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Prognostic factors of patients with AFP-positive colorectal cancer: a case-control study

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Background: α-Fetoprotein-positive colorectal cancer (AFPP-CRC) is a rare type of colorectal cancer (CRC), and there have been no comprehensive investigations on prognostic factors of AFPP-CRC. The aim of this study was to elucidate the prognostic factors of AFPP-CRC.

Method: During the years of 2010 to 2020, 127 CRC patients with preoperative elevated serum AFP level were collected, after excluding the diagnosis of hepatitis, hepatocirrhosis and hepatocarcinoma. The AFPP-CRC group was matched by 1:2 to the AFPN(α-Fetoprotein-negative)-CRC group after propensity score matching (PSM) analysis. Prognostic factors were investigated using univariate and multivariate Cox regression model. Kaplan-Meier curves were performed among the concerned prognostic factors as well. Logistic regression was used in studying associated factors between good and poor prognosis groups of AFPP-CRC.

Result: After adjusting for other confounding factors by PSM, AFP positivity and tumor stage were shown to be associated with poorer disease-free survival (DFS). The median of overall survival (OS) was 26.4 months versus 30.3 months when comparing AFPP-CRC group versus AFPN-CRC group (P=0.09). The median of DFS was 23.3 months in the AFPP-CRC group, as compared to 26.0 months in the AFPN-CRC group (HR:1.77, 95%CI:1.22-2.57, P=0.003). Among AFPP-CRC patients, those who also had poor prognosis were characterized by microsatellite stability even after considering other confounding factors (OR, 0.18; 95%CI, 0.04-0.63; P=0.01).

Conclusion: We found higher serum AFP level before surgery was associated with worse DFS in patients with CRC, even adjusting for tumor stage. Besides, AFPP-CRC with microsatellite stability might had a worse prognosis.

Key words: alpha-fetoprotein; colorectal cancer; clinicopathologic features; prognosis

Introduction

Alpha-fetoprotein (AFP) was first discovered in a human fetus in 1956(1). It is defined as a glycoprotein deriving from fetal liver, yolk sac and gastrointestinal tract(2), which is synthesized from 6 months of pregnancy to birth (3). AFP has a high concentration in the fetal blood circulation but decreases after birth, making it difficult to detect it in the blood(4). Elevated serum AFP level over 1 year old might indicate the presence of hepatocellular carcinoma (HCC) or yolk sac tumor, so elevated serum AFP level in adults is usually considered as abnormal(5). In clinical practice, AFP commonly serves as a well-known tumor marker to screen or monitor HCC and yolk sac tumor. However, apart from HCC and yolk sac tumors, other tumors are also associated with high serum levels of AFP, such as gastric cancers(6, 7), along with colorectal cancers (CRC)(8), gallbladder cancers(9), bladder cancers(10) and lung cancers(11).

Since Nakajima first detected AFP positive in a colorectal cancer with liver and lung metastasis in 1985(12), only a few cases of AFP-producing colorectal cancer have been reported(13-25). Ren et al. found that AFP-producing CRC had distinctive clinicopathological characteristics, showing an aggressive biological behavior and worse prognosis than traditional CRC(21). Besides, Ren et al. considered that serum AFP was significantly higher in patients with lymphatic metastasis than in patients without lymphatic metastasis. Feng et al. also found that AFP-positivity was a significant negative predictor of overall survival (OS) in patients with CRC as compared with stage-matched CRC not producing AFP(22). All these studies indicated that AFP-producing CRC had high malignant potential.

However, there is no standardized available definition as well as a few cases of AFP-positive CRC so far. Studies mentioned above were limited in the number of patients and the results were not convincing. Due to small and heterogeneous cohort size and significantly different baseline characteristics, most of previous reports could not provide data for a direct comparison between AFP-positive colorectal cancer AFPP-CRC and AFP-negative colorectal cancer (AFPN-CRC). Furthermore, neoadjuvant therapy of chemo/radiotherapy might affect the level of serum AFP. Herein, we attempted to elucidate the prognostic factors of AFPP-CRC after strict patients' inclusion and exclusion. To be noted, we used a statistical method called propensity score matching (PSM) to reduce the possible bias and the influence of mixed variables, so as to make the comparison between the experimental group and the control group more reasonable(26). The aim of this study was to elucidate the prognostic factors of AFPP-CRC.

Method

Patients

We studied patients diagnosed with colorectal adenocarcinoma in the Sixth Affiliated Hospital of Sun Yat-sen University from 2010 to 2020. Patients who were tested serum AFP preoperatively and without preoperative chemotherapy, radiotherapy or chemoradiotherapy were considered as AFP-positive(27) group when the level of serum AFP levels were greater than 8.78 ng/ml based on the reference of this variable in our hospital. The inclusion criteria were as follows: 1. Patients aged 18-90 years old who were diagnosed with colorectal adenocarcinoma without any treatment for carcinoma before; 2. Data on clinical stage T, clinical stage N, and clinical stage M were available; 3. Preoperative serum AFP was tested. Meanwhile, the exclusion criteria were as follows: 1. Patients who underwent chemotherapy, radiotherapy or chemoradiotherapy before the surgery; 2. Patients with concomitant diseases that were associated with increasing serum AFP levels (i.e., hepatitis, fatty liver, cirrhosis, alcoholic liver, hepatocellular carcinoma (HCC) or yolk sac tumor); 3. Pregnant women; 4. Patients were diagnosed other types of malignancy such as embryogenic tumor of gonad, gastric tumor, pancreatic tumor etc; 5. Patients who had incomplete clinical data. According to the above criteria, a total of 127 patients were identified as AFP-positive and enrolled in the present study. Propensity score matching (PSM) for reduction of intergroup disparities was performed using R 4.0.2 software. The AFPP-CRC group was matched by 1:2 to the AFPN-CRC group after propensity score matching (PSM) analysis. The flow chart of patient's selection was shown in Figure1.The research was approved by the Ethics Review Committee of the Sixth Affiliated Hospital of Sun Yat-sen University, as we anonymously retrieved data from electronic databases and therefore did not give informed consent.

Data collection and outcome definition

The clinicopathological characteristics, including age, sex, body mass index (BMI), preoperative serum level of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA199), carbohydrate antigen 12-5 (CA125) and AFP, number of primary tumors, tumor location, liver metastasis, pathologic stage, histological differentiation, tumor deposits, peritoneal implantation, serosal invasion, vascular infiltration, perineural invasion, microsatellite status, PIK3A mutation status, BRAF mutation status, KRAS mutation status and post-operative chemotherapy scheme were recorded. In our study, according to the BMI classification in China, we classified the patients as underweight (BMI <18.5 kg/m^2), normal body weight (BMI 18.5-23.9 kg/m^2), overweight and obesity (BMI ≥ 23.9 kg/m^2). Cut-off point for serum

CEA was 5 ng/ml, serum CA199 was 37 U/ml, and serum CA125 was 35 U/ml, all of which were the upper limit of normal reference value in our hospital laboratory. For the definition of tumor location, we stipulated that the right colon included cecum, ascending colon and 2/3 of proximal transverse colon, while the left colon included 1/3 of distal transverse colon, the descending colon and sigmoid colon. Staging was performed according to the American Joint Committee on Cancer (AJCC) TNM Staging Classification for Carcinoma of the Colon and rectum (Eighth Edition).(28) Overall survival (OS) was defined as the time from surgery to death or final follow-up. Disease-free survival (DFS) was defined as the time from surgery to metastasis or recurrence of tumor. Based on the definition of whether the OS is more than 3 years, the patients were divided into good prognosis group and poor prognosis group.

Follow-up

When it came to follow-up and outcomes, we conducted two rounds of telephone interviews to track vital status and cause of death for those who had no vital status information. We telephoned participants who did not answer at least 3 times separately were classified as missing and excluded them from data analysis. In our study, all patients answered our telephone interviews at last. The last follow-up time were March 23 at the year of 2021.

Statistical Analysis

We utilized a Person's χ^2 test to measure the differences among variables according to baseline characteristics. Survival curves were estimated by the Kaplan-Meier method and differences were evaluated by the log-rank test. Prognostic factors were investigated using univariate and multivariate Cox regression model. Furthermore, univariate analysis and multivariate logistic regression were used to estimate potential risk factors for good and poor prognosis in AFPP-CRC patients. Sensitivity analysis was used to verify the results. The accepted level of significance was $P < 0.05$. SPSS 22.0 package (IBM, Chicago, IL, USA) and R 4.0.2 software were used for statistical analyses.

Result

1. Baseline characteristics

A total of 127 patients with AFP positive were enrolled in the study. AFPP-CRC patients (n=127) were matched to AFPN-CRC patients (n=254) using the nearest neighbor matching within a caliper of 0.001 by PSM. Variables included in the PSM were age, sex, BMI, clinical stage T, clinical stage N and clinical stage M. The clinicopathological characteristics of those patients were summarized in Table 1.

After PSM, baseline characteristics (age, sex, BMI, clinical stage T, clinical stage N and clinical stage M) used for matching were well-balanced. As shown in Table 1, compared with AFPN-CRC, patients with AFPP-CRC had higher level of preoperative CEA (44.1% vs. 29.5%, *P*=0.005), CA199 (24.4% vs 13.4%, *P*=0.007), CA125 (15.7% vs. 8.3%, *P*=0.03). Notably, most of the tumors in AFPP- CRC patients were located on the left side (50.4%), while those in AFPN-CRC patients were located on the rectum (53.1%, *P*<0.001). Besides, the number of primary tumor were more in AFPP-CRC than in AFPN- CRC (8.7% vs.3.5%, *P*=0.04).

Moreover, we observed more AFPP-CRC patients received post-operative chemotherapy as compared with AFPN-CRC patients (66.1% vs. 50.0%), along with multi-chemotherapy (61.4% vs. 46.1%). No significant differences were found according to liver metastasis, TNM stage, histological differentiation, tumor deposits, peritoneal implantation, serosal invasion, perineural invasion, microsatellite status, PIK3CA mutation status, BRAF mutation status, and KRAS mutation status.

2. OS and DFS among the recruited patients

The median follow-up of the recruited 381 patients was 38.6 months. The median of OS was 26.4 months versus 30.3 months when comparing AFPP-CRC group versus AFPN-CRC group. There was no significant difference between AFPN-CRC and AFPP-CRC patients with regard to the OS (*HR*:1.59, 95%*CI*:0.93-2.73, *P*=0.09). (Fig.2A) The DFS in AFPP-CRC group (23.3 months) was lower as compared with the AFPN-CRC group (26.0months). The AFPP-CRC patients exhibited a trend toward worse DFS (*HR*:1.77, 95%*CI*:1.22-2.57, *P*=0.003). (Fig.2B) Besides, log-rank test confirmed DFS was significantly different between the two groups (*P* = 0.009). 5-year DFS rate was 15.8% for AFPN-CRC patients and 15.0% for AFPP-CRC patients.

We further investigated prognostic factors affecting DFS in those recruited patients. In univariate analysis, we found post-operative multi-chemotherapy (*P*=0.04), pre-operative serum CEA level (*P*<0.001), pre-operative serum CA199 level (*P*<0.001), pre-operative serum CA125 level (*P*<0.001), pre-operative serum AFP level(*P*=0.008), liver metastasis (*P*<0.001), pathologic stage III (*P*<0.001) & IV (*P*<0.001), poorly and undifferentiated histology (*P*<0.001), peritoneal implantation (*P*<0.001), serosal invasion (*P*<0.001), vascular infiltration (*P*<0.001), perineural invasion (*P*<0.001), tumor deposits (*P*<0.001), BRAF mutation status (*P*=0.01) were significantly negatively associated to DFS. Multivariate analysis revealed that post-operative chemotherapy, pre-operative serum AFP level and TNM stage were independent prognostic factors, as shown in Table 2.

3. DFS and OS among the AFPP-CRC patients

Prognostic factors of AFPP-CRC on DFS and OS were shown in Fig.3.We could clearly see that AFPP-CRC patients with higher level of pre-operative CEA had a worse OS and DFS than those with normal level of pre-operative CEA. Interestingly, AFPP-CRC patients with microsatellite stability (MSS) had a poorer OS and DFS than those with microsatellite instability (MSI). The patients in AFPP-CRC with liver metastasis had a worse OS and DFS than patients with no liver metastasis. In addition, the tendency with perineural invasion in AFPP-CRC patients showed a poorer OS and DFS than those with no perineural invasion. The figure revealed that the OS and DFS of AFPP-CRC with poorer stage was worse than with better stage. Also, the patients in AFPP-CRC with peritoneal metastasis had a worse OS and DFS than patients with no peritoneal metastasis.

5.Logistic regression analysis of good and poor prognosis in AFPP-CRC

Based on the definition of whether the OS was more than 3 years, we divided AFPP-CRC patients into good prognosis group (n=55) and poor prognosis group (n=72). Univariate analysis and multivariate logistic regression were used to estimate potential risk factors for the two groups. As shown in Table 3, univariate logistic analysis revealed that pre-operative serum CEA level (*P*=0.003), pre-operative serum CA199 level (*P*=0.01), pre-operative serum CA125 level (*P*=0.03), liver metastasis(*P*=0.02), stage IV(*P*=0.006), and microsatellite status (*P*=0.003) were prognostic factor for AFPP-CRC patients. However, multivariate logistic analyses revealed that the presence of MSI (*OR*:0.18, 95%*CI*:0.04-0.63, *P*=0.01) was the only independent prognostic factor for AFPP-CRC patients.

Since the positive value of AFP (>8.78 ng/ml) was defined according to the results of clinical laboratory examination in our hospital and the upper normal limits of AFP in many published studies was 10 ng/ml, sensitivity analysis was necessary to verify the results. We selected a total of 68 patients with AFPP- CRC whose serum AFP was greater than 10 ng/ml and divided them into a good prognosis group (n=23) and a poor prognosis group (n=45) according to the above definition. The result was similar, shown in the Supplementary Table 1.

Discussion

As known, AFPP-CRC is an extremely rare case among CRC. Interestingly, there is no standardized available definition for AFPP-CRC yet. Due to small and heterogeneous sample size and significantly different baseline characteristics, most of previous reports could not provide data for a direct comparison between AFP-positive and AFP-negative CRC. Our study provides unique data on AFPP-CRC before

surgery and chemo/radiotherapy through baseline characteristics. Moreover, this study involved a large sample which could more comprehensively clarify the prognostic factors of AFPP-CRC. We also introduced PSM to reduce the impact of possible bias in baseline characteristics between the two groups.

According to a small quantity of published case reports, compared with traditional CRC, AFP-producing CRC presented biologically and clinically aggressive and it was usually associated with liver and lymphatic metastases(18, 20-22). Besides, the OS was significantly shorter as compared to traditional CRC. Although the mechanism by which an increased serum AFP level was linked to worse outcomes was not fully understood, Feng et al. (22) who combined PSM and immunohistochemical staining demonstrated that AFP-positivity was a significant negative predictor of OS in patients with CRC, and the presence of liver metastasis was the only independent prognostic factor in AFP-producing CRC. Ren et al. found that AFP-producing CRC had distinctive clinicopathological characteristics, showing an aggressive biological behavior and worse prognosis than traditional CRC(21).

Previous publications only included case series studies or case control studies and did not exclude selection bias. Here, we used PSM, which is a well-established method of balancing significant differences in baseline characteristics between the two groups. PSM is a statistical method used to reduce the impact of possible bias and mixed variables in the observation study, so as to make a more reasonable comparison between the experimental group and the control group. There are even indications that PSM may produce results similar to those of randomized controlled trials(29). According to the ratio of 1: 2, AFPN-CRC were selected as the control group. After PSM, the baseline characteristics (age, sex, BMI, clinical T, clinical N, and clinical M) used for matching were well-balanced. Thus, this analysis demonstrates that the PSM created well-balanced cohorts.

The present study showed that a higher utilization of post-operative chemotherapy theme in the AFP-positive group compared with the AFP-negative group, along with a higher utilization of multi-chemotherapy. Based on the results of our study, AFPP-CRC patients were more likely to receive postoperative chemotherapy, especially multi-chemotherapy, than AFPN- CRC patients. It is generally acknowledged that chemotherapy drugs inevitably damage some normal tissues, such as hair follicles, bone marrow, digestive tract and so on, while killing cancer cells(30). Nausea and vomiting are the most common symptom after chemotherapy(31), which can cause dehydration and electrolyte disorder in patients with severe cases. However, the extent and duration of side effects of chemotherapy depend on the use of chemotherapy drugs and the doctors' countermeasures. Thus, chemotherapy may affect the

DFS and OS of patients to a certain extent.

The prognosis of AFP-producing CRC was reported to be poor. With regard to the OS, there was no significant difference between AFPN-CRC and AFPP-CRC patients. The reason may be that our follow-up was not long enough, and the median follow-up was 38.6 months. In our study, we observed that the AFPP-CRC patients exhibited a trend toward worse DFS as compared with AFPN-CRC. We further investigated prognostic factors affecting DFS in all recruited patients. Multivariable regression analysis demonstrated that post-operative chemotherapy, pre-operative serum AFP level and pathologic stage (TNM) were independent prognostic factors, in line with the study by Feng et al(22).In his study, he also found that AFP-positivity and poorer pathologic stage were significant negative predictor of overall OS in patients with CRC as compared with stage-matched AFPN-CRC.

When it comes to elucidate the prognostic factors of AFPP-CRC, we performed Kaplan-Meier curves. We found that AFPP-CRC patients with higher level of pre-operative CEA, poorer stage, liver metastasis, perineural invasion or peritoneal metastasis had a worse DFS and OS. Feng et al. (22)found that liver metastasis was the only independent prognostic factor in AFP-producing CRC. In our study, liver metastasis showed significant statistical significance in univariate Cox analysis of the prognosis of patients with AFPP-CRC rather than multivariate Cox regression. Similarly, liver metastasis showed significant statistical significance in univariate logistic regression analysis of good prognosis of AFPP-CRC instead of multivariate logistic regression.

As to microsatellite status, AFPP-CRC patients with MSI had a better OS, but there was not statistically significant in DFS. This may indicate that microsatellite status may not be strongly associated with recurrence and metastasis in AFPP-CRC patients. A recent study conducted a meta-analysis of 1,164 MSI-high patients with non-metastatic colorectal cancer. The results showed that BRAF V600E mutation was associated with the worst OS, but not with disease recurrence(32, 33). Since previous studies on patients with AFPP-CRC did not include microsatellite status factor, the related mechanism needs to be explored in subsequent experiments.

Moreover, we defined that OS was a good prognosis group for more than 3 years and found that poor prognosis group in AFPP-CRC characterized by higher pre-operative serum CEA, CA199, CA125 levels, stronger liver metastasis, poorer pathologic stage and MSS. The results were consistent with previous studies(21-23, 34, 35). Multivariate logistic analysis revealed that the presence of microsatellite status was the only independent prognostic factor as compared with the good and poor prognosis in AFPP-CRC.

More importantly, it is worth noting that AFPP-CRC with MSI has a good prognosis. In our study, comparison between good prognosis group and poor prognosis group patients with AFPP-CRC, multivariate logistic analysis revealed that the presence of microsatellite status was the only independent prognostic factor. DNA repair system is mainly used to repair mismatched bases that escape polymerase proofreading immediately during replication, so as to maintain genome stability, avoid cell mutations, and indirectly inhibit tumor occurrence(36, 37). Microsatellites are particularly prone to replication errors due to their repetitive structure, which are usually repaired by the mismatch repair (MMR) system(37, 38). Deficient MMR (dMMR) results in a strong mutator phenotype known as microsatellite instability (MSI)(39), which is characterized by extensive length polymorphisms of microsatellite sequences resulting from DNA polymerase slippage. Following the initial success of melanoma treatment, immune checkpoint inhibitor has become a new hot spot in cancer treatment in recent years, and it is a new treatment strategy for dMMR/MSI-CRC(38, 40, 41). Based on our study, the tendency with MSI in AFPP-CRC patients showed a better prognosis than with MSS in AFPP-CRC, which means that the use of checkpoint inhibitors can activate the anti-tumor immunity of human body to kill tumors.

There were some limitations in the present research. First, the population included in the studies was relatively small. Though, our inclusion criteria were strict, and only 127 AFPP-CRC patients among 5274 CRC patients were included for analysis. More research with a larger population size is needed to confirm the findings of the current research. Second, immunohistochemically staining for AFP was not performed in our study. The definition of AFP positive was limited to serum level. We could make further immunohistochemical analysis in the future. Third, the follow-up time is not long enough, and the median follow-up in our study was 38.63 months. Furthermore, although we have studied the clinicopathological features related to AFPP-CRC, the exact molecular mechanisms for explaining the invasive biological behavior of AFPP-CRC are still unclear and limited. We should further study invasive biological behavior at the cellular and molecular levels and develop effective multimodal therapies for AFPP-CRC.

Conclusion

We found higher serum AFP level before surgery was associated with worse DFS in patients with CRC, even adjusting for tumor stage. Besides, we found AFPP-CRC patients with MSI might had a good prognosis, which could help us to guide the treatment for this group of patients.

Figure legends

Figure 1 Flow chart of patient's selection.

Figure 2 Overall survival (OS) (Fig.2A) and disease-free survival (DFS) (Fig.2B) and related to AFP-positive and AFP-negative colorectal cancer after propensity score matching (PSM).

Figure 3 Overall survival (OS) related to CEA, microsatellite status, liver metastasis, perineural invasion, pathologic stage, and peritoneal implantation in AFP-positive colorectal cancer. (Fig.3A)

Disease-free survival (DFS) related to CEA, microsatellite status, liver metastasis, perineural invasion, pathologic stage, and peritoneal implantation in AFP-positive colorectal cancer. (Fig.3B)

Abbreviations

AFP: α-Fetoprotein; AFPN-CRC: α-Fetoprotein-negative colorectal cancer; AFPP-CRC: α-Fetoprotein-positive colorectal cancer; BMI: body mass index; CA125: carbohydrate antigen 12-5; CA199: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen; CRC: colorectal cancer; DFS: disease-free survival; dMMR: deficient MMR; HCC: hepatocellular carcinoma; *HR*= hazard ratio; MMR: mismatch repair; MSI: microsatellite instability MSS: microsatellite stability; OS: overall survival; PSM: propensity score matching; 95%*CI* = 95 percent confidence interval

Consent for publication

We reached an agreement of all participants in this study to publish this document.

Competing interests

The authors declared no financial conflict of interests.

Ethics statement

Our study was approved by Ethics Review Committee of the Sixth Affiliated Hospital of Sun Yat-sen University, as we anonymously retrieved data from electronic databases and therefore did not give informed consent.

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Figures

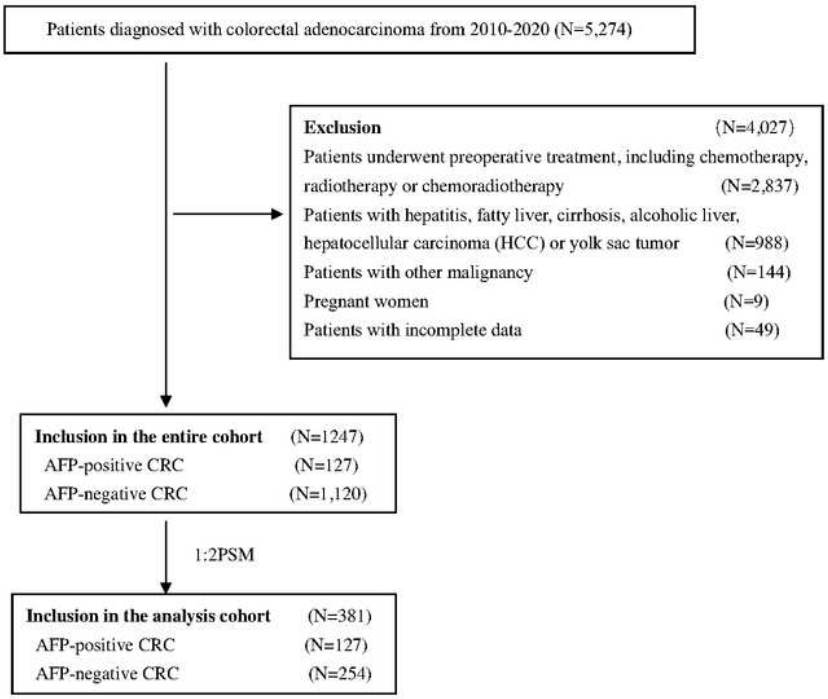

Figure 1

Flow chart of patient's selection.

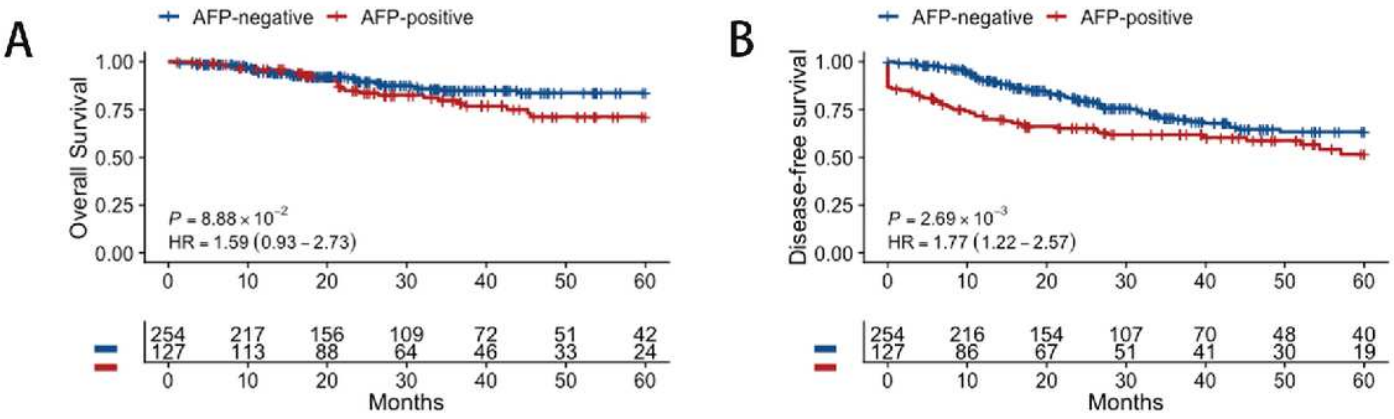

Figure 2

Overall survival (OS) (Fig.2A) and disease-free survival (DFS) (Fig.2B) and related to AFP□positive and AFP-negative colorectal cancer after propensity score matching (PSM).

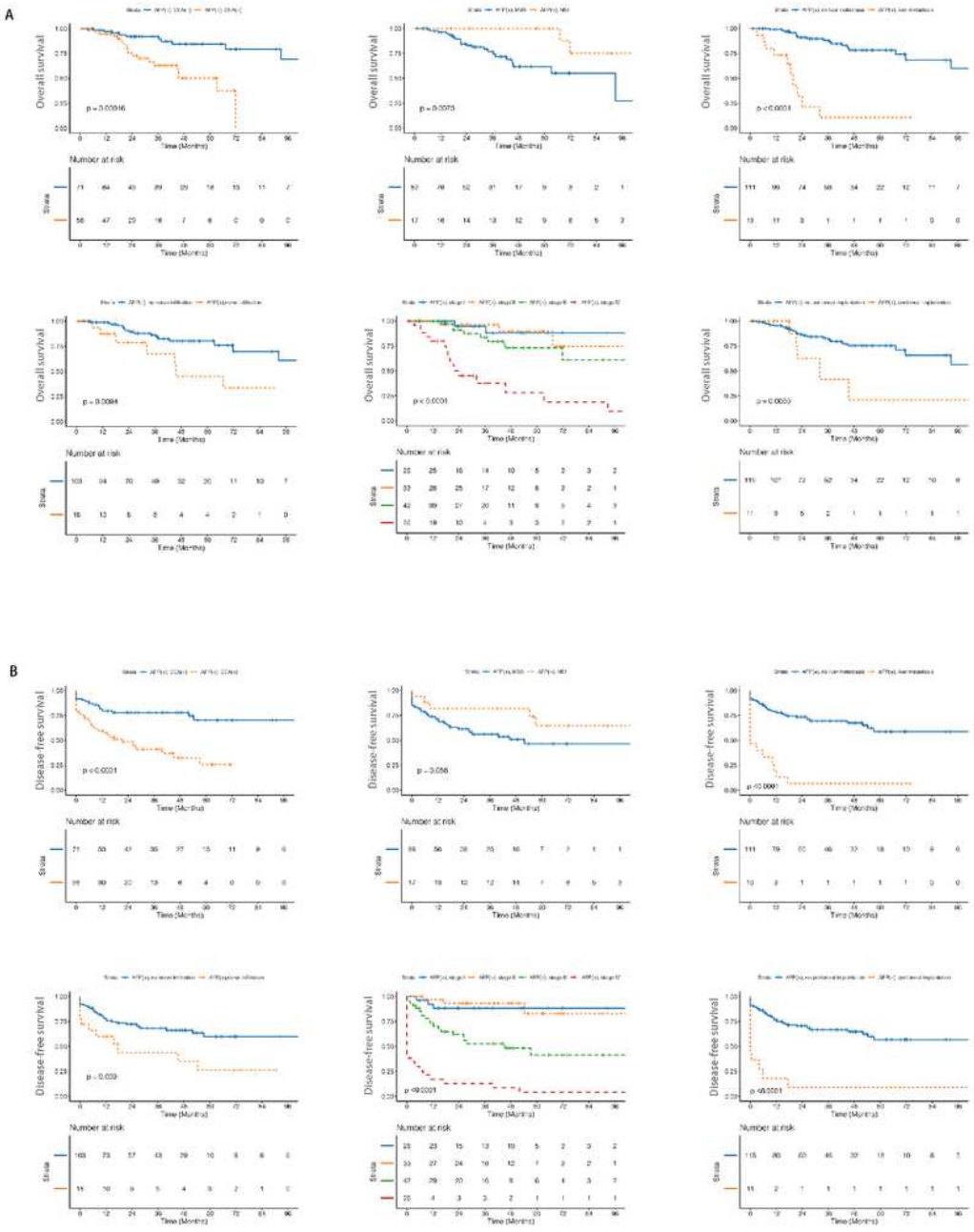

Figure 3

Overall survival (OS) related to CEA, microsatellite status, liver metastasis, perineural invasion, pathologic stage, and peritoneal implantation in AFP-positive colorectal cancer. (Fig.3A) Disease-free survival (DFS) related to CEA, microsatellite status, liver metastasis, perineural invasion, pathologic stage, and peritoneal implantation in AFP-positive colorectal cancer. (Fig.3B)

Supplementary Files

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- Table1.pdf
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