Serum Pepsinogen as a Biomarker of Gastrointestinal Stromal Tumors (GIST) in Stomach

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Abstract

Background

No biomarker was identified for gastric GISTs(GG) detection. We first observed that glands surrounding GG are regionally atrophic. We hypothesize that this local atrophy may mildly reduce pepsinogen Ⅰ(PGⅠ) but the PGⅠ/PGII ratio remains normal. To test our hypothesis, a retrospective analysis was conducted to evaluate the diagnostic efficiency of PG in GG detection.

Methods

We retrospectively analyzed a cohort of consecutive GG and gastric cancer(GC) patients at our center. Pathologic confirmed GG patients and GC patients with tested PG levels before medical intervention were included. Three criteria were assessed: 1.Serum PGI≤70ng/ml; 2.Positive-Gastric-GIST-PG(PGⅠ≤70 ng/ml and PGI/PGII ratio>3.0); and 3.Positive-Gastric-GIST-PG-CEA(Positive-Gastric-GIST-PG plus normal CEA). Sensitivity, specificity, positive predictive value(PPV), negative predictive value(NPV) and overall accuracy(OA) were calculated. A Chi-square test was applied to detect the differences.

Results

After screened 562 GG and 1090 GC, 100 GG and 174 GC samples were included. For PGI≤70ng/ml, the Positive-Gastric-GIST-PG and the Positive-Gastric-GIST-PG-CEA criteria discriminating GG from GC, the sensitivities were 75%(95%CI 66-82),70%(60-78) and 68%(58-76), respectively; the specificities were 50%(43-57), 70%(62-76), and 78%(71-83), respectively; the PPV were 46%(39-54), 57%(48-65), and 64%(55-73), respectively; the NPV were 78%(69-84), 80%(73-86), 81%(74-86), respectively; and the OA were 59%(53-65), 70%(64-75), and 74%(69-79), respectively. There was statistic difference in sensitivities between GG and GC for Positive-Gastric-GIST-PG-CEA criterion(68% for GG vs. 22% for GC, P<0.0001).

Conclusion

Serum PGs are useful for both detecting GG and distinguishing GG from GC. Integrating our criteria into current PG test scheme of gastric precancerous screening will be helpful for early GG detection without additional economic expense.

Introduction

Gastrointestinal stromal tumors (GISTs) are rare, but they are the most common mesenchymal tumors of the gastrointestinal (GI) tract, accounting for 5·7% of all types of sarcomas.[1] Most GISTs (50%-70%) arise in the stomach. With similar ultrastructure to interstitial pacemaker cells of Cajal, GISTs are recognized as predominantly associated with KIT or PDGFRA.[2] Immunohistochemistry (IHC) of KIT, discovered on GIST-1 (DOG-1) and CD34, has been proven to be reliable in GIST diagnosis.[3–5] The incidence of clinical GIST sharply increased to 10–22 per million per year worldwide with the improvement of pathological

understanding and the wide use of CD117 IHC staining since 2000.[6–11] However, the true incidence is much higher than that if pathological microscopic GISTs (< 1 cm) and minimal gastric GISTs (GGs) (< 2 cm) are included.[12–14] Complete resection avoiding tumor rupture is the mainstay strategy used to cure GISTs. Tyrosine kinase inhibitors (TKIs) increased the 5-year survival of GG from 46% to 66–90%.[10,15–18] However, most advanced stages, which account for approximately 20% of GGs at diagnosis, are still incurable.[15] Because most GGs (58%) are asymptomatic, they are hardly alert to medical consulting.[19] For GGs≤5 cm, which have a better prognosis than larger size GGs, 65% patients were asymptomatic.[19]

Pepsinogen (PG) is the precursor of pepsin specifically produced in the stomach. PGII is synthesized and secreted by chief cells in both gastric oxyntic glands at the corpus and pyloric glands in the gastric antrum, whereas PGI is secreted by only the corpus stomach mucosa. Although only 1% of PG is secreted into the bloodstream, the serum/plasma PG levels are quite stable within approximately 10 years in more than 90% of adults.[20] This high stability makes serum PG levels a good noninvasive biomarker for oxyntic gland population decrease in the stomach. It increases in the early stage of inflammation, Helicobacter pylori (*Hp.*) infection and gastric ulcers and decreases in mucosal atrophy. A decrease in the oxyntic gland population leads to a reduction in serum PGI, which may result in a lower PGI/PGII ratio. Serum PGI ≤ 70 ng/ml plus PGI/PGII < 3 is a widely used criterion for screening atrophic gastritis (AG).

In GG hematoxylin and eosin-stained (HE) slides, we found that some glands surrounding GG are atrophic, while no atrophy occurred in glands of resection margin. We also found obvious atrophy of the GIST-surrounding mucosa in the HE slides of an 8-year-old GG patient (Fig. 1). Because PG decreases with severe AG, we hypothesize that this local atrophy may reduce PG and but is not enough to reduce the PG/PGII ratio. To test our hypothesis, we first searched the data from June 1, 2012, to December 31, 2012, in our hospital database (PG tests were available at our hospital since June 2012). Patients who met the two criteria were included. First, GG was diagnosed by both HE and CD117 positivity. Second, PGI and PGII were tested before medical intervention. Ten patients fulfilled the criteria. Four patients showed both abnormal PGI levels (≤ 70ng/ml) and normal PGI/PGII levels (ratio > 3·0). The pathological slides of six patients were available. The slides of 2 patients had no surrounding gastric mucosa. Three of the remaining 4 patients showed obvious mucosal atrophy (Table S1). To further test our hypothesis, we conducted a retrospective analysis. Considering that gastric cancer (GC) is the most common epithelial tumor of the stomach, we compared PG and carcinoembryonic antigen (CEA) levels between GG and GC patients.

Methods

Study patients

We retrospectively screened GG inpatients and outpatients in our hospital from January 1, 2013, to April 30, 2019. GC patients were also screened from a database (those patients were tested for PG levels before medical intervention and confirmed to be diagnosed with adenocarcinoma at our hospital from

July 2012 to August 2016). The inclusion criteria were as follows: (1) patients of any age or sex diagnosed with GG or gastric adenocarcinoma and (2) patients with tested PG levels before medical intervention. The diagnosis of GIST was based on morphological and IHC findings. Positive CD117 and/or DOG1 and/or CD34 were determined by IHC. The exclusion criteria were as follows: (1) patients with renal failure or synchronous other GI cancer; (2) patients with previous gastric surgery; (3) patients with previous blood transfusion within 4 weeks before the PG test; (4) patients with a history of Hp. eradication; (5) patients who took proton pump inhibitor (PPI)/nonsteroidal anti-inflammatory drugs (NSAIDs) or traditional Chinese medicine (TCM) within 4 weeks before the PG test; and (6) patients with a gastric ulcer history. Serum PG and CEA levels were extracted from medical records. The serum PG status was defined as Positive-Gastric-GIST-PG when the criteria of both serum PG□ level ≤ 70 ng/ml and PG□/PGII ratio > 3·0 were simultaneously fulfilled. Positive-Gastric-GIST-PG-CEA was defined as Positive-Gastric-GIST-PG plus normal CEA (CEA ≤ 5 μg/L). HE and IHC slides were reviewed by two pathologists (Gao and Huang) for atrophy evaluation. If there were disagreements between them, a third pathologist (Sun) judged whether there was atrophy. Because there were clear boundaries between the tumor lesions and surrounding glands in GG rather than in GC, only GG patients were evaluated for gland atrophy. Blinding was not applied to the pathologists because it is easy to recognize GG on HE slides.

This retrospective study was approved by the institutional review board of Tianjin Cancer Hospital. The requirement for informed consent was waived because this was a retrospective study, and all patients were discharged. The authors vouch for the accuracy of the data. No commercial entity was involved.

Patient enrollment

We first reviewed the medical records to confirm the diagnosis and drug consumption of the patients for further assessment. Then, all participants or their family members were interviewed by telephone about their drug consumption and blood transfusion history before the PG test.

Sample collection and analysis

Following the handbook of our hospital, overnight fasting serum levels of PG□ and PGII were measured by using the chemiluminescence microparticle immuno assay method (ARCHITECT pepsinogen □ and pepsinogen □ reagent kit, Abbott, U.S.) in accordance with the instructions of the manufacturer. CEA was measured by Elecsys CEA kit (Roche Diagnostics GmbH, Germany).

Outcome measures and statistical analysis

Three criteria were assessed: 1. Serum PGI ≤ 70 ng/ml; 2. Positive-Gastric-GIST-PG; and 3. Positive-Gastric-GIST-PG-CEA. Subgroup analysis of both groups was also performed. The standard measures of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy (OA) with a two-sided 95% confidence interval (CI) were calculated with the Wilson score method.[21] A chi square test and odds ratio (OR) were used for detection differences of categorical variable.

The kappa value and its 95% CI were calculated to describe the agreement of two pathologists for adjacent gastric mucosa atrophy.

Patients

From January 1, 2013, to April 30, 2019, a total of 562 GG patients and 1090 GC patients were screened. After evaluation, 199 GG patients and 1022 GC patients were interviewed by telephone. In addition to loss to follow-up, PPIs usage was the main reason for exclusion (27 GG patients and 122 GC patients). Finally, 100 GG patients and 174 GC patients were included (Fig. 2). The median age of all included patients was 60 (range 27–83) years. The detailed clinicopathological data are shown in Table 1.

Table 1
Demographic and Clinical Characteristics of the Patients

		Gastric GIST	Gastric Cancer
		(N = 100)	(N = 174)
Age, median (range) — yr		59.5 (27.0–82.0)	61.0 (28.0–83.0)
Male sex — no. (%)*		32 (32)	126 (72)
Alcohol drinking-no. (%)			
	Current drinker	20 (20.0)	49 (28.2)
	Drinking quitters	1 (1.0)	6 (3.4)
	Nondrinker	72 (72.0)	112 (64.4)
	Unknown	7 (7.0)	7 (4.0)
Smoking-no. (%)			
	Current smoker	23 (23.0)	39 (22.4)
	Smoking quitters	2 (2.0)	13 (7.5)
	Nonsmoker	68 (68.0)	116 (66.7)
	Unknown	7 (7.0)	6 (3.4)
Tumor size, median (range)-cm		5.0 (0.5–23.0)	5.0 (0.8–19.0)
Tumor resection-no. (%) *			
	Yes	96 (96)	122 (70.0)
	No	4 (4)	52 (30.0)
Operative type*			
	Laparotomy-no. (%)	56 (56)	103 (59.2)
	Laparoscopic surgery -no. (%)	28 (28)	2 (1.1)
	ESD or EFR-no. (%)	12 (12)	0 (0)
	Palliative resection-no. (%)	0 (0)	15 (8.6)
	No surgery-no. (%)	4 (4)	54 (31.1)

* $P < 0.001$ for male sex, $P < 0.001$ for tumor resection, $P < 0.001$ for operative type, $P < 0.001$ for tumor location, $P < 0.001$for other distant metastases, $P = 0.014$ for abdominal and pelvic cavity metastasis.

ESD denotes endoscopic submucosal dissection, EFR, endoscopic full thickness resection and EGJ, esophagogastric junction

		Gastric GIST	Gastric Cancer
		(N = 100)	(N = 174)
Metastasis-no. (%)			
	Liver metastasis	5 (5)	19 (10.9)
	Lung metastasis	0 (0)	2 (1.1)
	Abdominal and pelvic cavity metastasis *	1 (1)	14 (8.0)
	Other distant metastases *	0 (0)	24 (13.8)
Tumor location-no. (%) *			
	EGJ	43 (43)	36 (20.7)
	Corpus	47 (47)	59 (33.9)
	Corner and antrum	9 (9)	61 (35.1)
	Whole stomach	0 (0)	6 (3.4)
	Location unknown	1 (1)	12 (6.9)
* $P < 0.001$ for male sex, $P < 0.001$ for tumor resection, $P < 0.001$ for operative type, $P < 0.001$ for tumor location, $P < 0.001$for other distant metastases, $P = 0.014$ for abdominal and pelvic cavity metastasis.			
ESD denotes endoscopic submucosal dissection, EFR, endoscopic full thickness resection and EGJ, esophagogastric junction			

As the clinical characteristics of GG and GC are different in the real world, several baseline factors were imbalanced between the two groups. The majority of GGs were located at the esophagogastric junction (EGJ), cardia, fundus and corpus (90/100) compared with 55% (95/174) of GC (P < 0·05). The GC group enrolled more males (72% vs. 32%, P < 0·05). More GC patients had distant metastasis, and fewer GC patients underwent radical surgery. Other factors, including age, alcohol consumption, smoking and tumor size (median size, 5·0 cm vs. 5·0 cm), were not significantly different between the two groups.

The positive rates were 95%, 98% and 98% for CD117, DOG-1 and CD34, respectively, in the GG group. Most patients had C-kit exon 11 mutations (41 out of 56 patients who had tested C-kit mutations). Forty-eight percent of GG patients were asymptomatic (Table S2).

Results

The analysis results of sensitivity, specificity, PPV, NPV and OA were shown on Table 2. PGI ≤ 70 ng/ml had the highest sensitivity (75%, 95% CI 66–82) but the lowest specificity, PPV, NPV and OA. Compared with serum PG□ ≤ 70ng/ml and Positive-Gastric-GIST-PG, the Positive-Gastric-GIST-PG-CEA criterion showed the highest specificity (78%, 95% CI 71–83), PPV (64%, 95% CI 55–73), NPV (81%, 95% CI 74–86)

and OA (74%, 95% CI 69–79) as well as a slightly lower specificity (68%, 95% CI 58–76). Compared with GC, the OR of Positive-Gastric-GIST-PG-CEA for GG detection was 7·6 (95% CI 4·3–13·1, P < 0.0001).

Table 2
Results of different criteria

Criteria		Gastric GIST	Gastric cancer	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Overall accuracy (95% CI)
Positive-Gastric-GIST-PG-CEA	+	68	38	0.68 (0.58–0.76)	0.78 (0.71–0.83)	0.64 (0.55–0.73)	0.81 (0.74–0.86)	0.74 (0.69–0.79)
	-	32	136					
Positive-Gastric-GIST-PG	+	70	53	0.70 (0.60–0.78)	0.70 (0.62–0.76)	0.57 (0.48–0.65)	0.80 (0.73–0.86)	0.70 (0.64–0.75)
	-	30	121					
PG≤70 ng/ml	+	75	87	0.75 (0.66–0.82)	0.50 (0.43–0.57)	0.46 (0.39–0.54)	0.78 (0.69–0.84)	0.59 (0.53–0.65)
	-	25	87					

PG denotes Pepsinogen, Positive-Gastric-GIST-PG, both serum PG level ≤ 70 ng/ml and a PG /PG ratio > 3.0 were simultaneously fulfilled and Positive-Gastric-GIST-PG-CEA, Positive-Gastric-GIST-PG plus normal carcinoembryonic antigen, PPV, positive predictive values, NPV, negative predictive values and OA, overall accurac

Subgroup analysis of sensitivity

GG group

As PG is produced by certain stomach locations and influenced by several factors, it is important to address whether the sensitivity is reproducible in subgroups. Subgroup analysis of the GG group was stratified by tumor location, size, recurrence risk, lesion growth pattern, initial symptoms, sex, age, and *Hp.* infection status. The results are listed in Table 3. The sensitivity of the Positive-Gastric-GIST-PG-CEA criterion was as high as 91% in tumors ≤ 1 cm (10 out of 11 patients) and in patients younger than 50 years (20/22). This criterion also detected 90% zero recurrent risk GG patients (14/17) and all moderate recurrent patients (9/9). The lesion growth pattern of 43 patients was obtained from computed tomography (CT) scan and endoscopy data. The analysis showed that there was no significant difference between exophytic and endophytic masses (20/29 vs. 9/14, P > 0·1). Two of four patients who showed no mass but limited thicker gastric wall on CT scan fulfilled both the Positive-Gastric-GIST-PG and Positive-Gastric-GIST-PG-CEA criteria. The sensitivities were not different between males and females (P > 0·05). All three criteria showed higher sensitivities in *Hp.* negative patients than in *Hp.* positive patients (P < 0·05). Correlations between PGI level and GIST recurrent risk or tumor size were not detected.

Table 3
Subgroup Analysis of Sensitivity in GG group

		No. of patients	PG Ⅰ≤70(ng/ml)	Positive-Gastric-GIST-PG	Positive-Gastric-GIST-PG-CEA
Tumor location-no. (%)					
	EGJ, cardia and fundus	43	34 (79)	31 (72)	30 (70)
	Corpus	47	34 (72)	32 (68)	31 (66)
	Corner and antrum	9	6 (67)	6 (67)	6 (67)
Tumor size-no. (%)					
	≤ 1cm	11	10 (91)	10 (91)	10 (91)
	>1 ≤ 2cm	6	4 (67)	4 (67)	4 (67)
	>2 ≤ 5cm	33	25 (76)	23 (70)	22 (67)
	>5 ≤ 10cm	35	24 (69)	21 (60)	21 (60)
	>10cm	14	11 (79)	11 (79)	11 (79)
Recurrent risk-no. (%)					
	None risk	17	14 (82)	14 (82)	14 (82)
	Very low risk	26	18 (69)	16 (62)	15 (58)
	Low risk	25	19 (76)	17 (68)	17 (68)
	Moderate risk	9	9 (100)	9 (100)	9 (100)
	High risk	15	9 (60)	8 (53)	8 (53)
Lesion growth pattern					
	Exophytic mass	29	21 (72)	20 (69)	20 (69)
	Endophytic mass	14	11 (79)	9 (64)	9 (64)

GG denotes gastric GISTs, PG Ⅰ pepsinogen Ⅰ, Positive-Gastric-GIST-PG, serum PG Ⅰ level ≤ 70 ng/ml and a PG Ⅰ/PG Ⅱ ratio > 3.0 were simultaneously fulfilled, Positive-Gastric-GIST-PG-CEA, Positive-Gastric-GIST-PG plus normal carcinoembryonic antigen, HP., Helicobacter pylori, and EGJ, esophagogastric junction

		No. of patients	PG Ⅰ≤70(ng/ml)	Positive-Gastric-GIST-PG	Positive-Gastric-GIST-PG-CEA
Initial symptoms-no. (%)					
	Symptomless	48	32 (67)	30 (63)	29 (60)
	Abdominal pain	15	10 (67)	8 (53)	8 (53)
	Abdominal distention	6	6 (100)	6 (100)	6 (100)
	Other epigastric discomfort	17	13 (76)	12 (71)	12 (71)
	Reflux	7	7 (100)	7 (100)	7 (100)
	Accidentally found when treating other diseases	4	4 (100)	4 (100)	4 (100)
	Diarrhea	2	2 (100)	2 (100)	1 (50)
	Gastrointestinal bleeding	1	1 (100)	1 (100)	1 (100)
Sex-no. (%)					
	Male	32	22 (69)	22 (69)	20 (63)
	Female	68	53 (78)	48 (71)	48 (71)
Age-no. (%)					
	≤ 39	5	4 (80)	4 (80)	4 (80)
	>39 ≤ 49cm	17	16 (94)	16 (94)	16 (94)
	>49 ≤ 59cm	28	21 (75)	20 (71)	20 (71)
	>59 ≤ 69cm	36	25 (69)	22 (61)	21 (58)
	>69	14	9 (64)	8 (57)	7 (50)
HP. infection status-no. (%)					
	Hp. negative	19	16 (84)	15 (79)	15 (79)
	Hp. positive	12	6 (50)	5 (42)	5 (42)

GG denotes gastric GISTs, PG Ⅰ pepsinogen Ⅰ, Positive-Gastric-GIST-PG, serum PG Ⅰ level ≤ 70 ng/ml and a PG Ⅰ/PG Ⅱ ratio > 3.0 were simultaneously fulfilled, Positive-Gastric-GIST-PG-CEA, Positive-Gastric-GIST-PG plus normal carcinoembryonic antigen, HP., Helicobacter pylori, and EGJ, esophagogastric junction

	No. of patients	PGⅠ≤70(ng/ml)	Positive-Gastric-GIST-PG	Positive-Gastric-GIST-PG-CEA
Hp. infection unknown	69	53 (77)	50 (72)	48 (70)

GG denotes gastric GISTs, PGⅠ pepsinogen Ⅰ, Positive-Gastric-GIST-PG, serum PG Ⅰ level ≤ 70 ng/ml and a PG Ⅰ/PG Ⅱ ratio > 3.0 were simultaneously fulfilled, Positive-Gastric-GIST-PG-CEA, Positive-Gastric-GIST-PG plus normal carcinoembryonic antigen, HP., Helicobacter pylori, and EGJ, esophagogastric junction

GC group

Subgroup analysis of the GC group was stratified by tumor location, size, grade of tumor differentiation, clinical stage, sex and age. The results are listed in Table S3. The sensitivity of Positive-Gastric-GIST-PG-CEA decreased from 38–11% with the clinical stages from stage Ⅰ to stage Ⅲ.

Mucosa atrophy

HE slides with both tumor and surrounding glands were available for 50 patients (7 patients did not have slides; 42 slides did not include the surrounding mucosa and 1 slide was post-neoadjuvant specimen). The kappa value was 0·88 (95% CI, 0·74 – 1·0). Twenty-nine out of 50 (58%) had atrophic mucosa.

Discussion

Even though GG patients have the best prognosis among all GIST patients, nearly 30% of GG cases eventually relapse or metastasize after curative resection of the primary tumor.[22] The most important recurrence factors are tumor size, tumor rupture and mitotic rate per 50 high-power fields. For example, the recurrence rate is 0% for GG patients with lesions ≤ 2 cm.[23] Generally, a smaller GG often means a lower mitotic rate, a lower chance of rupture, a higher chance of receiving less invasive surgery and a better prognosis with TKI therapy.[24] Early detection has been proven to improve the treatment outcomes of GG in Japan.[19] However, there is still a lack of a reliable noninvasive GG detection method. The reason may be that there are no specific symptoms or GG secreting factors. In fact, 48% of GG patients in our study were asymptomatic. Based on our Positive-Gastric-GIST-PG criterion, the serum PG test showed high sensitivities in both asymptomatic GG patients (63%) and small GG patients (14 out of 17 patients with lesions ≤ 2 cm). The benefit of detecting GGs ≤ 2 cm is still unclear. However, 11·4% of GISTs < 2 cm were metastatic (regional/distant).[25] Guidelines recommend different strategies for GG lesions < 2 cm. The National Comprehensive Cancer Network recommended conservative follow-up for GGs < 2 cm without high-risk features. The European Society for Medical Oncology and Japanese guidelines recommend resection for all GISTs < 2 cm. However, studies found that some GGs < 2 cm would enlarge after years of follow-up.[26,27]

Some medicines, such as PPIs and NSAIDs, and diseases (gastric ulcer, non-AG, *Hp.* infection) would increase the PG level. To minimize the drug impact on PG level, we excluded patients who received PPIs, NSAIDs, *Hp.* eradication therapy or TCM. Because most GG patients with GI bleeding received PPIs as first-aid medicine, only 1 patient with GI bleeding was included in our study. In fact, we also analyzed the impact of PPIs on the PG level of GG patients (data not shown). Only 2/11 and 3/16 patients fulfilled the Positive-Gastric-GIST-PG criterion among patients who received PPIs within 24 h and 2 weeks before the PG test, respectively. In clinical practice, PGI ≤ 70 ng/ml plus PGI/PGII < 3 are the most widely accepted values used in AG screening. The lower the PGI and PGI/PGII values are, the more severe the gastric atrophy. Therefore, our criteria can effectively discriminate GG from both AG and GC. As subjects routinely undergo endoscopy examination after PG levels suggest AG in health checkups, limited data about the PG level of endoscopically normal subjects were reported. One study conducted at an area of high incidence GC in China showed that only 1 out of 30 endoscopically normal subjects had PGI ≤ 70ng/ml.[28] These data indicate that our criteria can efficiently distinguish GG from endoscopically normal subjects.

The PG reduction in the Positive-Gastric-GIST-PG criterion is a result of local gastric atrophy. One-time PG test may not efficiently distinguish GG from other diseases of local atrophy, such as early GC and AG. A Japanese study identified 27 previously *Hp.* infected patients in 271 patients with gastric neoplasms (early GC and adenoma). All 27 patients had endoscopic atrophy in the gastric corpus. Twenty-four out of the 27 patients fulfilled our Positive-Gastric-GIST-PG criterion.[29] Considering that the true incidence of GG may be as high as 35% and early gastric neoplasms were undetectable, it is not surprising that another Japanese study found that 42·6% of asymptomatic middle-aged males with high-cancer risk fulfilled our Positive-Gastric-GIST-PG criterion.[29] However, following the Correa cascade, PG levels will stepwise reduce in the process from AG to GC. Based on the finding that the sensitivity of Positive-Gastric-GIST-PG decreased from stage □ to stage □ (from 38–11%) in the subgroup analysis of GC and that neither PG□ level nor PG□/□ differences were detected in different tumor size GG subgroups, periodic PG testing will help to discriminate GG from early AG and GC.

Based on the 22 per million prevalence of GISTs worldwide, the prevalence of clinically relevant GG should be 13·2 per million in the case of 60% of GISTs located in the stomach. According to Bayes' Theorem, the PPV and NPV were 0·0030799% and 99·9994429% with 70% sensitivity and 70% specificity. In fact, the actual incidence of all kinds of GGs is much higher than that. Three studies have reported that the incidence was 2·9%-35% for GGs ≤ 1 cm.[12–14] The incidence was 9·54% (74/776) when we summarized the data of the three studies. The PPV and NPV were 19·75% and 95·68%, respectively. If subjects were double positive, the PPV and NPV would change to 36·47% and 90·46%, respectively.

The baseline characteristics were not balanced between the two groups. However, our study samples represented most patients. In the real world, the prevalence of GC in male patients is 2·38 times that of female patients in China. GG was also slightly predominant in females. GC is more antrum involved and metastatic, while GG seldom metastasizes and rarely occurs in the antrum. The PG test is a noninvasive, repeatable, ray-free, economical and highly acceptable method. Future studies can focus on its role as a

biomarker to evaluate the effect of therapy and postoperative surveillance. Small GGs are often negative on CT scans and endoscopy. Endoscopic ultrasonography can be considered when subjects meet the Positive-Gastric-GIST-PG criteria twice, especially for subjects with high GG risk, such as those with familial GISTs and those with succinate dehydrogenase complex dysfunction.

There were some limitations in our study. The first limitation was its retrospective nature. The second limitation was that our study may still be influenced by selection bias, even though only 62 patients lost follow-up in the 199 followed GG patient. The third limitation of our results was that the PG tests were based on Abbot testing kits. Whether the criteria could be used with other manufacturer testing kits requires further study. A former study showed that the serum PG values using Japanese kits (LZ-test EIKEN; Eiken Chemical Co., Tokyo, Japan) were lower than those using the GastroPanel examination (Biohit Plc., Helsinki, Finland).[30] The fourth limitation was that we did not evaluate the atrophy status of all included GG patients because some slides were unavailable and some resection specimens did not have proffer adjacent mucosa. Despite these limitations, our study offered a new strategy for GG detection and deserved further prospective study. Furthermore, because PG has been applied for AG detection for decades, the PG > 70ng/ml is a standard for healthy cases, we did not think it was a definitely requirement to compare GG with healthy cases.

In summary, our study identified local gland atrophy surrounding the mucosa as a characteristic feature of most GGs. Positive-Gastric-GIST-PG-CEA can effectively distinguish the most common gastric stromal tumor (GG) from the most common GC. The criteria showed high sensitivities in all GG subgroups. Integrating our criteria into the current PG test scheme of gastric precancerous screening will be helpful for the early detection of GG without additional expense. Actually, the age at diagnosis of more than 90% of GG patients is older than 40 years, which is also the age of subjects suitable for Japanese government-sponsored AG screening by PG.

Declarations

Ethics approval and consent to participate

The ethical approval of this study was approved by the institutional review board of Tianjin Cancer Hospital. The informed consent was waived by the institutional review board of Tianjin Cancer Hospital because this was a retrospective study, and all patients were discharged.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors contribution

Likun Zhou: study design, data collation, patient follow-up, data analysis, data interpretation, pathological slides collection, article writing.

Zhiying Gao: data collation, data analysis, data interpretation, pathological slides reading, article writing.

Laizhi Luo: data collation, patients follow-up, pathological slides collection, article writing.

Yueting Han: laboratory results collection.

Yan Sun: pathological slides reading.

Yonghong Huang: pathological slides reading.

Shixia Li: study design comments.

Xinyun Chen: patients follow-up, pathological slides collection.

Huimin Yang: patients follow-up.

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Xi Wu: article writing

Huan Wu: article writing

Jing Bai: article writing

Wu Sun: article writing

Yi Ba: study design, data interpretation, article writing

All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Informed consent, ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the institutional review board of Tianjin Cancer Hospital. The requirement for informed consent was waived because this was a retrospective study, and all patients were discharged.

This manuscript has not been published previously and is not currently under consideration for publication elsewhere.

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Figures

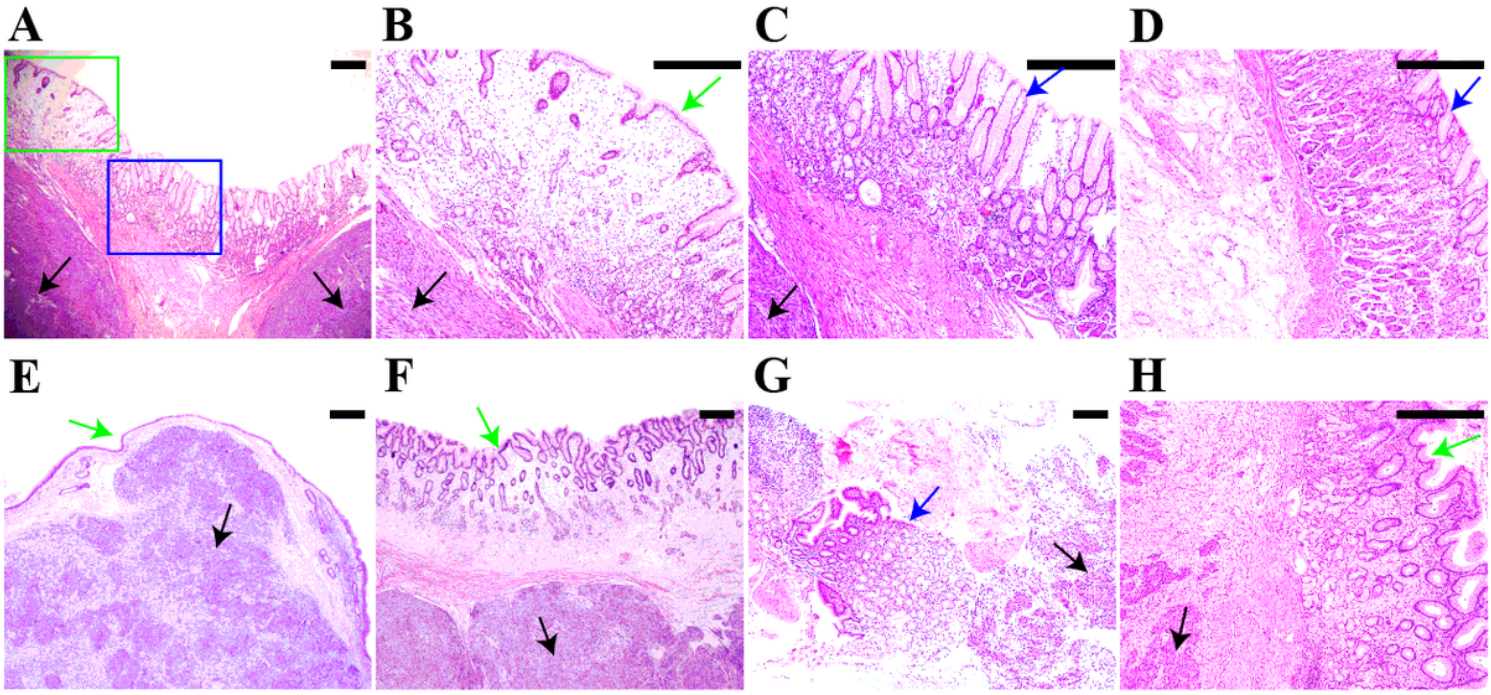

Figure 1

Hematoxylin and eosin-stained (HE) slides of glands surrounding gastric GISTs (GG) Green arrows showed atrophic glands, blue arrows showed non-atrophic glands and black arrows showed GG. (A) showed both atrophic and non-atrophic glands coexist. Glands in the green box were atrophic and glands in the blue box were non-atrophic (×40 times amplification). (B) 100 times amplification of the atrophic glands in green box. (C) 100 times amplification of the non-atrophic glands in blue box. (D) The glands in resection margin were not atrophic. (E) Glands surrounding GG almost disappear with thinner mucosa. (F) Atrophic glands existed in mucosa of normal thickness. Atrophy of the GIST-surrounding mucosa were detected in the resection slide of an 8-year-old GG patient (H), while no atrophy was founded in the biopsy slide (G). Scare-bars, 200 μm.

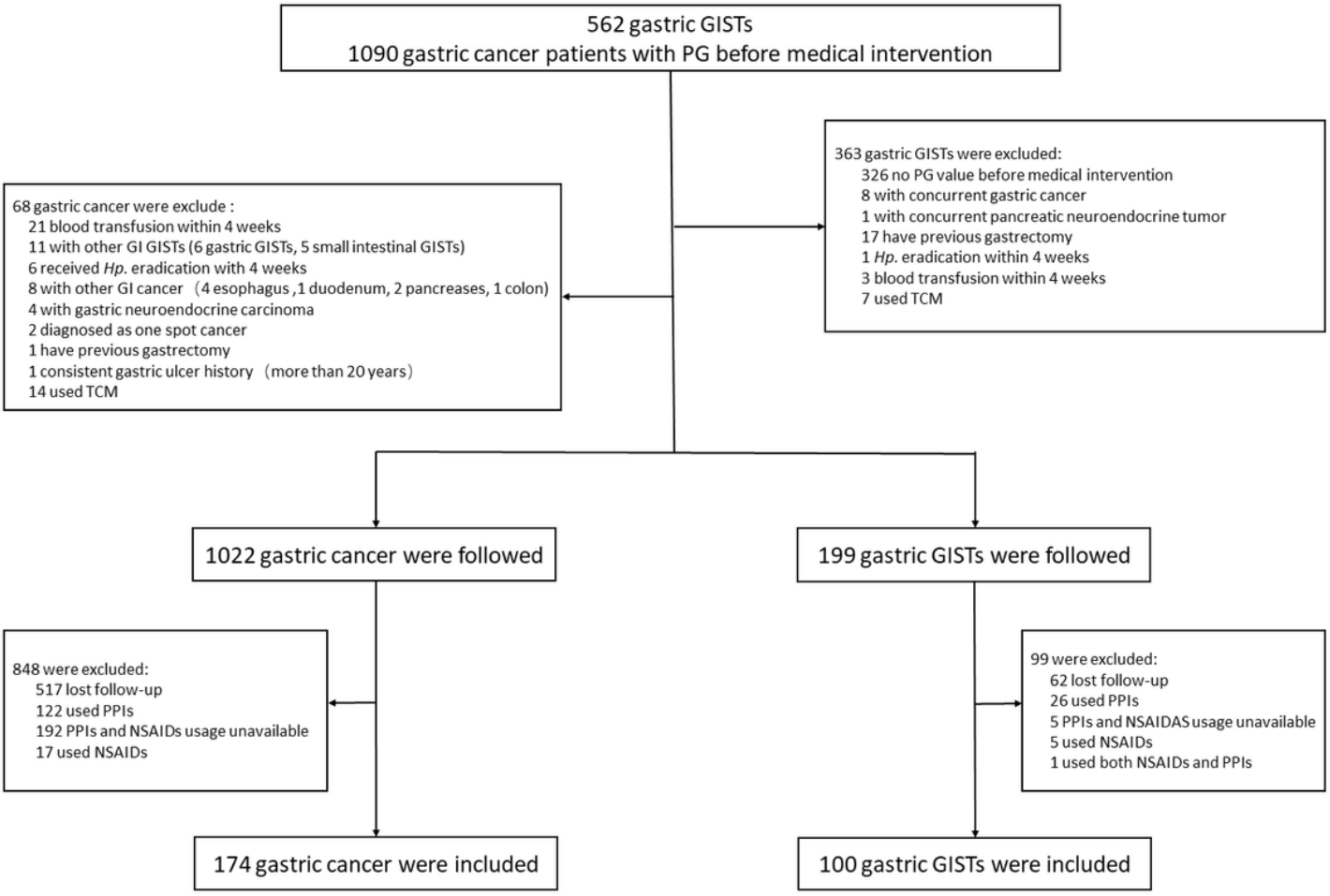

Figure 2

Study flowchart GISTs denotes gastrointestinal stromal tumors, PG, pepsinogen, GI, gastrointestinal, Hp., helicobacter pylori, TCM, traditional Chinese medicine, PPIs, proton pump inhibitors and NSAIDs, nonsteroidal anti-inflammatory drugs.

Supplementary Files

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