Quantification of Fibrinogen-to-Pre-Albumin Ratio Provides an Integrating Parameter for Differential Diagnosis and Risk Stratification of Early-Stage Colorectal Cancer

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Primary research

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Abstract

Background: Circulating fibrinogen to pre-albumin ratio (FPR) and albumin to fibrinogen ratio (AFR) are effective factors for predicting the prognosis of colorectal cancer (CRC). However, the role of these two ratios in diagnosing early-stage CRC and identifying the stage II CRC subgroup with high relapse risk remains unknown. This study aimed to assess the potential of FPR and AFR in differential diagnosis and risk stratification of early-stage CRC.

Methods: Two-stage cohorts (discovery and validation) including 895 patients with colorectal benign polyps patients and 714 patients with stage I-II CRC were enrolled in this study. Receiver operating characteristic curve (ROC), Kaplan-Meier curve, and time-dependent ROC were used to evaluate the diagnostic efficacy of AFR and FPR in the two cohorts and overall population, and the discriminating role of FPR in identifying clinical high-relapse risk patients in comparison with common clinical characteristics in stage II CRC patients.

Results: The area under the curve (AUC) of the preoperative circulating FPR was higher than that of AFR in the diagnosis of stage I-II CRC from colorectal adenomas, and colorectal benign polyps in the discovery and validation cohorts and overall population. Preoperative FPR combined with carcinoembryonic antigen (CEA) could effectively discriminate early-stage CRC from colorectal adenomas or benign polyps in the discovery and validation cohorts and overall population. Preoperative FPR could effectively distinguish stage II subgroups with high and low relapse risk, and was found to be superior to common clinical characteristics in identifying high-risk patients who could benefit from adjuvant chemotherapy (CT) post-surgery [time-dependent AUC: 0.637 *vs.* 0.511, $p<0.001$ for predicting recurrence-free survival (RFS); 0.719 vs. 0.501, $p<0.001$ for predicting overall survival (OS)]. Furthermore, patients with FPR>20 and stage II CRC treated with CT had the highest recurrence (31.16%) and death rates (21.88%), with similar highest recurrence (30.70%) and death (26.92%) rates found in non-CT-treated patients with FPR>20. Stage II CRC patients with 20≥FPR>15 could significantly benefit from postoperative CT, as the recurrence (33.30%) and death (35.71%) rates within non-CT treated patients were approximately five times higher than those of the CT-treated cases (6.77% and 7.41% for the recurrence and death rates, respectively). No significant difference in recurrence rate was observed between L-FPR (≤15) patients with (10.00%) or without CT (9.76%), indicating that these patients might not require to receive adjuvant CT after curative resection.

Conclusions: Preoperative FPR combined with CEA is superior to common tumor biomarker or either FPR, or AFR in distinguishing early-stage CRC from colorectal benign polys. Circulating FPR can be considered as an effective biomarker to identify high-risk patients and to choose suitable therapeutics for early-stage CRC.

Introduction

Colorectal cancer (CRC) is the second most common digestive malignancy and the fifth leading cause of cancer-related deaths in China [1], accounting for approximately 30% of all annually diagnosed CRC and disease-related deaths worldwide [2]. As the disease presents without clinical symptoms in the early stage, most patients are diagnosed with advanced disease, leading to a poor prognosis [3]. Colonoscopic polypectomy and surgical resection are the two primary methods for radical treatment of colorectal adenoma and stage I-II CRC [4]. Hence, detection and treatment of precancerous lesions and early-stage cancers can be highly effective in decreasing the morbidity and mortality caused by CRC.

Commonly, colorectal polyps can be histologically classified as adenomatous or non-neoplastic. Non-neoplastic polyps, such as hyperplastic, hamartomatous, and inflammatory polyps, are commonly considered to have no malignant potential. Neoplastic polyps including colorectal tubular and tubulovillous adenomatous polyps, and serrated hyperplastic polyps are capable of developing adenocarcinomas through the classic adenoma-carcinoma pathway and serrated pathway, respectively [5]. However, the colonoscopic appearance of malignant colorectal polyps that contain early-stage invasive CRC is not easily distinguishable from that of non-neoplastic and benign adenomatous polyps [6].

Accumulating evidence show that chronic inflammation and genetic variation play key roles in colorectal carcinogenesis via premalignant polyps [7]. Colorectal precursor lesions commonly harbor inflammatory histologic characteristics, while inflammation-promoted DNA damage has been commonly examined in cancer and precancerous lesions [8]. The inflammatory process in the microenvironment also stimulates angiogenesis, promotes cell proliferation, and inhibits apoptosis to promote the progression of polyps to CRC [9]. Our previous studies showed that circulating fibrinogen (Fib) to pre-albumin (pAlb)) ratio (FPR) and albumin (Alb) to fibrinogen ratio (AFR) are two sensitive biomarkers reflecting host chronic inflammation [10–13], where FPR had a better prognostic performance than the other inflammatory biomarkers for the localized CRC [14]. However, the differential diagnosis values of AFR and FPR in the subsets of colorectal polyps and early-stage CRC remain unknown, and there have been no study reporting their roles in identifying clinical high-relapse risk patients with early-stage CRC.

In our study, a discovery (155 patients with non-neoplastic polyps, 539 patients with colorectal adenomatous polyps, and 512 stage I-II CRC patients) and a validation (201 cases with colorectal polyps, and 202 cases with stage I-II CRC) cohort were enrolled. This study aimed to investigate: 1) the diagnostic efficacy of FPR and AFR in early-stage CRC and subsets of colorectal polyps and 2) the discriminating role of circulating FPR in identifying clinical high-risk patients with stage II CRC who may require adjuvant chemotherapy (CT) after surgery.

Patients And Methods

This study was approved by the ethics committee of the Second Affiliated Hospital of Nanchang University. Written informed consent was obtained from each patient, and all procedures were performed following the guidelines of the Declaration of Helsinki. A flowchart for the selection of eligible participants is shown in Figure 1. First, we screened for newly diagnosed cases of colorectal polyps (January of 2013 to December of 2019) and stage I-II CRC patients (January of 2013 to October of 2018) in the hospital. The eligible patients were screened according to the following inclusion criteria: a) clinical baseline, information, and the blood samples were provided; b) they did not receive any treatment nor were administered non-steroidal anti-inflammatory drugs before the clinical diagnosis; c) cases were confirmed by biopsy and pathological detection by two senior pathologists; and d) all the early-stage CRC patients

underwent curative operation, and cancer resected margins were negative. Second, the following patients were excluded according to the following criteria: a) loss of following-up in the first six months; b) diarrhea, vomiting, and presence of diseases including inflammatory bowel disease, hereditary polyposis, other malignancies, and polyps from other organs; and c) the patients harbored acute infection, autoimmune or chronic kidney disease, hematopathy, hepatopathy, or cardiovascular and cerebrovascular disease in the past month. Third, we classified the eligible patients into the five subgroups (inflammatory polyps, hyperplastic polyps, adenomatous polyps, stage I CRC; and stage II CRC) in the discovery cohort and into the two subgroups (benign polyp and early-stage CRC) within the validation cohort according to the inclusion and exclusion criteria.

Clinical baseline and pathological characteristics were collected from all the included patients. The laboratory-detected sample, 2-mL plasma and serum, were collected at the time of admission, and earlier than any other treatments at the hospital. Plasma fibrinogen (Fib) was detected by the Clauss assay using a SYSMEX CA-7000 machine (Sysmex, Tokyo, Japan). Bromocresol green staining method and immunological turbidimetry assays were used to measure the concentrations of serum albumin (Alb) and pre-albumin (pAlb) using the OLYMPUS AU5400 (Beckman Coulter, Tokyo, Japan), respectively. A chemiluminescence immunoassay was used to detect serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) using a Siemens ADVIA Centaur XP machine (Siemens, Erlangen, Germany). The inter- and intra-batch coefficients of these detections were less than 7.5%. We calculated Alb-to-Fib ratio (AFR=Alb/Fib) and the Fib-to-pAlb ratio (FPR=Fib*1000/pAlb) based on the results of detection.

Commonly, clinical characteristics such as poor histological differentiation (G3-4), T4 stage, vascular lymphatic infiltration, preoperative intestinal obstruction, or intestinal perforation, and number of lymph nodes detected in surgical specimens <12, are used to identify stage II CRC patients with clinical high-relapse risk [15]. In this study, we classified the stage II cases into clinical high- and low-risk patients in accordance with the criteria. A 3-year following-up, performed every 3 months in the first year and every 6 months in the second and third year, was conducted in the early-stage CRC subgroup. Recurrence-free survival (RFS) was the primary outcome in the present study, and was measured from the time of curative resection to the time of disease recurrence or the set deadline, the deadline was set in June of 2021. Overall survival (OS) was defined as the time from surgery to death or the deadline of the study, whichever was earlier. In the follow-up period, patients who were detected with extremely elevated (>2-fold) CEA or CA19-9, obvious recurrence imaging features, or colonoscopy observation, were considered to have recurrence or distal metastasis of the disease.

The prognostic cut-off values of FPR within stage I and II CRC were 14.0 and 16.5, respectively, as reported in our previous study [16]. Binary and continuous variables were summarized as numbers and frequencies, and medians and quartiles, respectively. Comparisons were analyzed using the Chi-square test, Fisher's exact test, Kolmogorov-Smirnov, and Mann-Whitney U tests. The survival differences between the comparisons were compared using the Kaplan-Meier curve (log-rank test) and Cox regression. Hazard ratios (HR) and 95% confidence intervals (CI) were used to assess the strength of association between them. Time-dependent receiver operating characteristic (ROC) curves, area under the curve (AUC), sensitivity (Sen), and specificity (Spe) were selected to evaluate the predicted efficacy on the 3-years RFS and OS. SPSS. 22.0 (IBM Corp, Armonk, NY, USA), R 3.5.1 (Institute for Statistics and Mathematics, Vienna, Austria) with packages of "tdROC", and GraphPad Prism 8.2.1 (GraphPad Software Inc, San Diego, USA) were used for the statistical analyses, and $p<0.05$ (two-sided) was recognized as statistically significant.

Results

According to the inclusion and exclusion criteria, 2256 patients were enrolled and screened to identify eligible patients. As a result, 155 cases of colorectal non-neoplastic polyps (88 inflammatory and 67 hyperplastic polyps patients), 539 cases of colorectal adenomatous polyps, and 512 early-stage CRC patients (110 stage I patients and 402 stage II cases) were enrolled as eligible cases in the discovery cohort. The validation cohort consisted of 201 patients with colorectal benign polyps (colorectal non-neoplastic and adenomatous polyps) and 202 patients with stage I-II CRC (Figure 1). The characteristics of the patients are summarized in Table 1. Significant sex and age distribution differences were observed between the colorectal benign polyps and early-stage CRC groups in the discovery cohort (all $p<0.01$). However, there was only a sex distribution difference in the validation cohort ($p=0.011$). All eligible patients underwent endoscopic resection or curative surgical operation, and 382 and 118 CRC patients received CT after surgery in the discovery and validation cohorts, respectively. Compared to the non-neoplastic and adenoma polyp subgroups, circulating Fib and FPR were significantly higher in the CRC subgroup (all $p<0.01$), conversely, Alb, pAlb, and AFR were extremely low in early-stage CRC patients compared to colorectal benign polyps cases in the two cohorts (all $p<0.01$).

Table 1
The baseline and clinicopathological characteristics of eligible patients in the discovery and validation cohorts.

		Discovery cohort			Validation cohort		
Variables		**Colorectal benign polyps (694)**	**Early-stage colorecta cancer (512)**	P-value	**Colorectal benign polyps (201)**	**Early-stage colorectal cancer (202)**	P-value
		N(%)	**N(%)**		**N(%)**	**N(%)**	
Gender	Male	433(62.39%)	312(60.94%)	<0.001	139(69.15%)	115(56.93%)	0.011
	Female	261(37.61%)	200(39.06%)		62(30.85%)	87(43.07%)	
Age	<60	410(59.08%)	238(46.48%)	<0.001	108(53.73%)	110(54.46%)	0.884
	≥60	284(40.92%)	274(53.52%)		93(46.27%)	92(45.54%)	
Smoking	Yes	152(21.90%)	100(19.53%)	0.317	31(15.42%)	32(15.84%)	0.908
	No	542(78.10%)	412(80.47%)		170(84.58%)	170(84.16%)	
Drinking	Yes	114(16.43%)	77(15.04%)	0.514	30(14.93%)	27(13.37%)	0.653
	No	580(83.57%)	435(84.96%)		171(85.07%)	175(86.63%)	
Diabetes	Yes	46(6.63%)	37(7.23%)	0.685	16(7.96%)	14(6.93%)	0.694
	No	648(93.37%)	475(92.77%)		185(92.04%)	188(93.07%)	
Hypertension	Yes	136(19.60%)	101(19.73%)	0.955	36(17.91%)	36(17.82%)	0.981
	No	558(80.40%)	411 (80.27%)		165(82.09%)	166(82.18%)	
TNM stage	I	-	110(21.48%)		-	49(24.26%)	
	II	-	402(78.52%)		-	153(75.74%)	
T stage	T1-2	-	110(21.48%)		-	52(25.74%)	
	T3-4	-	402(78.52%)		-	150(74.26%)	
Differentiation	G1-2	-	488(95.31%)		-	-	
	G3-4	-	24(4.69%)		-	-	
Radical surgery	Yes	-	512(100%)		-	202(100%)	
Chemotherapy	Yes	-	382(74.61%)		-	118(58.42%)	
	No	-	130(25.39%)		-	84(41.58%)	
CEA	<5	676(97.41%)	389(75.98%)	<0.001	198(98.51%)	145(71.78%)	<0.001
(>5ng/mL)	≥5	18(2.59%)	123(24.02%)		3(1.49%)	57(28.22%)	
CA199	<37	657(94.67%)	442(86.33%)	<0.001	197(98.01%)	176(87.13%)	<0.001
(>37U/mL)	≥37	37(5.33%)	70(13.67%)		4(1.99%)	26(12.87%)	
Fib (g/L)		2.45(2.10-2.81)	3.05(2.55-3.60)	<0.001	2.51(2.18-2.99)	3.07(2.54-3.83)	<0.001
Alb(g/L)		42.00(39.74-44.10)	40.68(38.50-42.64)	<0.001	41.73(39.72-44.13)	40.72(37.79-43.63)	<0.001
preAlb (mg/L)		261.72(218.74-305.10)	210.73(168.60-259.22)	<0.001	246.26(197.07-287.97)	181.40(135.45-229.21)	<0.001
AFR		17.11(14.87-20.02)	13.26(10.98-15.86)	<0.001	16.37(14.37-19.39)	13.40(10.09-16.13)	<0.001
FPR		9.54(7.68-11.72)	14.50(10.87-19.11)	<0.001	10.72(8.34-13.75)	17.05(12.12-26.41)	<0.001

Abbreviation and note: CRC: colorectal cancer; colorectal benign polyps include colorectal non-neoplastic and adenomatous polyps; Fib: Fibrinogen; Alb: albumin; pAlb: pre-albumin; FPR=Fib/pAlb ×1000; AFR=Alb/Fib; distribution differences of gender, age, smoking, drinking, diabetes, hypertension, CEA, CA199 between the groups were tested by Chi-square test; Fib, Alb, pAlb, AFR, FPR differences between groups were tested by rank sum test.

Among the colorectal non-neoplastic and adenomatous polyp subgroups, there were differences observed in circulating FPR (p<0.05) and AFR (p>0.05) in the discovery cohort (Figure 2A-B). Circulating FPR was significantly lower in patients with adenomatous polyps than in those with hyperplastic polyps (p<0.05) (Figure 2C). However, there was no difference in FPR between the inflammatory and hyperplastic polyp subgroups (Figure 2C). The AUCs of FPR, AFR, CEA, and CA19-9 for discriminating colorectal non-neoplastic and adenomatous polyps were 0.576, 0.549, 0.507, and 0.528, respectively (Table 2). Circulating AFR gradually decreased from colorectal adenoma to stage I and stage II CRC subgroups, and a significant difference in AFR was observed in patients with

colorectal benign polyps patients and early-stage CRC (Figure 2D). In contrast, circulating FPR was gradually increased in these subgroups (Figure 2E), and a significantly higher FPR was also observed in early-stage CRC than in colorectal benign polyps group (Figure 2F). In early-stage CRC, a significantly higher FPR was observed in the T3-4 subgroup than that in the T1-2 patients; however, no difference in FPR was observed in the comparisons of T1 *vs.* T2, T3 *vs.* T4 (Figure 2G) or in subgroups stratified by histological differentiation (Figure 2H).

Table 2
The diagnostic efficacy of preoperative FPR, AFR,CEA,CA199, and FPR combined with CEA and CA199 in patients with colorectal non-neoplastic polyps, adenomas, and early-stage colorectal cancer in discovery and validation cohorts and overall population.

Comparison		Biomarkers	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
Discovery cohort	Non-neoplastic polyps *vs.* adnomas	FPR	6.17	0.576	91.10%	14.20%	78.70%	31.40%	0.053
		AFR	22.69	0.549	12.90%	87.10%	77.78%	77.65%	0.027
		CEA	1.245	0.507	57.80%	48.10%	23.59%	80.48%	0.059
		CA199	8.165	0.528	70.40%	38.80%	80.60%	26.63%	0.095
	Adnomas *vs.* early-stage CRC	FPR	11.73	0.818	70.60%	79.70%	80.37%	69.67%	0.503
		AFR	14.90	0.767	76.10%	66.90%	66.11%	76.74%	0.430
		CEA	1.895	0.711	63.80%	68.30%	70.38%	61.58%	0.321
		CA199	16.895	0.577	41.60%	73.30%	64.67%	51.60%	0.149
		CEA+FPR	0.63	0.858	67.10%	90.90%	89.70%	70.11%	0.580
		CEA+CA199+FPR	0.62	0.858	67.10%	90.70%	89.46%	70.06%	0.578
	Colorectal benign polyps *vs.* early-stage CRC	FPR	12.47	0.792	65.10%	81.30%	72.25%	71.62%	0.464
		AFR	14.90	0.754	75.50%	33.10%	54.98%	55.44%	0.424
		CEA	1.875	0.711	63.80%	69.40%	65.83%	67.51%	0.332
		CA199	16.86	0.582	41.80%	73.60%	59.88%	57.86%	0.154
		CEA+FPR	0.50	0.835	68.30%	83.40%	79.11%	74.04%	0.525
		CEA+CA199+FPR	0.50	0.835	68.60%	83.90%	79.72%	74.30%	0.525
Validation cohort	Colorectal benign polyps *vs.* early-stage CRC	FPR	12.47	0.759	72.30%	68.20%	69.52%	79.98%	0.405
		AFR	14.90	0.703	70.60%	65.30%	67.14%	68.95%	0.359
		CEA	1.875	0.702	58.90%	68.20%	65.03%	62.27%	0.271
		CA199	16.86	0.579	43.60%	73.10%	61.97%	56.32%	0.167
		CEA+FPR	0.50	0.823	61.90%	83.60%	79.11%	68.57%	0.455
		CEA+CA199+FPR	0.50	0.823	61.90%	83.60%	79.11%	68.57%	0.455
Overall population	Colorectal benign polyps *vs.* early-stage CRC	FPR	12.47	0.780	67.20%	77.80%	69.08%	76.27%	0.450
		AFR	14.90	0.742	74.00%	66.40%	60.97%	75.70%	0.404
		CEA	1.875	0.709	62.40%	68.40%	59.29%	71.12%	0.308
		CA199	16.86	0.580	42.20%	73.50%	29.75%	38.33%	0.157
		CEA+FPR	0.50	0.829	63.20%	86.00%	76.96%	76.81%	0.492
		CEA+CA199+FPR	0.50	0.828	64.00%	85.70%	76.05%	76.38%	0.497

Abbreviation and note: CRC: colorectal cancer; colorectal benign polyps include colorectal non-neoplastic and adenomatous polyps; Fib: Fibrinogen; Alb: albumin; pAlb: pre-albumin; FPR=Fib/pAlb ×1000; AFR=Alb/Fib; PPV: positive predictive value; NPV: negative predictive value; AUC: area under curve.

In the discovery cohort, the AUCs of FPR, AFR, CEA, and CA19-9 were 0.818, 0.767, 0.711, and 0.577 for the differential diagnosis of early-stage CRC and colorectal adenomas polyps, respectively (Table 2). The Sen and Spe of FPR (cut-off=11.73, Sen=70.60%, Spe=79.70%) and AFR (cut-off=14.90, Sen=76.10%, Spe=66.90%) were better than those of CEA and CA19-9, respectively (Table 2). In discriminating CRC from colorectal benign polyps, the AUCs, Sen, and Spe were 0.792, 65.10% and 81.30%, for FPR, respectively, and 0.754, 75.50%, and 33.10% for AFR, respectively, and their AUCs were superior to CEA and CA19-9, respectively (Figure 3A **and** Table 2). Circulating AFR was negatively correlated with FPR in the overall population (Figure 2I). We selected FPR, CEA, and CA19-9 to evaluate the combined diagnostic efficacy in discriminating colorectal adenoma polyps and early-stage CRC. We observed that AUCs, Sen, and Spe of the combined CEA-FPR were 0.858, 67.10%, and 90.90%, respectively, which was similar to the combined CEA-CA19-9-FPR (Table 2). In colorectal benign polyps and early-stage CRC subgroups, the AUCs of the combined CEA-FPR and CEA-FPR-CA19-9 were 0.835 and 0.835, respectively, and their Sen were 68.30% and 68.60%, respectively, with Spe of 83.40% and 83.90%, respectively (Figure 3B **and** Table 2).

In the validation cohort, the AUC of FPR was significantly higher than that of AFR, CEA, and CA19-9 in diagnosing early-stage CRC from colorectal benign polyps (all p<0.01) (Figure 3C). The AUCs of FPR-CEA and FPR-CEA-CA19-9 were similar and were effectively improved compared to the single FPR (0.823 *vs.* 0.759, p<0.01) (Figure 3D **and** Table 2). AUC, Sen, Spe, positive predictive value, negative predictive value, and Youden's index of FPR were 0.780, 67.20%, 77.80%, 69.08%, 76.27%, and 0.450 for the diagnosis of early-stage CRC and colorectal benign polyps, respectively, which were better than the other single biomarkers in the overall population (Figure 3E **and** Table 2). The diagnostic efficacy of combined FPR and CEA was similar to that of FPR-CEA-CA19-9; however, the AUCs of combined FPR-CEA (0.829 *vs.* 0.780, p=0.011) and FPR-CEA-CA199 were significantly higher than those of FPR in the overall population (Figure 3F **and** Table 2).

According to the criteria of clinical high/low-risk patients, we divided the patients into clinical high- (572 cases) and low-risk (32 cases) groups. Although the recurrence rate in the non-CT-treated patients was higher than in CT-treated patients in clinical high-(21.79% vs. 15.73%, p=0.211) and low-risk (21.79% vs. 12.50%, p=0.512) subgroups, no statistical difference was observed between them (Figure 4A). Similarly, there was also no difference in the comparison of death rates between CT-treated and non-CT-treated high-risk patients and low-risk cases (15.38% vs. 8.75% vs. 5.88%, p=0.234) (Figure 4B). Furthermore, no survival (RFS and OS) differences were observed between them in the two subgroups (Figure 4C-D).

According to the cut-off value of FPR, stage II patients were classified into high-FPR (H-FPR) and low-FPR (L-FPR) subgroups. RFS and OS were shorter in H-FPR patients than in L-FPR patients in clinical CT-treated ($p_{log-rank}$=0.006 for RFS, $p_{log-rank}$=0.001 for OS) and non-CT-treated ($p_{log-rank}$=0.015 for RFS, $p_{log-rank}$=0.002 for OS) high-risk subgroups with the stage II disease (Figure 4E-H). However, no survival difference was observed between the clinical low-risk subgroup and high-risk patients with L-FPR regardless of treatment with CT (Figure 4E-H).

Clinical low-risk patients and non-CT-treated clinical high-risk patients with H-FPR harbored the highest recurrence rate (33.33%), while L-FPR patients with clinical high-relapse risk had the lowest recurrence rate regardless of treatment with CT (10.26% for non-CT treated patients; 10.53% for CT treated patients) (Figure 4I). In the clinical high-risk subgroup, the recurrence rate in H-FPR patients treated with CT was significantly lower than that in the non-CT-treated patients (22.61% vs. 33.33%, p<0.001), but was significantly higher than that in L-FPR patients treated with CT (22.61% vs. 10.53%, p<0.001) (Figure 4I). The lowest and highest death rates were observed in CT-treated high-risk patients with L-FPR (3.97%) and H-FPR patients with clinical low-risk (28.57%) and CT-treated H-FPR patients with clinical high-risk (30.77%) (Figure 4J). The death rate of CT-treated clinical high-risk patients with H-FPR was significantly lower than that of non-CT-treated clinical high-risk patients with H-FPR (15.18% vs. 30.77%, p<0.001) and clinical low-risk patients with H-FPR (15.18% vs. 28.57%, p<0.001) (Figure 4J). Additionally, the efficacy of FPR and common clinical characteristics predicted that the 3-years RFS and OS were 0.637 and 0.511, and 0.719 and 0.501, respectively. The AUC of FPR was significantly higher than clinical characteristics in predicting the prognosis (Figure 4K-L).

In patients with stage I CRC, the recurrence rate was only 3.85% in patients with L-FPR (≤15), and no deaths were observed in the two subgroups. In H-FPR (>15) patients, recurrence (18.18%) and death (15.15%) were observed at the 3-year follow-up. A significantly higher FPR was also observed in recurrence and death cases compared to non-recurrence and non-death cases (all p<0.01) in stage I CRC patients, respectively (Figure 5A-B). In H-FPR stage II CRC patients, recurrence and death rates of CT-treated patients with FPR>20, and non-CT treated patients with 20≥FPR>16.5 or FPR≥20 were 31.16% and 21.88%, 33.30% and 35.71%, 30.70% and 26.82%, respectively, and no difference was observed between them. However, the recurrence and death rates of CT-treated patients with 20≥FPR>15, L-FPR patients (≤15) with or without CT were 6.77% and 7.41%, 10.00% and 3.77%, and 9.76%, respectively, and the rates were significantly lower than those of CT-treated patients with FPR>20, and non-CT treated patients with 20≥FPR>15 or FPR>20 (Figure 5C-D).

Discussion

Cancer-related inflammatory biomarkers may aid in identifying early-stage disease, discriminating clinical high-risk stage II patients, and guiding therapeutics. In this study, we found significantly high FPR and low AFR in early-stage CRC compared to subsets of colorectal polyps. Preoperative FPR was superior to AFR and is considered a common tumor biomarker that may be used to diagnose early-stage CRC from colorectal benign polyps in the discovery and validation cohorts and the overall population. Preoperative FPR combined with CEA could effectively distinguish early-stage cancer from benign colorectal polyps, with an AUC, Sen, and Spe of 0.835, 68.30%, and 83.40% in the discovery cohort, 0.823, 61.90% and 83.60% in the validation cohort, and 0.829, 63.20%, and 86.00% in the overall population, respectively. Moreover, circulating FPR identified stage II patients with a high relapse risk after surgical operation, and its predicted efficacy was superior to that of common clinical characteristics. Additionally, preoperative FPR could help the clinicians choose suitable therapeutics for patients with stage I and II disease.

It is well known that the majority of CRCs, develop from colorectal adenomatous or serrated polyps [17, 18]. Screening, identification, and removal of the precancerous lesion and early-stage CRC can effectively reduce incidence and mortality[19]. The fecal immunochemical test (FIT) is the preferred and most used method to screen the early-stage CRC; however, its sensitivity needs further improvement, particularly in adenoma cases [20]. Combined multitarget stool or serum DNA methylation site tests and FIT can offer improved sensitivity [21–23]. The cost of testing restricts its wide use in the clinics, particularly in primary medical units. Although colonoscopy and sigmoidoscopy are significantly advantageous, most individuals are unwilling to undergo the procedures due to their invasive characteristics, especially during healthy check-ups [24].

Inflammation induces carcinogenic mutagenesis and regulates carcinogenesis of CRC [25–27]. Common inflammatory ratios such as neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, and lymphocyte to monocyte ratio show moderate diagnostic efficacies in distinguishing glioma, lung cancer, and healthy subjects, respectively [28, 29]. Our previous study also found that circulating FPR was superior to AFR and NLR in the diagnosis of stage I-IV CRC in healthy individuals [30]. In this study, the diagnostic AUCs of AFR and FPR were less than 0.60 in the diagnosis of colorectal non-neoplastic and adenomatous polyps, indicating that the two ratios could not differentially diagnose the subsets of colorectal polyps. Emir *et al.* also observed no significant differences in NLR and PLR in colorectal polyp cases and healthy individuals [31]. AFR and FPR were gradually decreased and increased in colorectal adonoma, stage I, and stage II CRC, respectively, and their diagnostic efficacies of them were high (up to 0.75), suggesting that the two ratios could effectively

distinguish early-stage CRC from adenoma and other subsets of colorectal polyps. The AUC of the combined CEA-FPR was equal to that of CEA-FPR-CA199, and their sensitivity and specificity were higher than those of the single biomarkers, showing that the combined CEA-FPR was superior to FPR or AFR in identifying early-stage CRC from colorectal benign polyps.

According to the CRC guidelines, majority of stage II cases are not recommended to receive adjuvant CT after surgery, except for clinical high-relapse risk patients [15]. In this study, we used the following clinical characteristics: poor histological differentiation (G3-4), T4 stage, vascular lymphatic infiltration, preoperative intestinal obstruction or intestinal perforation, and the number of lymph nodes detected in surgical specimens <12 to classify patients with stage II CRC into two subgroups with clinical high- and low-relapse risk. In CT-treated or non-CT-treated patients, we found clinical prognosis and recurrence rate to be the same between clinical high- and low-risk cases, and no difference in the rate was observed in each clinical high- and low-risk group with or without CT. Moreover, the predicted time-dependent AUCs of clinical characteristics were 0.511 and 0.501 for 3-year RFS and OS, respectively. These findings demonstrated that the common clinical characteristics could not effectively distinguish between clinical high- and low-risk patients, and that clinically high-risk patients could not benefit from adjuvant CT after surgical operation.

Different treatment efficacies in chemotherapeutic drug responses are related to the different grades of chronic inflammation in patients [32, 33]. The lowest recurrence rate was found in L-FPR stage II clinical high-risk patients regardless of treatment with CT. In contrast, the highest recurrence rate was found in non-CT-treated H-FPR patients regardless of clinical high or low risk, and the rate was approximately three times higher compared to patients treated with CT. Moreover, the rate was significantly decreased in H-FPR clinical high-risk patients receiving adjuvant CT compared to non-CT-treated patients. A similar result was also found in these patients with respect to death rate. L-FPR patients with stage II CRC had better survival than H-FPR patients in clinical high- and low-risk subgroups with or without CT. These results illustrated that H-FPR patients could benefit from CT, and L-FPR cases might not have received CT after surgery. Additionally, the predicted efficacy of FPR was much higher than the clinical characteristics for predicting the 3-year outcomes, indicating that FPR was superior to common clinical characteristics in identifying high-risk patients who can benefit from CT.

Our previous studies also showed that high-grade chronic inflammation could attenuate chemosensitivity, or even chemoresistance. Patients with low-grade FPR (≤15) showed complete response to CT; however, median-grade FPR (20≥FPR>15) and high-grade FPR (>20) implied chemosensitivity and chemoresistance in CRC patients [16]. In this study, we found that CT-treated FPR>20 patients harbored the highest recurrence and death rates, and similar highest recurrence and death rates were observed in non-CT-treated patients with FPR>20, indicating that the cases with FPR>20 might not benefit from CT, and that these patients may be treated with single-targeted therapy or onco-immunotherapy, combined CT after the surgery [34–36]. Moreover, the recurrence and death rates in non-CT treated patients with 20≥FPR>15 were approximately five times higher than those of the CT-treated cases, suggesting that patients with 20≥FPR>15 were suitable to receive CT and could significantly benefit from the treatment. Additionally, no significant difference in recurrence and death rates was observed between L-FPR patients (FPR≤15) with or without CT, indicating that these patients may undergo surgical operation only and may not need to receive adjuvant CT after curative resection (Figure 5E).

To the best of our knowledge, this study is the first study to investigate the role of FPR in distinguishing the early-stage cancer from benign polyps, and to identify patients with clinical high-relapse risk. Although preoperative FPR can effectively diagnose stage I-II CRC from colorectal benign polyps, it is not a specific biomarker for CRC. Therefore, FPR combined with CEA could improve the diagnostic efficacy for early-stage CRC. We also found that circulating FPR was superior to common clinical characteristics in identifying high-relapse risk patients who need to receive adjuvant CT; however, this study was only performed in a single center, and the findings should be validated by multi-center studies with large sample size.

Conclusion

Circulating FPR is an effective biomarker to distinguish early-stage CRC from subsets of colorectal polyps, to identify high-risk stage II CRC patients, and to choose suitable therapeutics. FPR combined with CEA can improve the efficacy and sensitivity of diagnosing early-stage CRC.

A List Of Abbreviations

CRC: colorectal cancer

FPR: fibrinogen to pre-albumin ratio

AFR: albumin to fibrinogen ratio

ROC: receiver operating characteristic curve

AUC: area under the curve

CEA: carcinoembryonic antigen

CT: chemotherapy

RFS: recurrence-free survival

OS: overall survival

L-FPR: low-FPR

H-FPR: high-FPR

Fib: fibrinogen

Alb: Albumin

pAlb: pre-albumin

HR: hazard

CI: confidence interval

Sen: sensitivity

Spe: specificity

Declarations

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Authors contributions

Hou-Qun Ying designed the study and prepared the manuscript; Wei Chen collected and detected the clinical samples, and performed follow-up; Cui-Fen Xiong screened and identified the eligible patients, performed the statistics; Yuanyuan Wang performed the following-up and carried out the statistics; Xiao-Juan Li checked the statistics and revised the manuscript; Xue-Xin Cheng designed and approved the study.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Ethical statement

This study was approved by the Ethics Committees of the Second Affiliated Hospital of Nanchang University. We obtained the written informed consent from each patient, and all the performed processes were carried out following the guidelines of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

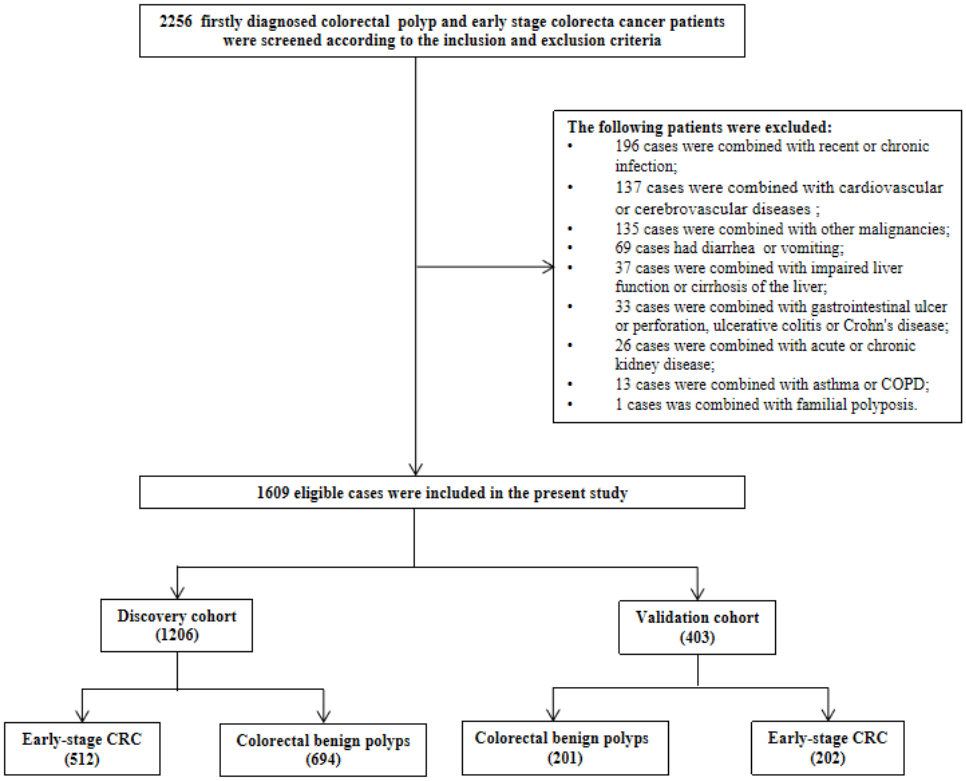

Figure 1

The screening and identifying flowchart of eligible patients in the present study.

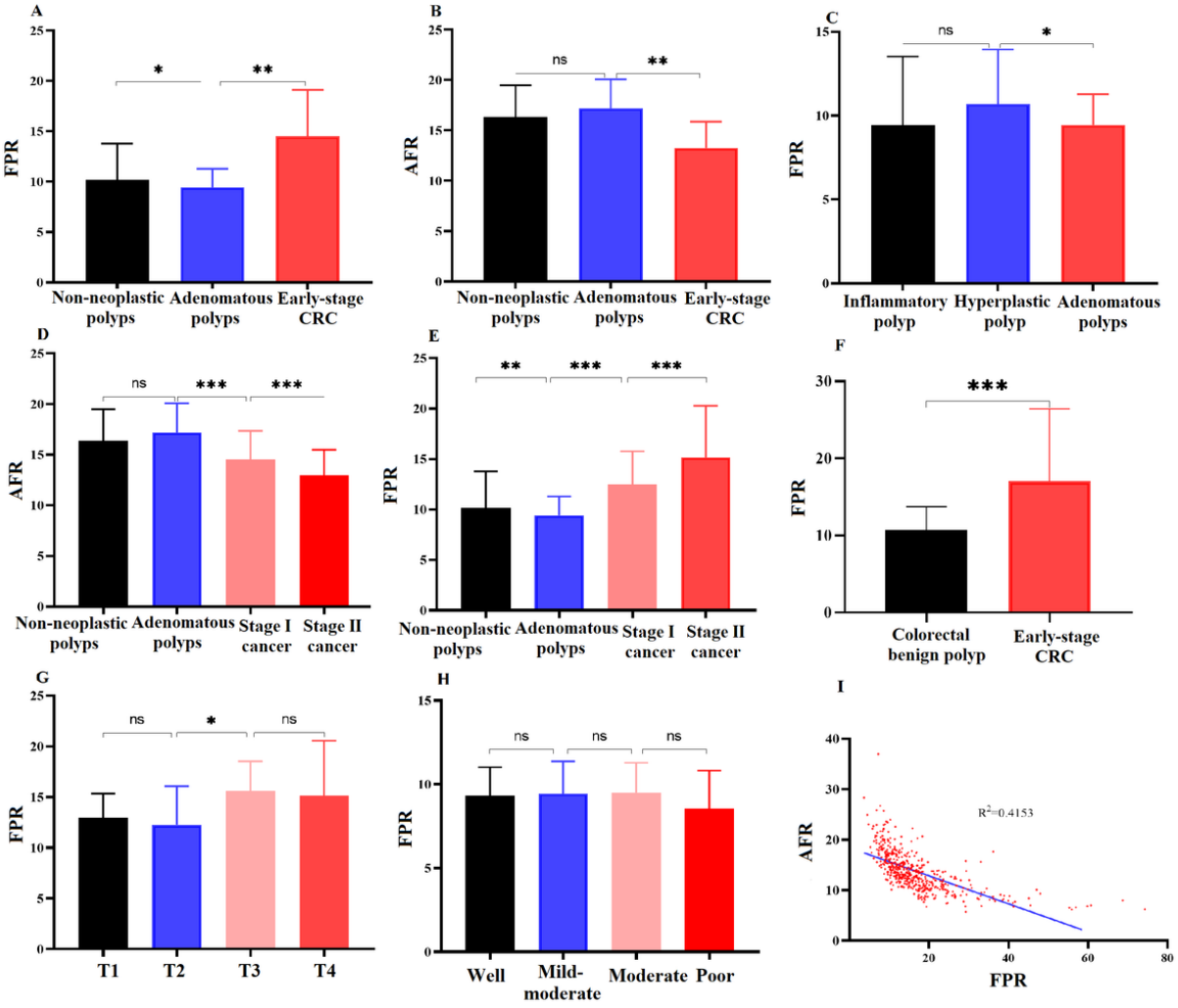

Figure 2

Circulating AFR and FPR in subsets of colorectal polyps and stage I-II colorectal cancer patients. Comparison of FPR (A) and AFR (B) in subsets of colorectal polyps and early CRC patients; comparison of FPR (C) in subsets of colorectal benign polyps; comparison of AFR (D) and FPR (E) in subsets of colorectal polyps, and stage I and stage II CRC patients; comparison of FPR in colorectal benign polyps and early-stage CRC (F) subgroups; comparison of FPR in T1-4 subgroups (G) and the patients with varied histological differentiation (H); scatter plot of circulating AFR and FPR in the overall population (I); *: p<0.05; **: p<0.01;***: p<0.001; ns: no significance.

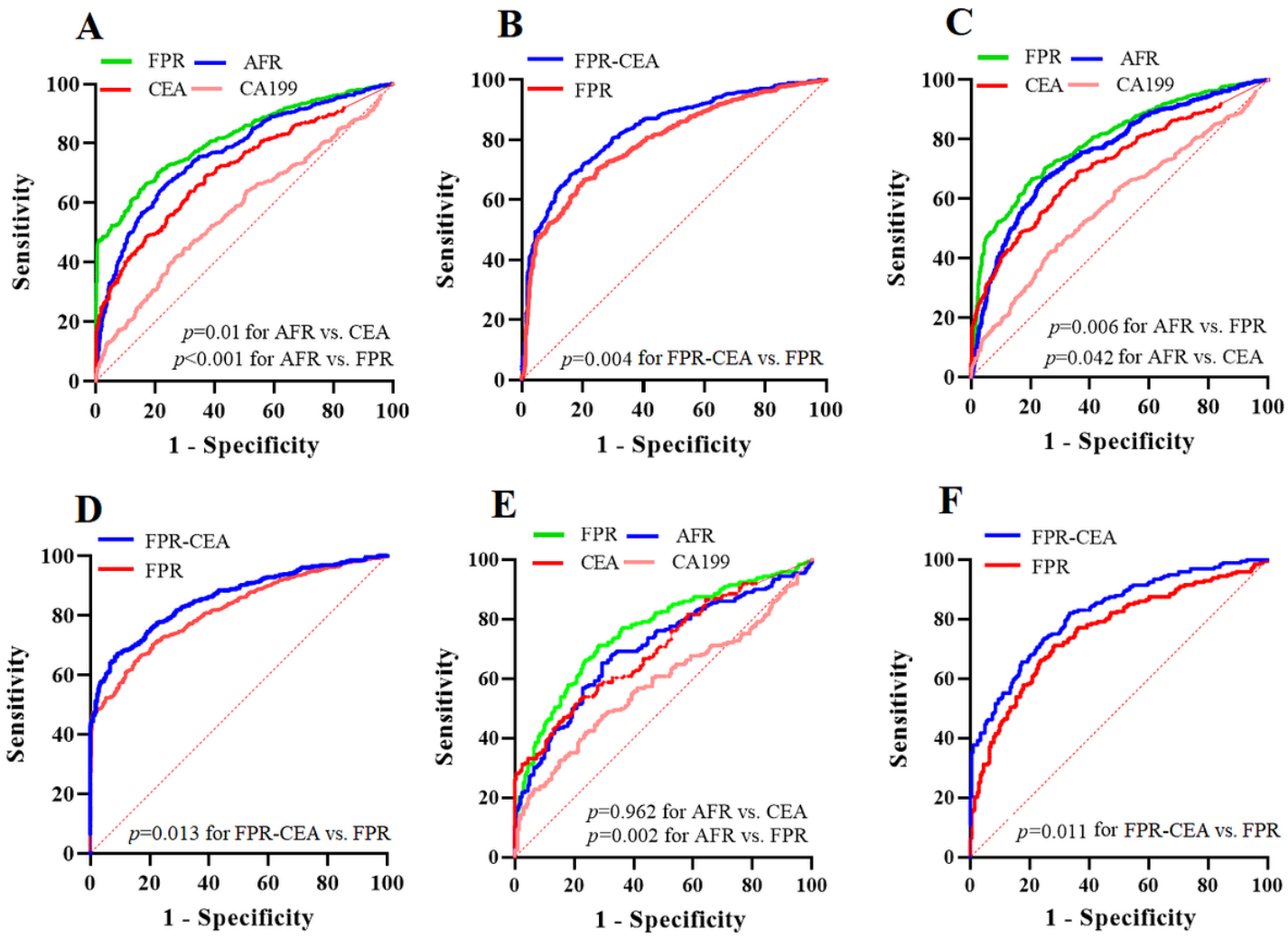

Figure 3

Diagnostic efficacy of circulating FPR, AFR, CEA, CA199, and combined FPR-CEA and FPR-CEA-CA199 in early-stage CRC, colorectal adenomas, and colorectal benign polyps. Receiver operation characteristic curve analysis (ROC) in early-stage CRC and colorectal benign polyps in the discovery cohort (A and B), in the validation cohort (C and D), and in the overall population (E and F).

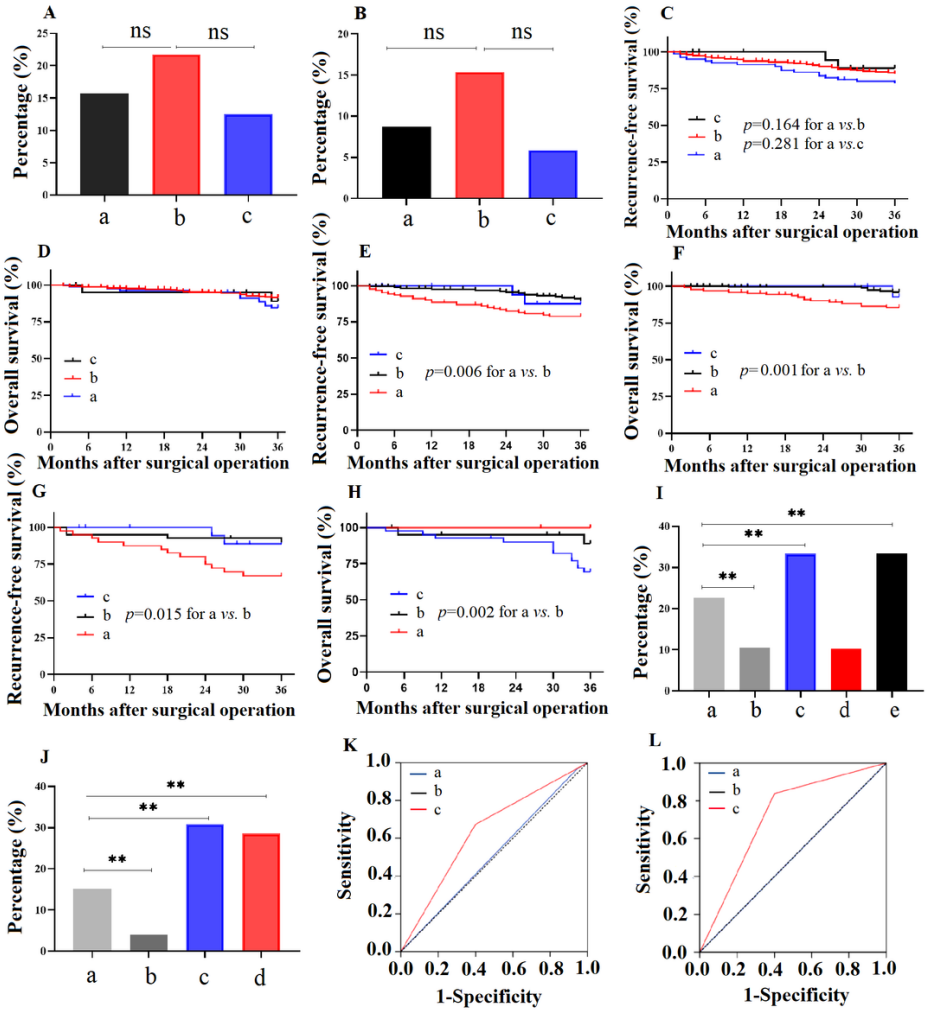

Figure 4

Prognosis and predicted efficacy of FPR and clinical common characteristics in identifying stage II CRC with clinical high-relapse risk. A-B: recurrence/death rate comparisons in chemotherapy (CT) (a) or non-CT-treated (b) clinical high-risk patients, and clinical low-risk patients (c); C-D: Kaplan-Meier curve of recurrence-free survival (RFS) and overall survival (OS) in non-CT-(a), CT-treated (b) clinical high-risk patients, and clinical low-risk patients (c); E-F: Kaplan-Meier curve of RFS and OS in clinical high (a)- and low-FPR (b) high-risk patients with CT and clinical low-risk subgroup (c); G-H: Kaplan-Meier curve of RFS and OS in clinical high (a)- and low-FPR (b) high-risk patients without CT and clinical low-risk subgroup (c); I-J: recurrence/death rate comparisons between CT treated clinical high-risk patients with H-(a) and L-FPR(b), clinical low-risk patients with H-FPR(c), and non-CT treated clinical high-risk patients with L-(d) and H-FPR(e); K-L: time-dependent ROC of FPR and clinical common characteristics in predicting the 3 year RFS and OS; a: clinical high/low risk; b: reference line; c:FPR, **: p<0.01; ns: no significance.

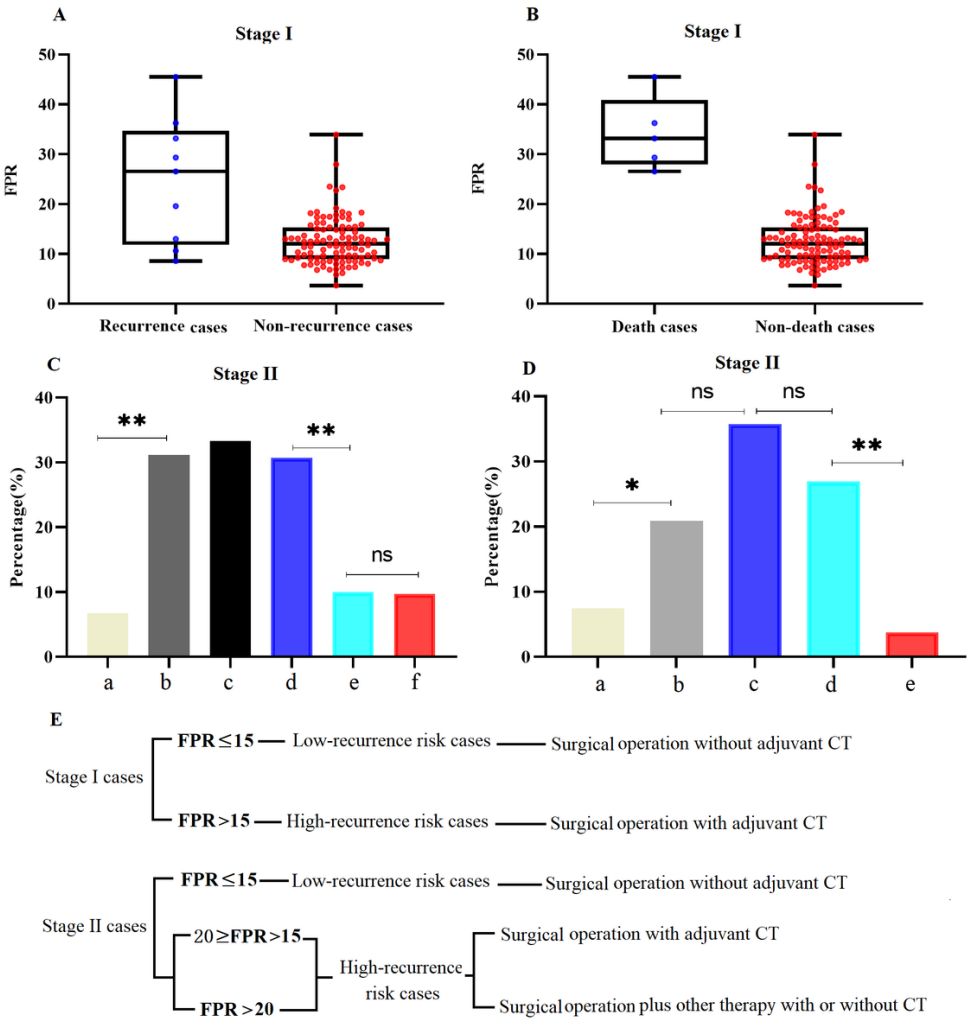

Figure 5

Circulating FPR, chemotherapy, and prognosis of early-stage CRC. A and B: FPR comparisons between stage I CRC patients with or without recurrence/death; C and D: recurrence and death rates of CT- and non-CT-treated stage II CRC patients with 20.0≥FPR>15 (a: CT-treated patients; c: non-CT-treated patients), or FPR>20 (b: CT-treated patients; d: non-CT-treated patients) and FPR≤15 (e: CT-treated patients; f: non-CT-treated patients); F: the therapeutic selection according to circulating FPR. **: p<0.01; ns: no significance.