DETECTION OF COLORECTAL CANCER AND ADENOMAS BY EPIGENETIC PROFILES OF CIRCULATING NUCLEOSOMES: A PILOT STUDY WITH 58 SUBJECTS

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SUMMARY

BACKGROUND:
Immunohistochemistry studies show genome-wide epigenetic changes in the chromatin of cancer tissue and have identified histo-oncoproteins - histone modifications and other epigenetic changes linked to cancer. In cancer patients, ctDNA circulates as nucleosome fragments of tumor chromatin consisting of short, less than 200 base pair, DNA sequences wrapped around four pairs of histone proteins. Nucleosome bound DNA fragments contain mutations found in cancer tissue suggesting a tumor chromatin origin for at least some circulating nucleosomes. Profiling of global levels of epigenetic alterations in circulating nucleosomes can provide disease specific diagnostic information.

MATERIAL and METHODS:
VolitionRx has developed serum ELISA assays that measure circulating nucleosomes containing specific epigenetic signals and used these to investigate global epigenetic profiles in colorectal cancer (CRC) and adenomas patients. The assays employ one antibody targeted to bind to a common nucleosome epitope and a second antibody targeted to bind to the epigenetic structure of interest.

Twelve circulating cfnucleosomes structures were measured in serum samples collected from 58 symptomatic subjects referred for a colonoscopy at the Academic Hospital, CHU UCL Namur (Belgium), using specific ELISA assays (NuQ®, Belgian Volition SA) and analysed using univariate and multivariate approaches. Linear models, based on a weighted sum of one to five variables were developed using Fisher's linear regression (LDA) optimised for the best Area Under the Curve

CONCLUSIONS:
Serum profiles of epigenetically altered circulating nucleosomes measured by ELISA can be used to detect CRC including early stage and precancerous bowel lesions in a simple blood test. Epigenetic nucleosome assays have the potential for improved patient compliance and accuracy in the early detection of CRC. Further studies in larger patients cohorts are warranted to validate the usefulness of these NuQ® biomarkers in CRC early diagnosis.

Conflict of interest:
MH, EJ, KS are employees of Belgian Volition SA.
JM is consultant of Belgian Volition SA.
MH, EJ, KS, JM have a financial interest in Belgian Volition SA.

EXAMPLE VOLITIONRX NUQ® ELISA

VolitionRx has developed five patent-protected families of NuQ® sandwich ELISA assays, each of which captures intact nucleosomes and labels (identifies) a specific structural feature:

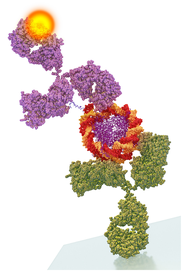
NuQ®-X specific DNA modifications

NuQ®-V histone variants

NuQ®-M histone modifications

NuQ®-A nucleosome-protein adducts

NuQ®-T total nucleosomes



Using a novel ELISA platform - Nucleosomics®, we evaluated 12 specific epigenetic features of circulating nucleosomes as potential blood-based biomarkers for colorectal cancer.

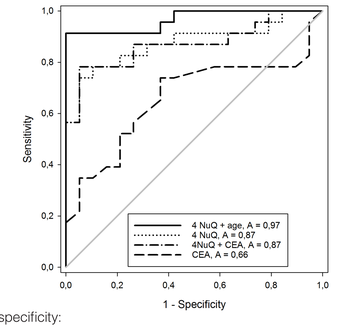
DEMOGRAPHIC OF THE STUDY GROUP

The study comprised 58 individuals above 50 years old at high risk, or displaying symptoms of colorectal cancer (CRC).
Patients were classified into three groups based on their coloscopy results: CRC patients, patients with colorectal polyps, healthy controls with normal epithelium.

Diagnosis	No. of patient	Mean Age (range)	Male:Female
CRC	23	76 (52-88)	16:7
stage 0-I	4	71 (62-84)	3:1
stage II	7	78 (70-84)	4:3
stage III	7	76 (52-88)	6:1
stage IV	4	80 (75-83)	3:1
unknown	1	64	0:1
Polyp	16	64 (50-88)	10:6
hyperplastic	6	59 (50-68)	1:5
dysplastic	10	65 (53-82)	9:1
Healthy	19	61 (50-71)	11:9

DETECTION OF UP TO 91% OF CRC CASES

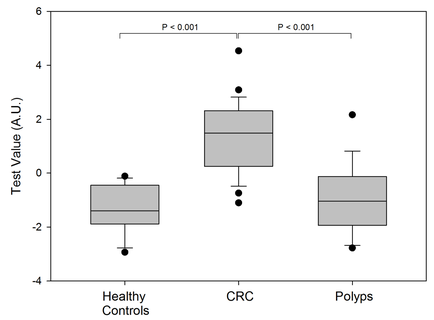
We evaluated the cumulative performance of cfnucleosome biomarkers alone, in combination with CEA and adjusted for age using a multivariate analysis.



At 90% of specificity:
The tumor marker CEA gave a sensitivity of **35%**
Combination of 4 cfnucleosome increased the sensitivity to **74%**
Age-adjusted cfnucleosome biomarker panel increased the sensitivity to **91%**

DISCRIMINATION COLORECTAL CANCER VS HEALTHY CONTROLS AND VS POLYP GROUP

Box Pot: Discrimination of four NuQ® assays in age-adjusted algorithm for CRC, polyps and healthy controls.



The score was significantly higher (p<0.001) in serum of patients with colorectal cancer compared to healthy controls and to the polyp group.

DETECTION OF EARLY STAGE CANCER

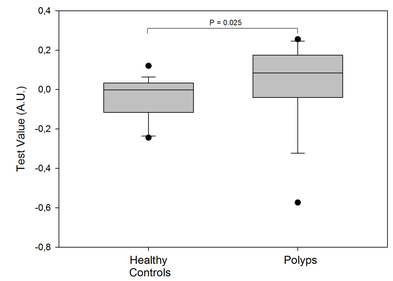
Table: Percentage of sensitivity at 90% specificity at the different CRC cancer stages for the established tumor marker Carcinoembryonic antigen (CEA) anf the cfnuclesomes biomarkers.

	% sensitivity at 90% specificity		
CRC	CEA	Combination of 4 NuQ® assays	Combination of 4 NuQ® assays age-ajusted
All stages	35	74	91
Stage I	0	75	75
Stage II	14	86	86
Stage III	17	71	100
Stage IV	75	50	100

The single biomarker CEA showed a relatively good sensitivity in stage IV but performed poorly in the earliest stages. Conversely, the 4 cfnucleosomes showed markedly increased sensitivity across all stages of CRC.

DETECTION OF 62% OF ADENOMA CASES

Box Plot: Discrimination of four NuQ® assays for patients with colorectal polyps and healthy controls.



A second algorithm, optimised for the discrimination between the polyp group and the healthy controls was developped. A combination of 4 cfnucleosome biomarkers significantly improved the discriminatiom of polyp vs healthy (p = 0.025).