

Combined Detection of Free Fatty Acid (FFA) and High-Density Lipoprotein Cholesterol (HDL-C) is a Promising Pretreatment Biomarker for Predicting the Overall Prognosis (OS) of Neuroendocrine Tumours (NETs) in the Colorectum: A Case-Control Study.

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Abstract

Purpose

The aim of the study was to evaluate the prognostic value of free fatty acid (FFA) and high-density lipoprotein cholesterol (HDL-C) in predicting colorectal neuroendocrine tumours (NETs).

Methods

One hundred patients with pathologically diagnosed colorectal NETs in 2011-2017 were enrolled, and the levels of FFA, HDL-C, low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs), cholesterol (CHOL), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) between colorectal NET patients and healthy controls matched by age and sex were compared. In addition, the association of clinicopathological characteristics and follow-up data with FFA and HDL-C was analysed.

Results

FFA was overexpressed (0.55 ± 0.23 vs. 0.48 ± 0.11 , $P = 0.006$) and HDL-C was underexpressed (1.31 ± 0.41 vs. 1.41 ± 0.29 , $P = 0.046$) in colorectal NETs. FFA ≥ 0.52 mmol/L predicted lymph node metastasis (LNM) ($\chi^2 = 5.964$, $P = 0.015$), and HDL-C ≤ 1.0 mmol/L predicted tumour size ≥ 2 cm ($\chi^2 = 5.647$, $P = 0.017$). No significant association was found between FFA and tumour size ($P = 0.142$) or HDL-C and LNM ($P = 0.443$). FFA ≥ 0.52 mmol/L ($\chi^2 = 6.016$, $P = 0.014$) and HDL-C ≤ 1.0 mmol/L predicted worse overall survival (OS) ($\chi^2 = 5.488$, $P = 0.019$). FFA ≥ 0.52 mmol/L in combination with HDL-C ≤ 1.0 mmol/L predicted an even worse prognosis in terms of OS ($\chi^2 = 4.818$, $P = 0.028$).

Conclusion

FFA ≥ 0.52 mmol/L and HDL-C ≤ 1.0 mmol/L were promising cut-off values in predicting LNM, tumour size and worse OS in colorectal NETs.

Introduction

Neuroendocrine tumours (NETs), previously described as "carcinoid tumours" in 1907, are heterogeneous neoplasms arising in secretory cells of the diffuse neuroendocrine system (Yao et al. 2008). The Epidemiology and End Results (SEER) database indicates that the incidence of NETs has increased significantly, approximately 5 times, to 5.25/100,000 cases/year; of these, colorectal NETs account for approximately 49.6% of primary NET sites in the digestive tract and have an incidence and prevalence inferior only to those of colorectal adenocarcinoma (Frilling et al. 2012; Lawrence et al. 2011; Modlin et al. 2003). Although endoscopic mucosal resection, endoscopic submucosal dissection, surgery, radiation

and chemotherapy have been used to treat localized and metastatic colorectal NETs, the 5-year survival rate of NETs with lymph node metastasis (LNM) or distant metastasis remains disappointing, with five-year overall survival rates of approximately 54–74% and 28–44.1%, respectively (Bertani et al. 2018; Dasari et al. 2017).

Free fatty acids (FFAs) are triacylglycerol (TG) precursors that are needed to replenish TG stores in adipose, liver and muscle tissue through esterification; when hepatic glycogen is low and muscles need energy, the TGs in adipose tissue are broken down into FFAs for energy (Stich and Berlan 2004). FFAs are overexpressed in colorectal patients (Zhu et al. 2021), and abnormal fatty acid metabolism is associated with tumour cell metastasis in various cancers, including colorectal cancer (Kawaguchi et al. 2016; Zhang et al. 2020). High-density lipoprotein (HDL) is responsible for the reverse transport of cholesterol from peripheral cells to the liver, and associated apolipoproteins and enzymes can also exert antioxidant, anti-inflammatory, antiangiogenic, antiapoptotic and immunomodulatory activities, resulting in overall antitumorigenic effects (Ganjali et al. 2019). HDL-C is involved in poor disease-free survival (DFS) and overall survival (OS) in colorectal cancer, and very low levels of HDL-C (<30 mg/dL) in women are significantly associated with cancer mortality (Penson et al. 2019; Wang et al. 2019).

However, evidence for the prognostic ability of FFAs and HDL-C and their relationship in colorectal NETs has never been reported. We designed this study to evaluate the prognostic ability of FFA and HDL-C in patients with colorectal NETs, compare FFA with HDL-C and analyse their combination.

Materials And Methods

Patients

Between 2011 and 2017, a total of 100 consecutive patients who received treatment for colorectal NETs in our hospital were enrolled. We constructed a database of retrospectively collected data from patient medical records, including clinical characteristics, pathological reports and survival during the follow-up period.

For local excision procedures, such as endoscopic submucosal dissection (ESD) and transanal excision (TAE), LNM was evaluated through computed tomography (CT) or magnetic resonance imaging (MRI) before the treatment and during the follow-up periods. The diagnosis of a metastatic lymph node was based on the following criteria: 1) Size criteria: short axis diameter greater than 8 mm for round lymph nodes and greater than 10 mm for ovoid lymph nodes; and 2) Morphological abnormalities: irregular contour and margin, unclear border, heterogeneous internal echoes or signal intensity. The tumour diameter refers to the longest diameter of the tumour according to pathology reports. For patients with distant metastases, tumour diameter was determined by endoscopic findings before treatment. The baseline characteristics of patients with colorectal NETs are listed in Table 1.

Table 1
Clinical and Histopathological
Characteristics of Colorectal NETs
(N=100)

Variables	N (%)
Age	
21-30	7(7%)
31-40	11(11%)
41-50	20(20%)
51-60	28(28%)
61-70	24(24%)
≥71	10(10%)
Gender	
Male	65(65%)
Female	35(35%)
BMI	
<23	41(41%)
≥23	59(59%)
Tumour location	
Rectum	28(28%)
Colon	72(72%)
Treatment	
ESD	14(14%)
Transanal excision	26(26%)
Radical resection	38(38%)
Multivisceral resection	8(8%)
Palliative resection	7(7%)
Systemic treatment	7(7%)
Tumour diameter (cm)	
<2	45(45.5)
≥2	54(54.5%)

Variables	N (%)
Lymph node metastasis	
Negative	45(46.9%)
Positive	51(53.1%)
Tumour grade	
G1	57(57%)
G2	14(14%)
G3	29(29%)
Syn	
-	5(5.4%)
±	15(16.3%)
+	72(78.3%)
CgA	
-	50(56.8%)
±	24(27.3%)
+	14(15.9%)
CD56	
-	19(22.6%)
±	10(11.9%)
+	55(65.5%)
CK	
-	2(3.0%)
±	3(4.5%)
+	61(92.4%)

The healthy controls were matched by age and sex one by one to patients with colorectal NETs.

Pathological Diagnosis

Tumour grade was defined numerically, in which low-grade (grade 1 [G1]) tumours have a mitotic rate from 0 to 1 per 10 high-power fields (HPFs) or a Ki-67 index from 0–2%, intermediate-grade (G2) tumours have a mitotic rate from 2 to 20 per 10 HPFs or a Ki-67 index from 3–20%, and high-grade (G3) tumours

have a mitotic rate greater than 20 per 10 HPFs or a Ki-67 index greater than 20% (Klimstra et al. 2010). The expression levels of chromogranin A (CgA) (N=88), synaptophysin (Syn) (N=92), cluster of differentiation 56 (CD56) (N=84) and cytokeratin (CK) (N=66) were scored according to the percentage of positive cells and the intensity of cell staining; no positive cells or negative cell staining intensity is labelled (-); a small amount of positive cells or weakly positive cell staining intensity is labelled (\pm); the majority of positive cells or strongly positive cell staining intensity is labelled (+).

Laboratory testing

FFA, HDL-C, TGs, cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) were measured routinely with a Cobas 8000 automatic biochemical analyser (Roche, Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. The enzyme endpoint method was applied to measure the levels of FFA, TG, CHOL, HDL-C and LDL-C. An immunoturbidimetric assay was applied to measure the levels of ApoA1 and ApoB. The FFAs that we detected were nonesterified fatty acids (Wako Pure Chemical Corporation, Japan); in addition, TG, CHOL, HDL-C, LDL-C, ApoA1 and ApoB were detected by original reagents (Roche, Diagnostics, Indianapolis, USA).

Inclusion Criteria

Patients who were treated in our centre for localized and metastatic colorectal NETs by pathological diagnosis from 2010 to 2017.

Exclusion Criteria

1. Colorectal NETs combined with other malignancies. 2. Insufficient clinical information or inappropriate pathology reports from outside hospitals.

Statistical Analysis

Statistical analysis was performed using SPSS for Mac, version 21.0 or GraphPad Prism version 8.0. Continuous data are described as the means \pm SDs in this study. The relationship between pathological characteristics and FFAs as well as HDL-C was assessed using Pearson's χ^2 or Fisher's exact test. Overall survival (OS) was analysed with the Kaplan–Meier method. Univariable and multivariable analyses for survival outcomes were conducted using the Cox proportional hazards model. Statistical significance was accepted for p values < 0.05.

Results

In this study, data from 100 colorectal NET patients and 100 control persons matched by sex and age were analysed. The age of the patients was 54.1 ± 13.7 years, and the male: female ratio was 65 (65.0%):35 (35.0%). The number of grade G1, G2, and G3 NETs were 57 (57.0%), 14 (14.0%) and 29 (29.0%), respectively. Of the 100 patients, 40 (40.0%) patients were resected locally, 14 (14%) by ESD and 26 (26%) by transanal excision. In addition, 53 (53.0%) NETs were surgically resected, including 38 (38.0%) radical

resections, 8 (8.0%) multivisceral resections and 7 (7.0%) palliative resections due to distant metastasis. The remaining 7 (7.0%) patients were treated by systemic treatment due to distant metastasis. The most commonly used chemotherapeutic regimen in our centre was the EP regimen (etoposide and cisplatin) as the first-line chemotherapy, and the second-line chemotherapy was variable and included the XELOX regimen (oxaliplatin and capecitabine); the FOLFOX regimen (oxaliplatin, calciumfolate and 5-FU); and everolimus, temozolomide, and tegafur/gimeracil/oteracil and their combinations. The tumour diameter was less than 2 cm in 45(45.5%) patients, and LNM was found in 51(53.1%) patients. The clinical and histopathological characteristics are summarized in Table 1.

The pretreatment levels of FFA (0.55 ± 0.23 vs. 0.48 ± 0.11 , $P=0.006$), CHOL (4.84 ± 0.87 vs. 4.56 ± 0.66 , $P=0.011$) and ApoB (0.96 ± 0.26 vs. 0.89 ± 0.16 , $P=0.03$) in patients with colorectal NETs were higher than those in controls. The pretreatment levels of HDL-C (1.31 ± 0.41 vs. 1.41 ± 0.29 , $P=0.046$) and ApoA1 (1.18 ± 0.43 vs. 1.54 ± 0.17 , $P<0.001$) were lower in patients than in controls. There was no significant difference in the levels of TGs (1.33 ± 0.80 vs. 1.24 ± 0.48 , $P=0.346$) or LDL-C (3.10 ± 0.78 vs. 2.98 ± 0.65 , $P=0.251$) between patients and controls. The data are illustrated in Figure 1.

The clinical and laboratory parameters, as well as the comparison between the high FFA and low FFA groups, are shown in Table 2; similar parameters and comparisons with regard to HDL-C are also shown. Pearson's χ^2 test showed significant associations between FFA and lymph node metastasis ($\chi^2 = 5.964$, $P=0.015$) and between HDL-C and tumour size ($\chi^2 = 5.647$, $P=0.017$). However, no significant association was found between FFA and tumour size ($P=0.142$) or between HDL-C and lymph node metastasis ($P=0.443$). Neither FFA nor HDL-C was found to be significantly associated with sex ($P_{\text{FFA}}=0.542$, $P_{\text{HDL-C}}=0.157$), age ($P_{\text{FFA}}=0.096$, $P_{\text{HDL-C}}=0.940$), BMI ($P_{\text{FFA}}=0.841$, $P_{\text{HDL-C}}=0.799$), tumour grade ($P_{\text{FFA}}=0.613$, $P_{\text{HDL-C}}=0.594$), Syn ($P_{\text{FFA}}=0.926$, $P_{\text{HDL-C}}=0.411$), CgA ($P_{\text{FFA}}=0.546$, $P_{\text{HDL-C}}=0.214$), CD56 ($P_{\text{FFA}}=0.460$, $P_{\text{HDL-C}}=0.662$), or CK ($P_{\text{FFA}}=0.321$, $P_{\text{HDL-C}}=0.083$).

Table 2
The serological levels of FFAs and HDL-C in colorectal NETs with different pathological characteristics.

	FFA N (%)		HDL-C N (%)	
	χ^2 value	P value	χ^2 value	P value
	<0.52 mmol/L	\geq 0.52 mmol/L	\leq 1.0 mmol/L	>1.0 mmol/L
Sex				
Male	32(32%)	33(33%)	15(15%)	50(50%)
Female	15(15%)	20(20%)	4(4%)	31(31%)
	0.371	0.542 ^a	2.006	0.157 ^a
Age				
<65	38(38%)	35(35%)	14(14%)	59(59%)
\geq 65	9(9%)	18(18%)	5(5%)	22(22%)
	2.773	0.096 ^a	0.006	0.94 ^a
BMI				
<23	23(23%)	27(27%)	9(9%)	41(41%)
\geq 23	24(24%)	26(26%)	10(10%)	40(40%)
	0.4	0.841 ^a	0.065	0.799 ^a
Tumour grade				
G1	27(27%)	30(30%)	9(9%)	48(48%)
G2	5(5%)	9(9%)	3(3%)	11(11%)
G3	15(15%)	14(14%)	7(7%)	22(22%)
	0.979	0.613 ^a	1.131	0.594 ^b
Lymph node metastasis				
Negative	28(29.2%)	17(17.7%)	7(7.3%)	38(39.6%)
Positive	19(19.8%)	32(33.3%)	12(12.5%)	39(40.6%)
	5.964	0.015^a	0.958	0.443 ^a
Tumour Size				
<2 cm	25(25.3%)	20(20.2%)	4(4.0%)	41(41.4%)

	FFA N (%)		HDL-C N (%)	
≥2 cm	22(22.2%)	32(32.3%)	15(15.2%)	39(39.4%)
	2.16	0.142 ^a	5.647	0.017^a
Syn				
-	2(2.2%)	3(3.3%)	2(2.2%)	3(3.3%)
±	8(8.7%)	7(7.6%)	2(2.2%)	13(14.1%)
□	34(37.0%)	38(41.2%)	14(15.2%)	58(63.0%)
	0.414	0.926 ^b	1.809	0.411 ^b
CgA				
-	24(27.3)	26(29.5%)	13(14.8%)	37(42%)
±	13(14.8%)	11(12.5%)	2(2.3%)	22(25%)
□	5(5.7%)	9(10.2%)	3(3.4%)	11(12.5%)
	1.21	0.546 ^a	3.138	0.214 ^b
CD56				
-	7(8.3%)	12(14.3%)	5(6%)	14(16.7%)
±	6(7.1%)	4(4.8%)	1(1.2%)	9(10.7%)
□	27(32.1%)	28(33.3%)	11(13.1%)	44(52.4%)
	1.54	0.46 ^b	0.969	0.662 ^b
CK				
-	0(0%)	2(3%)	1(1.5%)	1(1.5%)
±	1(1.5%)	2(3%)	2(3%)	1(1.5%)
□	33(50%)	28(42.4%)	11(16.7%)	50(75.8%)
	2.386	0.321 ^b	5.048	0.083 ^b

Univariable and multivariable analyses were performed to analyse the potential prognostic factors for survival. The median follow-up period of this cohort was 70 months (range: 1–130 months). Univariable analysis showed that higher serum FFA levels (≥ 0.52 mmol/l) ($P=0.014$), lower serum HDL-C levels (≤ 1.0 mmol/L) ($P=0.022$), the presence of tumour grades G2 ($P=0.003$) and G3 ($P<0.001$), positive lymph node metastasis ($P<0.001$), tumour size larger than 2 cm ($P<0.001$), and age older than 65 years ($P=0.046$) were significantly associated with shorter OS. Multivariable analysis showed that tumour grade

G3 and positive lymph node metastasis in the FFA group ($P_{G3}=0.013$, $P_{LNM}=0.048$) and HDL-C group ($P_{G3}=0.017$, $P_{LNM}=0.016$) were significantly associated with shorter OS. There was no significant association between shorter OS and tumour size/FFA/HDL-C. The hazard ratio (HRs) and corresponding 95% confidence intervals are shown in Table 3.

Table 3

Univariable and multivariable analysis of prognostic factors for OS in patients with colorectal NETs.

Parameters	Univariable analysis		Multivariable analysis (FFA)		Multivariable analysis (HDL-C)	
	HR (95%CI)	Pvalue	HR (95%CI)	P value	HR (95%CI)	P value
Sex (male vs. female)	1.325(0.729-2.408)	0.355				
Age (<65 vs. ≥65)	1.863(1.011-3.432)	0.046				
BMI (<23 vs. ≥23)	1.354(0.752-2.438)	0.313				
Tumour Grade	2.295(1.652-3.19)	<0.001				
G1	Reference		Reference	0.044	Reference	0.056
G2	3.639(1.554-8.524)	0.003	1.986(0.825-4.779)	0.126	1.837(0.750-4.498)	0.183
G3	5.487(2.756-10.926)	<0.001	2.518(1.216-5.216)	0.013	2.465(1.178-5.159)	0.017
LNM (Neg vs. Pos)	10.545(4.433-25.082)	<0.001	3.081(1.008-9.415)	0.048	3.944(1.295-12.015)	0.016
Tumour Size (<2 vs. ≥2 cm)	14.519(5.166-40.802)	<0.001	3.684(0.965-14.071)	0.056	2.950(0.752-11.574)	0.121
FFA (high vs. normal)	1.958(1.063-3.608)	0.014	1.804(0.960-3.388)	0.067		
HDL-C (high vs. normal)	0.484(0.254-0.922)	0.022			0.632(0.314-1.275)	0.2

Kaplan-Meier survival curves were plotted to analyse the different OS periods in colorectal NETs. The log-rank (Mantel-Cox) test showed that patients with high tumour grade ($\chi^2 = 28.69$, $P<0.001$), positive lymph node metastasis ($\chi^2 = 41.43$, $P<0.001$), bigger tumours ($\chi^2 = 44.36$, $P<0.001$), higher FFA level ($\chi^2 = 6.016$, $P=0.014$), lower HDL-C level ($\chi^2 = 5.488$, $P=0.019$), and both higher FFA and lower HDL-C in serum ($\chi^2 = 4.818$, $P=0.028$) had worse overall survival. The Kaplan-Meier curves are drawn in Figure 4.

We found that 5-year survival declined from grades G1 to G3 (78.6%, 42.9% and 27.6%) and was lower for LNM positive patients (29.4% vs. 88.8%) and those with bigger tumours (31.5% vs. 89.1%), a high level of FFA (50.9% vs. 70.2%), low level of HDL-C (47.4% vs. 63.0%), and a combination of high FFA with low HDL-C (40.0% vs. 62.2%). In addition, the median survival also declined from grades G1 to G3 (105.1 ± 6.2 , 59.6 ± 12.1 and 42.0 ± 7.8) and was poorer for patients with LNM positivity (43.6 ± 6.2 vs. 118.0 ± 4.6), larger tumour size (46.2 ± 6.2 vs. 121.2 ± 4.2), high level of FFA (67.8 ± 7.3 vs. 94.7 ± 7.3), low level of HDL-C (53.8 ± 9.7 vs. 87.1 ± 6.0), and high FFA in combination with low HDL-C (45.9 ± 14.3 vs. 85.2 ± 5.7). The data are shown in Table 4.

Table 4

Five-year survival and median survival months at different levels of FFAs, HDL-C, tumour grade, LNM, tumour size and FFA in combination with HDL-C.

Parameters	5-year survival (%)	Median survival months (Mean±SD)
Tumour Grade		
G1	78.6	105.1±6.2
G2	42.9	59.6±12.1
G3	27.6	42.0±7.8
Lymphnode metastasis		
Negative	88.8	118.0±4.6
Positive	29.4	43.6±6.2
Tumour Size		
<2 cm	89.1	121.2±4.2
≥2 cm	31.5	46.2±6.2
FFA		
<0.52 mmol/L	70.2	94.7±7.3
≥0.52 mmol/L	50.9	67.8±7.3
HDL-C		
≤1.0 mmol/L	47.4	53.8±9.7
>1.0 mmol/L	63.0	87.1±6.0
FFA in combination with HDL-C		
FFA ≥0.52 +HDL-C ≤1.0	40.0	45.9±14.3
Others	62.2	85.2±5.7
Abbreviation: SD, standard deviation.		

Discussion

From a follow-up period of up to 130 months, we collected consecutive colorectal NET patients by pathological diagnosis from 2011-2017 and found that FFA ≥ 0.52 mmol/L can be considered a cut-off point for predicting LNM and poor OS, HDL-C ≤ 1.0 mmol/L can be considered another cut-off point for predicting tumour size ≥ 2 cm and poor OS, and the combined detection of FFA and HDL-C complementarily predicted OS in our study.

Tumour grade in colorectal NETs refers to the proliferative activity of neoplastic cells, as measured by the mitotic rate and/or the Ki-67 index, and differentiation refers to the extent to which tumour cells resemble their normal counterparts (Klimstra et al. 2010). Some studies have suggested that worse survival was found for advanced tumour grade, consistent with our results (Wu et al. 2020). The risk of metastases is very low (<3%) in rectal NETs < 10 mm and very high (30%-80%) in rectal NETs \geq 20 mm in size, while between these two extremes, 4%-20% of patients with rectal NETs measuring 10-19 mm have synchronous or metachronous metastases (de Mestier et al. 2019). In addition, tumour size and lymph node metastasis have also been shown to be predictors of poor prognosis in colorectal NETs (Fields et al. 2019; Sohn et al. 2017; Wu et al. 2020), consistent with our study.

Traditionally, the level of FFA has been detected to assess lipid metabolism and has been associated with hypertension, cardiovascular disease, type 2 diabetes, and obesity (Ghosh et al. 2017). Similarly, HDL-C, LDL-C, TG, CHOL, ApoA1) and ApoB, all widely used, were analysed to assess lipid metabolism in the body as combined biochemical indicators. Recently, an increasing number of researchers have indicated that FFAs and HDL-C are involved in colorectal cancer. FFAs are overexpressed in colorectal cancer (Zhu et al. 2021), dietary palmitic acid promotes cancer metastasis (Pascual et al. 2021), and the reprogramming of fatty acid metabolism plays an important role in LNM of various cancers by the fatty acid-binding protein 5 (FABP5) pathway (Kawaguchi et al. 2016; Zhang et al. 2020). Interestingly, lymph node metastasis and poor prognosis were significantly associated with the pretreatment FFA level of colorectal NETs, consistent with our results. Oxidative modification of HDL results in compositional and functional changes, and following increased cholesterol ester transfer protein (CETP) activity in parallel with decreased lecithin-cholesterol acyltransferase (LCAT) activity, HDL particles become larger, and changes in HDL composition, such as enrichment with TG and reduced ApoA1, paraoxonase-1 (PON1) and apoM and increased serum amyloid A (SAA) proteins, occur. The interaction of overexpressed SAA with TLR2 in cancer cells leads to cancer progression through the NF- κ B-mediated pathway (Ganjali et al. 2021). In addition, HDL-C was related to poor prognosis in patients with colorectal cancer, and very low levels of HDL-C (<30 mg/dL) in women were significantly associated with cancer mortality (Penson et al. 2019; Wang et al. 2019). In our study, tumour size (\geq 2 cm) was related to pretreatment HDL-C level, and worse overall survival was found in colorectal NETs with larger tumour size, consistent with the above researchers.

CgA, Syn and CD56 are three neuroendocrine differentiation (NED) immunohistochemistry markers frequently used in NETs (MacIntosh et al. 2015; Modlin et al. 2010; Wiedenmann 1991). The results of immunohistochemistry are usually marked by semiquantitative scores to show positive cell percentages and positive cell staining intensities but are limited to qualification and by pathologist experience. Reports of CgA, Syn and CD56 are difficult to standardize, and it is difficult to predict prognosis by immunohistochemistry results directly. CgA in serum is an important biomarker in advanced pancreatic cancer and metastatic neuroendocrine tumours (Malaguarnera et al. 2009; Warner et al. 2011), but due to the lower incidence of NETs (Cives and Strosberg 2018) and the high cost of detecting reagents, the serological CgA test has not been widely performed. FFA and HDL-C are common biochemical biomarkers

detected in clinical laboratories, and they have promising applications in predicting LNM and tumour size and predicting the OS of colorectal NETs.

In our study, the patient numbers of FFA ≥ 0.52 mmol/L and HDL-C ≤ 1.0 mmol/L were very low, and thus there may be some statistical bias associated with their combined detection. It is worth noting that patients who had FFA ≥ 0.52 mmol/L accounted for almost half of the total colorectal patients, and those with HDL-C ≤ 1.0 mmol/L accounted for almost one-fifth of the total; interestingly, patients with FFA ≥ 0.52 mmol/L and HDL-C ≤ 1.0 mmol/L also made up one-fifth of the patients with FFA ≥ 0.52 mmol/L. The median survival of patients with FFA ≥ 0.52 mmol/L was 67.8 ± 7.3 months, the median survival of those with HDL-C ≤ 1.0 mmol/L was 53.8 ± 9.7 months, and the median survival of those with FFA ≥ 0.52 mmol/L and HDL-C ≤ 1.0 mmol/L was only 45.9 ± 14.3 months. The 5-year survival of patients with FFA ≥ 0.52 mmol/L was 50.9%, that of patients with HDL-C ≤ 1.0 mmol/L was 47.4%, and that of patients with FFA ≥ 0.52 mmol/L in combination with HDL-C ≤ 1.0 mmol/L was only 40.0%. Therefore, patient with high FFA and low HDL-C had a worse prognosis than patients with only high FFA, only low HDL-C and patients with low FFA or high HDL-C. Some researchers have suggested that FFA and HDL-C may promote cancer progression by different signalling pathways (Ganjali et al. 2021; Kawaguchi et al. 2016; Pascual et al. 2021; Zhang et al. 2020), so the combined detection of FFAs and HDL-C may have a complementary effect, consistent with our findings.

Mixed adenoneuroendocrine carcinoma of the colon and rectum are rare cancers; they are characterized by the presence of a combination of epithelial and neuroendocrine elements, where each component represents at least 30% of the tumour (Qiu et al. 2018), and are an unmet area where NETs need to be described and defined (Ramage et al. 2019). In our study, there were more colorectal NET patients with positive LNM than with negative LNM, which may be due to not excluding mixed adenoneuroendocrine carcinoma among the patient groups, which is composed of poorly differentiated neuroendocrine carcinoma and easily metastasizes (Milione et al. 2018; Qiu et al. 2018).

Reduced plasma levels of HDL-C are a hallmark of obesity and cardiovascular diseases (CVDs); similarly, reduced ApoA1 has also been associated with cardiovascular risk (Raitakari et al. 2013; Su and Peng 2020). Due to component differences between ApoA1 and HDL-C, a similar association was not found for LNM, tumour size or poor survival with HDL-C. However, the level of ApoA1 in colorectal NETs was significantly lower than that in controls, and the area under the receiver operating characteristic (ROC) curve was 82.2%. Interestingly, the potential diagnostic ability is worth analysing.

There were some limitations in our study. First, it was of a retrospective design and included a relatively small number of patients. However, we believe the results are reliable. Because this study lasted more than 130 months, we could investigate the long-term survival outcomes and prognostic factors after different treatments, even with the small number of patients. Second, progression-free survival (PFS) data were not collected, and prognostic results could not be predicted comprehensively. Finally, further studies should be performed to validate our main conclusions.

Conclusions

FFA, CHOL, and ApoB were overexpressed in colorectal NETs, and HDL-C and ApoA1 were underexpressed. FFA ≥ 0.52 mmol/L can be considered a cut-off point to predict LNM and poor OS, HDL-C ≤ 1.0 mmol/L can be considered another cut-off point to predict tumour size ≥ 2 cm and poor OS, and the combined detection of FFA and HDL-C complementarily predicts OS.

Declarations

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval This article does not contain any studies with human participants performed by any of the authors. It was approved by the Medical Ethics Committee of Fujian Medical Union Hospital.

Informed consent Not applicable.

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Figures

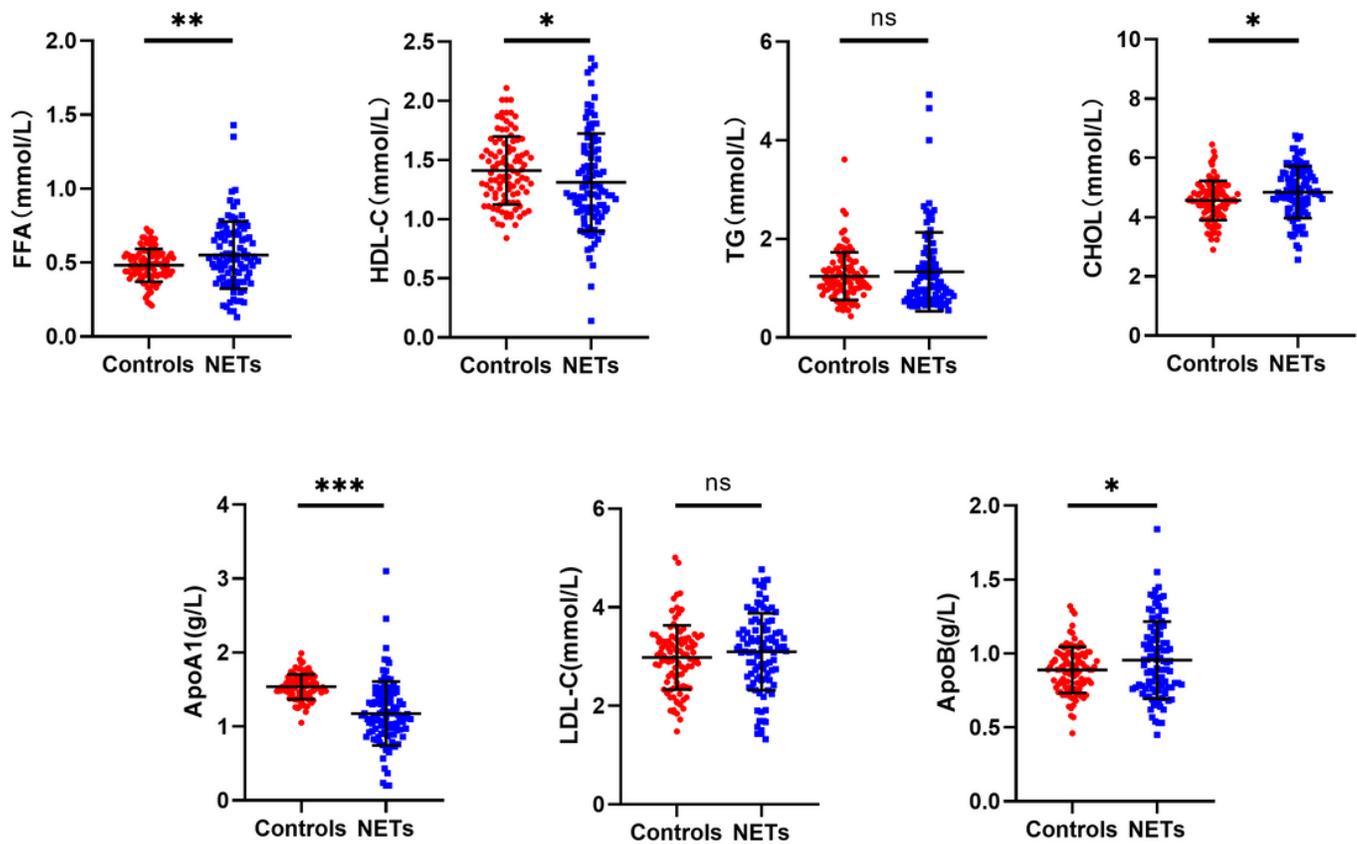


Figure 1

The pretreatment levels of FFA, HDL-C, TG, CHOL, ApoA1, LDL-C and ApoB in controls and patients with colorectal NETs. Controls: Normal persons matched by age and sex to patients with colorectal NETs one by one. Abbreviations: FFA, free fatty acid; HDL-C, how-density lipoproteincholesterol; TG, triglycerides;CHOL: cholesterol, LDL-C, low-density lipoproteincholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B.

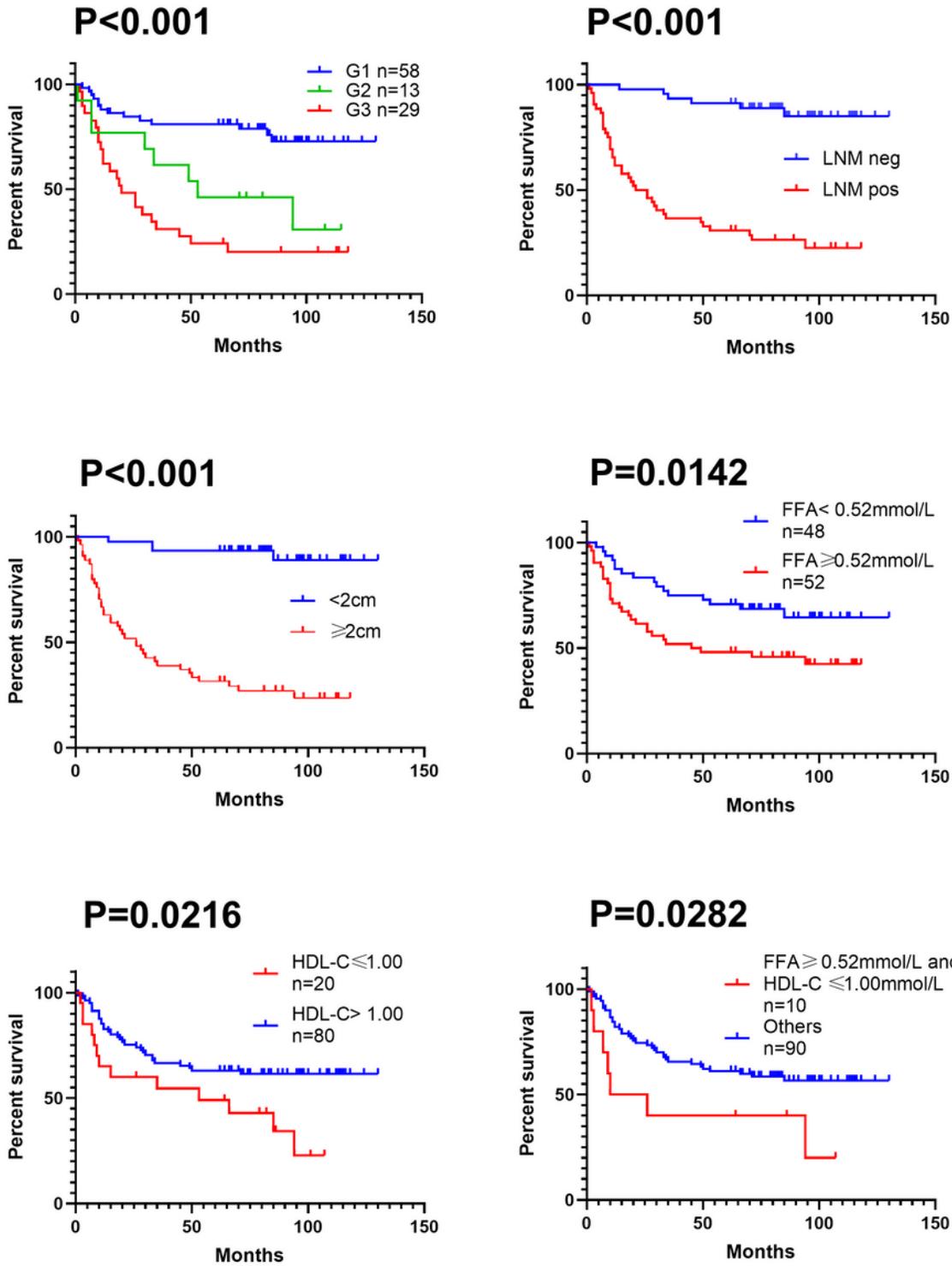


Figure 2

Kaplan-Meier survival curves for colorectal NET patients with different tumour grades, tumour sizes and lymph node metastases, as well as with respect to pretreatment FFA and HDL-C levels in serum.