FULL PAPER

BJC

British Journal of Cancer (2014) 111, 157–165 | doi: 10.1038/bjc.2014.279

Keywords: tumour stroma; tumour microenvironment; breast cancer; survival

The relationship between the tumour stroma percentage, clinicopathological characteristics and outcome in patients with operable ductal breast cancer

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Background: The percentage of tumour stroma (TSP) has recently been reported to be a novel independent predictor of outcome in patients with a variety of common solid organ tumours. The aim of this study was to examine the relationship between TSP, clinicopathological characteristics and outcome in patients with invasive ductal breast cancer, in particular node negative and triple negative disease.

Methods: A total of 361 patients with primary operable invasive ductal breast cancer were included in this study. The TSP was assessed visually on the haematoxylin and eosin-stained tissue sections. With a cutoff value of 50% TSP, patients with $\leqslant$50% stroma were classified as the low-TSP group and those with $>$50% stroma were classified as the high-TSP group.

Results: A total of 109 (30%) patients had high TSP. Patients with high TSP were old age ($P=0.035$), had more Her-2-positive tumours ($P=0.029$), low-grade tumour inflammatory infiltrate ($P=0.034$), low CD68+ macrophage infiltrate ($P<0.001$), low CD4+ ($P=0.023$) and low CD8+ T-lymphocytes infiltrate ($P=0.017$), tumour recurrence ($P=0.015$) and shorter cancer-specific survival ($P<0.001$). In node-negative patients ($n=207$), high TSP was associated with low CD68+ macrophage infiltrate ($P=0.001$), low CD4+ ($P=0.040$) and low CD8+ T-lymphocytes infiltrate ($P=0.016$) and shorter cancer-specific survival ($P=0.005$). In triple negative patients ($n=151$), high TSP was associated with high tumour grade ($P=<0.001$), lymph node positivity ($P=0.027$), low CD68+ macrophage infiltrate ($P=0.011$) and shorter cancer-specific survival ($P=0.035$). The 15-year cancer-specific survival rate was 79% *vs* 21% in the low-TSP group *vs* high-TSP group. In multivariate survival analysis, a high TSP was associated with reduced cancer-specific survival in the whole cohort ($P=0.001$), node-negative patients ($P=0.007$) and those who received systemic adjuvant therapy ($P=0.021$), independent of other pathological characteristics including host inflammatory response. However, TSP was not an independent prognostic factor for triple negative patients ($P=0.151$).

Conclusions: A high TSP in primary operable invasive ductal breast cancer was associated with recurrence and poorer long-term survival. The inverse relation with the tumour inflammatory infiltrate highlights the importance of the amount of tumour stroma on immunological response in patients with primary operable ductal breast cancer. Implementing this simple and reproducible parameter in routine pathological examination may help optimise risk stratification in patients with invasive ductal breast cancer.

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Received 1 February 2014; revised 7 April 2014; accepted 30 April 2014; published online 29 May 2014

Breast cancer is the commonest cancer and the leading cause of cancer death in women. It accounts for approximately 10th of all new cancers and 25% of all female cancers (Ferlay *et al*, 2010). With the advent of a screening programme, more than 70% of newly diagnosed invasive tumours present without nodal involvement (Health and Consumer Protection Directorate-General, 2006) and those women have 88% 10-year disease-free survival rate (Mirza *et al*, 2002).

In recent years, the simple concept that tumour progression depends solely on the intrinsic properties of cancer cells has recently given way to a more complex paradigm in which tumour progression depends on the interaction between tumour and host cells (Colotta *et al*, 2009; Hanahan and Weinberg, 2011). Recent evidence suggests that the tumour stroma profoundly influences tumour growth, angiogenesis and dissemination. Tumour stroma is thought to promote tumourigenesis by different mechanisms including remodelling of the extracellular matrix, suppression of immune response and alterations in stromal regulatory pathways affecting the motility and aggressiveness of cancer cells (Kim *et al*, 2005; Hu and Polyak, 2008; Cirri and Chiarugi, 2012).

Recently, it has been reported that tumour stroma has prognostic value in patients with colorectal (Mesker *et al*, 2007; West *et al*, 2010; Huijbers *et al*, 2013, Park *et al*, 2014) and oesophageal cancers (Staal *et al*, 2010; Wang *et al*, 2012). Also, the percentage of tumour stroma has been recently reported to have prognostic value in patients with triple negative (de Kruijf *et al*, 2011; Moorman *et al*, 2012) and node-negative breast cancer (Dekker *et al*, 2013). However, the relationship with other important determinants of outcome such as the lymphovascular invasion, necrosis and tumour inflammatory cell infiltrate is unclear.

Therefore, the aim of this study was to examine the relationship between the percentage of tumour to stroma, host inflammatory response, clinicopathological characteristics and outcome in patients with early breast cancer, in particular node negative and triple negative disease.

MATERIALS AND METHODS

Patients. Patients presenting with primary operable invasive ductal breast cancer at Royal Infirmary, Western Infirmary and Stobhill Hospital, Glasgow, between 1995 and 1998 were studied ($n = 361$). Clinicopathological data including age, histological tumour type, grade, tumour size, lymph node status, type of surgery and use of adjuvant treatment (chemotherapy, hormonal therapy and/or radiotherapy) were retrieved from the routine reports. ER, PR and Her-2 status were performed as previously described and the results of visual scoring were used in this study (Mohammed *et al*, 2012a,b). Tumour necrosis, general inflammatory infiltrate, CD68 + macrophage infiltrate, CD4 + T-lymphocyte infiltrate, CD8 + T-lymphocyte infiltrate were performed as previously described (Ikpatt *et al*, 2002; Klintrup *et al*, 2005; Mohammed *et al*, 2012c,2013). Ki67 was performed as previously described (Mohammed *et al*, 2012d).

In the present study, the proportion of patients with ER-negative tumours was relatively high in the TMA. This enabled specific examination of the prognostic value of tumour characteristics in a sub-cohort of patients with ER-negative ductal breast. Also, the patients included in this study did not receive neoadjuvant therapy or adjuvant anti-HER-2 therapy. The inclusion of ductal breast cancers only was to limit the potential confounding effects of other tumour types on the analysis in the present study.

Patients were routinely followed up following surgery. Date and cause of death was cross-checked with the cancer registration system and the Registrar General (Scotland). Death records were complete until 31st of May 2013 and that served as the censor date. Cancer recurrence was measured from the date of primary surgery until the date of first recurrence of breast cancer. Cancer-specific survival was measured from the date of primary surgery until the date of death from breast cancer.

Institutional Review Board approval for the use of human tissue in this study was given by the Research Ethics Committee of the North Glasgow University Hospitals NHS Trust.

Slide scanning and assessment of tumour stroma. The haematoxylin and eosin tumour sections stained according to standard histological protocols were scanned using a Hamamatsu NanoZoomer (Welwyn Garden City, Hertfordshire, UK) at objective magnification × 20. Visualisation and image analysis assessment was carried out using Slidepath Digital Image Hub, version 4.0.1, (Slidepath, Leica Biosystems, Milton Keynes, UK). Visual assessment of TSP was performed on a high-definition monitor and was carried out at the most invasive tumour area according to previously described criteria (Mesker *et al*, 2007). As Slidepath provides different levels of magnification similar to a conventional microscope, the most invasive tumour area to be analysed was identified visually and selected using a × 4 or × 5 magnification. The magnification was then set to × 10 at the selected area where both stroma and tumour tissue were available. Tumour cells must be present at all borders of the image field (north–east–south–west) (Figure 1). When necrotic and mucinous tissue was present within the selected area, the mucinous tissue was visually excluded for the scoring. Scoring percentages were given per 10-fold (10, 20, 30% etc.).

Cutoff at 50% TSP was used as described in previous reports (Mesker *et al*, 2007; de Kruijf *et al*, 2011) i.e., low stroma tumours

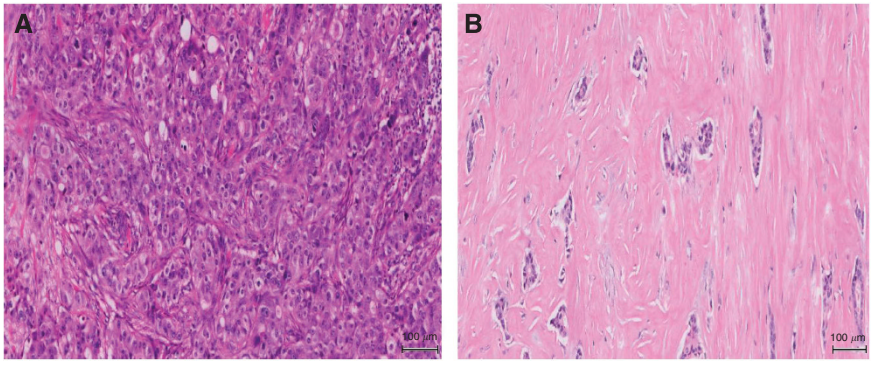

Figure 1. Haematoxylin and eosin-stained sections of invasive ductal breast tumours. (**A**) Tumour with low stroma (10%); (**B**) tumour with high stroma (80%). Magnificatiom × 10 objective and 100-μm scale.

were the presence of tumour stroma in ⩽50% of tumour area (Figure 1A), whereas high stroma tumours were the presence of tumour stroma in >50% of tumour area (Figure 1B).

A total of 40 specimens were independently estimated for TSP by two observers (FJAG and JE) blinded to the patient outcome and the other observer's score. The ICCC was 0.83 indicating excellent agreement. The author (FJAG) then scored the rest of slides.

Statistical analysis. Consistency between the observers was analysed using the ICCC value. Inter-relationships between variables were assessed using contingency table analysis with the χ^2 test for trend as appropriate. Univariate and multivariate survival analysis were performed using the Kaplan–Meier analysis and Cox proportional hazards model. A stepwise backward procedure was used to derive a final model of the variables that had a significant independent relationship with survival. All statistical analyses were two-sided and significance defined as *P*-value <0.05. Deaths up to May 2013 were included in the analysis. All statistical analysis was performed using the SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinicopathological characteristics. Table 1 shows clinicopathological characteristics of patients ($n=361$). The majority were older than 50 years (65%), had a grade III carcinoma (54%) equal or smaller than 2 cm (51%) with no axillary lymph node involvement (57%). The majority had ER-negative tumours (54%), PR-negative tumours (66%) and Her2-negative tumours (80%). A total of 30% had lymphovascular invasion and the majority had a high-grade tumour necrosis (64%). In total, 60% had low-grade general tumour inflammatory infiltrate with the cellular inflammatory infiltrates (CD68+ macrophage infiltrate, CD4+ T-lymphocyte infiltrate and CD8+ T-lymphocyte infiltrate) presented as tertiles (Table 1). In all, 81 (22%) patients received only hormonal therapy, 144 (40%) received only chemotherapy and 45 (13%) received both, and data on seven cases were not available.

The minimum follow-up of survivors was 142 months and the median follow-up was 168 months. During follow-up, 89 patients developed recurrence (25%), 172 patients died, 27% died of their cancer.

TSP. For all patients, TSP was evaluated on one section derived from the most invasive part of the tumour. The tumour specimens showed variety in TSP ranging from very solid tumours with little stromal involvement (Figure 1A) to tumours with large areas of stromal proliferation scattered with single and grouped tumour cells (Figure 1B). In total, 252 (70%) patients had low TSP (⩽50% stroma) and 109 (30%) patients had high TSP (>50% stroma). In node-negative patients, 153 (74%) patients had low TSP and 54 (26%) had high TSP. In triple negative patients, 115 (76%) patients had low TSP and 36 (24%) had high TSP.

Association of TSP with clinicopathological variables and outcome. The relationship between TSP, clinicopathological variables and host inflammatory response is shown in Tables 2–4. Patients with high TSP were old age women ($P=0.035$), had more Her-2-positive tumours ($P=0.029$), low-grade general inflammatory infiltrate ($P=0.034$), low CD68+ macrophage infiltrate ($P<0.001$), low CD4+ ($P=0.023$) and low CD8+ T-lymphocytes infiltrate ($P=0.017$), had tumour recurrence ($P=0.015$) and shorter cancer-specific survival ($P=0.001$). In node-negative patients ($n=207$), a high TSP was associated with low CD68+ macrophage infiltrate ($P=0.001$), low CD4+ ($P=0.040$) and low CD8+ T-lymphocytes infiltrate ($P=0.016$) and shorter cancer-specific survival ($P=0.005$). In triple negative patients ($n=151$), a high TSP was strongly associated with high tumour grade ($P=<0.001$), lymph node positivity ($P=0.027$), low CD68+ macrophage infiltrate ($P=0.011$), low CD4+ ($P=0.049$) and tended to have low CD8+ T-lymphocytes infiltrate ($P=0.071$) and shorter cancer-specific survival

Table 1. The clinicopathological characteristics of patients with early breast cancer ($n=361$)

Clinicopathological characteristics	Patients (*n*)
Age (⩽50/>50 years)	125 (35%) / 236 (65%)
Size (⩽20/21–50/>50 mm)	185 (51%) / 163 (45%) / 13 (4%)
Grade (I/II/III)	48 (13%) / 124 (34%) / 189 (52%)
Involved lymph node (0/1–3/>3)	207 (57%) / 120 (33%) / 34 (9%)
Oestrogen receptor status (no/yes)	194 (54%) / 167 (46%)
Progesterone status (no/yes)	238 (66%) / 123 (34%)
Her-2 status (no/yes)	290 (80%) / 71 (20%)
Lymphovascular invasion (no/yes)	251 (70%) / 110 (30%)
Tumour necrosis (no/yes)	131 (36%) / 230 (64%)
General inflammatory infiltrate (low/high)	215 (60%) / 146 (40%)
CD68+ macrophage infiltrate (tertiles)	82 (23%) / 115 (32%) / 103 (29%) / 61 (17%)[a]
CD4+ T-lymphocyte infiltrate (tertiles)	132 (37%) / 112 (31%) / 117 (32%)
CD8+ T-lymphocyte infiltrate (tertiles)	121 (34%) / 118 (33%) / 122 (34%)
Tumour stroma percentage (⩽50%/>50%)	252 (70%) / 109 (30%)
Loco-regional therapy (Lumpectomy+radiotherapy/mastectomy+radiotherapy)	130 (36%) / 231 (64%)
Systemic adjuvant therapy (hormonal/hormonal+chemotherapy/chempotherapy/none)	81 (22%) / 45 (13%) / 144 (40%) / 84 (23%) / 7 (2%)[a]
Tumour recurrence (no/yes)	272 (75%) / 89 (25%)
Alive/cancer death/non-cancer death	189 (52%) / 97 (27%) / 75 (21%)

[a]Number of patients when incomplete data available.

Table 2. The inter-relationship between clinicopathological characteristics and tumour stroma percentage in patients with invasive ductal breast cancer ($n = 361$)

	Tumour stroma percentage ⩽50 $n = 252$ (70%)	Tumour stroma percentage >50 $n = 109$ (30%)	*P*-value
Age (⩽50/>50 years)	96/156	29/80	0.035
Size (⩽20/21–50/>50 mm)	136/108/8	49/55/5	0.109
Grade (I/II/III)	35/77/140	13/47/49	0.289
Involved lymph node (0/1–3/>3)	153/78/21	54/42/13	0.052
Oestrogen receptor status (no/yes)	138/114	56/53	0.554
Progesterone status (no/yes)	168/84	70/39	0.653
Her-2 status (no/yes)	210/42	80/29	0.029
Lymphovascular invasion (no/yes)	180/72	71/38	0.234
Tumour necrosis (no/yes)	91/161	40/69	0.915
General inflammatory infiltrate (low/high)	141/111	74/35	0.034
CD68 + macrophage infiltrate (tertiles)	40/84/80	42/31/23	<0.001
CD4 + T-lymphocyte infiltrate (tertiles)	66/111/75	36/54/19	0.023
CD8 + T-lymphocyte infiltrate (tertiles)	71/80/101	73/46/26	0.017
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	93/159	37/72	0.591
Systemic adjuvant therapy (hormonal/hormonal + chemotherapy/chempotherapy/none)	55/35/97/61	30/21/35/23	0.104
Tumour recurrence (no/yes)	199/53	73/36	0.015
Alive/cancer death/non-cancer death	151/55/46	38/42/29	<0.001
Cancer-specific survival (months)[a]	176(168–186)	144(128–160)	<0.001

[a]Mean (95% confidence interval).

Table 3. The inter-relationship between clinicopathological characteristics and tumour stroma percentage in node-negative patients ($n = 207$)

	Tumour stroma percentage (⩽50) $n = 153$ (74%)	Tumour stroma percentage (>50) $n = 54$ (26%)	*P*-value
Age (⩽50/>50 years)	54/99	13/41	0.131
Size (⩽20/21–50/>50 mm)	90/60/3	30/23/1	0.709
Grade (I/II/III)	26/45/82	8/28/18	0.123
Oestrogen receptor status (no/yes)	84/69	26/28	0.394
Progesterone status (no/yes)	103/50	35/19	0.738
Her-2 status (no/yes)	128/25	41/13	0.208
Lymphovascular invasion (no/yes)	123/25	41/13	0.488
Tumour necrosis (no/yes)	62/91	21/33	0.488
General inflammatory infiltrate (low/high)	89/64	39/15	0.068
CD68 + macrophage infiltrate (tertiles)	27/49/46	24/13/10	0.001
CD4 + T-lymphocyte infiltrate (tertiles)	36/67/50	17/28/9	0.040
CD8 + T-lymphocyte infiltrate (tertiles)	41/52/60	22/20/12	0.016
Recurrence status (no/yes)	130/23	41/13	0.133
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	65/88	24/30	0.803
Systemic adjuvant therapy (hormonal/hormonal + chemotherapy/ chemotherapy/none)	35/12/55/49	15/8/16/15	0.251
Alive/cancer death/non-cancer death	102/20/31	124/35/48	0.002
Cancer-specific survival (months)[a]	192 (183–201)	164 (144–184)	0.005

[a]Mean (95% confidence interval).

($P=0.035$). A high TSP was not associated with hormonal status, tumour necrosis and lymphovascular invasion.

Sub-group analysis of the relationship between the TSP and Ki67 in different patient groups was performed (Table 5). Only 59% of patients from the whole cohort, 44% from node-negative group and 65% from triple negative group had Ki67 information available. There was no significant statistical difference between high-TSP and low-TSP groups in all sub-cohorts.

The 15-year cancer-specific survival rate was 79% *vs* 21% in the low-TSP group *vs* high-TSP group. Kaplan–Meier survival curves show that high TSP was significantly associated with poorer cancer-specific survival in the whole cohort ($P<0.001$), in node-negative patients ($P=0.005$) and in triple negative patients ($P=0.035$) (Figures 2A–C). In multivariate survival analysis, a high TSP was associated with reduced cancer-specific survival independent of other variables in the whole cohort (HR 2.12, 95% CI 1.37–3.29, $P=0.001$) and in node-negative patients (HR 3.11, 95% CI 1.53–6.33, $P=0.007$) but not in triple negative patients ($P=0.151$) (Tables 6–8).

The relationship between TSP, clinicopathological characteristics and survival in patients who underwent adjuvant systemic treatment was examined. In total, 270 (75%) patients received adjuvant systemic treatment. A high TSP was associated with shorter cancer-specific survival following adjuvant treatment in univariate analysis (HR 2.04, 95% CI 1.29–3.22, $P=0.002$). In multivariate analysis, a high TSP was associated with reduced cancer-specific survival (HR 1.86, 95% CI 1.10–3.15, $P=0.021$), independent of PR status, lymph node involvement, tumour necrosis and CD68 + T-lymphocyte infiltrate.

DISCUSSION

The results of the present study show that high TSP was consistently associated with low tumour inflammatory infiltrate. Furthermore, TSP was associated with poorer outcome in the whole cohort and in patients with node-negative disease with long-term follow-up. Taken together, the present results highlight the importance of the stroma in the tumour microenvironment and its impact on outcome.

Consistent with previous reports, the role of tumour stroma in breast cancer (de Kruijf *et al*, 2011; Moorman *et al*, 2012; Dekker *et al*, 2013) survival in the present study was significantly shorter in patients with high-TSP tumours. However, TSP was not independently associated with survival in triple negative patients, whereas de Kruijf *et al* (2011) reported that TSP was indeed a significant prognostic factor. The difference between these findings might be attributed to the differences in patients' characteristics or might be

Table 4. The inter-relationship between clinicopathological characteristics and tumour stroma percentage in triple negative patients with invasive ductal breast cancer ($n=151$)

	Tumour stroma percentage ⩽50 $n=115$ (76%)	Tumour stroma percentage >50 $n=36$ (24%)	*P*-value
Age (⩽50/>50 years)	49/66	14/22	0.694
Size (⩽20/21–50/>50 mm)	62/49/4	15/18/7	0.127
Grade (I/II/III)	0/17/98	3/11/22	<0.001
Involved lymph node (0/1–3/>3)	68/30/10	15/20/8	0.027
Lymphovascular invasion (no/yes)	77/38	20/16	0.214
Tumour necrosis (no/yes)	28/87	9/27	0.931
General inflammatory infiltrate (low/high)	44/71	19/17	0.124
CD68 + macrophage infiltrate (tertiles)	19/28/36	14/5/7	0.011
CD4 + T-lymphocyte infiltrate (tertiles)	19/48/48	11/15/10	0.049
CD8 + T-lymphocyte infiltrate (tertiles)	25/34/56	11/14/11	0.071
Tumour recurrence (no/yes)	83/32	21/15	0.119
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	41/74	18/18	0.125
Systemic adjuvant therapy (hormonal/hormonal + chemotherapy/chempotherapy/none)	13/7/67/25	6/4/19/7	0.296
Alive/cancer death/non-cancer death	64/33/18	13/16/7	0.103
Cancer-specific survival (months)[a]	176 (167–185)	147 (133–163)	0.035

[a]Mean (95% confidence interval).

Table 5. The inter-relationship between tumour stroma percentage and Ki67 in patients with invasive ductal breast cancer

All patients ($n=214$)	Tumour stroma percentage ⩽50 $n=115$ (76%)	Tumour stroma percentage >50 $n=36$ (24%)	*P*-value
Ki67 (low/high)	122/27	54/11	0.833
Node-negative patients ($n=120$)	89 (74%)	31 (26%)	
Ki67 (low/high)	75/14	28/3	0.407
Triple negative patients ($n=99$)	$n=76$ (77%)	$n=23$ (23%)	
Ki67 (low/high)	61/15	19/4	0.803

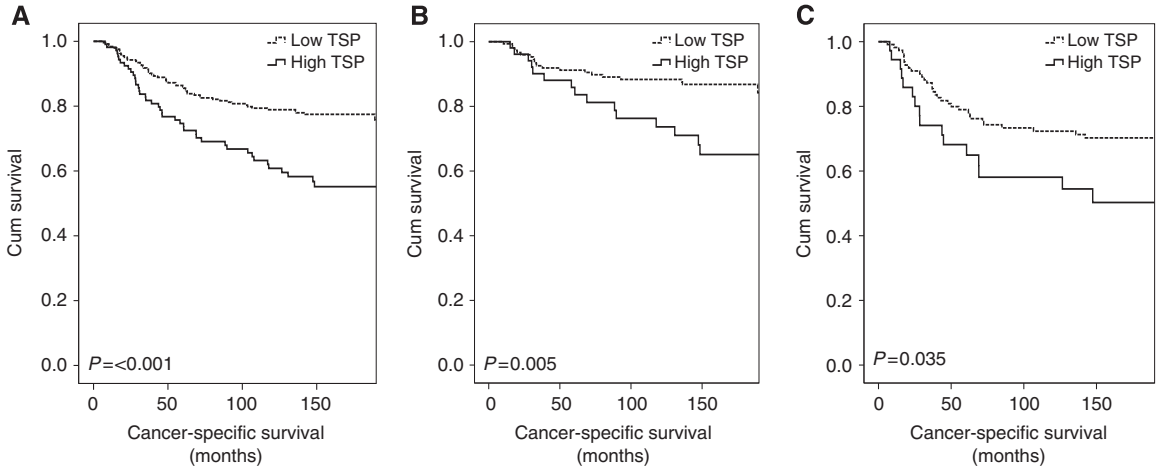

Figure 2. Kaplan–Meier survival curves Kaplan–Meier survival curves (log rank) of cancer-specific survival for tumour stroma percentage in the whole cohort (**A**), Node-negative patients (**B**) and triple negative patients (**C**).

Table 6. The relationship between clinicopathological characteristics and cancer-specific survival in patients with invasive ductal breast cancer ($n = 361$)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	***P*-value**	**Hazard ratio (95% confidence interval)**	***P*-value**
Age (⩽50/>50 years)	0.97 (0.64–1.46)	0.881		
Size (⩽20/21–50/>50 mm)	2.17 (1.54–3.07)	<0.001		0.142
Grade (I/II/III)	1.85 (1.3–2.58)	<0.001	1.72 (1.18–2.51)	0.005
Involved lymph node (0/1–3/>3)	1.97 (1.51–2.56)	<0.001	1.97 (1.46–2.66)	<0.001
Oestrogen receptor status (no/yes)	0.52 (0.34–0.79)	0.002		0.240
Progesterone status (no/yes)	0.44 (0.32–0.82)	0.006		0.184
Her2 status (no/yes)	1.44 (0.88–2.35)	0.145		
Lymphovascular invasion (no/yes)	2.07 (1.39–3.09)	<0.001		0.864
Tumour necrosis (no/yes)	1.97 (1.29–2.99)	0.002	2.49 (1.42–4.39)	0.001
General inflammatory infiltrate (low/high)	1.15 (0.77–1.73)	0.482		
CD68 + T-lymphocyte infiltrate (tertiles)	0.73 (0.55–0.96)	0.025		0.174
CD4 + T-lymphocyte infiltrate (tertiles)	0.46 (0.23–1.70)	0.075		
CD8 + T-lymphocyte infiltrate (tertiles)	0.64 (0.49–0.82)	<0.001	0.66 (0.46–0.80)	0.002
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	2.01 (1.27–3.19)	0.003		0.535
Systemic adjuvant therapy (hormonal/hormonal + chemotherapy/chempotherapy/none)	1.15 (0.71–1.87)	0.573		
Tumour stroma percentage (⩽50%/>50%)	1.89 (1.26–2.82)	<0.001	2.12 (1.37–3.29)	0.001
Systemic adjuvant therapy ($n = 207$)				
Size (⩽20/21–50/>50 mm)	1.44 (0.96–2.15)	0.080		0.462
Grade (I/II/III)	1.66 (1.13–2.43)	0.010		0.256
Involved lymph node (0/1–3/>3)	1.78 (1.31–2.40)	<0.001	1.99 (1.41–2.82)	<0.001
Oestrogen receptor status (no/yes)	0.47 (0.29–0.76)	0.002		0.181
Progesterone status (no/yes)	0.47 (0.27–0.83)	0.009	0.49 (0.26–0.89)	0.020
Lymphovascular invasion (no/yes)	1.99 (1.23–3.15)	0.003		0.160
Tumour necrosis (no/yes)	2.53 (1.41–4.52)	0.002	2.63 (1.40–4.96)	0.003
CD68 + T-lymphocyte infiltrate (tertiles)	0.63 (0.46–0.86)	0.004	0.61 (0.43–8.44)	0.003
CD8 + T-lymphocyte infiltrate (tertiles)	0.78 (0.56–1.03)	0.841		
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	1.93 (1.15–3.26)	0.013		0.841
Tumour stroma percentage (⩽50%/>50%)	2.04 (1.29–3.22)	0.002	1.86 (1.10–3.15)	0.021

Table 7. The relationship between clinicopathological characteristics and cancer-specific survival in patients with node-negative breast cancer ($n = 207$)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	*P*-value	Hazard ratio (95% confidence interval)	*P*-value
Age (⩽50/>50 years)	0.70 (0.36–1.36)	0.290		
Size (⩽20/21–50/>50 mm)	2.32 (1.25–4.31)	0.007	2.47 (1.23–4.95)	0.011
Grade (I/II/III)	1.64 (0.97–2.73)	0.062		0.217
Oestrogen receptor status (no/yes)	0.71 (0.36–1.40)	0.325		
Progesterone (no/yes)	0.75 (0.35–1.56)	0.437		
Her2 status (no/yes)	2.11 (1.03–4.31)	0.040		0.306
Lymphovascular invasion (no/yes)	1.62 (0.78–3.38)	0.198		
Tumour necrosis (no/yes)	1.97 (1.48–8.59)	0.005	2.51 (1.03–6.13)	0.043
General inflammatory infiltrate (low/high)	1.47 (0.76–2.86)	0.255		
CD68 + macrophage infiltrate (tertiles)	0.68 (0.43–1.07)	0.096		0.313
CD4 + T-lymphocyte infiltrate (tertiles)	0.88 (0.59–1.32)	0.520		
CD8 + T-lymphocyte infiltrate (tertiles)	0.89 (0.59–1.33)	0.558		
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	2.11 (1.01–4.39)	0.047		0.188
Systemic adjuvant therapy (no/yes)	1.18 (0.57–2.42)	0.657		
Tumour stroma percentage (⩽50%/>50%)	2.24 (1.29–4.97)	0.005	3.11 (1.53–6.33)	0.002

Table 8. The relationship between clinicopathological characteristics and cancer-specific survival in patients with triple negative breast cancer ($n = 151$)

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	*P*-value	Hazard ratio(95% confidence interval)	*P*-value
Age (⩽50/>50 years)	1.23 (0.69–2.18)	0.475		
Size (⩽20/21–50/>50 mm)	3.10 (1.91–5.04)	<0.001	2.53 (1.52–4.21)	<0.001
Involved lymph node (0/1–3/>3)	1.91 (1.34–2.71)	<0.001	1.64 (1.15–2.34)	0.007
Grade (I/II/III)	0.97 (0.54–1.74)	0.916		
Lymphovascular invasion (no/yes)	2.11 (1.20–3.70)	0.009		0.337
Tumour necrosis (no/yes)	4.63 (1.66–12.88)	<0.001	3.99 (1.43–11.13)	0.008
General inflammatory infiltrate (low/high)	0.84 (0.47–1.48)	0.534		
CD68 + macrophage infiltrate (tertiles)	0.79 (0.54–1.16)	0.227		
CD4 + T-lymphocyte infiltrate (tertiles)	0.85 (0.60–1.19)	0.342		
CD8 + T-lymphocyte infiltrate (tertiles)	0.76 (0.55–1.05)	0.098		0.253
Loco-regional therapy (Lumpectomy + radiotherapy/ mastectomy + radiotherapy)	2.38 (1.24–4.58)	0.009		0.176
Adjuvant therapy (no/yes)	1.00 (0.48–2.02)	0.997		
Tumour stroma percentage (⩽50%/>50%)	1.06 (1.03–1.12)	0.035		0.151

due to treatment regimen undertaken; though, in both studies patients did not receive neoadjuvant treatment. Irrespective of this, previous work has not determined whether the effect of an expanded tumour stroma on survival was independent of host inflammatory responses and other components of the tumour microenvironment.

Although the inter-relationships between the tumour stroma, tumour microenvironment and gross pathological characteristics are likely complex, the TSP remained independently and strongly associated with reduced cancer-specific survival. These results confirm the importance of tumour-host factors, such as the tumour microenvironment, in determining oncological outcome. In particular, node-negative patients with high TSP had a more than two-fold higher risk of breast cancer death compared with those with low TSP, independent and comparable with that of tumour size, lymph node status, grade and necrosis. Furthermore, survival was also significantly shorter in patients who received adjuvant therapy for high-TSP tumours. Thus, in addition to identifying high-risk patients, TSP may also select patients less likely to benefit from standard therapy and who should be considered for additional adjunctive treatment, potentially targeted at the stroma itself (Engels *et al*, 2012). These results confirm the importance of tumour-host factors, such as the tumour microenvironment in determining oncological outcome.

Despite recognition of the importance of the tumour stroma in cancer progression, its relationship with other components of the tumour microenvironment has yet to be fully characterised. In the present study, increased amount of stroma was associated with a weaker peritumoural inflammatory infiltrate, as measured by the Klintrup–Mäkinen score and by macrophages and T-cell subtypes. This is consistent with the recent observation that a high TSP trended toward a low peritumoural inflammatory infiltrate in patients with colorectal cancer (Park *et al*, 2014); however, the underlying mechanism is still unclear. In the present study, amount of tumour stroma was not associated with hormone receptors or proliferative marker such as Ki67, although it is of interest that Ki67 was recently shown to be significantly associated with tumour inflammatory infiltrates (Mohammed *et al*, 2012a,b,c,d).

It has previously been proposed that the tumour stroma may prevent effective tumour infiltration by immune cells (Ueno *et al*, 2004). The results from cell line experiments would also support these findings, namely that fibroblasts and myofibroblasts can modulate the ability of lymphocytes and macrophages to invade a tumour and may prevent penetration of immune cells within tumours, creating a physical barrier against an immune reaction while promoting tumour growth and progression, due to their contractile properties and their associated extracellular matrix (Lieubeau *et al*, 1999).

In the present study, although the cell markers of both innate and adaptive immune cells were examined in the present study, the effect of TSP on survival remained independent of local inflammatory responses, suggesting the presence of other mechanisms rather than a direct effect on immune cells. Indeed, tumour stroma may promote the development of a pro-tumour rather than anti-tumour immune infiltrate (Fridman *et al*, 2011). Stromal fibroblasts may also induce suppression of the immune response and produce immunosuppressive molecules such as TGF-β and VEGF, suggesting that CAFs may promote cancer immunoescape (Yaguchi *et al*, 2011; Engels *et al*, 2012). This may also implicate certain cell signalling pathways such as the common cell signalling pathway associated with inflammation; the JAK-State pathway (Yu *et al*, 2007, 2009). Therefore, further characterisation of the tumour inflammatory cell infiltrate and their association with tumour stroma and JAK-State signalling is warranted.

In the present study, amount of tumour stroma was not associated with hormone receptors or the proliferative marker Ki67. However, it is of interest that the hormone receptors were recently shown to be significantly associated with tumour inflammatory infiltrates. Patients with high-grade general inflammatory infiltrate were more likely to have ER-negative and PR-negative tumours. The expression of ER/PR was directly associated with the percentage of tumour lymphocyte infiltrate and inversely associated with CD68+, CD8+ and CD138+ infiltrates. Similarly, the expression of HER-2 was directly associated with CD8+ and inversely associated with CD138+ infiltrates (Baker *et al*, 2011; Mohammed *et al*, 2012c, 2013).

A potential limitation of the present study was that direct investigation of the effect of tumour stroma on the infiltration of inflammatory cells was not carried. This would require either cell line or animal models. Although cell line or animal models do have the advantage of allowing direct investigation of the effect of tumour stroma on inflammatory cell infiltration, they often lack clinical relevance to the patient with breast cancer with the consequent slow progress on immunotherapy for breast cancer. The present study highlights the importance of the amount of tumour stroma on immunological response in patients with primary operable ductal breast cancer.

In conclusion, the results of the present study show that a high TSP in primary operable invasive ductal breast cancer was associated with recurrence and shorter long-term survival. Implementing this simple and reproducible parameter in routine pathological examination may help optimise risk stratification in patients with invasive ductal breast cancer. The present study findings suggest that high TSP enables tumour cells to evade the immune response and promote tumour progression.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Ministry of Higher Education-Libya for funding this work. We also thank Clare Orange, who provided technical assistance.

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