Trefoil Factors as Screening Biomarkers for Gastric Cancer and Premalignant Lesions: A Cross-Sectional Population-Based Cohort Study

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Research

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Abstract

Background:

The lack of effective biomarkers for screening gastric cancer (GC) and premalignant lesions (PMLs) is a significant roadblock in the prevention and early intervention of GC. We aimed to identify noninvasive biomarkers to improve the screening of high-risk populations.

Methods:

We evaluated 25,000 adults residing in Wuwei. We collected baseline characteristics, GC risk indicators, including trefoil factors (TFF1–3), endoscopy diagnosis, and pathological information. We analyzed the data to determine the association of risk biomarkers with the progression of GC and the prediction capacities of these biomarkers using odds ratio (OR)-adjusted models and receiver operating characteristic (ROC) curve analyses.

Results:

TFF1 and TFF2 serum levels showed incremental changes from the PMLs to the GC group, with the highest serum TFF3 levels reported in the intestinal metaplasia group. TFF1 and TFF2 had significant predictive values in the PMLs and GC in the three OR-adjusted models but not in the non-atrophic gastritis group. Similar results were obtained after adjusting for all biomarkers and risk factors wherein the ORs (95 % confidence intervals) of TFF1 and TFF2 in the GC group were 2.71 (1.57–4.67) and 2.87 (1.75–4.71), respectively ($P < 0.001$). The combination of TFF1–3 showed the largest area under the curve across all four groups (chronic atrophic gastritis [0.74], intestinal metaplasia [0.79], low-grade intraepithelial neoplasia/dysplasia [0.79], and GC [0.84]), making it the best-fit ROC.

Conclusions:

TFF1, TFF2, and the combination of TFF1–3 can serve as sensitive, specific, and noninvasive biomarkers for detecting GC and PMLs, facilitating the early identification of these lesions.

Background

Although the incidence of gastric cancer (GC) has continuously decreased, it remains the second leading cause of cancer-related deaths worldwide [1]. In 2018, the incidence (32.05/100,000) and mortality (27.42/100,000) rates of GC in China were two to three times higher than the world average (13.54/100,000 and 10.25/100,000, respectively). Furthermore, the incident cases and deaths accounted for 44.1 % and 49.8 %, respectively, of the total GC occurrences worldwide [1, 2]. The high incidence and mortality rates of GC in China are serious public health issues and impose immense burdens on the health care system. Patients with GC have a poor prognosis mainly because most are diagnosed at advanced stages. Thus, early diagnosis is essential for reducing the GC mortality rate.

Endoscopy is currently the standard modality for the diagnosis and clinicopathologic evaluation of GC. Studies have reported a 47 % and 67 % reduction in the GC mortality by gastroscopy screening in Korea and Japan, respectively [3, 4]. However, population-based GC screening and endoscopy as the preferred screening modality in China is a formidable challenge due to its vast territory and large population; thus, the early diagnosis rate in China remains significantly low [5]. Furthermore, endoscopy is highly invasive and relies heavily on the availability of endoscopic

instruments and professional skills. These limitations make endoscopy less appropriate for large-scale population screening, especially in economically underdeveloped and low-risk areas. Thus, simple, efficacious, and noninvasive biomarkers for the high-risk population and subsequent endoscopic examination of identified at-risk individuals would be a reasonable strategy for the mass screening of GC.

Although pepsinogen (PG) and anti-*Helicobacter pylori* immunoglobulin G antibody (Hp-IgG) serum screening methods for GC have been in clinical practice for a long time [6, 7], limitations of its sensitivity and positive predictive value have been reported, and the panel has low reliability [8, 9]. Recent studies have reported a pivotal role of the trefoil factor (TFF) family in the oncogenic transformation, growth, and metastatic extension of common human solid tumors, including GC [10, 11]. The combination of serum TFF and PG can improve GC screening [12, 13]. However, previous research on TFFs was limited to patients with clinically confirmed GC, and their relationship with the significant stages in Correa's cascade of GC development has not been investigated. The purpose of GC screening is to detect early GC and to detect and monitor premalignant lesions (PMLs), which is highly significant and valuable in reducing the incidence and mortality of GC.

The Wuwei region in China has consistently reported high incidence and mortality rates of GC [14]. Thus, it was selected as the setting for a large-scale endoscopic GC screening program between March 2013 and April 2016. The Wuwei Cohort Study provides an opportunity to analyze the association between serum levels of TFFs and the different stages in Correa's cascade of GC development. In the end, we aimed to investigate the usefulness of serum TFFs as biomarkers for the screening of GC and PMLs.

Methods

Study design and subjects

This population-based screening study evaluated 25,000 adults aged 35–70 years residing in Wuwei (Wuwei Cohort) using the cluster sampling method (**Fig. 1**). Those who 1) initially declined consent (n = 1,544), 2) were physically unable to provide consent (n = 85), 3) were pregnant (n = 17), 4) were mentally ineligible (n = 8), or 5) had a medical history of malignancy (n = 83) were excluded. Of the remaining 23,263 participants, 2001 participants either declined or were ineligible for gastroscopy, and 21,262 participants underwent gastroendoscopy. After computer-generated random selection, 9,426 participants were selected to undergo serological testing. Among them, 3,986 participants who completed the survey and had complete data were evaluated in the final analysis.

The Ethics Committee of The First Hospital of Lanzhou University approved this study (approval number: LDYYLL2012001). All subjects provided written informed consent.

Study protocol

Personal information, including a family history of GC, past medical history, lifestyle, dietary habits, and smoking habits, was collected using a self-reported questionnaire survey. A 10-mL blood sample was drawn from the peripheral vein. The sample was immediately centrifuged, and the serum was stored at -80°C. No freeze-thaw cycles were performed before the assays. A certified gastroenterologist performed endoscopic examination (GIF-H260Z/H290Z, Olympus, Japan), and the diagnosis of GC was established independently by three experienced pathologists.

The Wuwei Cohort database was established and managed using Stata software (StataCorp LLC, College Station, Texas, United States). The participants were classified into six groups based on Correa's cascade of GC development:

normal gastric mucosa (NC; n = 124), non-atrophic gastritis (NAG; n = 649), chronic atrophic gastritis (CAG; n = 746), intestinal metaplasia (IM) with CAG (n = 1,002), low-grade intraepithelial neoplasia/dysplasia (LGD) accompanied by CAG and/or IM (n = 1,334), and GC or high-grade intraepithelial neoplasia/dysplasia (HGD; n = 131). HGD was defined as equivalent to carcinoma in situ [15, 16]. Gastric inflammation and atrophy were graded according to the Updated Sydney System [17], and a biopsy diagnosis of gastric epithelial neoplasia was established based on the World Health Organization classification [16].

Biomarker measurements

Serum levels of TFF1, TFF2, and TFF3 (TFF1–3), pepsinogen I (PGI), pepsinogen II (PGII), anti-*H. pylori* IgG antibody titer, high-sensitivity C-reactive protein (hs-CRP), and ^{14}C-urea breath test (^{14}C-UBT) were measured. For TFF1–3, measurements were conducted using enzyme-linked immunosorbent assay with a commercial kit (R&D Systems®, Minneapolis, Minnesota, USA), following the manufacturer's instructions. Duplicate negative and positive controls were included in each 96-well plate. Each TFF antibody reacted specifically and showed no cross-reactivity with other TFFs.

The status of *H. pylori* infection was determined by measuring serum levels of Hp-IgG and ^{14}C-UBT (Shenzhen Zhonghe Headway Bio-Sci & Tech Co., Ltd, Shenzhen, China). Positive infection was defined as Hp-IgG values > 15 AU/mL. Hp-IgG, PGI, and PGII were measured using a latex immunoturbidimetric assay with a commercial kit (Wantai DRD, Beijing, China). Hs-CRP was measured using the electrochemiluminescent immunoassay (Beijing Strong Biotechnologies, Beijing, China) on an automated clinical chemistry analyzer (Beckman Coulter AU5831, Brea, California, United States). Samples that yielded implausible values were retested.

Statistical analyses

Characteristics across the six disease groups were summarized using percentages for categorical data and means with standard deviations or medians (interquartile ranges) for continuous data. Trend tests across disease groups were performed using linear regression, Cuzick's nonparametric trend test, and the Cochran-Armitage test for trend, as applicable.

The association between biomarkers and outcomes was determined using logistic regression. Before entry into models, biomarker data were first log-transformed due to highly skewed distributions. Then, the distributions were standardized to a mean of 0 and a standard deviation of 1 to promote the contrast of effect sizes between the biomarkers. Three prediction models were established. Odds ratios (OR) per standard deviation and the corresponding 95 % confidence intervals (95 % CI) were calculated based on combinatorial adjustments of the variables to assess the associations between the biomarkers and NAG, CAG, IM, LGD, and GC. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, education, occupation, income, body mass index (BMI), smoking, alcohol consumption, eating hot food, eating fried food, eating fruits/vegetables, and medical history (gastritis, peptic ulcer, polyp, and high blood pressure). In Model 3, all biomarkers except PGII (due to multicollinearity) were adjusted based on Model 2.

Receiver operating characteristic (ROC) curves were constructed to evaluate the capability of these biomarkers to predict the risk of disease. The area under each ROC curve (AUC) was calculated to measure the discriminatory power of the biomarkers in the different groups. The potential of TFFs as a screening biomarker to discriminate GC and PMLs was analyzed by comparing them with traditional biomarkers (Hp-IgG, PGI, PGII, and the PGI/II ratio).

All statistical analyses were performed using Stata software (version 15.0). *P*-values < 0.05 were considered statistically significant. This was a pilot study; thus, sample size or power calculation was not required.

Results

Baseline characteristics

The average age of the participants was 51.8 years, and 54.7 % of the participants were male (Table 1). In total, 53.6 % of the participants had *H. pylori* infection as confirmed by the ^{14}C-urea breath test. The severity of gastric mucosal lesions gradually worsened from the NC to the GC group. The average age gradually increased from 50.0 years to 57.1 years, the proportion of males increased from 41.1 % to 74.8 %, and the proportion of smokers increased from 27.4 % to 48.9 %. Farmers accounted for 91.1 % of the population, with an average annual income of 32,000–20,000 RMB Yuan per family, representing a typical low-income status. The rate of alcohol consumption also increased from the NAG to the LGD group. However, lower alcohol intake was noted in the GC group. More than 60 % of the subjects in the GC and PML groups were used to eating hot food. Only 6.9 % of the subjects regularly consumed fruits and vegetables for six months. The medical history of gastritis, peptic ulcers, hepatitis, pancreatitis, and gastric polyps was statistically significant between the PML and GC groups (P for trend < 0.05).

Table 1
Baseline subject characteristics and biomarker parameters by group

Variables	NC n = 124	NAG n = 649	CAG n = 746	IM n = 1002	LGD n = 1334	GC n = 131	P trend
Age (years)	50.0 ± 7.6	50.8 ± 7.5	51.3 ± 7.8	52.4 ± 8.0	52.3 ± 8.1	57.1 ± 7.6	< 0.001
Male	51(41.1 %)	306 (47.1 %)	360 (48.3 %)	527 (52.6 %)	840 (63.0 %)	98 (74.8 %)	< 0.001
Married	115(92.7 %)	622 (95.8 %)	711 (95.3 %)	945 (94.3 %)	1,261 (94.5 %)	123 (93.9 %)	0.36
Education level							0.023
Illiteracy	19 (15.3 %)	108 (16.6 %)	153 (20.5 %)	226 (22.6 %)	281 (21.1 %)	25 (19.1 %)	
Primary	43 (34.7 %)	245 (37.8 %)	297 (39.8 %)	402 (40.1 %)	486 (36.4 %)	56 (42.7 %)	
Secondary	62 (50.0 %)	295 (45.5 %)	294 (39.4 %)	372 (37.1 %)	565 (42.4 %)	49 (37.4)	
Post-secondary	0 (0.0 %)	1 (0.2 %)	2 (0.3 %)	2 (0.2 %)	2 (0.1 %)	1 (0.8 %)	
Occupation (% farmer)	110 (88.7 %)	535 (82.4 %)	663 (88.9 %)	934 (93.2 %)	1,263 (94.7 %)	126 (96.2 %)	< 0.001
Income[a]	3.2 ± 1.5	2.8 ± 2.0	2.5 ± 1.8	2.1 ± 1.5	2.1 ± 1.5	2.0 ± 1.3	< 0.001
BMI (kg/m2)	23.7 ± 2.7	24.4 ± 3.1	24.1 ± 2.8	23.9 ± 2.9	23.9 ± 3.0	22.8 ± 2.9	< 0.001
Lifestyle habits							
Smoking[b]	34 (27.4 %)	227 (35.1 %)	260 (34.9 %)	399 (39.9 %)	643 (48.5 %)	64 (48.9 %)	< 0.001
Drinking[c]	5 (4.0 %)	30 (4.6 %)	60 (8.0 %)	55 (5.5 %)	101 (7.6 %)	6 (4.6 %)	0.11
Eating hot food[d]	48 (38.7 %)	407 (62.7 %)	487 (65.3 %)	660 (65.9 %)	808 (60.6 %)	86 (65.6 %)	0.20
Eating quickly[e]	20 (16.1 %)	149 (23.0 %)	168 (22.5 %)	227 (22.7 %)	292 (21.9 %)	32 (24.4 %)	0.70
Eating fried food[f]	0 (0.0 %)	14 (2.2 %)	1 (0.1 %)	1 (0.1 %)	7 (0.5 %)	2 (1.5 %)	0.038
Fruits/vegetables[g]	7 (5.6 %)	46 (7.1 %)	60 (8.0 %)	48 (4.8 %)	97 (7.3 %)	9 (6.9 %)	0.88
Medical history							
Gastritis	52 (41.9 %)	91 (14.0 %)	243 (32.6 %)	408 (40.7 %)	572 (42.9 %)	19 (14.5 %)	< 0.001
Peptic ulcer	2 (1.6 %)	8 (1.2 %)	29 (3.9 %)	36 (3.6 %)	77 (5.8 %)	11 (8.4 %)	< 0.001
Hepatitis	0 (0.0 %)	8 (1.2 %)	8 (1.1 %)	10 (1.0 %)	27 (2.0 %)	2 (1.5 %)	0.045

Variables	NC n = 124	NAG n = 649	CAG n = 746	IM n = 1002	LGD n = 1334	GC n = 131	P trend
Pancreatitis	0 (0.0 %)	2 (0.3 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0.041
Gallbladder diseases	18 (14.5 %)	81 (12.5 %)	102 (13.7 %)	137 (13.7 %)	200 (15.0 %)	18 (13.7 %)	0.23
Polyp	0 (0.0 %)	14 (2.2 %)	11 (1.5 %)	19 (1.9 %)	40 (3.0 %)	8 (6.1 %)	0.002
Hypertension	20 (16.1 %)	134 (20.6 %)	124 (16.6 %)	167 (16.7 %)	240 (18.0 %)	21 (16.0 %)	0.41
Diabetes	1 (0.8 %)	20 (3.1 %)	30 (4.0 %)	32 (3.2 %)	45 (3.4 %)	7 (5.3 %)	0.33
Anemia	0 (0.0 %)	8 (1.2 %)	5 (0.7 %)	16 (1.6 %)	14 (1.0 %)	1 (0.8 %)	0.62
Family history of GC	0 (0.0 %)	1 (0.2 %)	7 (0.9 %)	4 (0.4 %)	4 (0.3 %)	0 (0.0 %)	0.73
Biomarkers, median (Q1–Q3)							
TFF1 (pg/mL)	37.1 [15.5, 70.7]	44.4 [15.2, 104.0]	80.2 [31.7, 156.0]	117.4 [54.1, 206.2]	104.6 [54.5, 170.7]	134.0 [49.2, 239.5]	< 0.001
TFF2 (pg/mL)	1569.8 [895.2, 2726.7]	1866.0 [1069.0, 3103.1]	2732.9 [1848.1, 3968.1]	2729.5 [1869.6, 3949.2]	3058.7 [1973.5, 4426.2]	3647.9 [2485.9, 6164.2]	< 0.001
TFF3 (pg/mL)	6153.1 [4438.7, 7841.4]	6063.9 [3339.1, 7585.8]	7115.3 [5208.1, 9256.0]	7195.1 [5650.0, 9211.9]	6804.0 [5056.9, 9227.8]	6235.8 [4986.6, 8551.7]	< 0.001
Hp-IgG (AU/mL)	9.3 [1.9, 40.3]	28.4 [4.3, 57.1]	29.1 [5.0, 59.1]	32.6 [8.9, 61.0]	34.4 [10.0, 62.0]	29.8 [12.7, 54.3]	< 0.001
PGI (ng/mL)	60.8 [47.7, 82.2]	64.5 [47.6, 87.5]	69.8 [52.8, 91.5]	65.6 [47.4, 87.7]	70.2 [51.9, 90.2]	67.4 [47.4, 90.2]	0.016
PGII (ng/mL)	12.5 [6.9, 19.9]	14.1 [8.6, 22.6]	15.6 [9.7, 23.1]	16.1 [10.3, 23.5]	16.5 [10.9, 23.8]	18.3 [10.3, 25.4]	< 0.001
PGI/II ratio	5.1 [3.7, 7.2]	4.7 [3.4, 6.6]	4.5 [3.4, 6.3]	4.1 [3.0, 5.7]	4.3 [3.2, 5.7]	3.9 [2.9, 5.1]	< 0.001
Hs-CRP (mg/L)	0.7 [0.4, 1.4]	0.8 [0.4, 1.7]	0.8 [0.3, 1.8]	0.8 [0.3, 1.7]	0.8 [0.3, 1.6]	1.0 [0.5, 1.9]	0.85
H. pylori infection							
14C-UBT[h]	35 (28.2 %)	283 (43.7 %)	366 (49.1 %)	579 (57.9 %)	802 (60.2 %)	70 (53.4 %)	< 0.001
Hp-IgG[i]	52 (41.9 %)	391 (60.2 %)	451 (60.5 %)	690 (68.9 %)	928 (69.6 %)	95 (72.5 %)	< 0.001

Abbreviations: BMI, body mass index; CAG, chronic atrophic gastritis; CI, 95 % confidence interval; 14C-UBT, 14C-urea breath test; GC, gastric cancer; Hp-IgG, anti-Helicobacter. pylori immunoglobulin G antidody; hs-CRP, high-sensitive C-reactive protein; IM, intestinal metaplasia; Q, interquartile range; LGD, low-grade dysplasia; NAG, non-atrophic gastritis; OR, odds ratio; PG, pepsinogen; TFF, trefoil factor.

Data are expressed as the mean ± standard deviation, number (%), or median (IQR).

Variables	NC	NAG	CAG	IM	LGD	GC	P trend
	n = 124	n = 649	n = 746	n = 1002	n = 1334	n = 131	
NC, normal control; NAG, non-atrophic gastritis; CAG, chronic atrophic gastritis; IM, intestinal metaplasia; LGD, low-grade dysplasia; GC, high-grade dysplasia and gastric adenocarcinoma.							
[a]Income shown as 10,000 CNY per year per family.							
[b]Smoking was defined as whether the participants smoked at least one cigarette per day in the past 6 months or ever smoked.							
[c]Drinking was defined as consumption of at least 1,000 g of beer or 150 g of wine or hard liquor at least once per week during the past year.							
[d]Eating hot food was defined as the usual habit of eating hot food.							
[e]Eating quickly was defined as eating a bowl of noodles in less than 8 minutes.							
[f]Eating fried food was defined as eating a fried food meal at least 4 times a week.							
[g]Eating fruits/vegetables was defined as eating fruits/vegetables regularly for 6 months of the year.							
[h]H. pylori infection was detected by 14C-urea breath test.							
[i]H. pylori infection was detected by serum anti-H. pylori immunoglobulin G antibody titer.							

Serum biomarker distributions in each group

The serum biomarker distributions in the six groups are shown in Table 1. Serum levels of TFF1 and TFF2 showed an incremental trend from the NC, NAG, CAG, IM, and LGD groups to the GC group (TFF1 median [Q1–Q3] pg/mL: from 37.1 [15.5, 70.7] of the NC group to 134.0 [49.2, 239.5] of the GC group; TFF2 median [Q1–Q3] pg/mL: from 1569.8 [895.2, 2726.7] of the NC group to 3647.9 [2485.9, 6164.2] of the GC group) (P for trend < 0.001). However, the distribution of serum TFF3 levels differed across the six groups and was significantly higher in the IM group (median [Q1–Q3] pg/mL, 7195.1 [5650.0, 9211.9]). Serum levels of Hp-IgG and the rate of *H. pylori* positive-infection confirmed by ^{14}C-UBT gradually increased from the NC group (Hp-IgG: 9.3 [1.9, 40.3] AU/mL; ^{14}C-UBT: 28.2 %) to the LGD group (Hp-IgG: 34.4 [10.0–62.0] AU/mL; ^{14}C-UBT: 60.2 %); however, these values significantly decreased in the GC group (Hp-IgG: 29.8 [12.7–54.3] AU/mL; ^{14}C-UBT: 53.4 %) (P for trend < 0.001). In contrast, the rate of *H. pylor*i positive-infection confirmed by Hp-IgG gradually increased from the NC group (41.9 %) to the LGD group (69.6 %), and the highest value was observed in the GC group (70.5 %).

The serum levels of PGI differed among the groups (P for trend = 0.016). The PGI/II ratio showed a declining trend from the NC group to the GC group, while the serum levels of PGII showed a reversing trend (P for trend < 0.001). No significant association was observed between serum hs-CRP levels and the histological grade (P for trend = 0.85).

Age- and sex-adjusted correlations

The correlation coefficient analysis among biomarkers is shown in Table 2. After adjusting for age and sex, there was a strong positive partial correlation between PGI and PGII (r > 0.50, P < 0.001) and between PGII and Hp-IgG (r > 0.50, P < 0.001). There were moderate significant correlations between Hp-IgG and TFF1/TFF2, PGI and TFF2, PGII and TFF1/TFF2, and TFF1 and TFF2 ($0.20 < r \leq 0.50$, P < 0.001). Meanwhile, there were weak correlations between TFF1 and TFF3, TFF2 and TFF3, and PGI and TFF1 ($r \leq 0.20$, P < 0.001).

Table 2
Correlation among PGI, PGII, Hp-IgG, and TFF1–3 after adjusting for age and sex

Variables	PGI		PGII		Hp-IgG		TFF1		TFF2		TFF3	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
PGI	–	–	0.53	< 0.001	0.26	< 0.001	0.15	< 0.001	0.28	< 0.001	0.01	0.74
PGII	0.53	< 0.001	–	–	0.53	< 0.001	0.36	< 0.001	0.33	< 0.001	0.03	0.042
Hp-IgG	0.26	< 0.001	0.53	< 0.001	–	–	0.38	< 0.001	0.30	< 0.001	-0.02	0.20
TFF1	0.15	< 0.001	0.36	< 0.001	0.38	< 0.001	–	–	0.35	< 0.001	0.20	< 0.001
TFF2	0.28	< 0.001	0.33	< 0.001	0.30	< 0.001	0.35	< 0.001	–	–	0.20	< 0.001
TFF3	0.01	0.74	0.03	0.042	-0.02	0.20	0.20	< 0.001	0.20	< 0.001	–	–

Abbreviations: Hp-IgG, anti-Helicobacter. pylori immunoglobulin G antibody; PG, pepsinogen; TFF, trefoil factor.

r, partial correlation coefficient.

Association of the biomarkers with GC and PMLs

In Model 2, significant associations between all the serum biomarkers and GC and PMLs ($P < 0.05$) were observed. In Models 1 and 2, the OR of TFF1 and TFF2 were higher than those of PGI, PGII, the PGI/II ratio, and Hp-IgG in the PMLs and GC groups. Moreover, the results remained the same after adjusting for all biomarkers and risk factors in Model 3. Notably, in Model 3, the *P*-values of TFF1 and TFF2 were < 0.05 in the PMLs and GC groups; however, these values were > 0.05 in the NAG group. Furthermore, in Model 3, significant associations were evident between TFF1–3 and PMLs, Hp-IgG and LGD, TFF1 and GC, and TFF2 and GC ($P < 0.05$). Interestingly, TFF3 had a similar association with IM as TFF1 and TFF2. The ORs of TFF1, TFF2, and TFF3 in Model 3 of the IM group were 1.87 (95 % CI, 1.49–2.33), 2.02 (95 % CI, 1.54–2.64), and 1.95 (95 % CI, 1.40–2.72), respectively. Compared with other biomarkers, TFF1 and TFF2 also showed the highest OR in the GC group. The ORs (95 % CIs, *P*-values) of TFF1 and TFF2 in Model 3 of the GC group were 2.71 (1.57–4.67, $P < 0.001$) and 2.87 (1.75–4.71, $P < 0.001$), respectively. In Model 3 (**Supplementary Table 1, Additional File 1**), the OR of ^{14}C-UBT showed an advantage in the NAG (OR = 2.17, $P = 0.020$), CAG (OR = 1.68, $P = 0.043$), IM (OR = 2.35, $P = 0.001$), and LGD (OR = 3.14, $P < 0.001$) groups, but not in the GC group (OR = 2.37, $P = 0.10$).

Comparison of clinical ROC curves among different biomarker screening models

The ROC curves were plotted based on individual biomarker study models of serum TFF1, TFF2, TFF3, TFF1–3, Hp-IgG, PGI, PGII, and the PGI/II ratio across all five groups. The results of the AUC for the NAG, CAG, IM, LGD, and GC groups are presented in **Fig. 2a–e.** The ROC curves of these biomarkers, including ^{14}C-UBT, are shown in the **Supplementary Fig. 2a-e, Additional File 2** for the NAG, CAG, IM, LGD, and GC groups. TFF1–3 showed the largest AUC in the CAG, IM, LGD, and GC groups, which were 0.74, 0.79, 0.79, and 0.84, respectively, followed by TFF1 and TFF2.

The AUC of TFF1-3 was similar to the AUC of TFF1–3 combined with Hp-IgG and TFF1–3 combined with ^{14}C-UBT in each group. Furthermore, the best-fit ROC curve was TFF1–3, showing a significant increase in the AUC.

Discussion

Gastric carcinogenesis is a multifactorial, multistep process, usually described by a series of events known as the Correa's cascade, which begins from chronic active gastritis and progresses through a chain of mucosal changes including gastric atrophy, intestinal metaplasia, and intraepithelial neoplasia/dysplasia (also known as PMLs), finally developing into GC [15, 18]. Studies have reported that the detection sensitivity of serum TFF3 and PG test for GC is 80.0–80.4 % and 33.3–39.5 %, respectively [12]. Serum TFF3 was a better screening marker than PG, and the combination of TFF3 and PG can improve screening of GC in Japan [12, 13]. In particular, serum levels of TFF3 remained stable for the status and eradication of *H. pylori* infection [19]. Many such studies have motivated us to investigate the effectiveness of the TFF family as biomarkers in identifying GC and PMLs in a timely and accurate manner among the primary population in the high-risk area. This would function as an essential strategy for early inventions and help reduce the mortality and morbidity of GC. TFF peptides have a stable triple-loop structure that may have apparent resistance to protease hydrolysis, acid digestion, and heat treatment; thus, TFFs can maintain biological activity in the complex environment of the digestive tract [20, 21]. Commonly, TFF1 and TFF2 are expressed mainly in the mucosal epithelial cells of the gastric body and antrum, and TFF3 is expressed in goblet cells of the small intestine and the colon [22, 23]. However, under pathological conditions, the site-specific expression of TFFs is absent, and TFFs can be detected in any damaged mucosae as their expression is up-regulated to participate in gastrointestinal epithelial reconstruction and mucosal repair process [24, 25].

Moreover, in the development of GC, the expression of TFFs can be dynamically escalated [26, 27]; it is reasonable because, in Correa's cascade, the outline of the carcinogenesis process is characterized by gastric mucosal lesions and gland atrophy [15, 28]. In this study, from PMLs (i.e., CAG, IM, and LGD) to GC, the serum levels of TFF1, TFF2, and TFF3 changed with an almost incremental trend (P for trend < 0.05, Table 1), and the TFF3 level was remarkably high in the IM group. Moreover, the overall serum levels of TFF1, TFF2, and TFF3 were much more enhanced in the IM, LGD, and GC groups than in the NC and NAG groups, suggesting a significant association between the serum levels of TFFs and PMLs and GC.

The Wuwei Cohort study reveals a significant association between the severity of gastric mucosal lesions from PMLs to GC and sex (male), age, occupation, smoking, and medical history of gastritis, peptic ulcer, and polyps. Subsequent analyses were conducted based on a complete profile combining all the biomarkers, and these meaningful baseline characteristics were adjusted to eliminate mutual influence and explore relatively better independent biomarkers for clinical screening of high-risk populations.

The genes of three TFFs are adjacently located on chromosome 21q22.3 and share five regulatory sequences to coordinate expression [29, 30]. Taupin et al. indicated that TFF peptides could function as the instant early controllers fit for auto- and cross-acceptance of other trefoil peptides in gastric cell lines [31]. In addition, a diminished articulation of TFF1 and TFF2 was found in the TFF3 knockout (KO) mice. In addition, a decrease in TFF2 levels was observed in TFF1 KO mice [31, 32]. The cross-enlistment of TFFs requires enactment by means of phosphorylation of the epidermal growth factor receptor, the last being actuated by the intestinal TFF [33]. In Correa's cascade, *H. pylori* infection initiates gastric inflammation that may advance into PG-related gastric atrophy [34]. PG and Hp-IgG can be regulated by more than one trigger or by interaction with each other. Hp-IgG and PG were not independent indicators for high-risk GC. Infection with *H. pylori* is an established risk factor for GC. In this study, the trend showed a stepwise increase in PGII and a reduction in the PGI/II ratio from the PMLs to GC groups. The anti-*H. pylori* IgG antibody titer of

the GC group was lower than that of the PMLs group, which may be due to the extensive atrophy that could lead to the loss of *H. pylori* infection or the eradication of *H. pylori*. Additionally, lower antibody titers in advanced GC may be partly attributable to diminished immune response [28]. Studies have shown that the false-negative results of *H. pylori* infection in patients with anti-*H. pylori* IgG antibody titer of 3–9.9 U/mL as negative-high titer causes miss diagnosis of the high-risk GC populations [35]; hence, further examinations such as ^{13}C-UBT, stool antigen test, and endoscopy should be performed in order to overcome this limitation. However, cumbersome examinations do not meet the simple and effective population-based screening for GC. The reasons for individuals with positive anti-*H. pylori* IgG antibodies include current and/or former *H. pylori* infection or *Helicobacter* infections other than *H. pylori* infections [36, 37]. As a result, the positivity rate of *H. pylori* infection detected by Hp-IgG is higher than that detected by ^{14}C-UBT (Table 1). If anti-*H. pylori* IgG antibody is used as the screening marker, the increase in the false-positivity rate may cause a rise in the screening population. According to the results reported in Table 3 and **Supplementary Table 1, Additional File 1**, irrespective of whether the adjusted model included ^{14}C-UBT or Hp-IgG, the ORs of TFFs in the PML and GC groups were consistent, and the *P*-values of TFFs were less than 0.05, except that of TFF3 in the GC group. Moreover, the ORs of TFF1 and TFF2 were higher than those of PGI, PGII, the PGI/II ratio, and Hp-IgG, suggesting that elevated levels of TFF1 and TFF2 compared to that of Hp-IgG, PGI, PGII, and the PGI/II ratio may indicate an increased risk of GC and PMLs. The results of this study indicated that TFFs were independent biomarkers for predicting the risk of PMLs and GC, and TFFs were not affected by PGI, PGII, Hp-IgG, and ^{14}C-UBT. This was also observed in Model 3 after adjusting for all biomarkers and risk factors, indicating that TFF1 and TFF2 may be reliable, independent biomarkers for identifying individuals at a high risk of GC. Notably, TFF1 and TFF2 showed significant predictive values in the PML and GC groups (with all *P*-values < 0.05) but not in the NAG group (*P*-values > 0.05). These results further illustrate that TFF1 and TFF2 are more precise independent predictors of GC and PMLs. A study by Dhar et al. reported that both the TFF2 mRNA as well as the protein showed a high level of expression and a correlation with the clinicopathologic stage and/or prognosis of GC [38]. In other words, Model 3 confirmed TFF1 and TFF2 as more reliable biomarkers for identifying high-risk GC populations. If intestinal metaplasia is considered an intermediate step in the development of GC, the induction of TFF3 expression could be anticipated during the progression from a metaplastic epithelium to cancer [39]. The increased expression of TFF3 may be an early warning indicator of intestinal metastasis and intestinal GC. Therefore, the value of TFF3 lies in predicting the risk of intestinal metaplasia. It is also supported by this study which showed a higher OR of TFF3 (Table 3), and TFF3 has a significant and independent association with IM. However, in this study, we did not stratify because of the small sample size, which could underestimate the predictive value of TFF3 for GC.

Table 3
Association of the biomarkers with NAG, CAG, IM, LGD, and GC

Variables	Model 1a			Model 2b			Model 3c		
	OR	95 % CI	P	OR	95 % CI	P	OR	95 % CI	P
NAG (n = 649)									
TFF1	1.08	0.90–1.29	0.40	1.08	0.89–1.32	0.41	0.95	0.77–1.19	0.68
TFF2	1.05	0.91–1.21	0.54	1.10	0.93–1.29	0.26	1.00	0.84–1.20	0.99
TFF3	0.84	0.69–1.02	0.09	0.93	0.75–1.15	0.51	0.95	0.76–1.19	0.66
Hp-IgG	1.40	1.18–1.66	< 0.001	1.43	1.18–1.74	< 0.001	1.29	1.02–1.63	0.036
PGI	1.13	0.96–1.32	0.14	1.21	1.02–1.43	0.032	1.19	0.95–1.49	0.12
PGII	1.24	1.06–1.45	0.007	1.36	1.14–1.62	0.001	–	–	–
PGI/II ratio	0.86	0.72–1.01	0.07	0.82	0.67–0.99	0.035	0.85	0.68–1.05	0.13
CAG (n = 746)									
TFF1	1.69	1.40–2.04	< 0.001	1.83	1.49–2.24	< 0.001	1.49	1.18–1.89	< 0.001
TFF2	2.36	1.89–2.93	< 0.001	2.37	1.88–2.99	< 0.001	2.04	1.59–2.62	< 0.001
TFF3	1.46	1.19–1.79	< 0.001	1.61	1.29–2.01	< 0.001	1.52	1.19–1.93	< 0.001
Hp-IgG	1.51	1.26–1.81	< 0.001	1.49	1.23–1.80	< 0.001	1.04	0.80–1.35	0.76
PGI	1.30	1.09–1.55	0.003	1.43	1.18–1.73	< 0.001	1.19	0.93–1.53	0.17
PGII	1.46	1.23–1.74	< 0.001	1.53	1.27–1.84	< 0.001	–	–	–
PGI/II ratio	0.82	0.69–0.97	0.021	0.83	0.69–1.00	0.050	0.95	0.76–1.18	0.62
IM (n = 1002)									
TFF1	2.10	1.76–2.51	< 0.001	2.27	1.86–2.77	< 0.001	1.87	1.49–2.33	< 0.001
TFF2	2.58	2.07–3.23	< 0.001	2.56	2.02–3.25	< 0.001	2.02	1.54–2.64	< 0.001
TFF3	2.26	1.74–2.94	< 0.001	2.36	1.76–3.15	< 0.001	1.95	1.40–2.72	< 0.001
Hp-IgG	1.74	1.45–2.07	< 0.001	1.66	1.38–2.01	< 0.001	1.11	0.86–1.43	0.436

Abbreviations: CAG, chronic atrophic gastritis; CI, 95 % confidence interval; GC, high-grade dysplasia and gastric adenocarcinoma; Hp-IgG, anti-Helicobacter. pylori immunoglobulin G antidody; IM, intestinal metaplasia; LGD, low-grade dysplasia; NAG, non-atrophic gastritis; OR, odds ratio; PG, pepsinogen; TFF, trefoil factor.

Biomarker values were natural log transformed because of highly skewed distributions.

aModel 1 is adjusted for age and sex.

bModel 2 is adjusted for age, sex, education, occupation, income, body mass index, smoking, alcohol consumption, eating hot food, eating fried food, eating fruits/vegetables, and a medical history of gastritis, peptic ulcer, polyp, and high blood pressure.

cIn Model 3, all biomarkers except for PGII are adjusted based on Model 2.

Variables	Model 1a			Model 2b			Model 3c		
	OR	95 % CI	P	OR	95 % CI	P	OR	95 % CI	P
PGI	1.20	1.01–1.44	0.041	1.25	1.04–1.51	0.018	1.01	0.79–1.30	0.95
PGII	1.39	1.20–1.61	< 0.001	1.38	1.18–1.62	< 0.001	–	–	–
PGI/II ratio	0.79	0.68–0.90	< 0.001	0.81	0.70–0.95	0.007	0.97	0.79–1.20	0.79
LGD (n = 1334)									
TFF1	2.41	1.98–2.93	< 0.001	2.36	1.92–2.90	< 0.001	1.67	1.31–2.12	< 0.001
TFF2	2.10	1.78–2.49	< 0.001	2.06	1.73–2.46	< 0.001	1.63	1.34–2.00	< 0.001
TFF3	1.54	1.24–1.92	< 0.001	1.59	1.26–2.01	< 0.001	1.40	1.06–1.84	0.017
Hp-IgG	1.97	1.64–2.36	< 0.001	2.02	1.67–2.45	< 0.001	1.41	1.10–1.81	0.007
PGI	1.28	1.10–1.49	0.002	1.28	1.09–1.51	0.003	1.02	0.81–1.29	0.87
PGII	1.51	1.30–1.76	< 0.001	1.55	1.32–1.82	< 0.001	–	–	–
PGI/II ratio	0.74	0.63–0.87	< 0.001	0.73	0.62–0.87	0.001	0.96	0.76–1.18	0.64
GC (n = 131)									
TFF1	2.77	1.97–3.89	< 0.001	3.71	2.33–5.91	< 0.001	2.71	1.57–4.67	< 0.001
TFF2	2.97	2.09–4.22	< 0.001	3.73	2.41–5.76	< 0.001	2.87	1.75–4.71	< 0.001
TFF3	1.47	1.00–2.18	0.050	1.74	1.05–2.88	0.030	1.48	0.81–2.71	0.20
Hp-IgG	2.09	1.52–2.89	< 0.001	2.58	1.72–3.87	< 0.001	1.62	0.89–2.92	0.11
PGI	1.24	0.99–1.55	0.06	1.36	1.02–1.81	0.034	0.98	0.64–1.49	0.92
PGII	1.72	1.30–2.28	< 0.001	1.95	1.40–2.72	< 0.001	–	–	–
PGI/II ratio	0.72	0.55–0.94	0.015	0.71	0.54–0.94	0.016	0.83	0.57–1.19	0.31

Abbreviations: CAG, chronic atrophic gastritis; CI, 95 % confidence interval; GC, high-grade dysplasia and gastric adenocarcinoma; Hp-IgG, anti-Helicobacter. pylori immunoglobulin G antidody; IM, intestinal metaplasia; LGD, low-grade dysplasia; NAG, non-atrophic gastritis; OR, odds ratio; PG, pepsinogen; TFF, trefoil factor.

Biomarker values were natural log transformed because of highly skewed distributions.

aModel 1 is adjusted for age and sex.

bModel 2 is adjusted for age, sex, education, occupation, income, body mass index, smoking, alcohol consumption, eating hot food, eating fried food, eating fruits/vegetables, and a medical history of gastritis, peptic ulcer, polyp, and high blood pressure.

cIn Model 3, all biomarkers except for PGII are adjusted based on Model 2.

ROC curve analyses for GC and PMLs revealed that TFF1–3 yield the largest AUC. In the GC group, the AUC of TFF1–3 was 0.84, followed by the AUC of TFF1 and TFF2, which were 0.77 and 0.81, respectively. Aikou et al. reported that TFF3 had a high AUC in distinguishing GC patients from the health check subjects. Testing the combined levels of serum PGI/II and TFF3 could improve GC screening [13]. However, our studies support that TFF1 and TFF2 demonstrate a higher sensitivity and specificity than TFF3 in predicting the risk of GC and PMLs. On comparing the AUC of these biomarkers in the PML and GC groups, TFF1–3 had the largest AUC, which was similar to the AUC of the

combination of TFF1–3 and Hp-IgG and the combination of TFF1-3 and ^{14}C-UBT, although ^{14}C-UBT had a higher OR in the NAG and PML groups. Moreover, these results indicate that TFFs function as independent and stable biomarkers in the population-based screening for GC and are significantly associated with PMLs and GC. As reported above, TFFs are sensitive and specific predictors for GC and PMLs, making them more helpful in identifying high-risk GC populations.

The TFF family has been involved in the protective effect of the gastrointestinal tract against mucosal damage, and they play a crucial role in the progression of GC. TFFs influence disease pathogenesis by altering normal mucosal recovery, thus aggravating the ongoing inflammation [40]. TFFs can be involved in controlling the coordination of proliferation, apoptosis, and differentiation of gastrointestinal cells [41]. TFF overexpression is associated with increased cell migration and possibly increased GC invasion and angiogenesis [38, 42]. This, in turn, might be due to TFF gene mutations [43], chromatin remodeling, loss of heterozygosity, and promoter methylation [44]. The function of TFFs as regulatory factors for gastrointestinal cell differentiation has been well demonstrated. The presence of TFFs may help maintain the balance between proliferation and apoptosis of gastrointestinal cells during early tumorigenesis. Therefore, TFF levels may serve as an indicator for the progression from precancerous to cancerous lesions. TFF expression status also appears to be a promising prognostic marker in GC patients.

This study had some limitations. The cohort was a single-center population, and there were fewer participants in the normal healthy group than in the GC group. This may have possibly biased the results. Nevertheless, this study is the first to demonstrate that TFF1, TFF2, and TFF3 are sensitive and highly specific serum markers for GC and PMLs based on a large population. Prospective studies are needed to validate the association between TFF expression and clinical characteristics and outcomes of GC, and the role of TFF expression in cancer progression.

Conclusions

In conclusion, TFF1, TFF2, and TFF3 are sensitive and specific serum markers significantly associated with GC and PMLs compared to PG and Hp-IgG. The serum detection of TFFs is more straightforward, efficient, and feasible in the screening of high-risk GC population, which not only facilitates the early identification of the GC and PMLs in the population but can also provide a basis for developing early diagnostic and therapeutic intervention strategies and help gain insight into the carcinogenesis of GC.

List Of Abbreviations

GC
gastric cancer; PMLs:premalignant lesions; TFF:trefoil factors; OR:odds ratio; ROC:receiver operating characteristic; NC:normal gastric mucosa; NAG:non-atrophic gastritis; CAG:chronic atrophic gastritis; IM:intestinal metaplasia; LGD:low-grade intraepithelial neoplasia/dysplasia; HGD:high-grade intraepithelial neoplasia/dysplasia; Hp-IgG:anti-*Helicobacter pylori* immunoglobulin G antibody; *H. pylori*:*Helicobacter pylori;* PG:pepsinogen; PGI:pepsinogen I; PGII:pepsinogen II; hs-CRP:high-sensitivity C-reactive protein; ^{14}C-UBT:^{14}C-urea breath test; CI:confidence interval; BMI:body mass index; AUC:area under the curve; KO:knockout

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Hospital of Lanzhou University (approval number: LDYYLL2012001). All subjects provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no potential competing interests (financial, professional, or personal) relevant to the manuscript.

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Authors' contributions

XZ majorly contributed in the design and performance of the study, the analysis of data, and the writing of the manuscript.

ZC equally contributed to the investigation, the operation and the supervision of the project of the Wuwei cohort, the collection of data, and the endoscopic examination for subjects.

QG equally contributed to the investigation, the operation, and the supervision of the project of the Wuwei cohort, the collection of data, and the endoscopic examination for subjects.

YW equally contributed in the investigation and administration of the project of Wuwei cohort, and the collection and the collation of data.

ZZ equally contributed in the investigation and administration of project of Wuwei cohort, and the collection and the collation of data.

RJ equally contributed in the investigation of project of Wuwei cohort, the collection of data, and the endoscopic examination for subjects.

YZ equally contributed in the curation, analysis, and validation of data.

JZ equally contributed in the endoscopic examination for subjects the collection of data.

ZW equally contributed in the endoscopic examination for subjects the collection of data.

ML equally contributed in the pathological analysis of biopsy samples.

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QG equally contributed in the endoscopic examination for subjects and the collection of data.

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ML equally contributed in the endoscopic examination for subjects and the collection of data.

QR equally contributed in the endoscopic examination for subjects and the collection of data.

XH equally contributed feasibility analysis of the project of Wuwei cohort and the formulation of the cluster sampling method.

HL equally contributed in the endoscopic examination for subjects and the collection of data.

YW equally contributed in the endoscopic examination for subjects and the collection of data.

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RL equally contributed in the collection and the collation of data.

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JL equally contributed in the curation, analysis, and validation of data.

LQ equally contributed in the feasibility analysis of the project of Wuwei cohort, the formulation of endoscopic operation procedures and pathological analysis methods.

YZ majorly contributed in the design, the operation, and the supervision of the project of Wuwei cohort; was responsible for the names and order of authors, the verification of all data, figures, materials, and the submission and all substantive correspondence with editors.

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Figures

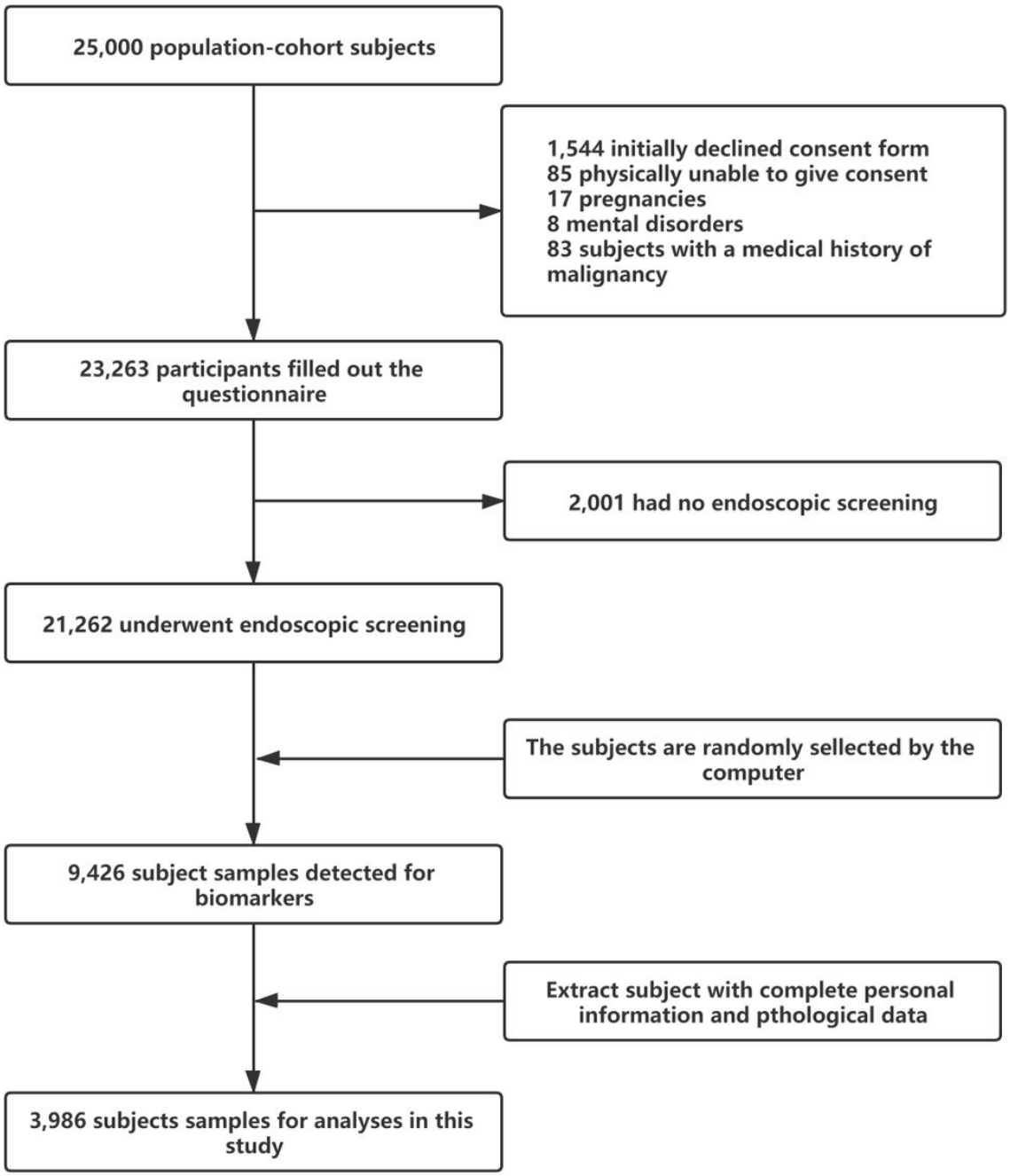

Figure 1

This population-based screening study evaluated 25,000 adults aged 35–70 years residing in Wuwei (Wuwei Cohort) using the cluster sampling method (Fig. 1). Those who 1) initially declined consent (n=1,544), 2) were physically unable to provide consent (n=85), 3) were pregnant (n=17), 4) were mentally ineligible (n=8), or 5) had a medical history of malignancy (n=83) were excluded.

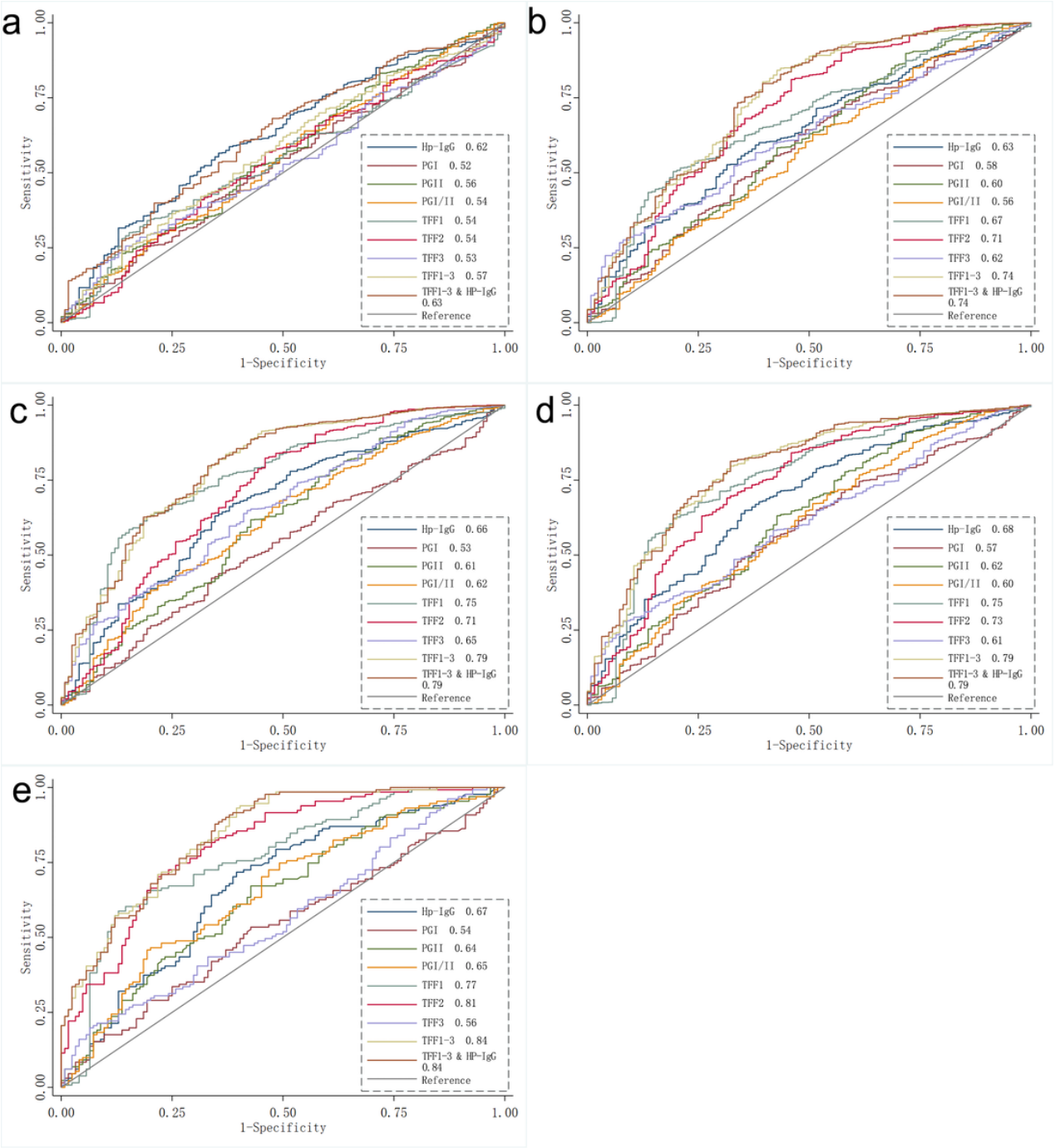

Figure 2

The ROC curves were plotted based on individual biomarker study models of serum TFF1, TFF2, TFF3, TFF1–3, Hp-IgG, PGI, PGII, and the PGI/II ratio across all five groups. The results of the AUC for the NAG, CAG, IM, LGD, and GC groups are presented in Fig. 2a–e.

Supplementary Files

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