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ILF2 protein is a promising serum biomarker for early detection of gastric cancer

Shao-Song Liu¹, Jin-Ke Wang¹, Mao-Sheng Liu¹, Ding-Fan Guo¹, Qi Wen¹, Yun-Hui Liang¹, Ting Wang^{1*} and Kun-He Zhang^{1*}

Abstract

Background Our previous small-sample study indicated that serum levels of interleukin enhancer binding factor 2 (ILF2) may have the potential for gastric cancer (GC) detection. The present study was conducted to further validate the diagnostic value of serum ILF2 protein for GC.

Methods Serum specimens and clinical data were collected from patients with GC ($n=99$) or benign gastric disease (BGD) ($n=49$) and healthy controls (HC) ($n=51$). Serum ILF2 levels were measured using enzyme-linked immunosorbent assay. The diagnostic performance of ILF2 was evaluated using the area under the receiver operating characteristic curve (AUC). The independence and synergy of ILF2 in GC diagnosis were analyzed by modeling with conventional blood indicators.

Results The median serum ILF2 level was higher in the GC group (227.8ng/mL) than in the BGD group (72.0ng/mL) and the HC group (56.8ng/mL) ($p < 0.001$), and no significant difference across GC subgroups. The AUCs of ILF2 were 0.915 (95%CI 0.873–0.957) for GC vs. HC, 0.854 (95%CI 0.793–0.915) for GC vs. BGD, 0.885 (95%CI 0.841–0.929) for GC vs. BGD+HC, and 0.888 (95% CI 0.830–0.945) for TNM I stage GC vs. BGD+HC, outperforming conventional blood indicators (corresponding AUCs ranging from 0.641 to 0.782). ILF2 was independent of and synergistic with conventional blood indicators in GC diagnosis, and a simple diagnostic model based on ILF2 and red blood cell count improved the diagnostic performance, with positive rates of approximately 90% in various subgroups of GC.

Conclusions Serum ILF2 protein is a novel and potential serum biomarker for the detection of GC, especially for early GC.

Keywords Gastric cancer, ILF2, Early diagnosis, Serum biomarker

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Introduction

Gastric cancer (GC) is the fifth most common cancer and the fourth leading cause of cancer-related death, with more than 1.08 million new cases and 0.76 million deaths annually [1]. The prognosis for GC remains poor, with a 5-year survival rate close to 30% [2]. Gastroscopy and biopsy [3] are effective but invasive and expensive methods for diagnosing GC [4], leading to a need for less invasive and more affordable diagnostic options.

Serum tumor biomarkers are clinically convenient approaches for cancer detection. In previous studies, traditional serum biomarkers have been extensively evaluated in the diagnosis of GC, such as carbohydrate antigen 72-4 (CA72-4) [5], carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), cancer antigen 125 (CA125), AFP (alpha-fetoprotein) [6], and carbohydrate antigen 242 (CA242) [7]. However, these traditional biomarkers are limited in the clinical application of GC due to low sensitivity and specificity [8, 9]. The discovery of novel biomarkers with improved diagnostic performance for GC is urgently needed.

Recent studies have identified some new types of tumor biomarkers [10], such as circulating tumor cells (CTC) [11], circulating tumor DNA (ctDNA) [12], and exosomes [13], which provide potential new approaches for GC detection [14]. However, these novel biomarkers have low serum levels and require further validation due to lack of standardized detection methods and limited real-world applications [15]. Proteins are important biomarkers in cancer liquid biopsy as they provide more detailed information than DNA/RNA in these samples [16]. Several protein biomarkers have been identified for GC, such as src kinase-associated phosphoprotein 1 (SKAP1) [17], carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) [18], as well as specific pepsinogen (PG), gastrin-17 (G-17) [19], and gastrokine 1 (GKN1) [20], but further clinical studies are needed to confirm their accuracy [21].

Previously, we identified a potential serum protein biomarker of GC, interleukin enhancer binding factor 2 (ILF2), using our aptamer-based serum proteomic data of GC and transcriptomic data of GC from public database, and the preliminary data showed that serum ILF2 levels were significantly elevated in GC patients compared with normal controls [22], suggesting that serum ILF2 protein may be a valuable biomarker for the detection of GC. Therefore, in this study, we further evaluated the diagnostic value of serum ILF2 protein in GC, especially in early stage GC.

Materials and methods

Subjects

The subjects of this study were patients with GC or benign gastric disease (BGD) and healthy controls (HC)

who received medical services at the First Affiliated Hospital of Nanchang University between 2013 and 2022. The inclusion criteria for the subjects were as follows: (1) GC patients were hospitalized for surgical treatment and did not have previously received antitumor therapy for GC; (2) BGD patients were hospitalized for the endoscopic or medical treatment of benign gastric diseases, including gastrointestinal stromal tumor (GIST) (only those with low or very low risk), gastric polyp, leiomyoma, ectopic pancreas, chronic gastritis, and peptic ulcer; (3) all patients were definitively diagnosed by pathology. Those whose blood laboratory results might be affected by disease or treatment were excluded: (1) with a history of blood transfusion in the past three months; (2) with malignant tumors of other organs; (3) with hematologic diseases or acute infectious diseases likely to affect the results of blood cell analysis. The HC group consisted of healthy individuals who underwent a health examination and did not have a history of gastric disease, epigastric symptoms, or abnormal routine laboratory and imaging findings. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University.

Collection of serum samples and clinical data

Leftover serum specimens (originally drawn for blood biochemistry) of the subjects were collected from the clinical laboratory department and stored at -80 °C. Clinical data of the subjects were collected from the hospital information system, including demographic data (age and sex), clinical laboratory data (blood cell analysis and conventional serum tumor markers), and pathological data (such as tumor histological type, pathological TNM tumor stage).

Measurement of serum ILF2 levels

Serum levels of ILF2 protein were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (SAB, USA) according to the manufacturer's instructions. Briefly, as follows: 100 µL of serum diluted 1:50 was incubated with capture antibodies in a 96-well plate at 37 °C for 2 h, followed by the addition of 100 µL of biotinylated antibody and incubation for 1 h, then the avidin conjugated to horseradish peroxidase (HRP) was added and incubated for 1 h after three washes. The liquid was discarded and the wells were washed five times. Tetramethylbenzidine (TMB) substrate was then added and incubated for 15 min in the dark, followed by the measurement of optical density (OD) values of each well at 450 nm using a JS-THERMO Varioskan Flash (Thermo Fisher Scientific, USA). Simultaneously, a standard curve was generated and used to determine the concentration of ILF2 in serum based on the OD values.

Statistical analysis

Data analysis was performed using R studio software (version 4.4.0). For numerical variables, Student t test or Wilcoxon rank sum test was utilized for comparisons between two groups, and one-way ANOVA or Kruskal-Wallis test was applied for comparisons between three groups, depending on data distribution and homogeneity of variance. Categorical variables were analyzed using chi-squared test, continuity-corrected chi-squared test, or Fisher's exact test depending on data characteristics. The receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of the variables, and the area under the curve (AUC) and diagnostic validity metrics (sensitivity, specificity, accuracy, etc.) were used to evaluate the diagnostic performance. The two-tailed

p-value was used, and a p-value < 0.05 was considered statistically significant.

Results

Clinical characteristics of the subjects

A total of 199 Chinese subjects and their clinical data were collected, including 99 cases of GC, 49 cases of BGD, and 51 cases of HC. GC consisted mainly of tubular adenocarcinoma (TAC) (75.8%) and poorly cohesive carcinoma (PCC) (20.2%); benign gastric diseases included low or very low risk gastrointestinal stromal tumor (GIST) ($n=17$, 34.7%), gastric polyp ($n=16$, 32.6%), leiomyoma ($n=7$, 14.3%), chronic gastritis ($n=4$, 8.2%), ectopic pancreas ($n=3$, 6.1%), and gastric ulcer ($n=2$, 4.1%). Table 1 shows the clinical data of these subjects. There

Table 1 Demographic, clinical and pathological characteristics of the subjects

	Result [n (%), Mean \pm SD, or Median (interquartile range)]						P
	n	GC	n	BGD	n	HC	
Sex, Male/Female	99	69 (69.7) / 30 (30.3)	49	22 (44.9) / 27 (55.1)	51	22 (43.1) / 29 (56.9)	0.001
Age (year)	99	65.0 (56.0, 71.0)	49	53.0 (48.0, 60.0)	51	33.0 (25.0, 39.5)	< 0.001
Blood cell analysis							
WBC ($\times 10^9/L$)	99	5.6 \pm 1.7	49	5.7 \pm 1.4	51	5.8 \pm 1.2	0.606
RBC ($\times 10^{12}/L$)	99	4.1 \pm 0.7	49	4.5 \pm 0.7	51	4.8 \pm 0.5	< 0.001
Hb (g/L)	99	121.4 \pm 23.1	49	134.4 \pm 18.9	51	140.8 \pm 25.1	< 0.001
HCT (%)	99	0.37 \pm 0.07	49	0.41 \pm 0.05	50	0.43 \pm 0.04	< 0.001
MCV (fl.)	99	89.9 \pm 8.2	49	89.3 \pm 7.1	51	89.9 \pm 6.6	0.896
MCH (pg)	99	29.4 \pm 3.5	49	29.4 \pm 3.0	51	30.3 \pm 1.9	0.067
MCHC (g/L)	99	325.1 \pm 16.6	49	328.2 \pm 13.0	51	328.8 \pm 47.4	0.677
RDW (%)	99	14.0 \pm 2.4	49	13.3 \pm 1.6	51	12.7 \pm 0.6	< 0.001
PLT ($\times 10^9/L$)	99	227.6 \pm 86.6	48	239.9 \pm 67.5	51	226.3 \pm 57.7	0.523
Tumor markers							
AFP (ng/mL)	83	2.5 (1.7, 3.8)	46	2.6 (1.9, 3.4)	26	2.1 (1.8, 2.9)	0.499
CEA (ng/mL)	97	2.1 (1.4, 3.2)	46	1.5 (0.9, 2.3)	27	1.3 (0.7, 1.7)	< 0.001
CA19-9 ($\mu g/mL$)	97	9.3 (6.0, 13.6)	48	10.7 (7.2, 17.1)	26	6.8 (5.4, 11.8)	0.054
CA125 ($\mu g/mL$)	96	9.5 (6.8, 14.5)	47	9.5 (7.3, 13.0)	26	12.9 (8.2, 19.4)	0.238
Lauren's type	96		/		/		/
Intestinal		72 (75.0)		/		/	
Diffuse		16 (16.7)		/		/	
Mixed		8 (8.3)		/		/	
Histological type	99		/		/		/
TAC		75 (75.8)		/		/	
PCC		20 (20.2)		/		/	
MAC		3 (3.0)		/		/	
PAC		1 (1.0)		/		/	
pTNM stage	97		/		/		/
I		43 (44.3)		/		/	
II		19 (19.6)		/		/	
III		33 (34.0)		/		/	
IV		2 (2.1)		/		/	

GC, gastric cancer; BGD, benign gastric disease; HC, healthy control; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width (coefficient of variation); PLT, platelet; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA125, cancer antigen 125; TAC, tubular adenocarcinoma; PCC, poorly cohesive carcinoma; MAC, mucinous adenocarcinoma; PAC, papillary adenocarcinoma; pTNM, pathological TNM

were significant differences in red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), red blood cell distribution width coefficient of variation (RDW), and CEA among the three groups.

Serum levels of ILF2 protein

The serum ILF2 protein level [median (interquartile range)] in the GC group was 227.8 (131.2, 521.8) ng/mL, which was significantly higher than that in the BGD group [72.0 (36.5, 127.4) ng/mL] and the HC group [56.8 (33.9, 84.7) ng/mL] ($p < 0.001$) (Fig. 1a). In addition, the ILF2 levels were higher in the BGD group than in the HC

group ($p < 0.05$). In various types of BGD (more than five cases), serum ILF2 levels were significantly lower than that in TNM stage I GC, but similar to the HC group (Fig. 1b), with 213.5 (147.4, 420.8) ng/mL in pTNM stage I GC, 137.2 (35.9, 183.1) ng/mL in leiomyoma, 49.29 (33.9, 111.8) ng/mL in GIST, and 68.71 (38.4, 112.8) ng/mL in gastric polyps. Additionally, serum ILF2 levels were higher in the leiomyoma and gastric polyp subgroups than in the healthy control group ($p < 0.05$). However, the ILF2 levels did not differ significantly within different histological types, Lauren's types, pT stages, pN stages, and pTNM stages (Fig. 1c-g).

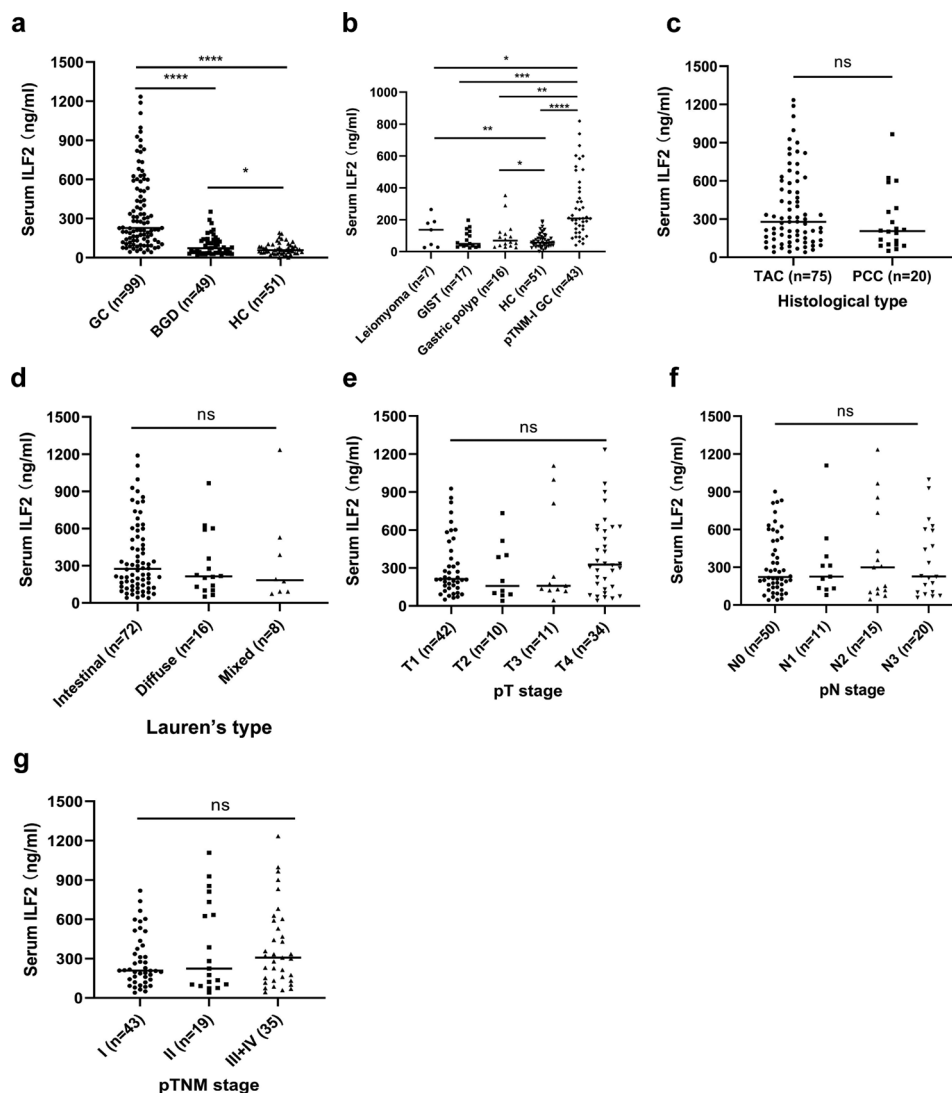


Fig. 1 Serum ILF2 protein levels in different groups of subjects and subgroups of gastric cancer. **a**: ILF2 levels in three groups of subjects; **b**: ILF2 levels in different types of BGD and their comparisons with early stage GC and HC groups; **c-g** ILF2 levels in various subgroups of gastric cancer. In the subgroup analysis, the following subgroups were not shown due to small sample size: chronic gastritis ($n = 4$), ectopic pancreas ($n = 3$) and gastric ulcer ($n = 2$) for BGD (**b**), and MAC ($n = 3$) and PAC ($n = 1$) for histological type (**c**); there were missing samples in the following subgroups: 3 cases for Lauren's type (**d**), 2 cases for pT stage (**e**), 3 cases for pN stage (**f**), and 2 cases for pTNM stage (**g**). ILF2, interleukin enhancer-binding factor 2; GC, gastric cancer; BGD, benign gastric disease; HC, healthy control; GIST, gastrointestinal stromal tumor; TAC, tubular adenocarcinoma; PCC, poorly cohesive carcinoma; pT, pathological T; pN, pathological N; pTNM, pathological TNM. * $p < 0.05$; **** $p < 0.0001$; ns, no significance

Diagnostic performance of ILF2 protein in GC

Using the ROC curve, we analyzed the diagnostic value of ILF2 and other significant blood laboratory indicators for GC. The results showed that ILF2 had high AUCs in differentiating GC patients, including early GC, from BGD patients and HC individuals, outperforming HCT, RBC, Hb, RDW and CEA, and the AUCs were similar between different stages of GC (Fig. 2).

Independence and synergy of serum ILF2 protein in the diagnosis of GC

The least absolute shrinkage and selection operator (LASSO) logistic regression analysis was performed using the “glmnet” R package to evaluate the independence of serum ILF2 and its synergic effect with significant blood laboratory indicators (HCT, RBC, Hb, RDW, and CEA) in the diagnosis of GC, in which 10-fold cross-validation and bootstrap resampling analyses were utilized to assess the robustness of the results. The LASSO logistic regression analysis identified two key indicators (Fig. 3a), ILF2 and RBC, and resulted in a diagnostic model: $Y=0.003ILF2-0.158RBC$, which had good discriminatory power between the GC group and the control group (Fig. 3b, c). The mean predicted values of the bootstrapped samples showed a normal distribution and a small standard deviation, especially in the control group (Fig. 3d, e). The mean AUC of the bootstrapped samples was high (0.914) and its standard deviation was very small (0.019), with a coefficient of variation of only 2.1% (Fig. 3f). The model outperformed ILF2 in overall diagnostic performance for GC (Fig. 4a-c). The improved diagnostic performance of the model was observed in TNM-II and TNM-III+IV GC, but not in TNM-I GC

(Fig. 4d-l). These results suggest that ILF2 is independent of the above blood indicators in the diagnosis of GC, and the combination of ILF2 with RBC improves the performance in the diagnosis of advanced GC and shows good robustness in the internal validation by bootstrap resampling.

The diagnostic accuracy metrics of the model, ILF2, and RBC (with the best diagnostic performance among these blood indicators) for the diagnosis of GC were calculated and shown in Table 2, and the model exhibited improved diagnostic accuracy for GC compared with ILF2 or RBC. The positive rates of the model and ILF2 were 84-94% and 57-73%, respectively, in various GC subgroups (with more than 15 cases), but were very low in the gastric polyp subgroup and the HC group (Fig. 5).

Discussion

In the present study, we further investigated the diagnostic value of serum ILF2 protein for gastric cancer based on our preliminary findings. The levels of ILF2 in the serum samples of GC and BGD patients and HC individuals were determined by ELISA and were significantly elevated in the GC group compared with the BGD and HC groups, and showed good diagnostic value for gastric cancer, including early-stage gastric cancer, and moreover, the diagnostic value of ILF2 was independent of conventional laboratory indicators and significantly improved in combination with RBC.

Currently, the early diagnosis of GC is suboptimal, leading to frequent diagnosis at advanced stages [23] and a median overall survival of less than one year [24]. We evaluated the serum levels and diagnostic performance of ILF2 protein in different stages of GC and found that

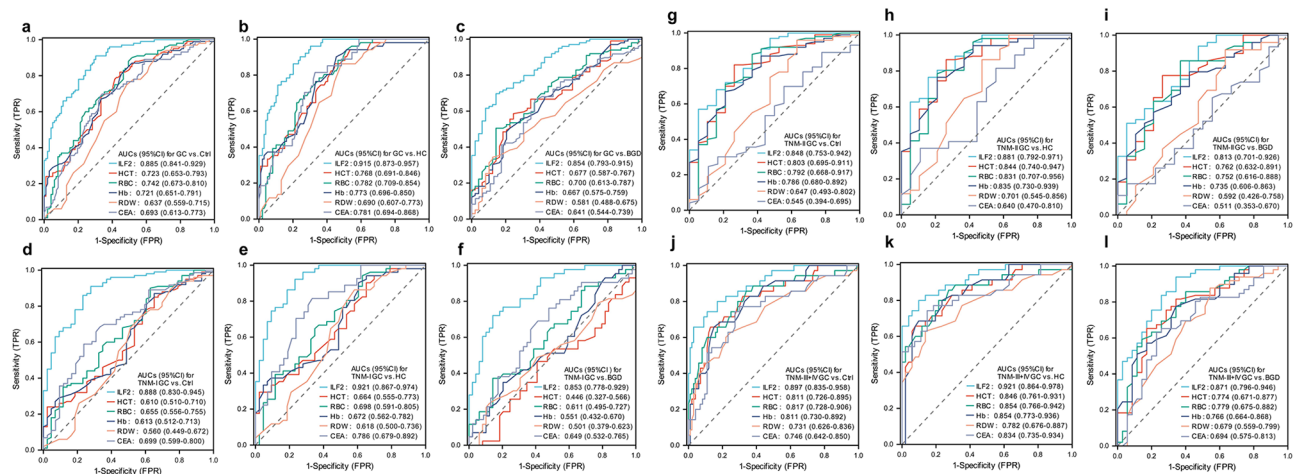


Fig. 2 Receiver operating characteristic (ROC) curves of serum ILF2 protein and significant blood laboratory indicators for the diagnosis of GC. **a-c**: Total GC group vs. control, HC and BGD groups; **d-f**: TNM-I GC subgroup vs. control, HC and BGD groups; **g-i**: TNM-II GC subgroup vs. control, HC and BGD groups; **j-l**: TNM-III + IV GC subgroups vs. control, HC and BGD groups. GC, gastric cancer; BGD, benign gastric disease; HC, healthy control; AUC, area under the curve; Ctrl, control (BGD + HC). ILF2, interleukin enhanced-binding factor 2; HCT, hematocrit; RBC, red blood cell; Hb, hemoglobin; RDW, red blood cell distribution width (coefficient of variation); CEA, carcinoembryonic antigen

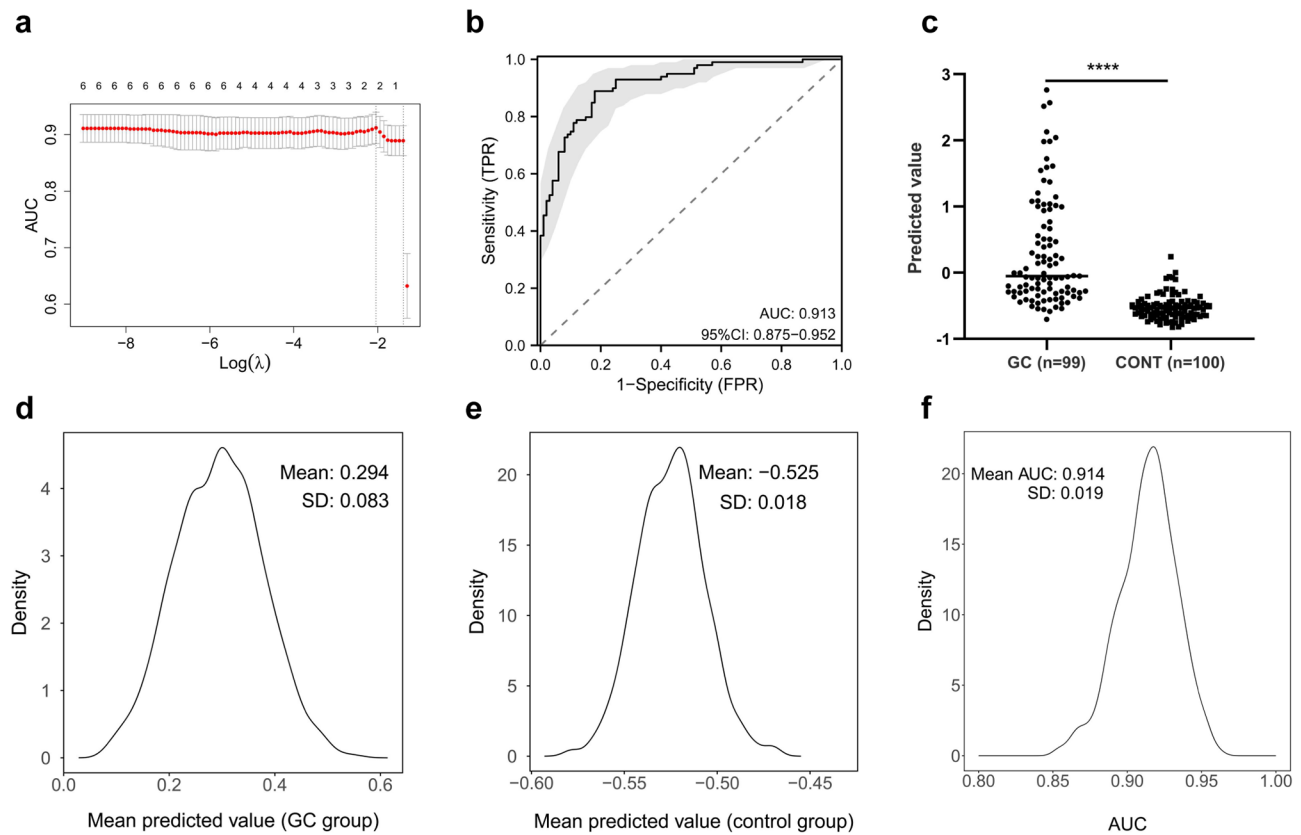


Fig. 3 Variable selection and internal validation of the model. **a**: Variable selection using the least absolute shrinkage and selection operator logistic regression and 10-fold cross-validation; **b**: The receiver operating characteristic curve of the model; **c**: Predicted values of the model for the GC and control (BGD+HC) groups; **d**, **e**: The distribution of mean predicted values of bootstrapped samples in the GC and control groups; **f**: The distribution of AUC values of bootstrapped samples. AUC, area under the curve; GC, gastric cancer; CONT, control; BGD, benign gastric disease; HC, health control; **** $p < 0.0001$

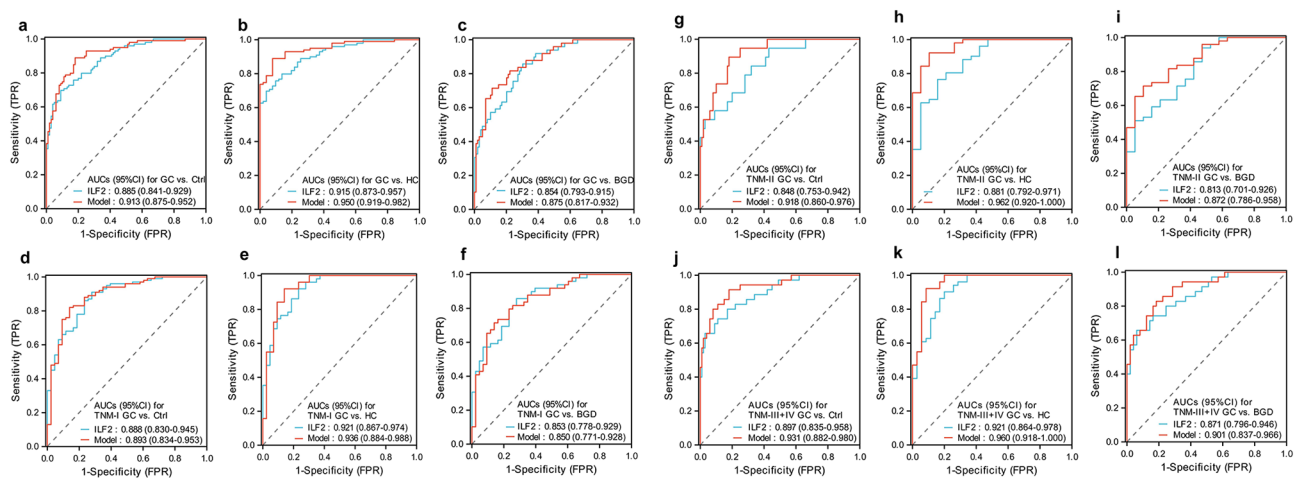


Fig. 4 Receiver operating characteristic (ROC) curves of serum ILF2 protein and the model for the diagnosis of GC. **a-c**: Total GC group vs. control, HC and BGD groups; **d-f**: TNM-I GC subgroup vs. control, HC and BGD groups; **g-i**: TNM-II GC subgroup vs. control, HC and BGD groups; **j-l**: TNM-III+IV GC subgroup vs. control, HC and BGD groups. AUC, area under the curve; Ctrl, control (BGD+HC); GC, gastric cancer; BGD, benign gastric disease; HC, healthy control; ILF2, interleukin enhancer-binding factor 2

Table 2 Diagnostic validity metrics of the model, ILF2 protein and RBC for GC

	Cut-off value	SEN (%)	SPE (%)	ACC (%)	PPV (%)	NPV (%)	PLR	NLR	DOR
Model	-0.43								
GC vs. Ctrl		88.9	82.0	85.4	83.0	88.2	4.94	0.14	35.29
GC vs. BGD		88.9	71.4	83.1	86.3	76.1	3.11	0.16	19.44
GC vs. HC		88.9	92.2	90.0	95.7	81.0	11.33	0.12	94.42
ILF2	158.30 (ng/mL)								
GC vs. Ctrl		69.7	91.0	80.4	88.5	75.2	7.74	0.33	23.45
GC vs. BGD		69.7	85.7	75.0	90.8	58.3	4.88	0.35	13.94
GC vs. HC		69.7	96.1	78.7	97.2	62.0	17.77	0.32	55.53
RBC	4.11 ($\times 10^{12}$ /L)								
GC vs. Ctrl		50.5	88.0	69.3	80.6	64.2	4.21	0.56	7.52
GC vs. BGD		50.5	85.7	62.2	87.7	46.2	3.54	0.58	6.10
GC vs. HC		50.5	90.2	64.0	90.9	48.4	5.15	0.55	9.36

SEN, sensitivity; SPE, specificity; ACC, accuracy; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio. ILF2, interleukin enhancer-binding factor 2; GC, gastric cancer; Ctrl, control (BGD+HC); BGD, benign gastric disease; HC, healthy control. RBC, red blood cell

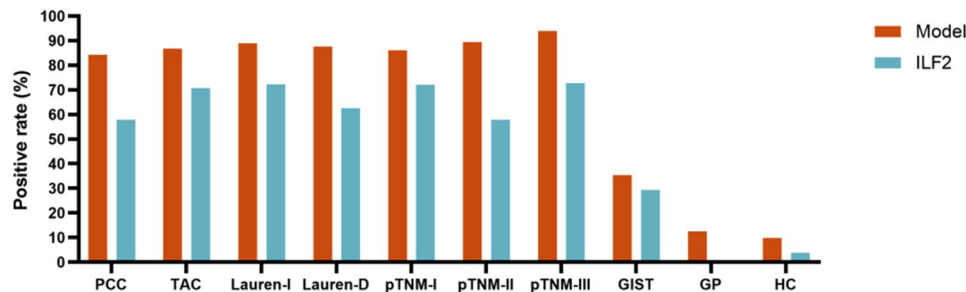


Fig. 5 Positive rates of the model and ILF2 protein in the GC and BGD subgroups and the HC group (only subgroups with more than 15 cases are shown). ILF2, interleukin enhancer binding factor 2; PCC, poorly cohesive carcinoma; TAC, tubular adenocarcinoma; Lauren-I, Lauren intestinal type; Lauren-D, Lauren diffuse type; GIST, gastrointestinal stromal tumor; GP, gastric polyp; HC, healthy control

ILF2 was valuable for the early detection of GC. The serum levels of ILF2 were not significantly different among TNM stages of GC and demonstrated similar diagnostic performance between early and advanced GC, with an AUC of 0.888 (95%CI 0.830–0.945) for TNM-I stage GC and much better than the simultaneously analyzed significant blood indicators (HCT, RBC, HB, RDW, and CEA, their AUCs ranging from 0.560 to 0.699). Compared with previous reports, ILF2 is also superior to some novel serum protein biomarkers for the diagnosis of early GC, such as anosmin 1 (ANOS1) (AUC=0.713) [25], mRNA export factor (RAE1) specific autoantibody (AUC=0.745) [26], and inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4) (AUC=0.839) [27].

Anemia is frequent and has some diagnostic and prognostic value in GC patients [28, 29]. In addition, traditional serum tumor markers (CEA, CA19-9, etc.) are widely used in the clinical detection of GC [6, 8], although their sensitivity and specificity are limited [30]. Therefore, in the present study, we simultaneously evaluated the diagnostic value of RBC-related indicators and traditional serum tumor markers and compared them with ILF2 protein. We found that RBC and some of its

derivatives (HCT, Hb, and RDW) had diagnostic value for GC, especially for advanced GC. Among the traditional serum tumor markers, only CEA showed weak value for the diagnosis of GC. However, both RBC-related indicators and traditional serum tumor markers are inferior to ILF2 in diagnostic performance.

In order to understand the independence and synergy of ILF2 protein with RBC-related indicators and CEA in GC diagnosis, we used multivariate analysis (LASSO regression) to select variables from ILF2 and blood indicators for the establishment of a diagnostic model, and found that only ILF2 and RBC remained in the model, indicating that ILF2 is an independent and synergistic indicator for the diagnosis of GC. The diagnostic model improved the diagnostic performance for GC compared to single ILF2 and RBC, especially for advanced GC, with AUC 0.893 for TNM-I GC and >0.9 for advanced GC, and positive rates approximately 90% for GC in different conditions, which was good enough for the simple model with only two variables. Compared with some of the reported diagnostic models, our model has either fewer variables or higher performance, or both [31–34].

The high diagnostic performance and ease of application indicate that our model has promising clinical usefulness.

The traditional modeling approach is to randomly divide subjects into a training set and a test set, where the training set is used to construct a diagnostic model and the test set is used to internally validate the generalizability of the model. Due to insufficient sample size, we did not use this training-and-test approach in our modeling, but instead built the model using the full dataset and internally validated the model using the bootstrap resampling approach. The internal validation by bootstrap resampling showed good robustness of the model at different levels (Fig. 3d-f). It has been reported that modeling with bootstrap validation can yield a better model than the traditional training-and-test method [35], while our internal validation method by bootstrap resampling in this study provides an alternative method for evaluating diagnostic models built in a relatively small cohort. However, external validation in an independent case cohort is essential to assess whether new diagnostic models are overestimated.

ILF2 protein was first identified as a 45 kDa transcription factor (also known as NF45) required for the expression of the interleukin-2 gene in T cells [36, 37]. Subsequent studies have shown that ILF2, as a component of the ILF2-ILF3 complex, is involved in various cellular processes and functions in different cell types, such as regulating mRNA abundances [38], mRNA processing and translation [39], DNA break repair [40], regulation of microRNA processing [41, 42], and regulation of cell growth during mitosis [43]. Notably, ILF2 gene and/or protein are involved in cancer development and progression through multiple mechanisms, such as regulating cell cycle and apoptosis [44, 45], participating in tumor metabolism [46], and maintaining tumor mitochondrial homeostasis [47]. In GC, ILF2 protein was overexpressed in tumor tissues and closely associated with tumor metastasis and poor prognosis [48, 49], and was involved in the DNA damage response mechanism of GC [50]. Our previous study showed that the expression level of ILF2 could regulate the growth of GC cells [22]. All these findings support ILF2 as a valuable biomarker for GC.

In our previous study, we analyzed serum ILF2 protein levels and its diagnostic performance in a small sample consisting only of GC and HC. In the present study, we not only increased the sample size of GC and HC, but also added benign gastric disease controls, which resulted in the evaluation of the diagnostic value of ILF2 for GC in a more representative study sample. However, the sample size of this study is still relatively small and further studies with a larger sample size and a more representative BGD group are warranted. Additionally, the fact that this study was conducted on inpatients may not be representative of the composition of gastric patients in the real

world, which may introduce bias into the study results. Furthermore, the diagnostic performance of serum ILF2 protein and the model may be overestimated due to the lack of validation in independent cohorts. Therefore, multi-center and multi-cohort validation studies are needed to more comprehensively and accurately evaluate the value of ILF2 and the model in GC detection.

Conclusions

In conclusion, this study validated elevated levels of ILF2 protein in the serum of patients with GC and demonstrated that ILF2 had good diagnostic value for GC, including early and advanced GC, outperforming conventional hematological indicators (serum tumor markers and RBC-derived indicators). Furthermore, the diagnostic performance of ILF2 was independent of and synergistic with conventional hematological indicators, and the combination of ILF2 with RBC could significantly improve the diagnostic efficiency. As a promising novel serum diagnostic biomarker for GC, ILF2 deserves further studies to validate its performance in the diagnosis of GC.

Abbreviations

AFP	Alpha-fetoprotein
AUC	Area under the curve
BGD	Benign gastric disease
CA125	Cancer antigen 125
CA19-9	Carbohydrate antigen 19–9
CA242	Carbohydrate antigen 242
CA72-4	Carbohydrate antigen 72–4
CEA	Carcinoembryonic antigen
CEACAM5	Carcinoembryonic antigen-related cell adhesion molecule 5
CTC	Circulating tumor cells
ctDNA	Circulating tumor DNA
ELISA	Enzyme-linked immunosorbent assay
G-17	Gastrin-17
GC	Gastric cancer
GIST	Gastrointestinal stromal tumor
GKN1	Gastrokine 1
Hb	Hemoglobin
HC	Healthy control
HCT	Hematocrit
HRP	Horseshoe peroxidase
ILF2	Interleukin enhancer-binding factor 2
LASSO	Least absolute shrinkage and selection operator
OD	Optical density
PCC	Poorly cohesive carcinoma
PG	Pepsinogen
RBC	Red blood cell count
RDW	Red blood cell distribution width coefficient of variation
ROC	Receiver operating characteristic
SKAP1	Src kinase-associated phosphoprotein 1
TAC	Tubular adenocarcinoma
TMB	Tetramethylbenzidine

Acknowledgements

This work was supported by the Key Laboratory Project of Digestive Diseases in Jiangxi Province (2024SSY06101), and Jiangxi Clinical Research Center for Gastroenterology (20223BCG74011).

Author contributions

KHZ, TW and SSL designed the study. SSL conducted the study. MSL and JKW participated in the experiments. DFG, QW and YHL participated in the data

analysis. SSL drafted the manuscript, and KHZ, TW revised the manuscript. All authors read and approved the final version of the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82160494).

Data availability

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

Declarations

Ethics approval and consent to participate

This research was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University. All examinations in humans were conducted according to the Declaration of Helsinki and its amendments. All procedures performed in studies involving human participants were approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University. All methods were carried out in accordance with relevant guidelines and regulations. The Ethics Committee of the First Affiliated Hospital of Nanchang University waived the requirement for patient informed consent because the assay for ILF2 used previously collected frozen leftover serum specimens from biochemical assays, not specifically drawn blood specimens.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 August 2024 / Accepted: 14 November 2024

Published online: 25 November 2024

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