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Clinical value of a diagnostic score for colon cancer based on serum CEA, CA19-9, cytokeratin-1 and mucin-1

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ABSTRACT

Background: Although established markers such as CEA and CA19-9 are important for diagnosing early stages of colon cancer, they are not ideal. Developing promising markers include cytokeratin 1 (CK1) and mucin-1 (MUC1), but the combined value of each of these markers is unclear. We therefore evaluated the value of a combined laboratory-based score of these four markers in the diagnosis of colon cancer.

Methods: Two hundred patients who had undergone colonoscopic examination (150 colon cancer, 50 benign growths) were recruited. The study was controlled by 35 healthy subjects. CEA, CA19-9, CK1 and MUC1 were measured by ELISA and evaluated for cancer diagnosis using area under the receiver operating characteristic curve (AUC).

Results: Serum levels of all four markers were increased in the order colon cancer > benign disease > healthy controls ($p < 0.001$). In multivariate analysis, CA19.9 ($p = 0.025$), CK1 ($p < 0.001$) and MUC1 ($p = 0.009$) were significant independent predictors of colon cancer. A score that gave the greatest power of discrimination for colon cancer was defined as 1.06 + [0.001 × CA19.9 result] + [0.003 × CEA result] + [0.03 × CK1 result] + [0.05 × MUC1 result]. The colon score provided superior discrimination, AUC, and sensitivity and specificity for colon cancer versus benign growth than each of the individual markers. Similarly, the colon score provided superior AUC, and sensitivity and specificity that each individual marker for tumour stage, lymph node invasion and distant organ metastases than each individual marker.

Conclusion: A colon score derived from serum CEA, CA19-9, CK1 and MUC1 is a potential valuable non-invasive index that could be used for detection and screening early stage colon cancer patients.

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Introduction

Worldwide, colon cancer is the third most commonly detected cancer, and being generally symptomatic, is often diagnosed in a late stage of development [1,2]. The probability of diagnosis and cure of treatable cancerous lesions is felt to be attributable to the early detection. Although colonoscopy is the primary clinical tool [3], it is expensive, invasive and not favourable for diagnosis of colon cancer [4]. Other imaging techniques such as magnetic resonance imaging and computed tomography have several drawbacks, such as expense, lack of sensitivity and (for the latter) radiation exposure [2]. Also, colon biopsy is an invasive method, unsuitable for the patients and sometimes prone to other greatest risks. Thus, alternative, non-invasive markers have been developed that, it is hoped, will improve colon cancer detection [5].

Tumour markers are used not only for diagnostic purposes but also to improve the predictive power of clinical and pathological factors [6]. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are the most frequently used markers, although sensitivities are low [7]. Thus, novel colon cancer biomarkers that will further enhance the detection and follow-up should be developed. Cytokeratin (CK) forms part of the cytoskeleton and the largest group of intermediate filament proteins. Two types are classified as acidic type I (cytokeratins 9–23) and basic type II (cytokeratins 1–8). High expression of these proteins is linked to cell transformation and epithelial tumourigenesis [8]. MUC1 is a glycoprotein expressed on the top borders of secretory epithelial cells. It is a transmembrane protein and its aberrant intracellular localisation, over expression, and glycosylation alters and confers tumourigenicity as reported in most human tumours [9].

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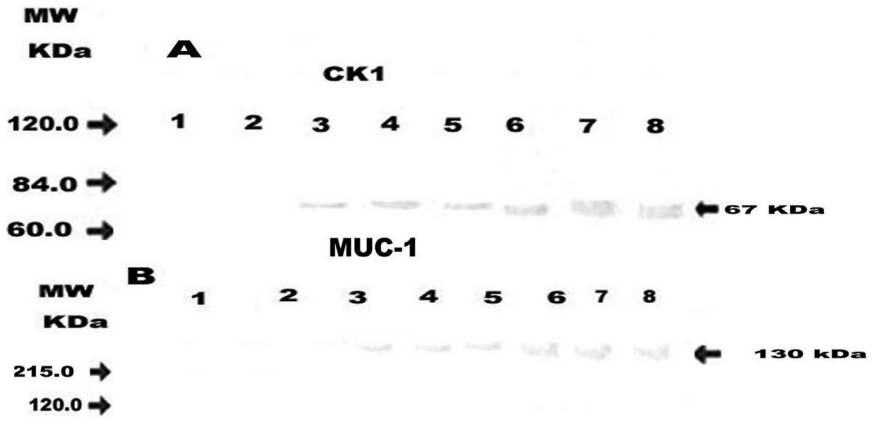

Figure 1. Identification of (A) CK1 and (B) MUC1 in sera of patients with colon cancer using western blot.
Notes: Lanes 1–2: serum from healthy individuals was used as negative controls. Lanes 3–5: serum from patients with benign growth. Lanes 6–8: serum from patients with colon cancer. Molecular weight marker was included myosin (215.0 kDa), phosphorylase B, (120.0 kDa), bovine serum albumin (84.0 kDa), ovalbumin (60.0 kDa). No reaction was observed in serum samples of healthy controls.

Table 1. Levels of CEA, CA 19.9, CK1 and MUC1.

Groups	CEA (ng/mL)	*p* Value	CA 19.9 (U/mL)	*p* Value	CK1 (µg/ml)	*p* Value	MUC1 (µg/ml)	*p* Value	Colon score	*p* Value
Studied groups										
Healthy ($n = 35$)	1.2 ± 0.3	<0.001	8.2 ± 0.4	<0.0001	0.1 (0.04–0.2)	<0.0001	1.2 ± 0.3	<0.0001	0.1 (0.04–0.2)	<0.0001
Benign ($n = 50$)	2.9 (1.6–5.9)[a]		14.1 ± 5.4[a]		0.5 (0.1–0.5)[a]		1.6 (1.2–3.3)[a]		1.21 ± 0.1[a]	
Cancer ($n = 150$)	4.8 (1.8–38.5)[a,b]		15.5 (9.8–48.9)[a,b]		2.0 (0.2–3.5)[a,b]		2.7 (2.2–3.6)[a,b]		1.4 ± 0.2[a,b]	
Tumour stage										
T1-T2 ($n = 57$)	2.9 (2.4–9.5)	0.31	15.5 (15.4–21.8)	0.06	2.2 (0.2–3.5)	<0.01	2.4 ± 0.7	<0.0001	1.3 ± 0.2	<0.01
T3-T4 ($n = 90$)	5.5 (1.8–38.5)		15.3 (9.8–49.9)		2.6 (0.5–3.5)		3.3 (2.4–5.3)		1.4 ± 0.2	
Lymph node invasion										
No ($n = 86$)	2.9 (1.7–7.5)	<0.01	15.5 (8.9–21.8)	<0.001	1.3 (0.2–3.5)	<0.0001	2.50 (2.0–3.3)	<0.0001	1.3 ± 0.2	<0.0001
Yes ($n = 64$)	10.1 (2.1–38.5)		48.9 (6.5–73.3)		3.5 (1.3–4.5)		3.4 (2.3–6.4)		1.5 ± 0.3	
Distant organ metastasis										
M0 ($n = 112$)	2.9 (1.7–9.4)	<0.0001	15.5 (8.9–43.0)	<0.001	2.0 (0.2–3.5)	<0.001	2.60 (2.0–3.3)	<0.0001	1.3 ± 0.2	<0.0001
M1 ($n = 38$)	38.5 (7.3–48.9)		48.9 (6.5–87.0)		3.5 (1.3–4.5)		4.3 (2.35–7.6)		1.6 ± 0.3	

Notes: Data are presented as Mean ± standard division or median with interquartile. *n*: Number of samples. Overall *p* value by ANOVA.
[a]Significant ($p < 0.05$) difference vs. control group [b]Significant ($p < 0.05$) difference vs. benign disease.

We set out to determine the value of the combination of serum CK1 and MUC1 with other established colon cancer markers (CEA, CA19.9) in increasing the overall sensitivity of the diagnosis of colon cancer.

Materials and methods

Subjects were recruited from the Oncology center, Mansoura, Egypt between January 2015 and July 2016. Blood samples were collected from 200 patients, of whom 150 were subsequently diagnosed with colon cancer and 50 with a non-cancerous (benign) growth. In addition, 35 blood samples for age- and sex-matched healthy individuals were included. Ethics and Scientific Committees of the Mansoura University Hospitals, Mansoura, Egypt approved this study. Informed consent was obtained from each participant. Depending on the cancer localisation, all patients had CT of the abdomen and pelvic MRI. Tumour infiltration and localisation the tumour site were visualised using colonoscopy. Targeted biopsy confirmed the diagnosis for all patients. The clinical data were recorded according to the Union International Contrele Cancer-Tumor-Node-Metastasis (TNM) Staging System [10].

Identification of CK1 and MUC1 was confirmed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot techniques. SDS-PAGE was carried out under non-reducing conditions according to Laemmli [11] and western blot analysis was performed according to Towbin et al. [12]

Table 2. Multivariate analysis of factors independently associated with colon cancer.

	Univariate analysis	Multivariate analysis	
Factor	*p* Value	OR (95% CI)	*p* Value
Sex	0.232	0.91 (0.4–2.2)	0.842
Age	0.07	0.98 (0.9–1.0)	0.278
CEA	0.001	0.96 (0.9–1.0)	0.187
CA19.9	<0.0001	0.95 (0.9–1.0)	0.025
CK1	<0.0001	0.43 (0.3–0.7)	<0.0001
MUC1	<0.0001	0.53 (0.3–0.9)	0.009

Notes: OR = Odds ratio, CI = Confidence interval.

Table 3. Diagnostic performance of markers and colon score.

	CEA (ng/mL)	CA 19.9 (U/mL)	CK1 (µg/ml)	MUC1 (µg/ml)	Colon score
Colon cancer vs. Benign growth					
AUC	0.58	0.60	0.75	0.73	0.84
95% CIs	0.49–0.67	0.52–0.68	0.68–0.83	0.64–0.82	0.78–0.90
Cut-off	4.00	13.10	0.35	2.00	1.21
Sensitivity (%)	54.2	64.2	70.8	74.2	87.5
Specificity (%)	68.7	70.6	73.7	76.0	91.2
Tumour stage (Early stage vs. late stage)					
AUC	0.51	0.54	0.63	0.67	0.72
95% CIs	0.41–0.63	0.43–0.65	0.53–0.73	0.58–0.77	0.64–0.82
Cut-off	4.00	13.10	2.75	2.17	1.32
Sensitivity (%)	57.4	61.8	70.6	72.1	76.5
Specificity (%)	67.7	71.2	72.0	75.4	80.1
Lymph node invasion (N1–N2 vs. N0)					
AUC	0.63	0.66	0.70	0.71	0.80
95% CIs	0.54–0.75	0.44–0.68	0.64–0.83	0.59–0.79	0.73–0.89
Cut-off	4.00	13.10	2.75	2.75	1.32
Sensitivity (%)	61.0	58.7	63.0	65.2	84.8
Specificity (%)	67.6	68.7	75.7	73.0	78.3
Distant organ metastasis (M1 vs. M0)					
AUC	0.75	0.58	0.63	0.66	0.80
95% CIs	0.64–0.87	0.42–0.74	0.52–0.74	0.53–0.79	0.72–0.89
Cut-off	9.75	13.10	2.75	2.17	1.32
Sensitivity (%)	76.9	61.5	57.8	69.2	80.8
Specificity (%)	71.0	68.6	66.3	70.0	81.4

Notes: AUC: Area under curve, *n*: number of patients. Reference range for serum CA19-9 < 37 U/mL. CI = Confidence interval.

with some modification for CK1 [13] and MUC1 [14]. Serum CK1 and MUC1 levels were determined using CK1 (ABC Diagnostics, New Damietta, Egypt) and MUC1 (ABC Diagnostics, New Damietta, Egypt) monoclonal antibodies and an ELISA as described in our previous studies [13,14]. Both CEA and CA-19-9 were determined using commercial ELISA kits (Monobind Inc. Lake Forest, CA92630, U.S.A.).

Statistical analysis was as follows. Continuous variables were expressed as mean with standard deviation (SD) and median (interquartile range) in case of non-normal data distribution. Statistical analyses were performed by SPSS software version 22.0 (SPSS Inc., Chicago, IL). Statistically significant differences were determined using ANOVA or Student *t* test was used in cases where 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 $p < 0.05$. Multivariate discriminant analysis was used to develop the optimum colon cancer diagnostic score. The diagnostic power was estimated using the area under the receiver operating characteristic (AUC) curves. Based on the receiver operating characteristic analysis (ROC), the best cut-off points were selected and diagnostic performances were determined.

Results

Sharp western blot bands of CK1 and MUC1 were observed at 67 and 130 kDa, respectively, in sera of colon cancer patients while no reaction was found in sera of healthy individuals (Figure 1). Serum levels of all four markers were increased in the order colon cancer > benign disease > healthy controls ($p < 0.001$; Table 1). In multivariate analysis, CA19.9, CK1 and MUC1 were significant independent predictors of colon cancer (Table 2). AUC, sensitivity and specificity data are shown in Table 3. Using these data, a score was calculated that gave the greatest power of discrimination for colon cancer. This score was defined as 1.06 + [0.001 × CA19.9 result] + [0.003 × CEA result] + [0.03 × CK1 result] + [0.05 × MUC1 result]. The colon score provided superior discrimination (Table 1), AUC, and sensitivity and specificity (Table 3) for colon cancer vs. benign growth than each of the individual markers.

CK1 and MUC1 levels increased with late stage *vs* early stage disease, and all markers were increased in those with lymph node invasion and with distant organ metastases (Table 1). The colon score displayed superior AUC, sensitivity and specificity for tumour depth, lymph node invasion and distant organ metastases (Table 3 and Figure 2).

Discussion

Biomarkers can be used for detecting early stage of colon cancer [15]. Higher levels of mucins and cytokeratins have been found in hepatocellular carcinoma, breast and colon cancers, and play important roles in diagnosis and prognosis of colon cancer [16,17]. In the present study, using western blot analysis CK1 was identified at 67 kDa and MUC1 was identified at 130 kDa in sera of patients with colon cancer and patients with a benign condition. Several authors reported that CK1 have high molecular weight range (40–68 kDa) [17–20]. MUC epithelial membrane antigen molecular weight ranging from 35 to 1500 kDa have been reported [13,21,22]. We report a significant increase in serum CK1 and MUC1 levels in patients with colon cancer compared with healthy controls and those with a benign growth. In the cancer patients, CK1 and MUC1 levels increased with late stage, positive lymph node invasion, and with distant organ

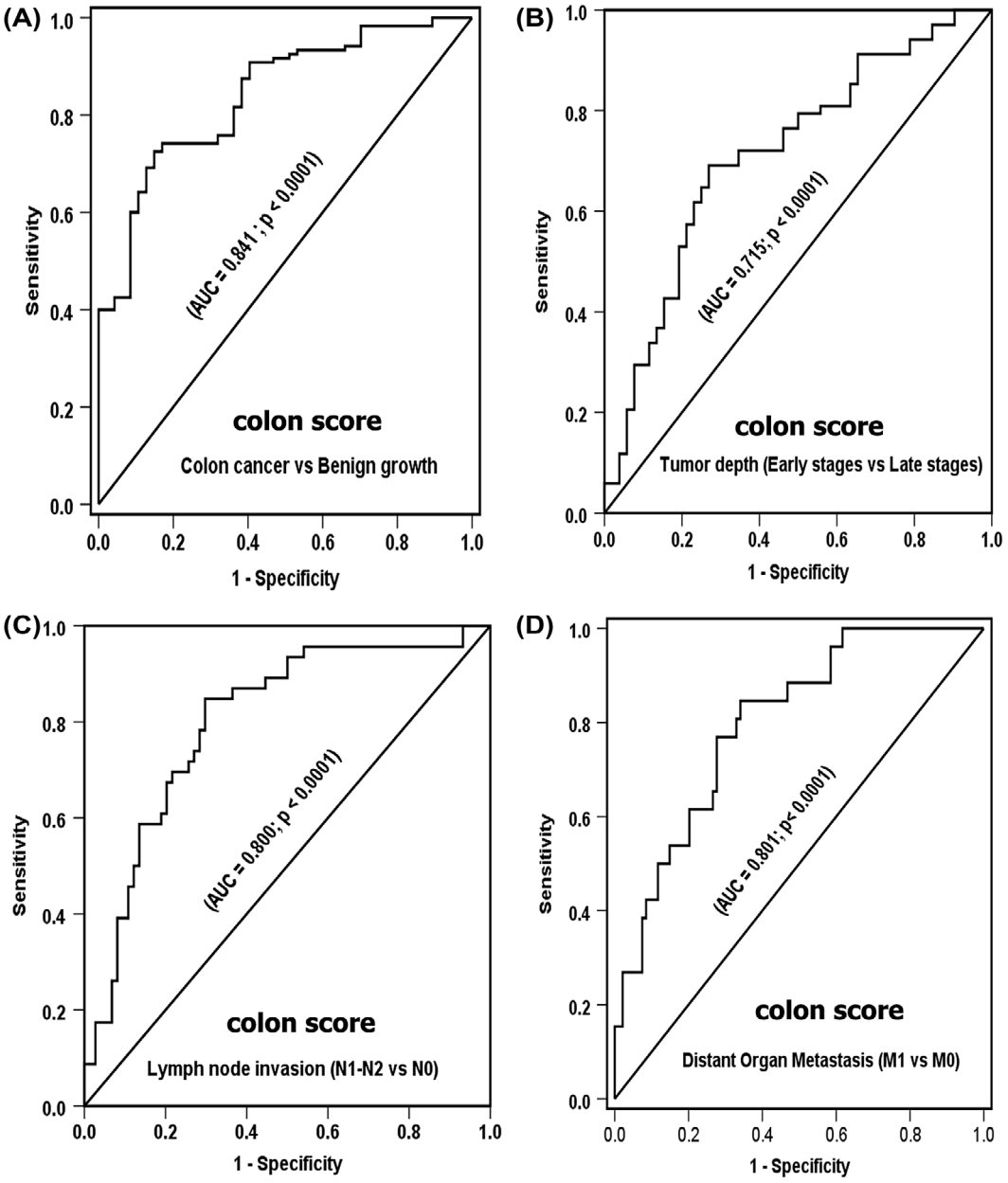

Figure 2. The ROC curve of colon score to differentiate (A) colon cancer patients from patients with benign growth, (B) colon cancer patients at late stages from those with early stages, (C) colon cancer patients with lymph node invasion from those without and (D) colon cancer patients with distant organ metastasis from those without.

metastasis. The high level of MUC1 may be explained in terms of inflammation and immunity, and have been previously reported in a small study [23].

There are several studies where serum markers have been to differentiate colorectal cancer. In the present study, CK1 and MUC1 had sensitivity 70.8 and 74.2% and specificity 73.7 and 76.0%, respectively, for differentiating patients with colon cancer from those with a benign growth, and are comparable to other markers. CEA is reported to have a sensitivity of 12–75% in the diagnosis of colon cancer [24], whilst cytokeratin 7 has 88% sensitivity and 82% specificity, and CK20 showed 82% sensitivity and 100% specificity. MUC5AC had lower sensitivity (70%) and 75% specificity [25]. Dressen et al. evaluated six markers for distinguishing colorectal cancer from benign colorectal diseases. AUC were 0.86 for CEA followed by CA 19-9, cytokeratin 19 fragments, IL-8, CA 125 and *osteopontin, where* AUCs ranged 0.70–0.74 [26]. The AUC of IL-6 for diagnosis of colorectal cancer was ranged from 0.72 to 0.79 [27].

Others have used combinations of markers. In one, the combination of cytokeratins with CEA, seprase, osteopontin, ferritin and anti-p53 gave 70% sensitivity and 95% specificity [28]. Krawczyk et al. evaluated two novel microRNAs in differentiating early stage colorectal cancer from healthy individuals with an AUC of 0.75, 0.74 and 0.75 for miR-506 and miR-4316 and combined two markers, respectively. The sensitivity and specificity were 61 and 77% for miR-506; for miR-4316 were 84 and 61% and for both were 77 and 75% [29]. The

sensitivity of combined four autoantibodies (anti-SLP2, p53, SEC61B and PLSCR1) was 64.1%, with a specificity of 80% that increased to 83.7% when CEA was added. Furthermore, the sensitivity of these four antibodies for early and advanced stages of colorectal was 66.7 and 62%, increasing to 88.3 and 84%, respectively, when CEA was added [30]. Against this background we report equivalent or better AUC (0.84), sensitivity (87.5%) and specificity (91.2%) of our colon score for colorectal cancer.

Therefore, we submit that this study represents in advance in biomedical science because it shows that a colon score, based on CK-1, Mucin-1, CEA and CA19-9, can be used for screening early stage colon cancer patients with high diagnostic accuracy.

Summary table

What is known about this topic:
- Disadvantages of colonoscopy have led to a drive towards identifying reliable biomarkers
- Many studies have been dedicated to the search of non-invasive markers
- However, these markers are useful for most severe colon cancer, but they have limited accuracy for lymph node invasion and other clinical features

What this work adds:
- The new colon score outperforms individual serum markers in recognising colon cancer
- The new score also predicts tumour stage, lymph node invasion and distant organ metastasis with superior AUCs of 0.72, 0.80 and 0.80, respectively.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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