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The prognostic value of proliferation in lymph-node-negative breast cancer patients is age dependent

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ABSTRACT

In lymph-node-negative invasive breast cancer patients <55 years, the proliferation marker mitotic activity index (MAI) has previously been shown to be the strongest prognosticator. In studies without age definition, MAI was not strongly prognostic. We investigated the age dependency of the prognostic value of proliferation for distant metastasis-free (MFS) and overall cancer-related survival (OS) in 1004 histologically diagnosed $T_{1-3}N_0M_0$ invasive breast cancers ($n = 516$, <55 years; $n = 322$, 55–70 years; $n = 166$, >70 years) without systemic adjuvant therapy and long follow-up (median: 108 months). The MAI decreases with age and the prognostic value of MAI varied by age group. For patients <55 years, hazard ratios (HR) for MAI $\geqslant 10$ *versus* <10 for MFS and OS were 3.1 and 4.4, respectively ($P < .0001$ for both), but only 1.9 and 1.9 ($P = .004$ and .006) for patients aged 55–70 years, while over 70 years, MAI was not significant ($P = .11$).The prognostic value of proliferation was age-dependent. Prognostic breast cancer studies must clearly indicate the age group being studied.

1. Introduction

The prognosis of patients with lymph-node-negative (LN–) breast cancers is relatively good (15–30% dying from recurrent disease) and survival improvement due to adjuvant systemic therapy (AST) will be less substantial than in lymph-node-positive patients. The typical 10-year survival for LN-cases may improve from 71% to 78% with AST. The benefits and side effects of this 7% improved survival from AST must be considered simultaneously.[1] Accurate and reliable prognostic markers can help in these decisions.

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Proliferation markers exceed the prognostic value of classical prognosticators.[2–15] The mitotic activity index (MAI) is an especially practical, strong, widely available, easily assessable, inexpensive, highly reproducible prognosticator.[5,8,10,12,13,15] We have recently shown that in women <55 years at diagnosis, without AST and with long follow-up, the MAI is the strongest independent prognostic factor and well suited to routine differentiation between high- and low-risk LN-breast cancer patients.[9,16] Moreover, in two independent studies, patients with rapidly proliferating tumours significantly benefited from adjuvant chemotherapy.[17,18] However, not all studies confirm the prognostic value of proliferation factors.[19,20] Possible explanations for this lack of prognostic agreement include differences in sample processing with possible changes in proliferation indices after tissue resection; methodological differences in MAI assessment; or varying selection of patient groups. Tissue processing as used in most pathology laboratories is an unlikely source of disparity; mitoses are stable and robust when certain simple, widely applied precautions are taken.[21] The MAI was well reproducible in a large multicenter study with strict adherence to well-validated protocol guidelines.[22] However, it never can be fully proven that MAI assessments in a particular study have been performed following strict protocols. Regarding the possibility of patient selection as a confounding influence, an important factor in our recent studies was the very large group of patients, all LN-women younger than 55 with operable breast cancer;[9,16] other prognostic studies with negative findings regarding the MAI have analysed small numbers, involved mixtures of both young and old patients, or did not define the patients' ages. Mixtures of different ages – and thus hormonally different conditions – may influence proliferation characteristics and consequently their prognostic value.

The question, therefore, is whether the prognostic value of proliferation and especially the MAI in node-negative patients is influenced by age. To answer this question, we investigated the prognostic value of proliferation for distant metastasis-free (MFS) and overall cancer-related survival (OS) in 1004 $T_{1-3}N_0M_0$ invasive breast cancers in different age groups. For this study, the material was used from the prospective, long-term nationwide Multicenter Mammary Carcinoma Project (MMMCP) in The Netherlands, which covers 34 hospitals[23] and includes almost 3500 consecutive women with breast cancer. This report compares the long-term prognostic value of different clinico-pathologic features in LN-breast cancer in women aged <55, 55–70 and >70 years without previous or concurrent malignancies. During the enrollment period, the general policy on breast cancer treatment in The Netherlands did not include AST for patients with LN- breast cancer. This allows to analyse the biological significance of proliferation, not influenced by cytostatic drugs.

2. Materials and methods

Details of the MMMCP protocol have been described elsewhere.[16,22,23] An important advantage of the MMMCP study is that participation bias of patients was excluded, as all consecutive patients with invasive breast cancer diagnosed as such in the 32 collaborating MMMCP hospitals were enrolled. These were both general and university hospitals, served by general pathology departments, thereby guaranteeing unselected participation of all patient ages. Another possible bias source regards *determination bias*, i.e. patients without biopsy, and hence without histologically confirmed breast cancer. These patients would have been missed in our study. However, this hypothetical factor is not relevant, as it would regard patients with a clinically very advanced disease, whereas our hospital-based study regards early stage node-negative T1-T3 patients.

Regarding the follow-up of the older patients, there were no indications that this was any different from the younger patients, with the exception that due to age, many more patients died from other diseases or age itself, and were therefore more frequently censored. This explains why the follow-up was inevitably shorter (median follow-up in the age group >70 years was 81 months versus 119 and 111 months in the <55 and 55–70 years age groups). The frequency of patients lost to follow-up was slightly higher in women >70, compared with those of 55–70 years (14% and 8%, $P = .044$) but the lost patients in the age groups were equally distributed over MAI <10 and $\geqslant 10$ ($P = .27$). Thus, this will not have influenced the prognostic value in the MAI subgroups analysed.

Of the 3479 MMMCP patients registered, 1744 had LN-breast malignancy. The following patients were excluded: 13 males; 86 women with carcinoma in situ (CIS) only or with extensive CIS with a micro-invasive part <1 mm (precluding MAI assessment, see below); 3 with sarcomas; 29 with inoperable cancer; 2 with distant metastases at diagnosis; 100 cases at stage T4; 108 with previous or synchronous malignancies (other than basal cell carcinoma of the skin or cervical CIS); 16 with double-sided carcinoma; 209 lost to follow-up; and 23 in which the slide quality was too poor for MAI assessment. The usual therapeutic regimens of many hospitals in The Netherlands in the 1980s meant that only a small minority (151) of the node-negative patients received systemic adjuvant hormonal or cytostatic therapies; these were also excluded. Thus, 1004 node-negative patients who had not received AST remained (516 < 55; 322 between 55 and 70 years; and 166 – 70 years). These patients received modified radical mastectomy or breast-conserving therapy (BCT), always with adequate axillary lymph-node dissection. Loco-regional radiotherapy was given in cases undergoing BCT or with medially localised tumours. Post-surgical tumour size was measured in the fresh specimens; the tumours were cut in 0.5-cm slices, fixed in buffered 4% formaldehyde, and embedded in paraffin. At least 6 (median: 11) lymph nodes were detected in the axillary lymph-node dissection specimens.

Paraffin sections, 4 μm thick, were stained with haematoxylin and eosin (H&E). Histologic type was assessed according to World Health Organisation criteria.[24] Grade was assessed during careful review by two pathologists with considerable experience in breast pathology, according to the Nottingham modification,[25,26] using MAI (see below) 0–5 = 1, 6–10 = 2 and >10 = 3; nuclear atypia as mild = 1, moderate = 2, or marked = 3; and tubule formation as majority (>75% = 1), moderate (10%-75% = 2), or little to none (<10% = 3). Grade is the sum of tubular formation + nuclear atypia + MAI class; Grade I (sum = 3–5), Grade II (sum = 6, 7) and Grade III (sum = 8, 9). Estrogen receptor (ER) value was assessed with

the ligand-binding charcoal technique (cutoff = 10 fmol/mg protein). The Sankt–Gallen criterion was used to identify as low-risk those tumours with combined Grade 1, tumour diameter <2 cm, and ER positivity. In women aged over 70, ER was not always known but the Sankt–Gallen criterion in all of these cases was unfavourable because of the value of the other two features.

For the assessment of the MAI, the MMMCP protocol[22,23] was used. With a black marker the most poorly differentiated peripheral area of the tumour was marked, avoiding necrotic, heavily inflamed, or benign areas. In this 'measurement area' (minimally 1×1 and maximally 5×5 mm in size), mitoses were counted in the most cellular area at ×400 magnification (objective 40, field diameter 450 μm at specimen level) in 10 consecutive neighboring fields of vision (representing a total area of 1.59 mm^2 at specimen level). Only definitive mitoses were counted; doubtful structures were ignored. The total resulting number of mitoses in these 10 fields of vision is the mitotic activity index. According to many previous studies, the most important prognostic threshold is 10, with MAI <10 indicating favourable prognosis and MAI $\geqslant$10 indicating poor prognosis.[5–8,10,16] If $5 < MAI < 15$, the MAI was assessed once more. In case of a discrepancy between two MAI assessments in which both values were either below or above 10, the higher of the two counts was used. However, in the very rare cases when the first MAI count was <10 and the other was $\geqslant$10, a third assessment was done by two observers simultaneously and the resulting MAI was then used for further analysis. An accurate MAI assessment takes approximately 3 min. Correction of the MAI for the percentage of tissue occupied by stroma or the number of tumour cells was not applied because this does not substantially improve the prognostic value of the MAI and is much more time consuming.[27]

2.1. *Statistical analysis*

Endpoints were distant recurrence (metastases-free survival, MFS), mortality due to distant metastases (overall survival, OS), and mortality due to any death. The latter endpoint includes deaths from non-breast cancer related causes, especially in older patients, and is therefore less specific to the study of prognostic factors. Nonetheless, it was used for comparison because this endpoint is often used in breast cancer studies. Recurrence was defined as any first recurrence at distant sites. All other patients were censored on the date of the last follow-up visit or deaths from causes other than breast cancer. Mortality was defined as any death resulting from distant metastases, evident from clinical, radiologic, histologic, or autopsy data; no patients died from loco-regional disease. If the cause of death was unknown but a metastasis was previously detected, then death was considered breast cancer related unless explicitly stated otherwise (in line with other studies).[1,16] SPSS version 13 (SPSS, Chicago, USA) was used for the analyses. Age, time to first recurrence and survival time were calculated. Survival curves were constructed using Kaplan–Meier techniques. Differences between groups were tested by log-rank tests or tests for trend. The relative importance of potential prognostic variables was tested using Cox-proportional hazard analysis and expressed as a hazard ratio (HR) with 95% confidence intervals. All variables were tested for proportionality, and continuous variables were checked for (non)-linearity and transformed or recoded if necessary or useful. To simulate age-corrected general mortality, we have standardised the three age groups (<55, 55–70 and >70), by a stratified analysis, thereby indicating that age might be a possible confounder. The hazard ratios were then recalculated for each single feature. In these stratified analyses, there was hardly any change in the hazard ratios of the different factors analysed.

3. Results

In the three age groups (<55, 55–70 and >70 years), median follow-up was 119, 111 and 81 months (range: 7–187), respectively; distant metastases occurred in 25%, 25% and 27%, whereas 17%, 23% and 21% died of metastases, respectively.

3.1. *Metastasis-free (MFS) and overall survival(OS)*

The prognostic value of the MAI differed among the three age groups studied. The findings for those under 55 years have been described before in detail and are only briefly mentioned here for reference.[9,16] Of the many factors analysed in these studies (age, operation type, oestrogen receptor, histological grade and type, tubular formation, nuclear atypia, MAI with different thresholds (<3, 3–9, >9; <6, 6–10, >10; <10, >9 and fibrotic focus)), MAI (as $\geqslant$10, <10) showed the strongest association with MFS (HR = 3.1; $P \leqslant .0001$) and OS (HR = 4.4; $P < .0001$). The absolute difference in 10-year Kaplan–Meier estimates of time to distant recurrence as well as survival was 22% (92% and 70%) between MAI <10 *versus* $\geqslant$10. A number of other factors were also prognostic, but none of these added to the MAI in multivariate analysis.

For patients aged 55–70 years, MAI was again the strongest prognostic factor for both MFS (HR = 1.9; $P = .004$) and OS (HR=1.9; $P = .006$), although the prognostic value was less than that for patients under 55 years. The absolute difference in 10-year survival between MAI <10 *versus* $\geqslant$10 (81% and 70%, respectively) was also lower at 11%. Tumor diameter (*i.e.* $\leqslant$2 *versus* >2 cm), ER and tubule formation (TF) were also prognostic (Table 1). In multivariate analysis, MAI and TF (*i.e.* <10% *versus* >10%) had independent prognostic value for distant metastases (HR = 2.1, $P = .001$; and HR = 2.0, $P = .048$, respectively). Neither tumour diameter, ER, nor nuclear atypia added to the prognostic power of the MAI. Further analysis showed that the additional prognostic value of TF was only relevant in the MAI <10 subgroup: if TF >10% (68/322, or 21% of all patients), 92% had a recurrence-free 10-year survival, contrasting to 72% if TF <10 (equal to patients with MAI $\geqslant$10).

In patients over 70, tumour diameter was the strongest prognosticator, followed by grade. MAI was not significant ($P = .11$; Table 2, Fig. 1). The absolute difference in 10-year Kaplan–Meier estimates of time to distant recurrence as well as survival between MAI <10 *v* $\geqslant$ 10 was 10% (82% and 72%, respectively). Separate multivariate analysis including the three grade constituents showed that nuclear atypia (mild *versus* non-mild) and TF (<10% *v* > 10%) were significant ($P < .05$ for both), but MAI was not ($P = .28$). Comparison of all features showed that in the current study, tumour diameter (<2 cm *versus* $\geqslant$2 cm) was the strongest prognosticator in this age

Table 1 – Node-negative patients 55–70 years: characteristics of patients and tumours in relation to distant recurrence and disease-related mortality

Characteristic	Recurrence			Disease-related mortality		
	Events/at risk	Log-rank *P* value	HR (95% CI)	Events/at risk	Log-rank *P* value	HR (95% CI)
Surgery						
BCT	43/179			39/179		
Mastectomy	39/143	.37	1.2 (0.8–1.9)	34/143	.53	1.2 (0.7–1.8)
Diameter						
⩽2 cm	39/181			34/181		
>2 cm	43/141	.03	1.7 (1.1–2.7)	39/141	.03	1.7 (1.1–2.7)
ER status						
Positive	50/215			41/215		
Neg/borderline	32/107	.04	1.6 (1.02–2.6)	32/107	.04	1.6 (1.0–2.6)
Grade						
I	12/61			11/61		
II	41/161		1.5 (0.8–2.8)	37/162		1.4 (0.7–2.8)
III	29/100	.35	1.6 (0.8–3.2)	25/99	.45	1.6 (0.8–3.2)
St. Gallen						
Low risk	4/31			4/31		
High risk	78/291	.21	1.9 (0.7–5.1)	69/291	.30	1.7 (0.6–4.6)
MAI						
<10	45/210			40/210		
⩾10	37/112	.004	1.9 (1.2–2.9)	33/112	.006	1.9 (1.2–3.0)
MAI						
0–5	32/169			28/169		
6–10	14/52		1.6 (0.8–3.0)	13/52	.18	1.6 (0.8–3.0)
>10	36/101	.002	2.4 (1.4–3.9)	32/101	.001	2.4 (1.5–4.1)
MAI						
<3	20/112			18/112		
3–9	25/98		1.4 (0.8–2.5)	22/98		1.4 (0.7–2.6)
⩾10	37/112	.009	2.2 (1.3–3.8)	33/112	.02	2.2 (1.2–3.9)
Nuclear atypia[a]						
Mild + moderate	37/159			34/159		
Marked	45/163	.32	1.2 (0.8–1.9)	39/162	.44	1.2 (0.8–1.9)
Tubule Formation[a]						
>10%	10/68			9/68		
<10%	72/254	.008	2.4 (1.2–4.6)	64/254	.01	2.4 (1.2–4.7)

Notes: HR values of >1 indicate an increased risk for the second (or third) category compared with the first category. Analysis standardised by age gave nearly the same hazard ratios and 95% confidence intervals.

Abbreviations: HR, hazard ratio; CI, confidence interval; P, probability of no significance; BCT, breast-conserving therapy; ER, oestrogen receptor; MAI, mitotic activity index.

a These thresholds gave the most significant results.

group. TF had borderline significant additional value ($P = .051$).

3.2. *'Any death' as end-point*

In patients under 55, analysis of the prognostic value of the different prognostic features using 'any death' as the mortality end-point gave the same results as for MFS and OS, in which MAI <10 *versus* ⩾10 was the strongest prognosticator. For patients aged 55–70, the MAI (again with the classical threshold of 10) was first selected by multivariate analysis. As in the analysis with 'dead of distant disease', the same features had some limited additional value (tumour diameter, TF and ER), but in this case ER and not TF was selected. The additional value of ER for survival prognosis was identified only in the MAI < 10 patients (overall: 84%, ER-positive 89%, ER-negative 71%). In patients over 70, none of the features were prognostic when 'any death' was used for events, contrasting the prognostic value with disease-specific survival (distant metastases). Fig. 1 summarises the prognostic value of the MAI for MFS and OS in the three age groups.

3.3. *Comparison of the MAI and tumour diameter in the three age groups*

Further comparison of the three age groups showed that the fraction of patients with tumours over 2 cm was higher in patients over 70 than in patients under 70 (53% *versus* 43%; $P = .009$), but it was the same in patients under 55 and ages 55 to 70 (43% and 44%). The mean MAI gradually decreased in the three age groups (from 12.8 to 11.0 to 8.7; differences between <55 *versus* 55–70 and <55 *versus* >70, $P = .08$ and .001, respectively) and the percentage of patients with MAI ⩾10 decreased (from 42% to 35% to 30%; differences between

Table 2 – Node-negative patients >70 years: characteristics of patients and tumours in relation to distant recurrence and disease-related mortality

Characteristic	Recurrence			Disease-related mortality		
	Events/at risk	Log-rank *P* value	HR[a] (95% CI[2])	Events/at risk	Log-rank *P* value	HR[a] (95% CI[2])
Surgery[3]						
BCT	13/47			9/47		
Mastectomy	32/119	.31	1.4 (0.7–2.8)	26/119	.27	1.5 (0.7–3.3)
Diameter						
⩽2 cm	11/78			7/78		
>2 cm	34/88	.01	2.4 (1.2–4.7)	28/88	.005	3.1 (1.3–7.1)
ER status						
Positive	26/95			22/95		
Neg/borderline	9/27	.05	2.2 (1.0–4.8)	6/27	.25	1.7 (0.7–4.4)
Grade						
I	5/33			3/33		
II	22/87		2.4 (0.9–6.5)	18/87		3.3 (1.0–11.5)
III	18/46	.05	3.3 (1.2–9.0)	14/46	.04	4.7 (1.3–16.3)
Grade						
I	5/33			3/33		
II + III	40/133	.03	2.8 (1.1–7.9)	32/133	.02	3.8 (1.2–12.6)
St. Gallen						
Low risk	1/11			0/11		
High risk	44/155	.19	3.8 (0.5–27.5)	35/155	.07	23.2 (0.1–>100)
MAI						
<10	27/116			21/116		
⩾10	18/50	.13	1.6 (0.9–2.9)	14/50	.11	1.7 (0.9–3.4)
MAI						
0–5	23/103			19/103		
6–10	5/18		1.1 (0.4–2.8)	3/18		0.8 (0.2–2.7)
>10	17/45	.21	1.8 (0.9–3.3)	13/45	.22	1.7 (0.9–3.5)
MAI						
<3	13/66			10/66		
3–9	14/50		1.4 (0.7–2.9)	11/50		1.4 (0.6–3.3)
⩾10	18/50	.23	1.9 (0.9–3.9)	14/50	.22	2.0 (0.9–4.6)
Nuclear atypia[a]						
Mild + moderate	25/106			19/106		
Marked	20/60	.08	1.7 (0.9–3.1)	16/60	.04	1.9 (0.9–3.7)
Tubule Formation[a]						
>10%	7/40			4/40		
<10%	38/126	.03	2.5 (1.1–6.1)	31/126	.02	3.2 (1.1–9.1)

Notes: HR values of >1 indicate an increased risk for the second (or third) category compared with the first category. Analysis standardised by age gave nearly the same hazard ratios and 95% confidence intervals.
Abbreviations: HR, hazard ratio; CI, confidence interval; P, probability of no significance; BCT, breast-conserving therapy; ER, oestrogen receptor; MAI, mitotic activity index.
a These thresholds gave the most significant results.

<55 *versus* 55–70 and <55 *versus* >70, *P* = .07 and .01, respectively) (Table 3).

3.4. *MAI ⩾10 indicates poor prognosis, irrespective of age*

In spite of the fact that the prognostic significance of the MAI decreased with increasing age, as described above, the survival probability of patients with MAI ⩾10 was poor in all three patient groups (30%, 30% and 28% died of disease in the <55, 55–70 and >70 groups, respectively). Interestingly, MAI < 10 indicated an excellent prognosis with 92% survival at the 10-year follow-up in patients under 55, but the survival probability of this subgroup was much lower in patients aged 55–70 (81%) and over 70 years (82%) (Table 3).

4. Discussion

The results of this prospective study in breast cancer patients show that the MAI is the strongest prognosticator in women under 71 years of age, but above 70 years the difference of 10% better survival in MAI < 10 compared with MAI ⩾10 is not significant (*P* = .11). Moreover, the prognostic strength of the MAI is less in women 55–70 than in patients younger than 55. The prognostic strength of the MAI therefore is age dependent (Table 3), in agreement with the findings of others that biomarker profiles of human breast cancer are age-associated.[28] The question that then arises is what causes this age-dependent prognostic value of MAI? MAI itself is an age-dependent phenomenon (Table 3), and it is therefore tempting to hypothesise that lower oestrogen levels in older patients are

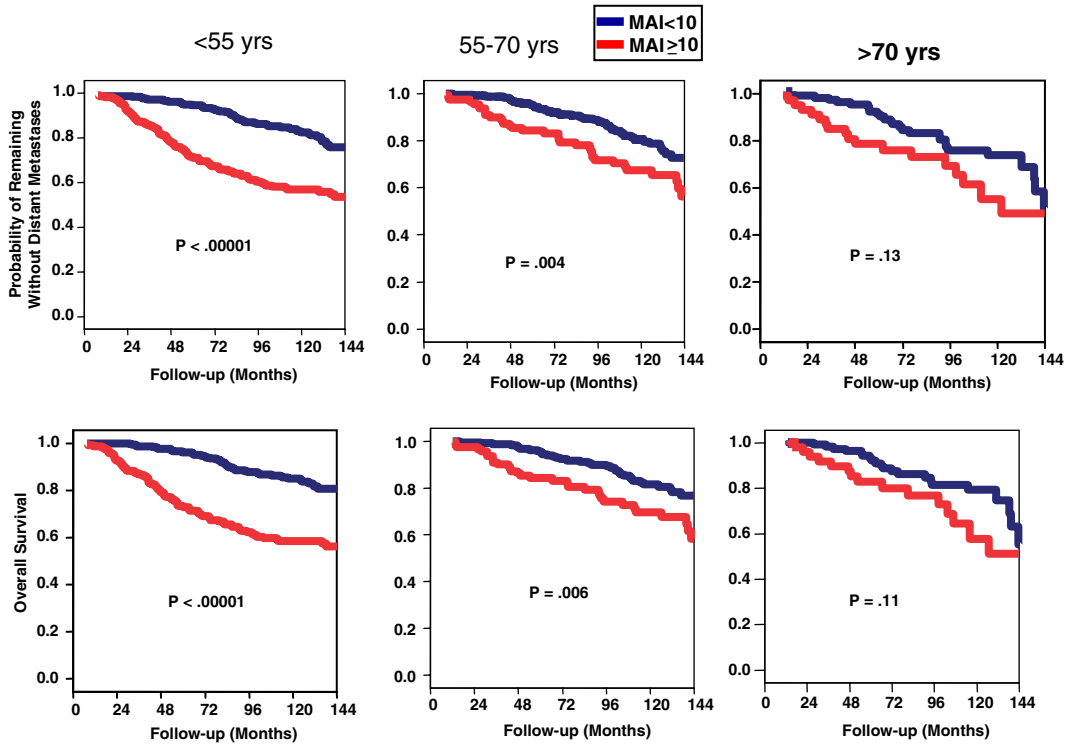

Fig. 1 – Comparison of the distant metastases: recurrence-free and overall survival of the MAI in the three different age subgroups.

Table 3 – Comparison of the number and percentages of patients with MAI <10 versus >9, and MFS and OS in patients with MAI <10 and >9, related to age group

	<55 years			55–70 years			>70 years		
	#DOD/ total	%DOD	% of patients	#DOD/ total	%DOD	% of patients	#DOD/ total	%DOD	% of patients
Tumor diameter									
⩽2 cm	67/290	23%	57%	34/181	19%	56%	7/78	9%	47%
>2 cm	69/222	31%	43%	39/141	28%	44%	28/88	32%	53%
MAI									
Mean (Range)		12.8 (0–121)			11.0 (0–126)			8.7 (0–102)	
MAI < 10	25/299	8%	58%	40/210	19%	65%	21/116	18%	70%
⩾10	65/217	30%	42%	33/112	30%	35%	14/50	28%	30%
10 year survival									
MAI < 10		92%			81%			82%	
MAI ⩾ 10		70%			70%			72%	
Probability and hazard		<.00001			.006			.11 (not significant)	
ratio of MAI < *versus* ⩾ 10		4.4			1.9			1.7	

Abbreviation: DOD, died of distant metastases.

an important cause. Estrogens increase proliferation in hormone receptor-positive breast cancers, so lower oestrogen levels would be expected to result in a decrease in proliferation, *i.e.* the MAI. Absolute ER values in fmol/mg protein and the percentage of receptor-positive cancers increase with age.[29,30] Likewise, in general, hormone receptor-positive tumours are better differentiated and have lower grades than receptor-negative tumours.[31] These factors may also explain the decreasing mean MAI values (12.8, 11.0 and 8.7 in the three age groups studied, respectively) and the lower fraction of tumours with a high MAI (⩾10) with increasing age, from 42% (<55 years) to 35% (55–70) and 30% (>70 years) (Table 3). Of course, it may be hypothesised that the higher ER levels result from receptors not blocked by oestrogens, but that does not change the basic concept that oestrogen effects at the molecular level could translate into these differences.

Considering the MFS and OS survival curves (Fig. 1), the decrease in prognostic value of MAI with age is mainly related to a decrease in the prognosis of patients with MAI < 10. Indeed, both the MFS and OS probabilities for patients with MAI $\geqslant$10 were poor in all three patient age groups (Table 3). The decreasing prognostic value of MAI with age is therefore not the result of an increasingly worse prognosis for patients with MAI $\geqslant$10, but rather results from the different behaviour of tumours with MAI < 10. The impact of this phenomenon increases with age with the increasing percentage of women with MAI < 10 and the decreasing percentage of cancers with MAI $\geqslant$10. There may be many reasons for a worse prognosis for older women with low proliferating tumours. As shown here, other prognostic factors may become more dominant than MAI. Indeed, women over 70 had larger tumours than the younger women, and tumour diameter in this group was of strong prognostic significance. In spite of this, slightly fewer patients over 70 died of distant metastatic disease (21%) than in the group of women 55 to 70 years of age (23%). However, the follow-up time was somewhat shorter in patients over 70 (median 81 months *versus* 111 in the younger patients). This shorter observation time for the older patients is in part a natural consequence of competing causes of death with increasing age. We cannot exclude with absolute certainty that this shorter follow-up time is a reason for the lack of prognostic value of MAI in this age group (*i.e.* that the patients over 70 simply did not live long enough to develop distant metastases). However, the fact that the survival curves of the MAI < 10 and MAI $\geqslant$10 almost overlap in the first 7 years of follow-up for this group (Fig. 1) makes this explanation unlikely. The larger tumour diameters in this oldest age group are most likely the result of reduced patient attention/awareness or less easy access to adequate medical care.

Another consideration includes major changes in the hormonal status of women in the two younger groups during follow-up. In our earlier study[16] of 516 women younger than 55, 283 (55%) were over 46 years. Virtually all women in this group, as well as some younger women and all premenopausal women in the 55–70 group, will have experienced natural menopause during the subsequent follow-up time, and consequently will have undergone a sharp decrease in blood oestrogen levels. For women with hormone receptor-positive tumours (about 60%), this decrease may act as adjuvant endocrine therapy and result in a marked prolongation of the MFS and OS. This 'natural endocrine therapy' is, of course, lacking in older women. Observing that the majority of tumours with MAI < 10 are receptor positive [in the former study, 211 of the 268 women (79%) under 55 with MAI < 10 were ER positive[16]], may in part explain the better prognosis of young women with node-negative, MAI < 10 tumours. Finally, different molecular pathways may be active in women of different age groups with consequent differences in the prognostic value of MAI.

The older patient is less likely to receive systemic chemotherapy once distant metastases have appeared. One could hypothesise that the lower prognostic effect of MAI in the elderly just reflects the tendency to treat them less aggressively, regardless of MAI in this age group, in case of recurrence. However, this is unlikely as the difference between the age groups is mostly in the patients with a low MAI, whereas the survival of the high MAI patients is very similar. Recent studies found that chemotherapy is effective in patients with a high proliferation rate, but not in patients with a low proliferation rate (as expected).[18,32] Thus, if the lower prognostic effect of the MAI in the elderly would just reflect the tendency to treat them less aggressively, one would expect a better survival in the young patients with a high MAI, not a lower survival of older patients with a low MAI, as we found. Clinically the most important group of women for receiving AST is the subgroup of patients under 55, for whom the MAI is the strongest prognostic factor for metastases-free survival.[9,16,32,33] In women over 54 and under 70, an MAI $\geqslant$10 identifies patients with a poor outcome; however, compared to women under 55, the outcome in MAI < 10 is less definitive, and it is in this subgroup that the prognostic value of MAI declines. It is therefore of utmost importance that in prognostic evaluations, predictive studies, and analyses of molecular pathways related to proliferation, the age of the patient group under investigation is clearly defined.

Conflict of interest statement

None declared.

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