Circulating Fibrinogen to Pre-albumin Ratio for Chemotherapy Efficacy Prediction and Prognosis of Colorectal Cancer Patients

Hou-Qun Ying
Nanchang University
Fan Sun
Nanchang Number 9 Hospital
Wei Wang
Nanchang University
Dan Cai
Nanchang University
Ying Yang
Nanchang University
Nuo-Wei Nie
Nanchang University
Ying Huang
Nanchang University
Xue-Xin Chen (cxxncu@163.com)
Nanjing Medical University Second Affiliated Hospital https://orcid.org/0000-0003-2241-2535

Research

Keywords: fibrinogen to pre-albumin ratio, chemotherapy, colorectal cancer, cancer-related inflammation, biomarkers

Posted Date: July 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-45688/v1

Abstract

Background

Evaluating chronic inflammation in colorectal cancer (CRC) may aid in identifying patients at the highest risk of recurrence or progression, and help inform clinical treatment decisions. Here, we report the effect of fibrinogen to pre-albumin ratio (FPR) in determining response to chemotherapy and reveal outcomes in CRC patients.

Methods

A total of 2917 eligible CRC patients from multiple-centers were enrolled, and the outcome of these patients was obtained by three years' follow-up. Circulating fibrinogen, albumin, pre-albumin, CEA, CA199 and FPR were detected and calculated in these patients. Kaplan-Meier curves, Cox regression, time-dependent ROC, Harrell's concordance index, calibration and decision curves were used to investigate the role of FPR in clinical outcome of CRC patients.

Results

Our results reveal significantly inferior outcomes in right- than left-sided patients with advanced CRC (stage III and IV), with preoperative FPR found to be a robust and independent prognostic factor for CRC at each stage. Moreover, prognostic nomograms, including FPR, effectively predicted clinical outcomes of the patients. Furthermore, preoperative FPR was significantly associated with chemotherapy efficacy. Specifically, low-grade (FPR < 15) and medium-grade (15 ≤ FPR < 20) FPR patients exhibited complete response to chemotherapy and attenuated chemosensitivity, respectively, whereas high-grade inflammation (FPR ≥ 20) conferred resistance to the treatment.

Conclusion

CRC-related inflammation affects response to chemotherapy and the resultant clinical outcomes. Circulating FPR is a simple, economically-friendly and robust independent prognostic factor for effectively predicting outcomes of CRC patients. Targeting chronic inflammation and its corresponding signaling pathway, coupled with measuring FPR, presents a novel approach for clinical management of CRC.

Introduction

Colorectal cancer (CRC) is one of the deadliest malignancies affecting both men and women worldwide [1]. Previous studies have implicated chromosome and microsatellite instability, CpG island methylator phenotypes and epigenetic alterations in CRC tumorigenesis and metastasis [2–4]. Studies have also reported distinct molecular features and varying gut microbiota in CRC patients [5–6], with analysis of clinical therapeutic responses and outcomes between the right- and left-sided disease yielding to contrasting results [7–9]. Furthermore, the role of inflammation in prognosis of CRC patients with different tumor locations, as well as possible causes of the heterogeneous outcomes are poorly understood.

Generally, chronic inflammation has emerged as a hallmark of different types of cancer, including CRC [10]. Particularly, clinically silent systemic inflammation is manifested during disease onset [11], whereas overt inflammation occurs throughout its progression [12]. Strong epidemiological evidence suggests that low-dose aspirin, and other non-steroidal anti-inflammatory drugs, are powerful chemo-preventive agents for reducing rates and cancer-related deaths [13–15]. Conversely, a dynamic alteration of circulating acute phase reaction proteins, such as fibrinogen (Fib), albumin (Alb) and pre-albumin (pAlb), has been implicated in prolonged inflammation and disease elevation. In fact, previous studies have demonstrated that ratios of Alb to Fib (AFR) and Fib to pAlb (FPR) are independent markers for predicting clinical outcomes in many kinds of solid malignancies, including non-small cell lung cancer [16], esophageal, hepatocellular and gastric cancers [17–19]. In our previous study, we demonstrated that preoperative FPR is closely associated with chemoresistance and outcomes of metastatic CRC [20]. However, the role of

quantitative FPR in impairment or resistance to chemotherapy remains unclear. In addition, the relationship between preoperative FPR and tumor laterality, as well as its dynamics and survival outcomes across stages (I-IV) of CRC progression are unknown.

The current study sought to evaluate circulating FPR across all CRC stages. Specifically, we determined survival outcomes in stage I-IV patients, based on different FPR and tumor location, involvement of circulating FPR in chemosensitivity and chemoresistance, and revealed the cut-off values for predicting efficacy of chemotherapy. Furthermore, we evaluated the use of a prognostic nomogram for effective determination of clinical outcomes in patients with stage I-IV CRC.

Materials And Methods

Eligible population

We performed this study on CRC patients enrolled across five surgery centers in Chinese University hospitals, between 2008 and 2016. The study was approved by the Ethics committees of the Second Affiliated Hospital of Nanchang University, and appropriate written informed consent obtained from each patient, or their legal surrogates, prior to enrollment. Patients who were hospitalized for at least one day, with CRC, were initially screened and enrolled in the study. Patient diagnosis and pathological staging were performed according to the criteria described in the 8th AJCC tumor classification [21]. Patients were enrolled in the study if they met the following criteria: i) they were newly diagnosed with stage I-IV CRC based on clinical characteristics, CT, MRI or histopathological detection; ii) those with stage I-III CRC received radical operation with histologically negative resection margins, and some of the metastatic patients underwent palliative surgery; iii) those who had not undergone hereditary polyposis and nonpolyposis CRC, emergency surgery, neoadjuvant chemoradiotherapy, or ulcerative colitis-associated cancer prior to clinical confirmation; iv) each patient had clinical characteristics data indicating they had not suffered from other malignancies, recent bacterial or viral infections, autoimmune, hematologic, cardiovascular or cerebrovascular diseases; v) each patient had normal liver and kidney function, had no diarrhea and were not taking drugs such as antibacterial agents, non-steroidal anti-inflammatory and anti-platelet or anticoagulant drugs, or intravenous albumin supplements in the recent three months. Right-sided CRC was defined as disease originating from ileocecum to transverse colon, whereas left-sided CRC comprised the disease occurring in the splenic flexure, descending and sigmoid colon, as well as the rectum.

Follow-up

In this study, 73.45% of stage II and 78.26% of stage III-IV patients received adjuvant chemotherapy following surgical operation. Patient follow-up was performed for 3 years. This was conducted by telephone, email and network questionnaires, every 3 months in the first two years, and every 6 months in the third year. The last follow-up was conducted in December 2019. The time from surgical operation to radiologic recurrence (local or distal recurrence of CRC) was considered as recurrence-free survival (RFS). Progression-free survival (PFS) was defined as time from the disease diagnosis to progression or censored at the deadline in stage IV patients. Overall survival (OS) was described as the time taken from operation/diagnosis to death in any cause or censored at the last follow-up.

Immunoassays

Peripheral blood, serum, and plasma samples (2 ml) were collected from all eligible patients, from 7 to 9 am, prior to the first clinical diagnosis, and used for laboratory detection. All the detections were completed within two hours of sample collection. Summarily, plasma Fib was measured by Clauss assay using SYSMEX CA-7000 machine (Sysmex, Tokyo, Japan), whereas serum Alb, pAlb, carcino-embryonic antigen (CEA) and carbohydrate antigen 199 (CA199) were measured using the Bromcresol green dye, immunoturbidimetric and chemiluminescence assays on OLYMPUS AU5400 (Beckman Coulter, Tokyo, Japan) and SIEMENS ADVIA Centaur XP (Siemens, Erlangen, Germany) machines. Inter- and intra-batch variation coefficients for Fib, Alb, pAlb, CEA and CA199 kits were less than 5%, therefore we calculated FPR in accordance with the detected results.

Statistical analyses

Continuous variables, with normal and skewed distribution, were expressed as means ± standard deviations (SD) of the mean, median and inter-quartile ranger (IQR) respectively. Comparisons differences among categorical variables were done using the Chi-square or Fisher's exact tests, whereas those across continuous variables with skewed distribution were done using the Mann-Whitney U test. Optimal FPR cut-off points across three years OS, at each disease stage were determined using the X-tile 3.6.1

software (Yale University, New Haven, CT, USA) as described in our previous studies[20, 28]. Differences in survival rates were calculated using Kaplan-Meier curves, with the log-rank test, whereas the prognostic role of clinical baseline characteristics and indicators detected in the laboratory were analyzed using the Cox proportional regression with hazard ratio (HR) at a 95% confidence interval (CI). Comparisons among baseline and pathological characteristics, treatment, and other confounder factors were done using multivariate analysis, by backward stepwise Cox regression modeling. Furthermore, FPR's prognostic predictive efficacy was assessed then compared using time-dependent receiver operating characteristic (ROC) curves, with significant characteristics and laboratory markers used to establish nomograms for predicting outcomes. In addition, predicted efficacies of Harrell's concordance index (c-index), time-dependent ROC, calibration and decision curves were analyzed using rms, survival, survivalROC, tdROC and rmda packages implemented in R software. All statistical analyses were performed in SPSS. version 22.0 (IBM Corp, Armonk, NY, USA), R version 3.6.3 (Institute for Statistics and Mathematics, Vienna, Austria) and GraphPad Prism version 8.2.1 (GraphPad Software Inc.,San Diego,USA). All analyses were two-sided, with values followed by $p < 0.05$ considered statistically significant.

Results

A summary of study overview and an outline of eligibility criteria is described in Fig. 1. Summarily, the inclusion and exclusion criteria allowed recruitment of 268, 998, 926, and 725 CRC patients with stage I, II, III and IV, respectively. A summary of their baseline characteristics, pathological and survival data are outlined in Table 1. All patients with stage I-III CRC received radical surgical operation, whereas 313 cases with stage IV underwent palliative resection. In addition, 733 and 799 patients with stages II, and III, respectively, underwent adjuvant chemotherapy following surgical resection, whereas 493 stage IV cases received the treatment after clinical diagnosis. The median follow-up time was 36 and 13 months for stages I-III and IV patients, respectively.

Table 1
The baseline characteristics of 2917 stage I- IV colorectal cancer patients in the study.

Variants	Stage I cases (n = 268)	Stage II cases (n = 998)	Stage III cases (n = 926)	Stage IV cases (n = 725)
Age, years	61(51–68)	62(51–71)	61(52–69)	60(49–69)
Sex				
Female	111(41.42%)	382(38.28%)	355(38.34%)	332(45.79%)
Male	157(58.58%)	616(61.72%)	571(61.66%)	392(54.21%)
Smoking				
Yes	35(13.06%)	166(16.63%)	113(12.20%)	125(17.24%)
NO	233(86.94%)	832(83.37%)	813(87.80%)	600(82.76%)
Drinking				
Yes	29(10.82%)	122(12.22%)	89(9.61%)	104(14.34%)
NO	239(89.18%)	876(87.78%)	837(90.39%)	621(85.66%)
Diabetes				
Yes	24(8.96%)	63(6.31%)	68(7.34%)	53(7.31%)
NO	244(91.04%)	935(93.69%)	858(92.66%)	672(92.69%)
Hypertension				
Yes	41(15.30%)	147(14.73%)	122(13.17%)	97(13.38%)
NO	227(84.70%)	851(85.27%)	804(86.83%)	628(86.62%)
pT stage				
T1	58(21.64%)	0	7(0.76%)	1(0.14%)
T2	210(78.36%)	0	77(8.31%)	10(1.38%)
T3	0	396(39.67%)	269(29.05%)	17(2.34%)
T4	0	602(60.32%)	573(61.88%)	283(39.04%)
NA	0	0	0	414(57.10%)
pN stage				
N0	268(100%)	998(100%)	0	99(13.66%)
N1	0	0	837(90.39%)	100(13.80%)
N2	0	0	89(9.61%)	104(14.34%)
NA	0	0	0	414(57.10%)
Distal Metastasis				
No	268(100%)	998(100%)	926(100%)	0
Yes	0	0	0	725(100%)
Histological grade				

Abbreviation: CRC: colorectal cancer; N1*: positive of node metastasis; NA: not available. Data are median (IQR) or n (%), Operation*: Radical resection for stage I-III CRC patients, palliative operation for stage IV cases.

Variants	Stage I cases (n = 268)	Stage II cases (n = 998)	Stage III cases (n = 926)	Stage IV cases (n = 725)
Good	32(11.94%)	43(43.10%)	62(6.70%)	9(1.24%)
Median	223(83.21%)	896(89.77%)	747(80.67%)	275(37.93%)
Poor	13(4.85%)	59(59.12%)	117(12.63%)	27(3.73%)
NA	0	0	0	414(57.10%)
Location				
Proximal colon	30(11.19%)	288(28.86%)	205(22.14%)	192(26.48%)
Distal colon	47(17.54%)	273(27.35%)	216(23.33%)	210(28.97%)
Rectum	191(71.27%)	437(43.79%)	505(54.53%)	323(44.55%)
Treatment				
Operation*	268(100%)	998(100%)	926(100%)	313(43.17%)
Chemotherapy	0	733(73.45%)	799(86.29%)	493(68.00%)
Radiology	0	53(5.31%)	83(8.96%)	53(7.31%)
Chemotherapy and radiology	0	50(5.01%)	78(8.42%)	15(2.07%)
Targeted therapy	0	0	0	47(6.48%)
Follow-up time, months	36	36	36(25–36)	13(7–26)
FPR	12.57(9.79–16.28)	15.50(11.45–21.18)	14.79(10.82–20.80)	23.34(15.11–34.75)
CEA (ng/mL)	1.74(1.15–3.20)	2.87(1.67–5.84)	3.45(1.72–9.22)	13.14(3.71–87.76)
CA199 (U/mL)	11.45(7.19–17.43)	13.71(7.30–24.77)	17.40(8.61–33.77)	43.49(1.52–375.18)

Abbreviation: CRC: colorectal cancer; N1*: positive of node metastasis; NA: not available. Data are median (IQR) or n (%), Operation*: Radical resection for stage I-III CRC patients, palliative operation for stage IV cases.

Based on tumor laterality, significantly higher recurrence rates were found in patients with right-sided than their left-sided counterparts. Specifically, right-sided patients with stage III CRC exhibited a 49.80% recurrence, compared to 40.50% ($p = 0.019$) in their left-sided counterparts, whereas recurrence rates of 82.70 and 73.70% ($p = 0.035$) were recorded in right- and left-sided stage IV patients, respectively (Fig. 2A). Similarly, right-sided patients exhibited significantly higher mortalities than left-sided counterparts, across stages III (30.70% *vs.* 23.40%; $p = 0.034$) and IV (78.10 *vs.* 70.90%; $p = 0.050$) (Fig. 2B). Comparison between left- vs. right-sided patients revealed similar overall survival rates at stage I ($p_{log-rank}$=0.615 for RFS; $p_{log-rank}$=0.582 for OS) and II ($p_{log-rank}$=0.224 for RFS; $p_{log-rank}$=0.371 for OS) (Table 2). However, Kaplan-Meier curves revealed significantly inferior survival rates in right-sided patients with stage III ($p_{log-rank}$=0.006 for RFS; $p_{log-rank}$=0.014 for OS) and IV ($p_{log-rank}$=0.033 for RFS; $p_{log-rank}$=0.011 for OS) than their left-sided counterparts (**Supplementary Fig. 1**). Adjusting for baseline and pathological characteristics, as well as FPR, it showed that right-sided location was still associated with RFS ($p = 0.027$, adjusted HR = 1.339, 95%CI = 1.033–1.735), albeit only in the stage III disease (Table 2).

Table 2 Cox analysis of tumor laterality and FPR in 2917 stage I- IV colorectal cancer patients.

Variant	Comparison	Outcome	Cox regression					
			Univariate		Multivariate			
					[1]		[2]	
			p-value	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value	HR (95%CI)
Stage I	Right vs. Left	RFS	0.622	1.347(0.413-4.399)	0.341	1.810(0.534-0.6138)	0.475	1.558(0.462-5.247)
		OS	0.582	1.532(0.336-6.992)	0.550	1.678(0.308-9.142)	0.532	1.735(0.308-9.771)
	H-FPR vs. L-FPR	RFS	<0.001	6.245(2.287-17.051)	0.003	4.705(1.690-13.101)	0.003	4.705(1.690-13.101)
		OS	0.019	6.535(1.357-31.459)	0.018	6.657(1.381-32.081)	0.018	6.657(1.381-32.081)
Stage II	Right vs. Left	RFS	0.238	1.182(0.896-1.560)	0.619	1.075(0.809-1.428)	0.911	1.016(0.765-1.351)
		OS	0.377	1.179(0.818-1.701)	0.401	1.172(0.809-1.697)	0.719	1.072(0.735-1.561)
	H-FPR vs. L-FPR	RFS	<0.001	3.144(2.267-4.361)	<0.001	2.742(1.965-3.826)	<0.001	2.742(1.965-3.826)
		OS	<0.001	4.756(2.985-7.579)	<0.001	4.173(2.601-6.693)	<0.001	4.173(2.601-6.693)
Stage III	Right vs. Left	RFS	0.006	1.370(1.093-1.716)	0.010	1.352(1.074-1.701)	0.027	1.339(1.033-1.735)
		OS	0.015	1.430(1.071-1.910)	0.039	1.366(1.016-1.836)	0.713	1.066(0.758-1.499)
	H-FPR vs. L-FPR	RFS	<0.001	2.439(1.947-3.056)	<0.001	2.162(1.717-2.723)	<0.001	2.023(1.592-2.569)
		OS	<0.001	2.761(2.079-3.666)	<0.001	2.216(1.648-2.979)	<0.001	2.216(1.648-2.979)
Stage IV	Right vs. Left	PFS	0.043	1.260(1.007-1.577)	0.031	1.286(1.023-1.617)	0.278	1.153(0.892-1.490)
		OS	0.013	1.270(1.051-1.535)	0.003	1.339(1.105-1.624)	0.327	1.117(0.895-1.394)
	H-FPR vs. L-FPR	PFS	<0.001	2.651(2.100-3.347)	<0.001	2.527(1.994-3.202)	<0.001	2.527(1.994-3.202)
		OS	<0.001	2.418(1.987-2.943)	<0.001	2.254(1.842-2.758)	<0.001	2.254(1.842-2.758)

Abbreviation: FPR: fibrinogen to prealbumin ratio; RFS: recurrence-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; [1]: was adjusted by gender, age, tobacco, alcohol, diabetes, hypertension, chemotherapy, radiotherapy, T, N, differentiation, cancer size, CEA, CA199 for tumor laterality or FPR; [2]: was adjusted by gender, age, tobacco, alcohol, diabetes, hypertension, chemotherapy, radiotherapy, T, N, differentiation, cancer size, CEA, CA199 and tumor laterality for H-FPR vs. L-FPR; was adjusted by gender, age, tobacco, alcohol, diabetes, hypertension, chemotherapy, radiotherapy, T, N, differentiation, cancer size, CEA, CA199 and FPR for right- vs. left-tumor location.

Our results also revealed significantly higher circulating FPR in left-sided than right-sided patients with stage II ($p < 0.001$), III ($p < 0.001$) and IV ($p < 0.001$) CRC (Fig. 2C). In addition, no differential circulating FPR was observed in patients with stages I-III, based on comparisons between T1 *vs.* T2 ($p = 0.290$), T3 *vs.* T4 ($p = 0.741$), and negative-metastasis of lymph nodes (LN-) *vs.* positive-metastasis of lymph nodes (LN+) ($p = 0.856$) (Fig. 2D-E). However, significantly high FPR was observed in T3 subgroup comparing

to T2 ($p < 0.001$) patients, and the similar significant result was observed between well ~ median and poor differentiated subgroups ($p = 0.01$) (Fig. 2D **and G**). Comparisons revealed significantly higher preoperative FPR in patients with stage IV relative to those with stages I-III or IIIB (T3/4N1M0) ($p < 0.001$) (Fig. 2F **and H**). A similar trend was also observed with regards to circulating FPR between patients with stage I and IIIA (T1/2N1M0) ($p = 0.104$) or those with stage II and IIIB (T3/4N1M0) ($p = 0.513$) (Fig. 2H). Furthermore, patients with stage II CRC exhibited significantly higher preoperative FPR than those with IIIA(T1/2N1M0) ($p < 0.001$) **(**Fig. 2H**)**. Moreover, significantly higher preoperative FPR was observed in patients with stage I-III, with a median of 5□10 cm ($p < 0.001$) and large cancer bulk (≥ 10 cm) ($p < 0.001$) relative to those with small (< 5 cm) bulk **(**Fig. 2I**)**. Additionally, median FPR gradually increased from the smallest (< 1 cm) to the largest cancer bulk (> 9 cm) (p-trend < 0.001) **(**Fig. 2J**)**.

A comprehensive analysis using the X-tile software, based on our previous studies, revealed optimal FPR cut-off values for stage I, II, III, and IV patients to be 14.0, 16.5, 19.5 and 22.8, respectively. Consequently, we divided the patients into high- and low-FPR groups across each stage. Results indicated significantly higher high-FPR proportions in right-sided than left-sided patients with stage III (50.90 vs. 40.70%; $p < 0.001$) and IV (66.00 vs. 47.20%; $p < 0.001$) (Fig. 2K). In addition, Kaplain-Meier curves and univariable Cox regression analyses showed that patients with high-FPR were significantly associated with poor survival rates than those with low-FPR at stage I ($p_{log-rank}$<0.001 and 0.048 for RFS and OS, respectively), II (all *plog-rank* < 0.001 for both RFS and OS), III (all *plog-rank* < 0.001 for both RFS and OS), and IV (all *plog-rank* < 0.001 for both RFS and OS) disease (Table 2: **Supplementary Fig. 2**). Adjusting for all baseline and pathological characteristics as well as tumor laterality, it indicated that low-FPR was still significantly associated with long clinical outcomes for patients with stage I (adjusted HR = 6.657, 95%CI = 1.381–32.081 for OS), II (adjusted HR = 4.173, 95%CI = 2.601–6.693 for OS), III (adjusted HR = 2.216, 95%CI = 1.648–2.979 for OS) and IV (adjusted HR = 2.254, 95%CI = 1.842–2.758 for OS) disease (Table 2). Time-dependent area under curves (AUCs) for FPR were 0.724 and 0.720 in stage I patients, 0.657 and 0.694 in stage II patients, 0.623 and 0.634 in stage III patients to predict RFS and OS, respectively, and were 0.714 and 0.723 for stage IV to predict three years' PFS and OS (Fig. 2L-N). FPR resulted in a better prediction efficacy relative to CEA, CA199 and tumor sidedness, since it resulted in the highest AUC for predicting survival rates across each substage (**Supplementary Fig. 2–3**).

To further understand efficacy of adjuvant chemotherapy on management of chronic inflammation, we analyzed recurrence rates and survival differences in stage III and IV patients under varying FPR concentrations. Summarily, 3-year follow-up revealed recurrence and progression rates of 66.15 and 93.33%, respectively, for the two stage non-chemotherapy treated patients regardless of FPR levels (Fig. 3A **and D**). On the other hand, recurrence and progression rates in the two stage patients, with low-grade FPR (< 15), were nearly 47.50 and 83.50%, respectively, although these gradually increased with medium-grade FPR (15 ≤ FPR < 20). However, higher stable recurrence rates, of 66 and 93% for stage III and IV cases, respectively, were observed when circulating FPR was larger than 20 (Fig. 3A **and D**). In addition, significant recurrence and progression rates as well as survival differences were also observed among patients with FPR ≥ 15 and ≥ 20 (Fig. 3A, C-D **and F**). A comparison with stage III chemotherapy untreated patients revealed significantly lower HR predicting recurrence in chemotherapy treated patients with FPR ≤ 15, was although this value gradually increased to nearly 1.0 for the treated cases with FPR ranging from 15 to 19. Conversely, HR remained stable, at 1.0, in patients with FPR ≥ 20. A similar HR trend was observed in patients with stage IV CRC, who exhibited low- (FPR < 15), medium- (15 ≤ FPR < 20) and high-FPR (FPR ≥ 20), relative to those without any treatment (Fig. 3B **and D**).

Furthermore, FPR and clinical risk factors were used to successfully establish relevant quantitative prognostic nomograms to predict survival rates in patients with stage I-III and stage IV CRC (Fig. 4, **Supplementary Fig. 4).** Summarily, C-indexes of 0.760 and 0.780 for stage I-III nomograms (FPR) to predict three years RFS and OS, respectively, and 0.671 and 0.718 for stage IV nomogram (FPR) to predict PFS and OS (Table 3). These were significantly higher than non-FPR nomograms, FPR and TNM. Moreover, time-dependent AUCs revealed that nomograms (FPR) (AUCs = 0.791 and 0.795 for 3 years RFS and OS in stage I-III population, respectively; AUCs = 0.777 and 0.821 for 3 years PFS and OS in stage IV population, respectively) were significantly higher than the non-FPR included ones, FPR and TNM stage for predicting one-, two- and three-year survival rates **(**Table 3, Fig. 4C **and F: Supplementary Fig. 4C and F)**. Calibration plots showed that the nomograms performed well compared with the performance of an ideal model (Fig. 4D **and G: Supplementary Fig. 4D and G)**. Additionally, results from decision curve analyses showed that nomogram (FPR) were better than non-FPR nomograms and FPR in predicting survival outcomes of stage I-III and IV CRC patients, respectively (Fig. 4E **and H: Supplementary Fig. 4E and H**).

Table 3
Comparison of predicted efficacy between prognostic nomogram and other prognostic biomarkers in stage I-III and IV CRC patients.

Stage	Outcome	Variants	12-month survival	24-month survival	36-month survival	
			AUROC(95%CI)	AUROC(95%CI)	AUROC(95%CI)	C-index(95%CI)
I-III	RFS	Nomogram (FPR)	0.790(0.761–0.823)	0.784(0.755–0.830)	0.791(0.756–0.839)	0.760(0.735–0.785)
		Nomogram (Non-FPR)	0.762(0.732–0.804)*	0.753(0.714–0.806)*	0.760(0.722–0.805)*	0.726(0.693–0.747)*
		FPR	0.664(0.599–0.709)**	0.646(0.601–0.685)**	0.653(0.622–0.689)**	0.634(0.601–0.667)**
		TNM	0.689(0.644–7.35)**	0.695(0.633–0.746)**	0.701(0.660–0.742)**	0.673(0.636–0.700)**
	OS	Nomogram(FPR)	0.772(0.722–0.813)	0.803(0.755–0.842)	0.795(0.751–0.849)	0.780(0.750–0.810)
		Nomogram (Non-FPR)	0.717(0.648–0.753)**	0.745(0.698–0.801)*	0.744(0.699–0.796)*	0.730(0.690–0.770)*
		FPR	0.713(0.655–0.752)**	0.714(0.644–0.766)**	0.714(0.668–0.742)**	0.694(0.652–0.736)**
		TNM	0.652(0.604–0.698)**	0.676(0.639–0.736)**	0.672(0.624–0.716)**	0.660(0.624–0.696)**
IV	PFS	Nomogram(FPR)	0.737(0.688–0.779)	0.773(0.724–0.822)	0.777(0.739–0.822)	0.671(0.641–0.701)
		Nomogram(Non-FPR)	0.644(0.594–0.696)**	0.670(0.644–0.724)**	0.693(0.652–0.752)**	0.615(0.581–0.649)**
		FPR	0.695(0.658–0.762)*	0.720(0.639–0.762)*	0.714(0.659–0.748)*	0.632(0.605–0.659)*
	OS	Nomogram(FPR)	0.779(0.739–0.812)	0.794(0.733–0.845)	0.821(0.766–0.853)	0.718(0.6935–0.743)
		Nomogram(Non-FPR)	0.694(0.638–0.751)**	0.719(0.647–0.751)**	0.741(0.701–0.749)*	0.678(0.650–0.705)*
		FPR	0.674(0.630–0.712)**	0.690(0.633–0.740)**	0.723(0.677–0.765)**	0.629(0.605–0.654)**

Abbreviation: FPR: fibrinogen to pre-Albumin ratio; AUC: area under curve; CI: confidence interval; AUROC: area under curve of receiver operating characteristic; *: $p < 0.05$ for Z-test; **: $p < 0.01$ for Z-test.

Discussion

CRC is one of the deadliest malignancies worldwide, mainly due to chemotherapy failure that results in recurrence and metastasis in patients [2]. To date, no simple, economically-friendly and practical CRC biomarker has been identified. Therefore, prospecting for new powerful biomarkers is necessary to guide development of chemotherapeutical approaches, alleviate the risk of recurrence and progression, as well enhance prognosis of the disease. In the present study, we found that preoperative FPR not only indicated disease burden, but also predicted complete response to chemotherapy, impaired chemosensitivity and chemoresistance. Specifically, high FPR was associated with poor survival of CRC patients, with circulating differential FPR accounted for survival differences between right- and left-sided patients with stage III and IV disease. Moreover, FPR was an independent prognostic factor for each stage of the disease, with nomogram containing FPR effectively predicting survival of CRC patients.

Previous studies have demonstrated the key roles played by Fib and pAlb in acute and chronic phases of malignancies such as CRC [22]. Particularly, Fib is a driver of chronic low-grade inflammation, owing to its effect on platelets, eukocyte migration and role in promoting carcinogenic properties. In addition, it has been shown to function as a scaffold for cancer growth, migration and

metastasis [23–25]. On the other hand, Alb and pAlb represent the main sources of energy and nutrition for tumor growth. Previous studies have demonstrated that inflammatory cytokines produced from CRC microenvironment and kupfer cells, including interleukin-6, effectively suppress Alb and preAlb synthesis by hepatocytes [26]. Consequently, CRC patients, especially those at advanced stages, have been found to commonly manifest malnutrition or hypoalbuminemia [20, 27]. In the present study, we found significantly higher circulating FPR in large cancer bulk and distal metastatic subgroups than those with small cancer bulk and non-distal metastatic patients, respectively, although this was gradually elevated according to increased cancer bulk. These findings indicate that circulating FPR is determined by the cancer, and can be attributed to an uncontrolled inflammatory response. Consequently, this factor can be used to evaluate cancer burden.

In our previous studies, we found an association between circulating high FPR and poor prognosis in stage II-III and IV CRC patients [20, 28]. In the current study, univariate Cox regression revealed a significant association between high FPR and poor disease outcomes across each stage. Adjusting for common confounders, CEA, CA199 and tumor location, it revealed that high FPR was still robustly associated with poor prognosis of patients across each disease stage. Although we found significant differences in survival outcome between left- and right-sided patients with stage III and IV disease, there was no association between them following adjustment for other confounders, including FPR. These findings indicated that preoperative FPR, and not tumor laterality, was an independent prognostic factor for CRC patients at each stage of disease progression. Moreover, we found significantly higher preoperative FPR in right- than left-sided patients with stages III and IV CRC, indicating presence of high-grade inflammation in right-sided cases. A significantly higher high-FPR distribution in stage III and IV of right-sided relative to left-sided patients, might have contributed to the observed differences in survival outcome. Additionally, time-dependent FPR AUCs were superior to CEA, CA199 and tumor location in predicting the prognosis at each stage of disease progression, whereas c-indexes and AUCs from prognostic nomograms (FPR) found to be significantly higher than those from single factor or non-FPR nomograms. This indicates that preoperative FPR was an effective biomarker for predicting disease outcomes, therefore it can be used to improve prediction efficacies of prognostic nomogram in patients with all stage of CRC.

Previous studies have shown that an interaction among cancerous or stem cells, different immune and inflammatory cells as well as various mediators, such as cytokines and gut microbiota, sharpens the inflammatory microenvironment and promotes initiation and progression of CRC [29–30]. For example, cancer-associated stromal and inflammatory cells, such as fibroblast, macrophages and neutrophils, as well as colon cancer cells, were found to activate mitogen-activated protein kinase (MAPK), NF-kB, PI3K signaling pathway thereby promoting resistance to various conventional chemotherapy agents [31–33]. Moreover, gut microbiota, such as Fusobacterium nucleatum, have been implicated in regulation of a molecular network of the Toll-like receptor, microRNAs, and autophagy, thereby promoting chemoresistance [34]. In the current study, chemotherapy-treated patients with preoperative FPR < 15 exhibited the best outcomes, whereas those with FPR ≥ 20 had the worst, in both stages III and IV of CRC. Moreover, clinical outcomes of chemotherapy-treated stage III and IV patients with FPR ≥ 15 were superior to those without chemotherapy as well as those who underwent chemotherapy with FPR ≥ 20. However, there were no significant differences between survival rates of chemo-treated stage III and IV patients with severe chronic inflammation (FPR ≥ 20) and those who did not undergo chemotherapy. These results indicated that FPR might be an indicator for effective chemotherapy in CRC patients. Particularly, FPR < 15 might indicate complete response to chemotherapy, $15 \leq$ FPR < 20 could imply impaired sensitivity to adjuvant chemotherapy, whereas FPR ≥ 20 might denote chemoresistance. Overall, these findings show that clinical outcomes of right-sided stage III and IV patients are worse than those of left-sided counterparts, and high FPR effectively predicts poor survival rates in CRC patients.

This is the first study reporting a comprehensive analysis of prognostic and predictive significance of preoperative FPR in chemotherapy across stages I-IV in CRC patients, with different tumor locations. Based on results from a large sample size, used herein, it is evident that high-grade chronic inflammation attenuated chemosensitivity or triggered chemoresistance, and conferred poor outcomes within patients with the stage III and IV disease. Preoperative FPR was a robust predictor and prognostic factor for CRC patients, following chemotherapy. However, other prospective studies are needed to validate our findings. Specifically, functional and mechanistic analyses should be carried out to elucidate the association between FPR, inflammation and chemoresistance.

Conclusion

CRC-related inflammation affects response to chemotherapy and the resultant clinical outcomes. Circulating FPR represents a simple, economically-friendly and robust independent prognostic factor for evaluating efficacy of chemotherapy at each stage of CRC progression. In addition, prognostic FPR-contained nomogram is superior to the non-FPR counterpart and FPR in predicting outcomes of CRC patients. Overall, we recommend FPR and FPR-contained nomogram during evaluation of chemotherapy efficacy, and decision-making for management of CRC patients.

Abbreviations

CRC
colorectal cancer; Fib:fibrinogen; Alb:albumin; pre-albumin:pAlb; FPR:ratio of fibrinogen to pre-albumin; AFR:ratio of albumin to fibrinogen; RFS:recurrence-free survival; PFS:progression-free survival; OS:overall survival; CEA:carcino-embryonic antigen; CA199:carbohydrate antigen 199; SD:standard deviations; IQR:inter-quartile ranger; HR:hazard ratio; CI:confidence interval; ROC:receiver operating characteristic curver; c-index:Harrell's concordance index.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics committees of the Second Affiliated Hospital of Nanchang University, and appropriate written informed consent obtained from each patient, or their legal surrogates, prior to enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

All the authors agreed to publish the study and declared no conflicts of interest.

Funding

This study was supported by the National Natural Science Foundation of China (grant number: 81702090), the Natural Science Youth Foundation of Jiangxi Province (grant number: 20171BAB215054), the Key Technology Research and Development Program of Jiangxi Province (grant number: 20171BBG70049) and 2020 Scientific Fund of Health and Family Planning Commission of Jiangxi Province (20204251).

Authors' contributions

Hou-Qun Ying, Fan, Sun and Wei Wang (co-first authors): conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing. Dan Cai and Ying Yang: statistical analysis of data. Nuo-Wei Nie and Ying Huang: collection and assembly of data, interpretation of results. Xue-Xin Cheng: conception and design, collection and assembly of data, data analysis and interpretation, manuscript revision and financial support.

Acknowledgements

The authors are grateful to freescience for providing language-editing services in present study.

Authors' information (optional)

[1]Department of Nuclear Medicine, Jiangxi Province Key Laboratory of Laboratory Medicine, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China. [2]Department of Laboratory Medicine, Jiangxi Province Key Laboratory of Laboratory Medicine, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China. [3]Jiangxi Medical College, Nanchang University, Nanchang 330006, Jiangxi, China. [4]Department of Science and Technology, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467–80.
3. Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nat Rev Gastroenterol Hepatol. 2020;17(2):111–30.
4. Kim SY, Kim TW. Current challenges in the implementation of precision oncology for the management of metastatic colorectal cancer. ESMO Open. 2020;5(2):e000634.
5. Merlano MC, Granetto C, Fea E, Ricci V, Garrone O. Heterogeneity of colon cancer: from bench to bedside. ESMO Open. 2017;2(3):e000218.
6. Zheng C, Jiang F, Lin H, Li S. Clinical characteristics and prognosis of different primary tumor location in colorectal cancer: a population-based cohort study. Clin Transl Oncol. 2019;21(11):1524–31.
7. You XH, Jiang YH, Fang Z, Sun F, Li Y, Wang W, et al. Chemotherapy plus bevacizumab as an optimal first-line therapeutic treatment for patients with right-sided metastatic colon cancer: a meta-analysis of first-line clinical trials. ESMO Open. 2020;5(2):e000605.
8. You XH, Wen C, Xia ZJ, Sun F, Li Y, Wang W, et al. Primary Tumor Sidedness Predicts Bevacizumab Benefit in Metastatic Colorectal Cancer Patients. Front Oncol. 2019;9:723.
9. Wang CB, Shahjehan F, Merchea A, Li Z, Bekaii-Saab TS, Grothey A, et al. Impact of Tumor Location and Variables Associated With Overall Survival in Patients With Colorectal Cancer: A Mayo Clinic Colon and Rectal Cancer Registry Study. Front Oncol. 2019;9:76.
10. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.
11. Tuomisto AE, Makinen MJ, Vayrynen JP. Systemic inflammation in colorectal cancer: Underlying factors, effects, and prognostic significance. World J Gastroenterol. 2019;25(31):4383–404.
12. Wang K, Karin M. Tumor-Elicited Inflammation and Colorectal Cancer. Adv Cancer Res. 2015;128:173–96.
13. Friis S, Riis AH, Erichsen R, Baron JA, Sørensen HT. Low-Dose Aspirin or Nonsteroidal Anti-inflammatory Drug Use and Colorectal Cancer Risk: A Population-Based, Case-Control Study. Ann Intern Med. 2015;163(5):347–55.
14. Kuo CN, Pan JJ, Huang YW, Tsai HJ, Chang WC. Association between Nonsteroidal Anti-Inflammatory Drugs and Colorectal Cancer: A Population-Based Case-Control Study. Cancer Epidemiol Biomarkers Prev. 2018;27(7):737–45.
15. Hua X, Phipps AI, Burnett-Hartman AN, Adams SV, Hardikar S, Cohen SA, et al. Timing of Aspirin and Other Nonsteroidal Anti-Inflammatory Drug Use Among Patients With Colorectal Cancer in Relation to Tumor Markers and Survival. J Clin Oncol. 2017;35(24):2806–13.
16. Li SQ, Jiang YH, Lin J, Zhang J, Sun F, Gao QF, et al. Albumin-to-fibrinogen ratio as a promising biomarker to predict clinical outcome of non-small cell lung cancer individuals. Cancer Med. 2018;7(4):1221–31.
17. Gao QF, Qiu JC, Huang XH, Xu YM, Li SQ, Sun F, et al. The predictive and prognostic role of a novel ADS score in esophageal squamous cell carcinoma patients undergoing esophagectomy. Cancer Cell Int. 2018;18:153.
18. Zhang L, Chen QG, Li SQ, Zhang J, Min QH, Gao QF, et al. Preoperative fibrinogen to prealbumin ratio as a novel predictor for clinical outcome of hepatocellular carcinoma. Future Oncol. 2019;15(1):13–22.
19. Zhang J, Li SQ, Liao ZH, Jiang YH, Chen QG, Huang B, et al. Prognostic value of a novel FPR biomarker in patients with surgical stage II and III gastric cancer. Oncotarget. 2017;8(43):75195–205.
20. Chen QG, Zhang L, Sun F, Li SQ, You XH, Jiang YH, et al. Elevated FPR confers to radiochemoresistance and predicts clinical efficacy and outcome of metastatic colorectal cancer patients. Aging. 2019;11(6):1716–32.
21. Weiser MR. AJCC 8th Edition: Colorectal Cancer. Ann Surg Oncol. 2018;25(6):1454–5.
22. Li SQ, You XH, Sun F, Xia ZJ, Fang Z, Wang W, et al. Albumin to fibrinogen ratio and fibrinogen to pre-albumin ratio are economical, simple and promising prognostic factors for solid malignancy. J Thorac Dis. 2019;11(Suppl 15):2036–8.

23. Jennewein C, Tran N, Paulus P, Ellinghaus P, Eble JA, Zacharowski K. Novel aspects of fibrin(ogen) fragments during inflammation. Mol Med. 2011;17(5–6):568–73.
24. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. Blood. 2019;133(6):511–20.
25. Kwaan HC, Lindholm PF. Fibrin and Fibrinolysis in Cancer. Semin Thromb Hemost. 2019;45(4):413–22.
26. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Mol Aspects Med. 2012;33(3):209–90.
27. Haskins IN, Baginsky M, Amdur RL, Agarwal S. Preoperative hypoalbuminemia is associated with worse outcomes in colon cancer patients. Clin Nutr. 2017;36(5):1333–8.
28. Sun F, Peng HX, Gao QF, Li SQ, Zhang J, Chen QG, et al. Preoperative circulating FPR and CCF score are promising biomarkers for predicting clinical outcome of stage II-III colorectal cancer patients. Cancer Manag Res. 2018;10:2151–61.
29. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. Nat Rev Clin Oncol. 2015;12(10):584–96.
30. Brennan CA, Garrett WS. Gut Microbiota, Inflammation, and Colorectal Cancer. Annu Rev Microbiol. 2016;70:395–411.
31. Zhang J, Roberts TM, Shivdasani RA. Targeting PI3K signaling as a therapeutic approach for colorectal cancer. Gastroenterology. 2011;141(1):50–61.
32. Grossi V, Peserico A, Tezil T, Simone C. p38alpha MAPK pathway: a key factor in colorectal cancer therapy and chemoresistance. World J Gastroenterol. 2014;20(29):9744–58.
33. Isidro RA, Appleyard CB. Colonic macrophage polarization in homeostasis, inflammation, and cancer. Am J Physiol Gastrointest Liver Physiol. 2016;311(1):G59–73.
34. Yu T, Guo F, Yu Y, Sun T, Ma D, Han J, et al. Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. Cell. 2017;170(3):548–63.

Figures

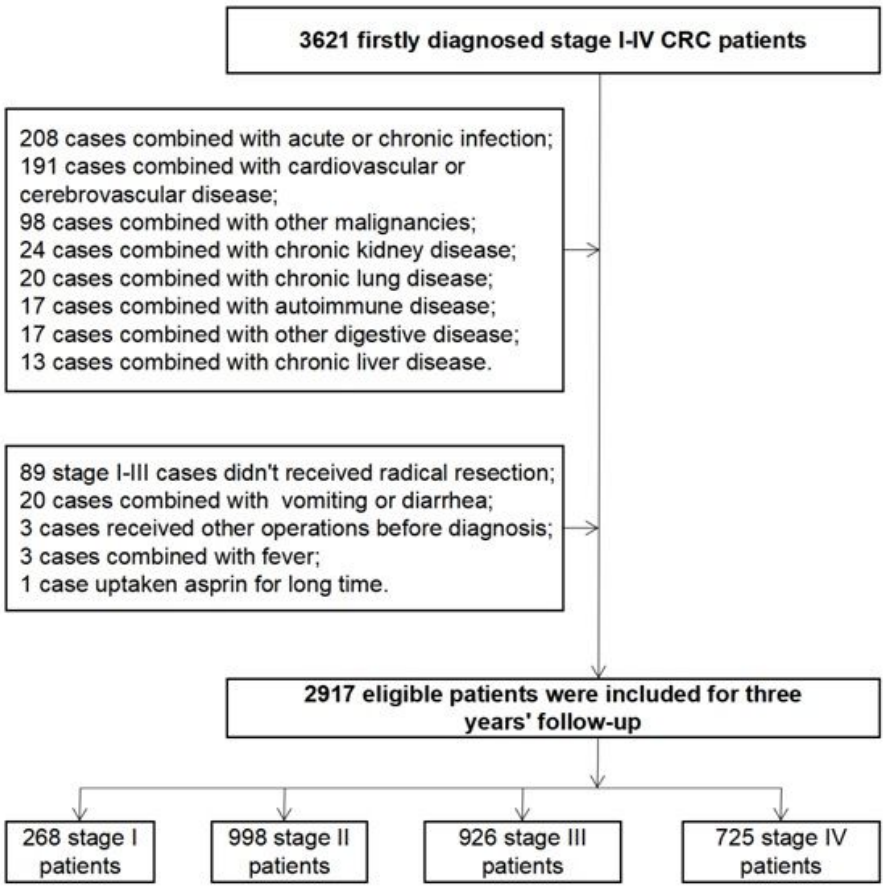

Figure 1

Selection flowchart of 2917 eligible colorectal cancer patients.

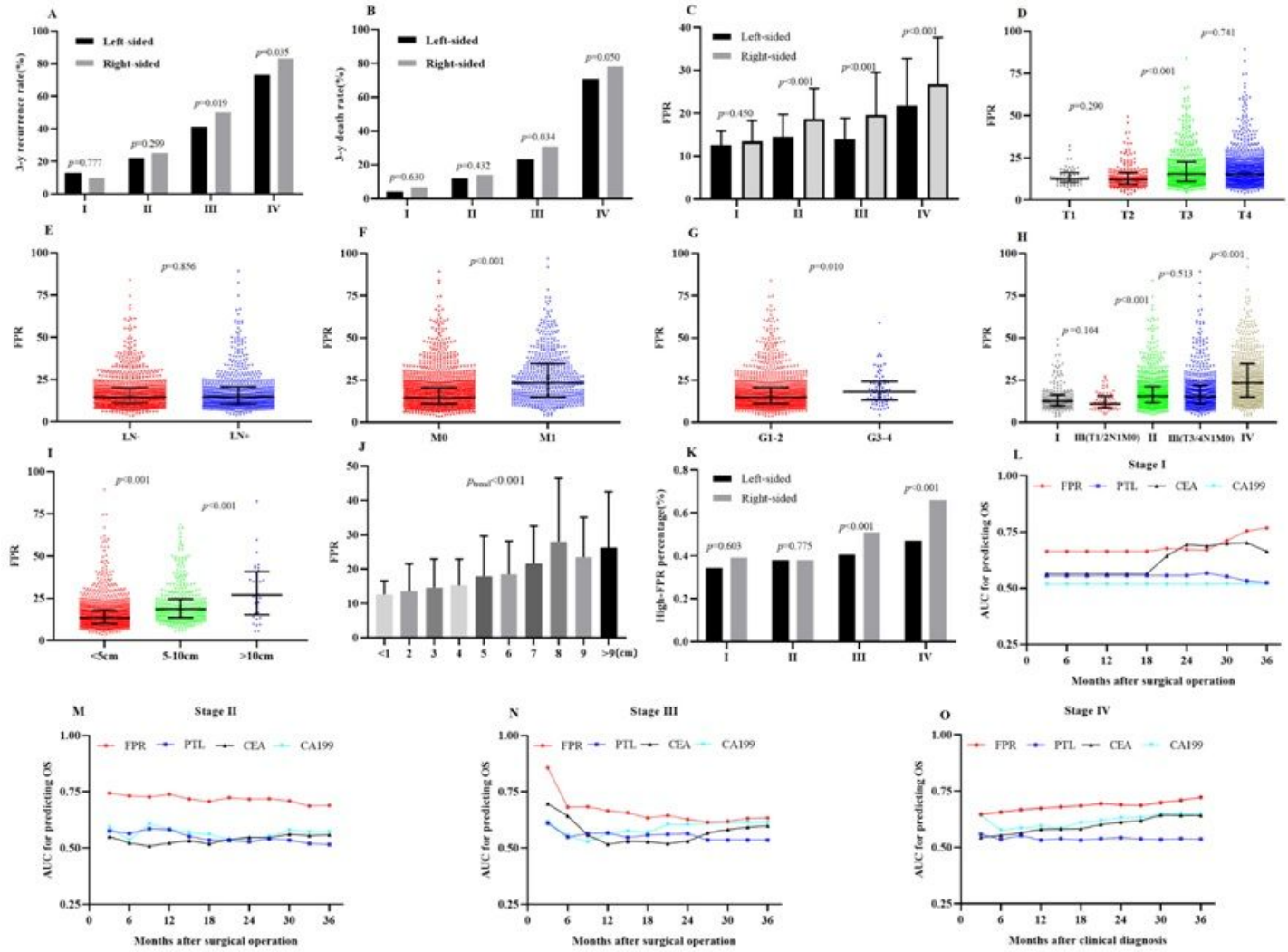

Figure 2

Primary tumor location, circulating FPR and survival in colorectal cancer patients. A: 3-years' recurrence rate in the right- and left-sided patients; B: 3-years' death rate in the right- and left-sided patients; C: circulating FPR in the right- and left-sided patients; D: circulating FPR in the T1~4 patients; E: circulating FPR in the patients with different lymph node status; F: circulating FPR in the patients with different distal metastatic status; G: circulating FPR in the patients with different cell differentiation; H: circulating FPR in different TNM stage; I-J: circulating FPR in the patients with different cancer bulk; K: high-FPR distribution in left- and right-sided patients with different TNM stage; L: time-dependentarea under curve (tdAUC) for predicting 3-years' overall survival(OS) in stage I patients; M:tdAUC for predicting 3-years' OS in stage II patients; N:tdAUC for predicting 3-years' OS in stage III patients; O:tdAUC for predicting 3-years' OS in stage IV patients.

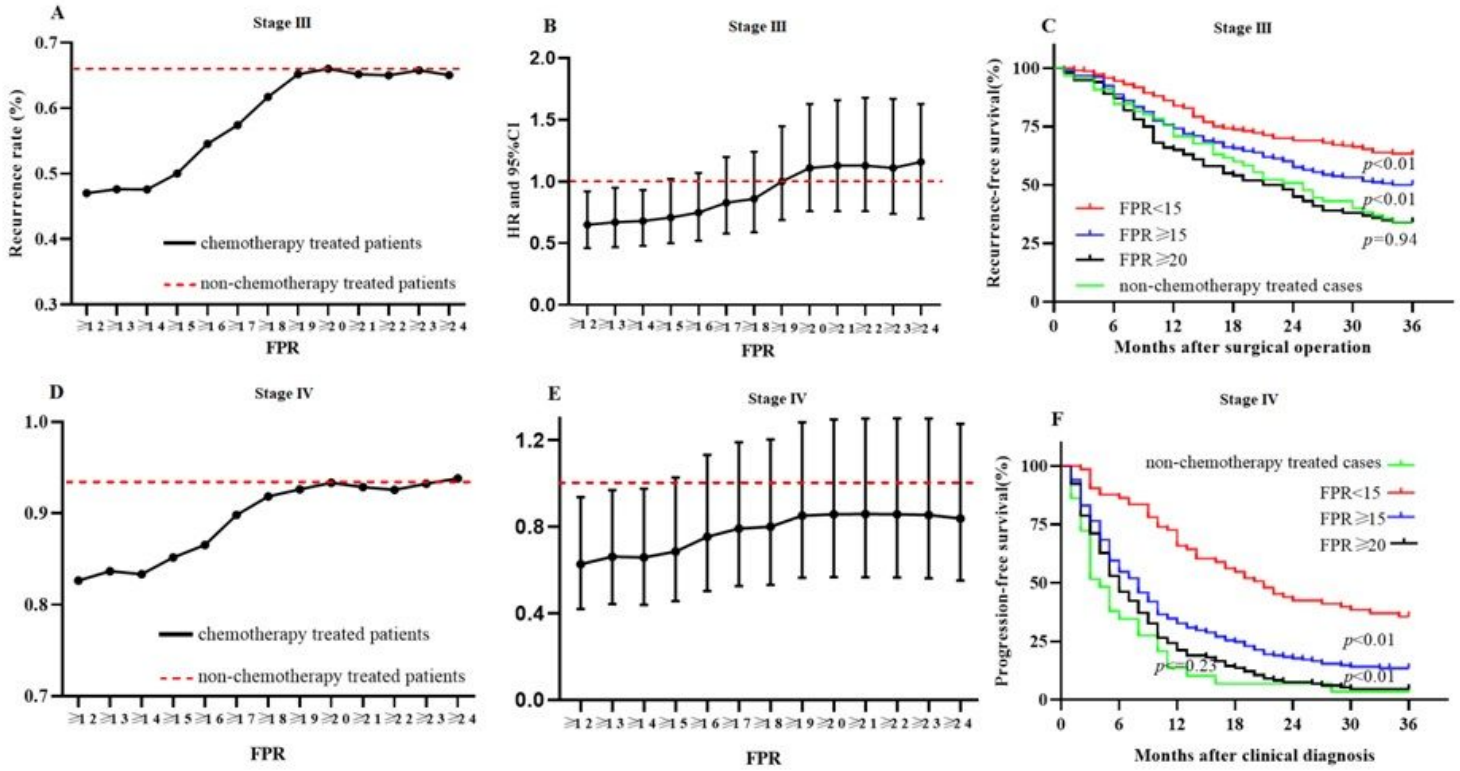

Figure 3

Circulating FPR, recurrence and progression in stage I-III and IV CRC patients. A: recurrence rate according to increased FPR; B: hazard ratio (HR) and 95% confidence interval (CI) change in recurrence-free survival comparison of stage I-III chemotherapy-treated patients with increased FPR and the cases without chemotherapy; C: Kaplain-Meier curve in stage I-III patients; D: progression rate according to increased circulating FPR; E:HR and 95%CI change in progression-free survival comparison of stage IV chemotherapy- treated patients with increased FPR and the cases without chemotherapy; F: Kaplain-Meier curve in stage IV patients.

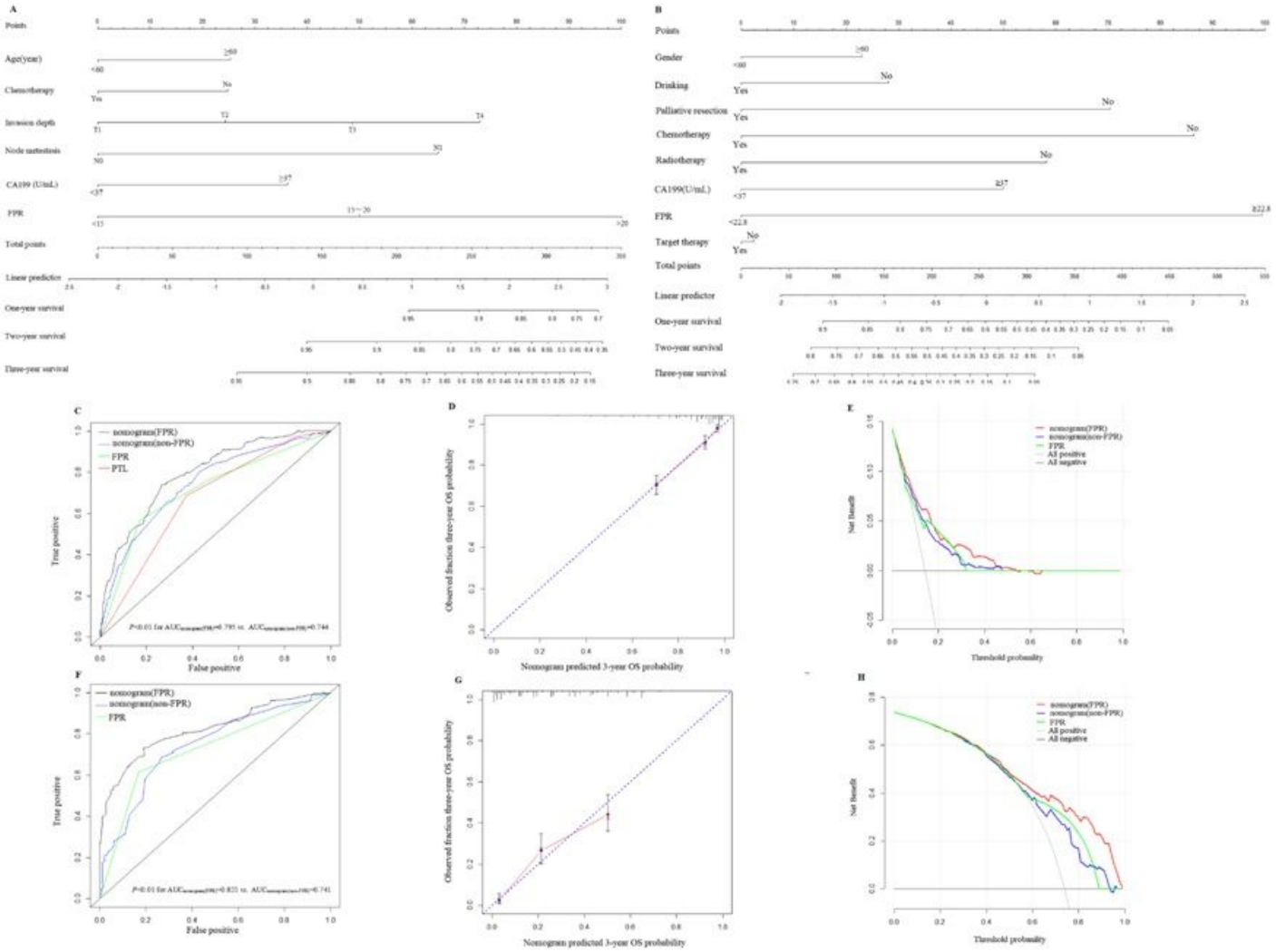

Figure 4

Building and evaluation of prognostic nomogram.A:overall survival predicted nomogram(FPR) in stage I-III operated CRC patients; B: overall survival predicted nomogram(FPR) in stage IV CRC patients; C: time-dependent ROC analysis in stage I-III operated CRC patients; D: calibration curve of overall survival predicted nomogram(FPR) in stage I-III operative CRC patients; E: decision curve overall survival predicted nomogram(FPR) in stage I-III operated CRC patients; F: time-dependent ROC analysis in stage IV CRC patients; G: calibration curve of overall survival predicted nomogram(FPR) in stage IV CRC patients; H: decision curve overall survival predicted nomogram(FPR) in stage IV CRC patients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure4.tif
- SupplementaryFigure4.tif
- SupplementaryFigure3.tif
- SupplementaryFigure3.tif
- SupplementaryFigure2.tif
- SupplementaryFigure2.tif
- SupplementaryFigure1.tif

- SupplementaryFigure1.tif