Tumour-stroma ratio and 5-year mortality in gastric adenocarcinoma: a systematic review and meta-analysis

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Tumour-stroma ratio (TSR) is a novel potential prognostic factor in cancers and based on the proportions of stroma and tumour area. The prognostic value of TSR in gastric cancer is incompletely known. The aim of this study was to estimate prognostic significance of TSR in gastric adenocarcinoma. A search of PubMed (MEDLINE), Web of Science, EMBASE, Cochrane and Scopus databases was performed. A meta-analysis was conducted on five-year survival in gastric cancer patients using inverse variance random-effects methods. The literature search yielded 5329 potential titles, of which a total of seven studies were eligible. Results of six studies including a total of 1779 patients were pooled in the meta-analysis. Only 23 (1.3%) of the patients received neoadjuvant therapy. All six studies had a cut-off of 50% for the proportion of stroma when dividing the patients into low- and high stroma groups. Low TSR (high amount of stroma) was strongly associated with increased five-year mortality (hazard ratio 2.19, 95% CI 1.69–2.85). In conclusion, TSR is a strong prognostic factor in gastric cancer. It could be used to estimate prognosis of gastric cancer patients not receiving neoadjuvant chemotherapy. Further studies including patients receiving neoadjuvant therapy are recommended.

Gastric cancer is the third most common cause of cancer death in the world1. The survival after the surgery of gastric cardia cancer has been improved during the past decades, while in non-cardia gastric cancer the improvement in survival been more modest2. TNM-classification for cancers provides prognostic information based on the degree of tumour progression, but does not take into account the tumour biology, and we still see recurrences and cancer death after surgery even in early-stage gastric cancer3. Additional, easy-to-replicate histological factors that could identify gastric cancer patients with highest risk of recurrence or mortality are needed.

Some tumour biology-related factors, such as tumour-stroma ratio (TSR), have been proposed to identify patients with high risk of cancer mortality. TSR is defined as the area of stroma compared to area of the tumour cells in the tumour and is strongly associated with survival in several cancer types, including colorectal cancer4, breast cancer5, and hepatocellular carcinoma6. TSR can be easily and routinely analysed from haematoxylin-eosin (HE) stained slides routinely used for diagnostic purposes7. Tumours that have high amount of stroma have low TSR, and tumours that have low amount of stroma have high TSR.

Some studies have suggested that low TSR is associated with poor survival in gastric cancer8–10. Despite that the prognostic impact of TSR in gastric cancer is currently poorly known. The aim of this meta-analysis was to identify all studies on tumour-stroma ratio in and estimate the prognostic value of TSR in gastric adenocarcinoma.

Methods

This study was a meta-analysis conducted according to the PRISMA guidelines11. The study followed a study protocol established a priori.

Search. The literature search was conducted in August 2018 using a keyword search on PubMed (MEDLINE), Web of Science, EMBASE, and Cochrane databases using the terms (stroma* OR Glasgow tumor microenvironment score) AND (gastri* OR gastrectomy OR gastroesophageal OR gastro-oesophageal OR oesophagogastric OR esophagogastric) AND (neoplas* OR cancer OR carcinoma OR adenocarcinoma) AND (prognos* OR
mortality OR survival). Scopus database was searched using terms (stroma*) AND (gastrointestinal* OR gastroesophageal OR gastro-oesophageal OR oesophagogastric OR esophagogastric) AND (neoplas* OR cancer OR carcinoma OR adenocarcinoma) AND (prognosis* OR mortality OR survival).

**Study selection.** The studies considered for inclusion had to be original articles written in English. They had to contain assessment of proportion of intratumoral stroma compared to tumour area and contain hazard ratios for survival, or Kaplan-Meier curves stratified by intratumoral stromal proportion.

Duplicates of studies identified in literature search were removed. Titles of studies left after removing duplicates were screened by one researcher, and studies that clearly did not fill the inclusion criteria were excluded. Abstracts of the studies left after reading titles were read by one researcher, and the studies clearly not fulfilling the inclusion criteria were excluded. If the study fulfilled the criteria or there was not enough information in the abstract to exclude the study, full texts of the articles were studied independently by two researchers. If there were disagreements, the studies were discussed with third researcher and consensus was reached.

**Data extraction.** The data necessary for this study were extracted independently by two researchers from the original studies. The data collected included the name of the first author, the study interval, the type of the study, the number of patients in the study, the country of the study population, the age and sex of patients included in the study, if the patient received chemotherapy or not and characteristics of the tumour the patient had (histological type, histological grade and TNM-stage). Study quality was assessed independently by the two researchers using the Newcastle-Ottawa scale, as included studies were cohort studies. Discrepancies on study quality were settled by consulting third researcher.

**Definition of exposure and outcome.** The exposure of this study was high amount of intratumoral stroma. The patient group with low amount of intratumoral stroma (high TSR) was considered as control group. The primary endpoint of this study was death during the five-year follow-up period after surgery. The primary outcome of this study was 5-year overall survival, as it is commonly used as outcome in studies considering prognosis in gastric cancer. The secondary outcome of this study was overall survival. The endpoint for overall survival was death during follow-up after surgery.

**Statistical analysis.** Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK) was used to perform statistical analyses. The estimates of hazard ratios for 5-year mortality and overall mortality were extracted from the studies, with preference to estimates from multivariable analyses to reduce confounding. If multivariable analysis was not available, univariable analysis was used instead. If the data was presented only as a Kaplan–Meier curve, the WebPlotDigitizer tool (http://arohatgi.info/WebPlotDigitizer) was used to extract proportion of surviving patients on 12, 24, 36, 48 and 60 months for both patient groups when estimating five-year mortality. When estimating overall mortality, the longest follow-up period available was used and datapoints were extracted every 12 months until the end of follow-up. Natural logarithms of hazard ratios and standard errors were then calculated based on those measurement points, according to the method described by Tierney et al. Generic inverse variance-method with random-effect models was used to calculate estimates of average hazard ratios with Review Manager. Publication bias was estimated by inspecting the funnel plots instead of statistical testing, given the small number of included studies.

**Ethics approval.** No ethical approval was sought for this systematic review and meta-analysis.

**Results**

**Study selection.** The study selection is summarized in Fig. 1. Some 5329 titles were found in the literature search, and 3057 of them were left after removing duplicates. After the exclusion of studies not fulfilling the inclusion criteria based on title and abstracts there were 32 titles left, of which full text versions were studied and seven of them filled the inclusion criteria. Of these, a Singaporean study compared the lowest tertile to the highest tertile of tumour stroma measured with computerized method without reporting any percentage cut-offs, and could not be included in the meta-analysis due to different methodology compared to the other studies. The details of the six studies fulfilling the inclusion criteria are presented in Table 1.

**Included studies.** The six studies selected included a total of 1779 patients. In all studies TSR was assessed from the resected specimen. Five of the included studies assessed the proportion of stroma from HE-stained slides, while one of the studies based its analysis of proportion of stroma on immunohistochemical stainings. All of the studies used 50% of stroma as cut-off between low and high TSR groups. One of the studies only included patients with diffuse type carcinoma. One study included only patients with T-grade 3 or 4. Two of the studies included some patients operated with palliative intent. Only one of the studies included patients with neoadjuvant chemotherapy. Further details of patients included in the studies are presented in Table 2 and 3.

**Study quality.** One of the studies received six points, four seven points and one eight points from quality assessment using Newcastle-Ottawa scale (Table 1). One of the studies provided multivariate hazard ratio with 95% confidence interval for five-year survival. One of the studies provided univariate hazard ratio with 95% confidence interval for five-year survival. Four of the studies didn’t provide hazard ratios for five-year survival but they provided Kaplan–Meier figures for overall survival, which were used to calculate hazard ratios and 95% confidence intervals for five-year survival.
Tumour-stroma ratio and 5-year mortality. The meta-analysis was conducted on five-year survival data. The forest plot is shown in Fig. 2. The hazard ratio for stroma-rich group compared to stroma-poor group was 2.19 (95% CI 1.69–2.85). The statistical heterogeneity was high (I² = 63%). The inspection of funnel plot showed some asymmetry, mainly caused by the smallest study in the meta-analysis8, suggesting small-study effects and a risk of publication bias (Supplementary Fig. 1). However, the removal of this study from the meta-analysis in a sensitivity analysis had only small effect on the estimate, resulting in a HR of 1.93 (95% CI 1.62–2.29), and low statistical heterogeneity (I² = 20%). The Singaporean study not included in the meta-analysis also suggested worse survival in patients in the stroma-rich groups14.

Table 1. Characteristics and quality of the studies included in the qualitative synthesis. n.d., not described; *two independent cohorts were studied.

| Study         | Country          | Study interval | Assessment | Type of study | Number of patients | Study quality |
|---------------|------------------|----------------|------------|---------------|--------------------|---------------|
| Ahn et al.15  | South-Korea      | 2009–2012      | Retrospective | Cohort       | 196                | poor (6/9)    |
| Aurello et al.8 | Italy           | 2004–2015      | Retrospective | Cohort       | 106                | good (7/9)    |
| Kemi et al.9  | Finland          | 1983–2016      | Retrospective | Cohort       | 583                | good (8/9)    |
| Lee et al.12  | South-Korea      | 2005–2008      | Retrospective | Cohort       | 175                | fair (7/9)    |
| Peng et al.10 | China            | 2002–2011      | Retrospective | Cohort       | 494                | good (7/9)    |
| Wu et al.14   | Singapore        | n.d.           | Retrospective | Cohort       | 131 + 153*         | fair (6/9)    |
| Zhou et al.16 | China            | 2000–2011      | Retrospective | Cohort       | 225                | good (7/9)    |

Table 2. Patient characteristics in studies included in the qualitative synthesis stratified by tumour-stroma ratio. TSR, tumour-stroma ratio; n.d., not described.

| Study         | Number of patients | Age of patients | Sex ratio (M:F) | Neoadjuvant therapy |
|---------------|--------------------|-----------------|-----------------|---------------------|
|               | High TSR | Low TSR | High TSR | Low TSR | High TSR | Low TSR | High TSR | Low TSR |
| Ahn et al.15  | 118      | 78      | 52 < 60 years | 66 > 60 years | 42 < 60 years | 36 > 60 years | 85:33 | 42:36 | None | None |
| Aurello et al.8 | 41      | 65      | mean 70.1 years | Mean 69.3 years | Mean 65.0 years | 57:49 | None | None |
| Kemi et al.9  | 241      | 342     | Mean 69.3 years | Mean 65.0 years | 154:87 | 198:144 | 5/241 | 17/342 |
| Lee et al.12  | 111      | 64      | 56 < 50 years | 55 > 50 years | 40 < 50 years | 24 > 50 years | 66:45 | 28:36 | None | None |
| Peng et al.10 | 254      | 240     | Median 58,8 years | Median 59,1 years | 183:71 | 166:74 | None | None |
| Wu et al.14 cohort LS-2 | 131 | Total median 68 years | Total 81:50 | None |
| Wu et al.14 cohort SG-3 | 153 | Total median 65 years | Total 95:53, 5 unknown | Total 1/153 |
| Zhou et al.16 | 139      | 86      | Total 99 < 60 years, 126 ≥ 60 years | Total 168:57 | None | None |

Tumour-stroma ratio and 5-year mortality. The meta-analysis was conducted on five-year survival data. The forest plot is shown in Fig. 2. The hazard ratio for stroma-rich group compared to stroma-poor group was 2.19 (95% CI 1.69–2.85). The statistical heterogeneity was high (I² = 63%). The inspection of funnel plot showed some asymmetry, mainly caused by the smallest study in the meta-analysis8, suggesting small-study effects and a risk of publication bias (Supplementary Fig. 1). However, the removal of this study from the meta-analysis in a sensitivity analysis had only small effect on the estimate, resulting in a HR of 1.93 (95% CI 1.62–2.29), and low statistical heterogeneity (I² = 20%). The Singaporean study not included in the meta-analysis also suggested worse survival in patients in the stroma-rich groups14.
Tumour-stroma ratio and overall mortality. The forest plot of the meta-analysis conducted on overall survival data is shown in Supplementary Fig. 2. The hazard ratio for stroma-rich group compared to stroma-poor group was 2.33 (95% CI 1.81–3.01). Similarly to meta-analysis of 5-year mortality, statistical heterogeneity was high ($I^2 = 57$%), which was mainly caused by the smallest study included. Removal of this study resulted in a HR of 2.13 (95% CI 1.72–2.63) with lower heterogeneity ($I^2 = 41$%).

Discussion

Based on this first systematic review and a meta-analysis on TSR in gastric cancer patients, those patients with low TSR experience on average worse outcomes than those with high TSR. Some strengths and limitations should be considered before conclusions on the results. The strengths of this study include broad literature search and stringent inclusion criteria, resulting in inclusion of all potentially eligible quality studies. The studies included have been performed in Caucasian and Asian populations, which increases the applicability of the pooled results. Only one study included patients treated with neoadjuvant therapy and even it included only 23 patients that received neoadjuvant therapy, which is known to cause fibrosis in tumours. Therefore, the results are not applicable to patients undergoing neoadjuvant treatment.

The quality of the studies was mostly good, but loss to follow-up was not described in four of the studies. As all of the studies are single-center studies, potential selection bias might reduce the applicability of the results. However, all the studies included had the same direction of the results, including the two cohorts in the Singaporean study that was excluded from the meta-analysis, with similar effect sizes, suggesting low selection bias. Lastly, analysis of 5-year mortality adjusted for confounders was not available in five of the six studies, which implies some residual confounding in the pooled estimate. However, the pooled estimate was very similar to the only study adjusted for confounders. Subgroup analyses or meta-regression could have provided additional ways of taking confounders into account, but neither could be performed. Meta-regression is recommended only if ten or more studies are available, while the six included studies in the present meta-analysis were clearly too few for a reliable meta-regression. Only one study provided subgroup analysis of patients with different histological types and

Table 3. Tumour characteristics in studies included in the qualitative synthesis stratified by tumour-stroma ratio. ‘T-stage due to no TNM-stage provided; TSR, tumour-stroma ratio; n.d., not described; AC, adenocarcinoma; SRC, signet ring-cell carcinoma.

| Study          | Histological type         | Histological grade of differentiation | Tumour stage |
|----------------|---------------------------|--------------------------------------|--------------|
|                | High TSR                  | Low TSR                              | High TSR     | Low TSR                  | High TSR                  | Low TSR                  |
| Ahn et al.     | Intestinal 68            | Intestinal 23                        | Diffuse 38   | Mixed 12                 | Well- or moderate 64      | Poor 54                  |
|                |                          | Diffuse 47                           | Mixed 8      |                          | Well- or moderate 20      | Poor 58                  |
| Aurello et al. | n.d.                      | n.d.                                 | n.d.         |                          | I-II: 15                  | III: 26                  |
| Kerri et al.   | Intestinal 174           | Intestinal 119                       | Diffuse 62   | Mixed 5                  | I-II: 190                 | III–IV: 51               |
| Lee et al.     | Diffuse (111)            | Diffuse (64)                         | n.d.         |                          | T-1: 2: 53*               | T-3: 4: 58*              |
| Peng et al.    | AC 206                    | AC 205                               | n.d.         |                          | I-II: 103                 | III: 151                 |
| Wu et al.      | Total Intestinal 91      | Total Well- or moderate 62           | Mixed 10     |                           | I-II: 89                  | III: 41                  |
| Wu et al.      | Total Intestinal 72      | Total Well- or moderate 55           | Diffuse 59   | Mixed/unclassifiable 22 | Total I-II: 52            | III–IV: 98               |
| Zhou et al.    | Total Intestinal 100     | Total Well or moderate 100           | Diffuse 125  |                           | Total I-II: 108           | III: 117                 |

Figure 2. Forest plot comparing five-year survival in low and high TSR groups.

Tumour-stroma ratio and overall mortality. The forest plot of the meta-analysis conducted on overall survival data is shown in Supplementary Fig. 2. The hazard ratio for stroma-rich group compared to stroma-poor group was 2.33 (95% CI 1.81–3.01). Similarly to meta-analysis of 5-year mortality, statistical heterogeneity was high ($I^2 = 57\%$), which was mainly caused by the smallest study included. Removal of this study resulted in a HR of 2.13 (95% CI 1.72–2.63) with lower heterogeneity ($I^2 = 41\%$).
only one study provided subgroup analysis of patients with different T-grades, preventing any subgroup analyses. Small study effects were seen in funnel plot with the smallest study included, but the sensitivity analysis without this study showed only small impact on the hazard ratio for five-year survival.

Other ways of measuring survival-progression-free survival (PFS) and disease-free survival (DFS), could not be analysed. None of the studies reported PFS. DFS was reported in three studies, but only one of them defined DFS as time before recurrence of the disease or death, while the other two studies defined DFS as time before recurrence of the disease, excluding death. Therefore, DFS could not be reliably analysed. However, due to the aggressive nature of gastric cancer, five-year- and overall survival should relatively accurately depict outcomes after surgery, while the assessment of PFS and DFS might not provide additional value to this study.

In recent years the knowledge on the stromal component of the tumours has increased. Cancer associated fibroblasts (CAFs) present in tumour stroma seem to be capable of contributing in turning normal tumour growth suppressing stromal microenvironment to one supporting tumour growth. CAFs contribute to tumour growth in several ways. For example they secrete cytokines that promote desmoplastic environment, they might promote angiogenesis and they might contribute to the chemoresistance of the tumour. Stroma-rich tumours might benefit more from the growth-promoting capabilities of tumour, which could explain the worse prognosis of patients with low TSR.

As the role of stroma in the growth of tumour has become more apparent, the interest in development of therapies that target stroma instead of tumour has grown. For example, studies on antibodies against fibroblast growth factor, a signalling molecule for CAFs, antibodies against transforming growth factor beta, a tumor growth enhancing factor produced by CAFs, and T-cell immunotherapies targeting the stroma are currently being developed in preclinical settings. While therapies targeting the stroma have not yet reached clinical use, they might become important in the future. If such therapies become available, it would be interesting to assess whether patients with low TSR would benefit more from these therapies than those with high TSR.

Based on the results of this meta-analysis, TSR is an important prognostic factor in gastric cancer. As it can be easily analysed from HE-stained slides, it could be used to estimate prognosis of patients after surgery and it is one factor that could be considered when planning adjuvant therapy. As the prognostic significance of TSR for patients treated with neoadjuvant chemotherapy is currently unknown, TSR should not be used to estimate prognosis of patients that received neoadjuvant chemotherapy.

In the future, large and prospective studies would be useful to estimate the additional value of TSR in clinical use. Studying TSR in patients before and after undergoing neoadjuvant therapy in relation to prognosis is necessary to apply the results in the neoadjuvant-treated population. New treatments targeting stromal component of the tumour are under development, and could further increase the value of assessing TSR in cancer patients.

In conclusion, this first meta-analysis on TSR and gastric cancer shows that TSR is a strong prognostic factor in gastric cancer. TSR could be easily used as an adjunct for biological aggressiveness when deciding on adjuvant treatment in postoperative gastric cancer patients that have not undergone neoadjuvant therapy.

Data availability
We are willing to share study data upon request.

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Author contributions
N.K. and J.H.K. conceived and designed the study; N.K., M.E. and J.H.K. acquired the data; N.K. and J.H.K. analysed the data; N.K. drafted the manuscript; All authors critically reviewed, edited and approved the manuscript. J.H.K. provided funding, supervised the study and is the guarantor of the study.

Competing interests
The authors declare no competing interests.

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