The efficiency of collagen III, metalloproteinase 1, carcinoembryonic antigen, and carbohydrate antigen 19.9 for colon cancer diagnosis

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ABSTRACT:

Background: Currently, blood markers are noninvasive methods for the diagnosis of patients with colorectal cancer (CRC). We evaluated four biomarkers (Carcinoembryonic antigen (CEA), carbohydrate antigen 19.9 (CA19.9), collagen III, and metalloproteinase 1 (MMP-1)) and their combination as a substitute method to enhance diagnosis of CRC. **Subjects and methods:** one hundred ninety-five patients who had undergone colonoscopy examination were included in the study (135 CRC and 60 benign growths). Also, 45 healthy individuals were included. Multivariate discriminant analysis (MDA) and area under the receiver operating characteristic curve (AUC) were applied for evaluating the diagnostic power of single and their combination. **Results:** levels of collagen III, CEA, and CA 19.9 increased while MMP-1 decreased with the progression of CRC (stages, positive lymph node invasion, distant organ metastasis, and high grades) with a significant difference (P <0.01- P < 0.0001). Combination of **M**MP-1, **C**EA, **C**A 19-9, and **C**ollagen III yielded MC3 index had diagnostic power greater than every single marker could achieve alone. When differentiating colon cancer from benign growth, the AUC of MC3 index was 0.91 yielded 85.2% sensitivity and 86.7% specificity. Moreover, the MC3 index for patients with late stages, lymph node invasion, organ metastasis, and high-grade had AUC = 0.81, 0.82, 0.80, and 0.80 higher than CEA and CA19.9. **Conclusion:** MC3 index is can be used as an effective index for early detection of CRC.

KEYWORDS: Colorectal cancer, Diagnosis, Blood markers, Extracellular matrix (ECM), Collagen, Metalloproteinase, Index,

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1. INTRODUCTION

CRC is a heterogeneous disease, there were about 1.8 million new cases of colorectal cancer in 2018 **(Bray et al., 2018)**. CRC screening may be divided into two main categories: the first category includes fecal, blood, and urine biomarkers and the second group includes image-based methods such as flexible sigmoidoscopy and colonoscopy, **(Dhaliwal et al., 2015)**. The restriction of fecal occult blood test (FOBT), it's being an indirect screening test. Sigmoidoscopy allows the physician to see the rectum and any tumor in the upper part of the colon will be not diagnosed **(Jones et al., 2010)**. Colonoscopy is the gold standard method for colon cancer screening because colon cancer can be both detected and removed during the same procedure. But it is an invasive and not suitable method **(Smith et al., 2012)**. Each of these methods has its shortcomings. Hence, a search for non-invasive convenient, and inexpensive tumor marker with a high degree of accuracy, is effective to monitor colon cancer **(Torre et al., 2015)**. CEA and CA19-9 are routine tumor markers used in the screening of patients with colon cancer **(Vukobrat-Bijedic et al., 2013)**. However, they are not suitable for the early detection of colon cancer, as their sensitivity and specificity are low **(Lech et al., 2016)**. Thus, there is an urgent demand for biomarkers that can serve as diagnostic and prognostic markers for colon cancer **(Hauptman and Glavac, 2017)**. Increased or decreased collagen levels are associated with CRC **(Fang et al., 2014; Levental et al., 2009)**. Also, MMP-1 is an important proteolytic enzyme for extracellular matrix (ECM) remodeling during organ development and tissue regeneration **(Ala-aho and Kahari, 2005)**. MMP-1 is degraded II and III collagens that represent the main ingredients of the interstitial stroma **(Said et al., 2014)**. Therefore, this work aimed to evaluate the diagnostic performances of CEA, CA 19-9, collagen III, and MMP-1 as single and combined markers associated with colon cancer clinicopathologic parameters.

2. METHODOLOGY

Subjects

Serum samples from 195 patients, 109 males and 86 females, with age ranged 22-79 years and Mean ± SD; 48.8±12.8 years collected from the Oncology Center, Mansoura University Hospitals, Mansoura, Egypt between May 2016 and July 2018. They classified into 60 patients with benign growth conditions and 135 patients with colon cancer using colonoscopy. The tumor features were registered according to the Union for International Contrele Cancer-Tumor-Node-Metastasis (TNM) Staging System **(Greene, 2003)**. Also, 45 healthy individuals (age- and sex-matched) were included. Neither of the patients with benign tumors nor of the healthy individuals had a record of any cancer. This study was approved by the ethics and scientific committees of Mansoura University Hospitals, Mansoura, Egypt. Informed consent was get from each individual to collaborate in the present study.

Blood samples collection

Serum samples were obtained after centrifuged blood samples at 4000 rpm for 15 minutes. The serum was stocked at −20 °C until use. CA19.9 and CEA were measured by ELISA assay (IRMA) (IBL, Germany) according to the industrial prescript.

Determination of collagen III and MMP-1 using ELISA

Collagen III and MMP-1 were determined using ELISA as described in our previous studies **(Attallah et al., 2017)**. Collagen III and MMP-1 levels were expressed as μ/ml. Samples and controls were tested in duplicate. Samples were considered to be invalid and repeated the assay if the difference between levels (μg/ml) was more than 25%.

Statistical analysis: Data was expressed as mean ± standard deviation (SD). Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS version, 24). ANOVA or t-test was used when the Kolmogorov–Smirnov test results were not significant, while the Mann–Whitney U test was used in cases with nonparametric variables. Significance is defined at P<0.05. Novel indexes were developed using multivariate discriminant analysis and area under ROC. Diagnostic performances [sensitivity, specificity, positive predictive value, and negative predictive value] were determined after selections of the best cutoff points.

3. RESULTS

### *Quantitation of collagen III and MMP-1 levels*

The 135 patients with colon cancer were presented according to tumor depth, lymph node invasion, and distant organ metastasis, and high grade. Levels of CEA, CA 19.9, and collagen III increased while MMP-1 decreased with late stages, positive lymph node invasion, distant organ metastasis, and high grades of colon cancer with a significant difference (P <0.01- P < 0.0001) as shown in **Table 1** and **Fig. 1**.

Table 1: The level of candidate markers in different stages of the disease

Groups	CA 19.9 (U/mL)	CEA (ng/mL)	MMP-1 (µg/ml)	Collagen III (µg/ml)
Benign growth *Vs* Colon cancer				
Benign (n=60)	14.1±0.7	4.0±0.3	4.4±0.2	3.1±0.5
Cancer (n=135)	27.0±2.3	19.4±2.7	3.6±0.2	11.0±0.8
***P* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Tumor depth (Early stages *Vs* Late stages)				
Tis-T2 (n=64)	17.6±1.4	10.4±2.4	4.3±0.2	8.1±0.8
T3-T4 (n=71)	35.2±4.0	27.7±4.7	2.9±0.2	12.8±1.2
***P* value**	< 0.001	< 0.0001	< 0.0001	< 0.0001
Lymph node invasion (N1-N2 *Vs* N0)				
N0 (n=84)	20.2±1.6	11.2±2.2	4.1±0.2	8.5±0.6
N1-N2 (n=51)	38.3±5.3	32.8±6.0	2.7±0.2	15.1±1.6
***P* value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Distant Organ Metastasis (M1 *Vs* M0)				
M0 (n=99)	21.4±1.7	13.3±2.5	3.8±0.1	8.7±0.6
M1 (n=36)	40.8±6.4	34.4±6.7	3.1±0.1	16.8±1.9
***P* value**	< 0.0001	< 0.0001	< 0.01	< 0.0001
Tumor grade (Low-grade *Vs* High grade)				
Low grade (n=80) G1-G2	23.1±2.6	10.8±2.3	3.9±0.2	8.3±0.6
High grade (n=55) G3-G4	32.5±4.3	32.2±5.6	3.0±0.1	15.4±1.5
***P* value**	< 0.05	< 0.0001	< 0.01	< 0.0001

Data presented as (Mean ± SEM). **SEM:** Standard error of the mean**, n:** Number of samples

***P* value** < 0.05 statistically significant difference. ***P* value** < 0.01 statistically high significant difference**.**

***P* value** < 0.0001 statistically extremely high significant difference**.**
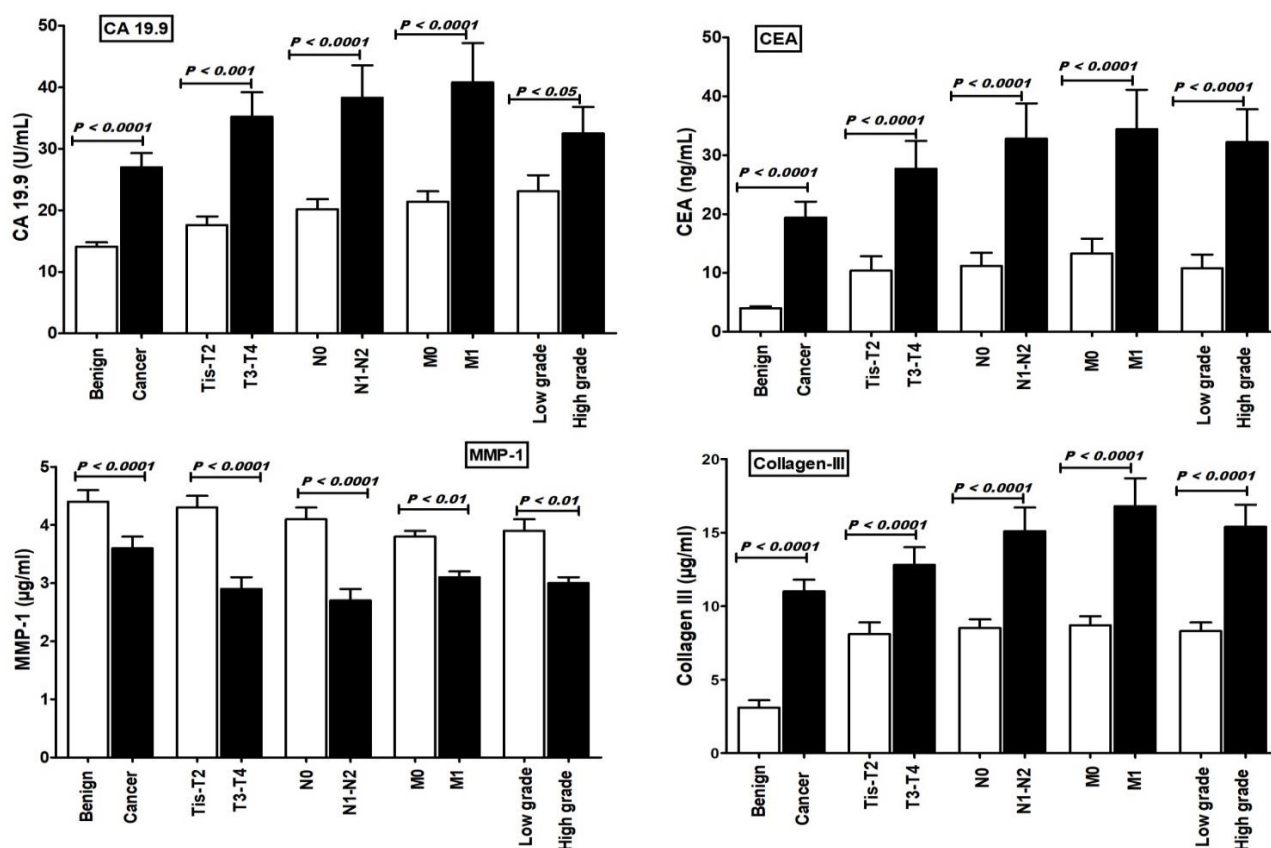
Figure 1: Levels of CA 19.9, CEA, MMP-1, and Collagen III in Benign growth *vs* Colon cancer, Tumor depth (early stages *vs* late stages), Lymph node invasion (N1-N2 *vs* N0) and Tumor grade (Low grade *vs* high grade).

Diagnostic performance of candidate markers

Using ROC curve analysis, we assessed and compared the diagnostic accuracy of CA 19.9, CEA, MMP-1, and collagen III in the diagnosis of colon cancer. The areas under ROC of candidate colon markers were in the order of CA 19.9 (0.58), CEA (0.61), MMP-1 (0.61), and collagen (0.84) to differentiate colon cancer from benign growth. Serum collagen was the most efficient marker for detecting patients with colon cancer (AUC=0.84), organ metastasis (AUC=0.70), and high grade from benign growth (AUC=0.69). While MMP-1 was the most efficient marker for detecting patients with early-stage (AUC=0.72) and lymph node invasion (AUC=0.75). The diagnostic performance of candidate markers associated with colon cancer clinicopathologic parameters was listed in **Table 2**.

Diagnostic performance of colon index

The multiple discriminant analysis (MDA) showed that CA19.9, CEA, MMP-1, and collagen III

were significantly ($P < 0.0001$) associated with colon cancer. Sex, age was not significantly ($P > 0.05$) associated with colon cancer. We developed a new index to differentiate between colon cancer patients and benign tumor named MC3 (**M**MP-1, **C**EA, **C**A19.9 and **C**ollagen) index = 0.02 x CEA + 0.02 x CA19.9 + 0.14 x Collagen - 0.2 x MMP-1. When differentiating colon cancer from benign growth, the AUC of MC3 index was 0.91 yielded 85.2%

sensitivity and 86.7% specificity at 0.3 cutoffs. As shown in **Fig. 2,** the AUC of the MC3 index was 0.81, 0.82, 0.80, and 0.80 for patients with late stages, lymph node invasion, organ metastasis, and high grade. The diagnostic performances of the MC3 index associated with colon cancer clinicopathologic parameters were listed in **Table 2**. The diagnostic performances of the MC3 index were greater than every single marker could achieve alone.

Table 2: Diagnostic performance of CA 19.9, CEA, MMP-1, Collagen III, and Index

Variables	Cut-off	Sensitivity	Specificity	PPV	NPV
Benign growth *Vs* Colon cancer					
CA 19.9 (U/mL)	15.3	59.4%	55.6%	74.8%	37.5%
CEA (ng/mL)	3.0	62.2%	60.0%	77.8%	41.4%
MMP-1 (µg/mL)	4.0	63.0%	68.4%	81.0%	44.4%
Collagen III (µg/mL)	2.5	76.0%	79.0%	88.8%	60.0%
MC3 Index	0.3	85.2%	86.7%	93.5%	72.2%
Tumor depth (Early stages *Vs* Late stages)					
CA 19.9 (U/mL)	15.3	60.7%	64.8%	59.7%	65.7%
CEA (ng/mL)	6.0	57.4%	55.7%	53.0%	60.6%
MMP-1 (µg/mL)	4.0	73.8%	74.6%	71.4%	76.8%
Collagen III (µg/mL)	6.2	73.2%	73.8%	70.7%	75.5%
MC3 Index	1.5	82.0%	82.3%	79.4%	84.0%
Lymph node invasion (N1-N2 *Vs* N0)					
CA 19.9 (U/mL)	15.3	58.8%	63.4%	72.1%	47.8%
CEA (ng/mL)	6.0	68.6%	68.0%	78.1%	56.4%
MMP-1 (µg/mL)	4.0	71.4%	78.0%	84.5%	62.5%
Collagen III (µg/mL)	6.2	66.7%	68.6%	77.8%	55.6%
MC3 Index	1.5	82.1%	82.4%	88.5%	73.7%
Distant Organ Metastasis (M1 *Vs* M0)					
CA 19.9 (U/mL)	15.3	66.7%	61.6%	76.7%	48.8%
CEA (ng/mL)	6.0	70.0%	67.0%	78.3%	56.5%
MMP-1 (µg/mL)	4.0	66.7%	66.2%	77.5%	55.5%
Collagen III (µg/mL)	6.2	75.4%	72.2%	84.0%	60.0%
MC3 Index	1.5	84.1%	86.1%	92.3%	73.8%
Tumor grade (Low-grade *Vs* High grade)					
CA 19.9 (U/mL)	15.3	66.2%	60.0%	70.0%	56.0%
CEA (ng/mL)	6.0	68.8%	67.3%	74.6%	66.6%
MMP-1 (µg/mL)	4.0	66.2%	65.5%	73.0%	63.0%
Collagen III (µg/mL)	6.2	70.0%	67.0%	77.8%	67.6%
MC3 Index	1.5	81.5%	83.8%	86.5%	72.7%

PPV: positive predictive value, **NPV:** negative predictive value
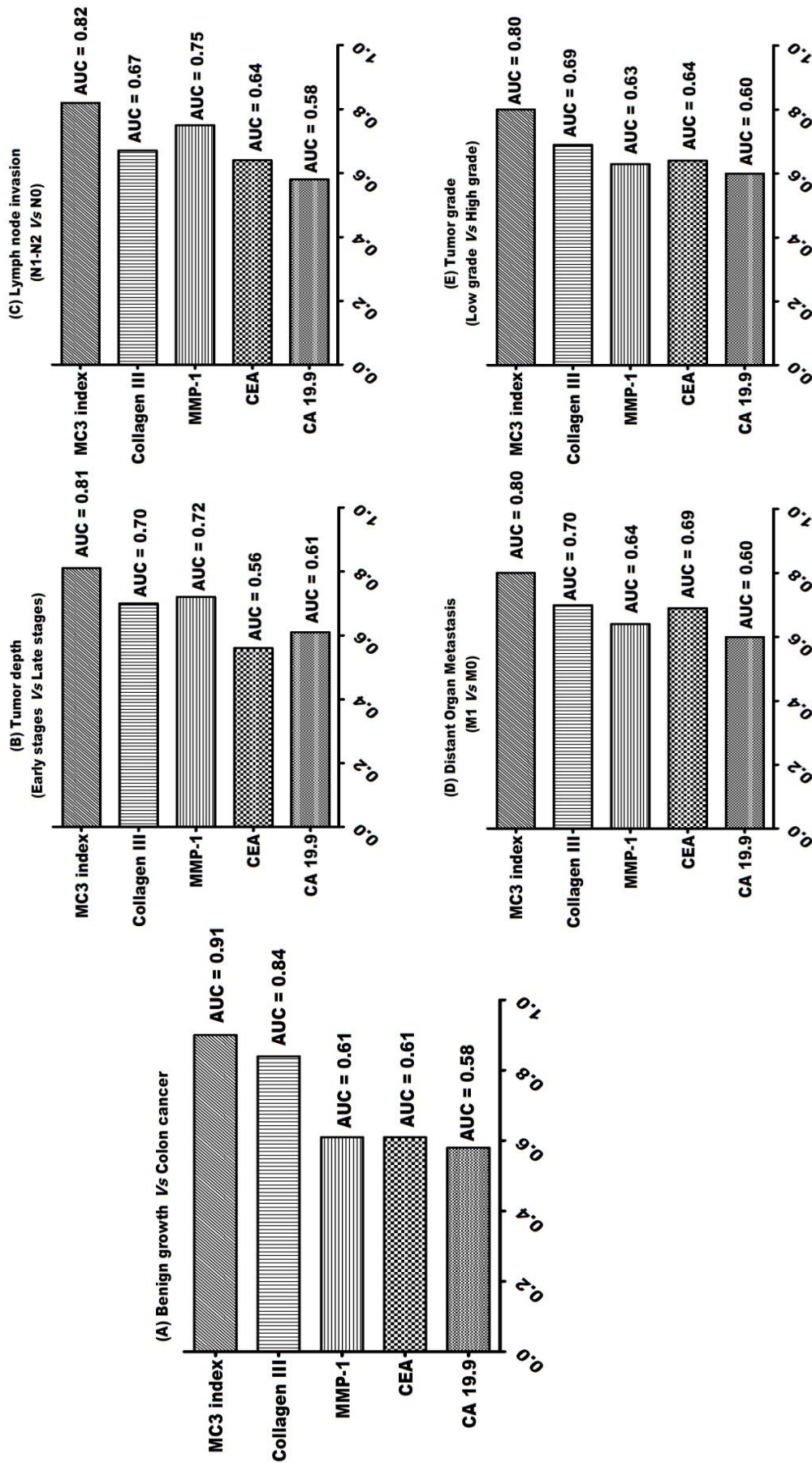
Figure 2: Diagnostic performance of CA 19.9, CEA, MMP-1, Collagen III and MC3 Index. (A) Benign growth *vs* Colon cancer. **(B)** Tumor depth (Early stages *vs* Late stages). **(C)** Lymph node invasion (N1-N2 *vs* N0). **(D)** Distant Organ Metastasis (M1 *vs* M0). **(E)** Tumor grade (Low grade *vs* High grade).

4. DISCUSSION

Colon cancer is a very common malignancy in the world **(Araghi et al., 2018)**. Colon blood markers examinations are accepted in public than stool examination. The majority of participants in CRC screening choose blood examination (83%) or stool examination (15%) instead of colonoscopy **(Adler et al., 2014)**. There is an urgent need for reliable blood markers or indexes to improve the diagnosis of colon cancer. MC3 index was based on four blood markers (MMP-1, collagen, CEA, and CA 19-9). These four candidate tests play a significant role in the development of colon cancer **(Mysliwiec and Ornstein, 2002; Prager et al., 2014; Shibutani et al., 2014; Shiozawa et al., 2000; Walker et al., 2018)**. In this study, colon cancer patients with advanced stages, +ve lymph node invasion, metastasis, and high grades were correlated with higher levels of CEA, CA 19-9 and collagen III and decrease the level of MMP-1 in contrast to benign colon diseases with a significant difference ($P < 0.01$- $P < 0.0001$). Cancer development associated with higher levels of collagen and other ECM molecules promotes cancer tissue rigidity **(Walker et al., 2018)**. The elevated levels of collagen III in our patients with colon were agreed with the results of Basso et al **(Basso et al., 2001)** . MMPs play an important function in the development of human carcinoma. The destruction of ECM, tumor invasion, and metastasis were mediated with MMPs. MMPs also have angiogenic activity and play role in tumorigenesis **(Mysliwiec and Ornstein, 2002)**. Shiozawa et al. **(Shiozawa et al., 2000)** reported that levels of MMP-1 were associated with the severity of CRC. CEA and CA 19-9 were used in diagnosing colon cancer **(Shiozawa et al., 2000)**. CEA affects the formation of CRC by promoting tumor cell survival and encourage tumor angiogenesis **(Prager et al., 2014)**. CA 19.9 plays important functions in a cell to cell recognition processes. The combination of CA 19.9 and CEA was used to diagnose patients with CRC **(Shibutani et al., 2014)**. Our results similar to those reported by Cosimelli et al, who found that elevated levels of CEA and CA 19-9, correlated with the depth of invasion and pathological stage **(Cosimelli et al., 1989)**. CEA and CA19-9 have a low diagnostic performance (sensitivity of 40% -70%) and (specificity of 73% -90%) **(Nikolaou et al., 2018)**. Epigenetic changes markers have been evaluated for colorectal diagnosis with 70%-96% sensitivity and 72%-96% specificity. DNA genetic and epigenetic biomarkers had sensitivity (30% - 87%) and 96% specificity **(Lech et al., 2016)**. Therefore, a combination of CEA, CA 19-9, collagen, and MMP-1 yielded a novel colon index. The AUC of colon index was 0.91 with 85.2% sensitivity and 86.7% specificity for differentiation of colon cancer from benign growths. Several authors combined single markers to raise the diagnostic performances for the diagnosis of colon cancer. The score based on six blood markers included CEA, ferritin, seprase, cytokeratin, osteopontin, and p53 gave sensitivity (70%) and specificity (95%) **(Wild et al., 2010)**. MC3 index for patients with late stages, lymph node and organ metastasis had AUC = 0.81, 0.82, 0.80 and 0.80 in comparison with 0.72, 0.80 and 0.80 for colon score **(Attallah et al., 2018)**. The combination of serum lipids (cholesterol, high-density lipoprotein) and cancer antigens (CEA and CA19) were rose PPV to 88% for the diagnosis of colon cancer **(Li et al., 2018)**. The AUC of B7-H4, osteopontin, tissue polypeptide-specific antigen, and CEA were 0.87, 0.81, 0.81, and 0.83, respectively. AUC of B7-H4 and CEA was 0.93 with high sensitivity (99%) and 80% specificity for diagnosis CRC from healthy individuals **(Wang et al., 2018)**.

In conclusion, a combination of serum CEA, CA 19-9, collagen, and MMP-1 yielded MC3 index with a diagnostic power greater than every single marker could achieve alone.

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