Metabolomic profiling on plasma reveals potential biomarkers for screening and early diagnosis of gastric cancer and precancerous stages

Lijing Du

School of Pharmacy, Shanghai Jiao Tong University

Shasha Li

The Second Clinical College of Guangzhou University of Chinese Medicine

Xue Xiao

Guangdong Pharmaceutical University

Jin Li

The 903rd Hospital of PLA

Huizi Jin

Shanghai Jiao Tong University

Zhaolai Hua

People's Hospital of Yangzhong City

Juming Ma

The 903rd Hospital of PLA

Xi Wang

The 903rd Hospital of PLA

Shikai Yan (✉ shkyan@sjtu.edu.cn)

Shanghai Jiao Tong University of pharmacy

Research article

Keywords: Gastric cancer, Metabolomics, Biomarkers, Pathway analysis, UPLC-Q-TOF/MS

Posted Date: March 20th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-18127/v1>

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Abstract

Background: Gastric cancer (GC) remains one of the most common cancers all over the world. The greatest challenge for GC is that it is often detected at advanced stages, leading to the loss of optimum time for treatment and giving rise to poor prognosis. Thus, there is a critical need to develop effective and noninvasive strategies for early diagnosis of the disease process.

Methods: In total, 82 participants were enrolled in the study, including 50 chronic superficial gastritis (CSG) patients, 7 early gastric cancer (EGC) and 25 advanced gastric cancer (AGC) ones. Metabolites profiling on patient plasma was performed using ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF/MS). Principal components analysis as well as orthogonal partial least squares-discriminant analysis was utilized to evaluate the variation on endogenous metabolites for GC patients and to screen potential biomarkers. Furthermore, the biomarker panels detected above were used to create logistic regression models, which discrimination efficiency and accuracy was ascertained by receiver operating characteristic curve (ROC) analysis. Metabolic pathways were carried out on MetaboAnalyst.

Results: Totally 50 metabolites were detected differentially expressed among CSG, EGC and AGC patients. L-carnitine, L-proline, pyruvaldehyde, phosphatidylcholines (PC) (14:0/18:0), lysophosphatidylcholine (14:0) (LysoPC 14:0), lysinoalanine were defined as the potential biomarker panel for the diagnosis among CSG and EGC patients. Compared with EGC patients, 6 significantly changed metabolites, PC(O-18:0/0:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) were found to be up-regulated, whereas L-proline, L-valine, adrenic acid and pyruvaldehyde to be down-regulated in AGC patients. ROC analysis demonstrated a high diagnostic performance for metabolite panels with area under the curve (AUC) of 0.931 to 1. Moreover, the metabolomic pathway analysis revealed several metabolism pathway disruptions, including amino acid and lipid metabolisms, in GC patients.

Conclusions: In this study, a total of six differential metabolites that contributed to GC and precancerous stages were identified, respectively. The biomarker panels further improve diagnostic performance for detecting GC, with AUC values of more than 93.1%. It indicated that the biomarker panels may be sensitive to the early diagnosis of GC disease, which can be used as a promising diagnostic and prognostic tool for disease stratification studies.

Introduction

The International Agency for Research on Cancer estimates that gastric cancer (GC) is the fifth most prevalent type of malignancy and the third mortality of cancer death all over the world [1]. Although surgery is the most preferred treatment, the majority of patients have recurrence after surgery, leading to a poor five year survival rate [2, 3]. The growth and proliferation of GC is involved in multi-gene and multi-factor. Correa [4] proposed a theory of human intestinal-type gastric carcinogenesis initiated by normal mucosa, followed by chronic superficial gastritis (CSG), then chronic atrophic gastritis, to intestinal

metaplasia, and finally by dysplasia and intestinal-type gastric cancer, which is generally accepted. More specifically, CSG and chronic atrophic gastritis are the critical stage in the development and proliferation of GC, which are accounted as important risk factors for gastric carcinogenesis. So early prevention on gastric precancerous lesions and diseases could reduce the incidence of GC. In recent decades, diagnostic methods based on endoscopic examination, pathological section and barium meal examination, have been widely applied to GC patients [5]. However, these screening methods are limited by various disadvantages, such as time-consuming, invasiveness, laborious and harmful. Thus, establishment of a sensitive, noninvasive examination method for early detection and prognosis prediction in GC patients is of significant importance.

Metabolomics is an emerging science involving the profiling changes in small-molecular metabolites produced by a biological system under certain conditions [6]. It seems to be a very promising method for biomarker discovery due to the dynamic responses of the metabolome that reflects upstream biological processes in the body. It has several major advantages, such as the readily availability, noninvasive and high sensitivity [7]. For the past few years, metabolomics has been utilized for analysis of metabolic alterations caused by cancers and other diseases, which has led to substantial advances in early diagnosis, mechanism clarification, and discovery of biomarkers [8].

Biomarkers are small-molecular intermediates and end products of active cellular processes, forming a correlation between molecular metabolic changes and phenotype [9, 10]. Therefore, they reflect alterations of the physiological state of a biological system (cell, tissue or organism) at a certain point in time. Early gastric cancer (EGC) is asymptomatic. There is no doubt that one of the greatest challenges for biomarker-related is discovering biomarkers that accurately distinguish cancer from precancerous stages, where overlapping signs and symptoms (unintentional weight loss or vague epigastric pain) make differential clinical diagnosis difficult [11]. Till now, few studies on metabolic changes for screening and early diagnosis of GC and precancerous stages have been applied in clinical practice, which are needed to be further explored.

In this work, we developed an ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF/MS) and biotransformation-based metabolomics profiling approach for determining GC staging and CSG. Briefly, multivariate analyses were utilized to identify differential metabolites that related to GC staging and CSG groups. On the basis of potential biomarkers, the related metabolic pathways and correlation networks were investigated and the global metabolic features were discussed.

Methods

Patient information and sample collection

The present study was approved by the ethics Committee of the People's Hospital of Yangzhong City (Yangzhong, China) and all participants provided written informed consent. A total of 86 individual

patients who had CSG and GC were recruited at the People's Hospital of Yangzhong City between July and December, 2015. All of tissue specimens were examined by gastroscopic biopsy or pathological examination after surgery. According to the results from pathologic diagnosis, the 86 samples were divided into three groups, including fifty cases of CSG (mean age $\pm$ SD, 52.1 ± 7.0 years), seven cases of EGC (mean age $\pm$ SD, 66.3 ± 11.9 years) and twenty-five cases of advanced gastric cancer (AGC, mean age $\pm$ SD, 67.0 ± 9.7 years). Among them, four cases of patients who had a history of chemotherapy or surgical treatment were excluded from this study. Details of basic information of patients with CSG and GC staging are shown in Table S1.

5 mL venous blood samples from all patients were collected under fasting conditions and added in heparin sodium anti-coagulated tube. The collected whole blood was refrigerated at 4 °C within 15 min and centrifuged at 2000 rpm for 15 min within 4 h. Plasma was carefully transferred to an Eppendorf tube, and stored at -80 °C until use.

Chemicals and reagents

Acetonitrile, methanol, formic acid and isopropanol were purchased from J. T. Baker Chemical Co. (Phillipsburg, New Jersey, USA). All chemical reagents were HPLC grade. Purified water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

Sample preparation

All frozen plasma samples were thawed completely at room temperature for 3 h. Then, 100 μ L plasma sample was transferred to an Eppendorf tube, and 300 μ L methanol/acetonitrile solution (v/v, 1:1) containing 2-Chloro-L-phenylalanine (5 μ g/mL) as internal standard was added. Mixed sample was vortex-mixed for 30 s and placed at 4 °C for 1 h. Vortex-mix again for 30 s and kept at 4 °C for 3 h to fully precipitate the protein in the plasma. Finally, the mixture was centrifuged at 15000 rpm for 10 min at 4 °C, and the clear supernatant was collected to injection vial for UPLC-Q-TOF/MS analysis.

UPLC-Q-TOF/MS analysis

The metabolomic analysis was carried out on an ACQUITY UPLC (Waters) system equipped with Micromass quadrupole-time-of-flight mass spectrometer (Q-TOF/MS) (Waters Corp., Milford, USA). Plasma metabolites were separated on a Acquity BEH-C18 column (100 mm $\times$ 2.1 mm i.d., 1.7 μ m; Waters, Milford, USA) with the column oven temperature maintained at 50 °C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B used the mixture of B-isopropanol, acetonitrile, methanol, formic acid (20: 40: 40: 0.1 (v/v)). The gradient program (A/B, v/v) was changed from 98/2 to 0/100 for 12.5 min with a constant flow rate of 0.4 mL/min. Injection volume was set at 3 μ L. MS parameters were as follows: mode, positive ion; capillary voltage, 3000 V; cone voltage, 35 V; collision energy, 3 eV; ion source temperature, 115 °C; desolvent gas temperature, 350 °C; desolvent gas flow, 600 L/h; full scan range, 50-1000 m/z. The mass spectrometric data were recorded with a scan time of 0.3 s and an inter-scan delay of 0.02 s. To evaluate data quality and reliability, a quality control (QC) sample was injected and analyzed once every 10 study samples.

Statistical analysis

The data collected from MS were processed using Progenesis Q1, and then analyzed by SPSS 18.0. Statistical significance differences were established at $P < 0.05$. Then, normalized MS data were imported into SIMCA-P software (14.0, Umetrics AB) to perform multivariate analysis and modeling, including principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA). Significantly changed metabolites between two groups were identified by variable importance in the projection (VIP) ≥ 1 and $p < 0.05$ in paired t-test. Finally, the structure information of differentially expressed metabolites was further identified based on MS² fragments and database of HMDB, METLIN, LIPID, SERUM and KEGG, etc. SPSS was used to draw receiver operating characteristic (ROC) curves to display the level and diagnosability of potential biomarkers directly and metabolome pathway maps were retrieved from online MetaboAnalyst 4.0 (<https://www.metaboanalyst.ca/>).

Results

Metabolites detection and identification

A full-scan detection of plasma metabolites was performed by UPLC-Q-TOF/MS, including 50 cases of CSG, 7 cases of EGC and 25 cases of AGC, which involved principal components that account for the majority of the differences in the data. In conjunction with the Progenesis Q1 package, UPLC-Q-TOF/MS analysis of plasma metabolites contained three typical total ion current (TIC) chromatograms, as shown in Fig. 1. A total of 2666 peaks were detected and 50 differential metabolites were authentically identified, including L-proline, L-isoleucine, L-leucine, L-valine, lysine alanine, lysophosphatidylcholines (LysoPC) (12), phosphatidylcholines (PC) (16), phosphatidylethanolamines (6), L-carnitine, creatine, cholesterol, cholic acid, tyramine, uric acid, capryl carnitine, pyruvaldehyde, docosatrienoic acid, malonaldehyde and 1-sphingosine phosphate, etc. The identity of metabolites based on the following criteria: VIP score > 1 , $P \leq 0.05$ in the EZinfo software, and metabolites match in the databases of HMDB, LIPID MAPS and SERUM.

Differential plasma metabolic profiles among groups

Metabolite differences in CSG and EGC

Using SIMCA-P software, PCA as an unsupervised method and OPLS-DA as a supervised method were performed to discriminate the overall metabolic profiles between CSG and EGC patients. The score plots of PCA and OPLS-DA are presented in Fig. 2A-B. Subjected to the inter-group PCA analysis, it was observed that there is no obvious clustering pattern between the two groups. OPLS-DA (CSG vs EGC) revealed a well gathering trend and complete separation in score plot. Of all peaks detected by UPLC-Q-TOF/MS, the peak areas of 30 peaks were statistically different between CSG and EGC patients (VIP ≥ 1 , $P \leq 0.05$), and these signals were identified by database of HMDB, LIPID MAPS and Serum. After that, the molecules responsible for these peaks were further identified by comparing the MS/MS spectra and

Metlin database. Six metabolites, named L-carnitine, L-proline, pyruvaldehyde, PC(14:0/18:0), LysoPC(14:0) and lysinoalanine were defined in metabolic profiles. Identification and statistic analysis indicated significant elevation of L-carnitine, L-proline, pyruvaldehyde, PC(14:0/18:0), LysoPC(14:0) and lysinoalanine were defined in metabolic profile, while revealing significant reduction of LysoPC(14:0) and lysinoalanine, in EGC compared with CSG, as shown in Table 1.

Table 1
Statistically significant metabolites for comparison of CSG and EGC patients

NO	Retention Time(min)	m/z	Compound	VIP value	P value	The trend of EGC
1	0.67	162.11	L-Carnitine	1	0.008	↑
2	2.04	138.06	L-Proline	1	5.41E-08	↑
3	0.68	114.07	Pyruvaldehyde	1	0.006	↑
4	10.30	734.57	PC(14:0/18:0)	1	0.018	↑
5	5.65	468.31	LysoPC(14:0)	1	0.005	↓
6	2.85	251.17	Lysinoalanine	1	0.021	↓

Metabolite differences in EGC and AGC

Similarly, a PCA analysis was used to explore the metabolic profiling differences between the EGC and AGC patients, and the results are presented in Fig. 2C. There were no distinctive differences between EGC and AGC groups. Then, the OPLS-DA model was launched (Fig. 2D). Based on the criteria of OPLS-DA (VIP > 1 and P < 0.05), 16 statistically differentially expressed metabolic molecules in total were screened out and finally 6 metabolic molecules were identified as potential metabolite biomarkers between the two groups. The significantly changed 6 metabolites listed in Table 2. PC(O-18:0/0:0) and LysoPC(20:4(5Z,8Z,11Z,14Z)) were found to be up-regulated, whereas L-proline, L-valine, adrenic acid and pyruvaldehyde to be down-regulated in AGC patients.

Table 2
Statistically significant differences in metabolite levels between EGC and AGC groups

NO	Retention Time(min)	m/z	Compound	VIP value	P value	The trend of EGC
1	2.04	138.06	L-Proline	1	0.018	↓
2	0.73	118.08	L-Valine	1	0.015	↓
3	4.31	355.26	Adrenic acid	1	0.038	↓
4	7.96	551.43	PC(O-18:0/0:0)	1	0.027	↑
5	5.91	544.34	LysoPC(20:4(5Z,8Z,11Z,14Z))	1	0.041	↑
6	0.68	114.06	Pyrualdehyde	1	1.50E-09	↓

Discriminant models establishment based on the ROC analysis

The receiver operating characteristic (ROC) analysis is generally considered as a standard method for effectiveness assessment of diagnostic biomarkers. In this study, an in-depth ROC curve analysis was performed for 6 potential biomarkers (CSG vs EGC) and possible biomarker combinations. The area under the ROC curve (AUC) represents the overall accuracy of GC diagnostic test, and the results (optimal cut-off values, sensitivities, specificities and AUC values) are depicted in Table 3. The four up-regulated metabolites including L-Carnitine, L-Proline, Pyrualdehyde and PC(14:0/18:0) provided AUC values of 0.723 (sensitivity 57.1%, specificity 86.0%), 0.820 (sensitivity 71.4%, specificity 88.0%), 0.794 (sensitivity 100.0%, specificity 52.0%) and 0.769 (sensitivity 100.0%; specificity 50.0%) respectively, which implied a good distinctive ability in predicting GC. Another two down-regulated metabolites also have good independent predictive potential with AUC values from 0.780 to 0.849 (Table 3). Because the GC is a complex disease involving biochemical dysfunction in multiple pathways, a single biomarker could not be powerful to discriminate in clinical practice. Therefore, identifying a combination of biomarkers, which had greater predictive power, was particularly important. The 6 selected metabolites as the independent variables were combined together passed through binary logistic regression model with ROC curves to build the best biomarker panel. As a result, all six metabolites were used, which was termed as Mixmodel 1. The performance was calculated according to the Eq. (1) as follow.

Table 3
ROC curve analysis of potential biomarkers in GC

No.	Groups	Metabolites	AUC	Sensitivity (%)	Specificity (%)
1	CSG-EGC	L-Carnitine	0.723	57.1%	86.0%
2	CSG-EGC	L-Proline	0.820	71.4%	88.0%
3	CSG-EGC	Pyruvaldehyde	0.794	100%	52.0%
4	CSG-EGC	PC(14:0/18:0)	0.769	100%	50.0%
5	CSG-EGC	LysoPC(14:0)	0.849	100%	62.0%
6	CSG-EGC	Lysinoalanine	0.780	100%	52.0%
7	CSG-EGC	Mixmodel 1	1	100%	100%
8	EGC-AGC	L-Proline	0.611	88.0%	42.9%
9	EGC-AGC	L-Valine	0.526	64.0%	57.1%
10	EGC-AGC	Adrenic acid	0.720	44.0%	100%
11	EGC-AGC	PC(O-18:0/0:0)	0.749	100%	57.1%
12	EGC-AGC	LysoPC(20:4(5Z,8Z,11Z,14Z))	0.731	76.0%	71.4%
13	EGC-AGC	Pyruvaldehyde	0.657	40.0%	100%
14	EGC-AGC	Mixmodel 2	0.931	76.0%	100%

$$\text{Logit}(P)_{\text{Mixmodel1}} = -1934.912 + 0.086 \times x_1 + 1.238 \times x_2 + 0.061 \times x_3 + 0.054 \times x_4 - 0.001 \times x_5 + 0.008 \times x_6 \quad (1)$$

where x_1 , x_2 , x_3 , x_4 , x_5 and x_6 are represent for the peak value of L-carnitine, L-proline, pyruvaldehyde, PC(14:0/18:0), LysoPC(14:0) and lysinoalanine, respectively.

$$P = \frac{e^{\text{Logit}(P)}}{1 + e^{\text{Logit}(P)}} \quad (2)$$

The calculated results showed that the proposed biomarker panel model had AUC value of 1 (Fig. 3A), which meant that the multivariate model showed 100% discrimination power to separate EGC patients from CSG patients.

Similarly, to confirm the diagnostic potential for the early detection of GC, we examined the AUC values in stage EGC to AGC patients. As listed in the Table 4, 3 metabolites show good discrimination ability, with an AUC value above 0.7, along with 3 metabolites above 0.5. In addition, the biomarker panel was also applied to distinguish AGC patients with EGC patients, mining the potential ability for staging diagnosis. On the basis of binary logistic regression analysis, the plasma biomarker panel consisting of three metabolites, including L-proline, adrenic acid and PC(O-18:0/0:0), was defined as Mixmodel 2. The performance was calculated according to the following Eq. (3).

$$\text{Logit}(P)_{\text{Mixmodel2}} = 2.475 - 0.002 \times y_1 + 0.237 \times y_2 - 0.001 \times y_3 \quad (3)$$

where y_1 , y_2 and y_3 are represent for the peak value of L-Proline, Adrenic acid and PC(O-18:0/0:0), respectively. Results show that the biomarker panel had better diagnostic abilities than any single metabolite alone in distinguishing between early stage EGC patients and AGC patients, with sensitivity, specificity, and AUC value of 0.931, 76.0%, and 100.0% at the best cut-off points (Fig. 3B, Table 3). As indicated by these results, the biomarker combinations presented herein serves not only to discriminate EGC from CSG patients, but is also capable of distinguishing stage I and II GC models with relatively high diagnostic accuracy.

Metabolic Pathway Analysis

On the basis of the detected differential metabolites, pathway analysis was performed by MetaboAnalyst 4.0 to uncover the global metabolic disorders in CSG and GC patients. Figure 4A-B presents the major impacted pathways in the CSG-EGC and EGC-AGC groups, indicated by the red and orange colors ($-\log(p) > 2$ or impact value > 0.1). As shown in Fig. 3, the amino acid metabolism was discovered to be strikingly disturbed, including glycine, serine and threonine metabolism, valine, leucine and isoleucine biosynthesis and so on. The perturbations of central carbon metabolism (e.g., pyruvate metabolism) and lipid metabolism (e.g., glycerophospholipid metabolism, linoleic acid metabolism, alpha-linolenic acid metabolism and ether lipid metabolism) were also observed. The changes of detected differential metabolites related to the abnormal metabolic pathways, providing clues for underlying the potential metabolic mechanism in GC.

Discussion

In this study, high-throughput metabolomics couple with UPLC-Q-TOF/MS technology was utilized to investigate GC-related metabolic alterations and elucidate potential diagnostic biomarkers. The present evaluation was performed on patients with CSG and two subgroups of EGC and AGC subjects to search for the correlates between the small molecule metabolites and the disease progression. Most of the metabolites identified were altered on statistically significant level, derived mainly from general biochemical pathways related to amino acid metabolism, energy metabolism and lipid metabolism.

Amino acids, as the substrates for protein synthesis, are crucial for cancer cell migration and proliferation. Previous studies have associated amino acid metabolism aberrations with cancer development [12–13]. It is involved in multiple cancers that regulate several signaling pathways, including protein synthesis, cell growth, lipid biogenesis, autophagy and so on [14]. In this study, L-proline was found to be up-regulated in EGC and AGC stage, and L-valine was also found significantly up-regulated in AGC stage. High levels of proline could promote cell proliferation, energy production and resistance to oxidative stress (act as an antioxidant) [15–18]. L-valine is an essential and important functional amino acid involved in many growth and metabolic processes, and is also a glucogenic amino acid for biosynthesizing macromolecules (e.g., proteins and lipids), which are vital to the growth of cancer cell [19]. The accumulation of amino acids could ascribe to the proliferation by cancer cells, suggesting cancer transformation is linked with adaptive increases in protein synthesis [20].

Except a number of biologic functions, amino acid metabolism is also considered as an essential energy metabolism pathway of cancer cells to meet the high energy requirement [21]. For example, valine could be transformed into pyruvate for energy supply through aerobic glycolysis, resulting in the significant increase in pyruvate [22]. Thus, the up-regulated of valine in GC indicates that cancer cell energy metabolism may be significantly increased during cancer progression.

Another important feature in GC progression was the apparent changes of lipids. It is well known that lipids play an important role at cellular and organismal levels, being the dominant structural components of biomembranes and energy storage entities [23]. Additionally, lipids participate in signal transduction and can be broken down into biologically active lipid mediators, which regulate some carcinogenic processes [24, 25]. In the present study, potential biomarkers analyses revealed significant alterations in plasma LysoPC and PC. The down-regulation of them may be mainly due to the increased demand for membrane constituents during malignant transformation, cancer invasion and metastasis. Dysregulation of choline phospholipid metabolism is associated with carcinogenesis and cancer progression, which has been verified in many biomarker studies [26–28], including studies of GC [29]. Thus, the abnormal levels of LysoPC and PC may be considered as important biomarkers for GC patients.

A comprehensive understanding of metabolic alterations associated with cancer stages would be helpful in the development of GC. Among the all differential metabolites, there were some metabolites that showed a good potential to differentiate with AUC values more than 0.8, such as L-proline, LysoPC(14:0) (Table 4). However, a question arises as to whether one molecule has sufficient potential in GC detection. Based on the results of previous cancer biomarker studies, it can be assumed that the most efficient sample discrimination will be obtained using metabolite panels. Therefore, we built logistic regression models consisting of multiple metabolites. The AUC values (> 0.931) of multivariate ROC curve were higher than that obtained for single metabolite (Table 3) and the metabolite panels model turned out to be sensitivity enough to distinguish patients correctly. Therefore, the application of a combination strategy allows for better early diagnosis of GC and precancerous stages.

In summary, this study suggests that the biomarker panels possessed the potential value for the diagnosis of GC stages. The identified potential biomarkers and biological pathways might provide new directions for further studies in cancer growth and development. However, the strict inclusion and exclusion criteria decreased the number of recruited patients, and further research should involve the inclusion of multicenter subjects with GC of different stages, to better evaluate the accuracy of the developed models in early diagnosis.

Conclusion

In this study, UPLC-Q-TOF/MS plasma metabolomics has been successfully established for biomarker studies in GC. Differential metabolites signatures were globally depicted, and biomarker panels were defined for the diagnosis of EGC with satisfactory discrimination performance, even for AGC (AUC > 0.931). Metabolic pathway analysis indicated that changes in most potential plasma biomarkers were correlated with general biochemical pathways (amino acid metabolism and lipid metabolism), implying enhanced energy production and cell proliferation. The study highlights the potential advantages of biomarker panels in real clinical diagnostics, which can be used as a promising tool for early-stage GC diagnosis and prognosis.

Abbreviations

AGC: Advanced gastric cancer; AUC: area under the curve; CSG: Chronic superficial gastritis; EGC: Early gastric cancer; GC: Gastric cancer; LysoPC: lysophosphatidylcholines; OPLS-DA: Orthogonal partial least squares-discriminant analysis; PC: phosphatidylcholines; PCA: Principal components analysis; QC: Quality control; ROC: Receiver operating characteristic curve; UPLC-Q-TOF/MS: Ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry; VIP: Variable importance in projection.

Declarations

Acknowledgements

Not applicable.

Ethical approval and consent to participate

According to the Declaration of Helsinki, this study project was evaluated and approved by Institutional Review Board of the 903rd Hospital of PLA (20140501; Hangzhou, China) and Institutional Review Board of People's Hospital of Yangzhong City (IRB201404; Yangzhong, China). Written informed consent was obtained from all participants.

Authors' contributions

SK Y and XW conceived and designed the study. XW, SS L, J L, JM M and ZL H collected the clinical data and performed the experiments. LJ D drafted the first version of the manuscript. SK Y, XX and HZ J revised the manuscript together. All authors contributed to the interpretation of the results, edited and approved the final manuscript.

Funding

This work was supported by the Hangzhou Science and Technology Commission (20140633B41) and the Medical Innovation Project of PLA Nanjing Military Area Command (2013MS150).

Availability of data and materials

All the necessary materials can be found in the text or supplementary materials. Due to the privacy policy, the confidential data materials could only be obtained with the permission of the corresponding authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interests

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Figures

Figure 1

Typical total ion current chromatograms of diseases (color-blue) and QC samples (color-yellow) in positive mode UPLC-Q-TOF/MS analyses. (A) CSG; (B) EGC; (C) AGC.

Figure 2

Multivariate statistical analyses to distinguish the metabolic phenotypes between different groups. (A) PCA score plot of targeted metabolites collected from CSG (light blue) and EGC (green) groups; (B) OPLS-DA score plot of targeted metabolites collected from CSG (light blue) and EGC (green) groups; (C) PCA score plot of targeted metabolites collected from EGC (green) and AGC (navy blue) groups; (D) OPLS-DA score plot of targeted metabolites collected from EGC (green) and AGC (navy blue) groups.

Figure 3

ROC curves based on binary logistic regression model with biomarkers combination. (A) Mixmodel 1. (B) Mixmodel 2.

Figure 4

Metabolic pathway analysis in GC. (A) CSG and EGC. 1) Glycerophospholipid metabolism; 2) Linoleic acid metabolism; 3) alpha-Linolenic acid metabolism; 4) Pyruvate metabolism; 5) Glycine, serine and threonine metabolism. (B) EGC and AGC. 1) Aminoacyl-tRNA biosynthesis; 2) Valine, leucine and

isoleucine biosynthesis; 3) Pantothenate and CoA biosynthesis; 4) Ether lipid metabolism; 5) Pyruvate metabolism; 6) Glycine, serine and threonine metabolism.

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