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*Original article*

# Prognostic Value of Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen (CA19-9) in Colorectal Cancer

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**Abstract**

*The present study analysed the tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19 9 (CA19 9) in correlation with clinicopathological variables and survival outcomes in Libyan patients with colorectal cancer (CRC). The clinicopathological variables of 397 patients with CRC diagnosed at the National Cancer Institute in Misurata, Libya, between 2008 and 2017 were retrospectively analyzed. Blood samples from these patients were analyzed for serum CEA and CA19 9 levels before treatment by electrochemiluminescence immunoassay (double antibody sandwich ELISA) on a Roche cobas e 602 modules. The relationships between CEA CA19 9 expressions (separately and combined) with clinicopathologic variables and survival outcomes were analyzed using the Kaplan Meier method, log rank test and Cox regression analyzes. Cut off values for serum CEA and CA19 9 levels were 5 ng/ml and 37 U/ml, respectively. The mean serum levels of CEA and CA19 9 for all CRC tumors were 70.0 ng/ml and 473.0 U/ml, respectively. Tumors with higher serum CEA and CA19 9 levels were found in 60.0% and 46.0 % of CRC cases. Higher CEA and CA19-9 expression were significantly associated with more indicators of a malignant phenotype, including a young age <50 years, high histological grade, large tumor size, positive lymph nodes, advanced stage and distant metastases. The median follow-up duration was 46 months and 44.3% of patients had died of CRC. Patients with higher expression of the biomarkers CEA and CA19-9 had shorter overall survival and lower disease-free survival. Patients with both tumour markers increased showed a remarkably shorter 5-year survival rate (29.3%) and lower disease-free survival rate (p<0.0001). The Cox regression analysis emphasizes these results (p value < 0.0001). The combination of CEA and CA19-9 appear as an independent prognostic marker for survival. More intensive therapy in patients diagnosed with an advanced CRC with combined elevation of tumor markers (CEA and CA19-9) is highly considered. Measuring CEA and CA19-9 preoperatively in CRC patients is highly significant and could be useful as a prognostic marker.*

***Keywords.*** *Colorectal Cancer, Tumor Markers, CEA, CA19-9, Prognosis.*

## Introduction

Globally, approximately 18.1 million patients are diagnosed with cancer and about 9.6 million patients die from their disease [1]. Colorectal cancer (CRC) still one of the leading causes of cancer-related deaths, with it being the fourth most common cause of cancer death after lung, stomach, and liver cancer [2]. Surgery and other multimodal therapy provides a cure for many CRC patients, when they are diagnosed at early stage [3]. However, patients with advanced and metastatic disease were associated with poor survival [4]. So, it is becoming highly important to detect the disease in early stages.

In CRC, many screening methods available for early tumour detection such as the occult blood test, digital rectal examination, colonoscopy, and images (Computed Tomography and/or Magnetic Resonance Imaging ) [5 and 6]. Anyhow, about 15% of patients experience a relapse of the disease after the radical surgery, mainly during the first 18 months [7].

In addition, early detection during the follow-up program is vital as a relapse is curable when treated early [8]. To early detect the disease relapse, physicians use an intense follow-up program for 5 years after complete tumour resection, which includes proper history, clinical examination, blood tests, including the search for biological tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), a colonoscopy, an abdominal ultrasound, and computed tomography [6].

CRC is a group of heterogeneous diseases with different genetic and biological behaviour that explain diverse tumour characteristics and outcomes [9]. The patient's outcome different greatly between the patients, and survival rates ranging from 10% to 90% based on disease stage and other variables [10]. The prognosis of CRC depends mainly on disease stage as considered by the Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) TNM staging classification. Anyhow, the differences in clinical outcomes and prognosis within patients of the same pathological stage are considerable [11]. The identification of prognostic and predictive markers may be important to identify patients at a high risk of recurrence or metastases for improved management of the disease.

In particular, serum tumour markers are non-invasive/cost-effective additional tools that are increasingly used in various cancers including CRC, to advance understanding of cancer pathophysiology, improve molecular stratification and thus achieve improved outcomes.

CEA, a glycoprotein with a molecular weight of 180-200 kDa, was first isolated from fetal colon and CRC tissue in 1965 [12]. CEA is overexpressed in several cancers, including colon, breast, lung and thyroid cancers [13]. Nevertheless, an elevation of CEA may also be seen in many non-malignant conditions, such as cigarette smoking, alcoholism diverticulitis, pancreatitis, and liver disease [14 and 15]

Since 1979, CA19-9 has been known and is used more frequently for early detection of pancreatic carcinomas nowadays [16]. CA 19-9 is a tumour associated antigen with a half-life of 48 days, whose epitope has been shown to be the sialylated Lewis antigen [17]. High serum CA19-9 can be found in malignant and benign conditions [18].

The sensitivity level for CEA ranging from 65% to 74% in CRC patients and CA19-9 only had sensitivity ranging from 26% to 48% [19,20]. Studies detected that CA19-9 correlates with the tumour marker CEA and may, therefore, improve the sensitivity of CEA [19,21,22]. CEA has been used as a tumour marker for the diagnosis and surveillance of colorectal cancer [23,24], and CEA is a risk factor for recurrence in patients with CRC [25]. CA19-9 has also been reported as a prognostic factor for CRC [14,15]. Therefore, the combination of these two tumour markers may provide a more sensitive biomarker for CRC.

The aim of the study was to find an increased informative value when interpreting both tumour markers together to increase the chances of survival of patients and to optimize treatment in the future.

## Methods

### *Clinicopathological data.*

Between 2008 and 2017, 690 patients were diagnosed with CRC in the surgical department at the National Cancer Institute, Misurata, Libya. Out of this patient collective, 230 patients were identified with incomplete follow-up and/or incomplete document record and 67 patients did not have any tumour markers documented and, therefore, had to be excluded from this study.

This study group consisted of 397 patients with availability of complete demographic ,clinicopathological data and preoperative biological markers. The study design are showed in Figure 1. Complete demographic and clinicopathological data included age at diagnosis, gender, family history, tumour location, lymph node status, stage, histological type, histological grade, type of treatment, and follow-up data. These clinicopathological data were obtained from the patients' records and are showed in **Table 1.** The mean age of the patients was 52.0 years (range, 22-90 years).

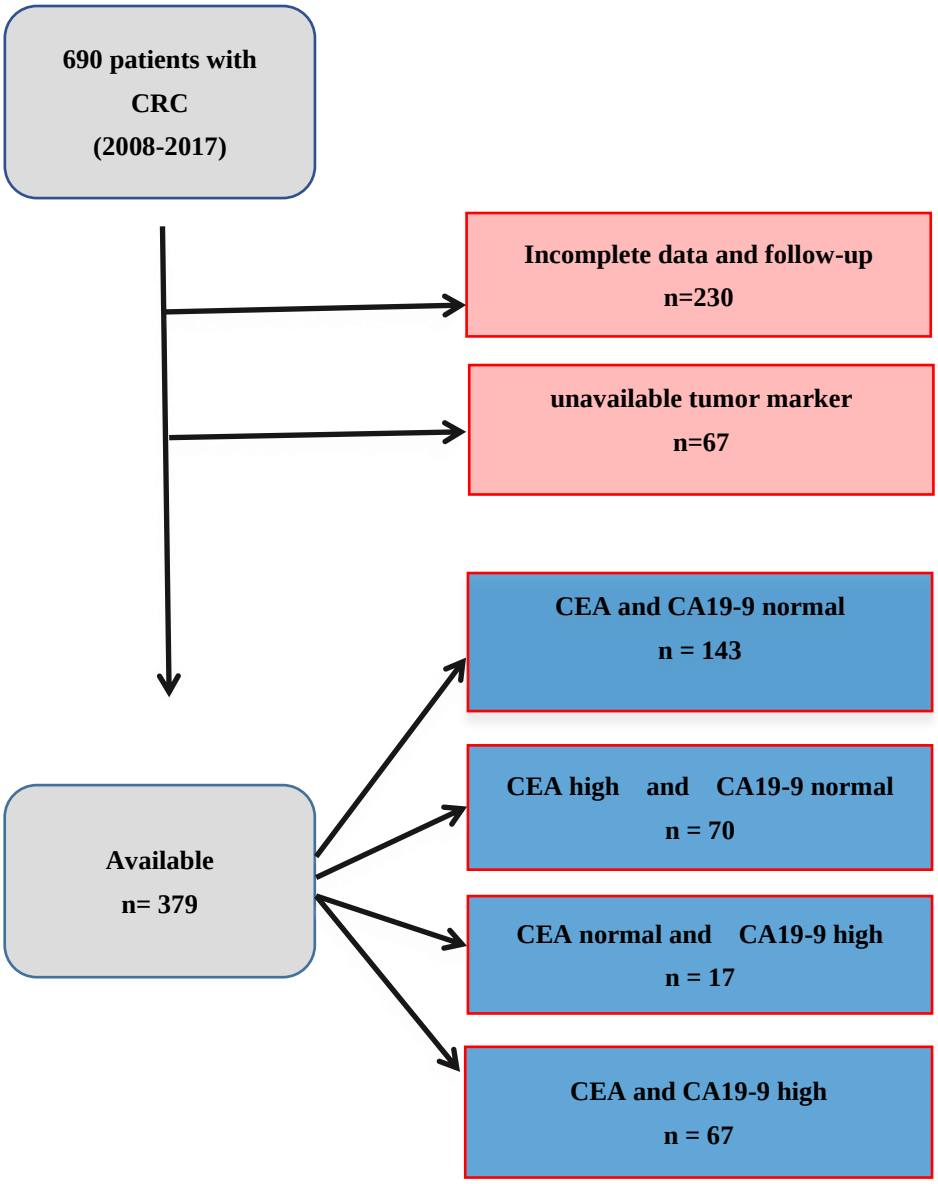
Biological markers included serum levels of CEA and CA19-9, which were determined in all patients before any treatment. CEA levels <5 ng/mL were defined as normal and ≥5 ng/mL was defined as increased levels, and for CA19-9, a level <37 U/mL was defined as normal and a level ≥ 37 U/ml was defined as an increased levels [ 26 and 27 ].

Tumour staging of CRC was evaluated according to the American Joint Committee on Cancer (AJCC), TNM classification [28]. Pan-colonoscopy and radiological staging by Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI) was performed in all patients to assess tumour extension. The extent of the tumour (local and distant) at the time of diagnosis was confirmed by imaging [CT, MRI, or Positron Emission Tomography (PET)].

**Table 1.** Correlations between the preoperative CEA expression (<5 ng/ml vs. ≥5 ng/ml) and clinicopathological variables in colorectal cancer patients (n=397).

| **Clinicopathological variables** | | **Number** | **CEA expressions (%)** | | ***p* value** |
|---|---|---|---|---|---|
| | | | **<5 ng/ml** | **≥ 5 ng/ml** | |
| Age /years | < 50 | 158 | 28.5 | 71.5 | **<0.0001** |
| | ≥ 50 | 239 | 48.1 | 51.9 | |
| Gender | Male | 201 | 41.8 | 58.2 | 0.540 |
| | Female | 196 | 38.8 | 61.2 | |
| Family history | Positive | 14 | 42.9 | 57.1 | 0.843 |
| | Negative | 383 | 40.2 | 59.8 | |
| Tumour site | Colon | 240 | 40.8 | 59.2 | 0.790 |
| | Rectum | 157 | 39.5 | 60.5 | |
| Histological type | Adenocarcinoma | 355 | 42.0 | 58.0 | 0.089 |
| | Mucinous carcinoma | 26 | 30.8 | 69.2 | |
| | Signet ring carcinoma | 16 | 18.8 | 81.3 | |
| Histological grade | Grade 1 | 51 | 70.6 | 29.4 | **<0.0001** |
| | Grade 2 | 239 | 39.3 | 60.7 | |
| | Grade 3 | 107 | 28.0 | 72.0 | |
| Depth of invasion T | T1 | 7 | 71.4 | 28.6 | **<0.0001** |
| | T2 | 24 | 70.8 | 29.2 | |
| | T3 | 220 | 51.8 | 48.2 | |
| | T4 | 65 | 32.3 | 67.7 | |
| | Tx | 81 | 3.7 | 96.3 | |
| Lymph modes N | N0 | 127 | 72.4 | 27.6 | **<0.0001** |
| | N1 | 90 | 42.2 | 57.8 | |
| | N2 | 97 | 27.8 | 72.2 | |
| | Nx | 83 | 3.6 | 96.4 | |
| Metastasis M | M0 | 275 | 55.6 | 44.4 | **<0.0001** |
| | M1 | 122 | 5.7 | 94.3 | |
| TNM Stage | Early stage (1 and 2) | 120 | 74.2 | 25.8 | **<0.0001** |
| | Late stage (3 and 4) | 277 | 25.6 | 74.4 | |
| Surgical treatment | Radical | 275 | 55.6 | 44.4 | **<0.0001** |
| | Palliative | 70 | 5.7 | 94.3 | |
| | No surgery | 52 | 5.8 | 94.2 | |

**Figure 1.** Study design of the data collection. The patients who were excluded from the study (the two red boxes). The patients that were available for the study (the blue boxes) and their classification into four arms: both tumour markers below cut-off value, only CEA increased, only CA19.9 increased, and both tumour markers increased.




***Treatment and follow-up.***

Radical surgery was done in 275 patients (69.3%), palliative surgery was done in seventy patients, and no surgical intervention for 52 patients had metastasis at the time of diagnosis. However, colonoscopy and/or sigmoidoscopy with biopsy were performed in these patients for histopathological diagnosis.

In the National Cancer Institute in Misurata the following guidelines were established: adjuvant combined chemotherapy based on FOLFOX (folinic acid, fluorouracil, and oxaliplatin) and/or XELOX (oxaliplatin and capecitabine) was given to 266 patients and 101 patients received palliative chemotherapy with FOLFIRI (folinic acid, fluorouracil, and irinotecan) and/or capecitabine. In addition, 30 patients were not eligible for chemotherapy, so these patients did not receive chemotherapy. Neoadjuvant concurrent chemoradiotherapy was given to rectal cancer patients (n=75).

Follow-up of patients was carried out every 3 months for 2 years, 6 months for 5 years, and thereafter every 1 year. Disease recurrence (local and distant metastases) was confirmed by colonoscopy and imaging (CT, MRI, or PET) performed when clinical symptoms suggestive

of disease recurrence were present. Patients' outcomes were considered as follows: overall survival, duration between the date of pathological diagnosis to the date of death and/or to date of the end follow up period; disease-free survival, duration between the date of pathological diagnosis to the date of diagnosis of recurrence (local and/or distant metastases) or death [29].
Patients were followed up until death or to the end of the observation period (until December 2021). The median follow-up duration was 46 months (range, 4-116 months). At the end of follow up period, 176 patients (44.3%) had died of CRC.

***CEA and CA19-9 measurement.***
Prior to each treatment, approximately 5 ml of peripheral fasting blood was drawn from the forearm veins. The blood was immediately taken to the central laboratory of the National Cancer Institute in Misurata and then routinely centrifuged for 10 min at speed of 1,792 x g at 20-22˚C temperature. The serum samples were first stored at 4˚C. Then they were placed in polypropylene vials and stored at -80˚C. The concentrations of CEA and CA19-9 in serum were determined using an electrochemiluminescence immunoassay (double antibody sandwich ELISA, cat. nos. TM E-4131 and TM E-4531 respectively; Labor Diagnostika Nord GmbH & CoKG) on a Roche cobas e 602 modules (Roche Diagnostics). This technology uses a sandwich chemilumines- cence immunoassay; Chemibeads contain a chemiluminescent dye and Sensibeads contain a photosensitizer dye. Biotinylated antibodies (1:100) and Chemibeads form sandwiches and immune complexes are formed by further addition of Sensibeads. A chemiluminescence reaction is initiated at 680 nm and finally the signal is detected at 612 nm (according to the manufacturer's instructions). The accuracy of internal and external quality controls was determined according to the guidelines of RiliBAeK [ 30 ]. The detection limit and blank limit were as follows: CEA: 0.2 and 0.12 ng/ml, CA19-9: 2.0 and 1.0 u/ml, CA15-3: 1.0 and 0.3 u/ml, respectively. Roche's original ancillary reagents were used, including streptavidin- coated magnetic beads, anti-CEA monoclonal antibody and biotinylated anti-CA19-9 and anti-CEA monoclonal antibody and Ru-labelled anti-CA19-9.

***Statistical analysis***
Statistical analysis. Continuous variables were calculated using SPSS 26.0 for Windows (IBM Corp.). Frequency tables were analyzed using the $\chi 2$ or Fisher's exact tests to evaluate the power of association between categorical variables. Kaplan-Meier curves were constructed for survival rate analysis and differences between curves were analyzed using the log-rank test. Multivariate survival analysis for the outcome [overall survival and disease-free survival] was performed using the proportional hazard Cox model in a backward stepwise manner with the log-likelihood ratio (L-R) significance test, using standard values for the entry and exclusion criteria. The cut-off point for CEA of 5 ng/ml and for CA19-9 of 37 U/ml was used to distinguish between high-expression and low-expression tumors as it provided the best results for prognosis prediction in this and other studies [26 and 27]. We compared the patients outcome [overall survival and disease free survival] by analyzing CEA and CA19-9 separately, and dividing the patient collective into two groups: first group with tumor marker below the cut-off value and other group with tuour marker equal and/or more than the cut-off value. In addition, we analyzed the overall survival and disease free survival for CEA and CA19-9 combined, and their subdivision into four groups: both tumor markers below cut-off value, only CEA increased, only CA19-9 increased, and both tumor markers increased. The assumption of proportional hazards was controlled by log-minus-log (LML) survival plots. $P<0.05$ was considered to indicate a statistically significant difference.

**Results**
***Patient demographic and clinicopathologic variables.***
The demographic and clinicopathologic variables are shown in Table I. The mean age of the patients was 52.0 years (range, 22-90 years) and the majority of patients (80.2%) were >50 years old. Regarding gender distribution, CRC was nearly the same frequent among males and females (50.6% and 49.4%, respectively). A total of 3.5% of patients had a family history of CRC. In 240 patients the tumours were located in the large bowel (60.5%) and in 157 patients the tumours were located in the rectum (39.5%). Most patients had tumours with adenocarcinoma type and moderate-grade (89.4% and 60.2%, respectively). The most common T stage was T3 (55.4%), followed by Tx and T4 (20.4% and 16.4%, respectively).

A total of 90 patients (22.7%) had positive lymph nodes and negative lymph nodes were detected in 127 patients, while lymph node status could not be assessed in 83 patients (20.9%). According to the AJCC staging system, 122 patients were at stage IV (30.7%), 152 patients were at stage III, 103 patients were at stage II and 20 patients were at stage I.

***General description of CEA and CA19-9 expression profiles.***
CEA and CA19-9 expressions at cut point of (5ng/ml and 37 U/ml, respectively) are shown in Table 2. The mean CEA expression was 70 ng/ml (range 1.1-882 ng/ml). CEA expression was low in 160 samples (<5 ng/ml) and high in 237 samples (≥ 5ng/ml). The mean value of CA19-9 was 473 U/ml (range 1- 10987 U/ml). CA19-9 was low in 213 samples (<37 U/ml) and high in 184 samples (≥37 U/ml). CEA expression was more frequent in tumours with high CA19-9 than in patients with low CA19-9 (p <0.0001).

**Table 2.** Univariate survival according to analysis of CEA expression (cut point of 5 ng/ml) and CA19.9 (cut point of 37 U/ml) in Libyan patients with colorectal cancer (n= 397).

| Variables | Threshold | No of patients | Survival analysis | | | *p*-value |
|---|---|---|---|---|---|---|
| | | | Median survival (months) | Mean survival (months) | Survival rate (%) | |
| All patients | | 379 | 45.87 | 45.64 | 55.7 | |
| CEA level | < 5 | 160 | 54.45 | 54.9 | 80.6 | **<0.0001** |
| | ≥ 5 | 237 | 36.85 | 39.39 | 38.8 | |
| CA19.9 level | < 37 | 213 | 53.20 | 53.01 | 74.6 | **<0.0001** |
| | ≥ 37 | 184 | 38.77 | 37.10 | 33.7 | |
| Both CEA and CA19.9 normal | CEA <5, CA19-9 <37 | 143 | 55.14 | 55.85 | 81.1 | **<0.0001** |
| CEA increased and CA19-9 normal | CEA ≥ 5, CA19.9 < 37 | 70 | 45.25 | 47.21 | 61.4 | **<0.0001** |
| CEA normal and CA19-9 increased | CEA< 5, CA 19-9 ≥ 37 | 17 | 44.33 | 46.88 | 76.5 | **<0.0001** |
| Both CEA and CA19.9 increased | CEA ≥ 5, CA19-9 ≥ 37 | 167 | 35.47 | 36.11 | 29.3 | **<0.0001** |

***Correlation of CEA expression with clinicopathological variables.***
The significant correlations between CEA expression (<5 ng/ml vs. ≥5 ng/ml) and clinicopathological variables are shown in Table 1. High CA expression was significantly associated with <50 years old patients (P<0.0001), high histological grade tumour (P<0.0001), large tumour size (P<0.0001), unevaluable lymph node status (P<0.0001), advanced stages (P<0.0001) and distant metastases (P<0.002). However, gender, family history, tumour type and tumour site showed no significant relationship with CEA expression.

***Correlation of CA19-9 expression with clinicopathological variables.***
The correlations between CA19-9 expression at the cut point of 37 U/ml and clinicopathological variables are shown in Table 3. High CA19-9 expression was more common in patients with young age <50 years, high histological grade (P<0.001), advanced T stage (P<0.0001), unevaluable lymph node status (P<0.0001), advanced stage (P<0.0001) and distant metastases (P<0.002). However, gender, family history, tumour type, and tumour site showed no significant association with CA19-9 expression.
Correlation of serum CEA and CA 19-9 expression patterns with patient survival outcomes.
Univariate survival analyzes (survival rates) with CEA expression at a cut-off point of 5 ng/ml and CA19-9 at a cut-off point of 37 U/ml are shown in Table 2.

To carefully analyze the prognostic value of CEA and CA19-9, First, we compared the survival outcomes of patients by analyzing CEA and CA19-9 separately, dividing the patients into two groups: one group with tumour marker below the cut-off value and other group with tumour marker equal and/or more than the cut-off value. The survival rate was 80.6% in patients with low CEA expression and 38.8% in patients with high expression profile (P<0.0001). The low CA 19-9 expression group had an improved survival rate than the high expression group (74.6 and 33.7%, respectively) (P<0.0001).
Kaplan-Meier survival curves for both CEA and CA19-9 levels showed that shorter survival was associated with high CEA and high CA19-9 levels (Figure. 2). On the other hand, patients with low CEA and CA19-9 levels were associated with a lower recurrence rate and therefore had longer disease-free survival (P<0.0001 and P<0.0001, log rank, respectively, (Figure. 3).

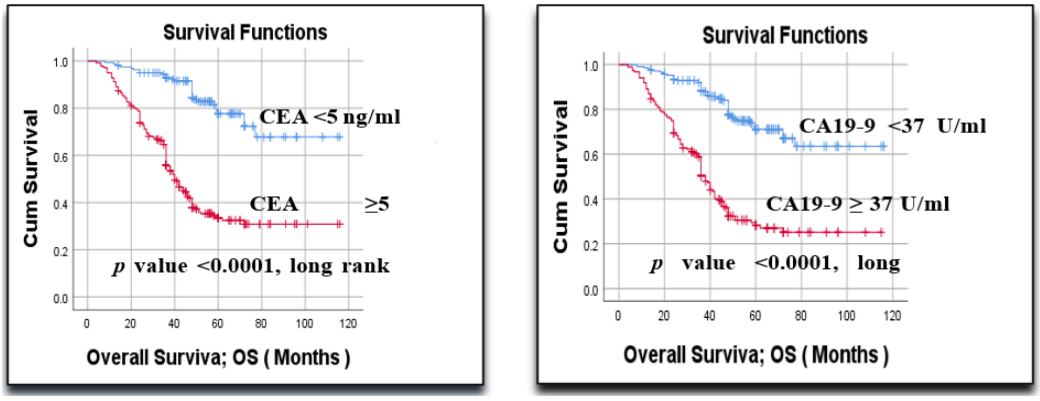



**Figure 2.** Overall survival (OS) according to analysis of CEA and CA19.9 expressions in colorectal cancer.

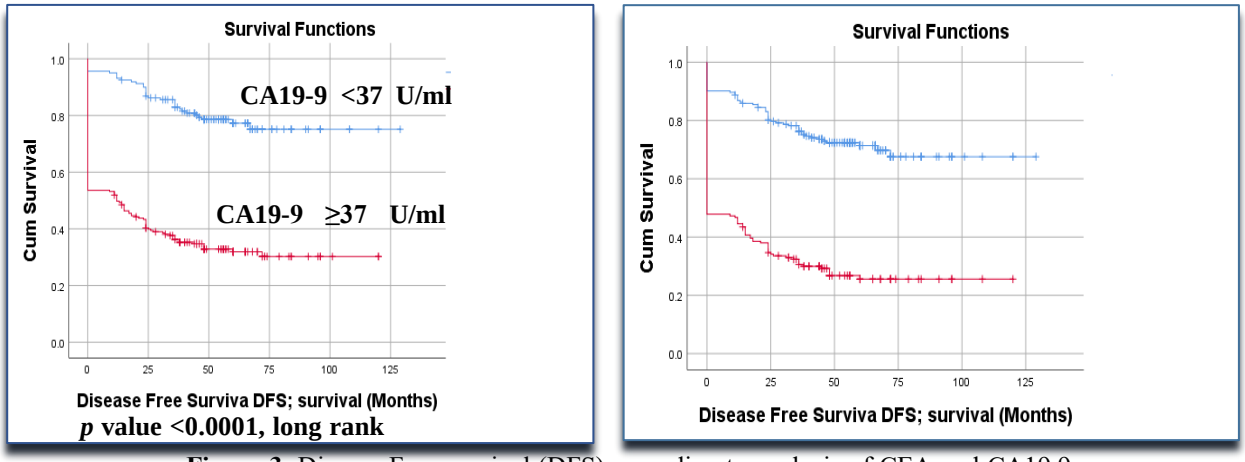



**Figure 3.** Disease Free survival (DFS) according to analysis of CEA and CA19.9 expressions in colorectal cancer.

Second, we analyzed the survival outcomes for CEA and CA19-9 combined, and their subdivision into four groups: both tumour markers below cut-off value, only CEA increased, only CA19-9 increased, and both tumour markers increased (Table. 3).
The 5-year survival rate for patients with both tumour markers below the cut-off value was 81.1%, 61.4 % for patients with only the tumour marker CEA elevated, and 76.5 % for patients with only the tumour marker CA19-9 elevated. However, patients with both tumour markers increased had an even shorter survival rate, with a 5-year survival rate of 29.3% (p<0.0001).
Kaplan-Meier survival curves showed that patients with both tumour markers below the cut-off value had the best outcome and patients with both tumour markers increased had a remarkably shorter overall survival (Figure. 4). On the other hand, patients with both tumour markers increased had a shorter disease-free survival and patients with both tumour markers decreased had a longer disease free survival (P<0.0001 and P<0.0001, log rank, respectively, (Figure. 5).

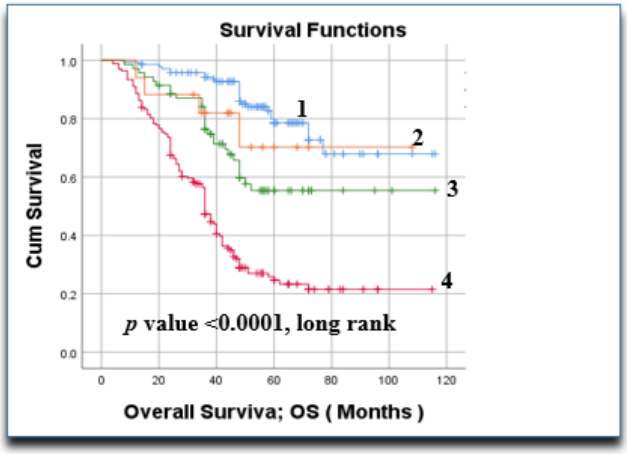



**Figure 4.** Overall survival (OS) according to analysis of both CEA and CA19.9 expressions in colorectal cancer.1 (blue line): both tumour markers normal. 2 (light red line): with normal CEA and increased CA19. 3 (green line): with increased CEA and normal CA19.9. 4 (dark red line): both tumour markers increased.

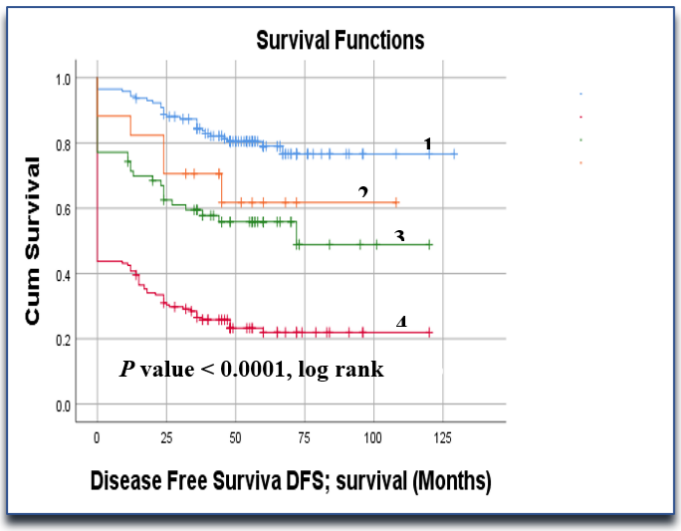



**Figure 5.** Disease free survival (DFS) according to analysis of both CEA and CA19.9 expressions in colorectal cancer.1 (blue line): both tumour markers normal. 2 (light red line): with normal CEA and increased CA19. 3 (green line): with increased CEA and normal CA19.9. 4 (dark red line): both tumour markers increased.

Cox regression analysis was performed, shown in Table 4, to prove the association between increased preoperative CEA and CA19-9 and patient outcomes (in term of OS and DFS). Therefore, Table 4 confirms the results emphasized in Figure 4 and 6, showing stage and both tumor markers as independent parameters for overall survival and disease free survival (p-value < 0.0001 and p< 0.0001. respectively).

**Table 3.** Correlations between the preoperative CA19.9 expression (< 37 U/ml vs. ≥ 37 U/ml) and clinicopathological variables in colorectal cancer patients (n=397).

| **Clinicopathological variables** | | **Number** | **CA19.9 expressions (%)** | | ***p* value** |
|---|---|---|---|---|---|
| | | | **<37 U/ml** | **≥ 37 U/ml** | |
| Age /years | < 50 | 158 | 46.2 | 53.8 | 0.015 |
| | ≥ 50 | 239 | 58.6 | 41.4 | |
| Gender | Male | 201 | 53.2 | 46.8 | 0.866 |
| | Female | 196 | 54.1 | 45.9 | |
| Family history | Positive | 14 | 57.1 | 42.9 | 0.789 |
| | Negative | 383 | 53.5 | 46.5 | |
| Tumour site | Colon | 240 | 40.8 | 59.2 | 0.564 |
| | Rectum | 157 | 39.5 | 60.5 | |
| Histological type | Adenocarcinoma | 355 | 54.1 | 45.9 | 0.391 |
| | Mucinous carcinoma | 26 | 57.7 | 42.3 | |
| | Signet ring carcinoma | 16 | 37.5 | 62.5 | |
| Histological grade | Grade 1 | 51 | 74.5 | 25.5 | **0.001** |
| | Grade 2 | 239 | 54.0 | 46.0 | |
| | Grade 3 | 107 | 43.0 | 57.0 | |
| Depth of invasion T | T1 | 7 | 71.4 | 28.6 | **<0.0001** |
| | T2 | 24 | 83.3 | 16.7 | |
| | T3 | 220 | 63.2 | 36.8 | |
| | T4 | 65 | 53.8 | 46.2 | |
| | Tx | 81 | 17.3 | 82.7 | |
| Lymph modes N | N0 | 127 | 83.5 | 16.5 | **<0.0001** |
| | N1 | 90 | 60.0 | 40.0 | |
| | N2 | 97 | 40.2 | 59.8 | |
| | Nx | 83 | 16.9 | 83.1 | |
| Metastasis M | M0 | 275 | 69.5 | 30.5 | **<0.0001** |
| | M1 | 122 | 18.0 | 82.0 | |
| TNM Stage | Early stage (1 and 2) | 120 | 84.2 | 15.8 | **<0.0001** |
| | Late stage (3 and 4) | 277 | 40.4 | 59.6 | |
| Surgical treatment | Radical | 275 | 69.5 | 30.5 | **<0.0001** |
| | Palliative | 70 | 22.9 | 77.1 | |
| | No surgery | 52 | 11.5 | 88.5 | |

**Table 4.** Multivariate analysis (Cox proportional hazard model) of prognostic factors in patients with colorectal cancer

| Variables | Overall survival model | | | Disease free survival model | | |
|---|---|---|---|---|---|---|
| | Hazard Ratio (95% CI) | Standard Error SE | *P value* | Hazard Ratio (95% CI) | Standard Error SE | *P value* |
| **Age** (<50 years / ≥50 years) | 0.777 (0.573-. 1.055) | 0.156 | 0.106 | 0.813 (0.608-1.088) | 0.148 | 0.164 |
| **Gender** (male / female) | 1.112 (0.561-1.501) | 0.153 | 0.487 | 1.051 (0.789-1.399) | 0.146 | 0.733 |
| **Tumour site** (Colon / rectum) | 0.769 (0.561-1.054) | 0.161 | 0.103 | .853 (0.634-1.146) | 0.151 | 0.291 |
| **Histology type** (adenocarcinoma /others) | 1.267 (0.781-2.056) | 0.247 | 0.338 | 1.032 (0.652-1.635) | 0.235 | 0.892 |
| **Clinical Stage** (early / late) | 12.716 (5.810-27.832) | 0.400 | **<0.0001** | 7.099 (3.744-13.462) | 0.326 | **<0.0001** |
| **Preoperative tumor markers** | | | | | | |
| CEA and CA19-9 normal | 0.833 (0.308-. 2.580) | 0.542 | **<0.0001** | 0.719 (0.295-1.755) | 0.455 | **<0.0001** |
| CEA increased and CA19-9 normal | 1.288 (0.443- 3.741) | 0.544 | | 1.192 (0.491-2.892) | 0.452 | |
| CEA normal and CA19-9 increased | 1,201 (0.421-2.541) | 0.531 | | 1.178 0.472-2.784 | 0.442 | |
| Both CEA and CA19-9 increased | 2.693 (0.979- 7.406) | 0.516 | | 2.226 (0.967-5.124) | 0.426 | |

**Discussion**

Numerous studies have investigated the efficacy of various biological markers as diagnostic, predictive and prognostic markers in CRC [14,15,19-33]. Among them, CEA and CA19-9 are the most commonly used tumor biomarkers.

The efficacy of CEA and CA19-9 in screening, diagnosis, follow-up, assess the treatment process and detect cancer residual disease has been studied in CRC patients since their discovery. Up to now, guidelines recommended only the use of CEA for determining prognosis and monitoring treatment. Due to the low sensitivity, CA19-9 is still not recommended as a useful marker in CRC patients [31–33].

Anyhow, the behaviour and efficacy of the combination of CEA and CA19-9 have not yet been studied enough to make any guideline-oriented recommendations. The present study performed a detailed retrospective analysis of 397 patients with CRC diagnosed and treated at the National Cancer Institute, Misurata, Libya. CEA and CA19-9 expression levels at cut-off points (5 ng/ml and 37 U/ml, respectively) were found to be the most promising discriminators of both clinicopathological variables and survival outcomes and was able to provide further information about the value of measuring both tumor markers separately and combined. Anyhow, there remain limitations to this study, such as the remaining bias as this study only included patients treated in a one center. Since this article is based on a retrospective study, prospective studies including patients from different centers is recommend.

In the cohort of present study, the mean value of CEA in serum was 70.0 ng/ml and high CEA expression was detected in 59.7% of patients. The mean value of CA19-9 in serum was 473.0 U/ml and high CA19-9 expression was detected in 46.3% of patients. This result

was higher than published data. Lakemeyer et al [27] and other reported that CEA positivity was found to be 34.0% and CA 19-9 positivity 18.0% in patients with CRC [34].
Patients with high expression of CEA and/or CA19-9 are often associated with a higher grad of malignancy such as advanced stages, positive lymph nodes and distant metastasis. Therefore, increased expression of CEA and CA19-9 might indicate that the tumors are already at an advanced stage. Moreover, tumor progression was associated with higher levels of these tumor markers. Consistent with these findings, Yayın et al [35] report that expression of CEA and CA19-9 is significantly associated with large tumor size, lymph node metastasis, advanced stages and tumor progression.
Our study show that high expression of CEA and CA19-9 were associated with high histological grade, with large tumour size, with positive lymph nodes, with advanced stages and with distant metastasis. On other hand low CEA and CA19-9 were more common with favorable prognostic variables. These findings confirm results of other studies [14,15,25,35,36] and these data suggest that patients with a higher CEA expression and CA19-9 value had a worse prognosis.
The most important finding of the present study was undoubtedly the significant correlation of CEA and CA19-9 expression (separately and in combined) with disease progression, especially overall survival and disease-free survival. Guidelines recommend CEA as a useful predictor of patient outcomes [31,37,38]. Our study was able to underline this assertion, as we were able to present CEA as a reliable predictor for overall survival and disease-free survival. A shorter 5-year survival rate of 38,3 % in patients with an elevated CEA compared to patients with a normal CEA (80.6%). Analysis using Kaplan-Meier curves also showed that short survival was more common in the group with high CEA levels, while the group with low CEA levels had longer disease-free survival. Our results similar to results of numerous studies using the same cut-off value of 5 ng/mL for the tumor marker CEA [26,39,40].
For CA19-9, guidelines do not recommend the use of CA19-9 in a prognosis setting [36.38,39]. Anyhow, our study was able to demonstrate the similar significance of CA19-9 as a predictor of survival compared to the tumor marker CEA. Patients with increased preoperative CA19-9 had a significantly poorer 5-year overall survival rate of 33.7 % compared to patients with a normal CA19-9 level (74.6%). Again, Analysis using Kaplan-Meier curves also showed that short survival was more common in the group with high CA19-9 levels, while the group with low CA19-9 levels had longer disease-free survival. Numerous other studies were able to present similar results using different cut-off values include 37 U/mL [21,22,26,39,40,41].
Up to now, a few studies have investigated patient outcomes in association with CEA and CA19-9 combined. Our study showed that a remarkably shorter 5-year survival rate of only 29.3% for patients with both tumor markers elevated compared to patients with either CEA or CA19-9 (CEA:61.4%, CA19-9: 76.5%) or no tumor marker (81.8%) increased.
In a current study, patients who had tumors with both tumor markers elevated were associated with short survival time and high rate of disease recurrence. On other hand, the best overall survival and disease-free survival was observed in patients who had both tumor markers normal. In addition, our study showed nearly equally outcomes in patients with either CEA or CA19-9 elevated. These results are in line with previous published data [27,31,37,38-40]. Overall survival rate for CRC patients with both tumor markers elevated was 23% and 71 % in patients with both tumor marker normal as reported by Lakemeyer et al [27 ]. Disease-free survival rate of patients with preoperative tumor markers elevated was remarkably shorter than in patients with only one tumor marker increased or with both tumor markers normal [22,42].
Cox regression analysis showed that stage was powerful an independent prognostic factor with combined tumor markers. The same finding was also reported by Lakemeyer et al, who showed by multivariate analysis that stage, CEA and CA19-9 CEA acts an independent prognostic factors [27].

**Conclusion**

The mean serum levels of CEA and CA19-9 for all CRC tumors were 70.0 ng/ml and 473.0 U/ml, respectively. Tumors with higher serum CEA and CA19-9 levels were found in 60.0 and 46% of CRC cases, respectively. Significantly, patients with higher CEA and CA19-9 serum levels had aggressive tumor grade, higher recurrence rate and shorter survival time and should be treated carefully. With carefully analyzing the preoperative tumor markers CEA and CA19-9 separately and combined in association with overall survival and disease

free survival, our study show that both biomarkers give significant prognostic information. In this study, increased CEA and CA19-9 levels evaluated separately already showed significant differences in overall survival and disease-free survival. Then, when analyzing both tumor markers combined in overall survival and disease-free survival, an even more significant result appeared, showing the association of increasing tumor markers resulting in lower life expectancy and recurrence-free survival. Since the combination of CEA and CA19-9 acts as an independent prognostic marker for survival. So, a more intensive therapy in patients diagnosed with an advanced CRC with combined elevation of tumor markers (CEA and CA19-9) is highly considered.

***Authors' contribution***

MA: study design and manuscript drafting; EE: statistical analysis, study design and collection of demographics and clinicopathologic data and data analysis. MG, MA, and AR and AG: data collection and analysis and drafting of manuscript and ME: data interpretation and analysis, drafting and proof reading of results and discussions; NE: preparation of figures, review of study, data interpretation, drafting and proof reading of manuscript, All authors critically reviewed and approved the final version of the manuscript.

***Ethical approval***

The study was done under research ethics approval by ethical committee at the National Cancer Institute, Misurata. All authors critically reviewed and approved the final version of the manuscript. Written informed consent was obtained from all patients for surgical treatment, pathologic examinations, and investigations performed according to the institutional guidelines of the National Cancer Institute, Misurata, Libya.

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