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

C L Downey, S A Simpkins, J White, D L Holliday, J L Jones, L B Jordan, J Kulka, S Pollock, S S Rajan, H H Thygesen, A M Hanby and V Speirs, 1

1Leeds Institute of Cancer and Pathology, University of Leeds, St James’s University Hospital, Leeds LS9 7TF, UK; 2Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London, London, UK; 3Department of Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK and 42nd Department of Pathology, Semmelweis University, Úllőjütt. 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 \( P = 0.008, \text{HR} = 0.2–0.7 \); RFS \( P = 0.006, \text{HR} = 0.1–0.6 \)) and male breast cancer (OS \( P = 0.005, \text{HR} = 0.05–0.6 \); RFS \( P = 0.01, \text{HR} = 0.87–5.6 \)), 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

Received 27 July 2013; revised 14 January 2014; accepted 16 January 2014; published online 18 February 2014

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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 × 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 mm² areas were sampled and compared in the female cohort: tumour-leading and non-leading edge. For the male cases, the 9 mm² non-leading edge was selected using a digital slide viewer (ImageScope version 8.0, Aperio Technologies). 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 (κ = 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 ≤ 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 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 (≥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).

![Table 1. Clinicopathological details of cohort](https://example.com/table1.png)

**Table 1.** Clinicopathological details of cohort

| Characteristics | Female (n = 118) | Male (n = 62) |
|-----------------|-----------------|--------------|
| Mean age (range) | 59 (27–85)      | 65 (43–89)   |
| Type            |                 |              |
| Ductal          | 96              | 60           |
| Lobular         | 6               | 0            |
| Other           | 16              | 2            |
| Grade           |                 |              |
| 1               | 47              | 9            |
| 2               | 47              | 32           |
| 3               | 24              | 21           |
| LN              |                 |              |
| N0              | 58              | 25           |
| N1–3            | 59              | 25           |
| NA              | 1               | 3            |
| ER              |                 |              |
| +               | 118             | 62           |
| –               | 0               | 0            |
| Size            |                 |              |
| ≤10 mm          | 21              | 12           |
| >10 mm          | 94              | 47           |
| Multifocal      | 3               | 0            |
| NA              | 0               | 3            |
| Treatment       |                 |              |
| 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.
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 area that is 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.

**ACKNOWLEDGEMENTS**

We are grateful to Breast Cancer Campaign for funding our collection of male breast carcinomas and for supporting DLH and...
SP. SAS was funded by the Wolfson Foundation and a Wellcome Trust Vacation Scholarship. HHT was funded by Cancer Research UK. We convey special thanks to members of the Leeds Breast Team for input and support at various stages of this project.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)