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Pedro Fernando Magalhães Valente

Epithelial dysplasia of the stomach with gastric immunophenotype shows features of biological aggressiveness

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Epithelial dysplasia of the stomach with gastric immunophenotype shows features of biological aggressiveness

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Original article

Epithelial dysplasia of the stomach with gastric immunophenotype shows features of biological aggressiveness

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ABSTRACT

Background: Gastric dysplasia is classified as adenomatous/type I (intestinal phenotype) and foveolar or pyloric/type II (gastric phenotype) according to morphological (architectural and cytological) features. The immunophenotypic classification of dysplasia, based on the expression of mucins, CD10 and CDX2, recognizes the following immunophenotypes: intestinal (MUC2, CD10 and CDX2); gastric (MUC5AC and/or MUC6, absent of CD10 and absent or low expression of CDX2); hybrid (gastric and intestinal markers) and null.

Methods: Sixty-six cases of non-polypoid epithelial dysplasia of the stomach were classified according to morphological features (histotype and grade) and immunophenotype. Immunohistochemical staining was performed with antibodies against MUC2, MUC5AC, MUC6, CD10, CDX2, chromogranin, synaptophysin, Ki-67 and TP53. HER2 alterations were analysed by immunohistochemistry and silver-enhanced *in situ* hybridization (SISH).

Results: By conventional histology, dysplasia was classified as adenomatous/intestinal (n=42; 64%) and foveolar or pyloric/gastric (n=24; 36%) and graded as low-grade (n=37; 56%) and high-grade (n=29; 44%). Immunophenotypic classification showed intestinal (n=22; 33.3%), gastric (n=25; 37.9%), hybrid (n=17; 25.8%) or null (n=2; 3.0%) phenotypes. In 20 cases a coexistent intramucosal carcinoma was identified.

The intestinal immunophenotype was shown to be significantly associated with low-grade dysplasia (p=0.001), high expression of CDX2 (p=0.015), TP53 (p=0.034), synaptophysin (p=0.003) and chromogranin (p<0.0001); the gastric immunophenotype was significantly associated with high-grade dysplasia (p=0.001), high Ki-67 proliferative index (p=0.05) and coexistence of

intramucosal carcinoma (p=0.013). HER2 amplification was observed in 3 cases, typed as gastric or hybrid.

Conclusions: Epithelial non-polypoid dysplasia of the stomach with gastric immunophenotype shows features of biological aggressiveness and may represent the putative precursor lesion in a pathway of gastric carcinogenesis originated *de novo* from the native gastric mucosa, leading to gastric type adenocarcinoma.

MINI ABSTRACT

Epithelial dysplasia of the stomach encompasses two major immunophenotypes, intestinal and gastric, the latter significantly associated with features of biological aggressiveness: high-grade, high proliferative index and coexistent carcinoma.

Key words: Gastric carcinogenesis; dysplasia; *HER2*; immunophenotype; mucins.

INTRODUCTION

At present, gastric carcinoma (GC) has a significant morbi-mortality impact, being the fourth most incident cancer worldwide and the second deadliest one (1).

According to Laurén's classification (2), there are two main subtypes of GC - intestinal and diffuse - that differ in epidemiology, pathogenesis, morphology and molecular features (2, 3). According to the Correa model, gastric cancer develops along a cascade of lesions encompassing *Helicobacter pylori* induced chronic superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and ultimately invasive adenocarcinoma (4). Gastric dysplasia is neoplastic in nature and is a direct precursor of gastric carcinoma, as well as a risk factor of carcinoma in other locations of the stomach (5, 6).

Dysplasia is graded as low- and high-grade on the basis of architectural and cell features. Further, according to the histomorphological profile, dysplasia may be classified as adenomatous/type I (intestinal phenotype) and foveolar or pyloric/type II (gastric phenotype). The two types may be distinguished by the immunoexpression of mucins, CD10 and CDX2 (intestinal/adenomatous: MUC2, CD10, and CDX2; gastric/foveolar: MUC5AC and/or MUC6, absence of CD10 and low or absent expression of CDX2) (7-9). Cases with hybrid differentiation may also occur as well as null cases in which there is no expression of the aforementioned markers (8).

A relationship has been reported between the histological grade and the immunohistochemical profiles of dysplasia: in one study, 81.8% of low-grade dysplasia expressed intestinal markers, and 72.2% of high-grade dysplasia showed markers of gastric differentiation with variable expression of intestinal

markers (10). In another study, foveolar and hybrid subtypes were also significantly associated with high-grade dysplasia (8).

In this study we aimed at analysing the relationship between different types of gastric dysplasia (based on histotypes and grading) and the immunohistochemical profile according to the expression of markers of cell differentiation (MUC5AC, MUC6, MUC2, CD10). The expression of CDX2, Ki-67, TP53, HER2 and neuroendocrine markers (chromogranin and synaptophysin) was also evaluated.

MATERIALS AND METHODS

A series of 66 cases of non-polypoid epithelial dysplasia of the stomach identified in Endoscopic Submucosal Dissection (ESD) specimens were retrieved retrospectively from the files of the Department of Pathology, Centro Hospitalar São João, between June/2010 and June/2013. In 20 cases a coexistent intramucosal carcinoma was identified. The study was approved by the Ethical Committee of the Hospital. The clinicopathological features of the cases are summarized in Table 1.

Tissues were fixed in neutral buffered 10% formalin, embedded in paraffin and cut into 3 µm-thick sections.

The lesions were classified in H&E stained slides as: adenomatous/type I (intestinal phenotype) and foveolar or pyloric/type II (gastric phenotype), according to the WHO classification (3). The adenomatous/intestinal subtype resembles colonic adenomas, with crowded, tubular glands lined by atypical columnar cells with overlapping, pencillate, hyperchromatic and/or pleomorphic nuclei, with pseudostratification and mucin. The foveolar or pyloric/gastric

phenotype is characterized by cuboid/low columnar cells, with round to oval nuclei and clear or eosinophilic cytoplasm.

The grade of the dysplasia was evaluated according to WHO 2010 criteria (3): low-grade dysplasia shows minimal architectural disarray and only mild to moderate cytological atypia; the nuclei are elongated/oval, polarized and basally-located and the mitotic activity is mild to moderate; high-grade dysplasia presents pronounced architectural disarray, such as complex branching or fusion of glands; the neoplastic cells are usually cuboidal, rather than columnar, with a high nuclear/cytoplasm ratio, high number of mitoses, occasionally atypical, and nuclei within the luminal zone of the epithelium with loss of polarity. The diagnosis of invasive carcinoma was performed when invasion of the lamina propria or deeper occurred.

Immunohistochemical staining was performed with antibodies against MUC2, MUC5AC, MUC6, CD10, CDX2, chromogranin, synaptophysin, Ki-67, HER2 and TP53 (Table 2). Samples were processed in the automatic equipment Benchmark ULTRA using the Ultraview Universal DAB kit (Ventana Medical Systems, Roche group). Each sample was heated and deparaffinized, followed by antigen recovery through heat and high-pH buffer solution. Each primary antibody was incubated in an individually optimized time and temperature, followed by application of the detection system and contrast with hematoxilin and bluing reagent from the same manufacturer.

Immunoreactivity was scored as follows: the immunoexpression of MUC2, MUC5AC, MUC6, CD10, synaptophysin and chromogranin was scored as positive when ≥5% of the dysplastic cells displayed immunoreactivity; HER2 immunoexpression was scored according to Fassan et al (11): 0 – absence of

immunoreactivity; 1+ – tumour cell cluster with faint or barely perceptible membrane reactivity irrespective of percentage of immunoreactive cells; 2+ – tumour cell cluster with weak to moderate (complete, lateral or basolateral) reactivity irrespective of the percentage of immunoreactive cells; 3+ – tumour cell cluster with moderate to strong (complete, lateral or basolateral) reactivity irrespective of the percentage of immunoreactive cells; for scoring purposes any nuclear or cytoplasmatic backgroung staining was disregarded. The immunoexpression of CDX2 was considered positive when ≥25% of the dysplatic cells displayed immunoreactivity (9); immunoexpression of Ki-67 and TP53 was classified as absent/low when immunoreactivity was displayed in <50% of the dysplastic cells, and high in the presence of ≥50% positive cells (12).

The detection of the number of copies of the *HER2* gene was performed in the cases scored as 2+ and 3+ by immunohistochemistry with SISH automatized technique using the BenchMark XT equipment and the INFORM™ HER2 SISH probe, manufactured by Ventana Medical Systems. The *HER2*/Chr17 ratio of each case was calculated using a minimum of 40 cells in two independent areas of dysplasia. Cases were assigned a score based on the ASCO/CAP guideline recommendations for HER2 testing in breast cancer as follows (13): negative - ratio HER2:Cr17<2.0 with <4 copies of *HER2* gene; borderline - ratio HER2:Cr17<2.0 with ≥4 and <6 copies of *HER2* gene; positive - ratio HER2:Cr17<2.0 with ≥6 copies of *HER2* gene or ratio HER2:Cr17≥2.0.

Statistical Analysis

Appropriate statistical methods were used regarding the type of sample and its distribution. The data was analysed with SPSS software v. 19.0 (SPSS Software, Chicago, IL, USA), using *chi-square* or Fischer's test. *P*-value <0.05 was considered statistically significant.

RESULTS

The study group was composed of 66 cases (Table 1), classified by conventional histology in H&E stained slides as foveolar or pyloric/gastric (n=24; 36%) or adenomatous/intestinal (n=42; 64%) (Fig.1) and graded as low-grade (n=37; 56%) or high-grade (n=29; 44%). According to the immunophenotype, the cases were classified as gastric type (n=25; 37.9%) (Fig. 2 – a, c, e, g, i), intestinal (n=22; 33.3%) (Fig. 2 – b, d, f, h, j), hybrid (n=17; 25.8%) or null (n=2, 3.0%). The latter were not considered for subsequent analysis.

Table 3 summarizes the expression of the different markers in the three immunophenotypes of gastric dysplasia. Statistically significant differences were observed between the immunophenotypes regarding the expression of MUC2 (p=0.002), CD10 (p<0.0001), MUC5AC (p<0.0001) and MUC6 (p<0.0001). Cases with low/absent expression of CDX2 were observed only in the gastric immunophenotype (p=0.015).

The frequency of cases with high expression of Ki-67 was significantly higher in the gastric and hybrid (84.0% and 94.1%, respectively) than in the intestinal (63.6%) immunophenotypes (p=0.05).

The frequency of cases with high expression of TP53 was significantly higher in the intestinal and hybrid (52.9% and 52.9%, respectively) than in the gastric (16.0%) immunophenotypes (p=0.034).

The expression of HER2 (2+ and 3+) was observed in 11 cases with gastric or hybrid immunophenotypes (28.0% and 23.5%, respectively) and not detected in the intestinal immunophenotype (p=0.029).

Amplification of *HER2* gene was observed only in three cases, immunophenotyped as gastric (n=1) and hybrid (n=2).

Regarding the neuroendocrine markers, the frequency of the expression of synaptophysin was significantly higher in intestinal (81.8%) than in hybrid and gastric (58.8% and 32.0%, respectively) immunophenotypes (p=0.003). Similar observations were made for the expression of chromogranin, displayed predominantly in intestinal (95.2%) in comparison with hybrid and gastric (70.6% and 28.0%, respectively) immunophenotypes (p<0.0001). In some cases, immunophenotyped as intestinal, small nests of neuroendocrine cells were observed.

Table 4 shows the relationship between the immunophenotypes and the histotypes of gastric dysplasia (adenomatous/intestinal and foveolar or pyloric/gastric), and grade (low- and high-grade). The frequency of high-grade dysplasia was significantly higher in the gastric immunopenotype (68.0%) than in the other immunophenotypes (47.1% and 13.6%, in hybrid and intestinal, respectively). Within cases with intestinal immunophenotype, dysplasia was graded as low in most cases (86.4%) (p=0.001). Gastric immunophenotype encompassed cases classified by conventional histology as gastric (72.0%) and intestinal (28.0%); hybrid immunophenotype encompassed cases classified by

histology as gastric (35.3%) and intestinal (64.7%) and all cases of the intestinal immunophenotype displayed features of the adenomatous/intestinal histotype ($p<0.0001$).

Table 5 shows the relationship between the presence of the coexistent intramucosal adenocarcinoma and the features of dysplasia (grade, histo and immunophenotypes). In 20 of 66 cases (30.3%), there was a coexistent carcinoma at the periphery of the dysplastic lesions, the latter displaying the following features: high-grade (75.0%; $p=0.001$); gastric histotype (60.0%; $p=0.024$); gastric immunophenotype (65.0%, 20.0% and 15.0% for gastric, hybrid and intestinal immunophenotypes, respectively; $p=0.013$). Gastric dysplasia at the periphery of invasive carcinoma, when compared with gastric dysplasia in the absence of invasive carcinoma, displayed significantly lower frequency of expression of synaptophysin and chromogranin (30.0% and 40.0%; $p=0.006$ and $p=0.025$, respectively).

DISCUSSION

Gastric carcinogenesis is a complex process, still requiring the elucidation of putative distinct pathways. According to the so-called Correa model (4), gastric carcinogenesis is a multistep and multifactorial process that, in many cases, appears to involve a progression from normal mucosa, through chronic atrophic gastritis and intestinal metaplasia, to dysplasia and invasive carcinoma. However, evidence from literature points to the possibility of the existence of alternative pathways in which intestinal metaplasia may not play a role. Evidence stems mainly from the study of tiny early gastric carcinomas arising in non-metaplastic mucosa, as described by Japanese authors (14, 15) as well as

the studies of the expression of markers of gastric differentiation in dysplasia and gastric adenocarcinoma (7, 10, 16, 17). The latter demonstrate that both types of lesions may express, predominantly or exclusively, markers of gastric differentiation, raising the possibility of an origin in native gastric mucosa, rather than in intestinal metaplastic lesions. It remains to be elucidated the role of spasmolytic polypeptide-expressing metaplasia (SPEM) in the pathogenesis of the lesions with gastric immunophenotype. Other evidences stem from hereditary gastric cancer models (Hereditary Diffuse Gastric Cancer and Gastric Adenocarcinoma and Proximal Polyposis of the Stomach – HDGC and GAPPS) in which gastric carcinoma, diffuse/poorly cohesive type and intestinal/tubular types, respectively, originate in non-metaplastic gastric epithelium (fundic gland polyps in the case of GAPPS) (18, 19).

Our study provides additional evidence in favour of *de novo* neoplastic transformation from native gastric mucosa (37.9% of the dysplastic lesions displayed “pure” gastric immunophenotype).

Another relevant issue is the risk of malignant transformation of the different types of gastric dysplasia. Our results show that within the group of cases immunophenotyped as gastric, the majority were classified as high-grade dysplasia (68.0%; p=0.001). At variance, within cases immunophenotyped as intestinal, low-grade dysplasia was the most frequent (86.4%; p=0.001). These findings are in keeping with those recently reported by Nishimura et al (14), but differ from the results reported by Abraham et al (20), the latter showing that intestinal-type adenomas were more likely than gastric-type adenomas to display high-grade dysplasia and adenocarcinoma in the polyps. A major difference from this stud concerns the fact that while the series studied by

Abraham et al (20) was constituted by polypoid adenomas, our series is constituted by non-polypoid dysplasia.

HER2 amplification was observed in three cases, immunophenotyped as gastric or hybrid. These findings show that *HER2* amplification may be an early event in gastric carcinogenesis as observed by Fassan et al (11).

The results herein obtained in dysplasia with gastric immunophenotype (higher frequency of high-grade lesions, expression and amplification of *HER2*) suggest that this type of dysplasia may be an important player in gastric carcinogenesis.

The high frequency of cases with high proliferative index (Ki-67) in gastric and hybrid immunophenotypes (84.0% and 94.1%, respectively) when compared with the intestinal immunophenotype (63.6%; p=0.05) is in keeping with the features of aggressiveness identified in dysplastic lesions with gastric differentiation. At variance with other studies (21, 22) we have not found a significant difference in the Ki-67 proliferation index according to the grade of dysplasia.

In this study, we observed that the expression of CDX2 is correlated with the intestinal immunophenotype (100% of the cases), in keeping with data previously reported (9). In accordance with Park et al (9), a decreased expression of CDX2 was observed in cases with gastric immunophenotype (80% of the cases; decreased intensity of immunoreactivity). However, there is controversy in the literature regarding the expression of CDX2 in gastric dysplasia, probably reflecting the lack of sub-typing of dysplasia in the different studies (9, 23, 24).

In some cases with intestinal immunophenotype small nests of neuroendocrine cells were observed, qualifying for neuroendocrine hyperplasia as reported in the literature (25, 26). It is likely that adenomatous/intestinal dysplasia and neuroendocrine hyperplasia both arise in the setting of chronic atrophic gastritis, as previously suggested in neuroendocrine hyperplasia within gastric hyperplastic polyps (27). However, further studies are needed to elucidate the biological meaning of this event.

In the present study we observed that higher expression of TP53 significantly correlated with the intestinal immunophenotype (p=0.034) and was also more frequently observed in high-grade dysplasia, though this association was not significant (p=0.070 – data not shown). In previous studies, it was observed an increased frequency of TP53 overexpression along the progress of gastric carcinogenesis. However, in these studies the immunohistochemical sub-typing of gastric dysplasia was not performed (28). Kushima et al (29) showed that the frequency of TP53 expression was significantly higher in intestinal-type adenomas than in gastric-type adenomas, in keeping with the present study, and was higher in high-grade dysplasia than in low-grade dysplasia, leading to the suggestion that TP53 alterations occur earlier in the carcinogenetic sequence along intestinal rather than gastric differentiation pathway (29).

Summing up, our results point to the existence of two major types of non-polypoid dysplasia in the stomach. The gastric immunophenotype is significantly associated with high-grade dysplasia (p=0.001), high proliferative index (Ki-67) (p=0.050) and coexistence of intramucosal adenocarcinoma (p=0.013). The intestinal immunophenotype was shown to be significantly associated with low-

grade dysplasia (p=0.001), overexpression of TP53 (p=0.034) and neuroendocrine markers (p=0.003 for synaptophysin and p<0.0001 for chromogranin).

Recently, gene expression profiling using mRNA consensus clustering has revealed three distinct gastric cancer subtypes – mesenchymal, proliferative and metabolic (30). The metabolic subtype is characterized by the expression of genes normally expressed in gastric mucosa, involved in metabolic processes and digestion, and the expression of trefoil peptides (30) that are co-expressed in normal mucosa of the stomach with gastric mucins. These data are in keeping with the results of our previous studies showing the expression of trefoil peptides (and gastric mucins) in a subset of dysplastic and adenocarcinomatous lesions of the stomach (7, 16, 17), supporting the existence of a pathway of gastric carcinogenesis with gastric differentiation.

In face of the evidence we collected and that from the literature, we feel tempted to suggest that non-polypoid epithelial dysplasia of the stomach with gastric immunophenotype may represent the putative precursor lesion in a pathway of gastric carcinogenesis originated *de novo* from the native gastric mucosa, leading to a subset of glandular gastric carcinomas with gastric differentiation.

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affiliate of Hoffmann–La Roche AG (Roche Pharmaceuticals & Roche Diagnostics - Portugal).

TABLES

Table 1. Clinicopathological features of the series of cases.

Age (y, mean ± SD)	65.95 ± 10.93
Sex	
Male	34
Female	32
Tumour size (cm, mean ± SD)	2.51 ± 2.23
Location*	
Body/fundus	20 (31.3%)
Antrum/pylorus	44 (68.7%)

* Missing data for location (2 cases).

Table 2. Primary antibodies and immunohistochemistry conditions used in this study.*

Antibody	Clone	Antigen Retrieval Conditions	Dilution	Incubation time (min) at 37ºC	Localization	Source
CDX2	EPR2764Y	64 minutes at 96ºC	Pre-diluted	28	Nuclear	Cell Marque, USA
MUC2	Ccp58	52 minutes at 96ºC	1:100	36	Cytoplasmatic	Novocastra, UK
MUC5AC	MRQ-19	36 minutes at 96ºC	Pre-diluted	24	Cytoplasmatic	Cell Marque, USA
MUC6	MRQ-20	36 minutes at 95ºC	Pre-diluted	28	Cytoplasmatic	Cell Marque, USA
CD10	SP67	64 minutes at 95ºC	Pre-diluted	40	Membrane (Brush border)	Ventana, USA
Chromogranin	NS55	52 minutes at 96ºC	1:300	36	Cytoplasmatic	Invitrogen, USA
Synaptophysin	SP11	36 minutes at 95ºC	1:150	32	Cytoplasmatic	Neomarkers, USA
Ki-67	SP6	36 minutes at 95ºC	1:400	32	Nuclear	Neomarkers, USA
HER2	4B5	36 minutes at 95ºC	Pre-diluted	12	Membrane	Ventana, USA
TP53	318-6-11	52 minutes at 96ºC	1:200	32	Nuclear	DAKO, Denmark

* Antigen retrieval performed with CC1 (Tris/borate/EDTA buffer with pH 8.4 - Ventana Medical Systems, catalogue number 950-124).

Table 3. Expression of the different markers according to the three immunophenotypes of gastric dysplasia.

	Immunophenotype			p-value
	Gastric	**Hybrid**	**Intestinal**	
MUC2				
<5%	25 (100%)	10 (58.8%)	14 (63.6%)	
≥5%	0	7 (41.2%)	8 (36.4%)	.002
MUC5AC				
<5%	3 (12.0%)	8 (47.1%)	22 (100%)	
≥5%	22 (88.0%)	9 (52.9%)	0	.000
MUC6*				
<5%	2 (8.7%)	1 (5.9%)	22 (100%)	
≥5%	21 (91.3%)	16 (94.1%)	0	.000
CD10				
<5%	24 (96.0%)	3 (17.6%)	2 (9.1%)	
≥5%	1 (4.0%)	14 (82.4%)	20 (90.9%)	.000
CDX2				
<25%	5 (20.0%)	0	0	
≥25%	20 (80.0%)	17 (100%)	22 (100%)	.015
Ki-67				
<50%	4 (16.0%)	1 (5.9%)	8 (36.4%)	
≥50%	21 (84.0%)	16 (94.1%)	14 (63.6%)	.050
TP53				
<50%	21 (84.0%)	8 (47.1%)	13 (59.1%)	
≥50%	4 (16.0%)	9 (52.9%)	9 (52.9%)	.034
HER2				
0, 1+	18 (72.0%)	13 (76.5%)	22 (100%)	
2+, 3+	7 (28.0%)	4 (23.5%)	0	.029
Synaptophysin				
<5%	17 (68.0%)	7 (41.2%)	4 (18.2%)	
≥5%	8 (32.0%)	10 (58.8%)	18 (81.8%)	.003
Chromogranin*				
<5%	18 (72.0%)	5 (29.4%)	1 (4.8%)	
≥5%	7 (28.0%)	12 (70.6%)	20 (95.2%)	.000

* Missing data for MUC6 (2 cases) and chromogranin (1 case).

Table 4. Relation between immunophenotype and the histotype and grade of dysplasia.

	Immunophenotype			p-value
	Gastric	**Hybrid**	**Intestinal**	
Histotype				
Gastric	18 (72.0%)	6 (35.3%)	0	
Intestinal	7 (28.0%)	11 (64.7%)	22 (100%)	.000
Grade				
Low-Grade	8 (32.0%)	9 (52.9%)	19 (86.4%)	
High-Grade	17 (68.0%)	8 (47.1%)	3 (13.6%)	.001

Table 5. Comparison of the features of gastric dysplasia as isolated lesion or at the periphery of intramucosal gastric adenocarcinoma.

	Adenocarcinoma		p-value
	Absent	**Present**	
Grade of Dysplasia			
Low-Grade	31 (70.5%)	5 (25.0%)	
High-Grade	13 (29.5%)	15 (75.0%)	.001
Histotype			
Gastric	12 (27.3%)	12 (60.0%)	
Intestinal	32 (72.7%)	8 (40.0%)	.024
Immunophenotype			
Gastric	12 (27.3%)	13 (65.0%)	
Hybrid	13 (29.5%)	4 (20.0%)	
Intestinal	19 (43.2%)	3 (15.0%)	.013
HER2			
0, 1+	40 (90.9%)	13 (65.0%)	
2+, 3+	4 (9.1%)	7 (35.0%)	.027
Synaptophysin			
<5%	14 (31.8%)	14 (70.0%)	
≥5%	30 (68.2%)	6 (30.0%)	.006
Chromogranin*			
<5%	12 (27.9%)	12 (60.0%)	
≥5%	31 (72.1%)	8 (40.0%)	.025

* Missing data for chromogranin (1 case).

FIGURE LEGENDS

Fig. 1 Histotypes of gastric dysplasia: (a) foveolar/gastric type, displaying cuboid/low columnar cells, with round to oval nuclei and eosinophylic cytoplasm (H&E, original magnification 100X); (b) adenomatous/intestinal type, displaying tubular glands lined by columnar cells with overlapping, pencillated nuclei with pseudostratification (H&E, original magnification 100X)

Fig. 2 Immunophenotypes of gastric dysplasia: gastric immunophenotype displaying (a) foveolar and pyloric/gastric histotype, high expression of (c) MUC5AC and (e) MUC6 and lack of expression of (g) MUC2 and (i) CD10 (H&E (a) and IHC (c, e, g and i), original magnification 40X); intestinal immunophenotype displaying (b) adenomatous/intestinal histotype, lack of expression of (d) MUC5AC and (f) MUC6, and expression of (h) MUC2 (inset: MUC2 is expressed in goblet cells) and (j) CD10 (inset: CD10 is exhibited at the apical pole of dysplastic cells) (H&E (b) and IHC (d, f, h and j), original magnification 100X)

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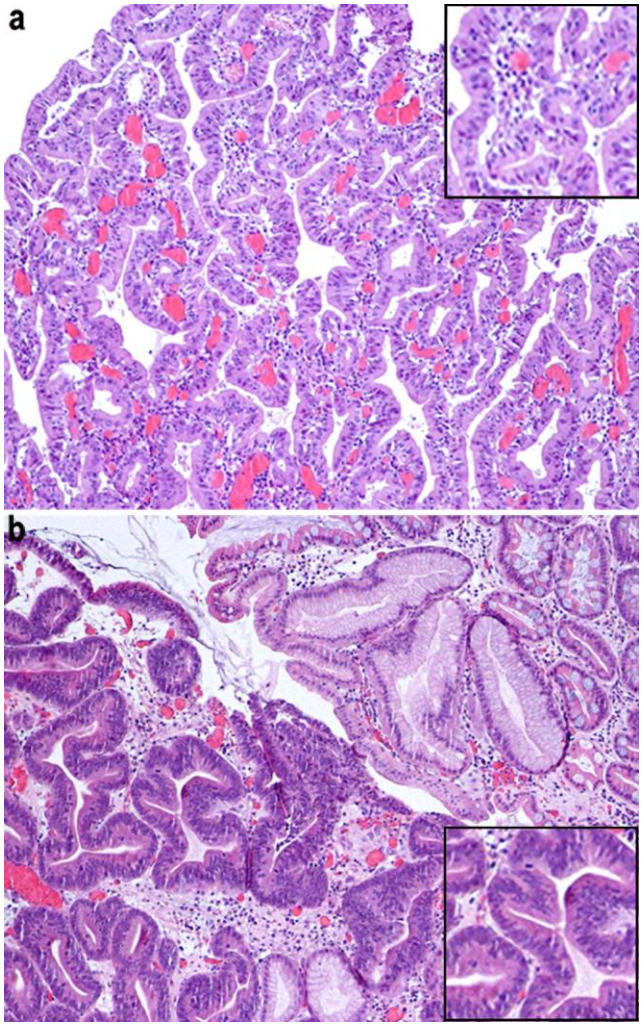
Fig. 1



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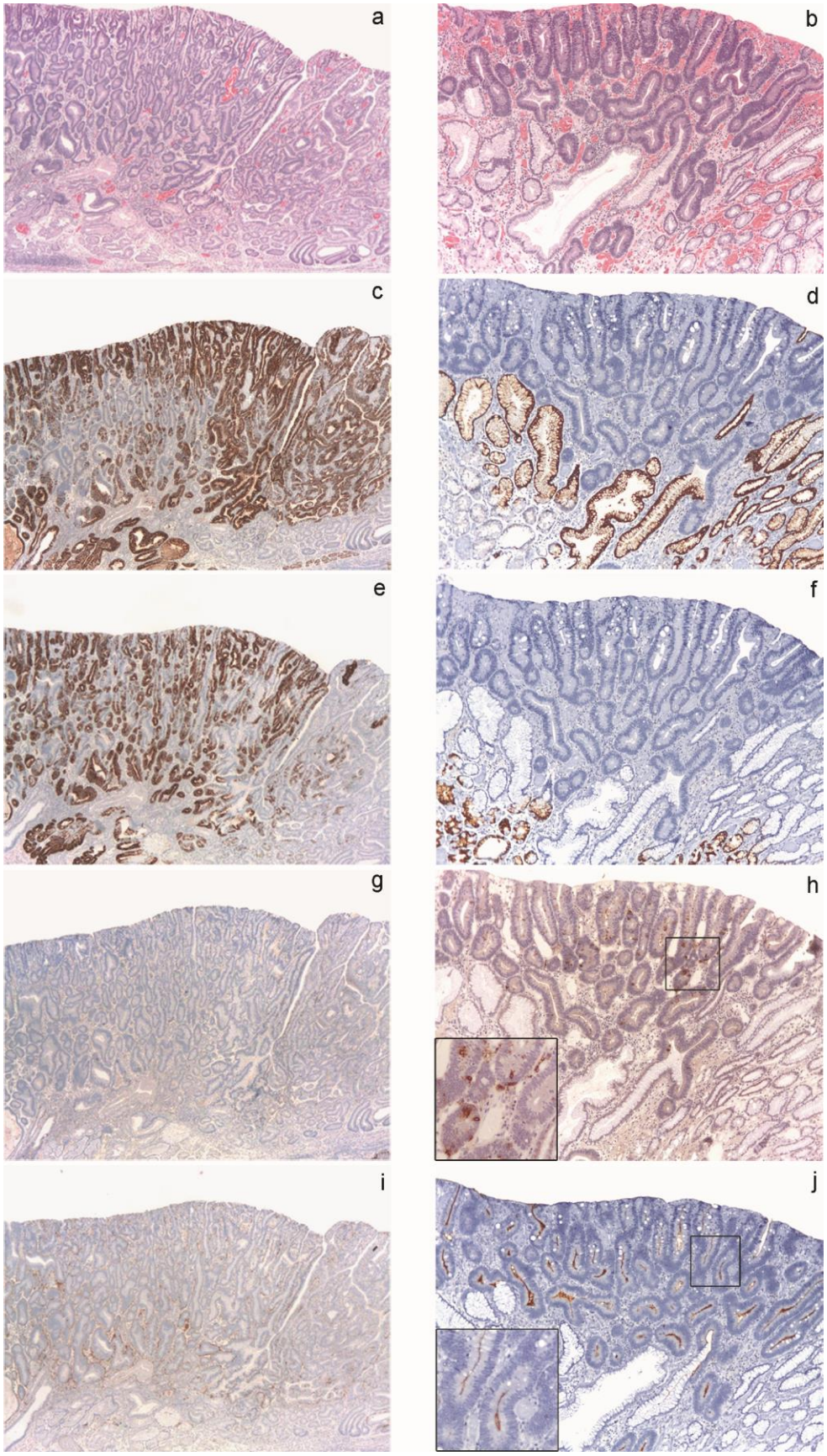

ANEXO

NORMAS DE PUBLICAÇÃO

“GASTRIC CANCER”

Gastric Cancer - Instructions to Authors

Revised February 1, 2014

Gastric Cancer, a joint official journal of the international Gastric Cancer Association and the Japanese Gastric Cancer Association, publishes significant studies related to stomach neoplasms. Original articles (up to 4000 words, excluding references, with no more than seven figures/tables), Case reports (up to 1500, excluding references, with no more than seven figures/tables), Short communications (up to 1500 words, excluding references, with no more than four figures/tables), and Technical notes (up to 1500 words, excluding references, with no more than seven figures/tables) will be peer-reviewed for publication on the understanding that the study has not been submitted simultaneously to or accepted by another journal. The criteria for acceptance are originality and high scientific quality. Review articles (up to 5000 words, excluding references, with no more than seven figures/tables) are in principle solicited by the Editor, but unsolicited manuscripts will also be considered. Letters to the Editor (up to 500 words, excluding references, with no figure/table) commenting on articles published in the journal or expressing views on topics of gastric cancer are welcomed. Meeting reports, at the request of the Editor, will include summaries of symposia or consensus achieved in the congresses of related associations.

Clinical Trial Registration

Any clinical trial for which patient enrollment began on or after January 1, 2014 must be registered. Authors have 6 months from the first patient enrollment to register the trial, but Gastric Cancer recommends registration prior to enrollment. This registration policy applies to prospective, randomized, controlled trials only.

Gastric Cancer follows the International Committee of Medical Journal Editors (ICMJE), which uses the World Health Organization's definition of a clinical trial. The ICMJE defines a clinical trial as "Any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes.' Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration."

The ICMJE lists the following registries as fully compliant:
Australian New Zealand Clinical Trials Registry
ClinicalTrials.gov
ISRCTN Register

UMIN Clinical Trials Registry
Netherlands Trial Register
Eudra CT
Brazilian Clinical Trials Registry (ReBec)
Chinese Clinical Trial Registry (ChiCTR)
Clinical Research Information Service (CRiS), Republic of Korea
Clinical Trials Registry - India (CTRI)
Cuban Public Registry of Clinical Trials(RPCEC)
EU Clinical Trials Register (EU-CTR)
German Clinical Trials Register (DRKS)
Iranian Registry of Clinical Trials (IRCT)
Japan Primary Registries Network (JPRN)
Thai Clinical Trials Registry (TCTR)
Pan African Clinical Trial Registry (PACTR)
Sri Lanka Clinical Trials Registry (SLCTR)

Upon submission, authors must provide the registration identification number and the URL for the trial's registry.
Authors can post their results in clinical trial registries as part of these requirements without it being considered previously published or overlapping publication.

Prerequisites for publication

Certification form: A certification form, which is available at: http://www.springer.com/10120, must be submitted to the journal's editorial office by uploading it as a PDF file at the same time you submit your manuscript via Editorial Manager.
IMPORTANT: The reviewing process starts only upon receipt of the Certification Form.

Manuscript submission via Editorial Manager

Authors should submit their manuscripts to Gastric Cancer online. Please log in directly at: https://www.editorialmanager.com/gcan and upload your manuscript following the instructions given on the screen. Please use the Help option to see the most recently updated system requirements.

Preparation of manuscript

Manuscript format and style should be in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (http://www.icmje.org). Type the manuscript double-space throughout with margins of at least 25mm. Number pages consecutively, beginning with the title page.

- Title page:

The title page should carry 1) the type of article (e.g., original article, case report, etc.) 2) the title of the article; 3) the names of authors; 4) the name of the department(s) and institution(s) to which the work should be attributed; 5) the name and address of the author responsible for correspondence about the manuscript, with phone and fax numbers and e-mail address; 6) the name and address of the author to whom requests for reprints should be addressed; 7) a short running head of no more than 40 characters (count letters and spaces); and 8) the word count of the article (please note the word limit for each type of article).

- Abstract, mini-abstract, and key words:

The second page should carry an abstract of no more than 250 words. In addition, a miniabstract summarizing the significant conclusion of the study within 30 words should be submitted to appear in the table of contents. The abstracts of Original articles should be structured into four paragraphs: Background, Methods, Results, and Conclusions. Authors should provide three to five key words using terms from the medical subject headings (MeSH) list of Index Medicus.

- References:

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in square brackets on the line, e.g., Ames et al. [1] reported...

Restrict citations, as far as possible, to papers written in English or with an English abstract. Use the style of the examples below, which are based on the formats used by the U.S. National Library of Medicine (NLM) in Index Medicus (http://www.nlm.nih.gov). List the first six authors followed by et al. The references must be verified by the authors against the original documents.

1. Journal article

Jacobson MA, Zegans M, Pavan PR, O'Donnell JJ, Sattler F, Rao N, et al. Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet. 1997;349:1443–5.

2. Journal article by DOI

Mitchell AJ, Vaze A, Rao S. Clinical diagnosis of depression in primary care: a meta-analysis. Lancet. 2009. doi: 10.1016/S0140-6736(09)60879-5.

3. Book chapter

Lefor AT, Flowers JL, Bailey RW. Laparoscopy in gastrointestinal malignancies. In: Wanebo HJ, editor. Surgery for gastrointestinal cancer: a multidisciplinary approach. 2nd ed. Philadelphia: Lippincott-Raven Publisher; 1997. pp. 145–59.

- Tables:

Type each table with double spacing on a separate page. Number tables consecutively in the order of their first citation in the text and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body. Explain in footnotes all nonstandard abbreviations that are used in each table. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

- Figures:

All figures should be cited in the text and numbered consecutively throughout. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication each item will still be legible. Figure parts should be identified by lowercase roman letters (a, b, etc.). If illustrations are supplied with uppercase labeling, lowercase letters will still be used in the figure legends and citations. Color figures will always be published in color in the online version. In print, however, they will appear in color only if the author agrees to make a contribution to printing costs (¥ 140,000 per article). For more information about preparing illustrations, please refer to the artwork guidelines available at the end of this document.

- Legends for figures:

Type legends for figures using double spacing, starting on a separate page, with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend.

- Units of measurement:

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. All hematologic and clinical chemistry measurements should be reported using the metric system of the International System of Units (SI).

- Abbreviations and symbols:

Use only standard abbreviations. Avoid abbreviations in the title and abstract. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Case report

Gastric Cancer publishes case reports with new findings. The following will be considered for publication. (A) Cases that have an important clinical impact: 1) Remarkable effects of a new therapy for gastric cancer; 2) Previously unreported adverse events in treatments for gastric cancer; 3) Novel suggestions or pitfalls in diagnosing gastric cancer; (B) Cases that may provide significant clues to the etiology or natural history of gastric cancer:

1) Combinations of gastric cancer and other diseases in which molecular biological findings provide new etiological information; 2) Previously unreported subtypes, syndromes, or familial accumulations of gastric cancer; 3) Untreated disappearance or long-term stable conditions of gastric cancer, including proof; 4) Previously unreported metastatic or progressive patterns of gastric cancer.

Reports of the following cases will not be accepted for publication. 1) Coincidental combination of gastric cancer and other diseases with no new etiological information;

2) Rare gastric cancer with no new findings;

3) Exceptional effects of conventional treatments without data explaining these results.

Provide an unstructured abstract of no more than 150 words. The text should not exceed 1,500 words (excluding references), and only essential figures and/or tables should be provided (no more than 5 items).

Authors of the report should be only those who made substantial contributions to the study. When histological findings play a key role in the report, a pathologist should be included as an author. Information that can identify patients must be omitted, or if in a figure, carefully masked.

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・Information that is more convenient in electronic form: sequences, spectral data, etc.

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