Original Article
A novel gastric juice index model for detecting early gastric cancer

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Abstract: Background: Early diagnosis of gastric cancer (GC) is crucial for improving patients’ outcome, but reliable biomarkers are scarce. This study measured gastric juice pH value, aromatic amino acid (AAA), and total protein concentrations to build a model using these parameters to diagnose GC. Method: We performed a case-control study by comparing the different levels of gastric juice parameters between GC and non-neoplastic gastric disease (NGD) patients. Gastric juice was collected from 200 consecutive patients who underwent gastroscopy at our institution. A total of 120 samples were divided into the derivation subgroup, and others into the validation subgroup by simple random sampling. The demographic and clinicopathological data were also assimilated. Results: Significantly higher levels of male to female ratio, pH value, and AAA content in gastric juice were observed in GC patients than in NGD individuals in the derivation subgroup (all \( P < 0.001 \)). By performing logistic regression analysis with these parameters, we developed a predicting model, defined as gastric juice index (GJI). For the detection of GC, the AUC of GJI was 0.897 (95% CI, 0.838-0.956) in the derivation subgroup and 0.805 (95% CI, 0.704-0.906) in the validation subgroup. Importantly, for the detection of early GC, its AUC was 0.848 (95% CI, 0.771-0.924). At the optimal cutoff value (8.995), its sensitivity, specificity, and accuracy were 75.0%, 78.9%, and 78.2%, respectively. Conclusion: The GJI model established with the above parameters is effective in predicting the existence of GC, and may be used as a diagnostic tool for early GC.

Keywords: Gastric cancer, gastric juice index (GJI), aromatic amino acid (AAA), pH value, liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Introduction

Gastric cancer (GC) is currently the fifth most common cancer and third leading cause of cancer-related deaths worldwide [1]. Early detection is critical for improving the prognosis of GC. But, the initial phase of GC is often asymptomatic and can be easily missed by conventional endoscopy [2]. Traditional plasma biomarkers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), are insufficient for the diagnosis of early gastric cancer (EGC) [3]. Therefore, new diagnostic markers with better sensitivity and specificity are urgently required.

As gastric juice contains metabolic information about the gastric epithelium, it can be utilized to obtain predictive information and develop valuable methods for GC screening. In previous studies, we established several endogenous fluorescence spectra of gastric juice for GC diagnosis and screening [4, 5], isolated and qualitatively identified three fluorescence candidates (gastric juice aromatic amino acids, AAAs) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance [6]. Gastric juice AAAs can be used to distinguish advanced gastric cancer (AGC) and EGC from benign gastric diseases through quantitative measurement using high-performance liquid chromatography [7]. However, the disadvantages of this method are that it requires derivatization and one run could last up to 30 min, which severely limits its detection efficiency. In the present study, we optimized the measuring method by LC-MS/MS, which facilitated an easy, rapid, and high-throughput detection of AAAs in gastric juice.
Gastric carcinogenesis is a multifactorial and multi-step process. Due to this, several prediction models are established for the diagnosis of GC, predicting lymph-node metastasis and evaluating the feasibility of endoscopic submucosal dissection for EGC [8, 9]. For example, stomach age model [10], which focused on eight factors (such as the pathological result of endoscopy, family history of GC, and other concomitant diseases) affecting the stomach age was established by logistic regression. However, stomach age was determined by fluorescence in situ hybridization analysis of stomach biopsy samples, which made the use of this method in large populations unrealistic. In the present study, we aimed to construct and certify a novel diagnostic model for GC, called the gastric juice index (GJI), based on male to female ratio, pH value, and AAAs’ concentrations in human gastric juice, promoting earlier diagnosis and treatment.

Patients and methods

Study design and ethical considerations

From December 2015 to August 2016, 283 gastric juice samples were collected from consecutive patients who underwent gastroscopy examinations at our institution. The demographic and clinicopathological data were also assimilated. Among them, 200 patients were finally included in our study, and the others were excluded for not fulfilling the inclusion criteria (n = 37) or meeting the exclusion criteria (n = 46). Based on the sequence number of the patients enrolled in the study, a random number was assigned to each patient using the SPSS software. Depending on the random order, the first 120 cases were taken as the training set to establish a diagnostic model using logistic regression analysis and the latter 80 as the validating set to verify its diagnostic performance, to ensure that the number of
patients in the latter part was no less than half of the former part. The flow diagram of the study is shown in Figure 1. The study protocol was approved by the Peking University Medical Ethics Committee. Informed consent was obtained from each patient, and the entire clinical investigation was conducted according to the Declaration of Helsinki.

Inclusion criteria

Patients (1) suspected with various benign and malignant gastric diseases diagnosed by mucosal biopsy and/or postoperative pathology, (2) >18 years of age, (3) whose gastric juice was exclusive of blood, bile, and gross food residue; without diluted in the process of gastroscopy and >5 mL, which is sufficient to conduct all the tests.

Exclusion criteria

Patients (1) who underwent surgery, chemotherapy, and/or radiotherapy, (2) with recurrent, metastatic, neuroendocrine carcinoma, or lymphoma in the stomach, (3) with gastrointestinal submucosal tumor or gastric polyp, (4) with lesions predominantly localized at the esophagus or duodenum, (5) with organic diseases or tumors in other organs diagnosed by clinical examination, (6) pregnant or lactating.

Diagnostic criteria

All patients were histologically confirmed by mucosal biopsy and/or postoperative pathology. Biopsies were performed at the antrum, corpus, and other suspicious sites (≥2). Abnormal lesions were sampled for histopathological examination. The diagnosis of each subject depended on the most severe lesion. In the case of pathological diagnosing discrepancy between biopsy and postoperative specimens, the diagnosis was mainly based on the latter. The classification of gastritis was according to the updated Sydney system, and the clinical stages of GC were updated according to American Joint Committee on Cancer guidelines in 2010 [11].

Sample collection, preservation, and preparation

Gastric juice (5-10 mL) was collected from the patients who underwent gastroscopy after overnight fasting. The samples were centrifuged at 3000 rpm, 4°C for 10 min. The supernatant was immediately used for pH detection and total protein assay, and then preserved in 2 mL aliquots at -80°C for subsequent analysis.

Chemicals and reagents

Tyrosine, phenylalanine, and tryptophan (purity 99.9%) were supplied by Sigma (St. Louis, MO, USA) and the isotope-labeled internal standard (IS) tyrosine-d2, phenylalanine-d5, and tryptophan-d8 (purity 98.0%; Figure 1) were supplied by CIL (Andover, MA, USA). HPLC-grade formic acid was obtained from Dikma (Lake Forest, USA), and methanol (HPLC grade) from Fisher Scientific (Fair Lawn, NJ, USA). Water purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used for the analysis.

Instrumentation

An SIL-20ACHT autosampler, CBM-20A system controller, and LC-20 AD VP pump were obtained from Shimadzu Corporation (Kyoto, Japan). An API-5500 Qtrap mass spectrometer from Applied Biosystems (Foster City, CA, USA) was used as the detector. Data were acquired and processed using Analyst v1.5.2 software (Applied Biosystems).

Sample treatment

The gastric juice specimens were thawed at room temperature. Before the chromatographic analysis, 50 µL samples were deproteinized by adding 500 µL methanol, vigorously agitated at 2000 rpm for 120 s, followed by centrifugation at 15000 rpm, 4°C for 5 min. 50 µL of the organic layer was further diluted with 250 µL of deionized water, and 2 µL was injected into the LC-MS/MS system.

Chromatographic conditions

A Phenomenex synergi Polar-RP 80A column (4 µm, 2.0 mm, 50 mm) was used for the separation. The column oven was maintained at 30°C. LC separation was performed using a mobile phase consisting of methanol (mobile phase A) and 0.1% formic acid in water (mobile phase B). The gradient employed was as follows: 0-2 min linear increase from 3-50% A, 2-3 min maintained at 50% A, 3-3.1 min from 50-3% A, and hold at 3% A for 3.4 min. The flow rate was 0.25 mL/min, and the run time for one sample was 6.5 min.
Mass spectrometry conditions

The LC-MS/MS data acquisition for tyrosine, phenylalanine, and tryptophan was conducted in a positive ionization mode. The ion source parameters were collision gas (CAD medium), curtain gas (CUR 40), ion source gas 1 (GS1 50), ion source gas 2 (GS2 40), and temperature (TEM 550°C). Other parameters included declustering potential (DP 70 V), entrance potential (EP 10 V), collision energy (CE 13 V), and collision cell exit potential (CXP 13 V) for tyrosine; DP 70 V, EP 10 V, CE 15 V, and CXP 13 V for phenylalanine; and DP 70 V, EP 10 V, CE 12 V, and CXP 16 V for tryptophan. The product ions were monitored in a single reaction monitoring mode. The monitored transitions were mass-to-change ratio (m/z) 182.0→165.1 for tyrosine, m/z 166.0→120.1 for phenylalanine, and m/z 205.1→188.1 for tryptophan (Figure S1).

Aromatic amino acid quantification

Standard solutions that contained equivalent concentrations of L-tyrosine, L-phenylalanine, and L-tryptophan (99%, Sigma) were prepared by diluting the stock solution using deionized water at 0.1 µg/mL, 0.2 µg/mL, 0.5 µg/mL, 1.0 µg/mL, 2.0 µg/mL, 5.0 µg/mL, 10.0 µg/mL, and 20.0 µg/mL for establishing calibration curves and were preserved at -20°C. Eight-point calibration curves were used and plotted with the peak area ratio of tyrosine/tyrosine-d2, phenylalanine/phenylalanine-d5 and tryptophan/tryptophan-d8 using a weighted (1/x²) quadratic fit.

Calibration curves, linearity, and retention time of the quantification system

The calibration curves showed excellent linearity for all three AAAs, with low interference and high sensitivity. The calibration curve, linearity, and working ranges were as follows: tyrosine, Y = 1.79X + 0.0236, r = 0.9994, 0.1-20.0 µg/mL; phenylalanine, Y = 1.14X + 0.0171, r = 0.9985, 0.1-20.0 µg/mL; and tryptophan, Y = 1.09X + 0.0493, r = 0.9988, 0.1-20.0 µg/mL. Y is the concentration of each amino acid in gastric juice, and X is the relative peak-area ratio of each amino acid/corresponding IS. The retention times of tyrosine, phenylalanine, and tryptophan were 2.9 min, 1.4 min, and 1.0 min, respectively (Figure S2).

Recoveries and precisions of the quantification system

The recoveries of tyrosine, phenylalanine, and tryptophan were 98.2-111.7%, 98.5-107.3%, and 101.6-101.8%, respectively. The precisions were 1.90-11.02%, 1.32-8.93%, and 2.63-7.04%, respectively. Taken together, these results showed that the present experimental system was stable and reliable for the quantification of AAAs in gastric juice.

If some of the AAAs fell beyond the measurement range, the samples were re-diluted before injection. In the event of extremely low concentration that was undetectable, the value was replaced with zero.

Gastric juice pH measurement

The pH of gastric juice samples was monitored using a digital pH meter (Thermo Orion, USA), according to the operation manual. Calibration was required before each test using standard buffer solution with pH value of 6.86 and 4.01, respectively. Only the slope is greater than 0.9, can the pH value of the sample be determined.

Quantification of total protein in gastric juice

The gastric juice supernatant was diluted 10-fold with double-distilled water (ddH₂O) and assayed using the bicinchoninic acid (BCA) protein assay kit (Beijing Applygen Technologies Inc., Beijing, China), according to the manufacturer's protocol. If the protein concentration was beyond the linear range (20-2000 µg/mL), then the sample was further diluted and reanalyzed.

Helicobacter pylori infection detection

H. pylori infection was determined by Warthin-Starry (WS) staining in all specimens using H. pylori detection kit (Beijing ShiJi HeLi Biotechnology Co., Ltd, Beijing, China) following the manufacturer’s instructions. The background tissue was stained to yellow and the nucleus was dyed to brown. If there is H. pylori infection, the bacteria would be stained to black.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).
Gastric juice index model for early gastric cancer

Continuous variables that conformed to normal distribution were expressed as mean ± standard deviation or else as median (25th to 75th percentile). Pearson’s Chi-square test was used to compare the difference in gender and H. pylori infection, independent t-test for the age, and Mann-Whitney U test for each observed indicator in gastric juice of patients between the two groups. Any parameter with statistical significance was analyzed by binary logistic regression, and a new variable established. The performance of the predictive model was assessed by the area under the curve (AUC). The Hosmer-Lemeshow test was used to predict the goodness of fit for the model. All P-values were two-sided, and P<0.05 was considered as statistically significant.

Enhanced levels of gastric juice parameters in the GC group as compared to the NGD group in the derivation subgroup

Table 3 shows that significantly higher pH values and free AAA levels in gastric juice were observed in the GC group as compared to the NGD group in the derivation subgroup (all P<0.001). However, no significant difference of TPC was observed between these two groups (P>0.05) (Figure 2A-C).

Establishment of the GJI model

Any parameter with statistical significance in the derivation subgroup was subjected to binary logistic regression analysis, and a unique diagnostic index was obtained. The adjusted

Table 1. Patients’ baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Derivation subgroup (n = 120)</th>
<th>Validation subgroup (n = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>GC (n = 31) NGD (n = 89)</td>
<td>GC (n = 27) NGD (n = 53)</td>
</tr>
<tr>
<td></td>
<td>61.4 ± 12.5 63.2 ± 12.4</td>
<td>58.7 ± 14.4 61.4 ± 13.6</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>21/10 36/53</td>
<td>21/6 26/27</td>
</tr>
<tr>
<td>H. pylori positive</td>
<td>6 17 17 14 27</td>
<td>9 7 12 46 36</td>
</tr>
</tbody>
</table>

Data on age are shown as mean ± standard deviation, other categorical data are shown as count. GC, gastric cancer; NGD, non-neoplastic gastric diseases; M/F, male/female. *Statistically significant difference (P<0.05) using independent t-test (a) or Pearson's Chi-square test (others).

Table 2. Patients’ disease constitution

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Patients</th>
<th>GC</th>
<th>NGD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivation</td>
<td>120</td>
<td>AGC</td>
<td>EGC</td>
<td>0.793</td>
</tr>
<tr>
<td>Validation</td>
<td>80</td>
<td>ATP</td>
<td>CAG/IM</td>
<td>0.753</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>CSG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data are shown as count. GC, gastric cancer; NGD, non-neoplastic gastric diseases; AGC, advanced gastric cancer; EGC, early gastric cancer; ATP, atypical hyperplasia; CAG/IM, chronic atrophic gastritis and/or intestinal metaplasia; CSG, chronic superficial gastritis. No statistically significant difference of disease constitution was found between the derivation and validation subgroups using Pearson’s Chi-square test.

Table 3. Gastric juice parameters measurement in the derivation subgroup

<table>
<thead>
<tr>
<th>Variable</th>
<th>GC</th>
<th>NGD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH value</td>
<td>4.586 (2.320-6.858)</td>
<td>1.826 (1.495-2.764)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Tyrosine (µg/mL)</td>
<td>11.400 (5.180-5.500)</td>
<td>3.870 (2.405-6.880)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Phenylalanine (µg/mL)</td>
<td>29.300 (7.320-68.000)</td>
<td>5.380 (3.300-9.075)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Tryptophan (µg/mL)</td>
<td>4.810 (1.610-22.200)</td>
<td>1.040 (0.702-2.715)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>TPC (µg/µL)</td>
<td>3.987 (2.528-6.669)</td>
<td>3.409 (2.396-4.590)</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Data are presented as median (25th to 75th percentile). GC, gastric cancer; NGD, non-neoplastic gastric diseases; TPC, total protein concentration. ***Statistically significant difference (P<0.001) using Mann-Whitney U test.

Results

The baseline characteristics of the study population are outlined in Table 1. No significant differences were observed in age and H. pylori infection rate between the GC and NGD groups in the derivation subgroup, except male/female ratio. No significant difference in age between the two groups in the validation subgroup was observed, but both gender and H. pylori infection rate were significantly different. As for the disease constitution, no significant difference was found between the derivation and validation subgroups (Table 2).
Gastric juice index model for early gastric cancer

logistic regression equation is: Logit $P = -1.637 * X_1 + 0.307 * X_2 - 0.135 * X_3 + 0.322 * X_4 - 0.327 * X_5 + 9.235$ (Table 4). The Logit $P$ is referred to as gastric juice index, GJI. Moreover, the calibration plot was drawn by the Hosmer-Lemeshow test, and its significance was 0.764, indicating an optimal fit for the logistic regression model (Figure S3).

**Validity of the five parameters for the detection of GC in the derivation subgroup**

ROC curve analysis was used to determine the values of these five parameters for discriminating between patients in the GC and NGD groups. For the detection of GC, the AUCs of each parameter are shown in Figure 2D. The cutoff value, sensitivity, specificity, and accuracy of each parameter are shown in Table 5.

**Validity of the GJI model for the detection of GC in the validation subgroup**

Table 6 shows that significantly higher pH values, free AAAs in gastric juice and GJIs were observed in the GC group as compared to the NGD group in the validation subgroup as well ($P<0.001$) (Figure 2E-G). For the detection of GC, the AUCs of each parameter are shown as Figure 2H. And the AUC of GJI was still higher than any individual parameter in the regression equation. Moreover, as compared to the optimal cutoff value (8.995) established from the derivation subgroup, the sensitivity, specificity, and accuracy of GJI

Figure 2. Scatter plots from the GC and NGD patients and ROC curves for the detection of GC with gastric juice parameters. Scatter plots showing the levels of gastric juice parameters from the GC and NGD groups in the derivation subgroup (A-C) and validation subgroup (E-G); ROC curves for the detection of GC with the above parameters were plotted in the derivation subgroup (D) and validation subgroup (H). GC, gastric cancer; NGD, non-neoplastic gastric diseases; TPC, total protein content; GJI, gastric juice index. Tyr-GC, tyrosine in the GC group; Tyr-NGD, tyrosine in the NGD group; Phe-GC, phenylalanine in the GC group; Phe-NGD, phenylalanine in the NGD group; Trp-GC, tryptophan in the GC group; Trp-NGD, tryptophan in the NGD group. ***Statistically significant difference ($P<0.001$) using Mann-Whitney U test. Error bars represent median ± interquartile range.
in the validation subgroup were 74.1%, 73.6%, and 73.8%, respectively. Although slightly lower than those in the derivation subgroup, they were significant, which illustrated that the GJI model was reproducible and robust.

Observation of the early diagnostic value using the GJI model in all patients

The median (25th to 75th percentile) of GJI in AGC, EGC and NGD group was 11.570 (9.438, 15.868), 10.762 (8.992, 13.071), and 8.337 (7.146, 8.888), respectively (Figure 3), which showed significantly higher levels of GJI in both AGC and EGC patients as compared to the NGD individuals (all \( P < 0.001 \)). But no difference was found between the AGC and EGC groups. For the detection of AGC and EGC, the AUCs of GJI were 0.876 (95% CI, 0.808-0.944) and 0.848 (95% CI, 0.771-0.924), respectively (Figure 4). At the optimal cutoff value (8.995), its sensitivity, specificity, and accuracy were AGC 83.3%, 78.9%, and 79.7%; EGC 75.0%, 78.9%, and 78.2%, respectively.

Discussion

Gastric cancer (GC) adds a heavy burden to global health. There were 260000 cases of cardia GC and 691000 cases of non-cardia GC worldwide in 2012 [1]. The mortality rate of GC has remained high, mainly due to its silent nature at the early stage, making timely diagnosis and treatment difficult. However, the usefulness of the conventional serological markers is limited. Therefore, new molecular markers with high accuracy are needed for diagnostic surrogates. In the past decade, our institute has isolated and identified three AAAs in gastric juice, which can be used to distinguish AGC and EGC from benign gastric disease [6, 7]. In this study, we constructed and validated a GJI model, based on the male to female ratio, pH values and three AAA concentrations in gastric juice by logistic regression analysis, which can be used to differentiate GC patients from NGD individuals. Moreover, the GJI model was also valuable for detecting EGC. Similarly, Xie et al [8] developed an electrochemical microfluidic chip combined with multiple antibodies against six biomarkers (CEA, CA19-9, H. pylori CagA protein, P53 oncoprotein, pepsinogen I (PG I), and PG II) and established a multi-index prediction model based on these biomarkers for predicting the risk of GC. The combined detection of these biomarkers would be helpful to enhance the accuracy of predicting GC risk. However, the final positive rate of the multivariate was not reported in the study.

We focused on the five factors that were involved in the logistic regression equation. Firstly, the gastric juice, as a source of gastric cancer biomarkers, is specific than other biofluids [12, 13]. Moreover, the diagnostic value of free amino acids is higher than the other gastric juice biomarkers [14, 15]. Probably because of being the end products, they are more stable than mRNAs and proteins. Herein, the enhanced production of three AAAs in GC corresponded to the results of our previous studies, which was proven [16] and approved [13, 17, 18] by other authors. Among the three AAAs, the best detection marker for GC was phenylalanine, which is also consistent with our previous studies [6, 7].

Several methods have been developed and used for the analysis of AAAs. Conventional methods for AAA estimation, such as liquid chromatography combined with UV [19] or fluorescence detection [20] rely on the determination of total proteinogenic amino acid concentrations by ion exchange chromatography and ninhydrin derivatization; and the completion of one analytical run may require 1 h or longer. Although Galba et al [21] developed and validated a sensitive ultra-high-performance liquid chromatography (UPLC) method for the analysis of phenylalanine, tyrosine, tryptophan, and kynurenine in rat plasma, the process of chemical derivatization is inevitable. Recently, nanoparticles have been proposed for detecting amino acids by selective attachment. Hazra et al [22] reported the use of Ln3+-doped nanocrystals to detect AAAs at nanomolar concentrations. Sui et al [23] reported a surface-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>B</th>
<th>S.E</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta X_1 ) Gender</td>
<td>1.637</td>
<td>0.638</td>
<td>0.010</td>
</tr>
<tr>
<td>( \Delta X_2 ) pH value</td>
<td>-0.307</td>
<td>0.169</td>
<td>0.069</td>
</tr>
<tr>
<td>( \Delta X_3 ) Tyrosine</td>
<td>0.135</td>
<td>0.069</td>
<td>0.049</td>
</tr>
<tr>
<td>( \Delta X_4 ) Phenylalanine</td>
<td>-0.322</td>
<td>0.109</td>
<td>0.003</td>
</tr>
<tr>
<td>( \Delta X_5 ) Tryptophan</td>
<td>0.327</td>
<td>0.116</td>
<td>0.005</td>
</tr>
</tbody>
</table>

S.E: standard error; Sig.: probability. Gender was calculated in binary, 1 represents male and 2 represents female.
enhanced resonance Raman scattering (SER-RS)-based approach coupled with azo coupling reaction for quantitative analysis of tyrosine and histidine. However, the availability of these platforms is poor, which limits their widespread utility. In this study, we analyzed gastric samples and detected significant changes of AAAs in gastric cancer patients by the LC-MS/MS method. The benefits of this method are as follows: 1. The pre-treatment method is relatively simple, and derivatization step is not required, which would dramatically reduce the separation and analytical time, and thus, an excellent sample throughput could be achieved. 2. Isotope-labeled internal standard method guarantees high sensitivity, and the matrix effects could be efficiently corrected.

Secondly, the median fasting pH is reported as 1.5, with a normal range of 0.3-2.9. The low level of hydrogen ion concentration ([H⁺]) in gastric juice is speculated to be strongly related to precancerous gastric conditions as well as chronic atrophic gastritis. If the lesions evolved into GC, the level might be lower [24]. In the present study, we found that the median (P25-P75) value of pH in GC patients was 4.586 (2.320-6.858), which is significantly higher than 1.826 (1.495-2.764) in NGD patients. At the optimal cutoff value (2.470) to differentiate between GC and NGD patients, its sensitivity, specificity, and accuracy were 71.0%, 71.9%, and 71.7%, respectively.

Thirdly, according to Globocan 2012 [1] estimates, males showed a higher rate of GC than females, particularly for gastric cardiac cancer (male-to-female ratio 3:1). In our study, the
male-to-female ratio was approximately 2:1 in GC patients, which is consistent with epidemiology.

In the present study, the random number was generated by the SPSS software, ascribing the first 120 patients as the training set and the latter 80 as the validating set. The prediction accuracy of the GJI model was established and evaluated. The credibility of the statistics according to the random number was clearly higher than the sequence number of the patients enrolled in the study. However, only one round of cross-validation was used. In order to reduce variability, 10-fold cross-validation should be performed.

The preferred method to detect EGC is gastroscopy followed by pathological biopsy. However, negative results are common, because tiny and insidious lesions are often missed, biopsied inadequately, or interpreted incorrectly by pathologists. The finding that the level of GJI can also be used to discriminate between EGC and NGD is exciting. Although the diagnostic performance of GJI in the EGC group was slightly lower than that in the AGC group, it was significant and similar to previously reported gastric juice AAAs [5, 7]. This demonstrated that metabolic and biochemical abnormalities accompanied or occurred even prior to visible morphological changes in the cascade of GC.

Therefore, the GJI model can be used to monitor the occurrence of metabolic perturbations and reveal early malignant transformations in the stomach.

**Limitations**

Although a panel assay of these gastric juice parameters may be used as an adjunct to histological assessment, there are a few drawbacks in this approach. Firstly, the use of less invasive procedures would be beneficial for screening purposes. The samples in this study were aspirated during gastroscopy, which would strictly limit the applicability of assessing gastric juice parameters for the early detection of GC. To increase the potential application of the GJI model, non-endoscopic methods should be introduced for sampling. For example, gastric juice can be safely and conveniently collected using endogastric capsule [25]. Next, the sample size in this study was relatively small, and thus, large-scale clinical cohorts are required to determine the normal reference range and optimized cutoff value. Lastly, this study was based on our preliminary findings, and only three AAAs in the gastric juice were measured. Therefore, for further exploration, the whole gastric juice free amino acid profiling in GC patients is an indispensable prerequisite. Finally, the subjects in our study were with gastrointestinal complaints or for endoscopic sur-
Gastric juice index model for early gastric cancer

In conclusion, our study built and tested a prediction model based on the male-to-female ratio, pH values, and AAA concentrations in gastric juice for GC. Interestingly, the level of the multi-index (GJI) begins to rise at the early stage of gastric carcinogenesis. Therefore, it has great potential for improving the early detection of GC.

Acknowledgements

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Disclosure of conflict of interest

None.

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References


Gastric juice index model for early gastric cancer


Gastric juice index model for early gastric cancer
Figure S1. Daughter ion mass spectra of three aromatic amino acids (A, C, E) and their isotope-labeled IS (B, D, F) in one sample.
Gastric juice index model for early gastric cancer
Figure S2. Chromatograms of three aromatic amino acids (A, C, E) and their isotope-labeled IS (B, D, F) in two samples.
Figure S3. Calibration plot of the gastric juice index (GJI) model. Calibration plot drawn by the Hosmer-Lemeshow test, indicated an optimal fit for the GJI model. The dashed lines demonstrate that the prediction and actual is exactly the same.