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RESEARCH ARTICLE

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The amount of signet ring cells is significantly associated with tumour stage and survival in gastric poorly cohesive tumours

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Abstract

Background and Objectives: The aim of this study was to evaluate whether the amount of signet ring cells (SRCs) affects clinicopathological characteristics and prognosis of poorly cohesive (PC) gastric tumours.

Study design: One hundred seventy-three patients with PC tumours treated at three European centres from 2004 to 2014 were reclassified in three categories: (a) pure SRC cancers (SRC1) (≥90% SRCs); (b) PC carcinoma with SRC component (SRC2) (>10%, <90% SRCs); (c) PC carcinoma not otherwise specified (SRC3) (≤10% SRCs).

Results: The percentage of SRCs was inversely related to the pT stage (Spearman's $\rho = -0.174$, $P < .001$) and the number of positive nodes coded as a continuous variable ($P = .009$). Five year cancer-related survival was significantly higher (58%, 95% confidence interval [CI]: 36%-75%) in SRC1 compared with SRC2 (39%, 95% CI: 28%-50%) and SRC3 (38%, 95% CI: 22%-53%), ($P = .048$). In multivariable analysis, the impact of PC categories on cancer-related survival was significant when controlling for sex, age, pT, pN, and curativity (hazard ratio [HR] of sSRC2 vs SRC1 = 2.08, 95% CI: 1.01-4.29, $P = .046$; HR of SRC3 vs SRC1 = 2.38, 95% CI: 1.05-5.41, $P = .039$).

Conclusion: The percentage of SRCs was inversely related to tumour aggressiveness, with long-term survival significantly higher in SRC1 compared with SRC2 and SRC3 tumours.

KEYWORDS

poorly cohesive gastric cancer, pure histology, signet ring cell

1 | INTRODUCTION

Gastric cancer (GC) is still one of the major causes of cancer-related death worldwide.[1] With the decrease of *Helicobacter pylori* infection, the incidence of intestinal-type tumours has been decreasing while that of diffuse-type tumours, especially the poorly cohesive (PC) types, has increased.[2] Controversies still exist regarding the biological behaviour and prognosis of PC tumours and no tailored treatment strategies have been identified so far. These controversies are mainly due to the lack of standardisation of histopathological classification and definitions used.[3-6] Indeed, the Laurèn classification[7] considers together the signet ring cell (SRC) and the other PC tumours as diffuse types, while according to the World Health Organisation classification[8]

a PC adenocarcinoma with most tumour cells displaying a signet ring morphology should be defined as SRC tumour. However, the terms "diffuse," "poorly cohesive," or "signet ring cells" have been indifferently used to describe the same category.

There is an urgent need to clearly evaluate the clinicopathological parameters affecting the prognosis of PC tumours, to identify tailored treatment strategies and allow a proper selection of cases in clinical trials and translational research.

The aim of the present study was to test the hypothesis that the clinical behaviour of PC tumours vary according to the percentage of SRCs, using the recently published classification by the European Chapter of the International Gastric Cancer Association, that divides PC tumours into three categories based on the relative amount of SRCs.[9]

2 | MATERIALS AND METHODS

The present observational multicentre retrospective study included 173 patients with PC tumours treated at three dedicated European surgical centres (University of Verona, CHRU Lille, and Hospital del Mar Barcelona) from 2004 to 2014. PC tumours were coded into three categories according to a recent classification by an European Consensus of experts[9]: (a) SRC1, "pure" SRC cancers having ≥90% of SRCs; (b) SRC2, PC carcinoma with SRC component between >10% and <90%; (c) SRC3, PC carcinoma with ≤10% of SRCs. In each centre, a panel including one expert pathologist, who had participated in the pathological consensus, reclassified the enrolled cases according to the new categories.

2.1 | Statistical analysis

Survival curves were estimated by the Kaplan-Meier method and the significance of differences among curves was evaluated by the Wilcoxon test. The impact of PC categories was further investigated by the Cox regression model, stratifying by the centre and controlling for sex, age, and tumor node mestases status.

3 | RESULTS

The main demographic and clinical characteristics of the 173 patients enroled in the three European centres are reported in Table 1, the surgical treatments in Table 2, and the pathological features in Table 3.

Women represented about half of the series. Most cancers had arisen from the antrum, and were advanced at clinical staging (cT3/cT4 and cN+). Patients from Barcelona were slightly older, while the Verona series comprised a larger proportion of cT4, cN+ and linitis plastica (Table 1). Neoadjuvant treatment and hyperthermic intraperitoneal chemotherapy were seldom used, while adjuvant treatment was administered to 40% of cases. Surgery was performed with curative intent in nearly all cases. Total gastrectomy was preferentially used in Barcelona and Lille, while subtotal gastrectomy in Verona. D2 was the most common lymphadenectomy in all centres, while D3 was performed in 30% of Verona patients. Postoperative morbidity was recorded in about half of patients from Barcelona and Lille, and four patients died in the postoperative period (Table 2). At pathological examination two-thirds of patients were pT3/pT4. The proportion of pT4 was particularly high in Verona. Nevertheless, surgery achieved curativity in about 80% of cases (Table 3).

Thirty-two patients were reclassified as SRC1, 98 as SRC2, and 43 as SRC3. The proportion of SRCs was inversely related to the depth of tumour invasion defined by pT (Spearman's $\rho = -0.174$, $P < .001$) and nodal status ($\rho = -0.154$, $P = .059$) (Table 4).

TABLE 1 Main demographic and clinical characteristics of patients enroled in the three European centres

	Barcelona (n = 51)	Lille (n = 38)	Verona (n = 90)	*P* value
Women (%)	28 (55%)	19 (50%)	45 (50%)	.835
Age (median, range)	66 (34-91)	58 (20-86)	61 (29-88)	.028
Tumour site				.004
Fundus	3 (6%)	3 (8%)	7 (8%)	
Body	10 (20%)	…	10 (11%)	
Antrum	32 (63%)	25 (68%)	60 (67%)	
Pylorus	4 (8%)	…	1 (1%)	
Entire stomach	2 (4%)	9 (24%)	12 (13%)	
cT				<.001
cT0	…	…	1 (1%)	
cT1	5 (10%)	2 (6%)	15 (17%)	
cT2	5 (10%)	12 (35%)	7 (8%)	
cT3	23 (47%)	17 (50%)	25 (28%)	
cT4	16 (33%)	3 (8%)	42 (47%)	
cN				.050
cN0	22 (45%)	20 (57%)	30 (33%)	
cN+	27 (55%)	15 (43%)	60 (67%)	
cM				.513
cM0	47 (96%)	35 (100%)	85 (94%)	
cM1	2 (4%)	…	5 (6%)	
Linitis plastica	6 (12%)	5 (14%)	39 (43%)	<.001
Adjacent organs involved	5 (10%)	8 (22%)	15 (17%)	.292
Ascites	4 (8%)	7 (19%)	10 (11%)	.282

Note: Age was missing in one subject from Barcelona and site in one subject from Lille. cT, cN, and cM were missing in two patients from Barcelona and one patient from Lille. Three, two, and two cases from Lille were classified as undetermined (cTx, cNx, and cMx, respectively). Information on linitis plastica was missing in one subject from Barcelona and one subject from Lille. Information on the involvement of adjacent organs and ascites was missing in one subject from Lille.

TABLE 2 Surgical treatment administered to patients enroled in the three European centres

	Barcelona (n = 51)	Lille (n = 37)[a]	Verona (n = 90)	*P* value
Neoadjuvant treatment	6 (12%)	4 (11%)	9 (10%)	.949
CT only	5 (10%)	4 (11%)	8 (9%)	
CRT	1 (2%)	…	1 (1%)	
Curative intent	45 (88%)	34 (92%)	82 (91%)	.794
Strategy changed	11 (22%)	9 (24%)	15 (17%)	.543
Gastrectomy				<.001
Subtotal	13 (25%)	4 (11%)	56 (62%)	
Total	38 (75%)	37 (89%)	34 (38%)	
Lymphadenectomy				<.001
D1	17 (33%)	7 (19%)	17 (19%)	
D2	34 (67%)	30 (81%)	46 (51%)	
D3	…	…	27 (30%)	
Transhiatal esophagectomy	5 (10%)	1 (3%)	1 (1%)	.043
Resection of other organs	9 (18%)	21 (57%)	20 (22%)	<.001
HIPEC	…	…	5 (6%)	.127
Postoperative complications	25 (49%)	22 (59%)	19 (21%)	<.001
Postoperative mortality	1 (2%)	3 (8%)	…	.018
Adjuvant treatment	26 (51%)	13 (34%)	37 (41%)[b]	.310
Radiotherapy	…	…	3 (3%)	
Chemotherapy	11 (21%)	11 (29%)	25 (28%)	
Chemotherapy + radiotherapy	15 (29%)	2 (5%)	…	

Abbreviations: CT, chemotherapy; CRT, chemoradiotherapy; HIPEC, hyperthermic intraperitoneal chemotherapy.
[a]Information on the type of treatment was missing in one patient from Lille.
[b]Information on the type of adjuvant treatment was missing in nine cases from Verona.

Specifically, 44% of SRC1 were at stage pT1 compared with only 16% of SRC2 and 14% of SRC3. The median of positive nodes was 0.5 (range: 0-30) in SRC1 tumours compared with 7 (0-62) in SRC2 and 4.5 (0-70) in SRC3 ($P = .009$) (Figure 1).

Five years of recurrence rate was significantly higher in SRC2 (62%) and SRC3 (59%) compared with SRC1 (34%) ($P = .026$) (Table 5).

The median follow-up in surviving patients was 69 months (interquartile range: 48-102 months). During the follow-up, comprising 586 person-years, overall 110 deaths were observed, 88 from cancer recurrence, 4 in the postoperative period, and 18 from other causes. Five years cancer-related survival was significantly higher (58%, 95% confidence interval [CI]: 36%-75%) in SRC1 compared with SRC2 (39%, 95% CI: 28%-50%) and SRC3 (38%, 95% CI: 22%-53%), ($P = .048$) (Figure 2). These findings were replicated in multivariable analysis. When controlling for sex and age, the hazard of cancer-related mortality in SRC2/SRC3 cases was increased by 150% with respect to SRC1 cases, and the effect was blunted only to a minor extent when controlling also for pT, pN, and curativity (Table 6). The results of multivariable survival analysis did not substantially change when patients undergoing neoadjuvant chemotherapy were excluded: with respect to SRC1, the hazard ratio (HR) of SRC2 was 2.55 (95% CI: 1.14-5.70, $P = .023$), while the HR of SRC3 was 2.52 (95% CI: 0.96-6.58, $P = .060$).

4 | DISCUSSION

In the present study, we observed that the amount of SRCs in PC gastric tumours is significantly associated with tumour stage at the time of diagnosis, with SRC1 cancers being more frequently detected at early stages. Accordingly, the long-term survival of PC tumours is directly related to the proportion of SRCs, even if when controlling for tumour stage and curativity.

Of note, recent reports on hereditary diffuse GC,[10] related to *CDH1* mutations, showed that in early stages tumours have a pure SRC morphology, while the morphology changes into PC similarly to SRC2 or SRC3 in advanced cancers, where an aggressive phenotype is detected with *TP53* mutations and high Ki67 proliferation index.[10] Another study suggested that in both hereditary and sporadic cases, early intramucosal lesions of PC cancers are mostly composed of well-differentiated SRCs, while neoplastic invasion beyond the gastric mucosa is associated with

TABLE 3 Pathologic characteristics of cancers in the series enroled in the three European centres

	Barcelona (n = 51)	Lille (n = 38)	Verona (n = 90)	*P* value
Depth of tumour invasion				.001
pT1	9 (18%)	11 (29%)	18 (20%)	
pT2	5 (10%)	3 (8%)	2 (2%)	
pT3	13 (25%)	11 (29%)	8 (9%)	
pT4	24 (47%)	13 (34%)	62 (69%)	
Nodal metastases				.772
pN0	16 (31%)	18 (47%)	27 (30%)	
pN1	4 (8%)	3 (8%)	9 (10%)	
pN2	7 (14%)	5 (13%)	11 (12%)	
pN3a	11 (22%)	7 (18%)	23 (26%)	
pN3b	13 (25%)	5 (13%)	20 (22%)	
pM+	6 (12%)	5 (13%)	13 (14%)	.958
Proximal margins (positive)	3 (6%)	3 (8%)	2 (2%)	.242
Distal margins (positive)	6 (12%)	7 (19%)	1 (1%)	<.001
SRC class				.003
90%-100%	4 (9%)	12 (32%)	16 (18%)	
11%-89%	22 (49%)	17 (45%)	59 (66%)	
0%-10%	19 (42%)	9 (24%)	15 (17%)	
Linitis plastica	5 (10%)	…	12 (13%)	.039
Lymphatic invasion	27 (53%)	5 (14%)	43 (48%)	<.001
Neural invasion	32 (63%)	7 (19%)	62 (70%)	<.001
Retrieved nodes	36 (18-80)	30 (6-58)	33 (3-88)	.058
Positive nodes	6 (0-70)	1 (0-29)	5.5 (0-62)	.081
Curativity				.211
R0	38 (75%)	45 (76%)	77 (86%)	
R1	6 (12%)	7 (12%)	9 (10%)	
R2	7 (14%)	7 (12%)	4 (4%)	

Note: One patient from Lille lacked information on proximal margins, distal margins, linitis plastica, lymphatic and neural invasion, retrieved and positive nodes, curativity. One patient from Verona lacked information on the lymphatic and neural invasion. Information on SRC class was missing in six patients from Barcelona.
Abbreviation: SRC, signet ring cell.

TABLE 4 pTNM status as a function SRC classification

	SRC ≥ 90% (n = 32)	SRC 11%-89% (n = 98)	SRC ≤ 10% (n = 43)	*P* value
Depth of tumour invasion				<.001
pT1	17 (53%)	16 (16%)	5 (12%)	
pT2	1 (3%)	6 (6%)	2 (5%)	
pT3	2 (6%)	15 (15%)	13 (30%)	
pT4	12 (37.5%)	61 (62%)	23 (53%)	
Nodal metastases				.059
pN0	17 (53%)	28 (29%)	14 (33%)	
pN1	5 (16%)	6 (6%)	4 (9%)	
pN2	4 (12.5%)	13 (13%)	5 (12%)	
pN3a	2 (6%)	29 (30%)	9 (21%)	
pN3b	4 (12.5%)	22 (22%)	11 (26%)	
pM+	2 (6%)	18 (18%)	3 (7%)	.092
Positive nodes (median, range)	0 (0-30)	7 (0-62)	5 (0-70)	.009

Note: P values were computed by Fisher's exact test.
Abbreviations: pTNM, pathological tumor node mestases; SRC, signet ring cell.

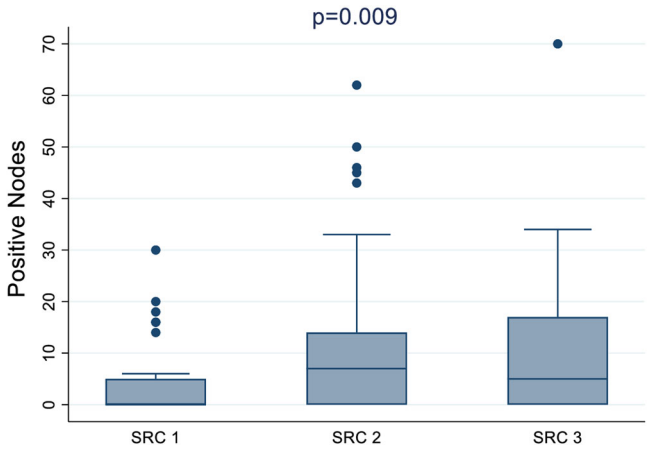

FIGURE 1 Box-and-whiskers plot of positive nodes, as a function of signet ring cell (SRC) categories [Color figure can be viewed at wileyonlinelibrary.com]

depletion of SRCs.[11] Interestingly, a recent report by Korean authors showed that SRC predominant tumours (containing more than 50% of SRCs) were associated with earlier tumour stage and better prognosis compared with PC tumours having more than 50% of neoplastic cells displaying PC non-SRC morphology.[12]

Our study reinforces the evidence suggesting that the proportion of SRCs is a marker of differentiation in PC subtype that can predict tumour aggressiveness and prognosis, and further suggests that the threshold for tumour aggressiveness is as low as 10% of dedifferentiated cells.

Our findings could partly explain the discrepancies in survival, recorded in a series of gastric SRC carcinoma from different geographic areas or with different tumour stages.[3-6] Indeed, it could be hypothesised that different series comprised different proportions of pure SRC type and other PC non-SRC types. Accordingly, in the recently published docetaxel, oxaliplatin, leucovorin, 5-fluorouracil (FLOT) trial[13] the

TABLE 5 Long-term outcomes of gastric cancer as a function of SRC classification

	SRC1 (n = 32)	SRC2 (n = 94)	SRC3 (n = 41)	*P* value
Recurrence	11 (34%)	58 (62%)	24 (59%)	.026
Death from recurrence	11 (34%)	50 (53%)	23 (56%)	.133

Abbreviation: SRC, signet ring cell.

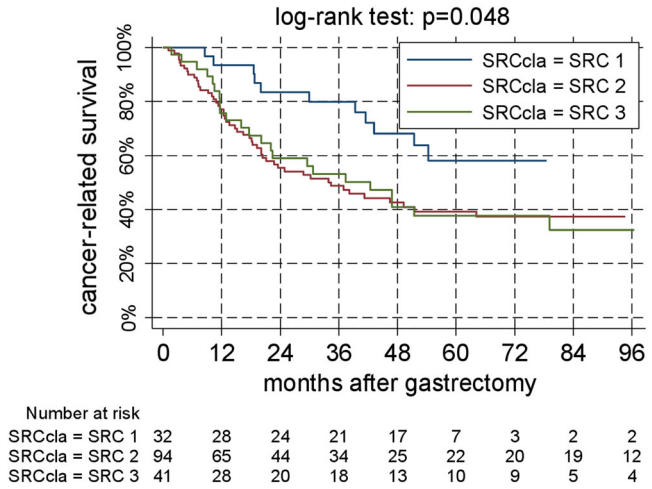

FIGURE 2 Kaplan-Meier survival curves, as a function of signet ring cell (SRC) category [Color figure can be viewed at wileyonlinelibrary.com]

survival benefit of FLOT regimen compared with ECF was significant when considering "SRC" tumours, but not when considering Laurèn diffuse cases, that likely include pure SRC and the other PC tumours. This observation suggests that tumours with different percentage of SRC may have a different response to chemotherapy.

To our knowledge, the present study is the first Western report to suggest that pure signet ring tumours and PC non-SRC types should be considered as separate entities in clinical ad translational studies. This difference could have a high clinical impact in the era of tailored treatments. However, we do not know yet the concordance between preoperative biopsies and final pathology of the surgical specimen according to this new classification. Evidence on this point should be provided to plan the proper treatment strategies based on tumour's characteristics starting from the time of diagnosis.

Our clinicopathological findings will help to deepen our knowledge of molecular pathways of invasiveness of PC/SRC tumours. Indeed, the comparison of differential gene expression profile between groups of pure SRC and PC tumours could enable to identify the molecular mechanisms of tumour progression in these GC subtypes.

TABLE 6 Influence of SRC classification on cancer-related survival

	Hazard ratio (95% CI)	*P* value
Adjusted for sex, age		
SRC2 vs SRC1	2.56 (1.28-5.11)	.008
SRC3 vs SRC1	2.59 (1.21-5.55)	.014
Adjusted for sex, age, pT, pN, curativity		
SRC2 vs SRC1	2.08 (1.01-4.29)	.046
SRC3 vs SRC1	2.38 (1.05-5.41)	.039

Note: Hazard ratios and *P* values were obtained by a multivariable Cox model, stratified by centre.
Abbreviations: CI, confidence interval; SRC, signet ring cell.

5 | CONCLUSIONS

The amount of SRCs is inversely related to tumour aggressiveness in PC gastric tumours with long-term survival significantly higher in "pure" SRC cases compared with other PC tumours.

DATA AVAILABILITY STATEMENT

Excel files containing all the data used for statistical analyses are available on Editor's or Reviewer's request.

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