UNITED EUROPEAN GASTROENTEROLOGY ueg week

Flinders Centre for Innovation in Cancer

ClinicalGenomics

Amsterdam UMC University Medical Centers

UNIVERSITY OF COPENHAGEN

2019 October 19–23 Barcelona, Spain

Performance Evaluation of a Blood Test Assaying for ctDNA Methylated in IRF4, BCAT1, IKZF1 for Detection of Colorectal Cancer

Susanne K Pedersen[1], Nicky Boulter[1], Gregor Tevz[1], Betty Yu[1], Michelle Chan[1], Hans J. Nielsen[2], Linnea Ferm[2], Ib J. Christensen[2], Evelien Dekker[3], Erin L Symonds[4,5], Graeme P Young[4], Lawrence LaPointe [1,4]

[1] Clinical Genomics Pty Ltd, Sydney, Australia, [2] Dept. of Surgical Gastroenterology, Hvidovre Hospital, University of Copenhagen, Hvidovre, Denmark, [3] Dept. of Gastroenterology & Hepatology, Amsterdam University Medical Centers, AMC, The Netherlands, [4]Flinders Centre for Innovation in Cancer, College of Medicine and Public Health, Flinders University of South Australia, Australia, [5] Bowel Health Service, Flinders Medical Centre, South Australia, Australia

Background & Aim

Blood-based testing for circulating tumor DNA (ctDNA) shows promise in screening for colorectal cancer (CRC). There is, however, a need to improve detection of early (asymptomatic) CRC to achieve clinical utility for population screening. The aim of this study was to determine the performance of a 3-gene ctDNA methylation assay in a study population with the full range of neoplastic and non-neoplastic pathologies encountered in the colon and rectum.

Methods

Test method: DNA was extracted from plasma, bisulphite converted and assayed using a multiplex, methylation-specific real-time PCR assay for detection of methylated *BCAT1*, *IKZF1* and *IRF4* DNA. The PCR assay also included an ACTB component used for QC purposes (Fig 1). Detection of any of the three methylation markers deemed a specimen positive for ctDNA. Testing was done by staff blinded to clinical diagnosis.

Testing cohort: Banked specimens sourced through observational, cross-sectional trials collecting blood either prior to a scheduled colonoscopy for standard clinical indications or prior to colonic surgery for primary CRC. Participating centres were located in Denmark (DK, Hvidovre Hospital), Australia (AU, Flinders), The Netherlands (NL, Amsterdam Medical Center) and Russia (RU, Proteogenex), Figure 1. Specimen selection was based on complete clinical and demographic data and plasma availability: 1,621 adults (median age, 64.2y (18-88y), 56% males).

Case classification was based on colonoscopy findings. CRC was staged according to AJCC 7th Edition. High-risk adenoma (HRA): size ≥20mm or ≥5 lesions, or ≥1 lesion(s) removed by piece-meal technique. Medium-risk adenoma (MRA): ≥10mm but < 20mm or ≥ 3 (but <5) lesions or villous histology or high-grade dysplasia. Low-risk adenoma (LRA): <10mm or < 3 lesions or tubular histology or low-grade dysplasia).

Figure 1. Test Cohort Details

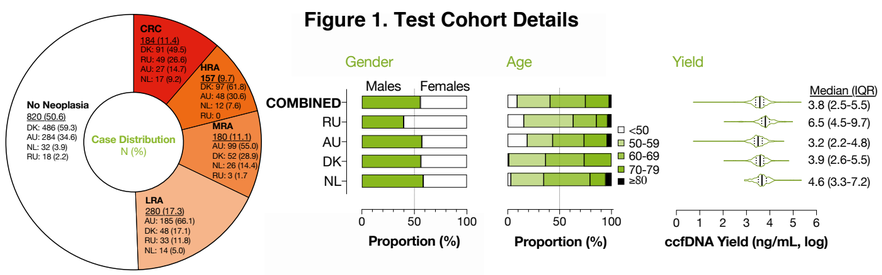
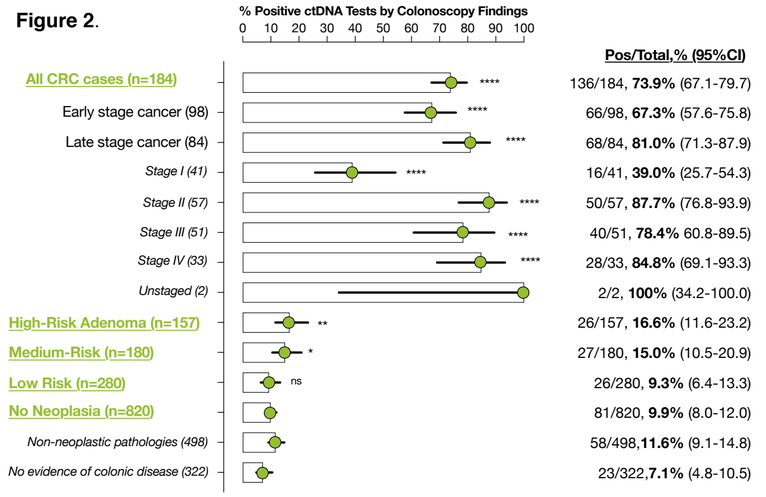


Figure 2.

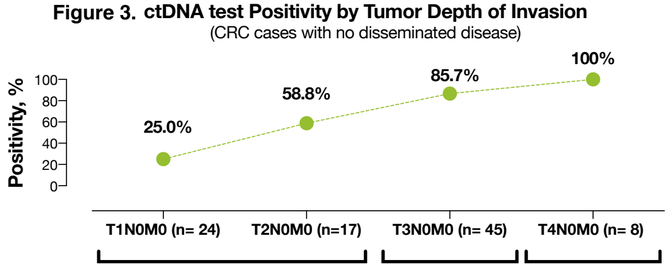


*Z-score two population test using No Neoplasia as Reference. ns: non-significant, *p<0.05, **p≤0.01, **** p≤0.0001.*

Table 1

		Total	Number of Positives/Total (%) *BCAT1*	*IKZF1*	*IRF4*
Colonscopy Finding					
Cancer		**184**	**87 (47.3)**	**109 (59.2)**	**92 (50.0)**
	Stage I	41	8 (19.5)	11 (26.8)	7 (17.1)
	Stage II	57	30 (52.6)	40 (70.2)	33 (57.9)
	Stage III	51	23 (45.1)	33 (64.7)	28 (54.9)
	Stage IV	33	25 (75.8)	23 (69.7)	22 (66.7)
	Unstaged	2	1 (50.0)	2 (100)	2 (100)
Adenoma	**High Risk**	**157**	**13 (8.3)**	**14 (8.9)**	**10 (6.4)**
	Medium Risk	180	16 (8.9)	15 (8.3)	10 (5.6)
	Low Risk	280	13 (4.6)	12 (4.3)	8 (2.9)
No Neoplasia		**820**	**44 (5.4)**	**35 (4.3)**	**18 (2.2)**
Non-neoplastic pathologies		498	32 (6.4)	25 (5.0)	16 (3.2)
No evidence of disease		322	12 (3.7)	10 (3.1)	2 (0.6)

Figure 3. ctDNA test Positivity by Tumor Depth of Invasion
(CRC cases with no disseminated disease)



Results

Figure 2 shows the positivity rates for the 3-gene methylation ctDNA assay by colonoscopy findings. Table 1 summarizes the positivity rates for each of the 3 markers.

Overall sensitivity for CRC: 73.9% (136/184, 95%CI: 67.1-79.1) with a better detection of later, 81.0%, (68/84; 71.3-87.9) versus earlier stage cancer, 67.3% (66/98; 57.6-75.8; p=0.0375).

Stage-dependent sensitivity for stage II, III and IV were 87.7% (50/57; 76.8-93.9), 78.4% (40/51; 60.8-89.5) and 84.8% (28/33; 69.1-93.3), respectively, and were not significantly different. However, the test had a reduced sensitivity for stage I (16/41, 39.0%; 25.7-54.3, p<0.0001).

Test sensitivity for adenoma detection was low (LRA, 9.3%, (26/280, 6.4-13.3); MRA 15.0% (27/180, 10.5-20.9); HRA, 16.6% (26/157, 11.6-23.2)), although a positive trend was observed with the HRA having the highest positivity rate of all the adenoma subtypes (p=0.0290).

The estimated specificity for cancer was 90.1% (81/820, 88.0-92.0), of which 82.7% (67/81) were methylation positive in a single gene only.

Other co-variables: Lesion location, gender, smoking, family history and age were not significant predictors of test positivity.

Test positivity versus invasiveness: To determine whether the apparent lower sensitivity for Stage I CRC was a biologically-determined issue, we examined the relationship between test positivity and tumor depth of invasion, Figure 3. Including only cases with no disseminated disease, a significant linear trend (p<0.0001) was observed between assay positivity and T stage determined at surgery: T1N0M0, 25% (6/24, 12.0-44.9); T2N0M0, 58.8% (10/17, 36.0-78.4); T3N0M0, 85.7% (42/49, 73.3-92.9); T4N0M0, 100% (8/8, 67.6-100).

Conclusion

With a specificity of 90.1%, the 3-gene methylation ctDNA blood test had an overall sensitivity for CRC of 73.9% and was positive for 67.3% of early stage cancer (stage I and II). The overall cancer positivity rate appears to be a function of tumor invasiveness. Based on the data reported herein, it is justified to proceed to validation of this ctDNA assay in true screening populations.

Funding from NHRMC Australia (ID#1006242).
Part funding from Clinical Genomics Pty. Ltd (Australia)