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ORIGINAL ARTICLE

Role of poorly differentiated cluster in gastric cancer: is it a new prognosis factor?

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ABSTRACT

Background: Poorly differentiated Clusters (PDCs) of tumor cells composed of more than five elements have been recently described in gastrointestinal cancers and correlate with a worse prognosis. Our study aims to investigate PDC occurrence in a series of patients with gastric cancer and correlate it with lymph node status and clinical outcome.

Material and Methods: 50 patients were included in the study; PDCs count was graduated as G1, G2, and G3 according to Ueno classification (PDCs count at 20× <5, 5–9 and ≥10 respectively). We collected several clinicopathologic variables such as tumor location, pTNM stage, vascular or perineural invasion, and lymph-node ratio for each case.

Results: The presence of PDCs was related to vascular invasion ($p < .013$) and recurrence event ($p < .027$). When the population was categorized according to the number of PDCs, a significant correlation was found with the presence of lymph node metastasis ($p < .000$), the Lymph Node Ratio ($p < .002$), WHO stage at the diagnosis ($p < .000$) and vascular invasion ($p < .001$). At the univariate and multivariate analysis, PDCs were found as an independent risk factor for recurrence (HR 1.94; CI 95% 1.209–3.121; $p < .006$ and HR 0.401; CI 95% 0.187–0.862; $p < .017$ respectively). The Kaplan–Meier curves for OS and DFS showed a significant association between PDCs and shorter time to recurrence or survival.

Conclusion: PDC is a strong prognostic factor in gastric cancer, easily detectable, and feasible. As far as we know, this is the first report in Literature of a strong correlation between PDC and survival in patients with operated gastric cancer.

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KEYWORDS
Gastric cancer; PDC; prognosis; lymph node metastases; prognostic factor; surgical oncology

Introduction

Gastric cancer is ranked sixth as incidence in both genders and fourth as mortality worldwide with a 5-year survival of about 25%. [1]. Italy is counted among the countries with intermediate incidence (10–20 cases per 100,000 inhabitants) with a decreasing trend in the last years, mainly for the distal forms [2]. Diagnosis and treatment of gastric cancer have been improved in recent years. Nevertheless, it is crucial to predict which patients will have a poor prognosis both in terms of overall survival (OS) and disease-free survival (DFS) in order to improve treatment and provide a more accurate follow-up [3]. Traditional histopathological variables such as tumor size and tumor stage do not assure sufficient prognostic information. Thus, it is necessary to employ the clinicopathological knowledge of tumor biology and tumor microenvironment to identify new prognostic factors [3].

Recent studies on colorectal carcinoma have shown a new histological grading system based on the presence of poorly differentiated tumor cell clusters (PDC) at the tumor mass front. PDCs clusters are composed of five or more cells without the glandular formation and are easily detectable at hematoxylin and eosin-stained slides (H&E) [4]. Based on their number of PDCs with the light microscope at 20× magnification, it is possible to obtain 3 degrees [4]: PDC G1 (<5 clusters), PDC G2 (5–9 clusters), and PDC G3 (≥10 clusters) (Figure 1). In colon cancer, the occurrence of PDCs is related to different unfavorable histological factors such as vascular invasion, tumor budding, and perineural infiltration. It is acquiring strength as an independent histological factor associated with unfavorable prognosis in terms of lymph nodes or distant metastases [4], reduction of the disease-free interval, specific cancer survival, and overall survival [5–7]. PDCs >10 correlates with tumor depth and the extension of tumor infiltration [7–9].

Few studies so far have evaluated PDCs in non-colic epithelial neoplasms, including breast cancers, small bowel tumors, and gastric cancer [10–12]. In particular, a recent study by Gurunluoglu et al. concerning 80 intestinal-type gastric cancer (GC) cases reported a strong correlation between the number of PDC and the number of metastatic lymph nodes, the ratio between metastatic lymph nodes,

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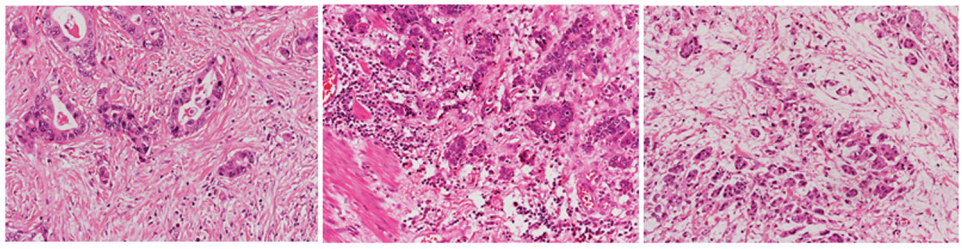

Figure 1. PDC G1, G2 and G3.

and the total number of removed lymph nodes and the pathological stage of lymph nodes (pN) as well as perineural invasion, and suggested that PDCs are relevant as a prognostic factor [12]. Data on diffuse-type GC and PDCs occurrence and survival are still lacking.

Hence in this study, we aimed to assess PDCs occurrence and PDCs grading in a selected cohort of patients affected by GC and compare its prognostic relevance with other histological parameters.

Materials and methods

Clinical and pathological features

This study included 50 gastric adenocarcinoma cases diagnosed between 2009 and 2015 and treated by gastric resection and lymph nodes D2 dissection [13].

For all cases, we reviewed the hematoxylin and eosin-stained slides representative of the whole tumor masses in order to collect the following data: tumor grading and Lauren histotype [14], type of growth (expansive or infiltrative), lymph node metastasis, vascular invasion, and poorly differentiated clusters (PDC). PDCs evaluation and count were performed following the Ueno et al. criteria [4]. According to this grading system, GC cases were subdivided into G1, G2, and G3, counting the maximum number of < 5, 5–9, $\geq$10 PDC, respectively, in an $\times$20 microscopic field.

Statistics

Continuous variables were reported as median and mean, whereas categorical variables were reported as absolute frequencies and percentages. The correlation between PDCs occurrence, PDCs grade, and other clinical or pathological categorical parameters was investigated using the Chi-square test. Overall survival and disease-free survival were calculated for all patients and served as primary end-points. They were defined as the survival duration from surgery to disease progression for DFS and death events for OS. The patients' disease-free survival and overall survival were assessed by the Kaplan–Meier method. The entry date as the date of primary surgery and the Log-rank test assessed statistical significance between groups. Univariate logistic regression analysis was used to evaluate each variable's individual ability to predict the disease's recurrence. Hazard ratios (HRs) with 95% confidence intervals (CIs) have been shown. A multivariate regression analysis was subsequently performed to identify variables that contributed independently to the risk of recurrence. For all tests, p-values $<$.05 were considered statistically significant. The statistical analysis was performed using SPSS software, version number 25 [SPSS Inc., Chicago, IL, USA].

Ethics statement

Histopathological data were anonymously collected and protected by a specific code, under the protocol code 1186-2018/OSS/AOUMO that was reviewed and approved by the Area Vasta Emilia Nord Ethics committee.

Results

The fifty patients elected for statistical analysis were 29 females (58%) and 21 males (42%); the patients' ages ranged from 34 years to 87 years with a mean age of 71. At histology examination, 28 tumors (56%) were classified as intestinal-type and graded in moderately differentiated (7 cases; 25%) and poorly differentiated (21 cases; 75%). Conversely, 22 cases (44%) exhibited the typical diffuse poorly cohesive morphology with a signet ring differentiation in 18 cases (38%) and mixed signet-ring and plasmacytoid pattern in 4 cases (6%). 66% of the tumors were pT3–T4, and 32% were T1–T2; at the onset, 24 (48%) had a neoplasm confined to the stomach in the absence of lymph node and distant metastases, whereas 26 patients (52%) had advanced disease (1 with peritoneal metastasis discovered intraoperatively – PCI = 1; 2%). The presence of PDCs was detected at the front of the advancement of 27 tumors (54%). According to Ueno grading, 12 cases (44%) were classified as G1, 8 cases as G2 (29.6%), and 7 cases as G3 (25.9%). The histological characteristics of the cases studied and the details of the PDCs counts are summarized in Table 1.

Concerning the presence or absence of PDCs, we found that the occurrence of lymph node metastasis and a higher lymph node ratio were statistically significant ($p < .0001$ and $<.002$, respectively). Moreover, vascular invasion ($p < .001$) and tumor stage at the diagnosis ($p < .0001$) were found significantly related to the presence of PDCs. The correlation between PDCs and recurrence events was found significant ($p < .027$). The DFS was significantly shorter in patients presenting PDCs, as shown in the Kaplan–Meier analysis ($p < .02$). On the contrary, neither the death event was related to the presence of PDCs, nor the OS curve showed statistical significance (Figure 2).

To further refine our analyses of PDCs categories in terms of the impact of each on survival, according to Ueno et al.

[4], survival curves of patients were at first separated into four categories: without PDCs, PDC G1 (from 1 to 4 clusters in each field), G2 (5–9 clusters) and G3 (>10 clusters). Curves of PDC 0 and G1 were substantially overlapping (Figure 3), so the analysis was limited to 3 categories of patient PDCs: Category 1 (both patients without PDCs and PDC G1), Category 2 (PDC G2), Category 3 (PDC G3).

70% of the patients were then graduated as G1, 16% as PDC G2, and 14% as PDC G3, respectively.

The characteristics of the population, thus categorized, are summarized in Table 2.

The stratification into the three categories (G1, G2, G3) showed that the increasing number of PDCs at the front of the tumor mass was significantly associated with vascular invasion ($p < .001$), advanced TNM stage at diagnosis ($p < .000$), lymph node metastases ($p < .000$), LNR ($p < .002$) (Table 2).

The DFS and OS curves, when applied to the three PDC categories, were found both significant ($p < .08$ and $p < .024$ respectively) (Figure 4).

In Table 3 the univariate and multivariate cox regression analyses are summarized. The univariate analysis revealed that PDC 3 categories (HR 1.94, C.I. 95% 1.209–3.121, $p < .006$), histotype (HR 1.733, CI 95% 1.050–2.861, $p < .031$), lymph node metastasis (HR 3.306 CI 95% 1.542–7.090 $p < .002$), LNR (HR 38.352, CI 95% 10.043–146.459, $p < .0001$), tumour grading (HR 4.037, CI 95% 1.065–15.303, $p < .040$), vascular invasion (HR 0.087, CI 95% 0.029–0.262, $p < .002$), tumor stage at diagnosis (HR 3.729, CI 95% 1.902–7.312, $p < .0001$), tumour size (HR 0.233, CI 95% 0.086–0.630, $p < .004$) were all significant risk factors for survival of patients with PDCs. Of these, the multivariate analysis emphasized as strong determinants of survival LNR (HR 17.931, CI 95% 1.676–191.646, $p < .017$), grade of the tumour (HR 5.701, CI 95% 1.092–29.765, $p < .039$), vascular invasion (HR 22.812, CI 95% 4.626–112.496 $p < .0001$) and finally PDC 3 categories (HR 0.401, C.I. 95% 0.187–0.862, $p < .017$).

Table 1. Clinicopathological characteristics according to presence or absence of PDC.

Characteristics	PDC−	PDC+	*p*-value
Cohort (50 pt)	23	27	
Age			Ns
<70 yr	6	17	
>70 yr	9	18	
Gender			Ns
Male	10	11	
Female	13	16	
Tumour site			Ns
GE junction	2	1	
Fundus	0	1	
Body	10	7	
Antrum	10	17	
Linitis Plastica	1	1	
Histotype			Ns
Intestinal	16	12	
Diffuse	6	12	
Mixed	1	3	
N			Ns
N−	7	16	
N+	6	21	
LNR			Ns
grading			Ns
G1	2	0	
G2	4	3	
G3	17	23	
Vascular invasion			.013
V−	13	10	
V+	6	21	
Staging			Ns
I	7	2	
II	8	7	
III	8	17	
IV	0	1	
T			Ns
T1–2	10	6	
T3–4	13	21	
Death event			Ns
D	15	21	
A	8	6	
Recurrence			.027
Yes	9	14	
No	19	8	

Discussion

Nowadays, gastric cancer is still a challenge in the matter of therapies and survival due to the high rate of recurrence and metastasis after surgery [15]. Among the unfavorable prognostic factors, the diffuse signet-ring histotype and the presence of lymph node metastases at the time of diagnosis represent some of the dismal significance [16]. In epithelial tumors, it is well known that multiple biological and

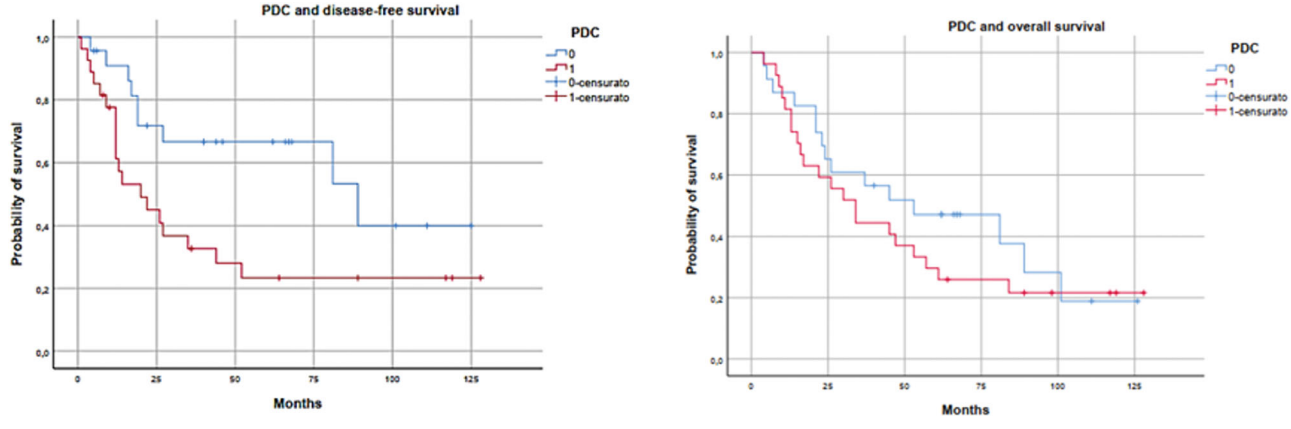

Figure 2. Kaplan–Meier DFS curve ($p < .02$) and OS curve ($p > .05$) according to the presence or absence of PDCs.

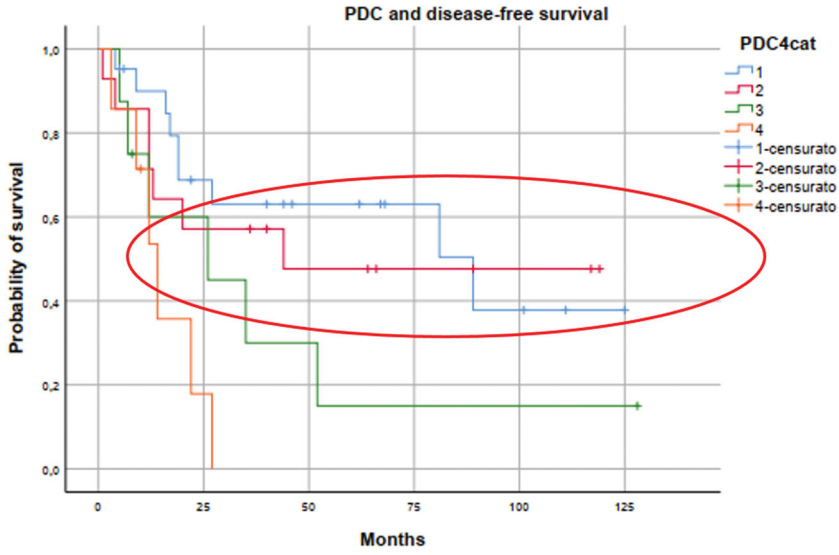

Figure 3. Kaplan–Meier DFS curves of group 0 and G1 substantially overlapping ($p < .020$).

Table 2. Clinicopathological characteristics according to PDC grading.

Characteristics	PDC G1	PDC G2	PDC G3	Chi-square
Cohort (50 pt)	35	8	7	
Age				Ns
<70 yr	9	2	4	
>70 yr	26	6	3	
Gender				ns
Male	14	5	2	
Female	21	3	5	
Tumour site				Ns
GE junction	2	1	0	
Fundus	0	0	1	
Body	15	0	2	
Antrum	16	7	4	
Linitis Plastica	2	0	0	
Histotype				Ns
Intestinal	24	3	1	
Diffuse	9	4	5	
Mixed	2	1	1	
N				.000
N–	13	0	0	
N+	22	8	7	
LNR				.002
0	13	0	0	
<0.1	5	1	0	
0.1–0.25	10	0	1	
>0.25	7	7	6	
Grading				ns
G1	2	0	0	
G2	6	1	0	
G3	26	7	7	
Vascular invasion				.001
V–	19	0	0	
V+	16	8	7	
Staging				.000
I	9	0	0	
II	15	0	0	
III	11	8	6	
IV	0	0	1	
T				ns
T1–2	14	2	0	
T3–4	21	6	7	
Death event				ns
D	13	1	0	
A	22	7	7	
Recurrence				ns
Yes	19	2	1	
No	16	6	6	

molecular factors promote the lymphatic spread of neoplastic cells. These factors include the spreading of neoplastic cells identified at the tumor's advancement and morphologically recognizable in tumor budding and clusters of poorly differentiated tumor cells (PDCs) [17]. PDCs have recently been described in gastric carcinoma in a cohort of 88 patients with intestinal-type tumors. It has been suggested to be a relevant prognostic factor associated with perineural invasion, lymph node metastasis, and high LNR [12].

In our study, we observed the prognostic behavior of PDC in patients operated with gastric cancer through a thorough analysis of risk factors comparing the population categorized in 3 different PDCs grading. As about the simple presence or absence of PDCs, a correlation between PDCs in the growth front and lymphatic or vascular invasion and the recurrence event became evident, thus confirming the role of PDCs in the progression and recurrence of gastric cancer. The correlation became even more relevant when we stratified the cases on the number of PDCs according to the Ueno classification [4]. G2 and G3 PDCs showed more vascular invasion and more lymph node metastases and higher LNR than G1 cases. Also, we observed that patients with high-grade PDCs presented higher WHO staging at diagnosis significantly. Moreover, the Kaplan-Meier curves showed statistically shorter OS and DFS times that worsens through grades of PDCs. Finally, PDCs at multivariate analysis were independent risk factors for poor prognosis together with well-known risk factors like LNR, grading, and vascular invasion.

PDCs, initially described by Ueno et al. in colorectal carcinomas [10], have been recently recognized by WHO among the unfavorable prognostic factors [18]. PDCs seem to be representative, even at the early stage, of tumor growth characterized by the spreading of cell clusters that tend to aggregate in groups of more than five cells without forming glands [6,10]. PDCs are readily detectable in H&E stained slides; the use of auxiliary immunohistochemical stains, such

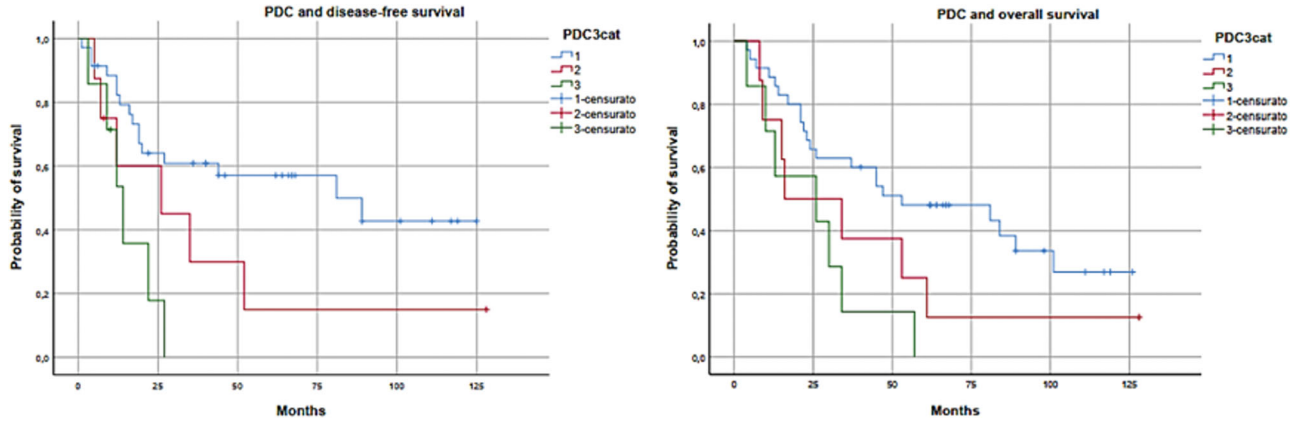

Figure 4. Kaplan–Meier DFS curve ($p < .008$) and OS curve ($p < .024$) according on the three grading categorization of PDC.

Table 3. Univariate and multivariate logistic regression of the main clinicopathological characteristics on DFS.

Characteristics	Univariate HR	95% CI	*p*	Multivariate HR	95%CI	*p*
Age	0.992	0.959–1.026	ns			
Gender	1.060	0.725–1.550	ns			
Tumour site						
Histotype	1.733	1.050–2.861	.031			
N						
LNR	38.352	10.043–146.459	.0001	17.931	1.676–191.646	.17
Grading	4.037	1.065–15.303	.040	5.701	1.092–29.765	.039
Vascular invasion	0.087	0.029–0.262	.002	22.812	4.626–112.496	.0001
Staging						
T	0.233	0.086–0.630	.004			
PDC– vs. PDC+	1.797	0.840–3.843	ns			
PDC 3 categories	1.94	1.209–3.121	.006	0.401	0.187–0.862	.017

as cytokeratins, is unnecessary even when the peritumoral desmoplastic tissue or masking accumulation of inflammatory cells is present in the peritumoral stroma [9–11].

Our study found PDCs mainly in intestinal types, especially in tumors invading the muscle wall and adipose tissue; in diffuse ring-cell histotype tumors, their identification was more difficult and was performed with much more accuracy. These tumors show a poorly cohesive growth, and therefore it is possible to erroneously consider growing tumor cells as PDCs, overestimating their number. This fact could become significant when PDCs are sought in biopsies because the fragmentation of the biopsy material could generate aggregates similar to PDCs that are misleading for diagnosis, as described in the experiences reported by Barresi V et al. in a series of biopsies from colon and rectum [9,19].

To our memory, our present report shows for the first time that PDCs retain a strong statistical significance as risk determinants for a worse prognosis in a population of patients with gastric cancer. Bearing in mind these results, the search for PDCs in gastric biopsies would be desirable as it could be useful in patients' treatment choices. Further studies should be performed on the estimation of PDCs as predictive response factors to preoperative cancer therapy.

Although this study showed that PDCs in gastric tumors have a significant correlation with the occurrence of lymph node metastases and disease progression, our results are preliminary with the limit of a small cohort of cases, albeit with an extended follow-up. Future studies with larger samples and possibly multicentric are eagerly expected as necessary to validate these novel data.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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