with the non-recurrent ones (13.9 mitotic figures/10 hpf and 3.9 mitotic figures/mm^2; p<0.02, p<0.01, respectively). In the same way, mitotic counts were higher in the patients who developed metastasis (21 mitotic figures/10 hpf and 5.3 mitotic figures/mm^2) than in those who did not by the end of the follow-up (13 mitotic figures/10 hpf and 3.9 mitotic figures/mm^2; p<0.0004, MAI; p<0.01, SMI, respectively). The mitotic counts were also higher in the advanced stages (21 mitotic figures/10hpf and 5.4 mitotic figures/mm^2) than in early stages (15 mitotic figures/10 hpf and 4 mitotic figures/mm^2) (p<0.04, MAI; p<0.05, SMI, respectively). There was also a significant association between mitotic count and response to treatment: the mean mitotic counts of patients with complete response (CR), partial response (PR) and progressive disease (PD) were 13 mitotic figures/10 hpf for MAI and 4 mitotic figures/mm^2 for SMI, 16 mitotic figures/10 hpf for MAI and 4.9 mitotic figures/mm^2 for SMI, and 19 mitotic figures/10 hpf for MAI and 5 mitotic figures/mm^2 for SMI (p<0.05, MAI; p<0.01, SMI), respectively.

The values of MAI and SMI were slightly higher in the LN$^+$ patients than in LN$^-$ patients (p<0.6, MAI; p<0.4, SMI, respectively). The same trend was observed between MAI/SMI and disease outcome, both being higher among the women who died of their disease as compared with those who were alive, although the difference did not reach significance (p<0.16, MAI; p<0.10, SMI, respectively). In contrast, there was no relationship between age and mitotic count, which was identical in the patients below and above the mean age of 47.5 years (p<0.84, MAI; p<0.40, SMI, respectively). Similarly, the mitotic count was associated with neither the involvement of the tumor margins (p<0.76, MAI; p<0.54, SMI, respectively) nor with tumor invasion to blood vessels or nerves (p<0.87, MAI; p<0.85, SMI, respectively).

Survival analysis. In the univariate (Kaplan-Meier) survival analysis, MAI (with mean as the cut-off) showed a trend towards being a predictor of DFS (log-rank p<0.3) (Figure 1). At 6 years, 20% of the patients with lower MAI showed recurrence, as compared to 33% of the patients with higher MAI (Figure 1). Mitotic count did not show any significant correlation with DSS (Figure 2, p<0.4).

Out of the variables entered in the multivariate regression model, response to therapy was the only independent predictor of DFS, with HR=3.42 (95% CI 1.77-6.61) for women with CR to be recurrence-free as compared to those with PR or PD. As with DFS, MAI or SMI were not independent predictors of DSS in the multivariate model, where none of the other variables proved to be independent predictors.

Discussion

A close correlation between the mitotic count and some of the clinicopathological features and also the disease outcome was shown in the BC patients. However, the biological

mechanisms responsible for these mitotic count variations in the tumor cells remain to be disclosed, although certain mutations in growth-regulating genes may contribute to the high mitotic activity seen (37). The significant factors observed in this study reflect the prognostic variables in the early stages of follow-up in Saudi BC. These mean values MAI and SMI were useful in separating the patients with favorable and unfavorable outcome of the disease in the present cohort. The proliferative activity of the Saudi material was within the ranges reported in a Finnish BC series where the corresponding values were 10.7 mitotic figures/10 hpf and 13.8 mitotic figures/mm^2 and in other European studies as well (38). However, these figures were much lower than in a Nigerian and African-American BC series (39). The Finnish premenopausal patients (40) had higher values of the proliferative indices than the postmenopausal patients which was in contrast to the Nigerian patients studied by Ikpatt *et al*. (39) where the mitotic count was higher in the postmenopausal patients. However, no statistically significant difference between the menopausal statuses was shown in the present study.

The different mitotic counts observed in the present series might reflect actual biological differences between BC in these populations. It is well known that significantly different tumor cell populations, clones, with dissimilar biology, exist in highly proliferating advanced BC. These different clones may have different *p53* status, DNA ploidy, proliferation rates and nuclear morphology (41).

The observation that tumors with higher mitotic counts were associated with the presence of LN metastasis was similar to other studies and requires further assessment. It would seem feasible that tumors with higher mitotic counts are more aggressive and more likely to be associated with LN involvement at diagnosis. In accordance with other similar cohorts (39, 42), the present study showed that the mitotic counts were correlated with the tumor grade and stage, high-grade tumors showing higher mitotic counts, which would be expected as the mitotic count is considered the most important constituent of the histological grade in individual tumors (21).

In the present study, a significant association was shown between the mitotic counts and the response to treatment: an objective response to adjuvant systemic therapy was observed among the patients with lower mitotic activity, in contrast to those with a higher proliferation rate who developed PD. This was in contrast to some other studies which showed that patients with rapidly proliferating tumors benefited from adjuvant systemic therapy more than patients with low proliferation rates (43, 44). The reasons for this discrepancy remain obscure at the moment.

In the present series, the mitotic count proved to be of some use in discriminating between patients with poor and favorable DFS in the univariate survival analysis with the patients with higher mitotic counts showing a higher rate of recurrence compared with those showing lower mitotic activity at baseline, although the difference did not reach statistical significance. As seen in Figures 1 and 2, the difference became more evident after mid- to long-term FU, and the full importance of these data merits confirmation in a larger series with extended (>10 years) FU. In the multivariate survival analysis, however, neither MAI nor SMI proved to be of any value as independent predictors of DFS or DSS.

In conclusion, increased cell proliferation in BC in Saudi Arabian patients correlates strongly with several indicators of poor prognosis, and the mean cut-off values of MAI and SMI can be applied as objective (quantitative) criteria to distinguish between BC patients with favorable or less favorable prognosis.

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