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ORIGINAL RESEARCH

Early Detection and Recurrence of Colorectal Adenomas by Combination of Eight Cancer-Associated Biomarkers in Plasma

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Introduction: Plasma levels of eight combined proteins have shown value as biomarkers for detection of colorectal cancer (CRC). However, their value in identifying colorectal adenoma needs further evaluation. The aim was to evaluate the eight proteins (AFP, CA19-9, CEA, CyFra21-1, Ferritin, Galectin-3, hs-CRP and TIMP-1) in detection of high-risk adenoma (HRA) and in prediction of recurrence of adenoma. Furthermore, the discrimination between HRA and low-risk adenoma (LRA) or CRC lesions was evaluated.

Methods: The study included 4698 individuals undergoing diagnostic colonoscopy. Automated ELISA platforms were used in the determination of protein levels in samples collected just before colonoscopy.

Results: Univariably, five proteins (AFP, CEA, CyFra21-1, hs-CRP and TIMP-1), respectively, significantly discriminated individuals with HRA from individuals with non-malignant findings. Multivariably, the combination of CEA and hs-CRP improved performance; AUC=0.63 (sensitivity=0.19 at specificity=0.90). CyFra21-1, Ferritin and TIMP-1 demonstrated significant discrimination between individuals with HRA and LRA in univariable analyses, respectively. Performance was improved in multivariable analysis; AUC=0.61 (sensitivity=0.13 at specificity=0.90). Discrimination between individuals with colorectal adenomas and healthy individuals was significant for CA19-9, CEA, hs-CRP and TIMP-1, respectively, in univariable analyses. Multivariable analysis improved performance; AUC=0.63 (sensitivity=0.17 at specificity=0.90). All proteins except AFP demonstrated significant discrimination between individuals with HRA and CRC. Combination of CEA, CyFra21-1, Ferritin, hs-CRP and TIMP-1 in multivariable analysis improved discrimination; AUC=0.78 (sensitivity=0.34 at specificity=0.90). Association between plasma levels of any of the eight proteins and recurrence of colorectal adenomas after endoscopic removal could not be demonstrated.

Discussion: The protein panel shows a promising potential in detection of colorectal adenomas in general, but specifically of HRA. However, improvements are needed for the panel to be valuable as a screening test. Finally, plasma levels of the eight proteins were not predictive of recurrence of colorectal adenomas.

Keywords: biomarkers, tumor, neoplasm recurrence, local, colorectal adenomas, colorectal neoplasms, alpha-feto protein, cancer antigen 19-9, carcino embryogenic antigen, cytokeratin fragment 21-1, Ferritin, Galectin-3, high sensitivity C-reactive protein, tissue inhibitor of metalloproteinases-1

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths.[1] Diagnostics and treatments of CRC have improved substantially during the last decade, but the

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disease continues to pose a major health challenge.[2] Early diagnosis of CRC increases overall survival,[3] and population-based screening programs have been implemented in many countries worldwide.[4] In addition, detection and removal of pre-cancerous lesions (colorectal adenomas) identified via screening has led to a decrease of CRC incidence,[5] which makes colorectal adenomas an important diagnostic target.

The natural history of CRC occurs over years and involves progression from low risk to high risk precancerous adenomas and, eventually, to carcinoma.[6] The lifetime-incidence of colorectal adenomas is approximately 35%,[7] and the majority of CRC cases develop through malignant transformation of high-risk adenoma (HRA).[8] It has been estimated that 10% of all adenomas and approximately 25% of HRA will progress to CRC;[9] diagnosis and removal of precancerous adenomas prevent progression to cancer.[10] Therefore, adenoma detection has a high priority in screening and early detection of colorectal neoplasia.

The predominant design of current screening programs includes an initial screening test to select a high-risk group for subsequent diagnostic colonoscopy.[4] Available clinical-approved screening tests for CRC include feces based tests (guaiac fecal occult blood test (gFOBT), fecal immunochemical test (FIT), multitarget stool DNA test (Cologuard)) and blood-based test for methylated SEPT9 DNA (mSEPT9) (Epi proColon). However, the performance of the screening tests in identification of advanced, high-risk adenomas (HRA) are not impressive; sensitivity=20-34% at specificity=91-97% for FIT,[11,12] sensitivity=9-14% at specificity=92-95% for gFOBT,[11,12] sensitivity=42-54% at specificity=90% for multitarget stool DNA test[13,14] and sensitivity=11-21% for mSEPT9 test (specificity not reported).[15,16] The inadequate performance of screening tests for detection of HRA combined with the fact that almost all adenomas are asymptomatic could lead to individuals that are not identified with HRA and thereby are not offered colonoscopy (false-negative result), and indeed colonoscopy examinations may be offered to individuals without adenomas by a false-positive result of the screening test. Furthermore, the determination of the malignant potential of adenomas by colonoscopy is challenging.[17,18] The ability to identify individuals with HRA and to differentiate between individuals with low-risk adenoma (LRA) and potentially malignantly transformable HRA, as well as to identify individuals at risk of recurrence of adenomas after polypectomy, would provide an opportunity to prioritize the diagnostic and follow-up colonoscopies offered to individuals in current and future screening programs.

The need for developing new screening options for early detection of CRC and HRA is urgent, and attention has been focused on identifying new blood-based biomarkers as the compliance associated with stool-based tests is estimated at 60–65%.[19–21]

A key element in the process of oncogenesis is genomic and transcriptional alterations, which consequently changes the expression of various proteins in tumor tissue and in the circulation. These proteins represent a major entity of blood-based biomarkers, and determination of proteins in the circulation, individually or in combination, may be used in detection of individuals with colorectal adenomas or CRC.[22–27] Various blood-based biomarkers have shown value although limited in discrimination between LRA and HRA[28,29] as well as in prediction of recurrence following polypectomy.[30] Of particular interest is a previous evaluation of a panel of eight blood-based proteins associated with CRC indicating a possible future role as biomarkers for early detection of CRC.[31] The eight proteins of the panel were chosen based on characteristics and current knowledge of their role in the carcinogenesis of CRC (Table 1). However, the accuracy of this specific protein panel in HRA detection and prediction of recurrence needs further elucidation.

The aim of the present study was to evaluate the value of these eight proteins (Table 1) in plasma, individually or combined, in detection of primary HRA and subsequently in prediction of adenoma recurrence. In addition, the aims included whether the proteins could be used to differentiate HRA lesions from LRA lesions, HRA lesions from CRC lesions and whether the proteins could differentiate between individuals with adenoma lesions from individuals with non-malignant findings or no findings (clean colorectum) at colonoscopy.

Materials and Methods

The study was a part of the Endoscopy II protocol.[31,47] In brief, blood samples were collected prospectively from individuals referred to diagnostic colonoscopy due to symptoms attributable to CRC. Blood for EDTA-plasma samples were collected before colonoscopy and handled and stored at −80°C within two hours according to a validated Standard Operating Procedure. The Endoscopy II protocol was initiated in 2010 and terminated in 2012 with inclusion of 4698 individuals with

Table 1 Characteristics and Current Knowledge in Association with CRC and HRA of AFP, CA19-9, CEA, CyFra21-1, Ferritin, Galectin-3, Hs-CRP and TIMP-1

Marker		References
AFP	• Tumor marker in hepatocellular carcinoma and embryonal testicular tumors. • Increased levels in the circulation are associated with cancer deriving from several organs (stomach, pancreas, lung, renal, ovary and colorectal cancer).	[32,33]
CA19-9	• Increased levels in the circulation are associated with CRC.	[33–37]
CEA	• Clinically approved for detection of metastatic disease or recurrence and monitoring response to treatment of CRC. • Limited sensitivity for screening in asymptomatic individuals.	[34–38]
CyFra21-1	• Diagnostic value in differentiating individuals with advanced colorectal adenomas from individuals with no colorectal adenomas or CRC. • Indicator of the neoplastic burden induced by carcinogenesis. • Increased levels in the circulation are associated with CRC.	[27,39,40]
Ferritin	• Increased levels in the circulation are associated with CRC. • Complex interactions; levels in the circulation are positive correlated to cancer-specific processes which could be antagonized by iron-deficiency anemia caused by chronic gastrointestinal bleeding.	[26,27,37,41,42]
Galectin-3	• Increased expression in tumor tissue of CRC and associated with advanced CRC.	[43]
hs-CRP	• Associated with CRC and carcinogenesis in general.	[27]
TIMP-1	• Increased levels in the circulation are associated with CRC.	[44–46]

Abbreviations: AFP, alpha-feto protein; CA19-9, cancer antigen 19–9; CEA, carcino embryogenic antigen; CRC, colorectal cancer; CyFra21-1, cytokeratin fragment 21–1; HRA, high risk adenoma; hs-CRP, high sensitivity C-reactive protein; TIMP-1, tissue inhibitor of metalloproteinases-1.

Table 2 Inclusion and Findings at Colonoscopy in the Endoscopy II Study

	Men	Women	Total	Median Age
Included individuals in Endoscopy II	2243	2455	4698	64 (18–96)
Individuals with CRC	306	206	512	
Stage I–II CRC	156	108	264	
Stage III–IV CRC	150	97	247	
No stage available	–	1	1	
Individuals with colorectal adenoma	384	305	689	
LRA	168	122	290	
• No recurrence at follow-up			249	
• Recurrence at follow-up			41	
• CRC at follow-up[a]			2	
HRA	216	183	399	
• No recurrence at follow-up			300	
• Recurrence at follow-up			99	
• CRC at follow-up[a]			9	

Notes: [a]Numbers of individuals with CRC at follow-up are included in numbers of individuals with recurrence at follow-up.
Abbreviations: CRC, colorectal cancer, LRA, low-risk colorectal adenoma, HRA, high-risk colorectal adenoma.

valid clinical and biomarker data. A previous publication based on the Endoscopy II protocol evaluated the value of plasma levels of the eight serological proteins in discrimination of individuals with CRC and HRA from the entire group of included individuals.[31] The present study was designed as a spin-off of the previous publication with the use of the same analyses methodologies and measurements of the eight proteins. Table 2 presents an overview of findings at colonoscopy in the study.

Data registered in the Endoscopy II database comprised age, gender, comorbidity and findings at colonoscopy including pathological and histological classification of colorectal adenomas (LRA/HRA) and CRC. The definition of LRA was one lesion <1cm, <3 lesions, tubular histology or low-grade dysplasia. The definition of HRA was one lesion ≥1 cm, ≥3 lesions, villous histology or high-grade dysplasia.

In the present study, recurrence of colorectal adenoma was recorded in individuals with a primary diagnosis of colorectal adenoma in a period from 12 months after the

primary diagnosis and until the end of December 2017 (median follow-up period of 75 (35–94) months).

For further statistical analysis, three endpoints were defined based on outcome at colonoscopy:

Endpoint 1: The discrimination of individuals with HRA from all other individuals with non-malignant findings (including LRA) or clean colorectum.

Endpoint 2: The discrimination of individuals with HRA from individuals with LRA.

Endpoint 3: The discrimination of individuals with HRA from individuals with CRC.

Endpoint 3 was subdivided into

Endpoint 3a: The discrimination of individuals with HRA from individuals with early-stage CRC (Stage I and II).

Endpoint 3b: The discrimination of individuals with HRA from individuals with late-stage CRC (Stage III and IV).

A secondary Endpoint was defined as the discrimination of individuals with HRA and LRA from all other individuals excluding individuals with CRC.

Plasma protein levels were determined by using the Abbott ARCHITECT® i2000 automated immunoassay platform (Abbott laboratory inc., Abbott Park, IL, USA) utilizing a two-step dual monoclonal immunoassay. All the laboratory analyses were performed by Abbott Center of Excellence at VU Medical Center (Amsterdam, The Netherlands), and Abbott in-house research prototype and on-market reagents were used for determinations. All achieved results from the analysis were included in the database.

Statistics

Initial univariable logistic regression analysis with the binary endpoints as the dependent variable and plasma levels of AFP, CA19-9, CEA, CyFra21-1, Ferritin, Galectin-3, hs-CRP and TIMP-1 as explanatory variables (log transformed (base2)) were performed. Individual biomarkers found to have significant univariable discrimination (p-value<0.05), as well as age and gender, were then used as explanatory covariates in multivariable logistic regression analysis reducing the number of explanatory covariates using a stepwise 10-fold cross-validation selection method. For each endpoint, a predictor (a linear combination of the significant explanatory covariates) has been established. The results are presented by the odds ratios (OR) with 95% confidence intervals (CI), sensitivity at 70, 80 and 90% specificity and receiver operating characteristic (ROC) curves with area under the ROC curve (AUC_{ROC}) as a measure of discrimination.

Analysis of time to adenoma recurrence has been done using the Cox proportional hazards model with the mean plasma values of the eight protein biomarkers as continuous covariates on the log scale (base 2). The resulting models were assessed using martingale residuals.

P-values less than 5% were considered significant. Database management and statistics were performed using SAS (v9.4, SAS Institute, Cary, N.C., USA). In addition, R was used for statistical calculations (R Development Core Team, Vienna, Austria, http://www.R-project.org).

Results

Descriptive statistics are presented in Table 2.

Data from the univariate analyses of Endpoint 1–3 and the secondary Endpoint are illustrated in Figure 1A and B.

In discrimination between individuals with HRA and all other individuals with non-malignant findings or clean colorectum (Endpoint 1), plasma levels of AFP (AUC_{ROC}=0.53, p-value=0.033, sensitivity=0.11 at specificity=0.90), CEA (AUC_{ROC}=0.57, p-value<0.001, sensitivity=0.13 at specificity=0.90), CyFra21-1 (AUC_{ROC}=0.54, p-value=0.010, sensitivity=0.14 at specificity=0.90), hs-CRP (AUC_{ROC}=0.57, p-value<0.001, sensitivity=0.11 at specificity=0.90) and TIMP-1 (AUC_{ROC}=0.58, p-value<0.001, sensitivity=0.14 at specificity=0.90), respectively, demonstrated significance. Plasma levels of all five proteins were increased in individuals with HRA compared to individuals with non-malignant findings or clean colon (OR=1.11–1.70).

Regarding Endpoint 2, univariable analyses showed that plasma levels of CyFra21-1 (AUC_{ROC}=0.55, p-value=0.018, sensitivity=0.15 at specificity=0.90), Ferritin (AUC_{ROC} =0.57, p-value=0.010, sensitivity=0.13 at specificity=0.90) and TIMP-1 (AUC_{ROC}=0.55, p-value=0.006, sensitivity=0.16 at specificity=0.90) were significantly different among individuals with HRA compared to individuals with LRA, respectively; Plasma levels of CyFra21-1 and TIMP-1 were decreased (OR=0.61–0.81), and plasma levels of Ferritin were increased (OR=1.15).

The results of the univariable analyses for the eight individual proteins in the discrimination of individuals with HRA from individuals with CRC (Endpoint 3) are presented for individuals with all stages of CRC (Endpoint 3), early-stage CRC (stage I+II) (Endpoint 3a) and late-stage CRC (stage III+IV) (Endpoint 3b).

A significant discrimination between individuals with HRA and individuals with CRC (all stages) was

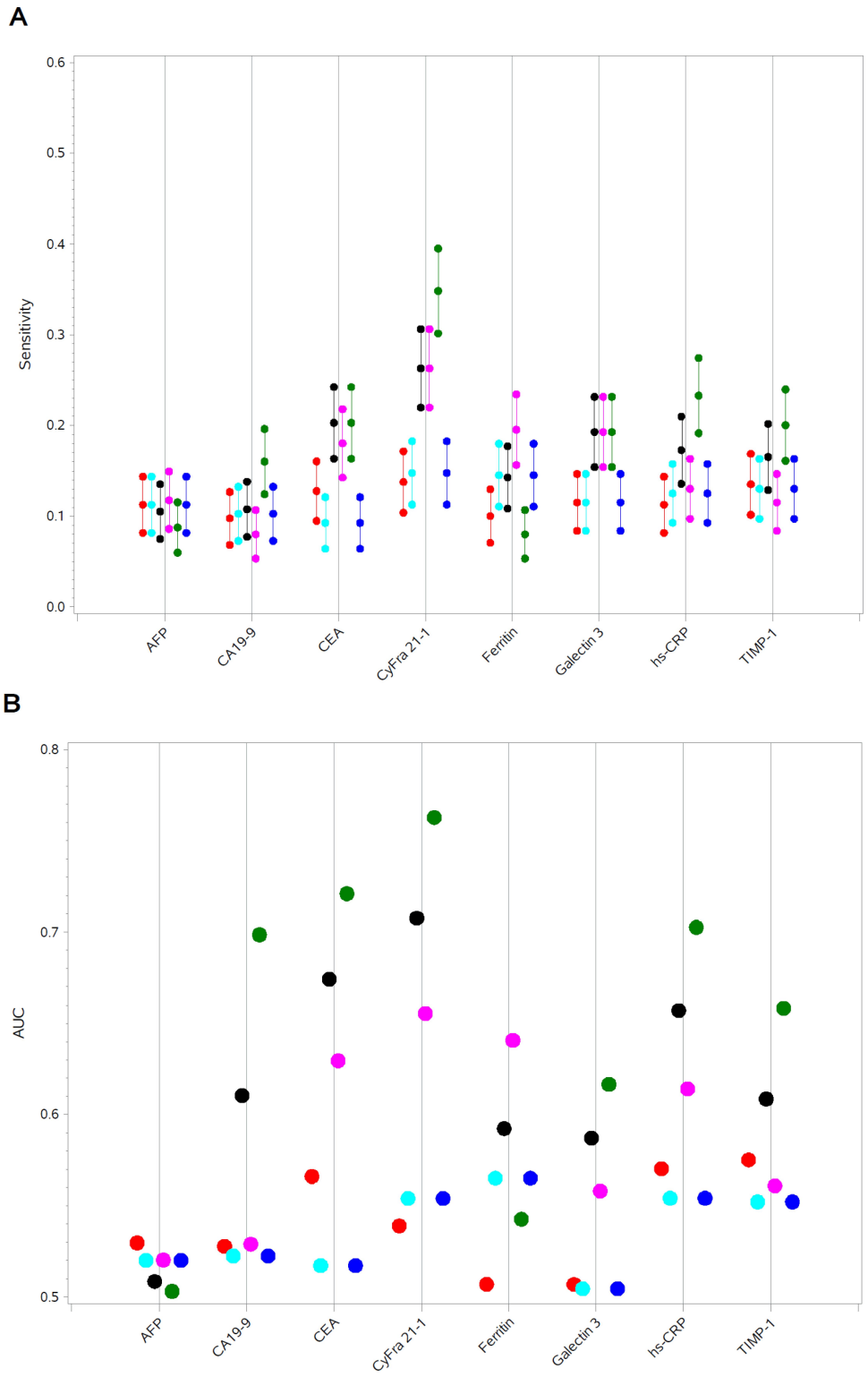

Figure 1 (**A**) Sensitivities at specificity=0.90 in univariate analyses of Endpoint 1–3 (including 3a and b) and secondary Endpoint. (**B**) AUC_{ROC} in univariate analyses of Endpoint 1–3 (including 3a and 3b) and secondary Endpoint.

Notes: Red: Endpoint 1, Turquise: Endpoint 2, Black: Endpoint 3, Magenta: Endpoint 3a, Green: Endpoint 3b, Blue: Secondary Endpoint.

Abbreviations: AFP, alpha-feto protein; CA19-9, cancer antigen 19–9; CEA, carcino embryogenic antigen; CyFra21-1, cytokeratin fragment 21–1; Hs-CRP, high sensitivity C-reactive protein; TIMP-1, tissue inhibitor of metalloproteinases-1.

demonstrated for plasma levels of all proteins, except AFP; CA19-9 (AUC_{ROC}=0.61, p-value<0.001, sensitivity=0.11 at specificity=0.90), CEA (AUC_{ROC}=0.67, p-value<0.001, sensitivity=0.20 at specificity=0.90), CyFra21-1 (AUC_{ROC}=0.71, p-value<0.001, sensitivity=0.26 at specificity=0.90), Ferritin (AUC_{ROC}=0.59, p-value<0.001, sensitivity=0.14 at specificity=0.90), Galectin-3 (AUC_{ROC}=0.59, p-value<0.001, sensitivity=0.19 at specificity=0.90), hs-CRP (AUC_{ROC}=0.66, p-value<0.001, sensitivity=0.17 at specificity=0.90) and TIMP-1 (AUC_{ROC}=0.61, p-value<0.001, sensitivity=0.17 at specificity=0.90). Plasma levels of CA19-9, CEA, CyFra21-1, Galectin-3, hs-CRP and TIMP-1 were decreased in individuals with HRA compared to individuals with CRC (all stages), (OR=0.48–0.76), and plasma levels of Ferritin were increased in individuals with HRA (OR=1.21).

Similar results were achieved when the analyses were restricted to discriminate individuals with HRA from individuals with late stage CRC (Endpoint 3b). Plasma levels of CA19-9 (AUC_{ROC}=0.70, p-value<0.001, sensitivity=0.16 at specificity=0.90), CEA (AUC_{ROC}=0.72, p-value<0.001, sensitivity=0.20 at specificity=0.90), CyFra21-1 (AUC_{ROC}=0.76, p-value<0.001, sensitivity=0.35 at specificity=0.90), Galectin-3 (AUC_{ROC}=0.62, p-value<0.001, sensitivity=0.19 at specificity=0.90), hs-CRP (AUC_{ROC}=0.70, p-value<0.001, sensitivity=0.23 at specificity=0.90) and TIMP-1 (AUC_{ROC}=0.66, p-value<0.001, sensitivity=0.20 at specificity=0.90) were significantly decreased in individuals with HRA (OR=0.37–0.68). Plasma levels of Ferritin were significantly increased (OR=1.09); AUC_{ROC}=0.54 with sensitivity=0.08 at specificity=0.90. Plasma levels of AFP did not reach significance.

Restricting the analyses to discriminate individuals with HRA from individuals with early stage CRC (Endpoint 3a), plasma levels of CA19-9 (AUC_{ROC}=0.53, p-value=0.021, sensitivity=0.08 at specificity=0.90), CEA (AUC_{ROC}=0.63, p-value<0.001, sensitivity=0.18 at specificity=0.90), CyFra21-1 (AUC_{ROC}=0.66, p-value<0.001, sensitivity=0.26 at specificity=0.90), Ferritin (AUC_{ROC}=0.64, p-value<0.001, sensitivity=0.20 at specificity=0.90), Galectin-3 (AUC_{ROC}=0.56, p-value=0.020, sensitivity=0.19 at specificity=0.90) and hs-CRP (AUC_{ROC}=0.61, p-value<0.001, sensitivity=0.13 at specificity=0.90) were persistently significant, but plasma levels of TIMP-1 lost significance. Plasma levels of CA19-9, CEA, CyFra21-1, Galectin-3 and hs-CRP were decreased (OR=0.54–0.87) and plasma levels of Ferritin were increased (OR=1.36) in individuals with HRA compared to individuals with early stage CRC.

For the secondary Endpoint (discrimination of individuals with HRA and LRA from all other individuals excluding individuals with CRC), the plasma levels of CA19-9 (AUC_{ROC}=0.54, p-value=0.004, sensitivity=0.13 at specificity=0.90), CEA (AUC_{ROC}=0.56, p-value<0.001, sensitivity=0.14 at specificity=0.90), hs-CRP (AUC_{ROC}=0.55, p-value<0.001, sensitivity=0.15 at specificity=0.90) and TIMP-1 (AUC_{ROC}=0.56, p-value<0.001, sensitivity=0.14 at specificity=0.90) were significantly decreased in individuals (OR=0.69–0.92) with adenomas compared to all other individuals.

Results of the multivariable analyses for Endpoint I, Endpoint 2, Endpoint 3 and the secondary Endpoint are presented in Table 3.

For Endpoint 1, the multivariable analysis included CEA and hs-CRP; AUC_{ROC}=0.63 with sensitivity=0.19 at specificity=0.90.

The multivariable analysis for Endpoint 2 included Ferritin and TIMP-1; AUC_{ROC}=0.61 with sensitivity=0.13 at specificity=0.90.

For Endpoint 3, the multivariable analysis included CA19-9, CEA, CyFra21-1, Ferritin, hs-CRP and TIMP-1 and showed AUC_{ROC}=0.78 with sensitivity=0.34 at specificity=0.90. Similar results were achieved when the discrimination was restricted to individuals with HRA from individuals with early-stage CRC (stage I+II) (Endpoint 3a); the analysis included CEA, CyFra21-1, Ferritin and hs-CRP; AUC_{ROC}=0.74 at sensitivity=0.28 at specificity=0.90. Restricting the control group to late-stage CRC (Endpoint 3b), CA19-9, CEA and CyFra21-1 were included in the multivariable analysis; AUC_{ROC}=0.80 at sensitivity=0.39 at specificity=0.90.

The multivariable analysis for the secondary Endpoint included AFP, CEA, CyFra21-1 and hs-CRP; AUC_{ROC}=0.63 with sensitivity=0.17 at specificity=0.90.

The potential use of plasma levels of the eight proteins in prediction of recurrence of colorectal adenomas after endoscopic removal was assessed. The hazard ratios (HR) calculated for individual plasma levels of all eight proteins and adenoma recurrence by multivariate analyses with 10-fold cross-validation were not significant for any of the proteins as shown in Figure 2. Adenoma pathology (LRA/HRA) as a predictor of recurrence was also included, and the presence of HRA showed significant increased HR (HR=3.01–3.11) compared to the presence of LRA (data

Table 3 Results of Multivariate Analyses of Endpoint 1–3 and Secondary Endpoint

	Odds Ratio	Lower CI	Upper CI	p-value	AUC_{ROC}	Sensitivity at 70% Specificity	Sensitivity at 80% Specificity	Sensitivity at 90% Specificity
Endpoint 1								
CEA	1.17	1.05	1.31	0.003	0.63	0.49	0.33	0.19
Hs-CRP	1.07	1.01	1.13	0.020				
Endpoint 2								
Ferritin	0.85	0.76	0.94	0.003	0.61	0.41	0.33	0.13
TIMP-1	1.60	1.11	2.32	0.012				
Endpoint 3								
CA19-9	0.89	0.80	1.00	0.048	0.78	0.70	0.56	0.34
CEA	0.70	0.61	0.80	<0.001				
CyFra21-1	0.60	0.50	0.73	<0.001				
Ferritin	1.34	1.22	1.48	<0.001				
Hs-CRP	0.82	0.75	0.89	<0.001				
TIMP-1	1.94	1.30	2.90	0.001				
Endpoint 3a								
CEA	0.71	0.61	0.83	<0.001	0.74	0.64	0.48	0.28
CyFra21-1	0.73	0.59	0.90	0.003				
Ferritin	1.43	1.28	1.59	<0.001				
Hs-CRP	0.86	0.78	0.95	0.002				
Endpoint 3b								
CA19-9	0.78	0.68	0.89	<0.001	0.80	0.67	0.58	0.39
CEA	0.71	0.60	0.83	<0.001				
CyFra21-1	0.55	0.44	0.67	<0.001				
Secondary Endpoint								
AFP	1.10	1.00	1.21	0.048	0.63	0.50	0.37	0.17
CEA	1.20	1.10	1.31	<0.001				
CyFra21-1	0.88	0.80	0.98	0.018				
Hs-CRP	1.05	1.01	1.10	0.024				

Abbreviations: AFP, alpha-feto protein; CA19-9, cancer antigen 19–9; CEA, carcino embryogenic antigen; CyFra21-1, cytokeratin fragment 21–1; Hs-CRP, high sensitivity C-reactive Protein; TIMP-1, tissue inhibitor of metalloproteinases-1.

not shown). In individuals with colorectal adenoma at baseline (inclusion), nine individuals with HRA and two individuals with LRA were diagnosed with CRC during the follow-up period (Table 2). Among these individuals, plasma levels of the eight proteins investigated in this study were not found significantly altered compared to levels in individuals with colorectal adenomas at baseline without CRC during the follow-up period (data not shown).

Discussion

In the present study, the value of eight plasma proteins was evaluated as individual or combined biomarkers for detection of HRA. Furthermore, plasma levels of the proteins determined at the time of primary diagnosis of colorectal adenoma were evaluated as predictors of adenoma recurrence. The study was spin-off from a previous publication based on the Endoscopy II protocol[31] with the primary aim to evaluate the value of the identical protein panel as biomarkers for early detection of CRC.

In the discrimination of individuals with HRA from individuals with non-malignant findings (including LRA) or clean colorectum, plasma levels of five proteins (AFP, CEA, CyFra21-1, hs-CRP and TIMP-1) were significant with increased plasma levels in individuals with HRA, but the discrimination was weak. The combination of two proteins (CEA and hs-CRP) in a multivariate analysis improved the performance, but the discrimination was still only moderate.

Accuracies of approved tests for CRC screening in discrimination of individuals with advanced adenoma

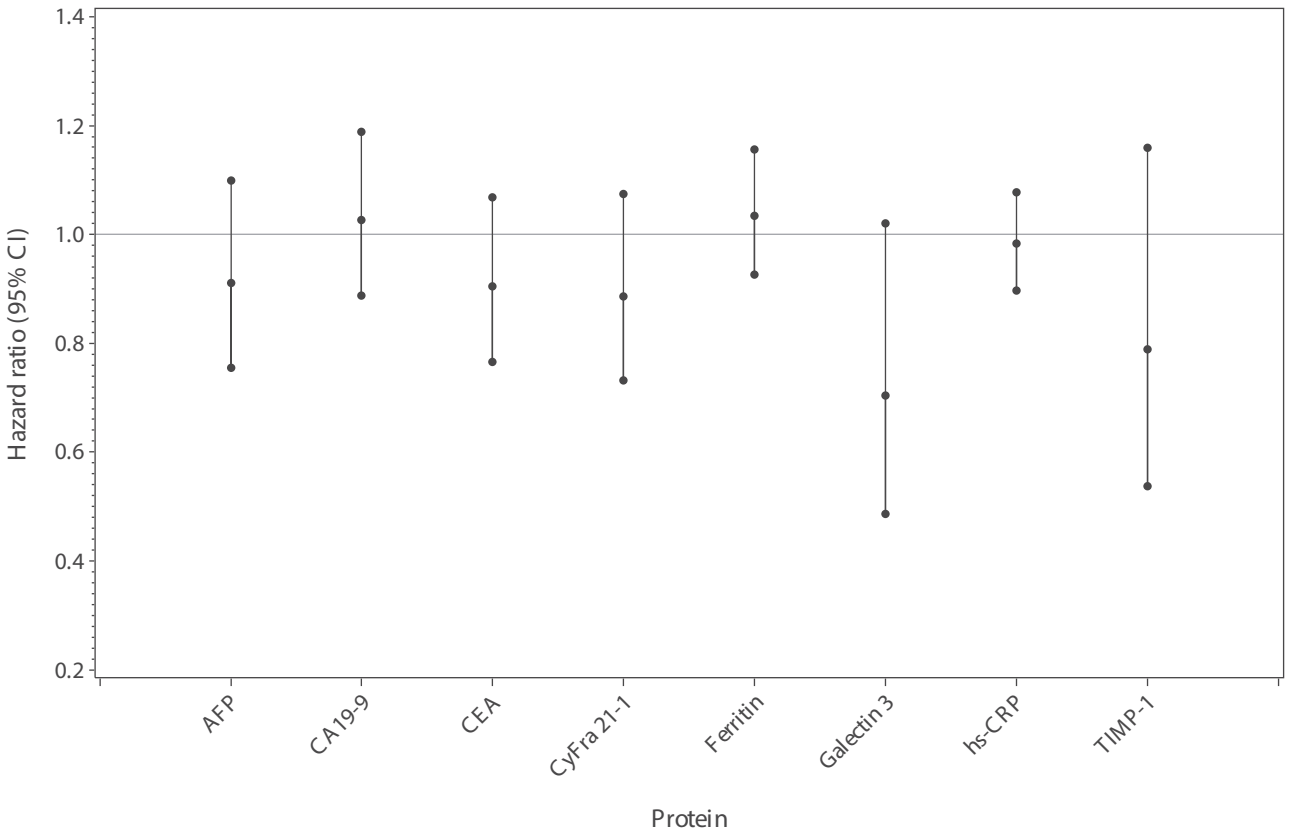

Figure 2 Hazard ratios for individual plasma levels of AFP, CA19-9, CEA, CyFra21-1, Ferritin, Galectin-3, hs-CRP and TIMP-1 and adenoma recurrence.
Abbreviations: AFP, alpha-feto protein; CA19-9, cancer antigen 19–9; CEA, carcino embryogenic antigen; CyFra21-1, cytokeratin fragment 21–1; Hs-CRP, high sensitivity C-reactive protein; TIMP-1, tissue inhibitor of metalloproteinases-1.

from individuals with non-malignant findings or clean colorectum are reported as sensitivities=20-34% at specificities=91-97% for FIT,[11,12] sensitivities=9-14% at specificities=92-95% for gFOBT,[11,12] sensitivities=42-54% at specificity=90% for multitarget stool DNA test[13, 14] and sensitivities=11-21% for mSEPT9 test (specificity not reported).[15,16] A direct comparison between these tests and the results of the present study is not possible due to differences in design and methods. However, it is indicated that the accuracy of the multivariable analysis of the current study in discrimination of individuals with HRA from individuals with non-malignant findings or clean colorectum are comparable to the accuracy of gFOBT and mSEPT9 tests, but are inferior to the accuracy of FIT and multi-target stool DNA test. Overall, it is concluded that the accuracy of the eight proteins evaluated in the study is too moderate to discriminate individuals with HRA from healthy individuals to be valuable in daily routine of screening for HRA.

The three proteins CyFra21-1, Ferritin and TIMP-1, respectively, were shown to statistically discriminate between individuals with HRA and individuals with LRA. However, the discrimination was weak. Plasma levels of Ferritin were increased in individuals with HRA, and levels of CyFra21-1 and TIMP-1 were decreased. Combination of two of the proteins (Ferritin and TIMP-1) in a multivariable analysis improved the discrimination slightly. In conclusion, plasma levels of the investigated proteins (individually or in combination) are not valuable as biomarkers for identification of individuals with HRA in a population with colorectal adenoma.

Furthermore, the performance in discrimination of individuals with colorectal adenomas from individuals with other non-malignant findings or clean colorectum at colonoscopy, whilst statistically significant, was not strong enough to be valuable as biomarkers, and the use in daily routines is therefore limited.

When evaluated individually, all proteins except AFP were found to significantly discriminate individuals with HRA from individuals with CRC (all stages) with a moderate discrimination. Similar results were obtained when the discrimination was restricted to individuals with

HRA from individuals with late-stage CRC, but the discrimination was improved. In the discrimination of individuals with HRA from individuals with early-stage CRC, TIMP-1 and AFP were not shown as valuable biomarkers. The discrimination using the remaining proteins was moderate and similar to the discrimination of individuals with HRA from individuals with CRC (all stages).

Plasma levels of six out the seven significant proteins (CA19-9, CEA, CyFra21-1, Galectin-3, hs-CRP, TIMP-1) were increased in individuals with CRC compared to individuals with HRA with the exception of Ferritin where plasma levels were decreased in individuals with CRC. The increased plasma levels could be explained by the positive correlation of the six proteins with colorectal neoplasia previously described in the literature where increasing levels during disease progression have been observed (Table 1). The interaction between plasma levels of Ferritin and colorectal neoplasia is, however, more complex. Even though possible positive correlations between levels of Ferritin and cancer-specific processes (including CRC) have been shown, iron deficiency anemia caused by chronic gastrointestinal bleeding might antagonize this effect.[41,42] The progression in colorectal neoplasia from HRA to CRC might cause increased gastrointestinal bleeding, which could explain why levels of Ferritin are decreased in individuals with CRC compared to individuals with HRA in the present study.

The combination of six significant proteins (CA19-9, CEA, CyFra21-1, Ferritin, hs-CRP and TIMP-1) in a multivariable analysis improved the discrimination of individuals with HRA from individuals with CRC (all stages). Restricting the control group to early-stage CRC, two proteins lost significance (CA19-9 and TIMP-1), and four proteins were included in the model (CEA, CyFra21-1, Ferritin and hs-CRP). Restricting the control group to late-stage CRC, three proteins lost significance compared to the discrimination from all stages (Ferritin, hs-CRP and TIMP-1), and three proteins (CA19-9, CEA and CyFra21-1) were included in the model.

The discrimination of individuals with HRA by a single significant protein or proteins in combination improved when the control group was restricted to individuals with late-stage CRC compared to individuals with early-stage CRC or all stages of CRC. This might also be explained by the positive correlation between the proteins and CRC as previously described (Table 1). Consequently, a potential difference in plasma levels of the proteins would be more pronounced as the carcinogenesis of CRC progresses which would improve the discrimination as observed in the present study.

However, the overall performance of the multivariable analyses in discriminating individuals with HRA from individuals with CRC in the present study, although the performance is promising, still needs improvements to be valuable as a screening test in daily routines.

The assessment of identifying individuals at risk of recurrence of colorectal adenoma based on plasma levels of the eight proteins at the time of diagnosis of the primary adenoma did not reach significance for any of the proteins. HRs for all eight proteins of this study are shown in Figure 2. The power of this analysis was sufficient to detect any clinically relevant differences, suggesting that there is no association between the plasma levels of the eight proteins and the probability of recurrence of colorectal adenoma. Similar evaluation of plasma levels of the specific proteins as predictors of adenoma recurrence has not yet been published.

The plasma proteins investigated in this study are not specific for colorectal adenoma or CRC, and altered expression in the circulation of the eight proteins can be observed in various biological processes including inflammation or carcinogenesis in other organs. The differential panel of the plasma proteins discriminating HRA from CRC and HRA from the remaining subjects suggests the possibility of developing a reflex algorithm, improving the identification of these outcomes. There is however a need for better and more specific biomarkers, in particular, for the separation of HRA and LRA.

Conclusion

In conclusion, combination of plasma levels of two (CEA and hs-CRP) of the eight proteins investigated in the present study shows a promising potential in detection of primary HRA (discrimination of individuals with HRA versus healthy individuals plus individuals with LRA). However, to be recommended and implemented as a screening test for HRA in daily routines, the achieved results by combination of biomarkers need improvements. Similarly, the detection of colorectal adenomas in general demonstrated potential, but the results are too limited to be implemented in clinical practice. Finally, plasma levels of the eight proteins could not significantly predict recurrence of colorectal adenomas.

Future perspectives to improve performance in detection colorectal adenomas, specifically HRA, may include combination of the protein panel of the present study with other experimental or established biomarkers (proteins or genetic/epigenetic markers) which may result in a high-performing model for a blood-based screening test.

Abbreviations

AFP, alpha-feto protein; AUC, area under the ROC curve; CA19-9, cancer antigen 19-9; CEA, carcino embryogenic antigen; CRC, colorectal cancer; CyFra21-1, cytokeratin Fragment 21-1; ELISA, enzyme-linked immunosorbent assay; HR, hazard ratio; HRA, high-risk adenoma; Hs-CRP, high sensitivity C-reactive protein; LRA, low-risk adenoma; OR, odds ratio; ROC, receiver operating characteristic; TIMP-1, tissue inhibitor of metalloproteinases-1.

Ethics Approval and Informed Consent

The Ethics Committee of the Capital Region of Denmark (H-3-2009-110) and the Danish Data Protection Agency (2007-58-0015) approved the study, which was performed according to the Declaration of Helsinki II. Informed consent was signed by all participating individuals on the day of the colonoscopy.

Data Sharing Statement

All data can be presented by contacting corresponding author.

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