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Multivariate prognostic evaluation of the mitotic activity index and fibrotic focus in node-negative invasive breast cancers

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Abstract

We validated with univariate and multivariate (Cox) analysis, the prognostic value of the mitotic activity index (MAI), the fibrotic focus (FF) and other prognosticators in 448 patients with lymph node-negative (LN−) invasive breast cancer <55 years without adjuvant systemic treatment (72.5 months median follow-up, range 4–119). Of these patients, 24.8% developed distant and 1.6% loco-regional recurrence. FF showed excellent inter-observer reproducibility ($\kappa = 0.93$). Strong prognosticators were MAI, grade, nuclear atypia, FF and the St. Gallen criterion (SG). The subgroup with excellent survival selected by SG was only 16% of all patients, implying over-treatment of more than 70% of all LN− patients when using SG as adjuvant therapy selection criterion. If MAI <10, 13% showed distant metastases, contrasting with 41% if MAI $\geqslant$10. FF was prognostic in the ductal and mixed ductal cancers, but not in the lobular and other subtype cancers. Patients with invasive (mixed) ductal cancers with FF absent, FF < 1/3 or FF > 1/3 of the tumour area, had distant metastasis rates of 17%, 35% and 48%; in MAI < 10 and FF absent, FF < 1/3 or FF > 1/3, metastasis rates were 11%, 13% and 42% and if MAI $\geqslant$ 10, metastasis rates were 31%, 48% and 50%, respectively. In the 12 patients with MAI < 10 and a large FF > 1/3, event-free survival was similar to patients with MAI $\geqslant$ 10. With multiple regression MAI < 10 *versus* $\geqslant$10 is the strongest prognosticator (also stronger than the SG). The FF may be important as it has additional prognostic value to the MAI in the small subgroup of invasive ductal or mixed-ductal breast cancer patients with combined MAI < 10 and an FF > 1/3 of the tumour area.
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Keywords: Breast cancer; Mitotic index; Fibrotic focus; Prognosis

1. Introduction

Adjuvant systemic therapy (AST) for early-stage lymph node-negative (LN−) breast cancer patients has gained acceptance since the 1988 Clinical Alert [1], and subsequent conferences have further defined treatment [2]. However, the St. Gallen consensus-guideline (SG) recommends no AST in 'low risk' patients (i.e. small grade 1 oestrogen receptor positive tumours [3]). This would imply that almost 85% of all pre-menopausal LN− patients would be treated with AST, while only 25–30% would develop distant metastases without AST. In addition, the reproducibility of grade is far from perfect between experts [4].

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In breast cancer, proliferative activity represents one of the biological processes most thoroughly investigated for its prognostic association [5–14] but prospective multicentre analyses with fixed thresholds have only recently started to yield evidence of a relationship between proliferation and response to systemic treatments [15,16]. One of the most decisive, yet simple, well reproducible proliferation-associated prognostic factors is the mitotic activity index (MAI) [17–19]. Its prognostic value has been shown in many retrospective and prospective studies using a fixed threshold ($MAI < 10$ favourable; $MAI \geqslant 10$ unfavourable) [17,20–24]. MAI is not sensitive to fixation delay [25] and is already part of different grading systems [26].

Another recently described promising prognostic factor is the fibrotic focus (FF), which is a scar-like area, consisting of fibroblasts and collagen in the centre of an invasive ductal breast carcinoma. FF has often been regarded as an inactive sclerotic part of the tumour, but the fibroblasts forming an FF are significantly more proliferative than those of invasive ductal carcinomas without FF [27]. FF presence is correlated with tumour size, higher histological grade, necrosis, c-erbB-2 overexpression, high stage and high microvessel density [28]. The usefulness of FF as a surrogate for quantifying angiogenesis was confirmed [29]. This may be important, since microvessel density (MVD) is the only morphological primary tumour characteristic that adds to the prognostic value of the MAI in LN− breast cancer [24]. On the other hand, the value of the MVD is not equivocal [30]. The FF is easily assessable in standard histological sections and may therefore be an important new prognosticator (Fig. 1). Indeed, the proliferative activity of intra-tumoural fibroblasts is closely correlated with locoregional and distant metastases [31]. Moreover, the presence of FF has been associated with decreased survival [32,33] and has also been proposed as an indicator of tumour aggressiveness [34,35], especially in early stage breast cancer [36]. In the latter study, the relative size (fibrotic focus/tumour ratio) of the FF was also associated with patient outcome. However, the prognostic FF studies mentioned used mixed LN+ and LN− patients of all ages (although patients <55 years have a worse prognosis), with and without adjuvant systemic therapy [35], or selected LN− patients with an either excellent and or very poor outcome [29]. This carries a serious risk of selection bias and may have significantly influenced the results. Moreover, the additional prognostic value of MAI and FF combined is not clear, as the FF studies mentioned did not take the MAI into account. Therefore, we endeavoured to analyse the prognostic value of the MAI and FF in an independent multicentre prospective population-based group of 448 consecutive LN− women, <55 years of age and no systemic adjuvant therapy. The MAI and other classical prognostic factors were determined prospectively, the FF retrospectively.

2. Patients and methods

2.1. Patients

The patients were enrolled in the national Dutch Prospective Multicenter Morphometric Mammary Carcinoma Project (MMMCP) [37]. All consecutive primary invasive breast cancer patients diagnosed in the 34 collaborating MMMCP hospitals from 1st October 1987

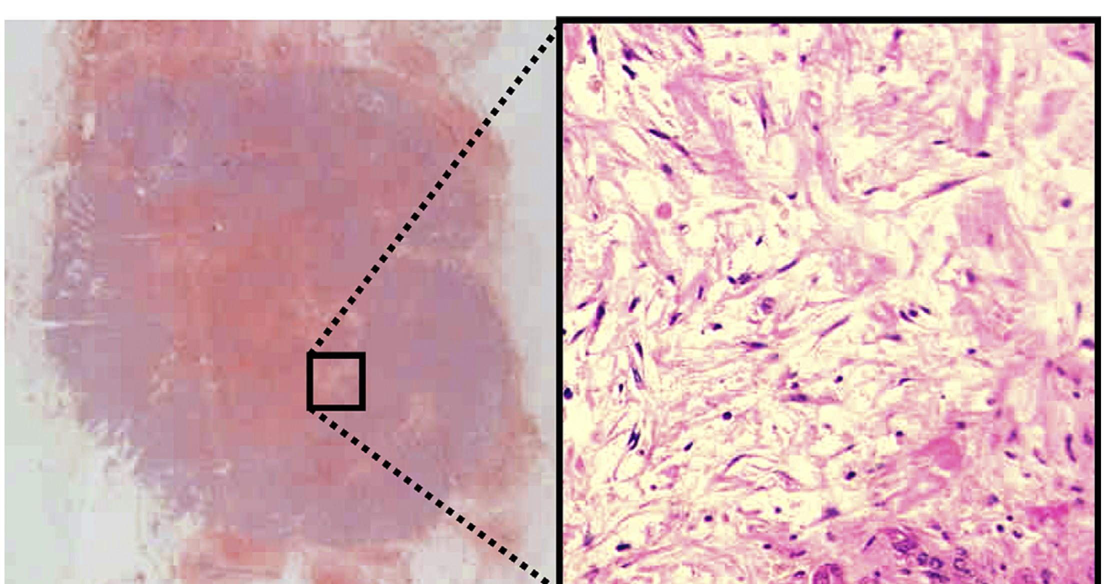


Fig. 1. A fibrotic focus is a scar-like area in the centre of an invasive carcinoma of the breast. It can be seen easily on the unmagnified haematoxylin-eosin stained tissue section.

to 1st January 1990 were enrolled. Follow-up was updated annually. Of the 3479 patients registered, 516 were <55 years, had no previous malignancies, had LN− invasive breast cancer of more than 2 mm invasion (thus excluding carcinoma *in situ* with microinvasion) and all the following data known: tumour size, oestrogen receptor, histological type and grade and its constituent features (tubular formation, nuclear atypia, mitotic activity) and adequate follow-up information. According to the 1998 guidelines of the Dutch Society of Medial Oncology, patients under 55 years with LN− yet high-risk invasive breast cancers are candidates for adjuvant chemotherapy [38,39] and therefore this age group was further studied here. Of these 516 patients with invasive breast cancer, 448 were selected because a section was available containing at least half of the tumour cross-sectional area allowing evaluation of an FF. All patients were treated with modified radical mastectomy ($n = 155$; 34.6%) or breast-conserving therapy (BCT, $n = 293$; 65.4%), always with adequate axillary lymph node dissection but no adjuvant systemic therapy. Loco-regional radiotherapy was given in cases that underwent BCT or had medially localised tumours. In principle, post-operative control was performed every 6 months for the first 5 years, and annually thereafter. This schedule was well kept, although small inter-institutional variations inevitably occurred. Median follow-up was 72.5 months (range 4–119 months).

2.2. Tumour characteristics, MAI, FF

The tumours were cut into 5 mm thick slices, fixed in buffered 4% formaldehyde and embedded in paraffin. Post-surgical tumour size was measured in the fresh specimens. At least 10 (median 14) lymph nodes were detected in the axillary lymph-node dissection specimens. Paraffin sections 4 μm thick were cut and stained with haematoxylin-eosin (H&E). Histological tumour type was assessed according to the World Health Organisation criteria [40]. There were 338 ductal carcinomas, 46 lobular, 16 tubular, 8 colloid, 2 medullary and 38 carcinomas of another type.

Following the MMMCP protocol, the total number of well-defined mitotic figures was prospectively counted in each of the participating laboratories by 27 different technicians, at 400× magnification (objective 40, field diameter 450 μm at specimen level) in 10 consecutive neighbouring fields of vision, representing a total area of 1.59 mm^2 in the most poorly differentiated peripheral tumour area. The technicians were instructed in detail at four training sessions held in the first three months, after which MAI assessments were well reproducible [18]. Necrotic or inflamed fields and doubtful structures were ignored. The resulting total number of mitoses is the mitotic activity index (MAI), an accurate assessment of which takes 3–5 min. Correction of the MAI for the stroma percentage or tumour-cell number does not improve its prognostic value and is much more time-consuming [23]. Tubular formation (<10%, 10–75%, >75%), nuclear atypia (mild, moderate and marked) and MAI were assessed according to the Nottingham modification [26], using MAI 0–5 as 1, 6–10 as 2, and >10 as 3.

Oestrogen receptor value was assessed in reference laboratories with the charcoal technique (positive ⩾10 fmol/mg protein, borderline 4–9 fmol/mg protein, negative <4 fmol/mg protein).

The presence of an FF was evaluated [33]. An FF appears as a radially expanding fibrosclerotic core (Fig. 1) and consists of either loose, dense or hyalinised collagen bundles, or a variable number of fibroblasts resembling the various stages of scar development in the healing process of necrotic tissue. Fibroblasts and collagen fibres are arranged in irregular or storiform patterns, a characteristic most useful in distinguishing FF from desmoplastic connective tissue, which shows a more orderly arrangement. Elastic tissue may be abundant in FF while remnants of necrotic tissue may also be found. Small FFs (<3 mm) do not contain carcinoma cells, while larger FFs sometimes do. A minimal diameter of 1 mm is required for a fibrosclerotic core to be called FF [27]. The area of coagulation necrosis of tumour cells within FF is smaller than that occupied by the fibroblasts and collagen fibres; necrosis of tumour cells not surrounded by proliferating fibroblasts and collagen fibres is insufficient to be called FF. FFs occupy various percentages of the tumour area. We evaluated not only the presence or absence of an FF, but also estimated its relative size or FF/tumour <1/3 and >1/3 of the tumour area [29]. FFs can be seen with the naked eye on a well-stained H&E slide (Fig. 1) so the FF/tumour ratio can be estimated by dividing the area of the FF by the area of the tumour, both viewed on the unmagnified H&E stained tissue section. We previously reported an inter-observer concordance of 85% for this estimated relative FF size [29]. When multiple fibrotic foci are present, only the largest one is taken into account. The reproducibility of the FF between observers was assessed by independent analyses in 27 randomly selected cases.

2.3. Statistical analysis

Statistical Package (SPSS) for Windows version 11 was used. The main endpoints were recurrence (defined as any first local, contralateral or distant disease recurrence) and mortality (any death due to breast cancer as evident from clinical, radiological, histological or autopsy data). If the cause was unknown, but a metastasis was previously detected, death was considered to be breast cancer-related unless explicitly stated otherwise. If a metastasis or loco-regional recurrence occurred without an exact date of first recurrence, the follow-up visit date was used as 'recurrence date'. Age, time to recurrence and survival time was calculated relative to the diagnosis date. If the latter was unknown, pathology-diagnosis date was used as cancer-diagnosis date. Two follow-up parameters were used. In recurrence-free survival (RFS) an event is defined as alive with distant metastasis, dead of other causes with distant metastasis, dead of disease with distant metastasis, alive with local recurrence, or dead of disease with local recurrence; a non-event is defined as alive and well, or dead of other causes without distant metastases. In distant metastases disease-free survival (DDFS) an event is defined as alive or dead of disease with distant metastasis and a non-event as alive and well, alive with local recurrence, dead of other causes, or dead of disease with local recurrence. Continuous variables were discretised using prognostically significant thresholds or the median, tertiles and quartiles to create 2, 3 or 4 groups of similar size. Kaplan–Meier survival curves were made. Differences between groups were tested by the log-rank test. Hazard ratio (HR) with 95% confidence intervals (95% CI) were calculated. The relative multivariate importance of potential prognostic variables was tested (Cox model). *P*-values below 0.05 were regarded as significant. For the MAI, different thresholds were tested but as before, the MAI with threshold 10 was the strongest prognostic factor and further described. The analyses were carried out on the whole group, and separately on the ductal, lobular and non-ductal cancers. As the hazard ratios of the RFS and DDFS were rather similar, only the DDFS is presented in Table 1. The κ statistic was used to assess the reproducibility of the FF.

3. Results

A total of 111 patients (24.8%) developed distant osseous, visceral, cerebral or multiple metastases and 7 (1.6%) a local recurrence. Mean age was 45.7 years (range 22.4–54.9). Table 1 shows the 8-year survival and hazard ratios for the tumour characteristics for DDFS. Strong prognostic factors were histological grade, MAI, nuclear atypia, FF and the St. Gallen guideline. For tubular formation, only >75% tubular structures versus <10% tubular structures were significant. Age, tumour diameter and oestrogen receptor were also significant, but with lower HRs. Coagulation necrosis occurred in 30% (48/158) of the FF, but this was not significant prognostically.

Separate analysis showed that the tubular ($n = 16$, 1 dead of disease (DOD)), colloid ($n = 8$, 1 DOD) and medullary carcinomas ($n = 2$, 1 DOD) did not have an FF. Eight of the 46 lobular invasive cancers had a (small) FF, but the presence of an FF was not associated with a worse outcome. In the mixed ductal-lobular cancers, FF had the same prognostic value as the pure ductal cancers.

All further analyses were therefore performed with the ductal and mixed ductal cancers only ($n = 376$). Table 2 shows the correlation between tumour diameter, grade, oestrogen receptor, FF and prognosis. Note that within tumours of a certain diameter, grade or oestrogen receptor content, the FF distinguishes subgroups of different outcomes. Multivariate analysis showed that MAI $\geqslant$ 10 *versus* <10 was the strongest prognostic factor for distant metastases (HR = 4.0, 95% CI 2.6–6.3). The presence of an FF (HR = 1.7, 95% CI 1.1–2.8 for FF absent *versus* FF < 1/3; HR = 2.4, 95% CI 1.4–4.2 for FF absent *versus* FF > 1/3) was the only factor with independent additional prognostic value to the MAI. For RFS, MAI $\geqslant$ 10 *versus* <10 was again selected first (HR = 3.7, 95% CI 2.4–5.6), followed by the presence of a FF (HR = 1.6, 95% CI 1.1–2.6 for FF absent *versus* FF < 1/3; HR = 2.5, 95% CI 1.4–4.0 for FF absent *versus* FF > 1/3). None of the other variables had independent prognostic value once these features were included.

Recurrence rate (both distant and local) for patients with MAI $\geqslant$ 10 was 42% and the risk of distant metastasis was 41%, compared with 14% and 13%, respectively, for patients with MAI < 10 (see Fig. 2). Table 3 and Fig. 3 show the influence of FF on DDFS in the MAI < 10 and MAI $\geqslant$ 10 subgroups. If MAI < 10, the distant metastasis risk increased from 11% when FF was absent or small to 42% when an FF > 1/3 was present, similar to patients with MAI $\geqslant$ 10. However, the latter group consists of 12 patients only. When MAI $\geqslant$ 10, the distant metastasis risk was 31% in the absence and 49% in the presence of an FF but the FF size was irrelevant (see Table 3). Assessment of the FF showed excellent inter-observer reproducibility ($\kappa = 0.93$, Table 4). Thus, the FF may be important as it has additional prognostic value to the MAI in the small subgroup of invasive ductal or mixed-ductal breast cancer patients with combined MAI < 10 and an FF > 1/3 of the tumour area. Comparison of the MAI and St. Gallen shows that the MAI is prognostically stronger (Fig. 4).

4. Discussion

The MAI is the strongest independent prognostic factor in the current prospective analysis of invasive cancers with more than 2 mm invasive carcinoma, which confirms the results of many earlier studies. MAI is also stronger than grade or its constituent features, which is in agreement with the data of Volpi and colleagues [41]. With multivariate analysis, FF presence is the only factor with independent additional prognostic value to the MAI. These findings are important, since both the MAI and FF are well reproducible and easy to perform on

Table 1
Distant metastases disease-free survival in lymph node-negative breast cancer patients <55 years

Characteristic	Distant metastasis				
	Event/at risk	Log-rank *P*-value	KM % censored	HR	95% CI
Age					
<45 years	59/192	4.8	69.3	0.7	0.4–1.0
>45 years	52/256	0.03	79.7		
Tumour diameter					
<2 cm	54/264	6.3	79.6	1.6	1.1–2.3
>2 cm	57/184	0.01	69.0		
Tumour diameter					
<2 cm	54/264	6.4	79.6	1.6	0.9–2.5
2–3 cm	28/90	0.04	68.9	1.6	1.1–2.6
>3 cm	29/94	(global)	69.2		
Oestrogen receptor					
Positive	57/259	11.4	78.0	1.9	1.3–2.8
Borderline/negative	51/152	0.0007	66.5		
Grade					
1	17/153	46.9	88.9	2.0	1.1–3.8
2	24/118	<0.0001	79.7	4.9	2.9–8.3
3	70/177	(global)	60.5		
MAI					
<10	33/256	51.8	87.1	4.0	2.7–6.0
⩾10	78/192	<0.0001	59.4		
0–2	10/136	59.1	92.7	2.9	1.4–6.1
3–10	24/126	<0.0001	81.0	7.6	3.9–14.7
>10	77/186	(global)	58.6		
0–5	26/202	51.8	87.1	1.1	0.5–2.5
6–9	7/54	0.0001	87.0	4.1	2.6–6.3
⩾10	78/192	(global)	59.4		
Tubular formation					
>75%	4/60	19.2	93.3	2.8	0.9–8.5
10–75%	16/98	0.0001	83.7	5.6	2.1–15.2
<10%	91/290	(global)	68.6		
Nuclear atypia					
Mild	12/100	21.0	88.0	2.3	1.2–4.4
Moderate	39/159	<0.0001	75.5	3.7	2.0–7.0
Marked	60/189	(global)	68.3		
Fibrotic focus					
Absent	49/290	41.6	83.1	2.5	1.6–3.8
Present <1/3	37/105	<0.0001	64.8	4.1	2.5–6.7
Present >1/3	25/53	(global)	52.8		
Necrosis in fibrotic focus					
Absent	42/110	0.38	61.8	1.2	0.7–2.0
Present	20/48	0.54	58.3		
St. Gallen classification					
Favourable	8/70	9.0	88.6	2.9	1.4–5.9
Unfavourable	103/378	0.003	72.8		

KM, Kaplan–Meier survival estimates; HR, hazard ratios; CI, confidence interval; MAI, mitotic activity index.

standard histological sections. The reproducibility of the FF as found in the present study confirms earlier independent reproducibility studies [36]. This can have important clinical consequences as application of the St. Gallen guideline to our patients selected a subgroup with excellent survival, but only $70/448 = 16\%$ of patients belonged to that category. Thus, when using the SG for AST selection, 84% of the patients would be treated, while only 30% of these developed metastases without AST (an over-treatment of 54%). Moreover, the relative survival improvement of AST in LN− breast cancer patients <55 years who develop metastases, is only 25% [42]. Consequently, AST could rescue 28 of the 111 patients with distant metastases, but at the ex-

Table 2
Ductal and mixed ductal cancers only ($n = 376$)

	Fibrotic focus			Overall
	Absent	<1/3	>1/3	
Tumour diameter <2 cm				
Events %	15%	29%	47%	22%
Events/total	22/143	17/58	9/19	48/220
Tumour diameter 2–3 cm				
Events %	20%	50%	50%	34%
Events/total	8/41	11/22	8/16	27/79
Tumour diameter >3 cm				
Events %	21%	33%	47%	30%
Events/total	9/42	6/18	8/17	23/77
Grade 1				
Events %	10%	11%	33%	11%
Events/total	8/82	2/18	2/6	12/106
Grade 2				
Events %	20%	40%	44%	29%
Events/total	18/89	15/37	7/13	40/139
Grade 3				
Events %	24%	40%	48%	35%
Events/total	13/55	17/43	16/33	46/131
Oestrogen receptor positive				
Events %	17%	32%	47%	23%
Events/total	22/131	19/60	7/15	48/206
Oestrogen receptor negative/borderline				
Events %	23%	50%	45%	35%
Events/total	17/73	15/30	15/33	47/136
Total				
Events %	17%	35%	48%	26%
Events/total	39/226	34/98	25/52	98/376

Correlation between tumour diameter, grade, oestrogen receptor, fibrotic focus and prognosis (in column 1, Events = number of events as dead of distant metastatic disease; total = number of cases; percentage = Events/total).

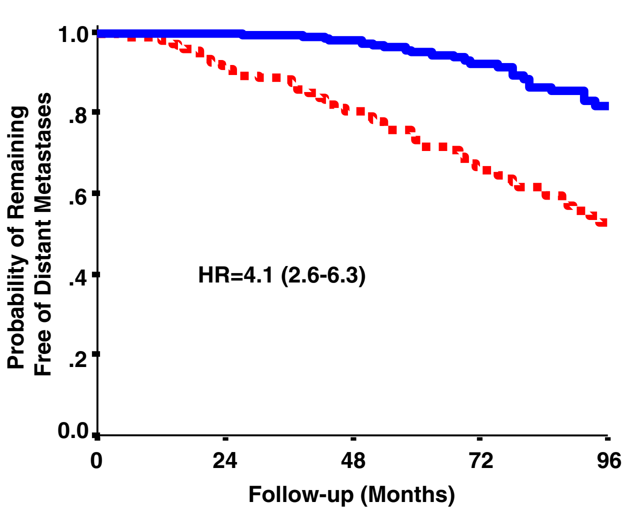
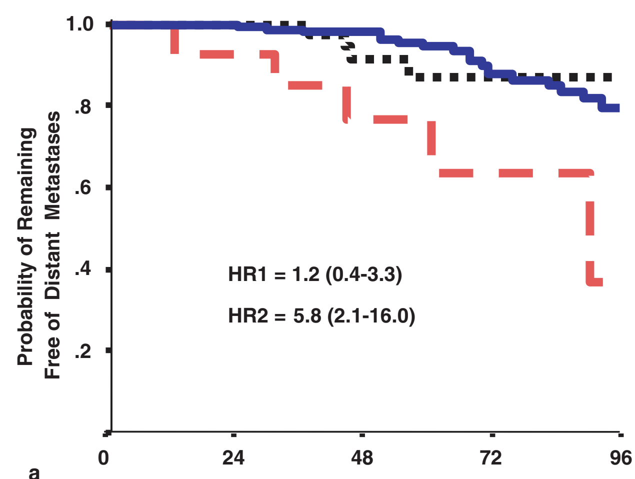


Fig. 2. Distant metastases-free survival curves of patients with mitotic activity index (MAI) < 10 (continuous line) *versus* ⩾10 (dotted line).

Table 3
Ductal and mixed-ductal invasive cancers only ($n = 376$)

	Fibrotic focus			Overall MAI
	Absent	<1/3	FF >1/3	
MAI < 10				
Event %	11%	13%	42%	13%
Events/total	(16/151)	(5/38)	(5/12)	26/201
MAI ⩾ 10				
Event %	31%	48%	50%	41%
Events/total	(23/75)	(29/60)	(20/40)	72/175
P-value	<0.0001	0.0009	0.64 (NS)	<0.0001
Overall FF	17%	35%	48%	26%
	39/226	34/98	25/52	98/376

NS, not significant.
Influence of mitotic activity index (MAI) and fibrotic focus (FF) on distant metastases disease free survival.



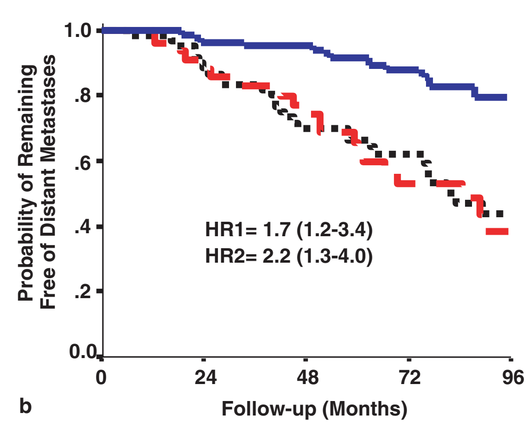


Fig. 3. Distant metastases-free survival curves of the patients with (a) mitotic activity index = MAI < 10 and (b) MAI ⩾ 10, both according to the absence of a fibrotic focus = FF (continuous line) *versus* the presence of a small FF occupying less than 1/3 (black dotted line) and more than 1/3 (red interrupted line).

pense of over-treating 267 non-metastatic women (i.e. 448–70 SG-favourable patients = 378–111 with distant metastases). The benefit ratio of AST in the SG-unfavourable group is thus (33 rescued)/(378 treated) = 9%

Table 4
Reproducibility of the fibrotic focus (FF) is very good ($\kappa = 0.93$)

	FF by observer #2			Total
	Absent	FF <1/3 diameter tumour	FF >1/3 diameter tumour	
FF by observer #1				
Absent	8	1	0	9
FF < 1/3 diameter tumour	0	8	0	8
FF > 1/3 diameter tumour	0	0	10	10
Total	8	9	10	27

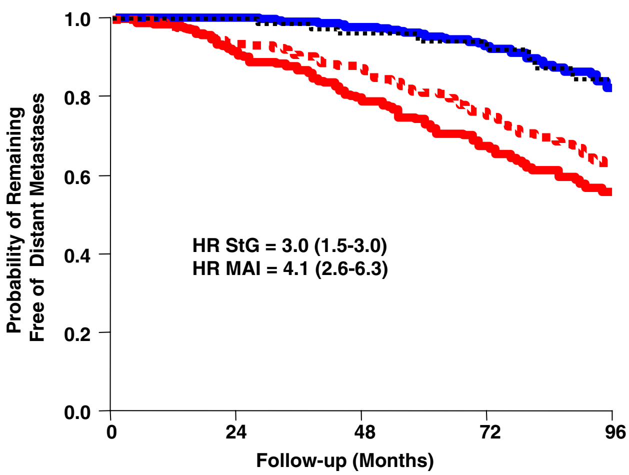


Fig. 4. Comparison of distant metastases-free survival curves of the patients according to the MAI < 10 (top continuous line), MAI ⩾ 10 (bottom continuous line) (hazard ratio = HR = 4.1) and St. Gallen favourable (top dotted line) and unfavourable (bottom dotted line) (HR = 3.0).

only, clarifying the need for prognostic factors other than SG in patient selection for AST. MAI ⩾10 is a highly suitable criterion to select high-risk patients for AST, forming 43% of all LN− patients, but in spite of being LN− such breast cancer patients have a high probability of distant metastases (41%), similar to young LN+ breast cancer patients [42]. The current results also show that the MAI is a stronger prognosticator than the St. Gallen criterion or its constituent characteristics tumour diameter, grade and oestrogen receptor, often used to select patients for adjuvant systemic treatment. Using the MAI rather than the SG, would save many unnecessary over-treatments. In our view, LN− breast cancer patients with high proliferation (either TLI or MAI ⩾ 10) should receive adjuvant systemic treatment (in agreement with Amadori [6]).

The situation in MAI < 10 patients is different, as only some 13% of the MAI < 10 patients still develop metastatic disease. The AST cost-benefit analysis in this subgroup ($n = 256$) would be even less favourable than described above for all St. Gallen unfavourable patients (as now widely applied), as follows. Only 33 develop distant metastases (see Table 3) and AST would rescue only 8. For this relatively favourable subgroup, 256 − 33 = 223 patients would have to be over-treated, resulting in a treatment-benefit ratio of 8/256 = 3% only. The FF is a potential candidate to better identify high-risk women in this MAI <10 subgroup. Hasabe and colleagues [35] showed that the FF was an unfavourable prognostic sign. However, the majority of his patients (74%) received adjuvant systemic therapy, thus carrying the risk of treatment bias. Moreover, the prognostic significance of the FF and the MAI were not compared. However, in the current prospective study of 448 LN− patients without AST, subgroup FF > 1/3 in the MAI < 10 was prognostically unfavourable. Although this regards a small number of patients only ($n = 12$), AST may be considered in these women.

With regards to the molecular and cell-biological background, specific amplifications and deletions occur in LN− breast cancers, which are correlated with prognosis [43] and high MAI values. Gene-specific studies point to a strong correlation between deletions on chromosome 1p [44]. A strong association has been found between the expression of the endogenous hypoxia marker carbonic anhydrase IX [45] and the presence of an FF [46]. Intra-tumoural hypoxia appears to be a key-regulatory tumour growth factor and many hypoxia-response pathway elements are candidates for therapeutic targeting [47]. Pathways that are regulated by hypoxia include angiogenesis, glycolysis, growth-factor signalling, immortalisation, genetic instability, tissue invasion and metastasis, apoptosis and pH regulation, all of which contribute to the malignant phenotype. Both cancer cells and normal cells are hypoxia-sensitive, but genetic and adaptive changes allow cancer cells to survive and even proliferate under hypoxic conditions. An important mediator of the cell's response to reduced oxygen levels is the hypoxia-inducible transcription factor-1 (HIF-1), which binds to the hypoxia-response elements of numerous oxygen-regulated genes, thereby activating their transcription. HIF-1 induces production of growth factors, which may indirectly promote the development of new blood vessels [48,49]. Stimulation of angiogenesis is one of the best-studied hypoxia responses. It is due to activation of vascular endothelial growth factor gene transcription by HIF-1 [50]. It has been suggested that FF can be used as a surrogate for quantifying angiogenesis

[29,51]. It is therefore not surprising that FF has additional independent prognostic value for certain MAI subgroups in LN− breast cancer. Several authors found that areas of highest intra-tumoural microvessel density and highest endothelial cell proliferation fractions are topographically close to the areas with the highest tumour cell proliferation fractions [52,53]. This can be explained by the reciprocal stimulation of tumour cell and endothelial cell growth through the release of important paracrine growth factors. However, other factors may also play a role [54–57]. Another aspect is the comparative prognostic value of the MAI and Ki-67 (MIB-1). In a detailed recent review article we have analysed the results of many studies comparing these two prognostic factors. The MAI is prognostically stronger than Ki-67 [58].

In conclusion, MAI < 10 *versus* $\geqslant 10$ is the strongest predictor of outcome. LN− patients with MAI $\geqslant 10$ are at risk for distant metastases, similar to LN+ patients. The presence of an FF adds to the prognostic value of the MAI but only if MAI < 10 . In the small subgroup of patients with MAI < 10 and FF $> 1/3$, event-free survival is 61%, much worse than in the MAI < 10 patients and similar to that in patients with MAI $\geqslant 10$. Both MAI and FF can easily and reproducibly be assessed in routine microscopic tissue sections and should be included in the pathology report of every breast carcinoma.

Conflict of interest statement

None declared.

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