ORIGINAL ARTICLE

Tumour size and vascular invasion predict distant metastasis in stage I breast cancer. Grade distinguishes early and late metastasis

P J Westenend, C J C Meurs, R A M Damhuis

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See end of article for authors' affiliations

Correspondence to: Dr P J Westenend, Pathologisch Laboratorium voor Dordrecht eo, Laan van Londen 1800, 3315 HG Dordrecht, The Netherlands; pwestenend@paldordt.com

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Background: Recent Dutch guidelines recommend adjuvant systemic treatment (AST) for women with high grade stage I breast carcinoma ⩾1 cm. High grade is defined as Bloom and Richardson grade 3 (B&R3), Nottingham modification, or mitotic activity (MAI) ⩾10/1.59 mm^2.
Aims: To investigate the validity of these histological prognostic factors as the exclusive defining criteria.
Materials/methods: Fifty patients with stage I breast carcinoma who developed distant metastases and 50 matched controls without metastasis were studied; none had received AST.
Results: Cases more often had tumours ⩾1 cm (p = 0,019), B&R3 tumours (p = 0.059), grade 3 nuclei (p = 0.005), and vascular invasion (p = 0.007). No differences were found for MAI ⩾10 (p = 0.46). In multivariate analysis, the only significant variables were vascular invasion and tumour size (odds ratios: 8.21 and 5.35, respectively). In a separate analysis, the 50 cases were divided into 25 patients with early and 25 with late metastasis. Those with early metastasis more often had B&R3 tumours (p = 0.009) and grade 3 nuclei (p = 0.006). No differences were found for tumours ⩾1 cm, vessel invasion, or MAI ⩾10. Using the present Dutch guidelines for AST, based on B&R3, 20 cases and 11 controls would have received AST. Based on MAI ⩾10, 14 cases and 11 controls would have received AST.
Conclusions: Tumour size and vessel invasion are the best prognostic factors for disease free survival in patients with stage I breast cancer. Dutch selection criteria for AST for these patients need to be improved. Some prognostic factors are time dependent, making their use as selection criteria for AST more complicated.

Adjuvant systemic treatment (AST) and/or hormonal treatment can improve survival in women with breast cancer, and this treatment is generally offered to all node positive patients. However, in node negative patients, the benefits of AST are smaller and must be balanced against the associated toxicity. This is especially the case in women with stage I (T1N0M0) breast cancer. Survival in these patients is good, with excellent 10 year overall survival.[1–4] Several histological factors have been described that identify patients with stage I breast cancer with a relatively worse prognosis who might benefit from AST as opposed to those with an excellent prognosis who could be spared the side effects of this treatment.[5] Among these factors are tumour size, tumour grade, mitotic index, and vascular invasion.

> ''Some histological prognostic factors, such as vessel invasion, that might be of importance, especially in node negative patients, have not been incorporated into the Dutch guidelines''

In the Netherlands, recent guidelines recommend AST and/or hormonal treatment for all node positive patients and those node negative patients with a tumour size larger than 3 cm.[6] AST is not recommended for node negative patients with a tumour smaller than 1 cm. For those node negative patients with a tumour size from 1 to 3 cm, adjuvant treatment is recommended for those with high grade tumours. Tumour grade is defined by the Nottingham modification of the Bloom and Richardson score or the mitotic activity index (MAI). The Nottingham modification of the Bloom and Richardson score is widely used and has already been reported.[7] It is a scoring system that looks at the growth pattern (tubule formation) of a tumour, the pleomorphism of the nuclei, and the mitotic activity. The scores are added and translated into three grades. In the Netherlands, the use of MAI ⩾ 10/1.59 mm^2, which is the same as a score of 3 points for mitotic activity in the Nottingham modification of the Bloom and Richardson score, has been advocated as a good alternative.[8] However, the MAI has not been widely studied and the counting of mitotic figures is not as simple as it seems.[9] Results are influenced by many factors, including time between removal of the tissue and fixation, speed of fixation, selection of fields for counting mitotic figures, the definition of a mitotic figure, and tumour heterogeneity. Another important issue is the setting of a threshold value.

Some histological prognostic factors, such as vessel invasion, that might be of importance, especially in node negative patients, have not been incorporated into the Dutch guidelines. Lymph vessel invasion has been incorporated as a prognostic factor into the Canadian guidelines for adjuvant treatment in node negative breast cancer.[10] However others, although acknowledging the prognostic value of histological factors such as tumour type and grade, seem to use only tumour size in small node negative breast cancer.[11] In addition, because breast cancer may have recurrences over a long period of time, prognostic factors that are significant in studies with a short term follow up might not be significant with longterm follow up. The aim of our present study was to assess the validity of the histological prognostic factors incorporated into the Dutch guidelines for adjuvant treatment and to test whether some of the factors have a

Abbreviations: AST, adjuvant systemic treatment; DCIS, ductal carcinoma in situ; DFS, disease free survival; MAI, mitotic activity index

Table 1 Univariate analysis of prognostic factors comparing women with stage I breast cancer who do (cases) or do not (controls) have distant metastases

Variable	Controls (n)	Cases (n)	p Value
Age			
<50	13	16	
50–69	30	24	
70+	7	10	1.000
Side			
Left	26	23	
Right	24	27	0.689
Tumour grade			
Well/moderate	13	9	
Poor/undifferentiated	22	28	
Unknown	15	13	0.452
Tumour size			
1: 2 cm	1	2	
1A: ⩽0.5 cm	1	2	
1B: >0.5 to ⩽1 cm	17	11	
1C: >1 to <2 cm	31	35	0.576
Type of surgery			
Mastectomy	19	25	
Lumpectomy	31	25	0.314
Radiotherapy			
Yes	33	26	
No	17	24	0.222
Screen detected			
No	33	39	
Yes	17	11	0.265
Incidence period			
1988–1991	14	12	
1992–1997	36	38	0.820

prognostic value that is dependent on the duration of follow up.

MATERIALS AND METHODS

Follow up data for patients with stage I breast cancer who were investigated at one of the local pathology laboratories (Pathologisch Laboratorium voor Dordrecht eo) were collected from the Rotterdam Cancer Registry, the Netherlands. This laboratory serves several hospitals in the area. Patients with a synchronous or metachronous breast carcinoma were excluded. Completeness of follow up was defined as a known date of death, last follow up visit after 1 January 1999, or a follow up period of at least 10 years. Patients were either treated by mastectomy or by lumpectomy, followed by radiotherapy. All patients underwent a complete axillary lymph node dissection and none of the patients received AST. Between 1988 and 1997, 561 patients were available in total. Complete follow up data were available for 96.3% of the patients. In this period, 77 patients died and 66 patients developed distant metastases.

Because of the low number of patients with distant metastases, a case–control study was designed for a detailed evaluation of histological prognostic factors. Cases were defined as patients with distant metastases. Controls were matched to cases for the time of diagnosis and the hospital where the patient underwent surgery. The follow up time for controls was always longer than for cases. In the case–control study, three pairs were excluded because the tumour size of the cases as measured from the slides was larger than 2 cm. One pair was excluded because in the case a micrometastasis was found on evaluation of the lymph nodes. In one pair the control was replaced because the tumour as measured from the slides was larger than 2 cm, and in another pair the control was replaced because a micrometastasis was found on evaluation of the lymph nodes. The final analysis was based on 50 pairs with a maximum tumour diameter of 2 cm as measured from the slides and no (micro)metastases on evaluation of the lymph nodes. Follow up data and the type of operation—mastectomy or lumpectomy—were taken from the Rotterdam Cancer Registry. In the study period, tumours were not always graded and there was no agreement upon which grading system should be used. Tumour size was determined macroscopically in the pathology specimen and was sometimes estimated instead of being measured. Therefore, all original slides were reviewed by one pathologist without knowledge of the outcome.

All tissues were formalin fixed and paraffin wax embedded. Slides from tissue that had been frozen were not used in our analysis. The size of the tumours was taken from the microscope slides and tumours larger than 2 cm were excluded from our study. The tumour type was recorded as ductal not otherwise specified or as a special histological type—for example, infiltrating lobular carcinoma. A histological grade was attributed to all tumours, including special

Table 2 Univariate analysis of prognostic factors after pathological revision

Variable	Controls (n)	Cases (n)	p Value
Tumour size			
Continuous variable			0.0012
Tumour size			
⩽1 cm	15	7	
>1 and ⩽1.5	25	24	
>1.5 and ⩽2 cm	10	19	0.017
Tumour size			
<1 cm	10	3	
⩾1 cm	40	47	0.074
BR grade			
1	19	11	
2	19	19	
3	12	20	0.043
BR grade			
1 or 2	38	30	
3	12	20	0.133
MAI			
Continuous variable			0.0393
MAI			
<10	39	36	
⩾10	11	14	0.644
Mitotic score (BR)			
1	32	26	
2	7	10	
3	11	14	0.292
Nuclear pleomorphism score (BR)			
1	8	5	
2	27	17	
3	15	28	0.020
Tubule formation score (BR)			
1	9	3	
2	10	9	
3	31	38	0.063
Tumour type			
IDC	41	43	
ILC	5	5	
Other	4	2	0.780
Multifocal			
No	46	45	
Yes	4	5	1.000
Vessel invasion			
No	49	40	
Yes	1	10	0.008
Extensive DCIS			
No	40	36	
Yes	10	14	0.482
DCIS grade			
0	40	36	0.074 (3 versus 0,
1 or 2	8	5	1, 2: 0.051 Fisher)
3	2	9	

BR, Bloom and Richardson; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; MAI, mitotic activity index.

histological types. If the pathology report noted that there was more than one tumour, this was recorded and the largest tumour was graded. The data from the pathology report were insufficient to make a distinction between multifocal and multicentric tumours. For all tumours the scores for tubule formation, nuclear pleomorphism, and the MAI were recorded and the final grade defined by the Nottingham modification of the Bloom and Richardson system.[7 12] In addition, the number of mitotic figures was recorded. Mitotic figures were counted in eight randomly selected high power fields (final magnification, ×400; field diameter, 0.5 mm) at the periphery of the tumour, and care was taken to avoid counting apoptotic figures. Vascular invasion was recorded as present or not present. Vascular invasion had to be outside the main tumour in spaces with endothelial lining and was mostly near small muscular blood vessels. The presence of ductal carcinoma in situ (DCIS) was recorded if 10 or more ducts were affected. In addition, DCIS was graded as described previously,[13] the presence or absence of comedo necrosis was recorded, and the presence or absence of calcifications in DCIS was also recorded. The number of lymph nodes was taken from the pathology report; all nodes were re-examined for the presence of metastasis and if present the patient was excluded from our study.

Univariate analysis was performed using χ^2 statistics. The Mantel-Haenszel χ^2 test was used for ordinal variables, the Kruskal-Wallis test for continuous variables, and the Fisher exact test for comparisons involving small cell numbers. Multivariate analysis was performed using conditional logistic regression. Only variables significantly improving the fit of the model ($p < 0.05$) were included in the final model. Odds ratios were calculated together with 95% confidence interval and represent the relative risk compared with the reference category.

RESULTS

We compared the data registered in the Rotterdam Cancer Registry of patients with distant metastases (cases) with controls—those patients without distant metastases (table 1). There were no significant differences between cases and controls for age at the time of diagnosis, side of involvement, tumour grade, tumour size, type of surgery, radiotherapy, method of detection, and incidence period. In this database, tumour grade and size are taken from the original pathology report. The median follow up time of the cases until distant metastasis was 3.7 years, the median follow up time of the controls was 6.6 years.

Table 2 shows the results of the univariate analysis of the pathological revision. Data were analysed in several ways for reasons of comparison with published data or the Dutch guideline for adjuvant treatment. Cases had significantly larger tumours than controls (table 2), whether size was analysed as a continuous variable or whether the data were subdivided into three or two groups. The Bloom and Richardson tumour grade was significantly different when analysed in three groups, as is customary, with cases having a higher grade. However, when grade 1 and 2 were collapsed into one grade, as is done in the Dutch guidelines, this resulted in no significant differences between cases and controls. The MAI was significantly different between groups when analysed as a continuous variable. However, when analysed in two groups (< 10 and $\geqslant 10$, as in the Dutch guidelines) or three groups (as in the mitotic score of the Nottingham modification of the Bloom and Richardson grading system), no significant differences were found. Significant differences were found for the nuclear pleomorphism score, with cases more often being high grade, but not for the tubule formation score. No significant differences were seen for histological tumour type or multifocal tumours. Vessel invasion was significantly different between cases and controls. There were no differences in extensive DCIS or DCIS grade between groups.

Table 3 shows the results of the multivariate analysis of data from the pathological revision, and only significant data are shown. As can be seen, only tumour size and vessel invasion were retained from the results in table 2.

The numbers of lymph nodes recovered from the axillary dissection were not different between cases and controls. For both groups the median number was 11 (range, 1–24), and the mean number was 10.9 for the cases and 11.3 for controls. In four cases and four controls the number of lymph nodes was five or less and in 18 cases and 17 controls the number was 13 or more.

The data from the pathological review were used to see which patients would have been selected for adjuvant treatment according to the Dutch guidelines (table 4). In these guidelines, adjuvant treatment is recommended for patients whose T1 tumours are 1 cm or larger, with a Bloom and Richardson grade 3 and/or MAI $\geqslant 10$. The selection of patients for adjuvant treatment based on Bloom and Richardson tumour grade was superior to selection based on MAI. Combining grade and MAI did not improve the selection of cases and selected more controls.

Because several authors have reported that prognostic factors can be time dependent, a separate analysis was performed for prognostic factors in cases. Cases were divided into two groups—those with early distant metastasis and those with late distant metastasis—and differences in prognostic factors were tested. Early metastasis was defined as distant metastasis occurring before the median time of 3.7 years, and late metastasis as metastasis occurring after 3.7 years. Table 5 shows the results of this analysis. There were striking differences between early and late distant metastasis for the Bloom and Richardson grade, whether analysed in three grades, as is conventional, or after collapsing grade 1 and 2 into one grade, as is done in the Dutch guidelines. High grade tumours were associated with early distant metastasis. Significant differences between early and late metastasis were seen for nuclear pleomorphism and to some extent for the mitotic score, but not for MAI. Interestingly, the type of tumour was also significantly different in this analysis, with the special types of tumour in the group of late distant metastasis. Other factors were not significantly different between the two groups.

Table 3 Multivariate analysis of pathological revision data

Variable	OR	95% CI
Vessel invasion		
No	1	
Yes	8.21	(1.015 to 66.417)
Tumour size		
For each 1 cm increase	5.35	(1.390 to 20.625)

CI, confidence interval; OR, odds ratio.

Table 4 Adjuvant treatment according to Dutch guidelines

Patient selection	Controls (n)	Cases (n)
Tumour size ⩾1 cm and BR grade 3 or MAI ⩾10	13	20
Tumour size ⩾1 cm and BR grade 3	11	20
Tumour size ⩾1 cm and MAI ⩾10	11	14

BR, Bloom and Richardson; MAI, mitotic activity index.

Table 5 Early versus late distant metastasis in cases, median 3.7 years

Variable	Early	Late	p Value
Tumour size			
⩽1 cm	2	5	
>1 and ⩽1.5	13	11	
>1.5 and ⩽2	10	9	0.547
Tumour size			
<1 cm	0	3	
⩾1 cm	25	22	0.235
BR grade			
1	2	9	
2	8	11	
3	15	5	0.008
BR grade			
1/2	10	20	
3	15	5	0.009
Mitotic score (BR)			
1	8	18	
2	7	3	
3	10	4	0.025
MAI			
<10	15	21	
⩾10	10	4	0.115
Pleomorphism score (BR)			
1	0	5	
2	6	11	
3	19	9	0.006
Tubule formation score (BR)			
1	2	1	
2	3	6	
3	20	18	0.588
Tumour type			
IDC	25	18	
ILC	0	5	
Other	0	2	0.009
Multifocal			
No	23	22	
Yes	2	3	1.000
Vessel invasion			
No	19	21	
Yes	6	4	0.725
DCIS extensive			
No	17	19	
Yes	8	6	0.753
DCIS grade			
0	17	19	
1 of 2	2	3	
3	6	3	0.648
Screen detected			
No	6	5	
Yes	19	20	1.000
Type of surgery			
Amputation	11	14	
Lumpectomy	14	11	0.572
Radiotherapy			
Yes	14	12	
No	11	13	0.777
Side			
Left	14	9	
Right	11	16	0.256
Age			
<50	10	6	
50–69	13	11	
70+	2	8	0.109
Incidence period			
1988–91	4	8	
1992–7	21	17	0.321

BR, Bloom and Richardson; DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; MAI, mitotic activity index.

DISCUSSION

Nowadays, patients with stage I breast cancer constitute a large group of patients with good prognosis. Nevertheless, adjuvant treatment is often considered in these patients, and several prognostic factors are used to select those who might benefit from this treatment. Our present study confirms the importance of several prognostic factors that can all be derived from a simple haematoxylin and eosin stained slide. In univariate analysis, we found significant differences for tumour size, Bloom and Richardson grade, nuclear pleomorphism, the number of mitotic figures, and vessel invasion, as has also been found by several other authors (recently reviewed by Mirza and colleagues[5]). In our present study, differences in disease free survival (DFS) cannot be explained by under staging because the number of lymph nodes examined was the same for both groups. Like others,[14] we could not confirm a disadvantage for a high number of negative lymph nodes, as found by Camp *et al*.[15]

Tumour size is a well established prognostic factor, and here we confirm a difference with respect to DFS between small and large T1 tumours, even using multivariate analysis. Others have made similar observations in T1 tumours with respect to DFS,[1 16–20] and also with respect to overall survival.[16 17 21]

The importance of tumour grade is more heavily debated.[22 23] Grade, using different criteria, as in our present study, has been reported as a prognostic factor in stage I carcinoma.[16 24] Grading has been made more reproducible and consistent by the Nottingham modification of the Bloom and Richardson grading system.[12] The reproducibility of this grading system has been tested and is acceptable,[25–27] and seems to have a prognostic value with respect to DFS and death in small node negative cancers.[1 3 19–22 27 28] However, some studies do not report a prognostic effect of grade in node negative tumours.[29] In univariate analysis of pathological prognostic factors we also found a significant difference for tumour grade, but this was lost in multivariate analysis, as was the case in other studies.[21 27] Studies that did retain grade after multivariate analysis differ from our study by including a smaller number of prognostic factors,[1] or by including only early relapses and no late relapses.[28]

> "Selection on the basis of size and tumour grade is superior to selection on the basis of size and mitotic activity index, which is thought to be equivalent"

In our study, significance on univariate analysis of the number of mitotic figures is dependent on how the data are represented, which may explain some of the apparently conflicting results in the literature: it is significant when analysed as a continuous variable, whereas it is not significant when analysed as a discrete variable, whether it is MAI ⩾ 10 versus < 10 or whether there are three mitotic count score groups. These findings are in accordance with other reports,[1 27 28] although yet other studies have found significant prognostic value in the analysis of non-continuous data.[21 30–33] These differences can be partly explained by the use of different thresholds for the number of mitotic figures, which were reported as between 0.2 and 30/10 high power fields, defining two and sometimes three groups of patients.[21 30 32 34] Another problem was the unbalanced distribution of cases according to the mitotic score, which adds to the difficulty of establishing a threshold.[32] This illustrates that counting mitotic figures is not as simple as it seems.[9] Nevertheless, a threshold value is needed if the mitotic activity is used in making therapeutic decisions.

Nuclear grade and tubule formation have been reported in the past as prognostic factors in stage I carcinoma.[35] In our present study, we confirmed the prognostic value of nuclear grade, but not of tubule formation. However, nuclear grade was lost as a prognostic factor in multivariate analysis, as has been found by others.[21] Genestie *et al* were also unable to confirm the prognostic value of these factors.[32] The methods of scoring both nuclear grade and tubule formation have been criticised as poorly reproducible.[36 37]

The Nottingham modification of the Bloom and Richardson grading system is used for usual ductal carcinoma and also for special types of breast cancer, such as invasive lobular carcinoma, tubular carcinoma, and colloid carcinoma. Nevertheless, some studies show a better prognosis for some of these special types.[16 38] We were unable to confirm these findings, probably because of the low number of special tumour types included in our study. Tumour type did not make a difference for early or late distant metastases.

The presence of DCIS is mostly studied as a prognostic factor for local recurrence. Some authors suggest that high grade DCIS with casting-type calcifications is also a prognostic factor for distant recurrence.[39 40] Tabar *et al* made this suggestion based on the presence of casting-type calcifications on mammography, without correlating these findings with the pathological slides.[39] The study of Zunzubegui *et al* included only 15 patients with high grade DCIS, precluding further analysis of their findings.[40] We were unable to confirm the prognostic value of high grade DCIS for DFS, as were Quiet *et al*.[4]

In our present study, vessel invasion is an important prognostic factor for DFS and it was still significant in multivariate analysis. Similar observations have been made by others in node negative breast cancer with respect to DFS,[4 16 17 20 28 41 42] and also for overall survival.[17 21 41–43] In the study of Rosen *et al*, the prognosis of patients with T1N0 disease and lymph vessel invasion was worse than that of patients with T1N1 disease, and they suggested that these patients should receive AST.[17] Lauria *et al* also found the relative risk of death for lymph vessel invasion in node negative patients sufficiently high to suggest that these patients should receive adjuvant treatment.[43] Vessel invasion is not always retained on multivariate analysis,[28] and in some studies is not a prognostic factor.[3]

Prognostic factors are used in the selection of patients, including patients with stage I disease, for AST and/or hormonal treatment. We applied the selection criteria put forward in recent Dutch guidelines for AST to our study to see which patients would have been selected.[6] Our results show that selection on the basis of size and tumour grade is superior to selection on the basis of size and MAI, which is thought to be equivalent. However, it can also be seen that the selection is not perfect because 30 of the 50 patients with distant recurrence would not have been selected for adjuvant treatment. In addition, 11 of the 50 control patients without distant recurrence would have been selected for AST. Offering adjuvant treatment to patients with a tumour size $\geqslant$ 1 cm, as has been suggested,[11] would select 47 of the 50 patients with distant metastases, but also 40 of the 50 patients without distant metastasis (table 2). It has to be remembered that we performed a case–control study, and that in the original population only 66 of the 561 patients with stage I disease had a distant recurrence, whereas 495 patients did not have a distant recurrence. This shows that the selection criteria for AST are still imperfect. A change in the threshold of the grade at which adjuvant treatment is offered was suggested as a solution to this problem.[26] However, in our study the overlap in grading values between patients with a recurrence and those without is considerable, which suggests that this is not an attractive solution. It has been suggested in the past that patients with node negative breast cancer 1.1–2.0 cm in size with lymphatic tumour emboli should receive AST.[16 43] This prognostic factor has been included in the Canadian guidelines for adjuvant treatment in node negative cancer.[10] Our data on vessel invasion support this strategy.

In a separate analysis of patients with distant metastases we used the median time of 3.7 years to distant metastasis to divide this group into cases with early and late metastasis.

Take home messages

- Tumour size and vessel invasion are the best prognostic factors for disease free survival in patients with stage I breast cancer
- The Dutch selection criteria for adjuvant systemic treatment for these patients need to be improved
- Some prognostic factors are time dependent, making their use as selection criteria for adjuvant systemic treatment more complicated

Comparison of these groups for Bloom and Richardson grade, mitotic index, and nuclear grade showed a highly significant difference, with grade 3, mitotic score 3, and nuclear grade 3 being associated with early distant metastasis. This suggests that these factors have a prognostic value depending on the duration of disease follow up. A similar observation was made by Page *et al* for tumour grade and mitotic index.[27] Interestingly, the risk of recurrence of breast cancer is also time dependent, with two peaks—an early peak at about 18 months after surgery and a second peak at about 60 months.[44] It has also been suggested that AST is effective in preventing some early recurrences, but is not effective in preventing late recurrences.[45]

In conclusion, our present study shows that tumour size and vessel invasion are the best prognostic factors for DFS in patients with stage I breast cancer. It also demonstrates that the selection criteria for AST for these patients need to be improved. In addition, we show that some prognostic factors are time-dependent, which makes the use of these factors as selection criteria for AST more complicated.

Authors' affiliations

P J Westenend, Pathologisch Laboratorium voor Dordrecht eo, Laan van Londen 1800, 3315 HG Dordrecht, The Netherlands
C J C Meurs, R A M Damhuis, Rotterdam Cancer Registry, PO Box 289, 3000 AG Rotterdam, The Netherlands

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