THE RELATIONSHIP BETWEEN TRIPLE TUMOR MARKER (CEA, CA 19-9, AND CA 125) AND COLORECTAL CANCER METASTASES AT MAKASSAR, INDONESIA

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ABSTRACT Introduction: Tumor markers combination (CEA, CA19-9, and CA125) can increase sensitivity due to interactions between several epitopes and tumor cells, especially in colorectal cancer (CRC) diagnosis. Therefore, this study aimed to assess the relationship between triple tumor markers (CEA, CA 19-9, CA 125) and CRC metastases. **Materials and Methods:** The research samples were CRC patients at Dr Wahidin Sudirohusodo Hospital, Makassar from November 2019 to April 2020. The histopathology data were collected through endoscopic or anal biopsy, CEA, CA 19-9, CA 125, Thorax X-Ray, Abdomen CT Scan, Surgery, and data processing. Furthermore, statistical tests were performed with the Mann Whitney difference test (significance = p <0.05). **Results:** 57 samples were obtained, consisting of 19 metastatic CRC and 38 non-metastatic cases with an age range of 18-82 years, and an average of 56.9 years. Furthermore, most of the patients were males with a ratio of 2:1, and the tumors are mostly located in the rectum by 68.5%, of which many are in stage II with 52.6%. The CEA value was obtained at $\geq$ 10 ng/ml (61.4%) and <10 ng/ml (38.6%), CA 19-9 was $\geq$ 10 ng/ml (61.4%) and < 10 ng/ml (38.6%), and CA 125 was $\geq$ 10 ng/ml (61.4%) and < 10 ng/ml (38.6%). Also, most metastases are located in the liver. Triple tumor marker significantly correlated with CRC (p <0.05), and the accuracy of the three combinations increased (87.7%), which indicates the markers can establish the presence or absence of metastasis in CRC patients. **Conclusion:** The markers (CEA, CA 19-9 and CA 125) can be used to establish the presence or absence of metastasis in CRC patients.

KEYWORDS Tumor Markers, Metastasis, Colorectal Cancer, CEA

Introduction

Colorectal cancer is the third most common malignancy after lung and breast, and it is the second leading cause of cancer death after lung [1]. The key to CRC survival is an early and accurate diagnosis. Furthermore, malignant tumors can spread through invasion, or move from its primary site to secondary sites in the body; this process is called metastasis. It is responsible for 90% of cancer patient deaths.

Early and precise diagnosis according to NCCN guidance 2019 consists of history taking, physical examination, tumor markers, colonoscopy, imaging (CT scan, MRI, Pet CT) and biopsy. However, these methods have several drawbacks such as failure of interpretation, the complexity of implementation, high costs, invasive, time-consuming procedures, and repeat requirements (3-5 years), which has led to bad reputation and

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poor patient compliance. Therefore, research is still needed on the use of tumor markers as a diagnostic tool for a non-invasive, quick, economical, and simple method of detecting metastasis [2,3].

These markers are detected through blood, urine, and body tissue (tumor tissue) of cancer patients. Furthermore, EGTM 2007, ASCO 2014, and NCCN 2019 on CRC often used and recommended CEA (Carcinoembryonic Antigen) marker. This is an oncofetal antigen, a high molecular weight glycoprotein produced by colon cells and functions as an intercellular adhesion molecule, which promotes CRC cell aggregation. Also, CA 19-9 acts as an intracellular adhesion molecule that affects cellular synthesis in various parts of the digestive system such as gastric, colorectal, and especially sensitive to the pancreas. Furthermore, CA (Carbohydrate Antigen) 125 is a sensitive marker in ovarian cancer, and it is also detected in gastrointestinal cancers such as gastric and colorectal. In addition, it is often used to detect peritoneal metastases in ovarian cancer [4].

According to Li et al. (2013), markers combination can increase sensitivity due to the interaction between several epitopes and tumor cells, which allows for clear cell identification [5]. Lalosevic, a pre-operative CEA combination and CA19-9 serum can be used to diagnose lymph node and liver metastases in CRC patients [6]. Furthermore, Li et al. (2017) found 4 markers in combination, causing increased sensitivity. According to Gao et al. (2018), CEA, CA19-9, CA72-4, CA125, and Ferritin serum can be used as diagnostic markers for CRC [3, 7]. Based on the facts above, researchers tried to ascertain the relationship between triple tumor markers and CRC metastases using easily obtained markers and often used in Indonesia, which are CEA, CA 19-9 and CA 125.

Patients and Samples

This was an observational study with a cross-sectional approach in the Department of Digestive Surgery, Dr Wahidin Sudirohusodo Hospital in Makassar, South Sulawesi. The research lasted for six months from 1 November 2019 to 31 April 2020.

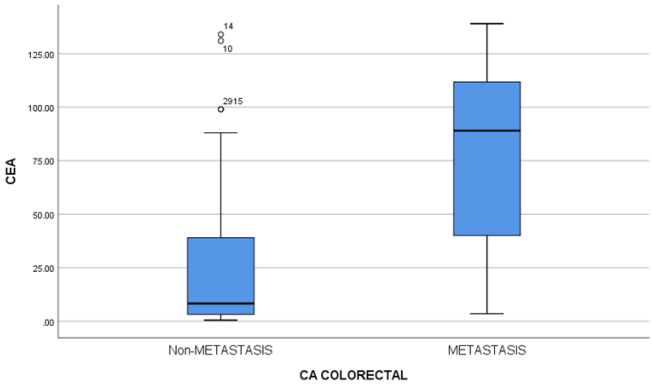
The population was CRC patients that had not undergone surgery, and with a biopsy of Lower Gastrointestinal Endoscopy (LGIE) and had a histopathological examination. The sample was the entire population that met the research criteria by consecutive sampling. Inclusion Criteria: Patients that did not experience total mechanical intestinal obstruction and perforation prior to surgery, and willing to be a research subject. Exclusion criteria: Patients that have undergone surgery, chemotherapy, radiotherapy, and had incomplete clinical and histopathological data.

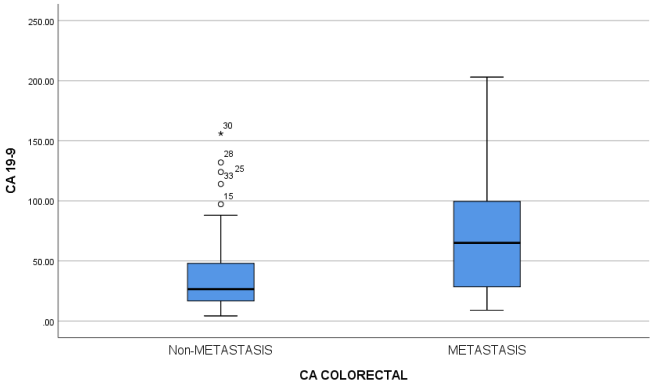
The principle of taking serum from the patient's blood

The cubital fossa area was disinfected with 70% alcohol swab, then 2 cc blood was taken with a disposable spout, followed by centrifugation at 200 rpm to get the serum.

Examinations of CEA, CA 19-9, and CA 125

This uses the electrochemiluminescent immunoassay (ECLIA) method from the Elicsys 2010 with Linearity of the tools: 0.200 - 1000 ng / ml (CEA), 0.200 - 3840 u/ml (CA 19-9), and 0.250 - 19,000 u / ml (CA 125). This technological method is for immunoassay detection, which uses antibodies to detect and measure specific analytes such as peptides, proteins, and hormones.



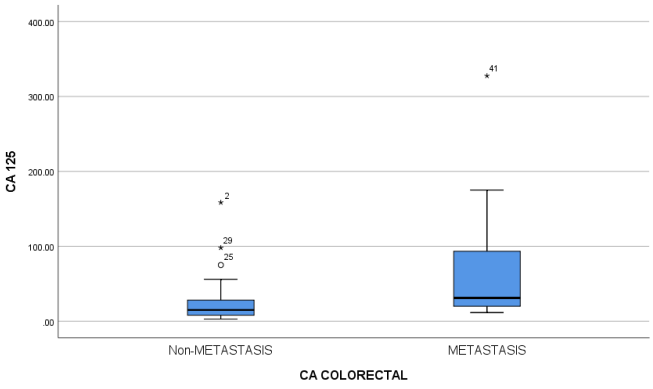


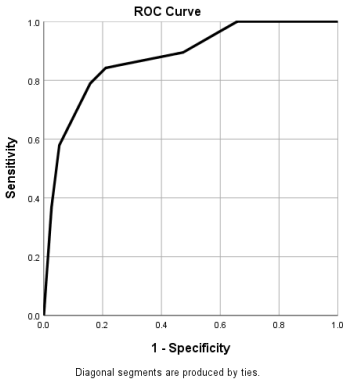
Research Processing and Analysis

The data was analyzed using SPSS version 22 (IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY). The statistical test used was the Mann Whitney difference test. Meanwhile, a significant result was obtained with P and lt value at 0.05.

Results

There were 271 subjects with only 57 meetings the criteria. 19 cases were metastatic, and 38 were non-metastatic with an age range of 20 - 60 years, and an average of 56.9 years. Furthermore, Male and female patients were 66.6% and 33.4% respectively, with a ratio of 2:1. In this study, the tumor was mostly found in the rectum of 68.5%, and the fewest was found in the transverse colon of 5.2%. According to the stage, patients with stage II, III, and IV were 52.6%, 14.1%, and 33.3% respectively and there was no patient with stage I. Based on CEA, 61.4% had a value of $\geq$





10 ng/ml while 38.6% had $\leq$ 10 ng/ml. Also, for CA 19-9, 61.4% had $\geq$ 10 ng / ml while 38.6% had $\leq$ 10 ng / ml. Based on the CA 125, 61.4% had $\geq$ 10 ng/ml while 38.6% had $\leq$ 10 ng / ml (Table 1). Based on location, the most liver metastases were 15 patients with CEA of $\geq$ 10 ng / ml 87%, CA 19-9 $\geq$ 37 u / ml 66%, and CA 125 $\geq$ 35 u / ml 40%. Also, CEA levels of <10 ng / ml was 13%, CA 19-9 <37 u / ml was 33%, and CA 125 <35 u / ml was 60%. Based on the location, pulmonary metastases were 2 patients, peritoneal 1, and bone was also 1 (Table 2).

**Mann Whitney test*

1. DIFFERENCES IN BIOMARKER LEVELS BETWEEN NON-METASTASTATIC AND METASTATIC CRC Based on table 3 results, the three markers (CEA, CA 19-9, CA 125) were significantly different (p <0.05) between non-metastatic and metastatic CRCs. Furthermore, the differences in CEA levels (figure 1), CA 19-9 (figure 2), CA 125 (figure 3) seem to be higher in metastatic than in non-metastatic. Also, it was found that the probability value (p <0.05) of CEA, CA 19-9, and CA 125 were <0.001, 0.019, and 0.002 respectively, which were significantly correlated. This means they have a strong relationship with metastasis. Figure 4 shows the combined ROC curves of biomarkers through multiple logistic regression analysis which indicates higher accuracy.

2. SENSITIVITY COMPARISON AND SPECIFICITY BETWEEN TRIPLE TUMOR MARKER AND SINGLE TUMOR MARKER IN CRC METASTASIS Table 4 shows the diagnostic test analysis results of each biomarker and a combination of the three. CEA, CA 19-9 and CA 125 have accuracy (81.8%, 69.2%, 75.9%), sensitivity (73.7%, 68.4%, 73.7%), specificity (84.2%, 68.4%, 63.2%), PPV (70.0%, 52.0%, 50.0%), and NPV (86.5%, 81.3%, 82.8%) respectively. Based on these values, CEA is the best. When the three biomarkers are combined, the accuracy, sensitivity, and NPV increase to 87.7%, 84.3%; and 90.9% respectively. However, the PPV and specificity decrease. Therefore, the results show an increase in sensitivity, a negative predictive value (NPV), a decrease in specificity and a positive predictive value (PPV) of triple tumor marker on CRC metastases.

Discussion

1. Research Characteristics Based on the age group, the subjects were 18-82 years old with a mean of 56.9 years, and those > 60 years old had the highest percentage level of 49.2%. Also, the tendency of CRC is high in the age group of 50-59 years and $\geq$ 60 years by 29.8% (17/57) and 49.2% (28/57), respectively. Furthermore, the percentage above and below 50 years was 79% (45/57) and 21% (12/57) respectively. In the past decade, there has been a trend change in Indonesia, where patients above 50 years experienced a decrease, and those below that age experienced an increase. This sharp increase was caused by (1) environmental factors influence which is either direct or indirect through dietary pattern change in young Indonesian people. This is a consequence of both increased prosperity and a shift towards the westerners' way of eating (westernization), which is high fat and low fiber foods. (2) genetic susceptibility factors, such as individuals born with mutations or genetic defects, for example, in hereditary groups such as HNPCC / Lynch syndrome [8].

   Based on gender, male and female patients were 66.6% (38/57) and 33.4% (19/57) respectively, with a ratio of 2: 1 (Table 7). According to Globocan 2018, CRC is more common in men than women, and 3-4 time common in developed countries. Also, men are more often affected than women with ratio of 7.7 / 100,000: 4.4 / 100,000 [1]. Based on location, the rectum is mostly affected 68.5% (39/57), the left colon of 15.8% (9/57), the right colon of 12.2% (7/57) and the fewest are in the transverse colon of 3.5% (2/57). Also, the patients were dominated by those with rectal cancer, probably due to the time factor.

   Based on stage, most of the patients were in stage II 52.6% (30/57), stage IV 33.3% (19/57), and the fewest were in stage III 14.1% (8/57), and none with stage I. Lusikooy 2013 reported that, out of the 159 CRC patients, the majority were still in stage IIb 27.7% (44/159), stage IIa 22.1% (35/159), stage IIIb 15.1% (24/159), stage IIIa13.2% (21/159), stage IV 20.7% (33/159) and stage Ia - Ib each 0.6% (1 / 159) [9]. Therefore, the results show that most CRC cases are still in stages IIa and IIb. This might be due to detection from colonoscopy investigations; thus, the cases can be diagnosed early.

   According to table 2, the liver is the most metastasis location, which is 78% (15/19). This is following Palaghia (2015) which stated that most CRC metastasis location is in the liver, at the time of initial diagnosis (20-25% of cases) or after primary tumor resection (40% of cases) [10].

2. RELATIONSHIP BETWEEN CEA AND CRC METASTASIS Figure 1 shows CEA levels in metastatic CRCs are higher than in non-metastatic. In the Mann Whitney test analysis, a Probability value of (Sig) <0.05 is <0.001 was found, which means the level has a significant correlation with CRC metastases. This is by Giovanni et al., in a long-term study in the form of 20 years surveillance, which stated that a serial CEA examination was performed on 125 of 239 patients and found that their sensitivity on the liver and non-hepatic metastases was 99% and 94% respectively. Therefore, they concluded that it is useful for grading preoperative colorectal tumors.

3. RELATIONSHIP BETWEEN CA 19-9 AND CRC METASTASIS Figure 2 shows CA 19-9 levels in metastatic CRC are higher than in non-metastatic. In the Mann Whitney test analysis, it was found that the Probability (Sig) value <0.05 was 0.019, which means the level was significantly correlated with CRC metastasis. This is in accordance with Malati et al. in 1996, which stated that CA 19-9 was also found to increase by 20-40% in colorectal cancers [11]. Fur-

Table 1 General Characteristics of Research Subjects.

Variable	Metastatic (n = 19)		Non-Metastatic (n = 38)		Total (n = 57)	
	n	%	n	%	n	%
Age Group (year old)						
< 20	-	-	1	2.6	1	1.7
20-29	-	-	1	2.6	1	1.7
30-39	1	5.2	3	7.8	4	7.1
40-49	1	5.2	5	13.1	6	10.5
50-59	8	42.2	9	23.7	17	29.8
$\geq$ 60	9	47.4	19	50.2	28	49.2
Gender						
Male	14	73.7	24	63.1	38	66.6
Female	5	26.3	14	36.9	19	33.4
Tumor Location						
Right Colon	2	10.5	5	13.1	7	12.2
Transversum Colon	1	5.2	1	2.7	2	3.5
Left Colon	4	21.1	5	13.1	9	15.8
Rectum	12	63.2	27	71.1	39	68.5
Stage						
I	-	-	-	-	-	-
II	-	-	30	78.9	30	52.6
III	-	-	8	21.1	8	14.1
IV	19	100.0	-	-	19	33.3
CEA (ng/ml)						
< 10	2	10.6	20	52.6	22	38.6
$\geq$ 10	17	89.4	18	47.3	35	61.4
CA 19-9 (u/ml)						
< 37	5	26.4	27	71.1	32	56.1
$\geq$ 37	14	73.6	11	28.9	25	43.9
CA 125 (u/ml)						
< 35	11	63.1	30	78.9	41	73.6
$\geq$ 35	8	36.9	8	21.1	16	26.4

Table 2 Characteristics of Metastasis based on marker levels.

Tumor Marker	Metastasis Location				
	Liver (n=15)	**Peritoneum (n='1)**	**Pulmonary (n=2)**	**Bone (n=1)**	**Brain (n=0)**
CEA (ng/ml)					
< 10	2 (13 %)	-	-	-	-
≥ 10	13 (87 %)	1 (100 %)	2 (100 %)	1 (100 %)	-
CA 19-9 (u/ml)					
< 37	5 (33 %)	-	-	1 (100 %)	-
≥ 37	10 (66 %)	1 (100 %)	2 (100 %)	-	-
CA 125 (u/ml)					
< 35	9 (60 %	-	2 (100 %)	-	-
≥ 35	6 (40 %)	1 (100 %)	-	1 (100 %)	-

Table 3 Differences in biomarker levels between Non-metastatic and Metastatic CRCs.

Biomarker	Group	Description			p*
		Min-Max	Mean (medium)	95% IK	
CEA	Non-Metastatic	0,5 – 134,0	26,47(8,25)	14,35-38,58	<0,001
	Metastatic	3.5 – 139,0	79,35(89,00)	57,68-101,02	
CA 19-9	Non-Metastatic	4,2-156,0	40,87(26,50)	28,19-53,54	0,019
	Metastatic	9,0-203,0	70,35(65,00)	9,00-203,00	
CA 125	Non-Metastatic	2,8-158,5	25,22(14,94)	15,21-35,23	0,002
	Metastatic	11,5-327,525	66,17(104,36)	27,98-104,36	

*Mann Whitney test

Table 4 Value of accuracy, sensitivity, specificity, and prediction of each biomarker and its combination.

Biomarker	Accuracy	Cut off	Sen(Spes)	NPP(NPN)
CEA	81,8%	54,0	73,7%(84,2%)	70,0/%(86,5%)
CA 19-9	69,2%	35,0	68,4/%(68,4%)	52,0%(81,3%)
CA 125	75,9%	20,0	73,7%(63,2%)	50,0%(82,8%)%
Combination-3	**87,7%**	**0,20**	**84,3%(78,9%)**	**66,7%(90,9%)**

thermore, Zhang et al. in 2015 concluded that it might be a tumor biomarker in addition to CEA for CRC [12].

4. RELATIONSHIP BETWEEN CA 125 AND CRC METASTASIS Figure 3 shows the CA 125 level in metastatic CRC is higher than in non-metastatic. In the Mann Whitney test analysis, it was found that the Probability value (Sig) <0.05 was 0.002, which means the level is significantly correlated with the CRC metastasis. This is in accordance with Bast et. al. in 1998, which stated that besides ovaries, it also increase in colorectal malignancies by 15.1% [13]. Furthermore, Huang et al in 2016 found an increase in CA 125 levels in men and women with CRC stage 4 (peritoneal metastasis) with a 57.1% sensitivity and 92.0% specificity [14].

5. RELATIONSHIP BETWEEN TRIPLE TUMOR MARKER (CEA, CA 19-9, and CA 125) AND CRC METASTASIS In Figure 5, the combined ROC curve of the three markers through multiple logistic regression analysis shows a higher accuracy. The markers combination was made possible based on Yang et al. in 2011, which found that they (CA19-9, CEA, and CA125) had an independent prognostic value to predict recurrence-free survival (RFS) in 5 years, in which patients with combined serum CA19-9, CEA and CA125 had the highest recurrence rate (100%), and the shortest RFS (median of 4 months) [15]. Therefore, combining CEA, CA19-9, CA72-4, CA125, and Ferritin can be used as a diagnostic marker for CRC [1].

6. COMPARISON OF SPECIFICITY AND SENSITIVITY BETWEEN TRIPLE AND SINGLE TUMOR MARKER IN CRC Table 4 shows the diagnostic test analysis results of each biomarker and a combination of the three. CEA, CA 19-9 and CA 125 have accuracy (81.8%, 69.2%, 75.9%), sensitivity (73.7%, 68.4%, 73.7%), specificity (84.2%, 68.4%, 63.2%), NPP (70.0%, 52.0%, 50.0%), and NPV (86.5%, 81.3%, 82.8%) respectively. Therefore, the diagnostic values show CEA as the best biomarker. Also, when the three are combined, the accuracy, sensitivity, and NPV increase to 87.7%, 84.3%, and 9 0.9%, respectively. However, the specificity and NPP decrease. This shows triple markers can determine the presence or absence of metastasis in CRC patients. This is following Li et al. (2013) which stated that markers combination could increase sensitivity because the interaction between several epitopes and tumor cells allows more clear identification [5]. Furthermore, Gao et al in 2018 found that several combinations (CEA, CA19-9, CA72-4, CA125, and Ferritin) can increase CRC diagnosis sensitivity and reliability, and assess the patient's pathological parameters [1].

Conclusion

This study shows triple markers (CEA, CA 19-9, and CA 125) can determine the presence or absence of metastasis in CRC patients.

Ethics committee approval

Ethics committee approval was received for this study from the ethics committee of Faculty of Medicine, Universitas Hasanuddin gave approval for this study (Ref.No.:1114/UN4.6.4.5.31/PP36/2019).

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Conflict of interest

There are no conflicts of interest to declare by any of the authors of this study.

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