Novel blood-based protein biomarker signature for early detection of colorectal cancer and advanced adenomas

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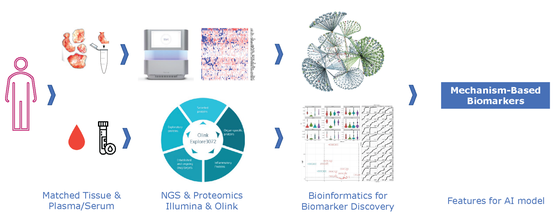
INTRODUCTION

- Colorectal cancer (CRC) is the second-leading cause of cancer-related mortality in Europe and the US
- Early detection boosts the 5-year relative survival rate to 91% (*American Cancer Society*, 2024)
- Colonoscopy remains the gold standard for screening, however, its invasiveness reduces adherence
- Less invasive stool- and blood-based biomarker strategies are needed to enhance accuracy, particularly for early-stage CRC and advanced adenomas (AA)

AIM: To discover novel blood-based protein biomarkers that are directly related to the CRC mechanism and can lead to the development of diagnostic tools for early detection of the disease.

METHODS



Analysis Pipeline

Initial Exploratory Analysis
Clustering, Covariate Analysis, Blood proteomics data of 296 participants

Biomarker Discovery
1. Differential protein expression analysis
2. Important predictors in Machine Lerning modeling (e.g. Elastic Net)
3. ROC analysis of single protein biomarkers

Biomarker Prioritization
1. Limit of Detection Filtering
2. Filtering using Normal ranges (IQR) in UKBiobank
3. Significant trend in clinical groups

Biomarker Evaluation
1. Optimal combination of biomarkers
2. Final evaluation of panel of protein biomarkers

RESULTS

CRC: Colorectal cancer, **AA**: Advanced Adenoma, **NAA**: Non advanced adenoma, **Healthy**: Participants with hyperplastic polyps or no findings, **Normal**: Healthy + NAA, **DEPs**: Differentially Expressed Proteins

- 212 unique DEPs uncovered as candidate biomarkers (Fig.1)
- 64 DEPs are common to CRC and AA versus Healthy
- Healthy + NAA (Normal) vs CRC yields 55 DEPs
- Biomarkers passing the UK Biobank criterion (Fig.2):
  - CRC: 84 ↑ / 30 ↓
  - AA: 138 ↑ / 55 ↓
- Top 21 markers show only weak to moderate correlation, supporting their independent diagnostic value (Fig.3)
- 12-plex combination outperforms single markers (Fig.4)
- 12-plex achieves CRC 98.1 % sensitivity (95 % CI 90.1–100), AA 50.0 % (37.0–63.0), and 93.1 % specificity for non-neoplastic (77.2–99.2) (Table 2)
- Probability distributions clearly separate CRC from Healthy/NAA (Fig.5)

Table 1. Discovery cohort characteristics

Group	# of Participants	Age (median)	Age (IQR)	Sex (%)
CRC	65	65.0	15.0	Female: 45.4 Male: 54.6
AA	106	65.0	13.0	Female: 40.9 Male: 59.1
NAA	50	63.5	10.8	Female: 47.6 Male: 52.4
Healthy	75	56.5	17.3	Female: 54.1 Male: 45.9

Table 2. Performance metrics of the 12-plex assay for different categories of CRC and AA, calculated considering disease state (CRC, AA, sub-categories) as true positives, and Normal samples as true negatives.

Comparison	AUC (%)	Sensitivity (%)	Sensitivity 95% CI	Specificity (%)	Specificity 95% CI	Accuracy (%)	Accuracy 95% CI
CRC vs Normal	95.9	98.1	90.1-100.0	93.1	77.2-99.2	96.4	89.8-99.2
SI vs Normal	96.9	100.0	71.5-100.0	93.1	77.2-99.2	95.0	83.1-99.4
SII vs Normal	97.6	100.0	75.3-100.0	93.1	77.2-99.2	95.2	83.8-99.4
SI/SII vs Normal	97.3	100.0	85.8-100.0	93.1	77.2-99.2	96.2	87.0-99.5
AA vs Normal	78.0	50.0	37.0-63.0	93.1	77.2-99.2	63.7	53.0-73.6
HGD vs Normal	74.7	43.5	23.2-65.5	93.1	77.2-99.2	71.2	56.9-82.9
CIS vs Normal	66.4	50.0	06.8-93.2	93.1	77.2-99.2	87.9	71.8-96.6
AA10 vs Normal	85.8	61.1	35.7-82.7	93.1	77.2-99.2	80.9	66.7-90.9
V vs Normal	74.1	50.0	21.1-78.9	93.1	77.2-99.2	80.5	65.1-91.2

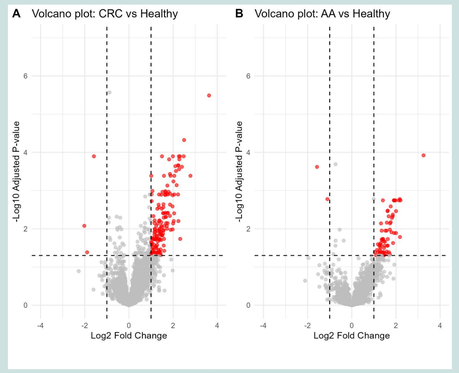


Figure 1. Volcano plots of differential protein expression. Dots = Candidate proteins. Red: DEPs with |log2FC|>1 and adj. p-value<0.05; Grey: not significant; Horizontal line: Statistical threshold; Vertical lines: $|\log_2 FC| = 1$; X-axis: $\log_2 FC$ values; Y-axis: Adjusted p-values in logarithmic scale.

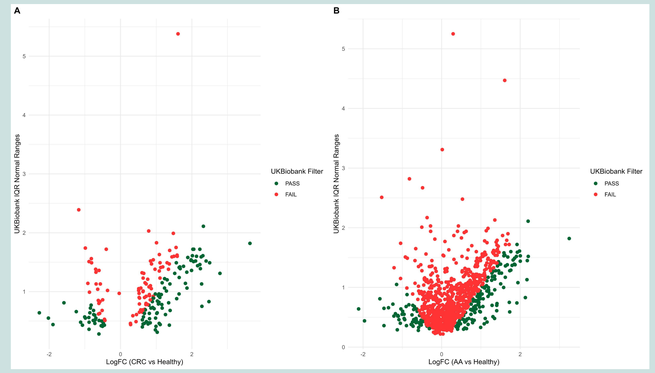


Figure 2. Scatter plots illustrating the association of CRC vs Healthy (A) and AA vs Healthy (B) biological effect size with the expected interquartile ranges from the UKBiobank normal cohort. Each dot represents a candidate marker, while different colors are connected if they passed the criterion (green: pass; red: fail). X-axis: log2FC values; Y-axis: Interquartile Normal Ranges in UKBiobank.

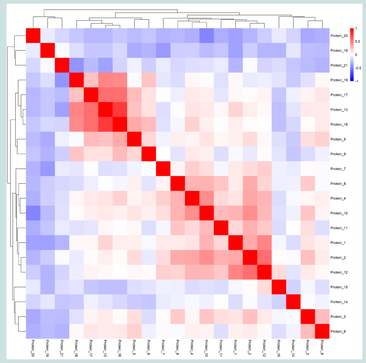


Figure 3. Heatmap of Pearson r among 21 early-detection biomarkers. Red = +1, blue = –1; hierarchical clustering groups correlated proteins.

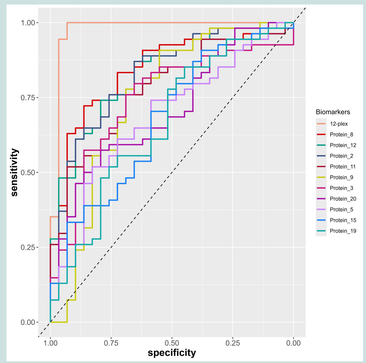


Figure 4. ROC curves: 12-plex model vs top-10 single markers for CRC detection (CRC = TP, Normal = TN). Diagonal dashed line = random classifier.

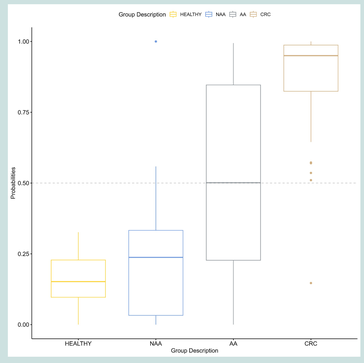


Figure 5. Elastic Net probability box-plots for Healthy, NAA, AA and CRC. Dashed line = cut-off 0.5 (> 0.5 = positive).

CONCLUSIONS

This protein biomarker signature could pave the way for more accurate, non-invasive CRC screening methods that detect both cancer and precancerous lesions at earlier, more treatable stages.

Future work is focused on developing multiplex immunoassays for these biomarkers to orthogonally validate the findings and assess the signature's analytical and clinical performance in a large participant cohort from the DIOPTRA validation studies (N=1600 participants).

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