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## Review

# Blood Markers for Early Detection of Colorectal Cancer: A Systematic Review

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## Abstract

**Background:** Despite different available methods for colorectal cancer (CRC) screening and their proven benefits, morbidity, and mortality of this malignancy are still high, partly due to low compliance with screening. Minimally invasive tests based on the analysis of blood specimens may overcome this problem. The purpose of this review was to give an overview of published studies on blood markers aimed at the early detection of CRC and to summarize their performance characteristics. **Method:** The PUBMED database was searched for relevant studies published until June 2006. Only studies with more than 20 cases and more than 20 controls were included. Information on the markers under study, on the underlying study populations, and on performance characteristics was extracted. Special attention was given to performance characteristics by tumor stage. **Results:** Overall, 93 studies evaluating 70 different markers were included. Most studies were done on

protein markers, but DNA markers and RNA markers were also investigated. Performance characteristics varied widely between different markers, but also between different studies using the same marker. Promising results were reported for some novel assays, e.g., assays based on SELDI-TOF MS or MALDI-TOF MS, for some proteins (e.g., soluble CD26 and bone sialoprotein) and also for some genetic assays (e.g., L6 mRNA), but evidence thus far is restricted to single studies with limited sample size and without further external validation.

**Conclusions:** Larger prospective studies using study populations representing a screening population are needed to verify promising results. In addition, future studies should pay increased attention to the potential of detecting precursor lesions. (Cancer Epidemiol Biomarkers Prev 2007;16(10):1935–53)

## Introduction

With more than 1 million new cases and about 530,000 deaths per year, colorectal cancer (CRC) is the third most common malignancy in the world (1). Given its slow development from removable precancerous lesions and from curable early stages, screening for CRC has the potential to reduce both incidence and mortality of the disease (2). However, the currently most reliable screening tool, screening colonoscopy, lacks compliance and widespread access. On the other hand, the currently most widely used noninvasive screening option, the test for occult blood in stool (FOBT), has important limitations, above all its low sensitivity. To overcome this problem, the search for novel biomarkers aimed at early detection of CRC is ongoing.

Apart from the development of novel stool markers, the development of tests based on the analysis of blood samples becomes a focus of current research. The latter may offer some advantages over stool testing. First,

sampling may be more convenient and acceptable for the patient. Furthermore, there is no microflora which could degrade the biomarker or hamper analysis, and sample processing may be easier.

The objective of this article is to provide an overview on studies aimed at evaluating blood markers for early detection of CRC and to summarize performance characteristics of the various approaches. Special attention is drawn to the composition of the underlying study populations and to sensitivity by tumor stage.

## Materials and Methods

The PUBMED database was searched for relevant articles published until June 2006. To identify pertinent studies, the terms "colorectal cancer," "detection," "diagnosis," "marker," "biomarker," "blood," "plasma," "serum," "protein," "DNA," and "assay" were used in the following combination: colorectal (and) cancer (and) [detection (or) diagnosis] and [serum (or) blood (or) plasma] (and) [marker (or) biomarker (or) DNA (or) protein (or) assay]. From the studies identified by this initial search, a further selection by relevance was done, first according to the title and in a second step according to the abstracts. In addition, the bibliographies of articles identified by the PUBMED search were checked for relevant citations.

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The search was limited to studies on humans published in English. Only full-text articles were included because abstracts did not provide enough information for a detailed review. Only studies examining both cases and controls were included, allowing the determination of both sensitivity and specificity. As regards sample size, only studies with more than 20 cases and more than 20 controls were included.

Studies fulfilling the above-mentioned inclusion criteria were divided into different groups according to the type of marker(s) under examination. To describe the study population, information on the number of cases and controls and their mean or median age was extracted from the articles (age was only listed when it was available for both cases and controls, allowing comparison between the groups). Cases were separated into CRC patients and patients bearing adenomas. For CRC cases, the distribution by tumor stage was considered. As regards controls, information on the setting of recruitment was collected. To describe performance characteristics of the markers under study, overall sensitivity and specificity was listed. Whenever information was available, performance characteristics were further stratified by relevant subgroups, e.g., by tumor stage among cases or by health status among controls. In case performance characteristics were not explicitly reported, information was extracted from available tables and graphs.

## Results

Overall, 93 studies investigating 70 different markers fulfilled the inclusion criteria. Types of markers under examination were proteins, cytologic markers, and DNA and mRNA markers. In some studies, different markers were analyzed in parallel. For example, combinations with carbohydrate antigens and carcinoembryonic antigens were very common. A total of 74 studies reported sensitivity by tumor stage, 42 studies by Dukes classification, 20 studies by the Unio Internationalis Contre Cancrum tumor-node-metastasis (TNM) classification, 5 studies by the American Joint Committee on Cancer classification, 2 studies by Astler-Coller classification, 3 studies by a classification into localized/metastasized disease, and 6 studies by a classification stage I to IV that was not further specified. The sensitivity to detect CRC was investigated in 92 studies, and the sensitivity to detect adenomas was investigated in 8 studies. In many studies, control groups were patients with benign gastrointestinal diseases ( $n = 29$ ) or other malignancies ( $n = 6$ ), only 59 studies used healthy controls. In 27 studies, otherwise characterized controls were used. Only 28 studies provided information on age for both cases and controls. Sample sizes varied from 20 cases and/or controls to 588 cases and/or controls. In 19 studies, overall sample size was below 100; in 58 studies, overall sample size was between 100 and 300, and in 16 studies, overall sample size was  $>300$  (including one study with the maximum overall sample size of 918 subjects). In addition to the detection of CRC, 24 studies investigated the potential of the respective marker to detect other forms of cancer.

**Proteins.** Table 1 lists studies evaluating protein markers. Overall, this group comprises 69 studies

examining 52 different markers. The group of markers was further subdivided into carbohydrate antigens, carcinoembryonic antigens, other antigens, antibodies, cytokines, other proteins, and chromatographic and spectrometrical assays.

Nineteen studies (3-22) investigated one or more carbohydrate antigen(s), including CA 19-9, CA 195, CA M26, CA M29, CA 50, CA 72-4, CA M43, and CA 242, which were primarily defined by monoclonal antibodies against colon carcinoma cell lines (refs. 23-25; see Table 1A). Thirteen studies evaluated CA 19-9. At the most common cutoff of 37 units/mL, overall sensitivity ranged 18% to 65%, and specificity was above 90% in most studies. Considering sensitivity by stage, estimates of 0% for Dukes A and up to 67% for Dukes D were reported. Sensitivities greater than 50% were only observed for nonlocalized disease. For other carbohydrate antigens, the observed sensitivity, its stage dependency, and specificity were comparable.

Nineteen studies (3-6, 8-15, 18, 19, 21, 26-29) reported performance characteristics for carcinoembryonic antigen (CEA; see Table 1B), which has been the first blood marker proposed in connection with CRC (30). Overall sensitivity varied between 43% and 69%. There was a clear increase of sensitivity by tumor stage, ranging from 8% for Dukes A up to 89% for Dukes D. Sensitivities greater than 50% were typically observed for non-localized disease only. Specificity was above 95% in four studies, between 95% and 90% in seven studies, and below 90% in eight studies. The latter category comprises all studies with a cutoff below 3 ng/mL. Overall, cutoff values varied between 2.4 and 10 ng/mL.

Further studies (10, 29, 31-37) examining various antigen markers are shown in Table 1C. Early approaches were the investigation of sialylated Lewis<sup>x</sup> antigen (SLEX) and of CO 29.11, another sialylated Lewis antigen. SLEX was originally found on tumor tissues by immunoperoxidase staining with monoclonal antibody CSLEX1 (38). CO 29.11 is expressed and shed by carcinoma cells of colon and other cancer types. Later studies investigated the potential of PA 8-15, another tumor-associated antigen that was originally observed in a pancreatic cancer cell line, of small intestinal mucin antigen (SIMA), and of urokinase-type plasminogen activator (u-PA). Furthermore, prostate-specific antigen (PSA), which is widely used as a serum marker for prostate cancer in men and is also present in other cancer sites, e.g., breast and ovarian cancer (39), has been studied in terms of detecting CRC. Neither of these markers showed a sensitivity above 50% except u-PA and TPA-M. For the former, Huber et al. (10) reported a sensitivity of 76% (82% and 73% for nonmetastasized and metastasized disease, respectively) with a specificity of 80%, using patients with Crohn's disease as controls. For the latter, Fernandes et al. (29) reported a sensitivity of 70% and a specificity of 96%.

Six studies (40-45) summarized in Table 1D assessed the potential of various circulating autoantibodies for the detection of CRC. Although specificity in this group of markers was 100% in all but one of these studies, sensitivities hardly reached 30%. For example, DEAD-box protein 48 (DDX-48) autoantibodies, which arose from a proteomics-based identification of markers for pancreatic cancer, showed a sensitivity of only 10% for CRC. Further approaches included autoantibodies

against p53, a tumor suppressor protein, and against sFasL, an autoantibody against the death receptor ligand of CD95.

Studies (46-51) evaluating cytokine markers, namely, vascular endothelial growth factor (VEGF), insulin-like growth factor II (IGF-II), IGF-binding proteins (IGFBP-2), stem-cell factor (SCF), and interleukin-3 (IL-3) are listed in Table 1E. Whenever specificity in this group of markers was between 90% and 100%, sensitivity was low or moderate. High values of sensitivity were reported for extremely low values of specificity only.

Table 1F summarizes studies (15, 19, 52-69) on protein markers, which cannot be assigned to the other groups. In this group of markers, observed sensitivities varied extremely. For example, a sensitivity of 16% was reported for hCG $\beta$  (human chorionic gonadotrophin; ref. 15), whereas sensitivity was 90% and 100% for sCD26 (soluble cluster of differentiation 26; ref. 58) and for BSP (bone sialoprotein; ref. 59), respectively. Promising results for both sensitivity and specificity were observed for CP (cancer procoagulant; ref. 54), sCD26 (58), fibrin degradation (63), and prolactin (64). Two studies evaluated the potential of tumor M2-pyruvate kinase (M2-PK), an isoform of the glycolytic enzyme pyruvate kinase, which has a regulative role in the glycolytic pathway of synthetic processes and energy production. In a study by Schneider et al. (66) with about 250 CRC cases and about 50 controls, sensitivity and specificity were 48% and 95%, respectively. In contrast, Zhang et al. (65) reported a higher sensitivity (69%) and a lower specificity (90%), which might be explained by the use of a lower cutoff value. By combining M2-PK with CEA (using fuzzy logic modeling) Schneider et al. were able to increase sensitivity to 58%, without lowering specificity (data not shown in the table).

Protein markers listed thus far in this review were analyzed by common standard procedures like ELISA, RIA, or activity assays. Table 1G lists studies (70-74) on protein markers analyzed by more complex assays. The various approaches included different chromatographic assays to determine high-molecular-mass alkaline phosphatase (HiMwALP), mass spectrometrical (MS) assays based on surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS using artificial neural networks with 3- to 10-fold cross-validation, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS using double cross-validation. Overall, performance characteristics reported for these assays were mostly promising, especially for the SELDI-TOF and MALDI-TOF MS assays, but sample sizes were comparably small (55 to 62 cases and 31 to 62 controls for SELDI-TOF MS and 63 cases and 50 controls for MALDI-TOF MS).

The potential of protein markers to detect adenomas was investigated in six studies (12, 15, 26, 45, 46, 50). CA 19-9 and CA 72-4 showed extremely low sensitivities (about 4%). CA 242 showed sensitivity between 15% and 21%, and CEA showed sensitivity between 7% and 29%. The same range of sensitivity was observed for sFasL, hCG $\beta$ , and VEGF. A high sensitivity of 94% could be achieved with IGF-II and IGFBP-2, but with a specificity of only 31%. Overall, sample sizes were very low (13-52 adenoma cases), with the exception of one study by Blake et al. (26), which examined 256 adenoma cases.

**Cytologic Assays.** Three studies investigated cytologic assays (see Table 2; refs. 75-77). One approach was an inhibition assay of *in vitro* leukocyte adherence by incubation with tumor antigens, conducted by Tataryn et al. (75) and Liu et al. (76). In both studies, sensitivity was above 70% for early stages, whereas sensitivity for more advanced stages was lower. However, in both studies, the number of cancers by stage was very small. Specificity for this assay ranged from 95% to 98%. Another approach was the detection of circulating tumor cells by membrane array (77), which yielded both a sensitivity and a specificity of 94%.

**DNA Markers.** Overall, four studies (78-81) were included investigating the potential of DNA markers for the early detection of CRC (see Table 3). Blood samples were analyzed for both genetic and epigenetic alterations of genes involved in the adenoma-carcinoma sequence (82). Sensitivity reported for this group of markers was about 60% and lower, whereas specificity ranged from 73% to 100%. The potential of detecting adenomas was investigated only for mutations in the *K-ras* gene in one study (78), which showed a sensitivity for adenomas of 35%.

**mRNA Markers.** Table 4 gives an overview of studies (83-102) applying reverse transcription-PCR to detect mRNA expressed in circulating tumor cells. Free mRNA is not stable in blood, but it can be isolated from circulating cells. In relevant studies, blood samples were analyzed for mRNA molecules coding for CEA, cytokeratin (CK) 8, 19, and 20, human telomerase reverse transcriptase (hTERT), guanylyl cyclase C (GCC), carcinoembryonic gene member 2 (CGM2), melanoma-associated antigen family A (uMAGE-A), tumor-associated antigen L6, mucins (MUC) 1 and 2, protease M (ProtM), and thymidylate synthase.

For mRNA coding for CEA and carcinoembryonic antigen-related cell adhesion molecule (CEACAM), which was examined in various studies between 1998 and 2006, sensitivities between 32% and 69% were reported, with a clear increase by stage. Specificities varied between 44% in controls with inflammatory bowel disease and 100% in healthy controls. For the combination of CEA mRNA and CK mRNA, sensitivity ranged from 56% to 83%, and specificity varied between 76% and 100%. The most promising performance characteristics in this group of markers were reported for GCC mRNA (98), showing a sensitivity of above 80% for early stages and a specificity of 95%, and for L6 mRNA (101), showing a sensitivity of about 80% (independent of stage) and a specificity of 100%. The analysis of the former marker (GCC mRNA), however, was only done in a small number of cases and controls (27 and 21, respectively). The potential of mRNA markers to detect adenomas was investigated in one study only. In this study, which tested for cytokeratins and mucins, only 3 of 30 patients with adenomas (10%) tested positive (95).

## Discussion

Our literature search identified 93 studies evaluating an overall of 70 different blood markers for CRC, including long discussed ones like carcinoembryonic antigen and carbohydrate antigens, as well as newly introduced ones

**Table 1. Protein markers**

Reference	Marker	Study population		Sensitivity		Specificity
		Cases	Controls	Carcinoma	Adenoma	
<b>(A) Carbohydrate antigens</b>						
Kuusela et al., 1984 (3)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 111 Dukes A/B/C/D/r 3/23/17/28/40	<i>n</i> = 37 benign colorectal diseases	36% A/B/C/D/r. 0/4/35/54/45%		97%
Wang et al., 1985 (4)	CA 19-9 cutoff >37.6 units/mL	<i>n</i> = 87 Dukes A/B/C/D 15/28/31/13	<i>n</i> = 40 hospital personnel	34% A/B/C/D  13/21/45/62% 65%		98%
Kornek et al., 1991 (5)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 72 Dukes A/B/C/D 3/21/15/33	<i>n</i> = 20 healthy controls <i>n</i> = 103 benign gastrointestinal disorders			100% healthy controls 42% benign gastrointestinal disorders 100%
Kuusela et al., 1991 (6)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 53 Dukes AB/CD/r 19/34/24	<i>n</i> = 29 benign colorectal diseases	34% AB/CD  16/44% 18% AB/CD 25/0% 60%		92%
Thomas et al., 1991 (7)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 34 asymptomatic A/B/C/D 16/8/7/3 <i>n</i> = 55 met. liver disease <i>n</i> = 81	<i>n</i> = 39 negative colonoscopy			90%
Nilsson et al., 1992 (8)	CA 19-9 cutoff >50 units/mL		<i>n</i> = 132 benign gastrointestinal diseases	23%		91%
Guadagni et al., 1993 (9)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 200 Dukes A/B/C/D 31/59/60/50; mean age 64 y	<i>n</i> = 100 benign colorectal diseases; mean age 53 y	27% A/B/C/D 7/15/27/54%		94%
Huber et al., 1993 (10)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 33 M <sub>0</sub> /M <sub>1</sub> 11/22; mean age 63 y	<i>n</i> = 53 Crohn's disease; mean age 32 y	52% M <sub>0</sub> /M <sub>1</sub> 27/64%		95%
Fernandez- Fernandez et al., 1995 (11)	CA 19-9 cutoff >51 units/mL	<i>n</i> = 127 Dukes A/B/C/D 11/53/40/23; mean age 71 y	<i>n</i> = 70 benign colorectal diseases; mean age 71 y	21%		96%
von Kleist et al., 1996 (12)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 119 Dukes A/B/C/D 24/24/42/29	<i>n</i> = 45 healthy controls	33% A/B/C/D 8/18/41/55%	0%	94%
Spila et al., 2001 (13)	CA 19-9 cutoff >37 units/mL	<i>n</i> = 429 A/B/C/D/met  23/154/81/45/98; mean age 63 y	<i>n</i> = 201 benign colorectal diseases mean age 55 y	30% A/B/C/D/met 22/16/23/44/55%		80%
Carpelan- Holmstrom et al., 2002 (14)	CA 19-9 cutoff >35 units/mL	<i>n</i> = 28 Dukes B/C/D/r 7/3/6/12	<i>n</i> = 161 benign gastrointestinal diseases	36% B/C/D/r 0/33/67/33%		98%
Carpelan- Holmstrom et al., 2004 (15)	CA 19-9 cutoff >35 units/mL	<i>n</i> = 204 Dukes A/B/C/D 31/70/49/54; median age 67 y	<i>n</i> = 77 benign colorectal diseases median age 67 y	26% A/B/C/D 6/11/29/54%	4%	

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Table 1. Protein markers (Cont'd)

Reference	Marker	Study population		Sensitivity		Specificity
		Cases	Controls	Carcinoma	Adenoma	
Kornek et al., 1991 (5)	CA 195 cutoff >10 units/mL	<i>n</i> = 72 Dukes A/B/C/D 3/21/15/33	<i>n</i> = 20 healthy controls <i>n</i> = 103 benign gastrointestinal disorders	71% A/B/C/D 33/52/80/81%		100% healthy controls 71% benign gastrointestinal disorders
Yedema et al., 1991 (16)	CA M26 cutoff >77 units/mL CA M29 cutoff >15 units/mL	<i>n</i> = 50 AJCC I/II/III/IV 13/7/17/13	<i>n</i> = 89 healthy controls	22% 12%		99% 99%
Holmgren et al., 1984 (17)	CA 50 inhibition assay cutoff >25%	<i>n</i> = 55 I+II/III+IV 18/36	<i>n</i> = 150 healthy blood donors <i>n</i> = 28 ulcerative colitis	67% I+II/III+IV 50/75%		99% healthy blood donors 93% colitis 97%
Kuusela et al., 1991 (6)	CA 50 cutoff >17 units/mL	<i>n</i> = 53 Dukes AB/CD/r 19/34/24	<i>n</i> = 29 benign colorectal diseases	40% AB/CD 26/47%		
Nilsson et al., 1992 (8)	CA 50 cutoff >35 units/mL	<i>n</i> = 81	<i>n</i> = 132 benign gastrointestinal diseases	24%		90%
Eskelinen et al., 1994 (18)	CA 50 cutoff >17 units/mL	<i>n</i> = 124 TNM I/II/III/IV/nd 28/30/18/33/15	<i>n</i> = 104 benign gastrointestinal diseases	30% I/II/III/IV 10/27/44/55%		86%
Pasanen et al., 1995 (19)	CA 50 cutoff >12 units/mL	<i>n</i> = 62 TNM I/II/III/IV 20/15/7/14 mean age 67 y	<i>n</i> = 97 benign gastrointestinal diseases; mean age 59 y	51%		51%
Guadagni et al., 1993 (9)	CA 72-4 cutoff >6 units/mL	<i>n</i> = 200 Dukes A/B/C/D 31/59/60/50 mean age 64 y	<i>n</i> = 100 benign colorectal diseases; mean age 53 y	43% A/B/C/D 3/31/53/70%		98%
Fernandez- Fernandez et al., 1995 (11)	CA 72-4 cutoff > 4.8 units/mL	<i>n</i> = 127 Dukes A/B/C/D 11/53/40/23 mean age 71 y	<i>n</i> = 70 benign colorectal diseases; mean age 71 y	40%		95%
Carpelan- Holmstrom et al., 2002 (14)	CA 72-4 cutoff >6 units/mL	<i>n</i> = 28 Dukes B/C/D/r 7/3/6/12	<i>n</i> = 161 benign gastrointestinal diseases	25% B/C/D/r 29/33/17/25%		96%
Carpelan- Holmstrom et al., 2004 (15)	CA 72-4 cutoff >6 units/mL	<i>n</i> = 204 Dukes A/B/C/D 31/70/49/54 median age 67 y	<i>n</i> = 27 <i>n</i> = 77 benign colorectal diseases; median age 67 y	27% A/B/C/D 3/17/27/56%	0%	95%
van Kamp et al., 1993 (20)	CA M43 cutoff >7.5 units/mL	<i>n</i> = 100 Dukes A/B/C/D 10/32/28/30 mean age 66 y	<i>n</i> = 138 healthy controls mean age 43 y <i>n</i> = 35 benign gastrointestinal diseases	42% A/B/C/D 0/41/22/77%		99% healthy controls 97% benign gastrointestinal diseases

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**Table 1. Protein markers (Cont'd)**

Reference	Marker	Study population		Sensitivity		Specificity	
		Cases	Controls	Carcinoma	Adenoma	Carcinoma	Adenoma
Kuusela et al., 1991 (6)	CA 242 cutoff >20 units/mL	<i>n</i> = 53 Dukes AB/CD/r 19/34/24	<i>n</i> = 29 benign colorectal diseases	55% AB/CD 47/59%		90%	
Nilsson et al., 1992 (8)	CA 242 cutoff >20 units/mL	<i>n</i> = 290 Dukes A/B/C/D 57/93/77/63	<i>n</i> = 174 benign diseases	39% A/BC/D 18/29/49/62%		90%	
Eskelinen et al., 1994 (18)	CA 242 cutoff >17 units/mL	<i>n</i> = 114 TNM I/II/III/IV/nd 26/25/18/32/13	<i>n</i> = 104 benign gastrointestinal diseases	30% I/II/III/IV 3/30/39/47%		88%	
Carpelan-Holmstrom et al., 1995 (21)	CA 242 cutoff >20 units/mL	<i>n</i> = 260 Dukes A/B/C/D 39/100/60/61	<i>n</i> = 92 benign colorectal diseases	39% A/B/C/D 26/26/40/67%		87%	
Pasanen et al., 1995 (19)	CA 242 cutoff >9.3 units/mL	<i>n</i> = 62 TNM I/II/III/IV 20/15/7/14;	<i>n</i> = 97 benign gastrointestinal diseases	39%		35%	
von Kleist et al., 1996 (12)	CA 242 cutoff >20 units/mL	mean age 67 y <i>n</i> = 119 Dukes A/B/C/D 24/24/42/29	<i>n</i> = 14 mean age 59 y <i>n</i> = 45 healthy controls	43% A/BC/D 25/36/42/62%	21%	89%	
Spila et al., 1999 (22)	CA 242 cutoff >20 units/mL	<i>n</i> = 384 A/B/C/D  49/112/78/114 mean age 63 y	<i>n</i> = 189 benign diseases  mean age 52 y <i>n</i> = 440 healthy controls mean age 44 y	35% A/B/C/D  16/20/33/59%		94% benign diseases  94% healthy controls	
Spila et al., 2001 (13)	CA 242 cutoff >20 units/mL	<i>n</i> = 429 A/B/C/D/met  23/154/81/45/98 mean age 63 y	<i>n</i> = 201 benign colorectal diseases  mean age 55 y <i>n</i> = 161 benign gastrointestinal diseases	33% A/B/C/D/met 26/17/29/51/58%		94%	
Carpelan-Holmstrom et al., 2002 (14)	CA 242 cutoff >20 units/mL	<i>n</i> = 28 Dukes B/C/D/r 7/3/6/12	<i>n</i> = 161 benign gastrointestinal diseases	46% B/C/D/r 0/33/83/58%		91%	
Carpelan-Holmstrom et al., 2004 (15)	CA 242 cutoff >20 units/mL	<i>n</i> = 204 Dukes A/B/C/D 31/70/49/54; median age 67 y	<i>n</i> = 27 <i>n</i> = 77 benign colorectal diseases median age 67 y	36% A/B/C/D 10/29/35/63%	15%	96%	
(B) Carcinoembryonic antigen							
Blake et al., 1982 (26)	CEA cutoff >5 ng/mL	<i>n</i> = 332 AJCC O/IA/IB/II/III/IV 19/18/82/ 30/72/111	<i>n</i> = 256 <i>n</i> = 323 benign diseases	57% O/IA/IB/ II/III/IV 16/22/27/ 60/67/86% 69%	10%	85%	
Kuusela et al., 1984 (3)	CEA cutoff > 2.5 ng/mL	<i>n</i> = 111 Dukes A/B/C/D/r 3/23/17/28/40	<i>n</i> = 37 benign colorectal diseases			70%	

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Table 1. Protein markers (Cont'd)

Reference	Marker	Study population		Sensitivity		Specificity
		Cases	Controls	Carcinoma	Adenoma	
Wang et al., 1985 (4)	CEA cutoff > 8.2 ng/mL	<i>n</i> = 87 Dukes A/B/C/D 15/28/31/13	<i>n</i> = 40 hospital personnel	52% 20/46/64/69%		88%
Kornek et al., 1991 (5)	CEA cutoff >5 ng/mL	<i>n</i> = 72 Dukes A/B/C/D 3/21/15/33	<i>n</i> = 20 healthy controls	54%		85% healthy controls
Kuusela et al., 1991 (6)	CEA cutoff >3 ng/mL	<i>n</i> = 53 Dukes AB/CD/r 19/34/24	<i>n</i> = 103 benign gastrointestinal disorders <i>n</i> = 29 benign colorectal diseases	57% AB/CD 32/71%		69% benign gastrointestinal disorders 83%
Nilsson et al., 1992 (8)	CEA cutoff >3 ng/mL	<i>n</i> = 290 Dukes A/B/C/D 57/93/77/63	<i>n</i> = 174 benign diseases	46% A/B/C/D 19/40/51/71%		90%
Guadagni et al., 1993 (9)	CEA cutoff >5 ng/mL	<i>n</i> = 200 Dukes A/B/C/D 31/59/60/50 mean age 64 y	<i>n</i> = 100 benign colorectal diseases mean age 53 y	43% A/B/C/D 29/22/42/78%		90%
Huber et al., 1993 (10)	CEA cutoff > 10 ng/mL	<i>n</i> = 33 stages M <sub>0</sub> /M <sub>1</sub> 11/22 mean age 63 y	<i>n</i> = 53 Crohn's disease; mean age 32 y	52% M <sub>0</sub> /M <sub>1</sub> 19/68%		98%
Eskelinen et al., 1994 (18)	CEA cutoff >2.5 ng/mL	<i>n</i> = 123 TNM I/II/III/IV/nd 28/29/18/33/15	<i>n</i> = 104 benign gastrointestinal diseases	63% I/II/III/IV 52/59/72/85%		88%
Paganuzzi et al., 1994 (27)	CEA cutoff >5 ng/mL	<i>n</i> = 61 Dukes B/C/met 24/14/23	<i>n</i> = 24 obese subjects <i>n</i> = 18 benign hepatic disease	54% B/C/met 46/43/70%		96% obese 100% benign hepatic disease 90%
Carpelan- Holmstrom et al., 1995 (21)	CEA cutoff >5 ng/mL	<i>n</i> = 260 Dukes A/B/C/D 39/100/60/61	<i>n</i> = 92 benign colorectal diseases	43% A/B/C/D 26/32/38/77%		
Fernandez- Fernandez et al., 1995 (11)	CEA cutoff >5.6 ng/mL	<i>n</i> = 127 Dukes A/B/C/D 11/53/40/23; mean age 71 y	<i>n</i> = 70 benign colorectal diseases; mean age 71 y	46%		95%
Pasanen et al., 1995 (19)	CEA cutoff >2.4 ng/mL	<i>n</i> = 62 TNM I/II/III/IV 20/15/7/14; mean age 67 y	<i>n</i> = 97 benign gastrointestinal diseases; mean age 59 y	58%		55%
von Kleist et al., 1996 (12)	CEA cutoff >4 ng/mL	<i>n</i> = 119 Dukes A/B/C/D 24/24/42/29	<i>n</i> = 14 <i>n</i> = 45 healthy controls	44% A/B/C/D 38/23/41/69%	29%	98%
Spila et al., 2001 (13)	CEA cutoff >5 ng/mL	<i>n</i> = 429 A/B/C/D/met 23/154/81/45/98; mean age 63 y	<i>n</i> = 201 benign colorectal diseases; mean age 55 y	44% A/B/C/D/met 17/34/42/60/69%		92%
Carpelan- Holmstrom et al., 2002 (14)	CEA cutoff >5 ng/mL	<i>n</i> = 28 Dukes B/C/D/r 7/3/6/12	<i>n</i> = 161 benign gastrointestinal diseases	54% B/C/D/r 29/33/83/58%		94%

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**Table 1. Protein markers (Cont'd)**

Reference	Marker	Study population		Sensitivity		Specificity
		Cases	Controls	Carcinoma	Adenoma	
Carpelan-Holmstrom et al., 2004 (15)	CEA cutoff >5 ng/mL	<i>n</i> = 204 Dukes A/B/C/D 31/70/49/54; median age 67 y	<i>n</i> = 27 <i>n</i> = 77 benign colorectal diseases median age 67 y	44% A/B/C/D 26/33/45/67%	7%	97%
Castaldi et al., 2005 (28)	fCEA cutoff >5 ng/mL	<i>n</i> = 72 TNM I/II/III/IV 24/19/15/14	<i>n</i> = 34 healthy blood donors	43% I/II/III/IV 8/37/73/79%		100% 94%
	CEA-IgM cutoff >200 units/mL			38% I/II/III/IV 29/37/53/36%		94%
	Either			65% I/II/III/IV 38/56/93/86%		
Fernandes et al., 2005 (29)	CEA cutoff >5 ng/mL	<i>n</i> = 169 mean age 62 y	<i>n</i> = 100 blood donors; mean age 42 y	56% I/II/III/IV 35%/23/34/69%		95%
(C) Other antigens						
Herlyn et al., 1985 (31)	CO 29.11 cutoff >35 units	<i>n</i> = 176 Dukes B/met 75/101	<i>n</i> = 151 healthy controls <i>n</i> = 132 inflammatory gastrointestinal diseases	41% B/met 34/76%		95% healthy controls 97% inflammatory gastrointestinal diseases
Kawahara et al., 1985 (32)	SLEX cutoff >20 units/mL	<i>n</i> = 65	<i>n</i> = 136 healthy controls	25%		96% healthy controls
Arai et al., 1990 (33)	PA 8-15 cutoff >55 units/mL	<i>n</i> = 29	<i>n</i> = 166 nonmalignant <i>n</i> = 338 healthy controls <i>n</i> = 114 benign diseases	45%		96% nonmalignant 95% healthy controls 87%
Pinczower et al., 1993 (34)	SIMA cutoff >12.3 units/mL	<i>n</i> = 113 Dukes A/B/C/D/nd 13/29/34/8/29	<i>n</i> = 97 healthy controls <i>n</i> = 21 benign gastrointestinal diseases	36% A/B/C/D/nd 10/37/32/71/22%		benign diseases 95% healthy controls
Eskelinen et al., 1995 (35)	SIMA I cutoff >12 units/mL	<i>n</i> = 73 TNM I/II/III/IV/nd 18/21/10/18/6	<i>n</i> = 104 benign gastrointestinal diseases	27% I/II/III/IV 11/43/20/39%		89% 89%
	SIMA II cutoff >9.8 units/mL			19% I/II/III/IV 6/33/10/28%		
Huber et al., 1993 (10)	u-PA cutoff >8.5 ng/mL	<i>n</i> = 33 stages M <sub>0</sub> /M <sub>1</sub> 11/22; mean age 63 y	<i>n</i> = 53 Crohn's disease; mean age 32 y	76% M <sub>0</sub> /M <sub>1</sub> 82/73%		80%
Kuroki et al., 1999 (36)	NCA-50/90 cutoff >100 ng/mL	<i>n</i> = 55	<i>n</i> = 246 healthy controls	35%		95%
Duraker et al., 2002 (37)	Free PSA cutoff >0.01 ng/mL	<i>n</i> = 75 TNM I+II/III+IV 43/32 only women; mean age 60 y	<i>n</i> = 30 healthy controls; only women; mean age 40 y	35% 20%		93% 93%

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**Table 1. Protein markers (Cont'd)**

Reference	Marker	Study population		Sensitivity		Specificity
		Cases	Controls	Carcinoma	Adenoma	
	Total PSA cutoff >0.34 ng/mL Free/total PSA cutoff >50%			I+II/III+IV 12/31% 20%		97%
Fernandes et al., 2005 (29)	TPA-M cutoff >72 units/mL	<i>n</i> = 169; mean age 62 y	<i>n</i> = 100 blood donors; mean age 42 y	I+II/III+IV 28/9% 70% I/II/III/IV 75/53/61/83%		96%
<b>(D) Autoantibodies</b>						
Yamaguchi et al., 1991 (40)	NCC-ST 439 cutoff >4.5 units/mL; males, females >50 y cutoff > 7 units/mL; females <50 y	<i>n</i> = 121 Dukes A/B/C/D 24/34/38/25	<i>n</i> = 36 benign diseases	27% A/B/C/D 0/18/26/68%		94%
Hammel et al., 1997 (41)	p53	<i>n</i> = 54 Dukes A/B/C 10/19/25	<i>n</i> = 24 non-malignant digestive diseases	26% A/B/C 0/32/32%		100%
Broll et al., 2001 (42)	p53	<i>n</i> = 130 TNM I/II/III/IV 41/33/29/27; mean age 68 y	<i>n</i> = 44 healthy controls; mean age 61 y	15%		100%
Chang et al., 2005 (43)	p53	<i>n</i> = 167 TNM I/II/III/IV 20/54/58/35	<i>n</i> = 40 healthy controls	28% I/II/III/IV 30/22/35/26%		100%
Xia et al., 2005 (44)	DDX-48	<i>n</i> = 30	<i>n</i> = 60 healthy controls	10%		100%
Reipert et al., 2005 (45)	sFasL	<i>n</i> = 21; mean age 64 y	<i>n</i> = 38 mean age 67 y	<i>n</i> = 38 healthy controls; mean age 67 y	33%	8% 100%
<b>(E) Cytokines</b>						
Hyodo et al., 1998 (46)	VEGF (plasma) cutoff >108 pg/mL	<i>n</i> = 26 TNM II+III/IV 8/18; mean age 60/66 y	<i>n</i> = 13 mean age 61 y	<i>n</i> = 20 normal controls; mean age 29 y	35% II+III/IV 0%/50%	8% 100%
Kumar et al., 1998 (47)	VEGF (serum) cutoff >217 pg/mL	<i>n</i> = 108 Dukes A/B/C 18/46/44	<i>n</i> = 136 healthy controls	90.7%		61%
Broll et al., 2001 (48)	VEGF (serum) cutoff >459 pg/mL	<i>n</i> = 122 TNM I/II/III/IV 41/27/30/24; mean age 68 y	<i>n</i> = 65 healthy controls; mean age 66 y	36%		95%
Tsai et al., 2006 (49)	VEGF (plasma) cutoff >148.6 pg/mL	<i>n</i> = 279 TNM I/II/III/IV 41/97/78/57	<i>n</i> = 20 hemorrhoids	64% I/II/III/IV 37/61/63/86%		95%
Renehan et al., 2000 (50)	IGF-II + IGFBP-2		<i>n</i> = 52 positive sigmoidoscopy; copy; mean age 60 y	<i>n</i> = 293 negative sigmoidoscopy; mean age 60 y		94% 31%
Mroczo et al., 2005 (51)	SCF cutoff >1,285 ng/mL IL-3 cutoff > 0,1 ng/mL	<i>n</i> = 75 AJCC II/III/IV 32/28/15; age 34-86 y	<i>n</i> = 40 healthy controls; age 21-66 y	89% 55%		17% 80%

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**Table 1. Protein markers (Cont'd)**

Reference	Marker	Study population		Sensitivity		Specificity
				Carcinoma	Adenoma	
		Cases	Controls			
		Carcinoma	Adenoma	Carcinoma	Adenoma	
<b>(F) Other proteins</b>						
Dudouet et al., 1990 (52)	Villin cutoff >20 ng/mL	<i>n</i> = 95 Dukes A/B/C/D 3/31/13/48	<i>n</i> = 421 healthy controls <i>n</i> = 40 Crohn's disease <i>n</i> = 80 extradigestive cancers	51% A/B/C/D 0/51/46/54%		97% healthy controls 87% Crohn's disease 95% extradigestive cancers
Severini et al., 1993 (53)	GST activity	<i>n</i> = 28 I/II/III/IV 1/9/10/8	<i>n</i> = 40 healthy controls <i>n</i> = 40 non-neoplastic	89% I/II/III/IV 0/89/90/100%		healthy controls 85% non-neoplastic 82%
Kozwicz et al., 1994 (54)	Cancer procoagulant cutoff >225 ng/mL	<i>n</i> = 83 I/II/III/IV/nd 2/8/15/7/50	<i>n</i> = 139 healthy controls <i>n</i> = 106 benign diseases	86% I/II/III/IV/nd 100/38/ 93/86/92%		healthy controls 83% benign diseases
Riedl et al., 1995 (55)	tenascin cutoff > 6 µg/mL	<i>n</i> = 118 TNM I/II/III/IV/nd 14/29/33/33/9	<i>n</i> = 51 healthy controls	25%		95%
Pasanen et al., 1995 (19)	TATI cutoff >21.3 ng/mL	<i>n</i> = 62 TNM I/II/III/IV 20/15/7/14 mean age 67 y	<i>n</i> = 97 benign gastrointestinal diseases mean age 59 y	74% I/II/III/IV 80/73/100/64%		34%
Ayude et al., 2000 (56)	α-L-fucosidase cutoff <5.6 units/mL	<i>n</i> = 137 Dukes A/B/C/D 13/69/40/15	<i>n</i> = 232 blood donors	69% A/B/C/D 69/70/72/53%		85%
Ayude et al., 2003 (57)	CD26 <sup>+</sup> α-L-fucosidase	<i>n</i> = 110 TNM I/II/III/IV 12/55/29/14	<i>n</i> = 46 blood donors	64% I/II/III/IV 75/66/66/43%		100%
Cordero et al., 2000 (58)	sCD26 cutoff >410 µg/L	<i>n</i> = 110 Dukes A/B/C/D 12/55/29/14	<i>n</i> = 52 blood donors	90%		90%
Fedarko et al., 2001 (59)	BSP cutoff 200-250 ng/mL OPN cutoff 400-500 ng/mL TIMP-1	<i>n</i> = 20	<i>n</i> = 77 normal patients	100% 30-65%		88-96% 56-85%
Holten- Andersen et al., 2002 (60)		<i>n</i> = 588 338CC/250RC Dukes A/B/C/D 58/218/175/137; median age 69 y	<i>n</i> = 108 healthy blood donors; median age 60 y	55% CC:65%/RC:42% early stages 58%/35%		95%
Kuru et al., 2002 (61)	Progesterone cutoff >0.9 ng/mL male cutoff >1.0 ng/mL female	<i>n</i> = 50 male Dukes B/C 22/28  <i>n</i> = 30 female Dukes B/C 13/17; mean age 58 y	<i>n</i> = 110 benign diseases  <i>n</i> = 80 male  <i>n</i> = 30 female; mean age 56 y	64% male;  57% female		37% male;  40% female

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Table 1. Protein markers (Cont'd)

Reference	Marker	Study population			Sensitivity		Specificity
		Cases		Controls	Carcinoma	Adenoma	
		Carcinoma	Adenoma				
Carpelan-Holmstrom et al., 2004 (15)	hCG $\beta$ cutoff > 2 pmol/L	<i>n</i> = 20 Dukes A/B/C/D 31/70/49/54	<i>n</i> = 27	<i>n</i> = 77 benign colorectal diseases	16% A/B/C/D 0/10/16/31%	15%	92%
Ferroni et al., 2004 (62)	sP-selectin cutoff > 75 ng/mL	<i>n</i> = 181 Dukes A/B/C/D/met 15/73/40/21/32; mean age 62 y		<i>n</i> = 181 healthy controls; mean age 60 y  <i>n</i> = 34 benign diseases; mean age 53 y	21% A/B/C/D/met 13/16/28/10/34%		99% healthy controls  94% benign diseases
Kerber et al., 2004 (63)	Fibrin degradation cutoff <0.7 $\mu$ g/mL	<i>n</i> = 30		<i>n</i> = 100 healthy blood donors	80%		93%
Soroush et al., 2004 (64)	prolactin cutoff > 20 ng/mL	<i>n</i> = 47 I/II/III/IV 9/19/10/9; mean age 55 y		<i>n</i> = 51 non-cancer controls; mean age 59 y	77%		98%
Zhang et al., 2004 (65)	M2-PK cutoff > 15 units/mL	<i>n</i> = 54 Dukes A/B/C		<i>n</i> = 20 healthy controls	69% A/B/C 72/79/59%		90%
Schneider et al., 2005 (66)	M2-PK cutoff >19.8 units/mL	<i>n</i> = 247 non-met/met 131/116		<i>n</i> = 53 without malignant disease	48% non-met/met 47/50%		95%
Melle et al., 2005 (67)	$\alpha$ -Defensins 1-3 cutoff > 14.8 ng/mL cutoff > 12.3 ng/mL	<i>n</i> = 26 <i>n</i> = 23		<i>n</i> = 22 healthy controls  <i>n</i> = 19 healthy controls	69% cutoff 14.8 ng/mL  65% cutoff 12.3 ng/mL		100% 100%
Saito et al., 2005 (68)	Laminin cutoff > 350 ng/mL	<i>n</i> = 205 Dukes A/B/C/D  49/37/57/22		<i>n</i> = 88 healthy controls	89%		88%
Roessler et al., 2005 (69)	Nicotinamide N-Methyltransferase	<i>n</i> = 109 TNM 0/I/II/III/IV/nd 3/33/23/21/23/6		<i>n</i> = 271 healthy employees <i>n</i> = 46 negative colonoscopy	51%		95%
(G) Chromatographic and mass spectrometric assays							
Wei et al., 1993 (70)	HiMwALP PAGE cutoff >17 units/L	<i>n</i> = 72 Dukes B/C/D 29/23/15; mean age 62 y		<i>n</i> = 38 patients with hemorrhoids; mean age 44 y	63%		89% 79%
Chen et al., 2004 (71)	HiMwALP DEAE SELDI-TOF MS Selected peaks at <i>m/z</i> of 4.476, 5.911, 8.817, 8.930	<i>n</i> = 55 Dukes A/B/C/D 8/22/13/12; median age 57 y		<i>n</i> = 92 healthy controls; median age 56	38% 91%*		93%*
Yu et al., 2004 (72)	SELDI-TOF MS Selected peaks at <i>m/z</i> of 5.911, 8.817, 8.922, 8.944	<i>n</i> = 55 Dukes A/B/C/D 8/22/13/12		<i>n</i> = 92 healthy controls	89% <sup>†</sup>		92% <sup>†</sup>

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**Table 1. Protein markers (Cont'd)**

Reference	Marker	Study population		Sensitivity		Specificity
		Cases		Controls		
		Carcinoma	Adenoma	Carcinoma	Adenoma	
Ward et al., 2006 (73)	SELDI-TOF MS Selected peaks at m/z of 6.44, 6.64, 8.94, 39.9, 50.7, 79.1	n = 62 Dukes AB/CD	n = 31 non-cancer controls	95% <sup>+</sup>		91% <sup>+</sup>
de Noo et al., 2006 (74)	MALDI-TOF MS <sup>§</sup>	27/35 mean age 67 y n = 63 median age 62 y	mean age 63 y n = 50 healthy controls	95% first test <sup>  </sup> 81% second test <sup>  </sup>		90% first test <sup>  </sup> 97% second test <sup>  </sup>

Abbreviations: AJCC, American Joint Committee on Cancer; CA, carbohydrate antigen; M<sub>1</sub>/M<sub>0</sub>, localized/advanced disease; met, metastatic; n, number of cases or controls; nd, not determined; TNM, tumour - lymph nodes- metastasis classification; r, recurrent; y, years; CEA, carcinoembryonic antigen; fCEA, free carcinoembryonic antigen; IgM, immunoglobulin-M; NCA, nonspecific cross-reacting antigen; PA, pancreatic antigen; PSA, prostate specific antigen; SIMA, small intestinal mucin antigen; SLEX, sialyted LewisX antigen; TPA, tissue polypeptide antigen; u-PA, urokinase-type plasminogen activator; DDX, dead box protein; sFasL, soluble Fas Ligand; IGF, insulin growth factor; IGFBP, insulin growth factor binding protein; IL, interleukin; n, number of cases or controls; SCF, stem cell factor; VEGF, vascular endothelial growth factor; BSP, bone sialoprotein; CC, colon cancer; CD, cluster of differentiation; GST, glutathione-s-transferase; hCG $\beta$ , human chorionic gonadotrophin beta; M2-PK, M2-pyruvatkinase; met, metastatic; OPN, osteopontin; RC, rectum cancer; sCD, soluble cluster of differentiation; sP-selectin, soluble P-selectin; TATI, tumor associated trypsin inhibitor; TIMP-1, tissue inhibitor of metalloproteinases 1; HiMwALP, high-molecular-mass alkaline phosphatase; MALDI-TOF MS, matrix assisted laser desorption ionization time-of-flight mass spectrometry; SELDI-TOF MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry.

\*Using artificial neural networks with 5-fold cross validation.

† Using artificial neural networks with 3-fold cross validation.

‡ Artificial neural networks with 10-fold cross validation.

§No peaks provided.

||First test in week 1, second test one week later; using double cross-validation.

like proteins identified through MS analysis. A broad timeframe with first studies from 1979 (75) to 2006 (49, 73, 74, 77, 81, 89-91, 102) was covered. The majority of studies evaluated protein markers, but in recent years, an interesting number of studies also evaluated genetic and epigenetic markers.

Overall, a broad range of sensitivity and specificity was reported for the various markers. A direct comparison of results from different studies is complicated due to the diverse populations used (different age, origin, "normal," or diseased controls), the diverse number of markers evaluated (single versus combined

**Table 2. Cytologic assays**

Reference	Marker	Study population		Sensitivity		Specificity
		Cases		Controls		
		Carcinoma	Adenoma	Carcinoma	Adenoma	
Tataryn et al., 1979 (75)	LAI cutoff NAI >30%	n = 115 Dukes A/B/C/D 6/20/23/66	n = 226 benign diseases + other cancer n = 41 inflammatory bowel disease	50% A/B/C/D 100/75/61/35%		98% benign disease 95% inflammatory bowel disease
Liu et al., 1989 (76)	LAI cutoff PAI >36%	n = 38 Dukes A/B/C/D/nd 9/6/10/10/3	n = 50 healthy blood donors n = 37 benign gastrointestinal diseases n = 32 other malignancies	68% A/B/C/D/nd 89/100/70/40/33%		96% healthy blood donors 95% benign gastrointestinal diseases 94% other malignancies
Wang et al., 2006 (77)	Circulating tumor cells (membrane array)	n = 88 TNM I/II/III/IV 11/27/38/12	n = 50 healthy controls	94% I/II/III/IV 73/93/100/100%		94%

Abbreviations: LAI, leukocyte adherence inhibition; n, number of cases or controls; NAI, nonadherence index; nd, not determined; PAI, percentage of adherence inhibition.

markers) and use of different cutoff points for the same marker (M2-PK and CEA). Furthermore, the majority of markers were evaluated in only one study, especially the newer ones. In addition, many studies had small sample sizes: overall sample size was <100 and between 100 and 300 in 19 and 57 studies, respectively, leading to rather imprecise estimates of performance characteristics. Only in 17 studies, overall sample size was above 300 subjects. Interpretation of many studies is further limited by the selection of cases and controls, which may differ from a screening population. In contrast to the screening situation, cases were typically identified by symptoms, and controls were often convenience samples, such as patients with other gastrointestinal diseases or employees from the same hospital with a much younger median age than the cases. The latter may have led to overoptimistic results, when the levels of the marker increase with age, irrespective of true disease status. Another concern refers to comparability of results across studies given potential differences in serum collection, processing and storage methods, and uncertainties in the stability of several biomarkers. Information provided on these issues is typically very limited.

Only very few studies examined sensitivity of markers among patients bearing adenomas. As regards controls,

they had often not undergone colonoscopy. These control groups most likely included a substantial proportion of adenoma carriers because the prevalence of adenomas among older adults is estimated to be about 20% to 30% (103-105). If markers are able to detect adenomas in addition to invasive CRC, specificity is underestimated in such studies.

In some studies, extraordinary high-performance characteristics were reported. For example, sensitivities above 77% and specificities above 90% were reported for sCD26 (58), BSP (59), fibrin degradation (63), prolactin (64), CTC by membrane assay (77), L6 mRNA (101), and for combinations of proteins (proteomic profiling) selected by SELDI-TOF MS (71-73) or MALDI-TOF MS (74). However, these markers were examined in one single study. Only one SELDI-TOF MS approach was examined in two studies (71, 72), which, however, turned out to be from the same group using the same study population. Furthermore, as regards approaches of SELDI-TOF MS and MALDI-TOF MS, subjects used for training and validation sets were the same, applying different techniques of cross-validation (including artificial neural networks). Further external validation is still outstanding. The seemingly high performance characteristics of MS-based techniques are subject to extensive discussion (106-117). In contrast to

**Table 3. DNA markers**

Reference	Marker	Study population		Sensitivity		Specificity	
		Cases	Controls	Carcinoma	Adenoma		
Mutations							
Kopreski et al., 2000 (78)	K-ras	<i>n</i> = 8	<i>n</i> = 62	<i>n</i> = 105 normal colonoscopy <i>n</i> = 65 non-neoplastic	63%	35%	73% normal colonoscopy 86% non-neoplastic
Wang et al., 2004 (79)	APC	<i>n</i> = 104 Dukes A/B/C/D 7/49/39/9	<i>n</i> = 50 healthy controls		14% A/B/C/D 0/20/45/33%		100%
	K-ras				15% A/B/C/D 0/22/46/50%		
	p53				13% A/B/C/D 0/20/43/57%		
	Either				34% A/B/C/D 0/22/49/67%		
Methylations							
Leung et al., 2005 (80)	APC	<i>n</i> = 49 TNM I/II/III/IV 5/15/17/8	<i>n</i> = 41 normal colonoscopy		6% I+II/III+IV 5/8%		100%
	hMLH1				39% I+II/III+IV 30/44%		98%
	HLTF				31% I+II/III+IV 25/36%		93%
	Either cutoff >0.01 pg/μL				57%		90%
DNA integrity							
Umetani et al., 2006 (81)	Free DNA concentration	<i>n</i> = 32 AJCC I/II/III/IV 3/14/6/9	<i>n</i> = 51 healthy controls		41%		90%
	Integrity of free DNA				56%		90%

Abbreviation: AJCC, American Joint Committee on Cancer; APC, adenomatous polyposis of the colon; DNA, deoxyribonucleic acid; hMLH1, human MutL homologue 1; HLTF, helicase-like transcription factor; *n*, number of cases or controls.

**Table 4. mRNA markers**

Reference	Marker	Study population	
		Cases	
		Carcinoma	Adenoma
Castells et al., 1998 (83)	CEA	<i>n</i> = 95 TNM I/II/III/IV 6/32/37/20	
Guadagni et al., 2001 (84)	CEA	<i>n</i> = 51 Astler-Coller A/B/C/D/r 6/15/8/17/5	
Sadahiro et al., 2001 (85) Silva et al., 2002 (86)	CEA CEA CK19 either	<i>n</i> = 121 TNM I/II/III/IV 20/48/34/18 <i>n</i> = 53	
Douard et al., 2005 (87)	CEACAM5/CEACAM7	<i>n</i> = 84 I/II/III/IV 11/28/27/18	
Vlems et al., 2002 (88)	CK20 4 times testing	<i>n</i> = 30 TNM II/III/IV 15/10/5	
Giribaldi et al., 2006 (89) Xu et al., 2006 (90)	CK20 CEA CK20 CK19	<i>n</i> = 99 non-met/met 49/50 <i>n</i> = 95 Dukes A/B/C/D 6/24/39/26 <i>n</i> = 46 A/B/C/D 4/12/15/15 <i>n</i> = 148 A/B/C/D 6/45/54/43	
Wang et al., 2006 (91)	CEA CK19 CK20 hTERT	<i>n</i> = 72 TNM I/II/III/IV 8/22/30/12	
Lledo et al., 2004 (92)	hTERT	<i>n</i> = 50 median age 74 y	
Denis et al., 1997 (93)	CK8/CK19/CK20	<i>n</i> = 23 Astler-Coller A/B/C/D 2/8/8/5, median age 64 y	
Wharton et al., 1999 (94) Hardingham et al., 2000 (95)	CEA/CK20 CK19/CK20/MUC1/MUC2	<i>n</i> = 100 Dukes AB/C/met 30/20/50 <i>n</i> = 94 Dukes A/B/C 16/47/31	
Schuster et al., 2004 (96)	ProtM/CEA/CK20/	<i>n</i> = 129	
Dandachi et al., 2005 (97)	CK20 cutoff >415	<i>n</i> = 82 LCC/MCC 40/42	
Bustin et al., 1999 (98)	GCC	<i>n</i> = 27 Dukes A/B/C/D 2/13/6/6, age 39-80 y	
Douard et al., 2001 (99)	CGM2	<i>n</i> = 78 Dukes A/B/C/D 8/21/30/19, mean age 69 y	
Miyashiro et al., 2001 (100) Schiedeck et al., 2003 (101) Garcia et al., 2006 (102)	uMAGE-A L6 Thymidylate synthase	<i>n</i> = 22 AJCC I+II/III/IV 3/4/15 <i>n</i> = 187 TNM I/II/III/IV 53/38/61/35 <i>n</i> = 88 Dukes A/B/C/D 5/49/32/2	

Abbreviations: AJCC, American Joint Committee on Cancer; CEA, carcinoembryonic antigen; CEACAM, carcinoembryonic antigen cell adhesion molecule; CGM2, carcinoembryonic gene member 2; CK, cytokeratin; GCC, guanylyl cyclase C; hTERT, human telomerase reverse transcriptase; LCC, localized colon cancer; MCC, metastasized colon cancer; met, metastatic; MUC, mucin; *n*, number of cases or controls; r, recurrent; uMAGE-A, human melanoma-associated antigen family A; y, years.

classic immunologic assays, it is possible to examine a panel of different biomarkers at once. However, initial discovery methods generally have been poorly suited for clinical applications. Points to be considered in this context are standardization, reproducibility, and quality assurance. Furthermore, identities and serum concentration of distinguishing molecules are often not known, and if identified, selected peaks sometimes corre-

sponded to highly abundant proteins, which have a much higher concentration than classic biomarkers. Also, these empirical MS-based profiling approaches may be subject to confounding and false-positive performance due to subject and/or sample bias without validation in independent studies and independent biomarker identification and quantitation, respectively (118-120).

Table 4. mRNA markers (Cont'd)

Controls	Sensitivity		Specificity
	Carcinoma	Adenoma	
<i>n</i> = 11 healthy controls <i>n</i> = 9 inflammatory bowel disease <i>n</i> = 11 other gastrointestinal cancer	41% I/II/III/IV 33/38/35/60%		100% healthy controls 44% inflammatory bowel diseases 91% other gastrointestinal cancer
<i>n</i> = 40 healthy controls <i>n</i> = 18 benign colorectal diseases	69% A/B/C/D/r 33/60/88/77/80%		95% healthy donors 100% benign colorectal diseases
<i>n</i> = 33 healthy controls <i>n</i> = 25 healthy controls	42% I/II/III/IV 45/29/53/56% 32% 74% 83%		82% 96% 80% 76%
<i>n</i> = 41 healthy controls <i>n</i> = 32 non-CRC <i>n</i> = 16 healthy controls <i>n</i> = 13 nonmalignant colorectal disorders <i>n</i> = 18 no malignancy + no colorectal disorder <i>n</i> = 150 healthy blood donors <i>n</i> = 30 healthy controls	61% I/II/III/IV 36/46/81/77% 30% II/III/IV 33/30/20 22% non-met/met 18/26% 36% A/B/C/D 17/29/39/42% 28% A/B/C/D 0/17/33/40% 42% A/B/C/D 33/42/41/44% 72% I/II/III/IV 13/77/73/100% 67% I/II/III/IV 38/64/73/75% 53% I/II/III/IV 13/59/60/50% 69% I/II/III/IV 25/72/77/75% 98%		100% healthy controls 100% non-CRC 81% healthy controls 69% nonmalignant colorectal disorders 83% no malignancy + no colorectal disorder 100% 97% 93% 97% 100% 97% 97% 100% 64%
<i>n</i> = 50 health care workers, median age 40 y <i>n</i> = 26 healthy controls <i>n</i> = 16 other gastrointestinal diseases, median age 40 y <i>n</i> = 70 patients with inguinal hernia <i>n</i> = 20 healthy controls <i>n</i> = 34 inflammatory bowel disease	52% A/B/C/D 0/25/75/80% 56% 20% A/B/C 13/9/42%		96% 100% healthy controls 88% inflammatory bowel disease 100% healthy controls 100% inflammatory bowel/infectious diseases
<i>n</i> = 45 healthy controls <i>n</i> = 13 inflammatory bowel/infectious diseases	13%	10%	78% healthy controls 40% inflammatory bowel disease 95%
<i>n</i> = 37 healthy controls <i>n</i> = 15 inflammatory bowel disease	55% LCC/MCC 55/55%		100% healthy controls 100% benign colorectal diseases 100% non-CRC
<i>n</i> = 21 healthy controls, age 26-61 y	74% A/B/C/D 100/85/67/50%		100% 100%
<i>n</i> = 62 healthy controls, mean age 41 y <i>n</i> = 16 benign colorectal diseases	59% Dukes A/B/C/D 38/43/77/58%		100% 100%
<i>n</i> = 31 non-CRC <i>n</i> = 20 healthy controls <i>n</i> = 45 healthy controls <i>n</i> = 27 healthy blood donors	32% I+II/III/IV 33/50/27% 79% I/II/III/IV 85/71/77/80% 47% A/B/C/D 40/35/66/50%		100% 100% 77%

When evaluating the potential of blood markers, a comparison with stool-based tests is indicated, not only with existing tests like FOBT, but also with novel approaches of stool testing. A first aspect is practicality. Both biological specimens are comparably easy to collect. Whereas stool sampling can be done at home, blood sampling has to be done by a health professional, but is often preferred by both patients and laboratory employees. Laboratory complexity and costs may be lower with blood samples because stool often requires extensive sample processing. Regarding performance characteristics, results for stool tests (121) also showed broad

variation. Like for blood tests, high overall levels of sensitivity up to 80%, with specificities up to 90% to 100%, were reported for some novel stool tests, but again, confirmation of the most promising results in large-scale studies conducted in a screening setting were missing. Nevertheless, preliminary results from both novel blood and stool tests suggests that performance characteristics for some tests may, by far, exceed those of guaiac-based fecal occult blood testing, the most widely used noninvasive CRC screening test thus far. In this context, it should also be deliberated to use serum biomarkers to screen subjects over time, as currently conducted with

FOBT and as being a promising tool for other cancer types (122).

The current stage of evidence calls for prospectively planned, systematic evaluations of both the most promising blood tests and the most promising stool tests in a well-defined, large-scale screening population, in which special attention is drawn to standardized sample collection, processing, and storage. A multicenter clinical context of screening colonoscopy might provide an ideal setting for such a study because it assures both representativeness of participants for a screening population and ascertainment of adenoma carriers. Although such studies may provide sufficient controls free of colorectal lesions and sufficient patients with premalignant adenomas, very large numbers of screening participants would have to be recruited, however, to include a reasonable number of CRC cases. Parallel recruitment of clinically well-characterized CRC cases in clinical settings (enriched for early-stage disease) using the same procedures of specimen collection and handling might therefore be required. Ideally, a large number of blood and stool tests should be evaluated in parallel in such studies. On the one hand, this would allow direct comparison of performance characteristics and practicality of single tests; on the other hand, the potential of combining different tests could be assessed. Enhanced performance by combining different serum markers was already reported by some studies included in this review (6, 8-16, 18, 20, 21, 28, 29, 33, 34, 40, 48, 49, 51, 56, 57, 60, 66, 70, 76, 84, 87, 90). A longitudinal design of future studies would allow to assess the potential of quantifying biomarkers over time to provide increased sensitivity for an emergent malignancy in individual patients, an approach that has shown promising results for ovarian cancer detection (122). Beyond the application of novel markers in the general screening context, specific study designs might be required for application in selected patients, e.g., in hereditary nonpolyposis colorectal cancer families, or for combination with novel techniques e.g., virtual colonoscopy (123, 124).

This review can only provide a general overview of the performance characteristics of the various tests, partly due to incomplete reporting of data in the original articles. For example, in many studies, characterization of the study population was rather scarce. Furthermore, this review represents only a narrative summary of studies. A meta-analysis with pooling of results of different studies was not conducted due to heterogeneity between studies. A possible source of bias may be incomplete selection of articles, because even with the sensitive selection strategy outlined in Materials and Methods, some articles may have been missed. Furthermore, reported sensitivities and specificities may provide an overoptimistic perspective because of publication bias, which may have led to selected publication of more promising results.

In conclusion, during the last years, many approaches have been reported to develop and evaluate new blood-based tests for the early detection of CRC. According to some recent reports, some tests may have the potential to improve current screening, but phased validation strategies resulting in large-scale prospective evaluation of the most promising candidates and final cancer control studies is needed (125). Further studies should

also focus on early stage, especially adenoma cases. Furthermore, practical issues and costs need to be considered because screening for CRC is typically implemented as a population-based, mass screening program.

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