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The prognostic value of tumour-stroma ratio in triple-negative breast cancer

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Abstract

Background: Triple-negative cancer constitutes one of the most challenging groups of breast cancer given its aggressive clinical behaviour, poor outcome and lack of targeted therapy. Until now, profiling techniques have not been able to distinguish between patients with a good and poor outcome. Recent studies on tumour-stroma, found it to play an important role in tumour growth and progression.
Objective: To evaluate the prognostic value of the tumour-stroma ratio (TSR) in triple-negative breast cancer.
Methods: One hundred twenty four consecutive triple-negative breast cancer patients treated in our hospital were selected and evaluated. For each patient the Haematoxylin-Eosin (H&E) stained histological sections were evaluated for percentage of stroma. Patients with less than 50% stroma were classified as stroma-low and patients with ≥50% stroma were classified as stroma-high.
Results: Of 124 triple-negative breast cancer patients, 40% had a stroma-high and 60% had a stroma-low tumour. TSR was assessed by two investigators (kappa 0.74). The 5-years relapse-free period (RFP) and overall survival (OS) were 85% and 89% in the stroma-low and 45% and 65% in the stroma-high group. In a multivariate cox-regression analysis, stroma amount remained an independent prognostic variable for RFP (HR 2.39; 95% CI 1.07–5.29; $p = 0.033$) and OS (HR 3.00; 95% CI 1.08–8.32; 0.034).
Conclusion: TSR is a strong independent prognostic variable in triple-negative breast cancer. It is simple to determine, reproducible and can be easily incorporated into routine histological examination. This parameter can help optimize risk stratification and might lead to future targeted therapies.

Keywords: Triple-negative breast cancer; Tumour-stroma; Survival; Prognostic value

Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide with 1.4 million new cases and 458 400 deaths in 2008.[1] While these numbers are alarming, we must keep in mind that breast cancer is a heterogeneous disease encompassing an extraordinarily diverse group of diseases in terms of presentation, morphology, biology, clinical behaviour and response to therapy.[2] Focussing on treatment options, breast cancer patients fall into three main groups: 1) patients with hormone-receptor positive tumours (the Luminal A and B); 2) the Her2 positive patients; and 3) those patients with hormone-receptor negative breast cancer. Worse outcomes are traditionally seen among woman with triple-negative breast cancer, which accounts for 10–17% of all breast carcinomas.[3–12] They primarily affect younger women, are more prevalent in African-American women, often present as interval cancers and are significantly more aggressive than tumours of the other molecular subtypes.[3–5,13] The peak risk of recurrence is between the first and third year following therapy and the

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majority of deaths occur in the first 5 years following therapy.[4] Triple-negative cancer represents one of the most challenging groups of breast cancer that currently lacks the benefit of a targeted therapy.[11] Molecular profiling techniques and prognostic algorithms, like Adjuvant Online, are unable to distinguish patients with low and high risk profiles.[8,14–16] In an attempt to make a more accurate assessment, we focused on the complex tumour microenvironment.

Recently, evidence suggests that the tumour-associated stroma and cancer-associated fibroblasts (CAF) may play an important role in tumour growth, angiogenesis and progression.[17–19] Stroma is the connective tissue that supports the deeper layer of breast tissue and if normal can in fact be protective in delaying or preventing tumour formation. In case of an invasive carcinoma, the epithelium has changed genetically, and as a result the stromal changes creating a permissive and supportive environment for tumour growth.[20,21] In more advanced stages the reactive stroma even stimulates invasion and metastases which inevitably results in diminished overall survival and relapse-free period.[22]

The amount of stroma has only recently been linked to a worse prognosis in cancer in a few studies. In a series of 122 colon cancer patients, the carcinoma-stromal composition appeared to be an independent prognostic variable. Patients with a high percentage of stroma had a worse overall survival and disease free period.[23,24] In a subsequent investigation of adenocarcinoma of the oesophagus[25] and in breast cancer patients[26] tumour-stroma ratio (TSR) also proved to be a significant prognostic variable.

The purpose of this study was to determine whether the amount of stroma is of prognostic value in triple-negative breast cancer. If the stromal component is indeed of prognostic value in this subgroup of breast cancer patients, it will not only be a candidate parameter for prognostification, but might also lead to the subsequent development of therapies targeting the stromal components.

Methods

Patient enrolment

This was a retrospective cohort study. During the period of January 2004–2008 all patients with triple-negative primary breast cancer who underwent surgery at the Hospital Group Twente, location Almelo and Hengelo, were selected. Hormone status was retrieved from the original patient files. Expression of oestrogen (ER), progesterone (PR) and human epidermal growth factor receptor 2 gene (HER2) were pre-determined by immunohistochemistry on formalin-fixed paraffin-embedded tumour material according to standard diagnostic procedure. Patients treated with neo-adjuvant therapy were excluded, since accurate evaluation of the tumour-stroma ratio was not possible in the final pathology. In case of known distant metastases at the time of surgery or recurrence within one month and patients with other malignancies at the time of presentation were also excluded.

Histopathology

The H&E stained sections from the primary tumour in the surgical specimen of all patients, were retrieved from the Pathology Laboratory East Netherlands. All samples were handled in a coded fashion, according to the National ethical guidelines ('Code for Proper Secondary Use of Human Tissue', Dutch federation of Medical Scientific Societies).

All pathological specimens were independently scored by two investigators [Moorman; Vink], who were not aware of the status of the patient. Slides from 128 resected tumours, varying from 1 section to up to 20 sections per tumour, were evaluated. The amount of stroma was quantified using a 5× objective lens to select the most invasive part of the tumour, then the 10× objective lens was used to score. Only fields were scored where both stroma and tumour cells were present, tumour cells had to be seen on all sides of the microscopic image field. In case of tumour heterogeneity, those areas with the highest stromal percentage were decisive (see Fig. 1).

Follow-up

Follow-up data was collected until March 2011. Overall survival (OS) was defined as the time between primary surgery and death or last follow-up. Relapse-free survival (RFS) was defined as the time between primary surgery and the first recurrence, metastases, death or until date of last follow-up.

Statistics

Statistical analysis was performed using SPSS software version 17.0. The stroma was scored per tenfold percentage. A 50% cut-off was used as previously determined in colon and breast cancer by maximum discriminative power, which was also confirmed in our breast cancer population.[24,26] Stroma-low was defined as $<50\%$ stroma, and stroma-high as $\geq 50\%$ stroma. The relationship between TSR (high versus low) and categorical data was assessed using the chi-square test or Fisher's exact test and the *T*-test or Mann–Whitney *U* test was used for continuous variables, depending on the distribution of the data. Variables included in multivariate analysis were the variables both related to TSR and to the outcome under investigation (both $p \leq 0.15$). Interobserver variability was analyzed using Cohen's kappa coefficient.

Analysis of the survival curves was performed using the Kaplan–Meier method and differences in survival distribution were tested with the Log Rank Statistic. The Cox proportional hazards model was used to determine the hazard

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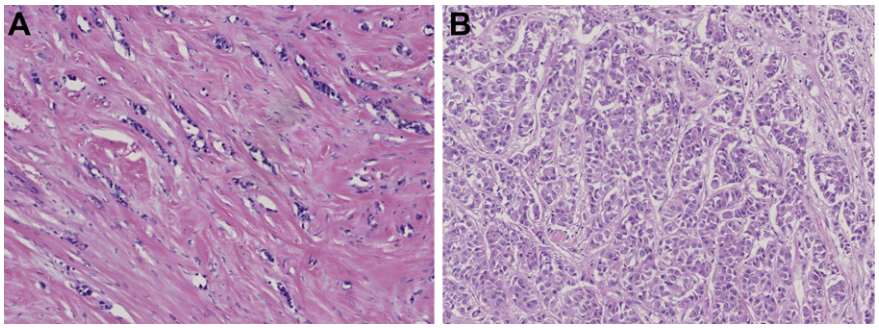

Figure 1. H&E stained sections of primary breast tumours. (A) tumour with large amounts of stroma, estimated as 80% with 10× objective; (B) tumour with low amount of stroma (30% with 10× objective).

ratio (HR) of explanatory variables on overall survival and relapse-free period. The results are given as hazard ratios with the 95% confidence interval (CI). *P*-values < 0.05 were considered statistically significant.

Results

Patient demographics

One hundred twenty four consecutive triple-negative breast cancer patients were selected for this study. The mean age of patients at the time of surgery was 56 years (range 23–87). The median time of follow-up was 37 months (4–84 months). A total of 25 patients died during this study. Nine patients were still alive with disease at the time of last follow-up. Patient and tumour characteristics are listed in Table 1.

Histopathology

TSR was assessed by two investigators. In 12 cases (9.5%) there was no agreement in TSR at first individual assessment (kappa 0.74; 90% concordance in classification): after re-evaluation by both investigators together there was total agreement.

Correlations TSR with prognosis

Fifty patients (40%) were classified as stroma-high (≥50% stroma) and 74 patients (60%) were classified as stroma-low (<50% stroma). In the stroma-low group 13.5% of patients (10/74) had a relapse and 9.5% (7/74) died of breast cancer during follow-up. In the stroma-high group 40% (20/50) had a relapse and 28.0% (14/50) died of metastasized disease following a relapse (both $p \leq 0.006$). Treatment and outcome characteristics are shown in Table 1. The 5-years RFP and OS were 85% and 89%, respectively, in the stroma-low group and 45% and 65%, respectively in the stroma-high group. Survival analyses showed that stroma-high patients had a significantly worse RFP (HR 2.93; 95% CI 1.37–6.26; $p = 0.004$) and OS (HR 2.56; 95% CI 1.03–6.35; $p = 0.035$) compared to stroma-low patients (Fig. 2).

Table 1
Patient, tumour, treatment and outcome characteristics grouped by tumour-stroma ratio (TSR).

	Stroma <50% ($n = 74$) No. pt. (%)	Stroma ≥50% ($n = 50$) No. pt. (%)	Chi-square test *P*-value
Age (y)			
<50	31 (41.9)	13 (26.0)	0.070
≥50	43 (58.1)	37 (74.0)	
Palpable tumour			
No	16 (21.6)	11 (22.0)	0.991
Yes	57 (77.0)	39 (78.0)	
Unknown	1 (1.4)	0 (0.0)	
Operation type			
Breast conserving surgery	38 (51.4)	22 (44.0)	0.422
Total mastectomy	36 (48.6)	28 (56.0)	
Histological type			
IDC	65 (87.8)	42 (84.0)	0.431
ILC	2 (2.7)	4 (7.8)	
Others	7 (9.3)	4 (7.8)	
Pathologic tumour stage			
pT1	32 (43.2)	17 (34.0)	0.515
pT2	37 (50.0)	30 (60.0)	
pT3 of 4	5 (6.8)	2 (4.0)	
Unknown	0 (0.0)	1 (2.0)	
Pathologic tumour grade			
1 (well)	0 (0.0)	2 (4.0)	0.024
2 (moderate)	10 (13.5)	13 (26.0)	
3 (poorly)	64 (86.5)	34 (68.0)	
Unknown	0 (0.0)	1 (2.0)	
Nodal status			
pN0	52 (70.3)	26 (52.0)	0.075
pN1	19 (25.7)	18 (36.0)	
pN2 or 3	3 (4.1)	6 (12.0)	
Family history			
Negative	8 (10.8)	12 (25.0)	0.100
Positive	22 (29.7)	10 (20.8)	
Unknown	44 (59.5)	26 (54.2)	
Extracapsular extension			
No	53 (96.4)	32 (88.9)	0.209
Yes	2 (3.6)	4 (11.1)	
Multifocality			
No	70 (94.6)	39 (78.0)	0.005
Yes	4 (5.4)	11 (22.0)	
Lymphovascular invasion (LVI)			
No	63 (85.1)	28 (56.0)	≤0.001
Yes	11 (14.9)	22 (44.0)	
Tumor free margin			
No	72 (97.3)	47 (94.0)	0.392
Yes	2 (2.7)	3 (6.0)	

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Table 1 (*continued*)

	Stroma <50% ($n = 74$) No. pt. (%)	Stroma ≥50% ($n = 50$) No. pt. (%)	Chi-square test *P*-value
Presence Ductal carcinoma in situ			
No	47 (63.5)	23 (46.0)	0.054
Yes	27 (36.5)	27 (54.0)	
Postmenopausal			
No	28 (38.4)	13 (27.1)	0.225
Yes	42 (57.5)	32 (66.7)	
Unknown	3 (4.1)	3 (6.3)	
Necrosis			
Absent	21 (28.4)	25 (50.0)	0.004
<30% necrosis	36 (48.6)	23 (46.0)	
≥30% necrosis	17 (23.0)	2 (4.0)	
Mitotic activity index (MAI)			
0–19/2 mm^2	27 (37.0)	22 (46.8)	0.059
20–39/2 mm^2	27 (37.0)	21 (44.7)	
>39/2 mm^2	19 (26.0)	4 (8.5)	
Local therapy			
BCS − Radiotherapy	3 (4.1)	5 (10.0)	0.278
BCS + Radiotherapy	34 (45.9)	17 (34.0)	
MST − Radiotherapy	29 (39.2)	10 (20.0)	
MST + Radiotherapy	7 (9.9)	1 (2.0)	
Chemotherapy			
No	24 (32.4)	18 (36.0)	0.720
Yes	49 (66.2)	32 (64.0)	
Unknown	1 (1.4)	0 (0.0)	
Event relapse			
No	64 (86.5)	30 (60.0)	0.001
Yes	10 (13.5)	20 (40.0)	
Death of disease			
No	67 (90.5)	36 (72.0)	0.006
Yes	7 (9.5)	14 (28.0)	

Abbreviations: Event relapse defined as recurrence, distant metastasis or death. BCS: breast conserving therapy; MST: mastectomy.

Age, nodal status, family history, multifocality, Lymphovascular invasion (LVI), ductal carcinoma in situ, necrosis and Mitotic Activity Index (MAI) were all related to TSR (all $p < 0.15$; see Table 1). In univariate Cox-regression analysis, nodal status, multifocality and LVI were also significantly related to RFP, as were nodal status, multifocality and necrosis to OS (all $p < 0.15$; see Table 2). In a multivariate Cox-regression analysis TSR remained an independent prognostic variable for both RFP (HR 2.39; 95% CI 1.07–5.29; $p = 0.033$) and OS (HR 3.00; 95% CI 1.08–8.32; $p = 0.034$). Multifocality remained an independent prognostic variable for RFP. For overall survival, nodal status and the presence of necrosis were independent prognostic variables (see Table 2). Multifocality showed a trend towards a worse survival, but was not significant in OS.

Strength of tumour-stroma ratio

The strength of the TSR is best illustrated, when compared to the routinely used variables for treatment strategies nowadays like multifocality, LVI and necrosis. The hazard ratios are respectively 2.39 (95% CI; 1.37–6.26) for stroma-high versus stroma-low, 2.18 (95% CI; 1.06–4.48) for nodal status pN1+ versus pN0, 2.10 (95% CI 0.89–4.91) for tumour size pT ≥ 2 versus pT1 and 0.53 (95% CI 0.70–3.91) for tumour grade 2 or 3 versus tumour grade 1 for RFP. Similarly, for OS the HR for stroma-high is 2.56 (1.03–6.30), for nodal status 2.87 (1.17–7.05), 1.54 for tumour size (0.59–4.03) and 0.31 (0.04–2.33) for tumour grade (Fig. 3).

Effect modification of chemotherapy

We formally investigated effect modification by chemotherapy in a multivariate cox regression with TSR, chemo and the interaction term TSR*chemo. The interaction was not statistically significant ($p = 0.32$).

Discussion

Our study shows that tumour-stroma ratio is an independent prognostic variable for patients with triple-negative breast cancer. Patients with a stroma-high tumour had a significantly worse relapse-free period (RFP) and overall survival (OS) in comparison with patients with stroma-low tumours. These results correspond to those found in other studies that investigated the TSR in cancer patients. Just

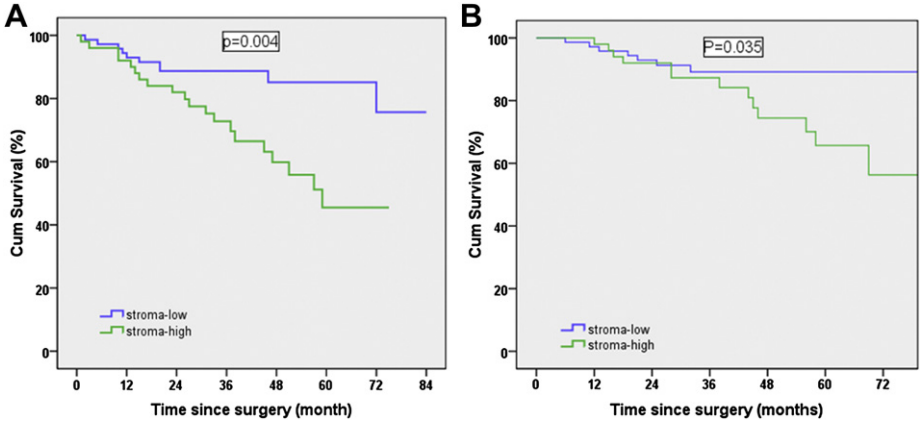

Figure 2. Kaplan–Meier curves for tumour-stroma ratio. Patients with stroma-high showed a significant worse relapse-free period, RFP (A) and overall survival, OS (B).

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Table 2
Univariate cox-regression analysis for RFP and OS for the variables significantly related to tumor stroma ratio ($p < 0.15$), and below the multivariate cox-regression analysis.

	Relapse-free period (RFP)			Overall survival (OS)		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Univariate analysis						
TSR						
Stroma-low	1.00			1.00		
Stroma-high	2.93	1.37–6.26	0.004	2.56	1.03–6.35	0.035
Age						
≤50 years	1.00			1.00		
>50 years	1.72	0.76–3.87	0.182	1.79	0.65–4.92	0.253
pN-status			0.006			0.011
pN0	1.00			1.00		
pN1	1.43	0.68–3.01	0.338	2.09	0.86–5.07	0.092
pN2/3	3.38	1.27–9.00	0.010	2.62	0.76–9.00	0.111
Family history						
Negative	1.00			1.00		
Positive	1.06	0.96–1.16	0.210	1.08	0.95–1.22	0.186
Multifocality						
No	1.00			1.00		
Yes	3.39	1.54–7.47	0.001	3.16	1.25–8.00	0.011
LVI						
No	1.00			1.00		
Yes	2.461	1.19–5.07	0.012	1.57	0.62–3.95	0.326
In situ comp						
No	1.00			1.00		
Yes	1.34	0.65–2.75	0.416	1.45	0.60–3.51	0.401
Necrosis			0.726			0.142
Absent	1.00			1.00		
<30% necrosis	0.648	0.31–1.34	0.242	0.494	0.19–1.22	0.121
≥30% necrosis	1.28	0.49–3.36	0.608	2.35	0.85–6.51	0.088
MAI			0.547			0.769
0–19/2 mm^2	1.00			1.00		
20–39/2 mm^2	0.842	0.29–2.43	0.751	0.77	0.30–1.96	0.584
>39/2 mm^2	0.85	0.40–1.83	0.693	1.43	0.473–4.37	0.519
Multivariate analysis						
TSR						
≤50%	1.00			1.00		
>50%	2.39	1.07–5.29	0.033	3.00	1.08–8.32	0.034
Multifocality						
No	1.00					
Yes	2.47	1.08–5.66	0.032			
pN-status				1.00		0.054
pN1				3.22	1.13–9.14	0.028,
pN2 and pN3				4.24	0.97–18.56	0.055
Necrosis				1.00		0.016,
<30% necrosis				0.86	0.30–2.41	0.774,
≥30% necrosis				5.37	1.37–21.02	0.016

Abbreviations: HR: hazard ratio; CI: confidence interval; TSR: tumour-stroma ratio; pN: pathological nodal status; LVI: lymphovascular invasion; MAI: mitotic activity index.

recently, de Kruijf et al.[26] found TSR to be a significant prognostic variable for RFP (HR 1.87) in triple-negative breast cancers. Their results are similar to those found in our study. For stage I–II colon cancer, the TSR also discriminated between patients with a poor and a better outcome,[23] which was further validated in a subsequent study.[24] A comparable study in oesophageal cancer gave similar results, with a hazard ratio of 2.00 for overall survival and 1.55 for RFP of stroma-high tumours compared to stroma-low.[25]

Besides the TSR, multifocality also appeared to be an independent prognostic variable for RFP, as were nodal status and presence of necrosis for OS. Compared to these variables and others, TSR proved to be a strong indicator. Despite being a relatively new variable, which only recently has been studied in cancer patients, it seems promising.

Determination of tumour-stroma ratio

Determination of TSR proved to be a relatively quick and simple procedure that can easily be included in the routine pathological examination. It can be done on routine H&E sections without the necessity for further staining. The interobserver agreement kappa value was high (0.74). In the cases without agreement at first assessment, almost all involved tumours with extensive central sclerosis leaving little tumour margin to evaluate. Despite this, the kappa was still substantial. Other studies also prove that it is a reproducible method. For colorectal cancer, the interobserver agreement varied between 0.60 and 0.70,[23,24] in oesophageal cancer the kappa was 0.86[25] and in a previous breast cancer study the kappa was 0.85.[26] In addition to being quick, simple and reproducible, it does not lead to additional costs.[24]

Risk stratification

The purpose of performing a risk assessment in breast cancer patients, is to differentiate between patients with good and poor prognosis, ultimately allowing for optimal therapy decisions. Various classification systems are available nowadays to estimate the risk for locoregional relapse, distant metastasis and death in breast cancer patients. Most common are the Nottingham Prognostic Index,[27] the Sankt Gallen classification[28] and Adjuvant Online.[29] The last one is the most commonly used and has the advantage of giving an estimate of the survival benefit and prevention of relapse given the standard therapy.[30] The estimations have proven to be fairly accurate, except for certain subtypes including our own.[31] A recent phase II study on Poly ADP ribose polymerase (PARP) inhibitors reported significant improvements in response rate and RFP,[32] but no results are available from subsequent studies. To this day, there are no specific guidelines for triple-negative breast cancer. Since these tumours commonly have an aggressive clinical behaviour and lack the benefit of a targeted therapy, it is a subgroup of great interest. Since triple-negative breast cancer constitutes a heterogeneous group with different pathological and clinical features,[11] a better understanding of these features might enable us to better select patients for future specific therapies.

Tumour microenvironment

In order to understand the growth and progression of cancer, research has focused on the complex microenvironment of the tumour. One of the components of the microenvironment is the stroma, the connective tissue of the breast.[19,33–36] Stroma is

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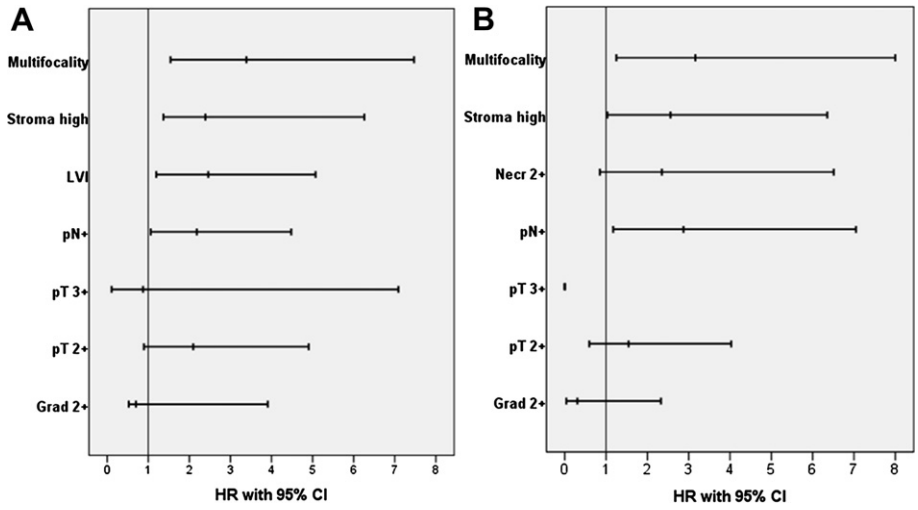

Figure 3. Strength of the tumour-stroma ratio, Multifocality and LVI/necrosis compared to the routinely used variables nodal status, tumour size and tumour grade on univariate cox-regression analysis for relapse-free period (A) and overall survival (B). Abbreviations: HR: hazard ratio; CI: confidence interval; LVI: lymphovascular invasion; Necr 2+: ≥30% tumour necrosis; pN+: positive nodes on pathology; pT3+: Tumour size ≥ 20 mm; pT2+: tumour size ≥ 10 mm; Grad 2+: tumour grade 2 or 3. *statistics for pT3 for overall survival were not possible, since no events occurred in that subgroup.

thought to promote tumour activity by multiple mechanisms including an increased number of fibroblasts, manipulation of the extracellular matrix, enhanced capillary density, recruitment of inflammatory cells, and alterations in stromal regulatory pathways.[37,38] The overall effect of these mechanisms is still not fully understood, but it strongly suggests that tissue architecture is a significant participant in tumour growth and progression. Especially the so-called cancer-associated fibroblasts (CAFs) were found to have a predominant role in tumour growth and progression.[38–40] This was also found in genetic mouse models, where the contribution of stromal fibroblasts led to tumour initiation and progression.[41,42] Another study investigated cultured primary breast epithelial cells in combination with stromal elements. The addition of the stromal elements caused the tumour to spread and become invasive, with a proportional effect on tumour growth with increasing concentrations of stromal elements.[37] Focussing on the stroma might therefore lead to better prognostication in cancer patients and provide new targets for therapy.

As far as we know, our study is the second performed in breast cancer patients, and in particular triple-negative breast cancer. More studies and larger study populations are necessary to further validate this parameter. Other variables, like growth pattern and lymphocytic infiltrate might also be of influence on the prognosis and TSR. Though, before these parameters can be properly evaluated, better definitions and assessment strategies are warranted. Current strategies are too vulnerable to subjectivity.

Conclusion

TSR is a strong independent prognostic variable in triple-negative breast cancer. Patients with a stroma-rich tumour were found to have an almost 2.5 fold increased chance of relapse or distant metastasis and a 3 fold increased chance of death when compared to patients with a stroma-low tumour. The TSR is easy to determine, reproducible and does not lead to additional costs. It can easily be incorporated into routine histological examination. This parameter can help optimize risk stratification in this subgroup of patients, where no definite prognostic parameters are available yet.

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Conflict of interest statement

All authors disclose to have no financial and personal relationships with other people or organisations that could inappropriately influence this study.

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