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# The prognostic significance of tumour–stroma ratio in oestrogen receptor-positive breast cancer

C L Downey<sup>1</sup>, S A Simpkins<sup>1</sup>, J White<sup>1</sup>, D L Holliday<sup>1</sup>, J L Jones<sup>2</sup>, L B Jordan<sup>3</sup>, J Kulka<sup>4</sup>, S Pollock<sup>1</sup>, S S Rajan<sup>1</sup>, H H Thygesen<sup>1</sup>, A M Hanby<sup>1</sup> and V Speirs<sup>\*,1</sup>

<sup>1</sup>Leeds Institute of Cancer and Pathology, University of Leeds, St James's University Hospital, Leeds LS9 7TF, UK; <sup>2</sup>Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London, London, UK; <sup>3</sup>Department of Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK and <sup>4</sup>2nd Department of Pathology, Semmelweis University, Üllőiút. 93, Budapest 1091, Hungary

**Background:** A high percentage of stroma predicts poor survival in triple-negative breast cancers but is diminished in studies of unselected cases. We determined the prognostic significance of tumour–stroma ratio (TSR) in oestrogen receptor (ER)-positive male and female breast carcinomas.

**Methods:** TSR was measured in haematoxylin and eosin-stained tissue sections (118 female and 62 male). Relationship of TSR (cutoff 49%) to overall survival (OS) and relapse-free survival (RFS) was analysed.

**Results:** Tumours with  $\geq 49\%$  stroma were associated with better survival in female (OS  $P=0.008$ , HR=0.2–0.7; RFS  $P=0.006$ , HR=0.1–0.6) and male breast cancer (OS  $P=0.005$ , HR=0.05–0.6; RFS  $P=0.01$ , HR=0.87–5.6), confirmed in multivariate analysis.

**Conclusions:** High stromal content was related to better survival in ER-positive breast cancers across both genders, contrasting data in triple-negative breast cancer and highlighting the importance of considering ER status when interpreting the prognostic value of TSR.

It is now well recognised that cancer initiation, growth and progression is dependent on tumour microenvironment of which tumour–stroma is an integral part. More recently, attention has focused on the potential prognostic value that tumour–stromal ratio (TSR) described by some as proportion of tumour may have an increasing number of different cancer types. As a result, TSR is fast emerging as a significant prognostic indicator in different cancer types. Collectively, many of these document an association of high stromal content with worse prognosis, for example, in cancers of the breast (de Kruijf *et al*, 2011; Ahn *et al*, 2012; Moorman *et al*, 2012), lung (Maeshima *et al*, 2002), prostate (Yanagisawa *et al*, 2007), stomach (Wu *et al*, 2013), and colon and rectum (Mesker *et al*, 2007; West *et al*, 2010). However, there are notable inconsistencies, particularly in breast cancer. Previous

studies in TSR in breast cancer have largely focused on triple-negative disease, that is, negative for ER, PR and HER2. Moorman *et al* (Moorman *et al*, 2012) showed that a high percentage of stroma predicts poor survival in triple-negative breast cancers. This finding supports a preceding paper that showed TSR to be an independent prognostic factor for relapse-free survival (RFS) in breast cancer patients, especially in those with triple-negative disease (de Kruijf *et al*, 2011). A recent validation study by the same group examining 403 assessable cases from 674 node-negative pre-menopausal breast cancer patients in the EORTC peri-operative chemotherapy trial (10 854) confirmed this finding (Dekker *et al*, 2013). However, this prognostic value is diminished in studies of unselected breast cancers (Ahn *et al*, 2012).

\*Correspondence: Professor V Speirs; E-mail: v.speirs@leeds.ac.uk

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These data suggest that the importance of TSR in breast cancer may be dependent on key molecular determinants of tumour subtype of which ER status is pre-eminent. We aimed to determine the prognostic significance of the TSR in a cohort of ER-positive male and female breast cancers.

## MATERIALS AND METHODS

**Ethical approval.** Ethical approval was granted by Leeds (East) Research Ethics Committee (06/Q1206/180; 06/Q1205/156).

**Patients.** Archival tissue blocks and clinicopathological data from 118 oestrogen receptor (ER)-positive female breast cancer cases diagnosed at the Leeds Teaching Hospitals (1994–1997) were obtained. We also included 62 ER-positive male breast carcinomas diagnosed in Leeds, Ninewells Hospital, Dundee, St Bartholomew's, London and Budapest, Hungary (1988–2010).

**Clinicopathological data.** Histopathological and treatment data were obtained from pathology reports and are summarised in Table 1. RFS and overall survival (OS) were available for each patient.

**Measurement of stromal density.** Four-micrometer-thick haematoxylin and eosin-stained tissue sections were prepared. Each slide was scanned at  $\times 20$  magnification (Aperio XT, Aperio Technologies, Vista, CA, USA), and an area for analysis was selected using a digital slide viewer (ImageScope version 8.0, Aperio Technologies). Two separate  $9\text{ mm}^2$  areas were sampled and compared in the female cohort: tumour-leading and non-leading edge. For the male cases, the  $9\text{ mm}^2$  non-leading edge was sampled. Areas containing large amounts of necrosis or which had been sampled for tissue microarray construction (most of the male cases) were avoided. A grid with a systematic random sample of 300 points was then superimposed on the selected area using virtual graticule software (RandomSpot, University of Leeds, Leeds, UK). The number of measurement points was consistent with that found accurate by previous studies (West *et al.*, 2010). The histopathological category under each point was recorded (Supplementary Figure 1) and the number of points attributable to each category was counted. Categories used were tumour, stroma and non-informative (unclassifiable). Points falling on areas of lumen, necrosis, blood vessels, inflammation or blank areas fell into the latter category. Cases were scored by CLD, SAS and JW, and guided by a breast histopathologist (AMH). To assess inter-observer variation, a subset of 63 cases were double scored by CLD and JW ( $\kappa = 0.7$ ). Discordant results were reviewed jointly to reach consensus. TSR was expressed as a percentage of all the informative points per section.

**Statistical analyses.** This was performed using GraphPad Prism (version 6.00 for Windows, GraphPad Software, La Jolla, CA, USA), R version 2.15 SPSS version 21 (Chicago, IL, USA) and the Survival package for R (SPSS version 21). The optimal cutoff value for TSR was calculated thus for each tumour proportion value occurring in the data set; a log-rank test was performed based on a comparison of the group of patients with a TSR  $\leq$  that value, and the patients with  $P$ -values above that. Primary end points were RFS and OS. Univariate and multivariate analyses were performed. Differences between the groups were assessed using the log-rank test and Cox regression analysis.  $P$ -values  $< 0.05$  were considered to be statistically significant.

## RESULTS

In order to determine optimum cutoffs to distinguish high and low TSR, log-rank tests were performed to compare groups of patients

Table 1. Clinicopathological details of cohort

Characteristics	Gender	
	Female (n = 118)	Male (n = 62)
Mean age (range)	59 (27–85)	65 (43–89)
<b>Type</b>		
Ductal	96	60
Lobular	6	0
Other	16	2
<b>Grade</b>		
1	47	9
2	47	32
3	24	21
<b>LN</b>		
N0	58	25
N1–3	59	25
NA	1	3
<b>ER</b>		
+	118	62
–	0	0
<b>Size</b>		
$\leq 10\text{ mm}$	21	12
$> 10\text{ mm}$	94	47
Multifocal	3	0
NA	0	3
<b>Treatment</b>		
Endocrine		
Yes	93	55
No	20	6
Unknown	5	1
Chemotherapy		
Yes	21	15
No	97	46
Unknown	0	1
Radiotherapy		
Yes	36	23
No	82	38
Unknown	0	1

Abbreviations: ER = oestrogen receptor; LN = lymph node; NA = not applicable.

with TSR below or equal to that value, with patients with TSR above that, the cutoff points that led to the smallest  $P$ -value were 0.490–0.493 (Supplementary Figure 2). A cutoff point of 49% was used to stratify patients with high ( $\geq 49\%$ ) and low ( $< 49\%$ ) stromal content.

High stromal content was associated with better survival in both females and males. For the female group, this finding was irrespective of area sampled (leading edge: OS  $P = 0.001$ , HR = 0.3–2.9; RFS  $P$ -value = 0.001, HR = 0.4–2.8; non-leading edge: OS  $P = 0.008$ , HR = 0.2–0.7; RFS  $P$ -value = 0.006, HR = 0.1–0.6). Figure 1 illustrates survival curves of female cases with high and low stromal content when the area sampled originated from the leading edge. These observations were confirmed in a cohort of ER-positive male breast cancers, which also showed that high stromal content was associated with better OS ( $P = 0.005$ , HR = 0.05–0.6) and RFS ( $P = 0.01$ , HR = 0.8–5.6).

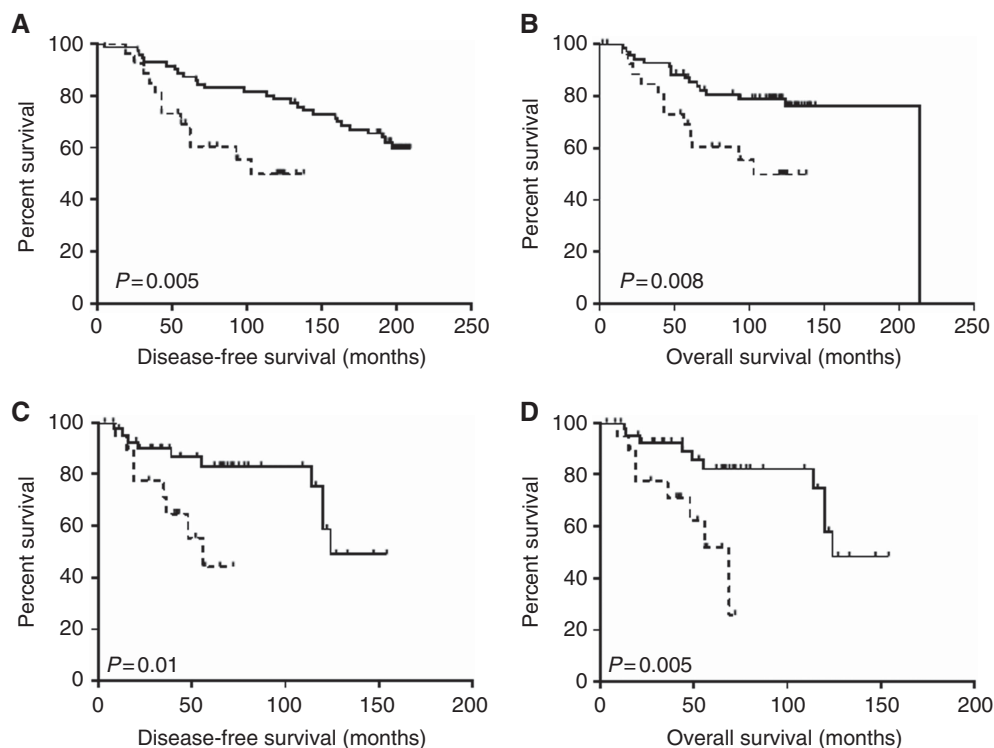


Figure 1. Kaplan–Meier survival curves showing relapse-free survival (A, C) and overall (B, D) survival after stratification by TSR in ER-positive female (A, B) and male (C, D) breast cancer. Solid line, high stroma; dotted line, low stroma.

When adjusted for tumour size, grade and lymph node status this remained significant for OS upon multivariate analysis in the male cohort ( $P=0.004$ , HR = 1.3–5.0) and in female breast tumours <20 mm ( $P=0.03$ , HR = 0.23–0.93). We were unable to compare leading and non-leading edge in the male cohort, as many of these cases had been sampled previously for TMA construction; however, data from the female cohort showed no difference in area sampled in relation to clinical outcome.

## DISCUSSION

The role of the stroma in carcinogenesis is receiving increased attention with recognition that cancer initiation, growth and progression is dependent on tumour microenvironment, of which tumour–stroma is an integral part. More recently, attention has focused on the potential prognostic value TSR may have in an increasing number of different cancer types that point toward worse prognosis in cancers that have a high stromal content relative to tumour epithelium (Maeshima *et al*, 2002; Mesker *et al*, 2007; Yanagisawa *et al*, 2007; West *et al*, 2010; de Kruijf *et al*, 2011; Ahn *et al*, 2012; Moorman *et al*, 2012; Wu *et al*, 2013).

In breast cancer, these studies have mainly focused on triple-negative breast cancers, which generally have a poor prognosis, or in unselected groups. To our knowledge, this is the only work thus far examining TSR in relation to prognosis in ER+ breast cancer. In direct contrast to published data on triple-negative breast cancer, this study showed that a high stromal content, equivalent to a low TSR, is related to better survival in ER+ breast cancer across both genders.

Much interest has arisen in recent years around the tumour-type specificity of carcinoma-associated fibroblasts that co-exist in breast cancer stroma. Molecular profiling has shown that for different molecular subtypes of breast cancer, the carcinoma-associated fibroblast molecular profiles also differ (Tchou and Conejo-Garcia, 2012). Thus, our observations that ER-positive (effectively luminal A and B) tumours are different in the relationship of TSR to outcome is most probably a mirror to the

effect of diverse molecular phenotype underpinning the biological differences.

The choice of the tumour area selected to define TSR can be subjective and is not well described in all published studies. Dekker *et al* (2013) used a compass system to ensure only areas that were surrounded by tumour cells in all directions, were sampled and avoided peripheral regions. In light of this, we postulated that the area sampled may influence the reliability of our results; hence, we applied our algorithm to two areas in our female cohort. The first area sampled was tumour-leading edge that was selected on the basis that the advancing front of a tumour may be more proliferative and the metabolic activity of tumour cells in this area is not compromised by a potential lack of nutrients. We then examined a central area, taking care to avoid areas with obvious necrosis. Identical results were obtained with both sampling methods giving confidence in our results and strengthening the evidence for an ER-dependent significance of TSR.

Our findings suggest that differences in stromal biology may exist between breast cancer subtypes and highlights the importance of knowing ER status when interpreting the prognostic value of TSR. This is important as it has been suggested that TSR be implemented into routine daily pathology practice (de Kruijf *et al*, 2011; Dekker *et al*, 2013). Future studies are warranted to confirm our findings in larger independent cohorts to validate the scope and potential significance of TSR in ER-positive breast cancer. With the stroma receiving increased attention as a point of potential additional therapeutic intervention in breast and other cancers, elucidation of biological differences in stroma of different tumour subtypes will help more fully understand its role during breast carcinogenesis.

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## REFERENCES

- Ahn S, Cho J, Sung J, Lee JE, Nam SJ, Kim K-M, Cho EY (2012) The prognostic significance of tumor-associated stroma in invasive breast carcinoma. *Tumour Biol* **33**(5): 1573–1580.
- de Kruijf EM, van Nes JGH, van de Velde CJH, Putter H, Smit VTHBM, Liefers GJ, Kuppen PJK, Tollenaar RAEM, Mesker WE (2011) Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple-negative carcinoma patients. *Breast Cancer Res Treat* **125**(3): 687–696.
- Dekker TJA, van de Velde CJH, van Pelt GW, Kroep JR, Julien J-P, Smit VTHBM, Tollenaar RAEM, Mesker WE (2013) Prognostic significance of the tumor-stroma ratio: validation study in node-negative premenopausal breast cancer patients from the EORTC perioperative chemotherapy (POP) trial (10854). *Breast Cancer Res Treat* **139**(2): 371–379.
- Maeshima AM, Niki T, Maeshima A, Yamada T, Kondo H, Matsuno Y (2002) Modified scar grade. A prognostic indicator in small peripheral lung adenocarcinoma. *Cancer* **95**: 2546–2554.
- Mesker WE, Junggeburst JMC, Szuhai K, de Heer P, Morreau H, Tanke HJ, Tollenaar RAEM (2007) The carcinoma–stromal ratio of colon carcinoma is an independent factor for survival compared to lymphnode status and tumour stage. *Cell Oncol* **29**: 387–398.
- Moorman AM, Vink R, Heijmans HJ, van der Palen J, Kouwenhoven EA (2012) The prognostic value of tumour-stroma ratio in triple-negative breast cancer. *Eur J Surg Oncol* **38**(4): 307–313.
- Tchou J, Conejo-Garcia J (2012) Targeting the tumor stroma as a novel treatment strategy for breast cancer: shifting from the neoplastic cell-centric to a stroma-centric paradigm. *Adv Pharmacol* **65**: 45–61.
- West NP, Dattani M, McShane P, Hutchins G, Grabsch J, Mueller W, Treanor D, Quirke P, Grabsch H (2010) The proportion of tumour cells is an independent predictor for survival in colorectal cancer patients. *Br J Cancer* **102**(10): 1519–1523.
- Wu Y, Grabsch H, Ivanova T, Tan IB, Murray J, Ooi CH, Wright AI, West NP, Hutchins GG, Wu J, Lee M, Lee J, Koo JH, Yeoh KG, van Grieken N, Ylstra B, Rha SY, Ajani JA, Cheong JH, Noh SH, Lim KH, Boussioutas A, Lee JS, Tan P (2013) Comprehensive genomic meta-analysis identifies intra-tumoural stroma as a predictor of survival in patients with gastric cancer. *Gut* **62**(8): 1100–1111.
- Yanagisawa N, Li R, Rowley D, Liu H, Kadmon D, Miles BJ, Wheeler TM, Ayala GE (2007) Stromogenic prostatic carcinoma pattern (carcinomas with reactive stromal grade 3) in needle biopsies predicts biochemical recurrence-free survival in patients after radical prostatectomy. *Hum Pathol* **38**: 1611–1620.

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