

Blood based epigenetic profiling of circulating cell free nucleosomes (cf-nucleosomes) in symptomatic individuals with Advanced Colorectal Adenomas – A novel approach to reducing the impact of Colorectal Cancer.



Marielle Herzog, Dorian Pamart, Brieuc Cuvelier, Eléonore Josseaux, Mark Eccleston and Jake Micallef (Volition, Namur, Belgium). Ib Jarle Christensen and Hans J. Nielsen (Hvidovre University, Denmark)

INTRODUCTION

Colorectal cancers (CRC) develop via precancerous adenomas and removal of high risk adenomas is impacting CRC incidence where screening is offered¹. However, limited compliance for colonoscopy and poor detection rates for adenomas with alternate screening approaches, predominantly stool testing in Europe, limit the potential for further reductions.

Blood based testing may improve screening compliance² but recently approved, Septin 9 only detects 11% of advanced adenomas (at 91.5% specificity)³. It also performs better in later stage cancers. Here we demonstrate the potential for cf-nucleosomes to identify high risk polyps.

HYPOTHESIS

A significant amount of circulating cell free DNA exists as mono or oligomeric nucleosomes and contains the same genetic mutations as matched cancer tissue⁴ suggesting a tumor origin for at least some circulating nucleosomes.

Volition has developed a novel, immunoassay platform Nucleosomic[®], to identify clinically distinct epigenetic profiles of cell free nucleosomes in blood from patients with colorectal cancers compared to healthy patients.

As epigenetic signals are altered early in tumorigenesis⁵ we hypothesized that epimutations may be present within precancerous lesions.

Methods

Volition have developed ELISAs (NuQ[®]) for specific epigenetic features of circulating cf-nucleosomes including:

- NuQ[®]-X specific DNA modifications
- NuQ[®]-V histone variants
- NuQ[®]-M histone modifications
- NuQ[®]-A nucleosome-protein adducts
- NuQ[®]-T total nucleosomes

Global levels of 18 cf-nucleosome structures were measured in serum samples (10µl in duplicate) collected from 530 patients with symptoms of colorectal diseases (Hvidovre Hospital, Denmark) using specific ELISAs comprising a coated capture antibody against a conserved nucleosome epitope and various biotinylated profiling antibodies

Samples were collected prior to colonoscopy verification of disease status Patients were classified into three groups based on colonoscopy:

Diagnosis	No of patients	Mean Age (range)	Male:Female
Adenoma	246	64.8 (24.0 to 88.8)	133:113
High Risk	172	65.7 (24.0 to 88.5)	93:79
Low Risk	74	62.7 (27.2 to 88.8)	40:34
CRC	98	70.2 (42.1 to 91.8)	51:47
Stage I	49	69.3 (49.2 to 91.8)	27:22
Stage II	48	80.0 (42.1 to 88.4)	24:24
No Evidence of Disease	186	52.3 (21.0 to 91.3)	72:114

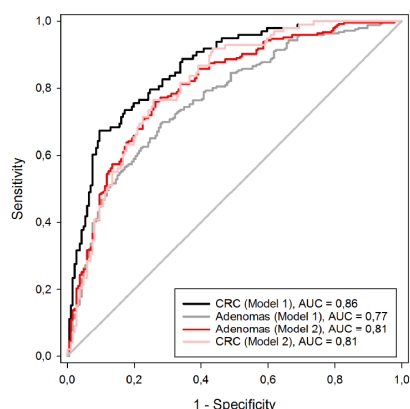
Linear models based on a weighted sum of one to 5 variables, were developed using Linear Discriminant Analysis (LDA) optimised for the best AUC for CRC vs NED (Model 1) or Adenoma vs NED (Model 2)

Results

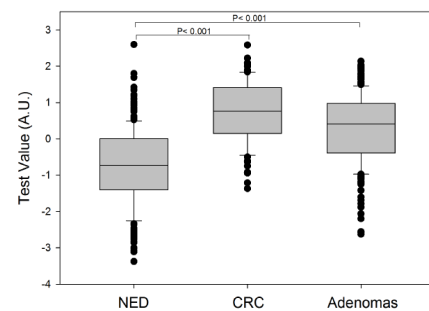
Table: Percentage sensitivity at 80% specificity for adenoma and cancer stages

Diagnosis	Model 1 (CRC trained)	Model 2 (Adenoma trained)
Adenoma	59	66
High risk	64	67
Low risk	47	62
CRC	76	65
Stage I	78	63
Stage II	73	67

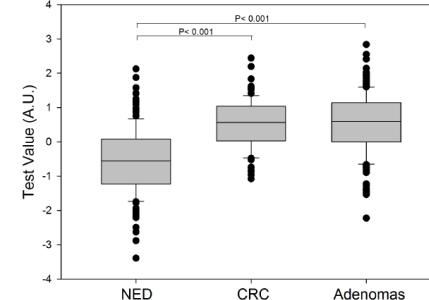
ROC curve for discrimination of CRC vs NED or Adenoma vs NED from a panel optimised for CRC vs NED (Model 1) or Adenomas vs NED (Model 2)



Box Plot : Discrimination of 4 normalised NuQ[®] assays in age-adjusted algorithm optimised for CRC vs No Evidence of Disease (NED) (Model 1)



Box Plot : Discrimination of 4 normalised NuQ[®] assays in age-adjusted algorithm optimised for Adenomas vs No Evidence of Disease (Model 2)



CONCLUSION

Serum profiles of epigenetically altered circulating nucleosomes measured by ELISA can be used to detect precancerous bowel lesions and early stage CRC in a simple NuQ[®] blood test.

Blood based epigenetic nucleosome assays could improve patient compliance for screening and accuracy in the early detection of individuals with high risk Colorectal adenomas as candidates for early surgical or therapeutic intervention.

Multivariate analysis shows significantly increased sensitivity and specificity compared to univariate analysis of circulating cell free nucleosome based biomarkers with potential to optimise either for Advanced Adenoma or early stage cancer detection.

References

- 1) Holme Ø et al. Flexible sigmoidoscopy versus faecal occult blood testing for colorectal cancer screening in asymptomatic individuals. *Cochrane Database Syst Rev* 2013;9:CD009259
- 2) Adler A et al. Improving compliance to colorectal cancer screening using blood and stool based tests in patients refusing screening colonoscopy in Germany. *BMC Gastroenterology* 2014;14:183-191
- 3) Church TR et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 2014;63:317-325.
- 4) Bazan V et al. Molecular detection of TP53, KRAS and p16 promoter methylation in serum of patients with colorectal cancer and its association with prognosis. Results of a 3 years GOIM (gruppo oncologico dell'Italia meridionale) prospective study. *Ann. Oncol.* 2006;17:784-790.
- 5) Jones AP & Baylin SB. The fundamental role of epigenetic events in cancer. *Nature Reviews Genetics* 3, 2012; Jun: 415-428