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In primary breast cancer the mitotic activity yields similar prognostic information as the histological grade: a study with long-term follow-up

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Abstract We evaluated with long-term follow-up, the prognostic value of the mitotic activity index (MAI) and the volume corrected mitotic index (M/V-index) compared with that of the histological grade in breast cancer patients not treated with adjuvant systemic therapy. Of 739 consecutive patients living in the city of Nijmegen, the Netherlands, 477 patients with primary unilateral breast cancer were not treated with adjuvant systemic therapy and eligible for the study. In multivariate survival analyses the MAI and M/V-index showed similar hazard ratios (HRs) compared to HRs of histological grade for overall survival (OS) (HR: 1.45, 1.48, and grade II versus grade I (GII/GI) 1.34, grade III versus grade I (GIII/GI) 1.53, respectively)

and for breast cancer specific survival (BCSS) (HR: 1.27, 1.57, and (GII/GI) 1.57 (GIII/GI) 2.32, respectively). Other independent prognostic variables for OS and BCSS were age at diagnosis, tumour size, and number of positive lymph nodes. In the present study with long term follow-up, we compared the prognostic value of mitotic activity with that of histological grade and found no advantage for the mitotic activity in predicting either BCSS or OS and concluded that histological grade and the mitotic activity were equally informative in predicting patient outcome. As histological grade is a well established and widely used prognosticator we do not have arguments to replace the histological grade by the mitotic indices MAI or M/V-index.

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Introduction

In order to predict overall survival (OS) and breast cancer specific survival (BCSS) of breast cancer patients a number of prognostic factors have been studied in the past. As a result, the lymph node status, tumour size, histological grade, oestrogen-receptor (ER) status, and age are established prognostic factors, and worldwide used in decision-making for selecting patients for adjuvant systemic therapy [1–4].

Although histological grade proved to be an independent prognostic factor, some groups have advocated the replacement of histological grade by the mitotic activity index (MAI) [5] or the volume corrected mitotic index (M/V-index) [6, 7], as these would be better prognosticators than histological grade. These latter prognosticators have indeed proven to be able to divide primary breast cancer patients into groups with different prognosis. The MAI, the number of mitoses in ten consecutive high power fields (HPFs), can differentiate into a good prognosis group when the MAI is <10 and a poor prognosis group when the MAI is ≥ 10 . This counts for the total group of breast cancer patients as well as for both the node-negative (N0) and node-positive (N+) subgroups [8–12]. Also an intermediate prognosis group can be discerned when the MAI is divided in <10 (good), 10–20 (intermediate), and >20 (poor) [13]. An alternative for the MAI is the M/V-index, the number of mitoses per mm^2 tumour [7, 14, 15]. However, follow-up in most of the studies with these mitotic indices varies considerably and is usually relatively short. Besides, some of these studies did not include multivariate analyses to address the independent prognostic effect.

In present study, based on the Nijmegen breast cancer archive, we had the opportunity to evaluate the prognostic impact of the MAI and the M/V-index compared with that of the histological grade on survival in a cohort of consecutive breast cancer patients who were not treated with adjuvant systemic therapy, with up to 28 years follow-up (median 12 years). We hypothesized that the mitotic indices would be better prognosticators than the histological grade.

Patients and methods

REMARK criteria for tumor marker studies

This study is reported using the REMARK criteria for tumor marker studies as described by McShan et al. [16].

Patients

In the period 1975–1986 a total of 739 consecutive primary invasive breast cancer patients, living in the city of Nijmegen, were treated in the Radboud University Nijmegen Medical Centre and the Canisius Wilhelmina Hospital, in Nijmegen, the Netherlands. Seventy-two cases (10%) were excluded from analyses because of various reasons (no histology of the primary tumour, poor quality of the histological slides, histological slides not available, invasive tumour too small to determine all prognostic factors, only small incisional biopsy or skin biopsy, and very extensive necrosis of the tumour). From the remaining 667 patients 121 patients (18%) received adjuvant systemic therapy and from 26 patients (4%) it was unknown if adjuvant systemic therapy was given. Adjuvant systemic therapy was gradually introduced during the inclusion period for N+ disease. None of the N0 patients received adjuvant systemic therapy. Additionally, from the 520 patients who did not receive adjuvant systemic therapy (neither chemotherapy, nor endocrine therapy), 36 patients (7%) had synchronous or metachronous bilateral breast cancer and seven patients (1%) had T4 breast cancer, leaving 477 patients eligible for analyses in this retrospective study.

Pathology review

One pathologist (PB) reviewed all cases, unaware of the follow-up data. For reviewing, the original pathology report, microscopical slides of the primary tumour, and, if present, the surgical specimen X-rays were retrieved from the archive. The following pathological variables were evaluated: histological tumour type, largest pathological tumour diameter, and histological grade with estimation of tubule formation, nuclear pleomorphism, and mitotic count (based on the grading system of Elston and Ellis [2]), with the following scores for the mitotic count (per 2.16 mm^2): 0–9: 1 point, 10–19: 2 points, and ≥ 20 : 3 points, the MAI and M/V-index, oestrogen receptor (ER) status, and progesterone receptor (PgR) status (were biochemically assessed with the ligand binding assay, in fmol/mg protein [$<10 \text{ fmol/mg protein}$: negative; $\geq 10 \text{ fmol/mg protein}$: positive] [17]), and number of positive axillary lymph nodes.

Mitotic activity index and volume corrected mitotic index

The MAI [5] in this study was determined as the number of mitoses in 10 consecutive HPFs (2.16 mm^2), including clear prophase figures, but counting anaphase and telophase mitotic figures as one.

The M/V-index was calculated using the formula: $M/V\text{-index} = 200/[\pi r^2 (\sum_1^{10} MI/\sum_1^{10} V)]$. r = the radius of the circular microscopical field/HPF, MI = number of mitoses in a HPF, and V = volume fraction of the invasive tumour (in percentage) as estimated by the area of the invasive tumour in a HPF. The M/V-index used was a modification of that described by Haapasalo and Pesonen [18]. The M/V-index was determined per 2 mm² tumour area. The tumour volume percentage per HPF was subjectively estimated in rates of ten (e.g. 50, 60, till 100%). This tumour volume percentage was simultaneously recorded with the MAI.

Statistical analysis

Only patients living in the city of Nijmegen were included in this study, because the follow-up data (alive or not) could be retrieved with high reliability from the city administration. When a patient died, the medical record was retrieved from the archive and the cause of death was determined. If necessary the medical doctors involved were consulted. All women who were alive at the end of follow-up or were lost during follow-up were right censored.

Survival curves were computed using the Kaplan–Meier technique. Differences between curves were statistically tested using the log-rank test.

The prognostic impact of the variables age at diagnosis, tumour size, MAI, M/V-index, histological grade, ER and PgR status, and number of positive lymph nodes, were first analysed univariately by estimation of the hazard rate ratio (HR) of having an event [death by any cause (OS) and death from breast cancer (BCSS)], along with its 95% confidence interval by applying the Cox proportional hazards method.

Independence of prognostic variables was assessed by a multivariate Cox proportional hazards model in which the variables were included that proved to be of independent prognostic value in the univariate survival analysis. We compared a number of competing models for the modelling of OS and BCSS. All models included the univariately independent variables and the MAI (two or three categories), or M/V-index (two or three categories), or the histological grade (three categories). As mitotic counting is one of the three items determining the histological grade, histological grade was not included in the multivariate analyses together with the MAI or M/V-index. Likewise, the MAI and M/V-index were not included in the multivariate analyses together as they were highly interrelated. The Akaike's Information Criterion (AIC) [19] was applied as a measurement of the prognostic influence of the MAI, or M/V-index, or histological grade in the multivariate models. The AIC shows which variable is the strongest. The lowest AIC means the strongest variable in the model.

Thus, the variable (the MAI, or M/V-index, or histological grade) resulting in the lowest value of the AIC was considered the most powerful prognostic factor in the model. Finally, a multivariate Cox proportional hazards model was performed for OS and BCSS including the variable (the MAI, or M/V-index, or the histological grade) with the lowest value for the AIC in the model for BCSS.

The cut-off value of the MAI in two categories was set on 15 and the cut-off values of the MAI in three categories was set on ≤ 15 , 16–30, and >30 (in 2.16 mm²) based on the method of counting and the published cut-off values [10, 13].

The cut-off value of the M/V-index in two categories was set on 30 and the cut-off values of the M/V-index in three categories was set on ≤ 30 , 31–60, and >60 (in 2 mm²) based on the method of counting and published cut-off values [15, 18].

We used the cut-off value of 15 mm for tumour size based on the publication of Tabár et al. [20].

All cut-off values of the variables were defined based on published data. No cut-off value searching strategies were carried out.

Results

One patient died shortly after diagnosis and treatment, resulting in a follow-up of 0 months. All other patients had a follow-up of at least 2 months. Thirteen patients (3%) were lost during follow-up. The follow-up period ended April 2003. The median age at diagnosis of the 477 patients was 61 years. Most patients (92%) were treated with modified radical mastectomy. Adjuvant locoregional radiotherapy was given to 262 patients (55%). The median follow-up time was 143 months (range: 0–339 months). Patient characteristics are summarized in Table 1.

For the pathology review of the primary tumour more than 12,000 microscopical slides were reviewed. The pathological variables are summarized in Table 2. The median tumour size was 20 mm. Most tumours were of invasive ductal type (75%) or invasive lobular type (13%). The median MAI was 34 and the median M/V-index was 64. Thirteen percent of the tumours were well differentiated, 34% moderately, and 53% poorly differentiated. Fifty-nine percent of tumours were ER and 42% PgR receptor positive.

In a part of the patients with clinically negative axillary lymph nodes (no suspicion of metastases on palpation) the axilla was treated with radiotherapy instead of surgery according to a study protocol. Of these 161 patients (34%) no histology of the axillary lymph nodes was available.

Table 1 Patient characteristics

Variable		N (%)
Age at diagnosis (years)	Median 61; range 25–93	477 (100)
	25–35	9 (2)
	36–49	76 (16)
	50–74	321 (67)
	≥75	71 (15)
Type of operation	Mastectomy	437 (92)
	Breast conserving therapy	38 (8)
	Operation refused	2 (0)
Detection	Screen-detected	195 (41)
	Non-screen-detected	282 (59)
Adjuvant locoregional radiotherapy ^a	None	162 (34)
	Given	262 (55)
	Unknown	53 (11)

^a Radiation of breast, or thoracic wall, or internal mammary chain, or axilla, or supraclavicular area, or combinations of these modalities

Kaplan–Meier curves for overall survival

The prognostic impact of tumour size, histological grade, MAI (two categories), and number of positive lymph nodes for overall survival (OS) is presented in Fig. 1a–d, respectively. Patients with tumours of 15 mm or smaller had a better prognosis compared to those with tumours larger than 15 mm (Fig. 1a; $P < 0.0001$). Grade I tumours did much better than the Grade II and III tumours, and the intermediate group (grade II) lain close to and partly overlapped the survival curve of patients with grade III tumours (Fig. 1b; $P = 0.0009$). If the MAI was low (≤ 15) prognosis was better than when it was high (>15) (Fig. 1c; $P = 0.0006$). Histological N0 patients showed a better survival compared to N+ patients. Within the N+ group the patients with 1–3 positive lymph nodes had a much better prognosis than the patients with ≥ 4 positive lymph nodes (Fig. 1d; $P < 0.0001$).

Kaplan–Meier curves for breast cancer specific survival

The difference between the BCSS curves for tumour size was comparable to that of OS (Fig. 2a; $P < 0.001$). For histological grade the three categories (grade I, II, and III) were more clearly separated from each other, especially when grade II and grade III are considered compared to OS (Fig. 2b; $P = 0.001$). The differences between the curves for the MAI (two categories) were comparable with the differences between the survival curves for OS (Fig. 2c; $P = 0.017$). The BCSS curves for the number of positive lymph nodes showed more deviations between the curves for 0, 1–3, and ≥ 4 positive lymph nodes than in OS (Fig. 2d; $P < 0.0001$).

Table 2 Pathological variables of the 477 breast cancers

Pathological variable		N (%)
Tumour size (mm)	Range 1–85	477 (100)
	≤ 15	156 (33)
	>15	321 (67)
	≤ 20	268 (56)
	>20	209 (44)
Histological tumour type	Invasive ductal carcinoma	360 (75)
	Invasive lobular carcinoma	60 (13)
	Mixed invasive carcinoma (ductal/lobular)	12 (3)
	Tubular carcinoma	9 (2)
	Mucinous carcinoma	17 (3)
	Medullary carcinoma	3 (1)
	Other	16 (3)
Mitotic activity index	Range: 1–989	477 (100)
	≤ 15	131 (27)
	>15	346 (73)
M/V-index	Range: 2–1476	477 (100)
	≤ 30	131 (27)
	>30	346 (73)
Histological grade	Well differentiated	62 (13)
	Moderately differentiated	163 (34)
	Poorly differentiated	252 (53)
Oestrogen receptor status ^a	Positive	281 (59)
	Negative	87 (18)
	Unknown	109 (23)
Progesterone receptor status ^a	Positive	201 (42)
	Negative	133 (28)
	Unknown	143 (30)
Number of positive lymph nodes ^b	0	238 (50)
	1–3	63 (13)
	≥ 4	15 (3)
	Not examined histological ^c	161 (34)

M/V-index, volume corrected mitotic index

^a In fmol/mg protein, <10 fmol/mg protein is negative, ≥ 10 fmol/mg protein is positive

^b Based on histological examination

^c The axilla was treated with radiotherapy, instead of surgery

The survival curves of the M/V-index for both OS and BCSS are highly comparable to the survival curves of the MAI (not shown).

Univariate and multivariate analyses

Table 3 summarizes the univariate survival analysis for OS and BCSS for age at diagnosis, tumour size, MAI (two categories), M/V-index (two categories), histological grade, ER status, PgR status, and number of positive lymph nodes.

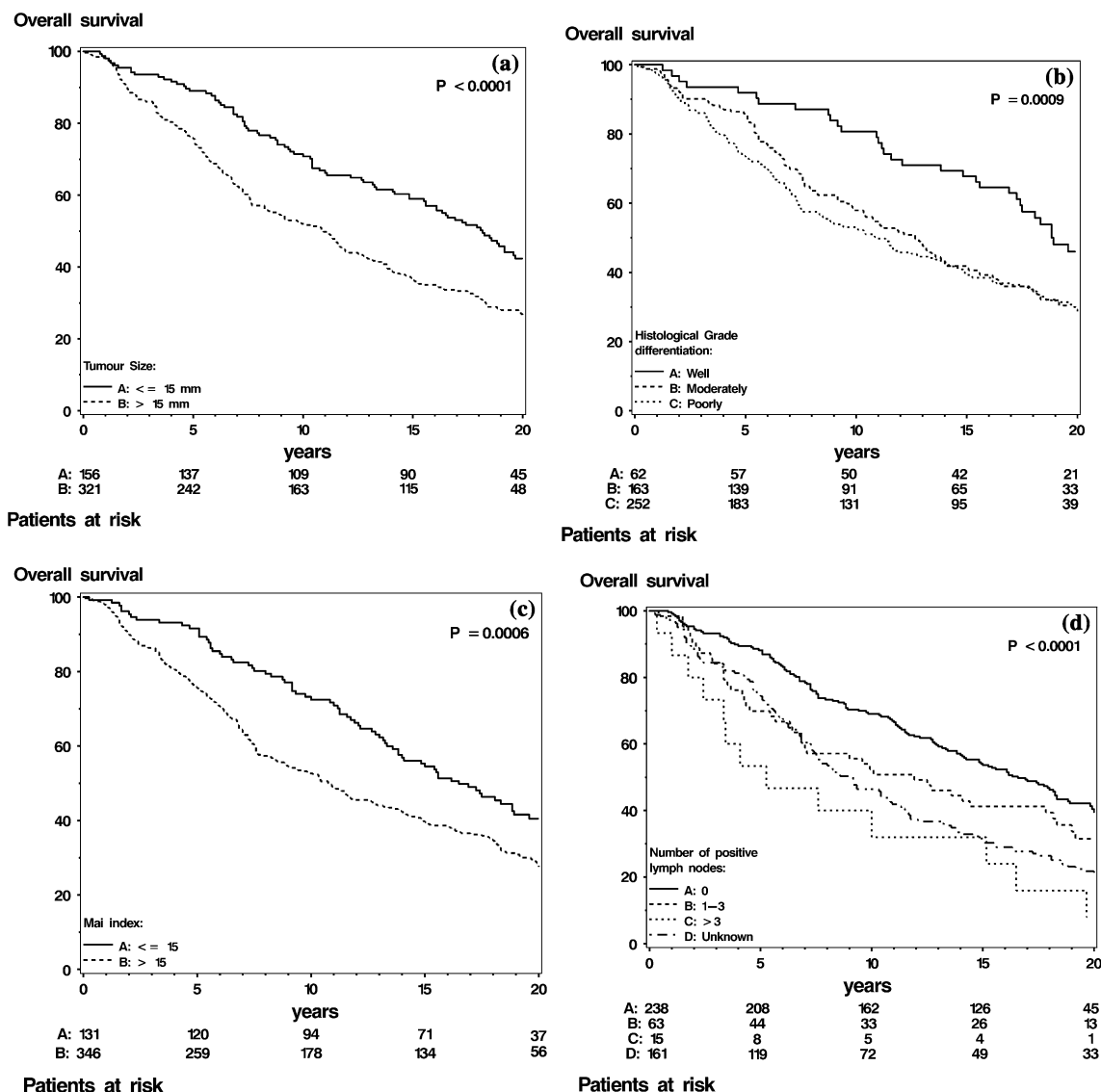


Fig. 1 Kaplan–Meier curves for overall survival for tumour size (a), histological grade (b), MAI (two categories) (c), and number of positive lymph nodes (d)

Except for ER and PgR status, all variables showed statistical significant differences for OS and BCSS.

The results of the multivariate analysis for OS and BCSS for the independent prognostic variables age at diagnosis, tumour size, and number of positive lymph nodes with histological grade are presented in Table 4. Age at diagnosis, tumour size, and the number of positive lymph nodes were independent predictors of OS and BCSS. Histological grade was nearly an independent predictor of OS ($P = 0.059$) and a significant predictor of BCSS ($P = 0.012$).

The results of multivariate survival analysis for OS and BCSS for the MAI (two categories), M/V-index (two categories), and histological grade, when they were included separately in the model (with age at diagnosis, tumour size,

and number of positive lymph nodes), are summarized in Table 5.

For OS the MAI, M/V-index, and histological grade (poorly differentiated vs. well differentiated) were all independently associated with OS, with a HR of approximately 1.5. According to the AIC, the M/V-index was slightly more powerful variable than histological grade and practically equally powerful as MAI (AIC: 3658, 3663, and 3659, respectively).

For BCSS histological grade (poorly differentiated vs. well differentiated) and the M/V-index were independently associated with survival. According to the AIC, histological grade was a slightly more powerful variable compared to the MAI or M/V-index (AIC: 1523, 1529, and 1525, respectively).

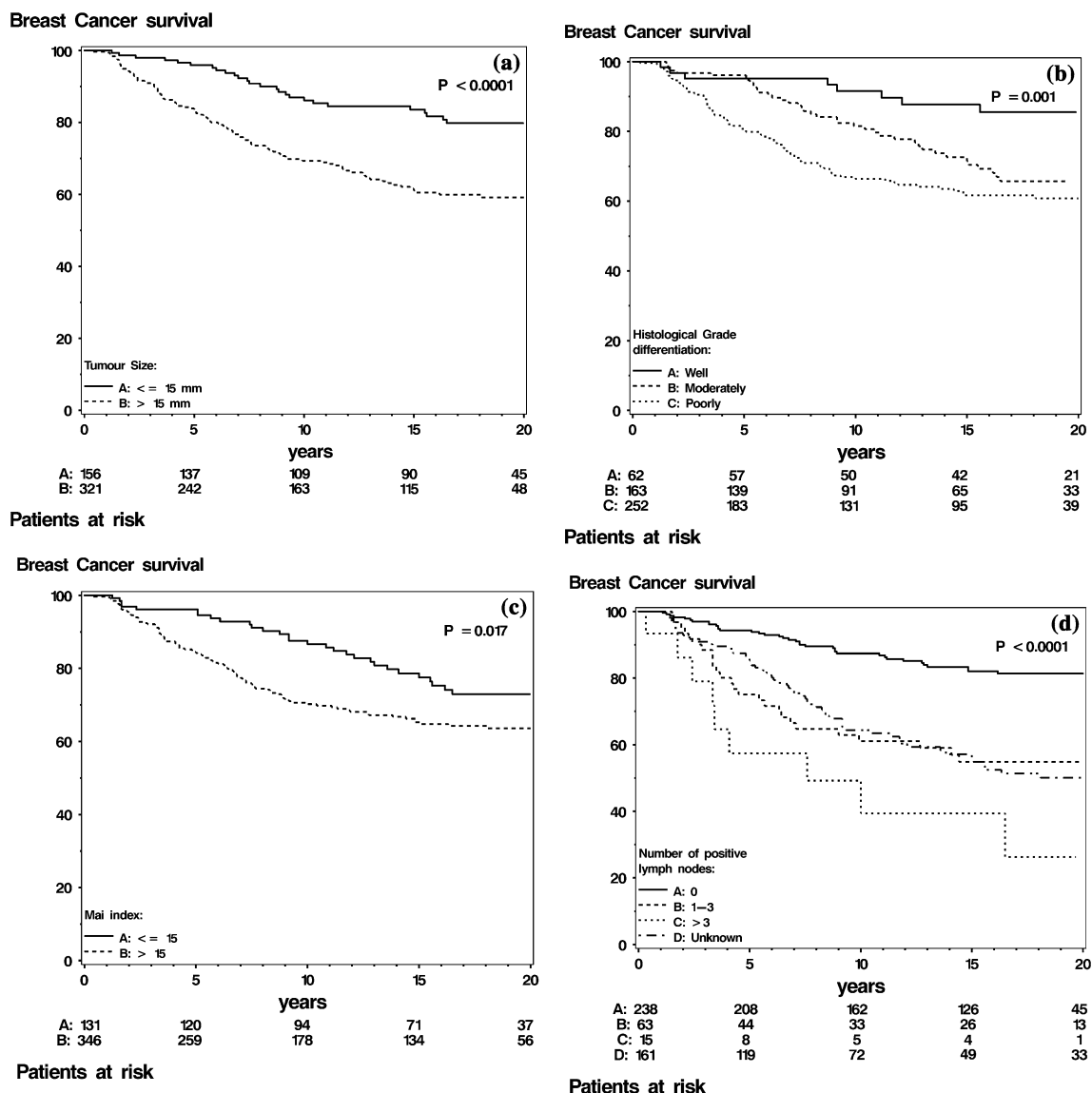


Fig. 2 Kaplan–Meier curves for breast cancer specific survival for tumour size (a), histological grade (b), MAI (two categories) (c), and number of positive lymph nodes (d)

The above described analyses were carried out also with the three tiered MAI and M/V-index (≤ 15 , 16–30, and > 30 and ≤ 30 , 31–60, and > 60 , respectively). According to the AIC, the M/V-index was the strongest variable for OS and the histological grade for BCSS (Table 6), similarly to the results of the two tiered MAI and M/V-index analyses (Table 5).

Discussion

In present study, we have confirmed the independent prognostic relevance of the classical prognostic factors age, histological grade, tumour size, and number of positive lymph nodes with up to 28 years follow-up (median

12 years) of patients not treated with adjuvant systemic therapy. The prognostic value of histological grade in breast cancer patients has been long ago established [2]. Some research groups, however, have advocated replacing the histological grade by the MAI or the M/V-index. In this study we evaluated the prognostic value of both the histological grade and the mitotic indices MAI and M/V-index for OS and BCSS in 477 primary breast cancer patients. We demonstrated that, although the MAI and M/V-index were able to discriminate in good versus poor prognosis, they were not more informative than histological grade in predicting survival in patients with primary breast cancer. So, we have to dismiss our predefined hypothesis that mitotic indices would be better prognosticators than histological grade.

Table 3 Results of the univariate analyses of the prognostic variables related to overall survival and breast cancer specific survival

Prognostic variable	Overall survival		Breast cancer specific survival	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age at diagnosis (years)		<0.0001		0.007
25–35 vs. 50–74	1.83 (0.90–3.70)		3.36 (1.56–7.24)	
36–49 vs. 50–74	0.54 (0.38–0.76)		0.95 (0.61–1.46)	
≥75 vs. 50–74	2.70 (2.03–3.58)		0.62 (0.30–1.29)	
Tumour size		<0.0001		<0.0001
>15 vs. ≤15 mm	1.72 (1.35–2.18)		2.49 (1.64–3.77)	
Mitotic activity index		0.0007		0.02
>15 vs. ≤15	1.53 (1.20–1.96)		1.61 (1.09–2.40)	
M/V-index		0.0006		0.002
>30 vs. ≤30	1.54 (1.20–1.96)		1.96 (1.29–2.97)	
Histological grade		0.01		0.002
Moderate vs. well	1.51 (1.06–2.16)		2.04 (1.02–4.07)	
Poor vs. well	1.70 (1.21–2.39)		3.00 (1.56–5.79)	
Oestrogen receptor status ^a		0.76		0.05
Negative vs. positive	1.05 (0.78–1.40)		1.52 (1.00–2.31)	
Progesterone receptor status ^a		0.64		0.64
Negative vs. positive	1.07 (0.82–1.39)		1.10 (0.72–1.68)	
Number of positive lymph nodes ^b		<0.0001		<0.0001
1–3 vs. 0	1.47 (1.06–2.04)		3.38 (2.07–5.53)	
≥4 vs. 0	2.72 (1.57–4.71)		6.24 (3.01–12.94)	
Unknown vs. 0	1.72 (1.35–2.18)		3.21 (2.14–4.82)	

P values of univariate proportional hazards method computed using the $-2 \log$ likelihood ratio test; M/V-index, volume corrected mitotic index

^a In fmol/mg protein, <10 fmol/mg protein is negative, ≥10 fmol/mg protein is positive

^b Based on histological examination

Table 4 Results of the multivariate analyses of the significant prognostic variables for overall survival and breast cancer specific survival

Prognostic variable	Overall survival ^a		Breast cancer specific survival ^a	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age at diagnosis (years)		<0.0001		0.012
25–35 vs. 50–74	1.77 (0.85–3.66)		3.19 (1.45–7.01)	
36–49 vs. 50–74	0.52 (0.37–0.73)		0.88 (0.57–1.35)	
≥75 vs. 50–74	2.57 (1.93–3.42)		0.51 (0.25–1.06)	
Tumour size		0.0009		0.0005
>15 vs. ≤15 mm	1.55 (1.20–2.00)		2.21 (1.42–3.45)	
Histological grade		0.059		0.012
Moderate vs. well	1.34 (0.93–1.94)		1.57 (0.77–3.19)	
Poor vs. well	1.53 (1.06–2.20)		2.32 (1.16–4.64)	
Number of positive lymph nodes ^b		<0.0001		<0.0001
1–3 vs. 0	1.41 (1.01–1.96)		3.05 (1.86–5.00)	
≥4 vs. 0	2.55 (1.45–4.47)		4.98 (2.38–10.42)	
Unknown vs. 0	1.72 (1.35–2.19)		3.58 (2.38–5.39)	

P values overall test minus covariate with >2 categories

HR hazard ratio, CI confidence interval

^a Multivariate analyses performed on all 477 cases

^b Based on histological examination

Table 5 Results from multivariate analyses for the prognostic variables MAI in two categories, M/V-index in two categories, and histological grade in three categories when included separately in the multivariate model (with age at diagnosis, tumour size, and number of

positive lymph nodes) for overall survival and breast cancer specific survival with hazard rate ratios (HRs) with their 95% confidence intervals (CI) and the Akaike's Information Criterion (AIC) for each model

The prognostic variables MAI, M/V-index, histological grade included in the multivariate model	Overall survival ^a			Breast cancer specific survival ^a		
	HR (95% CI)	<i>P</i>	AIC	HR (95% CI)	<i>P</i>	AIC
MAI			3658.720			1528.547
>15 vs. ≤15	1.45 (1.12–1.89)	0.005		1.27 (0.84–1.93)	0.26	
M/V-index			3657.747			1525.441
>30 vs. ≤30	1.48 (1.14–1.92)	0.003		1.57 (1.02–2.43)	0.04	
Histological grade			3663.235			1523.086
Moderate vs. well	1.34 (0.93–1.94)	0.12		1.57 (0.77–3.19)	0.22	
Poor vs. well	1.53 (1.06–2.20)	0.02		2.32 (1.16–4.64)	0.02	

P values based on Wald-statistics for comparison with the reference group in the multivariate proportional hazards method

^a Multivariate analyses performed on all 477 cases

Table 6 Results from multivariate analyses for the prognostic variables MAI in three categories, M/V-index in three categories, and histological grade in three categories when included separately in the multivariate model (with age at diagnosis, tumour size, and

number of positive lymph nodes) for overall survival and breast cancer specific survival with hazard rate ratios (HRs) with their 95% confidence intervals (CI) and the Akaike's Information Criterion (AIC) for each model

The prognostic variables MAI, M/V-index, histological grade included in the multivariate model	Overall survival ^a			Breast cancer specific survival ^a		
	HR (95% CI)	<i>P</i>	AIC	HR (95% CI)	<i>P</i>	AIC
MAI (three categories)			3660.396			1528.696
16–30 vs. ≤15	1.53 (1.11–2.11)	0.009		1.00 (0.57–1.75)	0.99	
>30 vs. ≤15	1.41 (1.07–1.87)	0.016		1.39 (0.90–2.15)	0.14	
M/V-index (three categories)			3659.464			1526.174
31–60 vs. ≤30	1.41 (1.02–1.94)	0.04		1.34 (0.79–2.27)	0.29	
>60 vs. ≤30	1.52 (1.15–2.01)	0.003		1.71 (1.08–2.70)	0.02	
Histological grade (three categories)			3663.235			1523.086
Moderate vs. well	1.34 (0.93–1.94)	0.12		1.57 (0.77–3.19)	0.22	
Poor vs. well	1.53 (1.06–2.20)	0.02		2.32 (1.16–4.64)	0.02	

P values based on Wald-statistics for comparison with the reference group in the multivariate proportional hazards method

^a Multivariate analyses performed on all 477 cases

In a previous study, we already demonstrated that the MAI was an independent prognostic factor for recurrence free survival (RFS), but not for OS [21]. To obtain more robust data, we initiated this study with a longer follow-up and larger number of patients. In addition, we reviewed all cases pathologically, including the microscopic slides of the tumours and used BCSS instead of RFS as relevant disease outcome.

As was shown by others [7–15, 22] the MAI was a significant prognostic factor in univariate analysis. We could confirm the independent prognostic significance of the MAI as well in multivariate analysis for OS, but not for BCSS, in contrast to a number of other studies [5, 9, 10, 12, 13, 15]. With respect to the M/V-index we could confirm the findings of the group of Aaltomaa et al. [7, 14, 22, 23]

who showed that the M/V-index was an independent prognostic factor for BCSS and was a better prognosticator than the MAI, but less powerful than histological grade.

Histological grade is a widely used prognostic variable for decision making and management of patients with primary breast cancer and has been repeatedly been advised in Europe [3, 24, 25], whereas the use of the MAI is limited to some centres, most in the Scandinavian countries and in the Netherlands.

Considering the workload for the different methods; the average time to determine the MAI takes about 10 min. Approximately the same time is needed to evaluate histological grade, as the mitotic count is also a part of the histological grade and the other two components, nuclear grade and tubular formation, are determined during

scanning the histological slides without the need of additional time. Thus, in respect to efforts to determine the MAI and histological grade no differences occur.

Stuart-Harris et al. [26] have recently conducted a systemic review and meta-analysis on proliferation markers related to survival in early breast cancer. They included in addition to the mitotic index (MI), Ki-67 (including MIB1), proliferating cell nuclear antigen (PCNA), and labelling index (LI) (including thymidine labelling index and bromodeoxyuridine labelling index) in their review. The authors concluded that the examined markers were associated with survival outcome in early breast cancer. However, it was not possible to identify the most powerful marker and it remained unclear if these markers provide additional prognostic information over the established prognostic factors.

We have performed a retrospective study in which unexpected biases may occur. For example, we only included patients living in the city of Nijmegen and these patients may not be representative for all patients. On the other hand, as follow-up was very reliable, only 3% of patients were lost to follow-up.

As nowadays most patients receive adjuvant systemic therapy, new studies examining the pure prognostic significance of the mitotic indices will be very hard to perform.

In conclusion, our study with a long-term follow-up, confirms that OS and BCSS of breast cancer patients is independently predicted by the established prognostic factors of age at diagnosis, tumour size, histological grade, and lymph node status. We showed in addition, that the MAI and M/V-index were equally informative in predicting outcome as histological grade. As histological grade is a well established and widely used prognosticator we do not have arguments, nor based on this study, nor on published data, to replace the histological grade by the mitotic indices MAI or M/V-index. Thus, both methods could be used for assessing prognosis in primary breast cancer.

Results of either or both methods should be part of the pathology report.

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