Serum based multiplex protein assay for early detection of colorectal cancer and precancerous lesions in a FIT positive population.

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Introduction: Colorectal cancer (CRC) is the second leading cancer worldwide in terms of incidence, 5-year prevalence and mortality for both women and men ages 45 years old and up. The current screening method for many countries with organized screening programs is the fecal immunochemical test (FIT) for fecal occult blood; however, this test can result in false positive rates as high as 65%. A FIT reflex test could reduce unnecessary colonoscopies while reducing wait times for those patients that need confirmatory colonoscopies the most.

Methods: Danish FIT positive colonoscopy confirmed serum samples (n = 1,981) were divided into training and validation sets (Fig. 1) maintaining approximately equivalent percentages of 40% clean colonoscopy, 16% low risk adenomas (LRA), 19% medium risk adenomas (MRA), 13% high risk adenomas (HRA), 5% stage I CRC, 2% stage II CRC, 4% stage III CRC, and 0.5% stage IV CRC. Proteins were quantified by a custom 16-plex immunoassay utilizing the Luminex xMAP® platform. Univariate analysis and support vector machines (SVM) supervised machine learning (ML) algorithms were utilized.

Results: Univariate analysis was performed on each of the 16 biomarkers tests (Table 1). Five biomarkers were selected for ML modeling. An SVM algorithm was trained with 5 biomarkers plus age and FIT concentration using 1,317 samples for the outcome MRA, HRA, and CRC versus LRA and clean colonoscopy. Then this algorithm was tested on a blind 664 sample validation set (Fig. 2 & Table 2). The performance of the SVM model was consistent between the training set and validation set (Fig. 2 & Table 2).

Conclusions: This study demonstrates feasibility of a novel blood-based multiplex protein immunoassay for use as a reflex to FIT positive results in population wide screening. It detected nearly all adenomas and carcinomas while reducing FIT false positives and thus unnecessary colonoscopies by more than 20%. A FIT reflex test could alleviate endoscopy burden experienced in countries with organized cancer screening programs, while providing better patient outcomes by detecting polyps and early-stage CRC with high sensitivity.

Table 1 Univariate Analysis of 16 biomarkers, outcome MRA, HRA, & CRC

Marker	Odds Ratio	OR Lower cl	OR Upper cl	P-value	AUC	Sensitivity at 30% specificity	Sensitivity at 70% specificity	Sensitivity at 80% specificity	Sensitivity at 90% specificity
AFP	1.01	0.93	1.11	0.7687	0.50	0.68	0.34	0.23	0.10
Cathepsin-D	1.31	1.12	1.53	0.0006	0.56	0.80	0.36	0.22	0.11
CD44	1.01	0.80	1.27	0.9331	0.49	0.67	0.29	0.19	0.11
CEA	1.14	1.03	1.26	0.0101	0.54	0.77	0.34	0.22	0.12
Ferritin	0.97	0.90	1.05	0.4911	0.51	0.71	0.32	0.23	0.13
GDF-15	1.35	1.16	1.57	0.0001	0.58	0.84	0.38	0.23	0.09
Hepsin	1.01	0.89	1.16	0.8301	0.49	0.70	0.28	0.17	0.09
IL-8	1.26	1.12	1.42	0.0002	0.56	0.77	0.38	0.26	0.15
Keratin 1/10	0.99	0.92	1.05	0.6812	0.52	0.73	0.31	0.19	0.08
L1CAM	0.95	0.82	1.1	0.5154	0.51	0.72	0.30	0.22	0.11
MIA	1.09	0.82	1.46	0.5382	0.50	0.72	0.29	0.21	0.12
MIDKINE	1.24	1.04	1.47	0.0147	0.54	0.75	0.38	0.22	0.11
NSE	1.1	0.93	1.29	0.2544	0.53	0.75	0.33	0.23	0.11
Osteonectin	1.24	1.01	1.51	0.0367	0.53	0.74	0.33	0.22	0.14
TWEAK	1.01	0.80	1.26	0.9543	0.50	0.70	0.31	0.21	0.11
YKL-40	1.11	1.01	1.22	0.0327	0.54	0.76	0.34	0.21	0.11

Fig. 1 Hvidovre Hospital ENDO III Cohort.

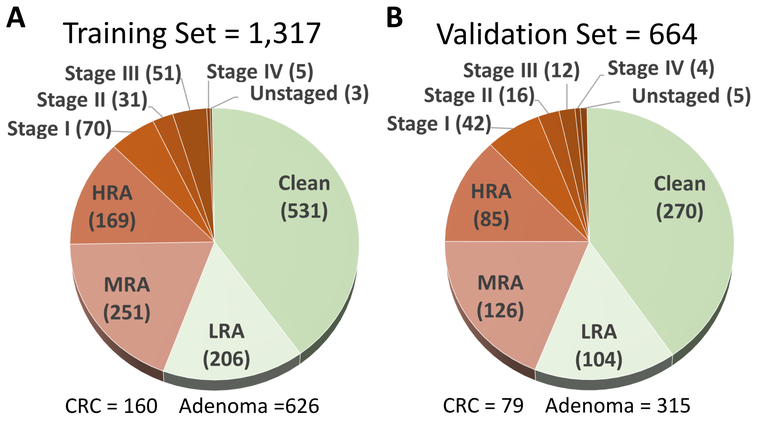


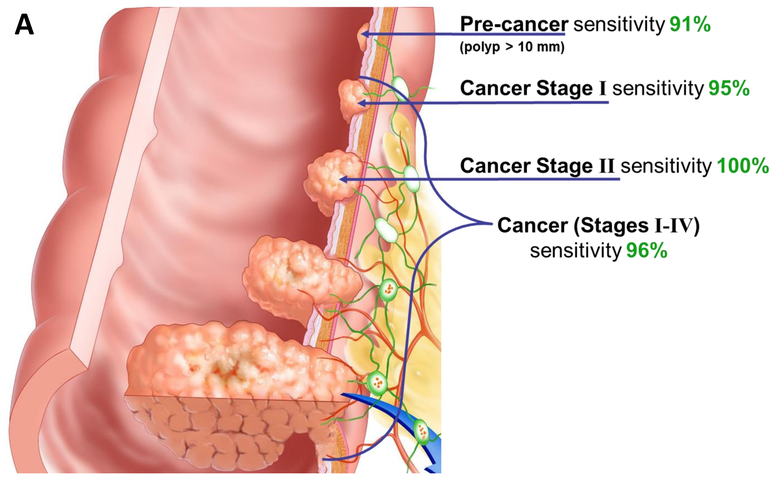
Fig. 2 SVM ROC curves.

Table 2 SVM 5 biomarkers plus age & FIT, Outcome MRA, HRA & CRC

Dataset	P-value	N	AUC	Sensitivity at 20% specificity	Sensitivity at 30% specificity	Sensitivity at 50% specificity	Sensitivity at 70% specificity	Sensitivity at 80% specificity	Sensitivity at 90% specificity
Training	<0.0001	1317	0.673	0.93	0.88	0.71	0.54	0.42	0.32
Validation	<0.0001	664	0.686	0.93	0.88	0.74	0.57	0.47	0.35

Fig. 3 SVM 5 biomarkers plus age & FIT sensitivity by stage.

A



B

CRC	Pos	Neg	TPR	
Unstaged	5	0	100%	96%
IV	4	0	100%	
III	11	1	92%	
II	16	0	100%	
I	40	2	95%	

C

Adenoma	Pos	Neg	TPR	
HRA	81	4	95%	91%
MRA	112	14	89%	

D

Low Risk	Pos	Neg	FPR	
LRA	88	16	85%	80%
Clean	211	59	78%	