The Ratio of Free Fatty Acids (Ffas) Divided By High-Density Lipoprotein Cholesterol (HDL-C) Is A Promising Pre-Treatment Biomarker For Predicting Worse Overall Survival (OS) of Neuroendocrine Tumours (Nets) In The Colorectum: A Retrospective Study.

Bin Zhu
Fujian Medical University Union Hospital https://orcid.org/0000-0001-7365-1371
Dan Wu
Fujian Medical University Union Hospital https://orcid.org/0000-0001-7161-0445
Yuanyuan Yang
Fujian Medical University Union Hospital https://orcid.org/0000-0002-1072-1835
Pingli Yu
Fujian Medical University Union Hospital https://orcid.org/0000-0001-7780-4443
Haobo Huang
Fujian Medical University Union Hospital https://orcid.org/0000-0003-0613-802X
Haiyan Cai
Fujian Medical University https://orcid.org/0000-0002-2818-4679
Yingping Cao (caoyingping918@sina.com)
Fujian Medical University Union Hospital https://orcid.org/0000-0002-0281-1515

Research Article

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Abstract

Background: free fatty acids (FFAs) and high-density lipoprotein cholesterol (HDL-C) were associated with various malignancy. However, whether FFA, HDL-C and FFA/HDL-C can play a potiential role in predicting patients with colorectal neuroendocrine tumours (NETs) was unclear. Meanwhile, FFA/HDL-C has a superior prognosis ability was unknown, too.

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, HDL-C and FFA/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) (P=0.015), HDL-C ≤1.0 mmol/L predicted tumour size ≥2 cm (P=0.017), and FFA/HDL-C>0.75 predicted tumour grade (P=0.030), LNM (P=0.014), and tumour size(P=0.018). No significant association was found between FFA and tumour grade (P=0.613) or HDL-C and tumour grade (P=0.594) or FFA and tumour size (P=0.142) or HDL-C and LNM (P=0.443). FFA ≥0.52 mmol/L (P=0.014) and HDL-C ≤1.0 mmol/L predicted worse overall survival (OS) (P=0.019). FFA/HDL-C predicted an even worse prognosis in terms of OS (P<0.001).

Conclusion: FFA ≥0.52 mmol/L HDL-C ≤1.0 mmol/L and FFA/HDL-C>0.75 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” in1907, are heterogeneous neoplasms arising in secretory cells of the diffuse neuroendocrine system(1). The Surveillance, 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 (2-4). 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(5, 6).

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(7). FFAs are overexpressed in colorectal patients (8), and abnormal fatty acid metabolism is associated with tumour cell metastasis in various cancers, including colorectal cancer (9, 10). High-density lipoprotein (HDL) is responsible for the reverse

transport of cholesterol from peripheral cells to the liver,andassociated apolipoproteins and enzymes can also exert antioxidant, anti-inflammatory, antiangiogenic, antiapoptotic and immunomodulatory activities, resulting in overall antitumorigenic effects(11). 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(12, 13).

According to the updated guidelines for colorectal NETs in 2016(14), tumour grade and size were only significant in univariate analysis, size remains a less than totally reliable discriminator of prognosis, and factors predicting LNM remain more researched. Currently, more reliable serological indicators that can predict prognosis in colorectal NETs have not been deeply demonstrated. In clinical work, we found that in some cancer patients, traditional tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were elevated tens of times compared with the upper limit of the healthy reference, while the standard deviations of lipid metabolism biomarkers in patients and controls were relatively similar. Is there a lipid biomarker with an advantage similar to that of traditional tumour markers? In addition, evidence for the prognostic ability of FFAs, HDL-C, and FFA/HDL-C and their relationship in colorectal NETs has never been reported. We designed this study to evaluate the prognostic ability of FFA, HDL-C and FFA/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.

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% to 2%, intermediate-grade (G2) tumours have a mitotic rate from 2 to 20 per 10 HPFs or a Ki-67 index from 3% to 20%, and high-grade (G3) tumours have a mitotic rate greater than 20 per 10 HPFs or a Ki-67 index greater than 20%(15). 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 was labelled (-); a small number of positive cells or weakly positive cell staining intensity was labelled (±); and the majority of positive cells or strongly positive cell staining intensity was labelled (+).

Laboratory testing

FFAs, 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 FFAs, TGs, 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 in appropriate 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 ± 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 numbers 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 pre-treatmentlevels of FFAs (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), ApoB (0.96±0.26 vs. 0.89±0.16, P=0.030) and FFA/HDL-C (0.51±0.61 vs. 0.38±0.11, P=0.027) in patients with colorectal NETs were higher than those in controls. The pre-treatment 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 (χ^2=5.964, P=0.015) and between HDL-C and tumour size (χ^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). There were significant associations of FFA/HDL-C with tumour grade (χ^2=4.734, P=0.030), lymph node metastasis (χ^2=6.032, P=0.014), and tumour size (χ^2=5.583, P=0.018). Neither FFA, HDL-C nor FFA/HDL-C was found to be significantly associated with sex (P_{FFA}=0.542, P_{HDL-C}=0.157, $P_{FFA/HDL-C}$=0.297), age (P_{FFA}=0.096, P_{HDL-C}=0.940, $P_{FFA/HDL-C}$=0.331), BMI (P_{FFA}=0.841, P_{HDL-C}=0.799, $P_{FFA/HDL-C}$=1.0), tumour grade (P_{FFA}=0.613, P_{HDL-C}=0.594), Syn (P_{FFA}=0.926, P_{HDL-C}=0.411, $P_{FFA/HDL-C}$=0.854), CgA (P_{FFA}=0.546, P_{HDL-C}=0.214, $P_{FFA/HDL-C}$=0.534), CD56 (P_{FFA}=0.460, P_{HDL-C}=0.662, $P_{FFA/HDL-C}$=0.254), or CK (P_{FFA}=0.321, P_{HDL-C}=0.083, $P_{FFA/HDL-C}$=0.071).

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), higher serum FFA/HDL-C levels (>0.75) (P<0.001), 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), HDL-C group (P_{G3}=0.017, P_{LNM}=0.016) and FFA/HDL-C group (P_{G3}=0.021, P_{LNM}=0.037) were significantly associated with shorter OS. There was no significant association between shorter OS and tumour size/FFA/HDL-C/FFA divided by HDL-C. The hazard ratios (HRs) and corresponding 95% confidence intervals are shown in Table 3.

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 (χ^2=28.69, P<0.001), positive lymph

node metastasis (χ^2=41.43, P<0.001), larger tumours (χ^2=44.36, P<0.001), higher FFA level (χ^2=6.016, P=0.014), lower HDL-C level(χ^2=5.488, P=0.019), both double-expressed patients (higher FFA and lower HDL-C) (χ^2=4.818, P=0.028) and higher FFA/HDL-C level (χ^2=12.528, P<0.001) had worse overall survival. The 5-year survival of FFA/HDL-C in different score groups (0.75-1, 0.5-0.75, 0.25-0.5, 0-0.25) decreased gradually (P=0.004). 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 larger tumours (31.5% vs. 89.1%), a high level of FFAs (50.9% vs. 70.2%), a low level of HDL-C (47.4% vs. 63.0%), double-expressed patients (high FFAs with low HDL-C) (40.0% vs. 62.2%) and FFAs/HDL-C (30.0% vs. 63.3%). 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), high double-expressed patients (high FFA with low HDL-C) (45.9±14.3 vs. 85.2±5.7) and FFA/HDL-C (35.3±13.7 vs. 86.7±5.5). The data are shown in Table 4.

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 mml/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 FFA/HDL-C >0.75 predicted tumour grade, LNM, tumour size and even worser OS in our study.

According to the updated guidelines for colorectal NETs in 2016(14), tumour grade, size, symptoms, and treatment modality were only significant in univariate analysis, and stage was the strongest predictor of survival in multivariate analysis; on the one hand, tumour size and depth can predict LNM; on the other hand, size remains a less than totally reliable discriminator of prognosis, and factors predicting LNM remain more researched. Currently, whether serological indicators can predict prognosis in colorectal NETs has not been deeply demonstrated. In our study, we identified a new biomarker of FFA/HDL-C that has a promising ability to predict prognosis as well as tumour grade, size and LNM. In addition, the predictions of FFA, HDL-C and FFA/HDL-C were relatively independent and complementary, and the OS prognosis of FFA/HDL-C was even worse than that of FFA or HDL-C. Combined detection of FFA and HDL-C is a promising biomarker in colorectal NETs, and it may benefit new guidelines in the future.

In clinical work, we found that in some cancer patients, traditional tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were elevated tens of times compared with the upper limit of the healthy reference, while the standard deviations of lipid metabolism biomarkers in patients and controls were relatively similar. In our study, we found that the FFA/HDL-C ratio of cancer patients has more scatter points than other lipid metabolism biomarkers, as shown in Figure 1. These scatter points, which have similar changes in FFA/HDL-C to CEA and CA19-9, account for almost one tenth of the scatter points. Similarly, we found that one-tenth of patients whose FFA/HDL-C >0.75 had a worse prognosis than the others, as shown in Figure 2. Interestingly, these scatter points may play an important role in predicting tumour grade, size, LNM and poor prognosis.

Furthermore, the patient numbers of FFAs≥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/Land 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, patients 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. FFA/HDL-C predicted an even worse prognosisin terms of OS. Some researchers have suggested that FFAs and HDL-C may promote cancer progression by different signalling pathways(9, 10, 16, 17), so the combined detection of FFAs and HDL-C may have a complementary effect, consistent with our findings.

Traditionally, the level of FFAshas been detected to assess lipid metabolism and has been associated with hypertension, cardiovascular disease, type 2 diabetes, and obesity(18).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 (8), dietary palmitic acid promotes cancer metastasis (17), and the reprogramming of fatty acid metabolismplays an important role in LNM of various cancers by the fatty acid-binding protein 5 (FABP5) pathway (9, 10).Interestingly, lymph node metastasis and poor prognosis were significantly associated with the pre-treatment 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 throughthe NF-κB-mediated pathway (16). 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(12, 13). In our study, tumour size (≥2 cm) was related to pre-treatment HDL-C level, and worse overall survival was found in colorectal NETs with larger tumour size, consistent with the above researchers.

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(19, 20). 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. In addition, no significant association with pathological characteristics was found in CHOL, ApoA1 and ApoB in our study.

CgA (chromogranin A), synaptophysin (Syn) and CD56 are three neuroendocrine differentiation (NED) immunohistochemistry markers frequently used in NETs (21-23). 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(24, 25), but due to the lower incidence of NETs(26) 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.

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(27) and are unmet areas where NETs need to be described and defined(28). In our study, there were more colorectal NET patents 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(27, 29).

There were some limitations in our study. First, it was of a retrospective design and included are 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 mml/L can be considered a cut-off point to predict LNM and poor OS, HDL-C≤1.0 mmol/L can be considered a cut-off point to predict tumour size ≥2 cm and poor OS, and FFA/HDL-C >0.75 can be considered another cut-off point to predict tumour grade, tumour size, LNM and poor OS.

Abbreviation

FFA: free fatty acid; HDL-C: How-density lipoprotein cholesterol; TG: triglycerides; CHOL: cholesterol; LDL-C: low-density lipoprotein cholesterol; ApoA1: apolipoprotein A1; ApoB: apolipoprotein B; OS: overall survival; PFS: progression-free survival; ROC: receiver operating characteristic; NETs: neuroendocrine tumours; SEER: Surveillance, Epidemiology and End Results; LNM: lymph node metastasis; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; CgA: chromogranin A; Syn: synaptophysin; FABP5: fatty acid-binding protein 5; CETP: Cholesterol ester transfer protein; LCAT: lecithin–cholesterol acyltransferase; PON1: paraoxonase-1 ; SAA: serum amyloid A.

Declarations

Acknowledgements

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Authors' contributions

Bin Zhu, Haobo Huang and Yingping Cao wrote and edited the manuscript; Yingping Cao provided study material; Bin Zhu, Dan Wu, Pingli Yu, Haobo Huang, Haiyan Cai and Yuanyuan Yang analysed and interpreted the data; all authors reviewed and approved the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Fujian Medical University Union Hospital (2021KJCX007). The consent from patients to participate in this study was waived since the present study assessed retrospective data and did not affect the treatments of patients.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests. The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

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Tables

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%)
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%)

Abbreviation: NET: neuroendocrine tumour; BMI, body mass index; ESD:endoscopic submucosal dissection; Syn: synaptophysin; CgA□chromogranin A; CD56: cluster of differentiation 56; CK: cytokeratin.

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

Parameters	FFA N (%)		HDL-C N (%)		FFA/HDL-CN (%)	
	X^2 value	P value	X^2 value	P value	X^2 value	P value
	<0.52 mmol/L	≥0.52 mmol/L	≤1.0 mmol/L	>1.0 mmol/L	>0.75	≤0.75
Gender						
Male	32(32%)	33(33%)	15(15%)	50(50%)	8(8%)	57(57%)
Female	15(15%)	20(20%)	4(4%)	31(31%)	2(2%)	33(33%)
	0.371	0.542[a]	2.006	0.157[a]	1.088	0.297[b]
Age						
<65	38(38%)	35(35%)	14(14%)	59(59%)	6(6%)	67(67%)
≥65	9(9%)	18(18%)	5(5%)	22(22%)	4(4%)	23(23%)
	2.773	0.096[a]	0.006	0.94[a]	0.943	0.331[b]
BMI						
<23	23(23%)	27(27%)	9(9%)	41(41%)	5(5%)	45(45%)
≥23	24(24%)	26(26%)	10(10%)	40(40%)	5(5%)	45(45%)
	0.4	0.841[a]	0.065	0.799[a]	0	1.0[a]
Tumour grade						
G1	27(27%)	30(30%)	9(9%)	48(48%)	2(2%)	55(55%)
G2	5(5%)	9(9%)	3(3%)	11(11%)	3(3%)	11(11%)
G3	15(15%)	14(14%)	7(7%)	22(22%)	5(5%)	24(24%)
	0.979	0.613[a]	1.131	0.594[b]	4.734	**0.030[b]**
Lymph node metastasis						
Negative	28(29.2%)	17(17.7%)	7(7.3%)	38(39.6%)	1(1.0%)	44(45.8%)
Positive	19(19.8%)	32(33.3%)	12(12.5%)	39(40.6%)	9(9.4%)	42(43.8)
	5.964	**0.015[a]**	0.958	0.443[a]	6.032	**0.014[b]**
Tumour Size						
<2 cm	25(25.3%)	20(20.2%)	4(4.0%)	41(41.4%)	1(1.0%)	44(44.4%)
≥2 cm	22(22.2%)	32(32.3%)	15(15.2%)	39(39.4%)	9(9.1%)	45(45.5%)
	2.16	0.142[a]	5.647	**0.017[a]**	5.583	**0.018[b]**

Syn						
-	2(2.2%)	3(3.3%)	2(2.2%)	3(3.3%)	0(0%)	5(5.4%)
±	8(8.7%)	7(7.6%)	2(2.2%)	13(14.1%)	2(2.2%)	13(14.1%)
+	34(37.0%)	38(41.2%)	14(15.2%)	58(63.0%)	8(8.7%)	64(69.6%)
	0.414	0.926[b]	1.809	0.411[b]	0.034	0.854[b]
CgA						
-	24(27.3)	26(29.5%)	13(14.8%)	37(42%)	7(8.0%)	43(48.9%)
±	13(14.8%)	11(12.5%)	2(2.3%)	22(25%)	2(2.3%)	22(25.0%)
+	5(5.7%)	9(10.2%)	3(3.4%)	11(12.5%)	1(1.1%)	13(14.8%)
	1.21	0.546[a]	3.138	0.214[b]	0.387	0.534[b]
CD56						
-	7(8.3%)	12(14.3%)	5(6%)	14(16.7%)	4(4.7%)	15(17.9%)
±	6(7.1%)	4(4.8%)	1(1.2%)	9(10.7%)	1(1.2%)	9(10.7%)
+	27(32.1%)	28(33.3%)	11(13.1%)	44(52.4%)	5(6.0%)	50(59.5%)
	1.54	0.46[b]	0.969	0.662[b]	1.302	0.254[b]
CK						
-	0(0%)	2(3%)	1(1.5%)	1(1.5%)	1(1.5%)	1(1.5%)
±	1(1.5%)	2(3%)	2(3%)	1(1.5%)	1(1.5%)	2(3.0%)
+	33(50%)	28(42.4%)	11(16.7%)	50(75.8%)	5(7.6%)	56(84.8)
	2.386	0.321[b]	5.048	0.083[b]	4.982	0.071[b]

a: It was detected by Pearson's chi-squared test; b:It was detected by Fisher's exact test. Abbreviations: NET: neuroendocrine tumour; BMI, body mass index; Syn: synaptophysin; CgA: chromogranin A; CD56: cluster of differentiation 56; CK: cytokeratin. G1:a mitotic rate from 0 to 1 per 10high-power fields (HPFs) or a Ki-67 index from 0% to 2%; G2: a mitotic rate from 2 to 20 per 10 HPFs or a Ki-67 index from 3% to 20%; G3: a mitotic rate greater than 20 per 10 HPFs or a Ki-67 index greater than 20%.

Table 3 Univariable and multivariable analysis of prognostic factors for OS in patients with colorectal NETs.

Parameters	Univariate analysis		Multivariate analysis (FFA)		Multivariate analysis (HDL-C)		Multivariate analysis (FFA/HDL-C)	
	HR (95%CI)	P value	HR (95%CI)	Pvalue	HR (95%CI)	Pvalue	HR (95%CI)	Pvalue
Gender (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	Reference	0.069
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	1.826 (0.746-4.469)	0.187
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**	2.370 (1.137-4.938)	**0.021**
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**	3.296 (1.078-10.083)	**0.037**
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	3.438 (0.848-13.933)	0.084
FFA (high vs. normal)	1.958 (1.063-3.608)	**0.014**	1.804 (0.960-3.388)	0.067				
HDL-C	0.484	**0.022**			0.632	0.2		

(high vs. normal)	(0.254-0.922)		(0.314-1.275)		
FFA/HDL-C	3.630	**<0.001**		2.015	0.083
(high vs. normal)	(1.685-7.819)			(0.913-4.445)	

Abbreviations: BMI, body mass index;LNM: lymph node metastasis;HR: hazard ratio.

Table 4 Five-year survival and median survival months atdifferent levels of FFAs, HDL-C,FFA/HDL-C, tumour grade, LNM, tumour size and FFA in combination with HDL-C.

Parameters	5-year survival (%)	Median survivalmonths (Mean±SD)	P value
Tumour Grade			
G1	78.6	105.1±6.2	
G2	42.9	59.6±12.1	
G3	27.6	42.0±7.8	**<0.001**
Lymphnode metastasis			
Negative	88.8	118.0±4.6	
Positive	29.4	43.6±6.2	**<0.001**
Tumour Size			
<2 cm	89.1	121.2±4.2	
≥2 cm	31.5	46.2±6.2	**<0.001**
FFA			
<0.52 mmol/L	70.2	94.7±7.3	
≥0.52 mmol/L	50.9	67.8±7.3	**0.027**
HDL-C			
>1.0 mmol/L	63.0	87.1±6.0	
≤1.0 mmol/L	47.4	53.8±9.7	**0.024**
Double-expressed patients			
Others	62.2	85.2±5.7	
FFA ≥0.52 +HDL-C ≤1.0	40.0	45.9±14.3	**0.026**
FFA/HDL-C			
≤0.75	63.3	86.7±5.5	
>0.75	30.0	35.3±13.7	**<0.001**

Abbreviation: SD, standard deviation.

Figures

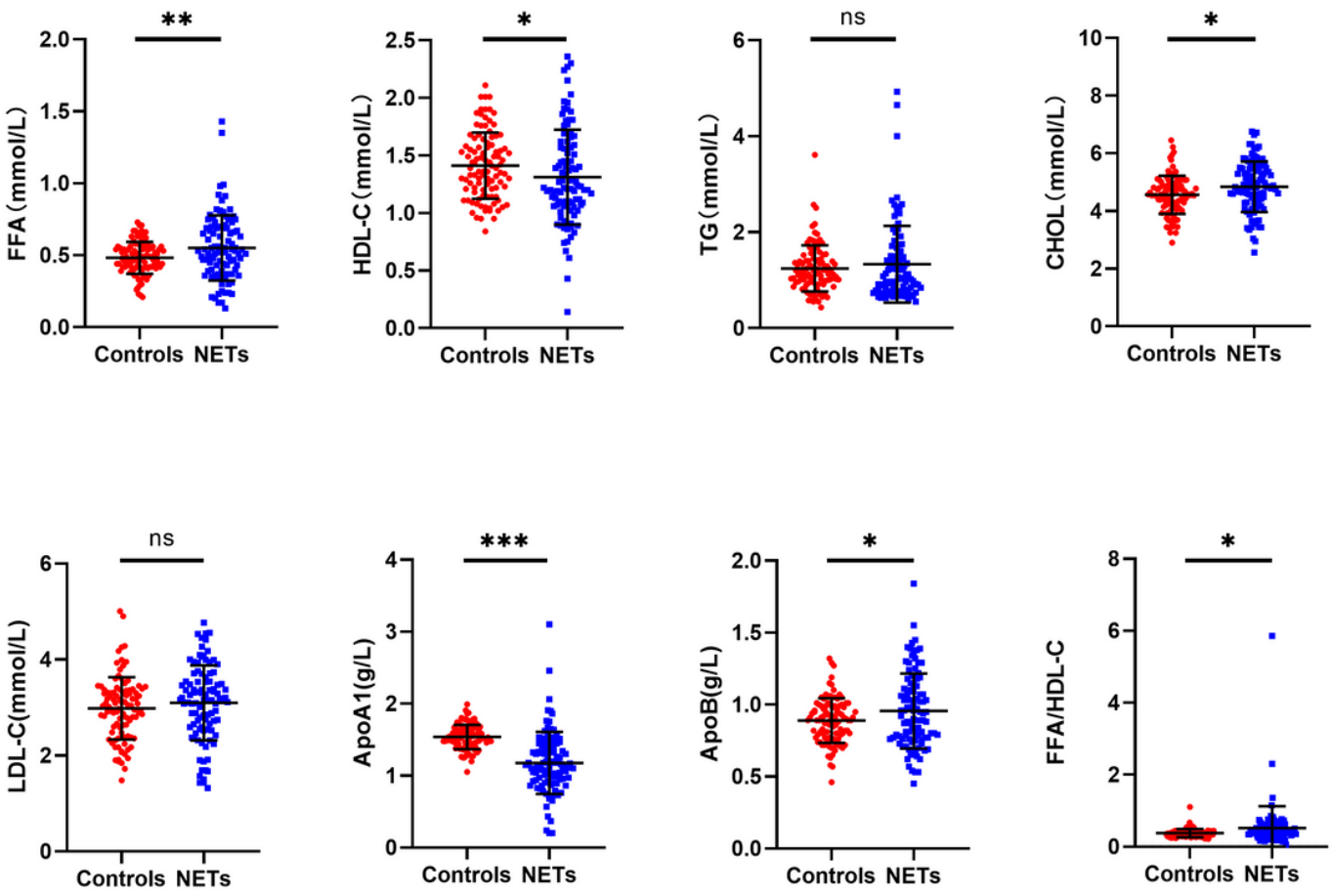

Figure 1

The pre-treatment levels of FFA, HDL-C, TG, CHOL, ApoA1, LDL-C and ApoB in controls and patients with colorectal NETs.

Controls: Normal persons were matched by age and sex to patients with colorectal NETs one by one. Abbreviations: FFA, free fatty acid; HDL-C, How-density lipoprotein cholesterol; TG, triglycerides; CHOL: cholesterol, LDL-C, low-density lipoprotein cholesterol; 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 pre-treatment FFA, HDL-C and FFA/HDL-C levels in serum; the relationship between 5-year survival and FFA/HDL-C level was also drawn above.