Efficacy of circulating microRNA-130b and blood routine parameters in the early diagnosis of gastric cancer

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Abstract. Patients with gastric cancer (GC) have a poor prognosis, which is mainly due to the low rate of early diagnosis. The present study aimed to evaluate whether circulating microRNA-130b (miR-130b) and blood routine parameters [neutrophil count (N#), lymphocyte count (L#), monocyte count (M#), neutrophil percentage (N%), lymphocyte percentage (L%), monocyte percentage (M%), hemoglobin (Hb) level, hematocrit (Hct), red blood cell distribution width (RDW), platelet count, platelet distribution width (PDW), mean platelet volume (MPV), MPV to platelet count ratio (MPV/PC), monocyte to lymphocyte ratio (MLR), neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR)] are useful biomarkers for GC, early stage GC (EGC) and precancerous lesion (Pre) detection, and to identify more effective diagnostic models by combining circulating blood markers. Circulating levels of M#, M%, RDW-coefficient of variation (RDW-CV), MPV, PDW, MLR and NLR were significantly higher, and the levels of Hb and L% were significantly lower in patients with GC and Pre compared with those in healthy controls (NCs) (all P<0.05). The N#, N% and PLR in patients with GC were significantly higher and the Hct was significantly lower than those in the NCs (all P<0.05). The values of MPV/PC were significantly higher in the Pre cohort compared with those in the NCs. The area under the curve (AUC) of the receiver operating characteristic curve of potential biomarkers for GC was 0.634-0.887 individually, and this increased to 0.978 in the combination model of miR-130b-PDW-MLR-Hb. Additionally, the values for RDW-CV, PLR, NLR, N# and N% were positively correlated with cancer stage, while the values for MPV, L#, L%, Hb and Hct were negatively correlated with cancer stage. Furthermore, the circulating levels of miRNA-130b, and the values for NLR, RDW-CV, PDW, M%, red blood cell count, Hct, Hb and MLR differed between the EGC and NC groups. The AUC values of these biomarkers were 0.6491-0.911 individually in the diagnosis of EGC, and these increased to 0.960 in combination. In addition, the AUC values for miR-130b, RDW-CV, MPV/PC ratio, MLR, NLR, PDW, L%, M%, M# and Hb in the diagnosis of Pre were 0.638-0.811 individually. The dual-model of miR-130b-PDW manifested the largest AUC of 0.896 in the diagnosis of Pre, and the sensitivity and accuracy were increased when miR-130b and PDW were combined. All these results suggested that circulating miR-130b and blood routine parameters might be potential biomarkers, and combinations of measurements of these biomarkers may improve the GC, EGC and Pre diagnostic accuracy.

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Introduction

It has been reported that gastric cancer (GC) is the second leading cause of cancer-associated mortality worldwide (1). Although extensive studies have been conducted, few risk factors have been confirmed and there are no effective biomarkers or screening tools for early detection in asymptomatic individuals (2,3). Conventional tumor markers, such as CEA, CA72-4 and CA19-9, are useful only for identifying cases of advanced GC and monitoring GC recurrence (3). However, these serum markers lack sufficient sensitivity and specificity (4). Currently, endoscopy and the barium meal test are the main methods used for the clinical diagnosis of GC. However, due to their invasiveness, patient compliance with this procedure is poor (1). Chronic inflammation is not an important cause of GC; however, it is considered to be involved in the pathogenesis of 25% of all cancer cases worldwide (5). Blood routine parameters have been introduced as biomarkers for the diagnosis of numerous cancer-related diseases associated with inflammatory processes (6-10). However, to the best of our knowledge, the longitudinal changes of these parameters in different stages of GC progression have not been studied comprehensively at present.

MicroRNAs (miRNAs/miRs) are members of the endogenous, non-coding single-stranded RNA family, and are released from tissues to extracellular biofluids after receiving inflammatory stimulation (11). Specific miRNAs, which have carcinogenic or tumor-suppressive activities, may be mediators for inflammation to induce carcinogenesis (12), and have been detected in numerous types of cancer, including breast cancer, hepatocellular carcinoma and other cancer types (13-15). Numerous studies have demonstrated that non-invasive circulating miRNA recognition is valuable and useful in diseases, including GC (16,17). However, these studies have not included patients with early stage GC (EGC) or patients with a precancerous lesion (Pre), although an ideal non-invasive marker should be able to identify both of these stages.

A recent study has reported that the combination of miR-650 and CA211 can distinguish between benign and malignant GC (18). Therefore, we hypothesized that the combined assessment of miRNAs and blood routine parameters may reveal novel insights for the diagnosis of GC. The present study examined the circulating levels of miR-130b and blood routine parameters in 90 patients with GC, 90 patients with a Pre and 45 healthy individuals. Subsequently, statistical analysis was performed to compare the diagnostic value of these markers for GC. The present study indicated that plasma miR-130b and complete blood count parameters might be promising non-invasive biomarkers for the early detection of GC, and combined utilization of these markers could improve the efficacy of the early diagnosis of GC.

Materials and methods

Patients and healthy control characteristics. The present study included 90 patients with GC, 90 patients with Pres and 45 healthy controls (NCs) who received treatment at the Affiliated Liutie Central Hospital of Guangxi Medical University (Liuzhou, China) between January 2014 and March 2019. Patients with GC and Pres were selected according

to gastroscopy combined with histological examination and the blood samples were collected prior to any surgery and therapy. Patients who had received radiation therapy or chemotherapy were excluded from the study.

The histological type and tumor stage were identified according to the Union of International Cancer Control TNM system, 7th edition (19). The histology of all patients was assessed according to World Health Organization criteria (20). NCs had no history of diabetes, heart disease, hypertension or cancer, and attended the Affiliated Liutie Central Hospital of Guangxi Medical University for routine health checks. Approval for the study was obtained from the Ethics Committee of the Affiliated Liutie Central Hospital of Guangxi Medical University, and written informed consent was issued by all study participants.

Plasma preparation and miRNA extraction. Peripheral blood samples were drawn using EDTA anticoagulative tubes prior to surgical treatment. Cell-free plasma samples were centrifuged at 1,520 x g for 5 min to prevent contamination by cellular nucleic acids and stored at -80˚C until miRNA extraction or kept at -20˚C for conventional tumor marker determination.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR) assay. Total RNA was isolated from 200 μl plasma using a Blood (Serum/Plasma) MicroRNA Extraction and Purification kit (spin column) (Novland Co., Ltd.; http://www.novland.com.cn/). The concentration of total RNAs was quantified using a NanoQ micro-volume Spectrophotometer (CapitalBio Technology, Inc.). Circulating miRNA-130b expression was determined by RT-qPCR using a One Step qRT-PCR Kit (with Taqman probes; catalog no. LK-0106B) with 2 μl initial template, while miR-16 served as an internal control (21). Thermocycling conditions were 45˚C for 30 min for reverse transcription, then 94˚C for 2 min, followed by 40 cycles of 94˚C for 15 sec, 55˚C for 45 sec and 72˚C for 60 sec. RT-qPCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences for PCR were as follows: miR-130b forward, 5'-GACACUCUUUCCCUGUUGCAC UACU-3' and reverse, 5'-CAGTGCGTGTCGTGGAGT-3'; and reference miRNA (hsa-miR-16) forward, 5'-GTCGTA TCCAGTGCAGGGTCCGAGTCGCACTGGATACGACCG CCAA-3' and reverse, 5'-GTATCCAGTGCAGGGTCCGAG GT-3'. Expression levels of target miRNAs were calculated using the quantification cycle (Cq) values with SDS 2.0 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). The relative expression levels of miR-130b were calculated using the $2^{-\Delta\Delta Cq}$ method (22), in which $\Delta Cq = Cq(miR-130b) - Cq(miR-16)$.

Blood parameter testing. Hematological parameters, including red blood cell count (R#), hemoglobin (Hb), hematocrit (Hct), white blood cell count (WBC), neutrophil count (N#), lymphocyte percentage (L#), monocyte count (M#), platelet count (PLT) and mean platelet volume (MPV), were measured before radical surgery using the automatic blood analyzer 800i (Sysmex Corporation) according to the manufacturer's protocols. Conventional tumor markers, including CA125, CA211 and CA50, were detected using a Roche cobas E170 analyzer (Roche Diagnostics).
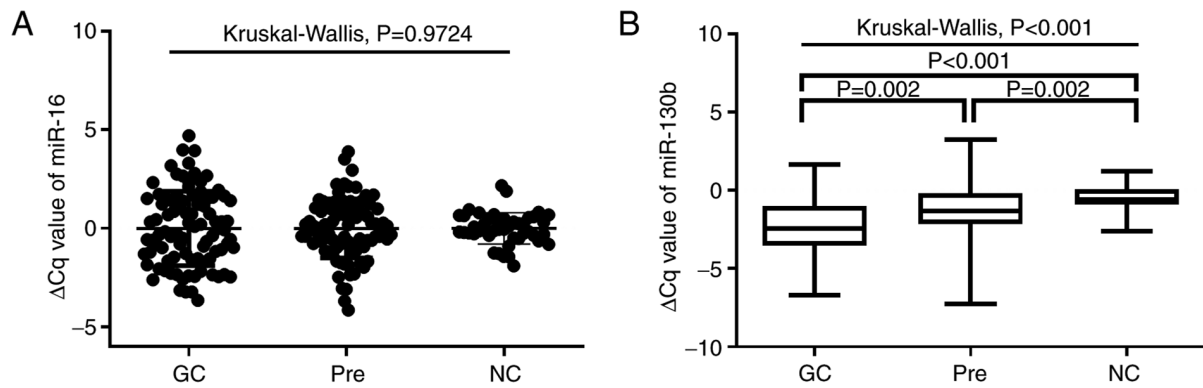
Figure 1. miR-130b expression in different groups. (A) Differential expression levels of reference miR-16 (P>0.05). (B) ΔCq values of miR-130b in different groups (P<0.05). The ΔCq values denote the normalized Cq value by subtracting the Cq value of miR-16 from that of miR-130b. A lower ΔCq value indicated a higher level of miR-130b expression. The Kruskal-Wallis H test was used to compare variables between three groups, and the Bonferroni method was used to correct for post hoc pairwise comparisons of significance levels. GC, gastric cancer; miR, microRNA; NC, healthy control; Pre, precancerous lesions.

Statistical analysis. All experiments were repeated three times independently, and the data are expressed as the mean ± SD. Differences in relevant indicators between two groups were compared using an unpaired t-test, or the Mann-Whitney U test when the conditions for the Student's t-test were not satisfied. Differences in relevant indicators among three groups were analyzed using one-way ANOVA, or Kruskal-Wallis H test when the conditions for one-way ANOVA were not satisfied. Tukey's honest significant difference test was used as a post hoc test after one-way ANOVA, and the Bonferroni method was used to correct for post hoc pairwise comparisons of significance levels after the Kruskal-Wallis H test. Differences in sex distribution among the three groups were analyzed using the χ^2 test. All statistical analyses and generation of images were performed using SPSS 20.0 software (IBM Corp.) or GraphPad Prism 8.0 Software (GraphPad Software, Inc.). Correlations between circulating biomarkers in GC were analyzed using Pearson's correlation. Correlations between blood biochemical indexes and cancer stage in GC were analyzed by Spearman's correlation. Sensitivity and specificity were defined by receiver operating characteristic (ROC) curves, and differences in the area under the curve (AUC) and diagnostic accuracy parameters, including sensitivity, specificity, negative likelihood ratio (LR⁻), positive likelihood ratio (LR⁺) and diagnostic odds ratio (DOR) were detected using MedCalc version 19.5.3 (MedCalc Software, Ltd.). For all analyses, P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of miR-130b, complete blood count parameters and conventional tumor markers in GC, Pre and NCs. A total of 90 patients with GC, 90 patients with Pres and 45 NCs were included in the present study. Plasma miR-130b expression was examined by RT-qPCR. As shown in Fig. 1A, no significant differences in miR-16 expression were observed among the three groups (P=0.9724). As shown in Fig. 1B, plasma miRNA-130b levels in patients with GC and Pres were significantly higher than those of NCs (all P<0.05). Furthermore, the plasma miRNA-130b levels in GC were significantly higher

than those in the Pre group (P=0.002). As shown in Table I, the values for M#, monocyte percentage (M%), red blood cell distribution width-coefficient of variation (RDW-CV), MPV, monocyte to lymphocyte count ratio (MLR), neutrophil to lymphocyte ratio (NLR), CA211 and CA50 in patients with GC and Pres were significantly higher than those in NCs (all P<0.05). Notably, patients with GC and Pres had significantly lower Hb, L% and platelet distribution width (PDW) than NCs (GC vs. NC, P<0.05; Pre vs. NC, P<0.05; Table I). The values for N#, neutrophil percentage (N%), platelet to lymphocyte ratio (PLR) and CA125 in patients with GC were significantly higher, while Hct was significantly lower, compared with those in the normal group (all P<0.05). Furthermore, the MPV to platelet count ratio (MPV/PC) was significantly higher in the Pre cohort (0.04±0.02) compared with NCs (0.03±0.01; P=0.004). In addition, the values for WBC and PLT did not differ significantly among the three groups.

Diagnostic values of tumor markers for GC based on AUC analysis. To further assess the diagnostic value of potential diagnostic biomarkers for GC, ROC analyses were subsequently performed, and the key indicators, including sensitivity, specificity, positive predictive value, negative predictive value, cut-off value and AUC, were measured as shown in Fig. 2 and Table II. ROC analyses suggested that the AUC was calculated as 0.887 for miR-130b, 0.828 for Hb, 0.764 for Hct, 0.763 for RDW-CV, 0.667 for MPV, 0.757 for NLR, 0.814 for MLR, 0.712 for PLR, 0.840 for PDW, 0.709 for N%, 0.715 for M%, 0.782 for L%, 0.634 for N#, 0.680 for M# and 0.719 for lymphocyte count (L#). At a cut-off value of 0.18, circulating miR-130b exhibited the highest diagnostic accuracy and sensitivity compared with any other parameters, highlighting its potential as an effective biomarker for GC. Our previous study also assayed CA211 and CA50 levels in the same plasma samples, and at cut-off values of 2.2 ng/ml for CA211 and 7.96 ng/ml for CA50, the sensitivity and specificity values were 80.65 and 95.65%, and 55.84 and 85.29%, respectively (23).

Integrative diagnosis model for detecting GC. To further assess the effect of circulating biomarkers, an integrative diagnosis

Table I. Blood biochemical examination results of subjects.

Variables	GC No. of patients	GC Mean ± SD	Pre No. of patients	Pre Mean ± SD	NC No. of patients	NC Mean ± SD	P-value[a]	GC vs. Pre P-value	GC vs. NC P-value	Pre vs. NC P-value
WBC, 10^9/l	89	6.41±2.98	76	6.25±2.68	45	6.01±1.28	0.2846	-	-	-
N#, 10^9/l	89	4.55±2.86	76	3.84±2.61	45	3.61±0.98	0.0445	>0.999	0.039	0.228
L#, 10^9/l	89	1.34±0.66	76	1.66±0.76	45	1.93±0.73	<0.0001	0.002	0.001	0.169
M#, 10^9/l	89	0.31±0.21	76	0.31±0.17	45	0.24±0.4	0.0018	>0.999	0.002	0.015
N%	89	67.81±14.75	76	63.45±12.65	45	61.23±7.99	0.0003	0.052	0.001	0.260
L%	89	22.74±11.4	76	29.31±11.63	45	31.90±7.57	<0.0001	0.025	0.016	0.001
M%	89	5.31±3.75	76	5.05±2.17	45	3.71±1.35	0.0003	0.237	0.001	0.023
Hb, g/l	89	116.10±32.22	76	128.04±21.67	45	138.46±16.05	<0.0001	0.001	0.01	0.001
Hct, %	89	35.70±9.07	76	39.21±6.18	45	40.43±3.92	<0.0001	0.001	0.001	>0.999
RDW-CV, %	89	14.01±3.24	76	12.75±1.92	45	12.31±1.40	<0.0001	0.001	0.001	0.048
PLT, 10^{12}/l	89	256.00±111.43	76	231.50±63.38	45	248.00±54.68	0.0704	-	-	-
PDW, %	89	11.65±2.77	76	11.91±2.51	45	15.90±0.38	<0.0001	>0.999	0.001	0.001
MPV, fl	89	9.62±1.20	76	9.91±1.14	45	8.90±1.39	<0.0001[b]	0.046	0.003	0.001
MPV/PC	89	0.04±0.04	76	0.04±0.02	45	0.03±0.01	0.0047	0.105	0.428	0.004
MLR	89	0.24±0.41	76	0.17±0.18	45	0.11±0.98	<0.0001	0.006	0.001	0.003
NLR	89	2.94±9.33	76	2.20±6.09	45	1.96±0.63	<0.0001	0.001	0.001	0.001
PLR	89	198.05±286.52	76	131.03±112.07	45	134.36±76.12	<0.0001	0.001	0.001	0.031
CA125, U/ml	68	59.22±92.72	53	12.36±7.78	45	11.07±4.05	0.0078	0.024	0.028	>0.999
CA211, ng/ml	40	16.15±44.84	62	2.95±1.38	45	2.09±0.48	<0.0001	0.045	0.001	0.001
CA50, U/ml	48	38.01±99.02	77	8.18±5.97	34	4.91±4.46	<0.0001	0.001	0.001	0.001

[a]P-values indicated for differences among three groups. [b]The only P-value calculated using ANOVA while all other P-values were calculated using Kruskal-Wallis. P<0.05 was considered to indicate a statistically significant difference. Some data are missing due to retrospective data collection. GC, gastric cancer; Pre, precancerous lesions; NC, healthy control; WBC, white blood cell count; N#, neutrophil count; L#, lymphocyte count; M#, monocyte count; N%, neutrophil percentage; L%, lymphocyte percentage; M%, monocyte percentage; Hb, hemoglobin; Hct, hematocrit; RDW-CV, red blood cell distribution width-coefficient of variation; PLT, platelet count; PDW, platelet distribution width; MPV, mean platelet volume; MPV/PC, mean platelet volume/platelet count ratio; MLR, monocyte to lymphocyte count ratio; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte count ratio.

model was generated for discriminating GC. The circulating markers with AUC >0.80 (miR-130b, Hb, MLR and PDW) were further considered for combinations. For the two-dimensional models, PDW-Hb yielded the greatest AUC (0.945), followed by miR-130b-Hb (0.937), miR-130b-PDW (0.922), miR-130b-MLR (0.892), PDW-MLR (0.843) and MLR-Hb (0.835). The AUC values of the dual-models miR-130b-PDW, miR-130b-Hb and PDW-Hb were all greater than those of any of the one-dimensional models. However, there was no statistically significant difference in AUC between the PDW-MLR and MLR-Hb dual-models compared with the corresponding one-dimensional models. In addition, the AUC of the dual-model miR-130b-MLR was significantly different from that of the miR-130b one-dimensional model, while miR-130b-MLR was not statistically significantly different compared with the MLR one-dimensional model. For the miR-130b-PDW-MLR three-dimensional model, the AUC (0.955) was greater than those of the miR-130b-MLR or the PDW-MLR two-dimensional models (all P<0.05; Table III). Similarly, the AUC (0.976) of the three-dimensional model miR-130b-PDW-Hb was greater than those of the

miR-130b-Hb or the PDW-Hb two-dimensional models. The AUC (0.942) of the miR-130b-MLR-Hb three-dimensional model was greater than those of the miR-130b-MLR or the MLR-Hb two-dimensional models. For the PDW-MLR-Hb three-dimensional model, the AUC (0.947) was greater than those of the PDW-MLR or MLR-Hb two-dimensional models. Furthermore, for the four-dimensional model miR-130b-PDW-MLR-Hb, the AUC (0.978) was greater than those of the three-dimensional models miR-130b-MLR-Hb and PDW-MLR-Hb (all P<0.05; Table III). These results indicated that the combination of different biomarkers can improve the diagnostic efficiency to some extent.

Diagnostic value of four-dimensional biomarkers in GC. The corresponding diagnostic accuracy parameters, including sensitivity, specificity, LR⁻, LR⁺ and DOR, are shown in Table IV. miR-130b exhibited a sensitivity and specificity of 91.11 and 71.11%, PDW had a sensitivity and specificity of 70.45 and 97.78%, MLR exhibited a sensitivity and specificity of 80.90 and 75.56%, and Hb had a sensitivity and specificity of 68.54 and 84.44%, respectively. miR-130b-PDW
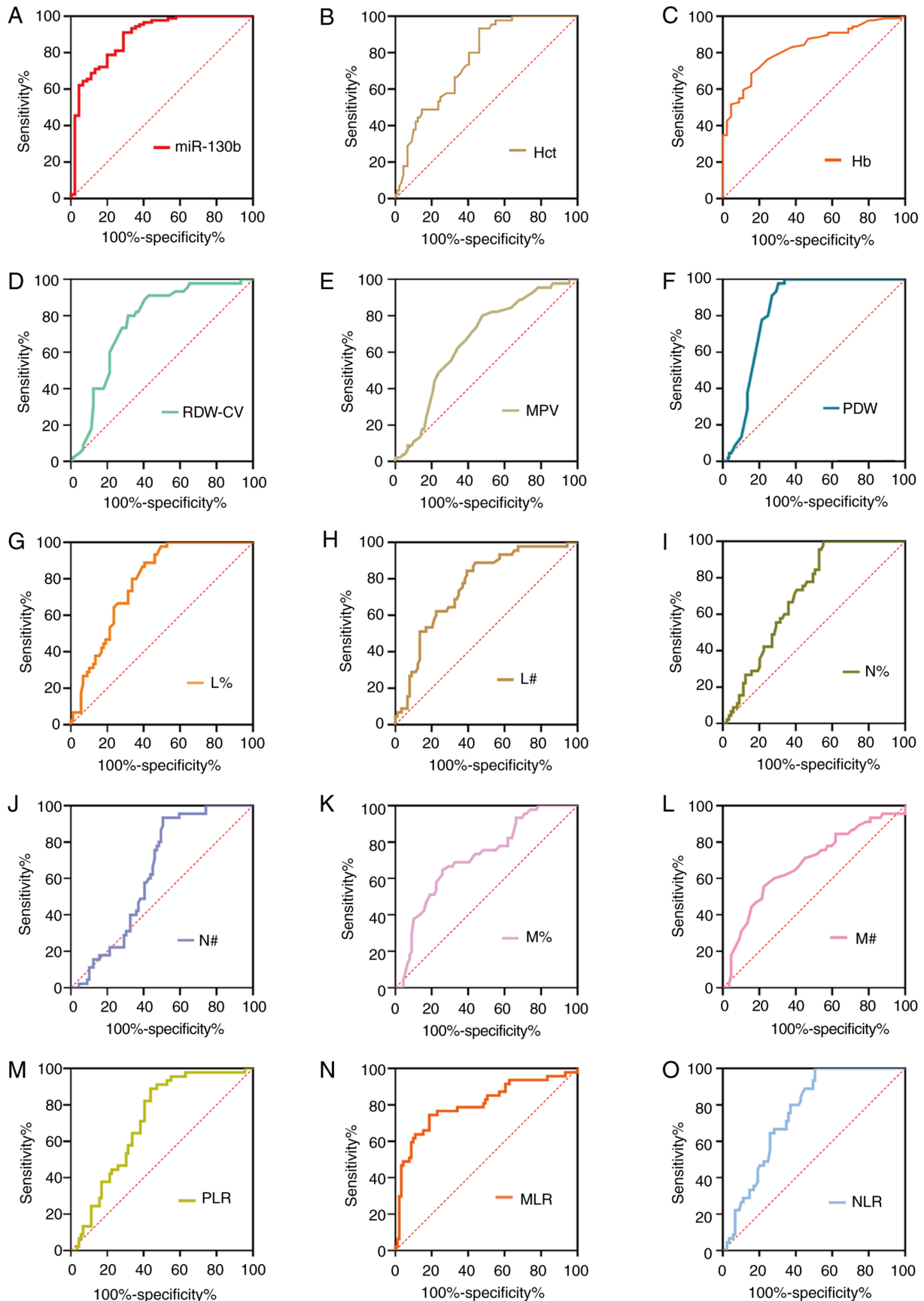
Figure 2. ROC curve analyses of tumor markers for gastric cancer. ROC curve analysis of biomarkers (A) miR-130b, (B) Hct, (C) Hb, (D) RDW-CV, (E) MPV, (F) PDW, (G) L%, (H) L#, (I) N%, (J) N#, (K) M%, (L) M#, (M) PLR, (N) MLR and (O) NLR for discrimination between gastric cancer and healthy controls. Hb, hemoglobin; Hct, hematocrit; L%, lymphocyte percentage; L#, lymphocyte count; miR-130b, microRNA-130b; MLR, monocyte to lymphocyte ratio; MPV, mean platelet volume; M%, monocyte percentage; M#, monocyte count; N%, neutrophil percentage; N#, neutrophil count; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; PLR, platelet to lymphocyte ratio; RDW-CV, red blood cell distribution width-coefficient of variation; ROC, receiver operating characteristic.

Table II. Diagnostic value of different indicators for gastric cancer.

Variable	AUC (95% CI)	Cut-off index J	Youden	Sensitivity, %	Specificity, %	Accuracy, %	PPV, %	NPV, %	P-value
miR-130b	0.887 (0.821-0.935)	0.18	0.6222	91.11	71.11	84.44	14.00	35.99	0.001
Hb, g/l	0.828 (0.753-0.888)	127.00	0.5298	68.54	84.44	73.87	8.00	39.35	0.001
Hct, %	0.764 (0.682-0.833)	36.11	0.4727	53.93	93.33	67.16	4.00	43.02	0.001
RDW-CV, %	0.763 (0.681-0.832)	12.80	0.4854	68.54	80.00	72.39	10.00	37.28	0.001
MPV, fl	0.667 (0.580-0.747)	9.50	0.3227	52.27	80.00	61.65	10.00	36.85	0.001
NLR	0.757 (0.675-0.827)	3.13	0.4944	49.44	100.00	66.42	1.00	46.00	0.001
MLR	0.814 (0.738-0.876)	0.15	0.5645	80.90	75.56	79.10	11.99	36.00	0.001
PLR	0.712 (0.628-0.787)	188.98	0.4507	56.18	88.89	67.16	5.99	41.02	0.001
PDW, %	0.840 (0.766-0.898)	15.31	0.6823	70.45	97.78	79.70	1.99	45.69	0.001
N%	0.709 (0.624-0.784)	70.40	0.4494	44.94	100.00	63.43	1.00	45.91	0.001
M%	0.715 (0.630-0.789)	4.21	0.3860	74.16	64.44	70.90	17.00	30.25	0.001
L%	0.782 (0.703-0.849)	25.22	0.4844	59.55	88.89	69.40	5.99	41.11	0.001
N#, 10^9/l	0.634 (0.547-0.716)	4.63	0.4277	49.44	93.33	64.18	4.00	42.93	0.0044
M#, 10^9/l	0.680 (0.594-0.758)	0.24	0.3308	77.53	55.56	70.15	20.99	26.25	0.0004
L#, 10^9/l	0.719 (0.635-0.793)	1.46	0.4067	60.67	80.00	67.16	10.00	37.02	0.001

Sensitivity, specificity, PPV, NPV, accuracy and Youden index J were calculated based on the numbers of positive and negative cases. AUC, area under the curve; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; miR-130b, microRNA-130b; Hb, hemoglobin; Hct, hematocrit; RDW-CV, red blood cell distribution width-coefficient of variation; MPV, mean platelet volume; NLR, neutrophil to lymphocyte ratio; MLR, monocyte to lymphocyte count ratio; PLR, platelet to lymphocyte count ratio; PDW, platelet distribution width; N%, neutrophil percentage; M%, monocyte percentage; L%, lymphocyte percentage; N#, neutrophil count; M#, monocyte count; L#, lymphocyte count.

had a sensitivity of 82.02% and a specificity of 88.89%, miR-130b-MLR had a sensitivity of 75.28% and a specificity of 86.67%, miR-130b-Hb had a sensitivity of 89.89% and a specificity of 86.67%, PDW-MLR had a sensitivity of 71.26% and specificity of 97.78%, PDW-Hb had a sensitivity of 89.66% and a specificity of 88.89%, and MLR-Hb had a sensitivity of 73.03% and a specificity of 84.44%. The three-dimensional model of miR-130b-RDW-MLR yielded a sensitivity of 94.25% and a specificity of 91.11% at the optimal cut-off point, miR-130b-PDW-Hb yielded a sensitivity of 90.80% and specificity of 97.78% at the optimal cut-off point of 0.77 as the optimal cut-off point, miR-130b-MLR-Hb yielded a sensitivity of 91.01% and a specificity of 86.67% at the 0.52 as the optimal cut-off point, and PDW-MLR-Hb yielded a sensitivity of 89.66% and a specificity of 91.11% at 0.55 as the optimal cut-off point. Furthermore, the four-dimensional model miR-130b-PDW-MLR-Hb had a sensitivity of 91.95% and a specificity of 97.78% at 0.76 as the optimal cut-off point. Furthermore, the combination of miR-130b-PDW-MLR-Hb yielded the largest accuracy, LR+ and DOR values.

Association between the levels of circulating biomarkers and the risk of GC. To further determine whether the circulating marker levels for GC were associated with the presence of GC, logistic regression analysis was performed with the biomarkers as dependent variables (Table V). The crude odds ratio was obtained from logistic regression analysis, and the adjusted odds ratio was evaluated by adjusting for age and sex. The results demonstrated that the increase of miR-130b, RDW-CV, MPV, NLR, PLR, N% and M% was positively associated with the presence of GC (all P<0.05; Table V). The levels of PDW, Hb, Hct, L% and L# were negatively associated with the risk of GC (all P<0.05), while the levels of MLR, N# and M# were not associated with the incidence of GC (all P>0.05; Table V).

Association between circulating biomarkers and conventional GC markers. Correlation analysis was performed as shown in Fig. 3. Correlation analysis demonstrated that the value of RDW-CV was positively associated with the concentrations of CA50 (R=0.2424; P=0.0337; Fig. 3A) and CA125 (R=0.3426; P=0.0042; Fig. 3B). Pearson's correlation analysis indicated that PLR (R=0.2568; P=0.0346; Fig. 3C) and N% (R=0.2886; P=0.0170; Fig. 3D) were positively associated with the CA125 concentration, and L% (R, -0.367; P=0.0022; Fig. 3O) was negatively correlated with the CA125 concentration. Furthermore, the analysis demonstrated that MPV, N#, NLC and M# were positively associated with the CA211 concentration (all P<0.05; Fig. 3E-H). Particularly, Hb and Hct were negatively correlated with the concentration of CA125, CA211 and CA50 (all P<0.05; Fig. 3I-N). However, miR-130b, MLR and PDW were not correlated with CA125, CA50 or CA211 (all P>0.05; data not shown).

Levels of circulating biomarkers are associated with severity of GC. Correlations between cancer stage and RDW-CV, PLR, NLR, MPV, PDW, N#, M%, miR-130b, N%, M#, L#, L%, Hb, Hct and MLR in patients with GC are shown in Fig. 4. Correlation analysis revealed that RDW-CV, PLR, NLR, N# and N% were positively correlated with cancer

Table III. Receiver operating characteristic analysis of circulating biomarkers individually and combined for gastric cancer detection.

Models	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	Youden index J	P-value
One-dimensional model						
miR-130b	0.887 (0.821-0.935)	0.18	91.11	71.11	0.6222	
PDW,%	0.840 (0.766-0.898)	15.31	70.45	97.78	0.6823	
MLR	0.814 (0.738-0.876)	0.15	80.90	75.56	0.5645	
Hb, g/l	0.828 (0.753-0.888)	127.00	68.54	84.44	0.5298	
Dual-model						
miR-130b-PDW	0.922 (0.863-0.961)	0.74	82.02	88.89	0.7091	<0.0001[a], 0.0005[b]
miR-130b-MLR	0.892 (0.827-0.939)	0.76	75.28	86.67	0.6195	<0.0001[a], 0.1541[c]
miR-130b-Hb	0.937 (0.882-0.972)	0.56	89.89	86.67	0.7655	<0.0001[a], 0.0014[d]
PDW-MLR	0.843 (0.769-0.900)	0.53	71.26	97.78	0.6904	0.5030[b], 0.5662[c]
PDW-Hb	0.945 (0.891-0.977)	0.50	89.66	88.89	0.7854	0.0003[b], 0.0001[d]
MLR-Hb	0.835 (0.761-0.893)	0.64	73.03	84.44	0.5748	0.6893[c], 0.0940[d]
Tri-model						
miR-130b-PDW-MLR	0.955 (0.905-0.984)	0.61	94.25	91.11	0.8536	0.0921[e], 0.0091[f], 0.0005[g]
miR-130b-PDW-Hb	0.976 (0.933-0.995)	0.77	90.80	97.78	0.8858	0.0602[e], 0.0218[h], 0.0341[i]
miR-130b-MLR-Hb	0.942 (0.888-0.975)	0.52	91.01	86.67	0.7768	0.0095[f], 0.0697[h], 0.0012[j]
PDW-MLR-Hb	0.947 (0.894-0.978)	0.55	89.66	91.11	0.8077	0.0004[g], 0.2198[i], 0.0002[j]
Tetrad-model						
miR-130b-PDW-MLR-Hb	0.978 (0.936-0.996)	0.76	91.95	97.78	0.8973	0.0651[k], 0.1465[l], 0.0277[m], 0.0316[n]

[a]Compared with the AUC of miR-130b. [b]Compared with the AUC of PDW. [c]Compared with the AUC of MLR. [d]Compared with the AUC of Hb. [e]Compared with the AUC of miR-130b-PDW. [f]Compared with the AUC of miR-130b-MLR. [g]Compared with the AUC of PDW-MLR. [h]Compared with AUC of miR-130b-Hb. [i]Compared with the AUC of PDW-Hb. [j]Compared with the AUC of MLR-Hb. [k]Compared with the AUC of miR-130b-RDW-MLR. [l]Compared with the AUC of miR-130b-PDW-Hb. [m]Compared with the AUC of miR-130b-MLR-Hb. [n]Compared with the AUC of PDW-MLR-Hb. AUC, area under the curve; 95% CI, 95% confidence interval; miR-130b, microRNA-130b; PDW, platelet distribution width; MLR, monocyte to lymphocyte count ratio; Hb, hemoglobin.

Table IV. Accuracy of miR-130b, PDW, MLR and Hb individually and combined for gastric cancer detection.

Variable	Cut-off	Sensitivity, %	Specificity, %	Accuracy, %	LR+	LR-	DOR
miR-130b	0.18	91.11	71.11	84.44	3.15	0.13	25.23
PDW,%	15.31	70.45	97.78	72.39	3.43	0.39	8.71
MLR	0.15	80.90	75.56	79.11	3.31	0.25	13.09
Hb, g/l	127.00	68.54	84.44	73.88	4.40	0.37	11.82
miR-130b-PDW	0.74	82.02	88.89	84.33	7.38	0.20	36.49
miR-130b-MLR	0.764	75.28	86.67	79.11	5.65	0.29	19.80
miR-130b-Hb	0.56	89.89	86.67	88.80	6.74	0.12	57.81
PDW-MLR	0.53	71.26	97.78	80.17	32.10	0.29	109.21
PDW-Hb	0.50	89.66	88.89	89.40	8.07	0.12	69.38
MLR-Hb	0.64	73.03	84.44	76.89	4.69	0.32	14.69
miR-130b-PDW-MLR	0.61	94.25	91.11	93.18	10.60	0.06	167.99
miR-130b-PDW-Hb	0.77	90.80	97.78	93.18	40.90	0.09	434.71
miR-130b-MLR-Hb	0.52	91.01	86.67	89.55	6.83	0.10	65.82
PDW-MLR-Hb	0.55	89.66	91.11	90.15	10.09	0.11	88.87
miR-130b-PDW-MLR-Hb	0.76	91.95	97.78	93.94	41.42	0.08	503.10

LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio; miR-130b, microRNA-130b; PDW, platelet distribution width; MLR, monocyte-lymphocyte count ratio; Hb, hemoglobin.

Table V. Crude odds ratio and adjusted odds ratio between circulating tumor marker levels and the risk of gastric cancer.

Indicators	β	Crude odds ratio	HR (95% CI)	Sig	β	Adjusted odds ratio[a]	HR (95% CI)	Sig
miR-130b	0.900	0.406	0.301-0.548	0.000	0.869	0.419	0.293-0.601	0.000
RDW-CV,%	0.578	10.782	10.326-20.393	0.000	0.574	1.776	1.197-6.744	0.004
MPV, fl	0.519	10.680	10.173-20.405	0.005	0.710	2.034	1.272-7.309	0.003
NLR	0.802	20.229	10.460-30.404	0.000	1.006	2.733	1.484-5.974	0.001
PLR	0.008	10.008	10.003-10.013	0.001	0.007	1.007	1.002-5.951	0.008
PDW,%	-0.769	0.464	0.334-0.643	0.000	-0.702	0.496	0.353-0.696	0.000
N%	0.054	10.055	10.022-10.090	0.001	0.057	1.059	1.014-6.421	0.010
M%	0.415	10.514	10.194-10.918	0.001	0.412	1.511	1.126-2.027	0.006
N#,10⁹/l	0.026	10.027	0.930-10.133	0.601	0.051	1.052	0.963-1.150	0.262
M#,10⁹/l	0.494	10.639	0.398-60.753	0.494	0.499	1.646	0.342-7.926	0.534
Hb, g/l	-0.063	0.939	0.914-0.964	0.000	-0.070	0.933	0.903-0.964	0.000
Hct,%	-0.191	0.826	0.757-0.902	0.000	-0.201	0.818	0.737-0.907	0.000
MLR	-0.219	1.245	0.607-2.554	0.550	-0.188	0.829	0.477-1.439	0.505
L%	-0.103	0.902	0.802-0.943	0.000	-0.100	0.905	0.858-0.954	0.000
L#,10⁹/l	-1.041	0.353	0.198-0.630	0.000	-0.923	0.397	0.203-0.776	0.007

[a]Adjustment for age and sex. HR, hazard ratio; 95% CI, 95% confidence interval; miR-130b, microRNA-130b; RDW-CV, red blood cell distribution width-coefficient of variation; MPV, mean platelet volume; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; PDW, platelet distribution width; N%, neutrophil percentage; M%; N#, neutrophil count; M#, monocyte count; Hb, hemoglobin; Hct, hematocrit; MLR, monocyte to lymphocyte ratio; L%, lymphocyte percentage; L#, lymphocyte count.

stage and MPV, L#, L%, Hb and Hct were negatively correlated with cancer stage. However, PDW, M%, M#, MLR and miR-130b were not correlated with cancer stage. In addition, the levels of circulating RDW-CV, PLR, NLR, and N% were significantly higher in advanced stages (III and IV) than in the earlier pathologic stages (I and II) of GC. By contrast, the levels of circulating L%, Hb, Hct and L# were significantly lower in advanced stages than in the earlier pathological stages of GC. However, there was no difference in miR-130b between the two groups (Fig. 5). Since the levels of RDW-CV, PLR, NLR and N% were positively correlated with tumor pathological characteristics (TNM stage), it is reasonable to hypothesize that these biomarkers may be associated with GC metastasis.

Integrative diagnosis model for discriminating EGC. To evaluate the role of circulating biomarkers in the diagnosis of early GC (TNM I-II), the present study further analyzed the levels of these markers (Fig. 6; Table VI). As shown in Fig. 6A-C, G-I and M-O, the circulating levels of mi-130b, NLR, RDW-CV, PDW, M%, R#, Hct, Hb and MLR differed significantly between the early stage group and the NC group. The results in Fig. 6D-F, J-L and P-R demonstrate the diagnostic efficiency of circulating miR-130b, NLR, RDW-CV, PDW, M%, R#, Hct, Hb and MLR individually for discriminating patients with EGC from controls. As one-dimensional models, miRNA-130b, RDW-CV, NLR, PDW, M%, R#, Hb, Hct, and MLR individually yielded AUC values of 0.9110, 0.7130, 0.6491, 0.8037, 0.7367, 0.7019, 0.7745, 0.6935 and 0.7610, respectively. These results indicated that miR-130b, NLR, RDW-CV, PDW, M%, R#, Hct, Hb and MLR could be used as novel biomarkers for the early diagnosis of GC.

To further explore the diagnostic efficacy of joint markers, the biomarkers with an AUC >0.77 (miR-130b, PDW and Hb) were further considered for combinations. Among the two-dimensional models (i.e., the models with two biomarkers), miR-130b-PDW yielded the greatest AUC (0.956), followed by miR-130b-Hb (0.923) and PDW-Hb (0.906). The combination of miR-130b with Hb had a significantly larger AUC value (0.923; 0.833-0.974) compared with Hb alone (P=0.0181), and the combination of PDW and Hb had a significantly larger AUC value (0.906; 0.812-0.963) compared with Hb alone (P=0.0464). The tri-model (miR-130b-PDW-Hb) had an AUC of 0.960 (0.883-0.993). However, there were no significant differences in the AUC values between the tri-model and dual-models (P=0.2250, P=0.4331 and P=0.0940).

Diagnostic performance of tri-dimensional biomarkers for patients with EGC. The corresponding diagnostic accuracy parameters, including sensitivity, specificity, accuracy, LR⁺, LR⁻ and DOR, are shown in Table VII. miR-130b and Hb had high sensitivities (95.56 and 83.33%, respectively), RDW-CV, PDW, R# and Hct had high specificities (80.00, 97.78, 97.78 and 93.33%, respectively), and miR-130b, PDW and R# had high accuracy (82.15, 91.31 and 88.27%, respectively) for distinguishing early stage cancer from NCs. The specificity and accuracy increased when miR-130b and Hb were combined, and the sensitivity and accuracy increased when miR-130b and PDW were combined. The combination of miR-130b-PDW-Hb could not improve the sensitivity, specificity and accuracy for the diagnosis of EGC in comparison with the two-dimensional model of miR-130b-PDW. Furthermore, the combination of miR-130b and PDW yielded the largest sensitivity, specificity, accuracy, LR⁺ and DOR values, and the lowest LR⁻ value.
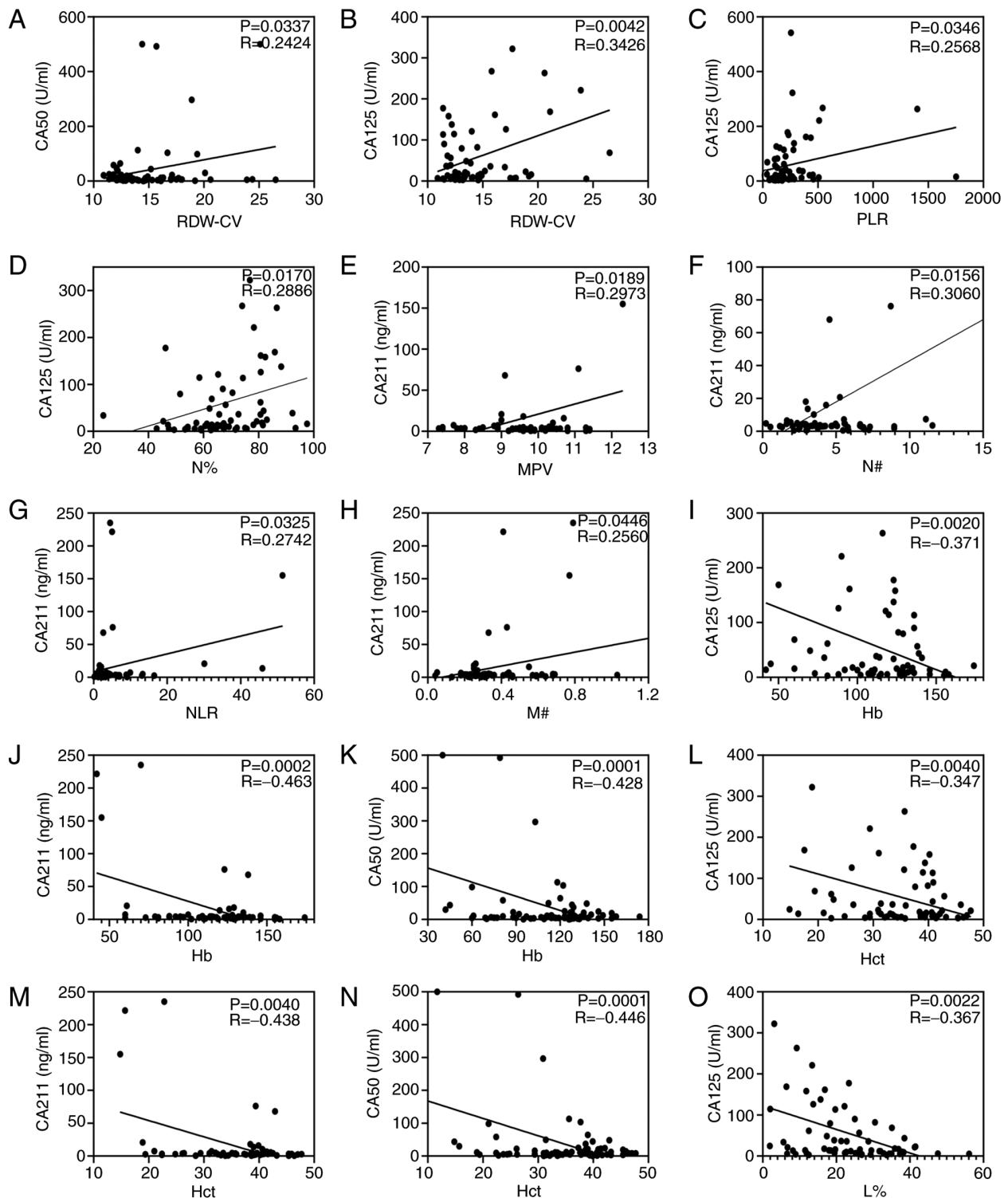
Figure 3. Circulating biomarkers and conventional gastric cancer markers analyzed using Pearson's correlation. Correlation analysis of RDW-CV with (A) CA50 and (B) CA125. Correlation analysis of CA125 with (C) PLR and (D) N%. Correlation between (E) MPV, (F) N#, (G) NLR and (H) M# and CA211. Continuous data for Hb with (I) CA125, (J) CA211 and (K) CA50. Continuous data for Hct with (L) CA125, (M) CA211 and (N) CA50. (O) Correlation analysis of L% with CA125. Hb, hemoglobin; Hct, hematocrit; L%, lymphocyte percentage; MPV, mean platelet volume; M#, monocyte count; N#, neutrophil count; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RDW-CV, red blood cell distribution width-coefficient of variation.

These results indicated that miR-130b-PDW had great diagnostic value for EGC and could be used as a non-invasive diagnostic model for EGC.

Integrative diagnosis model for discriminating precancerous gastric lesions. Based on the differences in RDW-CV, MPV/PC ratio, MLR, NLR, PDW, L%, M%, M# and Hb between the Pre and NC groups (Table I), the diagnostic analyses of single or combinations of selected differentially expressed biomarkers were conducted (Table VIII; Fig. 7). As shown in Table VIII, the AUC values for miR-130b, RDW-CV, MPV/PC ratio, MLR, NLR, PDW, L %, M%, M#
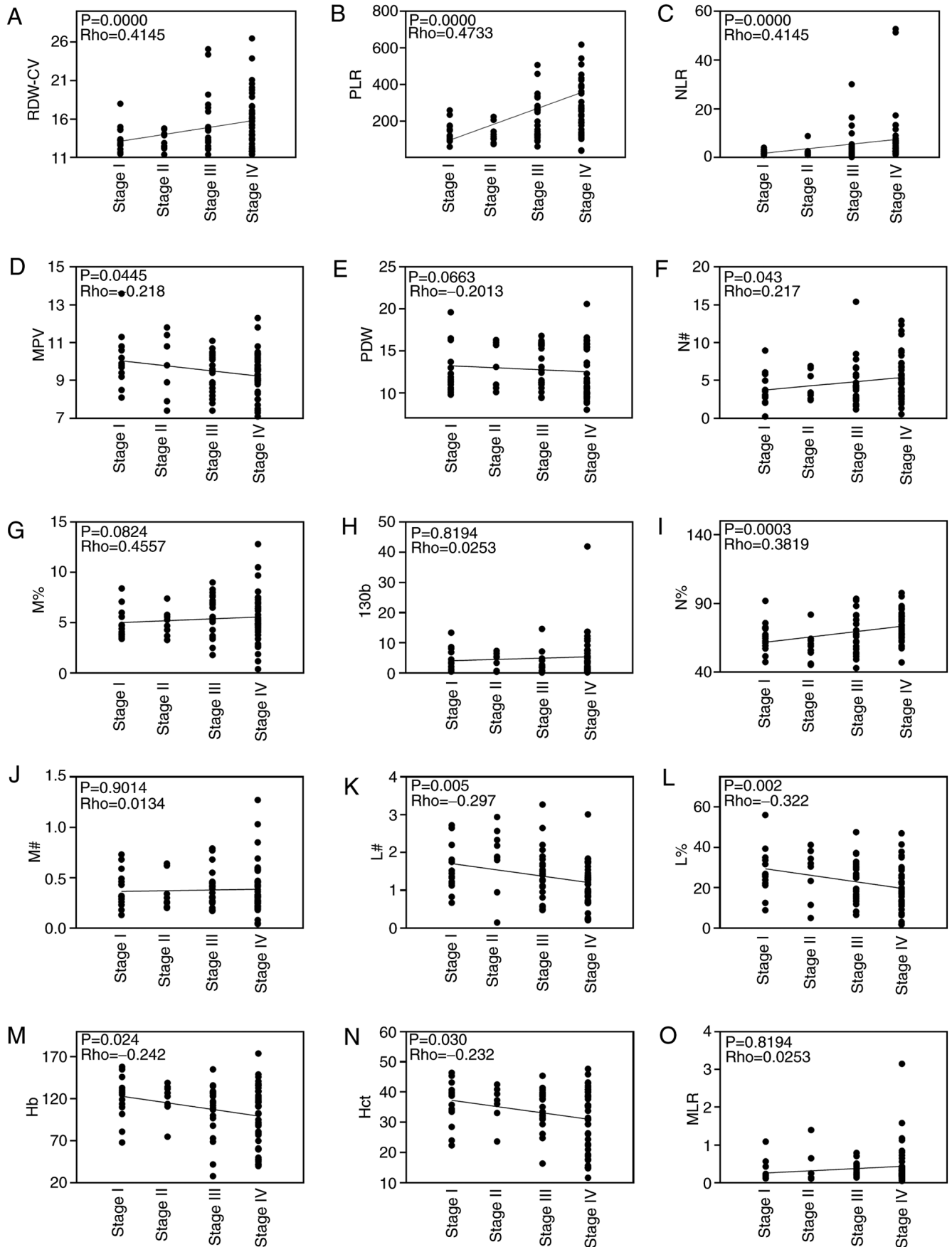
Figure 4. Spearman's correlation analysis of blood biochemical indexes and cancer stage. Analysis of correlation between blood biochemical indexes, including (A) RDW-CV, (B) PLR, (C) NLR, (D) MPV, (E) PDW, (F) N#, (G) M%, (H) miR-130b, (I) N%, (J) M#, (K) L#, (L) L%, (M) Hb, (N) Hct and (O) MLR, and cancer stage. Hb, hemoglobin; Hct, hematocrit; L%, lymphocyte percentage; L#, lymphocyte count; miR-130b, microRNA-130b; MLR, monocyte to lymphocyte ratio; MPV, mean platelet volume; M%, monocyte percentage; M#, monocyte count; N%, neutrophil percentage; N#, neutrophil count; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; PLR, platelet to lymphocyte ratio; RDW-CV, red blood cell distribution width-coefficient of variation.
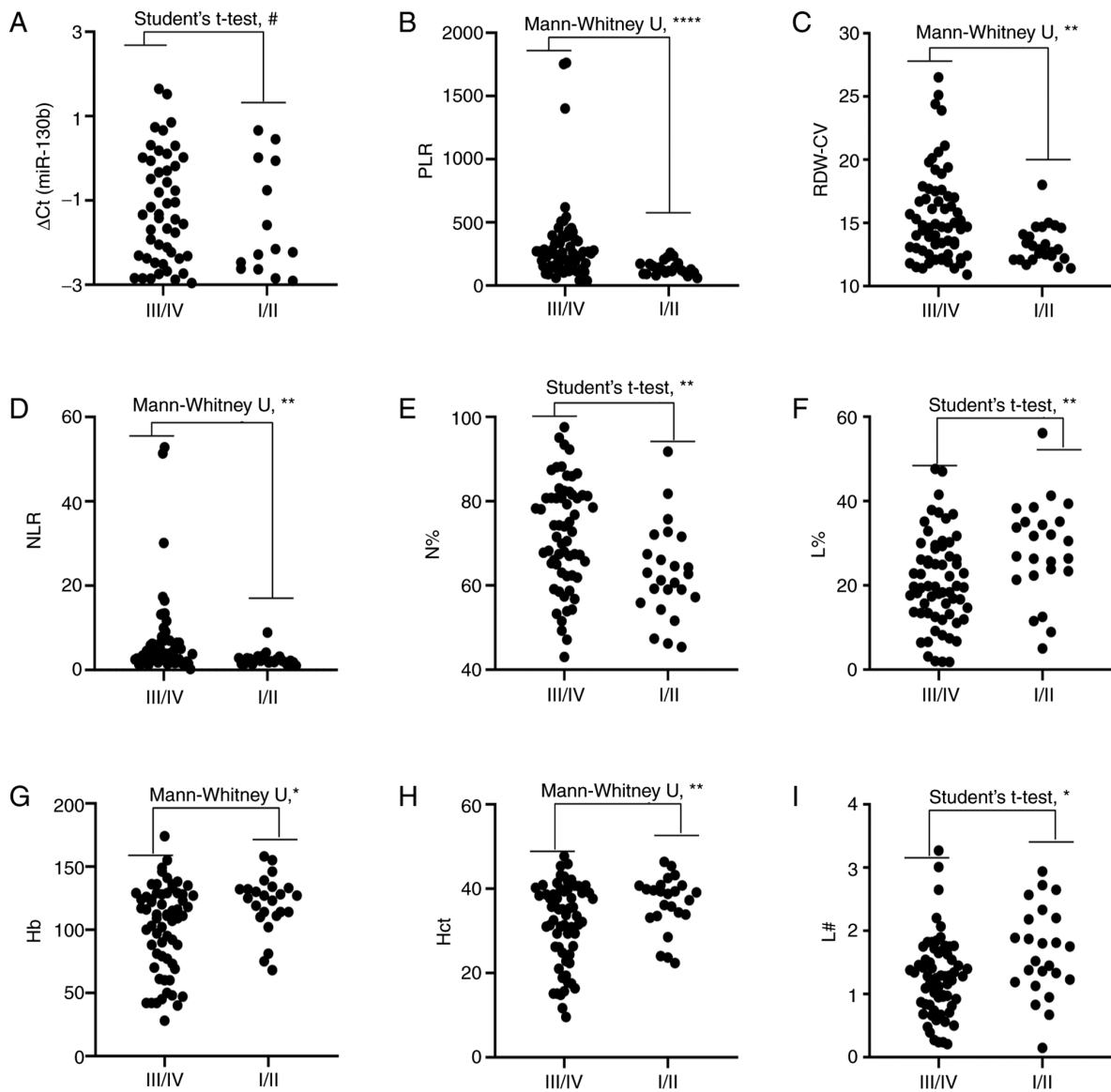
Figure 5. Comparison of circulating biomarker expression between the early stages (I/II) and advanced pathological stages (III/IV) of gastric cancer. (A) ΔCq value of miR-130b, (B) PLR, (C) RDW-CV, (D) NLR, (E) N%, (F) L%, (G) Hb, (H) Hct and (I) L#. Unpaired t-test or Mann-Whitney U test was used to determine statistical significance at ($^#P>0.05$, $^*P<0.05$, $^{**}P<0.01$ and $^{****}P<0.0001$). Hb, hemoglobin; Hct, hematocrit; L%, lymphocyte percentage; L#, lymphocyte count; miR-130b, microRNA-130b; N%, neutrophil percentage; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; RDW-CV, red blood cell distribution width-coefficient of variation.

and Hb were 0.700, 0.649, 0.687, 0.713, 0.616, 0.811, 0.638, 0.649, 0.657 and 0.679, respectively. To further explore the diagnostic efficacy of joint models, the biomarkers with an AUC >0.70 (miR-130b, PDW and MLR) were further considered for combinations. Among the two-dimensional models (i.e., the models with two biomarkers), miR-130b-PDW yielded the greatest AUC (0.896), followed by MLR-PDW (0.816) and miR-130b-MLR (0.687). The dual-model miR-130b-PDW had a significantly larger AUC 0.896 (0.827-0.944) compared with miR-130b and PDW, respectively. The tri-model (miR-130b-PDW-MLR) yielded an AUC value of 0.896 (0.827-0.944), which was larger than those of the dual-models of miR-130b-MLR and MLR-PDW. However, there were no significant differences in the AUC values between the tri-model (miR-130b-MLR-PDW) and the dual-model (miR-130b-PDW).

Diagnostic performance of tri-dimensional biomarkers for patients with Pres. The corresponding diagnostic accuracy parameters, including sensitivity, specificity, accuracy, LR+, LR- and DOR, are shown in Table IX. MLR and PDW had high sensitivities for distinguishing Pres from NCs (77.92 and 72.37%, respectively), miR-130b, NLR and L% had high specificities (93.30, 100.00 and 100.00%, respectively), and PDW had high accuracy (81.74%). The sensitivity and accuracy increased when miR-130b and PDW were combined. Furthermore, the combination of MLR-PDW yielded the largest LR+ and DOR values. However, the combination of miR-130b-MLR-PDW could not improve the sensitivity, specificity and accuracy for the diagnosis of precancerous gastric lesions. These results indicated that the dual-model of miR-130b-PDW was an appropriate and non-invasive diagnostic model for Pres.
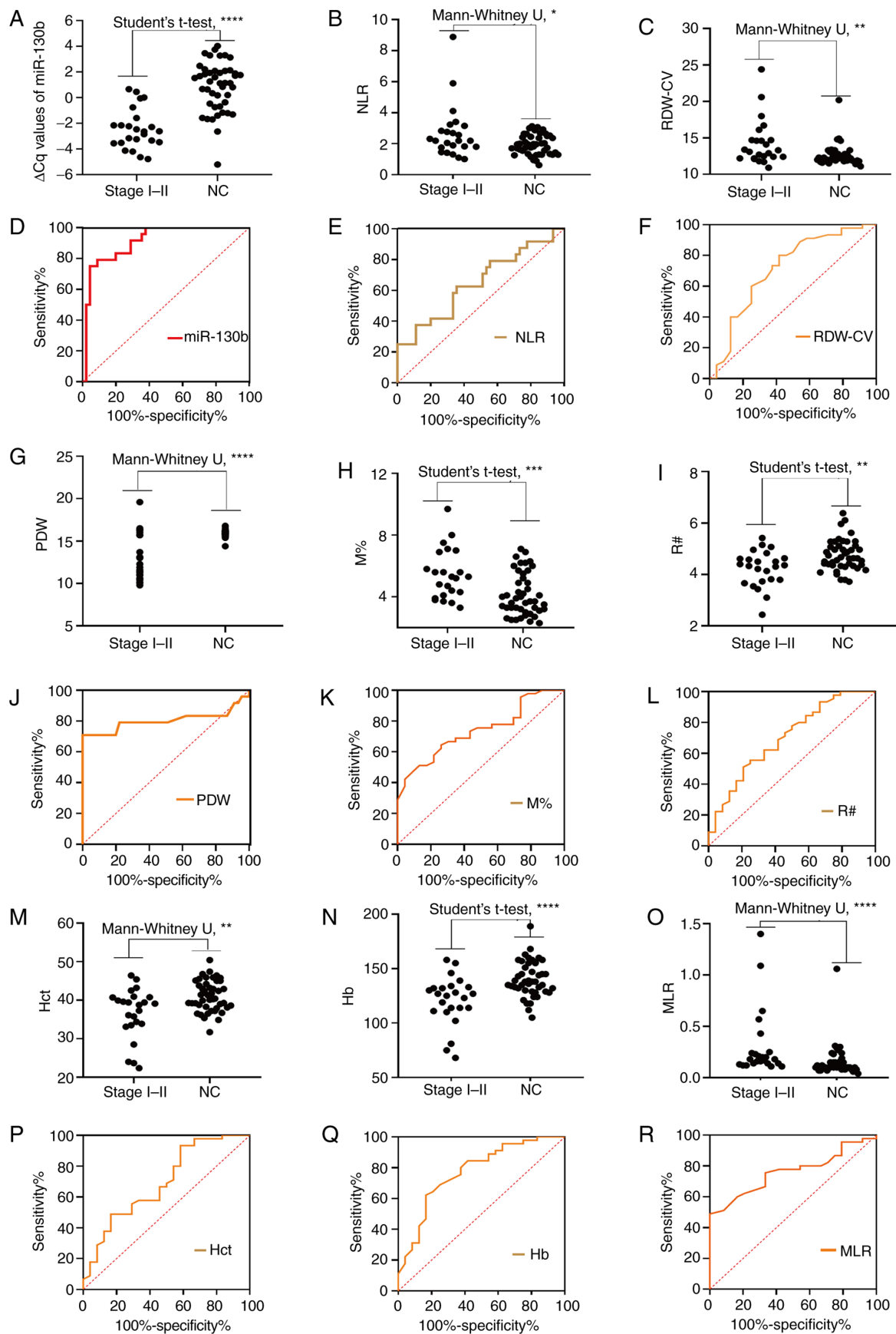
Figure 6. Diagnosis of early stages (I/II) of gastric cancer. Comparison of circulating biomarker expression between the early stages (I/II) of gastric cancer and NCs. (A) ΔCq value of miR-130b, (B) NLR, (C) RDW-CV, (G) PDW, (H) M%, (I) R#, (M) Hct, (N) Hb and (O) MLR. Unpaired t-test or Mann-Whitney U test was used to determine statistical significance (*P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001). Area under the curve of the expression of circulating biomarkers for early gastric cancer diagnosis. (D) ΔCq value of miR-130b, (E) NLR, (F) RDW-CV, (J) PDW, (K) M%, (L) R#, (P) Hct, (Q) Hb and (R) MLR. Hb, hemoglobin; Hct, hematocrit; miR-130b, microRNA-130b; MLR, monocyte to lymphocyte ratio; M%, monocyte percentage; NC, healthy control; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; R#, red blood cell count; RDW-CV, red blood cell distribution width-coefficient of variation.

Table VI. Receiver operating characteristic analysis of circulating biomarkers individually and combined for early stage gastric cancer detection.

Models	AUC (95% CI)	P-value
One-dimensional model		
miR-130b	0.9110 (0.8417-0.9805)	
RDW-CV,%	0.7130 (0.5770-0.8490)	
NLR	0.6491 (0.5082-0.7900)	
PDW,%	0.8037 (0.6616-0.9458)	
M%	0.7367 (0.6196-0.8539)	
R#,10^{12}/l	0.7019 (0.5715-0.8322)	
Hb, g/l	0.7745 (0.6563-0.8928)	
Hct,%	0.6935 (0.5601-0.8269)	
MLR	0.7610 (0.6430-0.8560)	
Dual-model		
miR-130b-PDW	0.9560 (0.877-0.991)	0.3775[a]; 0.0576[b]
miR-130b-Hb	0.9230 (0.833-0.974)	0.7412[a]; 0.0181[c]
PDW-Hb	0.9060 (0.812-0.963)	0.1731[b]; 0.0464[c]
Tri-model		
miR-130b-PDW-Hb	0.960　(0.883-0.993)	0.2250[d]; 0.4331[e]; 0.0940[f]

[a]Compared with the AUC of miR-130b. [b]Compared with the AUC of PDW. [c]Compared with the AUC of Hb. [d]Compared with the AUC of miR-130b-PDW. [e]Compared with the AUC of miR-130b-Hb. [f]Compared with the AUC of PDW-Hb. AUC, area under the curve; 95% CI, 95% confidence interval; miR-130b, microRNA-130b; RDW-CV, red blood cell distribution width-coefficient of variation; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; M%, monocyte percentage; R#, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MLR, monocyte to lymphocyte ratio.

Table VII. Accuracy of miR-130b, PDW and Hb individually and combined for early stage gastric cancer detection.

Variable	Cut-off	Youden index J	Sensitivity, %	Specificity, %	Accuracy, %	LR+	LR-	DOR
miR-130b	-2.15	0.71	95.56	75.00	82.15	3.82	0.06	64.57
RDW-CV,%	12.80	0.38	58.33	80.00	72.46	2.92	0.52	5.60
NLR	2.04	0.27	62.50	64.44	63.77	1.76	0.58	3.02
PDW,%	15.31	0.77	79.17	97.78	91.31	35.66	0.21	167.41
M%	4.20	0.38	73.91	64.44	67.64	2.08	0.40	5.13
R#,10^{12}/l	4.61	0.31	70.45	97.78	88.27	31.73	0.30	105.01
Hb, g/l	134	0.46	83.33	62.22	69.56	2.21	0.27	8.23
Hct,%	36.10	0.35	41.67	93.33	75.36	6.25	0.62	10.00
MLR	0.10	0.49	100.00	48.89	66.67	1.95	-	-
miR-130b-PDW	0.26	0.94	95.83	97.78	97.10	43.17	0.04	1,012.19
miR-130b-Hb	0.40	0.74	83.33	91.11	88.40	9.37	0.18	51.23
PDW-Hb	0.31	0.79	83.33	95.56	91.31	18.77	0.17	107.59
miR-130b-PDW-Hb	0.29	0.94	95.83	97.78	97.10	43.17	0.04	1,012.19

LR+, positive likelihood ratio; LR-, negative likelihood ratio; DOR, diagnostic odds ratio; miR-130b, microRNA-130b; RDW-CV, red blood cell distribution width-coefficient of variation; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; M%, monocyte percentage; R#, red blood cell count; Hb, hemoglobin; Hct, hematocrit; MLR, monocyte to lymphocyte ratio.

Baseline characteristics. A total of 90 patients with GC were included in the present study, including 46 men and 44 women, with a mean age of 65 years (range, 36-89 years). The age range was 29-88 (mean, 64.5 years)

years for the 90 patients with Pres, including 48 male patients and 42 female patients. A total of 45 NCs (age range, 39-80 years) with a mean age of 65 years, including 21 male patients and 24 female patients, were included. In

Table VIII. Receiver operating characteristic analysis of circulating biomarkers individually and combined for precancerous lesion detection.

Models	AUC (95% CI)	P-value
One-dimensional model		
miR-130b	0.700 (0.613-0.788)	
RDW-CV, %	0.649 (0.557-0.733)	
MPV/PC ratio	0.687 (0.597-0.768)	
MLR	0.713 (0.624-0.791)	
NLR	0.616 (0.524-0.702)	
PDW, %	0.811 (0.730-0.877)	
L%	0.638 (0.546-0.723)	
M%	0.649 (0.558-0.733)	
M# 10^9/l	0.657 (0.565-0.741)	
Hb, g/l	0.679 (0.588-0.761)	
Dual-model		
miR-130b-MLR	0.687 (0.597-0.768)	0.8154[a]; 0.7273[b]
miR-130b-PDW	0.896 (0.827-0.944)	<0.0001[a]; 0.0028[c]
MLR-PDW	0.816 (0.736-0.881)	0.1069[b]; 0.0832[c]
Tri-model		
miR-130b-MLR-PDW	0.896 (0.827-0.944)	0.0001[d]; 0.6849[e]; 0.0049[f]

[a]Compared with the AUC of miR-130b. [b]Compared with the AUC of MLR. [c]Compared with the AUC of PDW. [d]Compared with the AUC of miR-130b-MLR. [e]Compared with the AUC of miR-130b-PDW. [f]Compared with the AUC of MLR-PDW. AUC, area under the curve; 95% CI, 95% confidence interval; miR-130b, microRNA-130b; RDW-CV, red blood cell distribution width-coefficient of variation; MPV/PC, mean platelet volume to platelet count ratio; MLR, monocyte to lymphocyte count ratio; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; L%, lymphocyte percentage; M%, monocyte percentage; M#, monocyte count; Hb, hemoglobin.

Table IX. Accuracy of circulating biomarkers individually and combined for Pre detection.

Pre vs. NC	AUC (95% CI)	Cut-off	Youden index J	Sensitivity, %	Specificity, %	Accuracy, %	LR⁺	LR⁻	DOR
RDW-CV	0.649 (0.557-0.733)	12.40	0.2808	63.64	64.44	63.94	1.79	0.56	3.17
MPV/PC ratio	0.687 (0.597-0.768)	0.04	0.2973	51.95	77.78	61.48	2.34	0.62	3.78
MLR	0.713 (0.624-0.791)	0.12	0.3792	77.92	60.00	71.31	1.95	0.37	5.29
NLR	0.616 (0.524-0.702)	3.12	0.3506	35.06	100.00	59.01	-	0.65	-
PDW	0.811 (0.730-0.877)	15.31	0.7015	72.37	97.78	81.74	32.60	0.28	115.37
L%	0.638 (0.546-0.723)	21.71	0.3377	33.77	100.00	58.20	-	0.66	-
M%	0.649 (0.558-0.733)	4.50	0.2547	57.14	68.89	61.47	1.84	0.62	2.95
M#	0.657 (0.565-0.741)	0.25	0.2974	69.74	60.00	66.15	1.74	0.50	3.46
Hb	0.679 (0.588-0.761)	127.00	0.3250	48.05	84.44	61.47	3.09	0.62	5.02
miR-130b-MLR	0.687 (0.597-0.768)	0.68	0.4343	54.55	88.89	67.22	4.91	0.51	9.60
miR-130b-PDW	0.896 (0.827-0.944)	0.51	0.7623	82.89	93.33	86.77	12.43	0.18	67.79
MLR-PDW	0.816 (0.736-0.881)	0.51	0.7146	73.68	97.78	82.64	33.19	0.27	123.30
miR-130b-MLR-PDW	0.896 (0.827-0.944)	0.51	0.7623	82.89	93.33	86.77	12.43	0.18	67.79

Pre, precancerous lesions; NC, healthy control; AUC, area under the curve; 95% CI, 95% confidence interval; LR⁺, positive likelihood ratio; LR⁻, negative likelihood ratio; DOR, diagnostic odds ratio; miR-130b, microRNA-130b; RDW-CV, red blood cell distribution width-coefficient of variation; MPV/PC, mean platelet volume to platelet count ratio; MLR, monocyte to lymphocyte count ratio; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; L%, lymphocyte percentage; M%, monocyte percentage; M#, monocyte count; Hb, hemoglobin.

the present study, no significant differences were observed in age and sex (P=0.9598 and P=0.7658, respectively). The clinical characteristics of all patients are summarized in Table X.
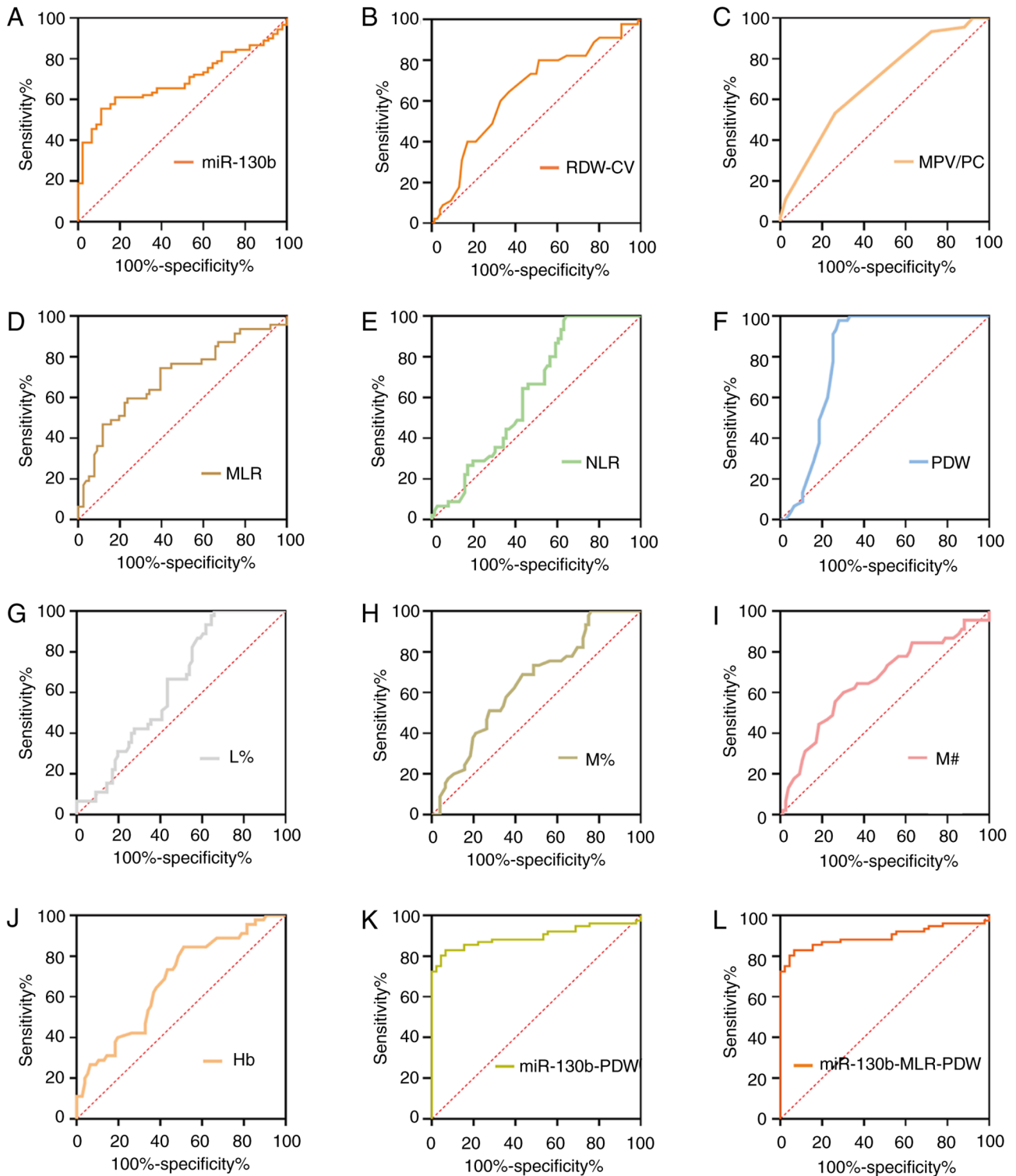
Figure 7. ROC curve analyses of circulating biomarkers for Pres. ROC analysis for detection of Pres using (A) miR-130b, (B) RDW-CV, (C) MPV/PC, (D) MLR, (E) NLR, (F) PDW, (G) L%, (H) M%, (I) M#, (J) Hb, (K) miR-130b-PDW and (L) miR-130b-MLR-PDW. Hb, hemoglobin; L%, lymphocyte percentage; miR-130b, microRNA-130b; MLR, monocyte to lymphocyte ratio; MPV/PC, mean platelet volume to platelet count ratio; M%, monocyte percentage; M#, monocyte count; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; RDW-CV, red blood cell distribution width-coefficient of variation; ROC, receiver operating characteristic; Pres, precancerous gastric lesions.

Discussion

The early diagnosis of GC is of great significance. In the present study, an integrated analysis was performed and it was revealed that plasma miR-130b and partial parameters of blood routine, such as PDW, RDW, MLR, NLR, and PLR, were dysregulated in Pres, EGC and GC. Furthermore, the early diagnostic value of miR-130b and blood routine parameters alone or in combination was explored and an optimal diagnostic model for GC, EGC and Pre was identified.

Table X. Clinicopathological characteristics of all individuals by subgroup.

Characteristics	GC (n=90)	Pre (n=90)	NC (n=45)	P-value
Mean age ± SD, years	65±12.7	64.5±14.4	65±5.1	0.9598[a]
Sex, male/female	46/44	48/42	21/24	0.7658[b]
TNM stage, n				
I	22			
II	8			
III	13			
IV	47			
Differentiation degree, n				
High	19			
Moderate	46			
Poor	25			
Histological type, n				
Adenocarcinoma	68			
Mucinous carcinoma	6			
Signet ring cell carcinoma	5			
Adenocarcinoma with signet ring cell carcinoma	11			
Histological type, n				
Intestinal metaplasia		82		
Atypical hyperplasia and other type		8		

[a]P-value was calculated using one-way ANOVA. [b]P-value was calculated using the χ^2 test. GC, gastric cancer; Pre, precancerous lesions; NC, healthy control.

Previous studies have reported that miR-130b (24) is dysregulated in various cancer types, including GC (25-29). It has been reported that miR-130b promotes cell proliferation, migration and invasion, and serves as a biomarker of a poor prognosis in lung cancer (25). Zhu et al (28) reported that miR-130b is upregulated in esophageal squamous cell carcinoma tumor tissues and cells, acting as a tumor promoter by targeting SAM and SH3 domain containing 1. In ovarian cancer, miR-130b downregulation has been associated with progression, multidrug resistance and poor histological differentiation (30). In breast cancer and lung cancer, miR-130b contributes to chemoresistance by activating the phosphoinositol 3 kinase/protein kinase B and Wnt/β catenin signaling pathways (31,32). Furthermore, it has been documented that miR-130b upregulation may be associated with enhanced epithelial-mesenchymal transition and angiogenesis in colorectal cancer (33). Lai et al (29) revealed that miR-130b expression is higher in gastric tissues compared with that in matched normal tissue, and this decreases the growth suppressive potential of RUNX family transcription factor 3 and contributes to tumorigenesis. Similarly, the present results demonstrated that circulating miRNA-130b was highly expressed in patients with GC, Pres and EGC, and it was significantly associated with the TNM stage of GC. Furthermore, the present study revealed that the AUC value of miR-130b was 0.887 for the diagnosis of GC, 0.911 for the diagnosis of EGC and 0.700 for the diagnosis of Pres, suggesting that miR-130b may contribute to the development of GC and could be a useful screening biomarker for EGC and Pres.

As an important parameter reflecting the heterogeneity of erythrocyte volume, RDW is often used in the diagnosis and observation of the curative effect of iron deficiency anemia, the differential diagnosis of small cell hypochromic anemia and the differential diagnosis of anemia (34). Beyazit et al (9) revealed that elevated RDW can serve as a useful biomarker for differentiating benign from malignant causes of biliary obstruction, with a sensitivity of 72% and a specificity of 69%. Spell et al (10) reported that RDW yielded 84% sensitivity and 88% specificity for distinguishing between right-sided colon cancer cases and healthy subjects. In breast cancer diagnosis, Seretis et al (35) also suggested that RDW may be a novel biomarker inversely associated with the tumor grade. The present study revealed that elevated RDW in the peripheral blood was a useful biomarker for the early diagnosis of GC, which was in line with results from a previous study (8). A previous study revealed that RDW levels in GC might be influenced by inflammation and iron loss associated with chronic inflammatory status (36). An earlier study revealed that response to elevated levels of pro-inflammatory factors in cancer, erythropoietic activity, and therefore iron metabolism and homeostasis, are impaired (37). In addition, previous studies have revealed that RDW is positively associated with the conventional tumor markers CEA and CA19-9 in colorectal cancer (38), and tumor stage in ovarian cancer (39). The present study demonstrated that RDW was positively associated with circulating markers CA50 and CA125, and the pathological staging of GC. Therefore, the increased RDW may be associated with the occurrence, development and prognosis of GC, and it might be an excellent circulating marker for the early diagnosis of GC.

Anemia is a common complication of cancer, which is associated with the energy level and quality of life score of patients (40). As a prognostic predictor, Hct has been reported in renal cell carcinoma, lung cancer and triple-negative breast cancer (41-43). Zhang et al (44) demonstrated that lower levels of Hb predict worse survival rate of patients with advanced GC. However, to the best of our knowledge, the value of Hb and Hct in the diagnosis of GC has not been reported. To the best of our knowledge, the present study was the first to reveal that both Hb and Hct could be useful biomarkers for GC, especially for EGC. As is well known, advanced stage is associated with a poor prognosis of GC (2). In further analysis in the present study, it was revealed that Hb and Hct were negatively correlated with cancer stage, which suggested that Hb and Hct may be associated with the severity of GC. It should be noted that numerous clinicopathological factors can affect Hct levels. For example, low Hct is more likely to occur in patients with advanced stage, anemia and low albumin (45). Therefore, these interference factors should be excluded when low Hct is used as a prognostic indicator.

PDW is a parameter reflecting the variation of platelet volume in the blood, and it is considered to be an indicator of platelet activation (46). A previous study demonstrated that PDW levels in patients with GC and Pres were lower than those in NCs (47). However, in the present study, there was no significant difference among the GC, Pre and NC groups, and the reasons for this require further study. MPV is an early indicator of platelet activation. In a study from China, Yun et al (48) stated that decreased levels of MPV in GC could be a potential biomarker for follow-up independently of tumor stage. The present study revealed higher levels of MPV in the GC and Pre groups than in the NC group, which was similar to a previous study (49). MPV/PC has been preferentially proposed as a predictor of patients with cancer (50). However, the association between MPV/PC and cancer is inconsistent. Sun et al (51) reported a higher MPV/PC ratio in patients with hepatocellular carcinoma compared with that in the control group. By contrast, a decrease in the MPV/PC ratio was detected in patients with advanced non-small cell lung carcinoma (52). In the present study, although the MPV/PC ratio of Pres was higher than that of the normal group, there was no difference between patients with GC and the control group. Additionally, the MPV/PC ratio has been recommended to be measured to differentiate iron deficiency anemia from other types of anemia (51). Therefore, the MPV/PC ratio could have potential clinical value in the identification of patients with GC. However, further investigations should be conducted to clarify the pathogenic mechanisms of the MPV/PC ratio in GC.

The other inflammatory parameters in the present study were L#, L%, N#, N%, M#, M%, NLR, PLR and MLR. It has been reported that platelet count, MPV, RDW, NLR and PLR can be used as circulating tumor markers to discriminate GCs from NCs (8). As reported by Chen et al (6), MLR can be used as a predictor to assess the survival rate of patients with advanced GC receiving chemotherapy. A previous study also reported that MLR may be a potential biomarker for predicting the overall survival rate of patients with advanced GC (53). The present results appear to be similar to these previous reports (6,8). Studies have demonstrated that high levels of NLR and PLR are associated with a poor

prognosis in colon cancer, ovarian cancer and GC (7,8). The present results agree with the findings of Aldemir et al (54), Yu et al (55) and Lian et al (56). The direct mechanism is not entirely understood. However, it seems most likely that increased neutrophil- and platelet-associated inflammation and decreased lymphocyte-dependent antitumor cellular immune response are closely associated with tumorigenesis. These changes result in an increase in neutrophil and platelet levels, and a decrease in lymphocyte levels (57). In addition, the present study was one of the few studies to focus specifically on the diagnostic value of NLR and PLR in patients with GC compared with past research (58). In the present study, NLR and PLR levels in GC were higher than those in NCs, and they were independently associated with the presence of GC. Notably, the data also revealed that NLR and PLR were positively correlated with GC staging and traditional tumor markers.

Combinations of biomarkers, such as circulating miR-19a-3p and miR-483-5p, can enhance the diagnostic performance in various cancer types (59-62). Notably, Chen et al (18) recently demonstrated that miR-650 combined with CA211 could be an effective diagnostic indicator for screening of the incidence of GC. Sun et al (63) demonstrated that the combination of serum multi-dimensional biomarkers could further improve the specificity and sensitivity of GC detection, thus achieving higher diagnostic efficiency. In the present study, a predictive diagnostic model of association for GC, EGC and Pres was developed. Different combinations were tested to elucidate subsets of potential biomarkers used to separate early and advanced stage patients. Furthermore, the present study evaluated the diagnostic performance of a four-dimensional biomarker panel for GC detection using AUC analysis. Overall, the combination of four-dimensional biomarkers improved the diagnostic accuracy for GC, with an accuracy of 93.94%, a DOR of 503.10 and an LR^+ of 41.42, which were better than those of one-, two- or tri-dimensional biomarkers. It confirmed that such four-dimensional biomarkers had potential value as a warning sign of GC. Subsequently, the combinations of miR-130b with PDW and Hb had larger AUC values for EGC compared with one- or two-dimensional biomarkers, suggesting that using these combined markers may improve the early detection of GC. Finally, the present study assessed the diagnostic performance of a three-dimensional biomarker panel for Pre detection using AUC analysis. Results demonstrated that the combination of miR-130b and PDW was better than any other single indicator or combination of indicators. Based on the aforementioned analysis results, it was hypothesized that circulating miR-130b and blood routine parameters were effective early warning indicators for early diagnosis of GC.

Although the results of the present study have certain clinical value, there were several limitations. Firstly, in view of the small number of included cases, although valuable results have been achieved in the present study, the time point at which these indicators are measured is unclear and their specific application has not been assessed. Therefore, clear consequences cannot be obtained, and further research is required. Secondly, Helicobacter pylori is an important carcinogen of GC. The present study did not investigate the relationship between H. pylori and mir-130 in GC, which is also a focus of further research. Finally, there was no specific

genotyping in patients with GC, and miRNA expression might be different between gene subtypes. Therefore, the significance of miR-130b and blood routine parameters in the early diagnosis of GC requires further study.

In conclusion, miRNA-130b, which was upregulated in GC, may be a novel early diagnostic marker for GC. Furthermore, using ROC curve analysis, the optimal cut-off values of the markers could be determined and the diagnosis of GC could be improved based on these blood routine markers. Novel screening models (miR-130b combined with blood routine parameters) were developed in the present study, and the optimal application of these circulating tumor markers could promote the clinical screening and diagnosis of GC, which patients may be more willing to accept for the detection of GC over more invasive tests.

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Availability of data and materials

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

Authors' contributions

JC conceived the idea, designed the study protocol and performed the experiments. ZL, GG, YM, HZ, WH, LW, XH, JD, CL, HL, JF and YS carried out the data collection and data analysis, and provided the critical revision. XG analyzed and interpreted the data, was involved in drafting and revision of the manuscript and gave final approval of the version to be published. JC, ZL and YS confirm the authenticity of all the raw data. All authors read and approved the final manuscript, and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by The Ethical Review Committee of Affiliated Liutie Central Hospital of Guangxi Medical University (Liuzhou, China). All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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