Performance Comparison of the Methylated *BCAT1/IKZF1* ctDNA Test (Colvera) with the CEA Assay for Detection of Recurrent Colorectal Cancer

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BACKGROUND

Routine surveillance using imaging and carcinoembryonic-antigen (CEA) following curative therapy for colorectal cancer (CRC), improves chance of long-term survival by early detection of operable metastatic disease.

STUDY SYNOPSIS

Objectives: To compare the sensitivity and specificity of a quantitative assay for epigenetic circulating tumor DNA (ctDNA) (methylated *BCAT1/IKZF1*) with that of CEA testing for detection of recurrent CRC following treatment of primary CRC.

Study Design & Methods: Observational study measuring the concentration of CEA and methylated ctDNA in blood samples obtained at a single time point closest to confirmation of recurrence status using the LIAISON CEA test (Diasorin) and the COLVERA™ ctDNA test (Clinical Genomics, [1]), respectively in patients undergoing surveillance for recurrence. The mass of methylated ctDNA was reported as the total sum of methylated *BCAT1* and *IKZF1* per processed specimen. Receiver operator characteristic (ROC) analyses were applied for determination of optimal quantitative threshold criterion for Colvera. A threshold of 5ng/mL was used for CEA as recommended by manufacturer. Radiological imaging was used to determine clinical status of recurrence.

Table 1. Characteristics of patients included in primary analysis | N = 144

		Recurrence (n=50)	No recurrence (n=94)	P value
Age at diagnosis, median (IQR)		65.1 (55.7-73.7)	62.5 (51.1-72.3)	0.284
Gender, Male, No. (%)		34 (68.0)	58 (61.7)	0.454
Stage at diagnosis, No. (%)	Stage I	0 (0)	21 (22.3)	0.0003
	Stage II	13 (26.0)	37 (39.4)	0.109
	Stage III	30 (60.0)	32 (34.8)	0.004
	Stage IV	7 (14.0)	4 (4.4)	0.040
Location, No. (%)	Right[1]	12 (24.0)	37 (39.4)	0.064
	Left	16 (32.0)	30 (31.9)	1.000
	Rectum	22 (44.0)	27 (28.7)	0.065
T stage at diagnosis, No. (%)	T1	0 (0)	12 (12.8)	0.008
	T2	2 (4.0)	14 (14.9)	0.048
	T3	26 (52.0)	51 (54.3)	0.798
	T4	20 (40.0)	17 (18.1)	0004
	Tx	2 (4.0)	0 (0)	0.515
Months elapsed between diagnosis and verified recurrence status, median (IQR)		24.2 (16.1-35.0)	18.2 (13.0-28.9)	0.010
Location[2] of recurrence, No. (%)	Local	13 (26.0)		
	Distant	37 (74.0)		

[1]Right colon includes cecum, ascending colon, hepatic flexure, transverse colon, [2] Recurrence was defined distant when present in liver, lung, peritoneum, bone, brain or adrenal gland. All other recurrences were defined as local.

RESULTS

1. **Study cohort:** 144 of 389 CRC patients had a blood sample for analysis and had a definitive radiological assessment following treatment for primary disease. A total of 50 recurrences and 94 no-recurrences were included for analysis, Table 1.

2. **The nature of recurrences**: Distant recurrence to the lungs was more frequent than recurrence to the liver. Local recurrence was more likely to undergo surgery with curative intent (76.9%) compared to cases with distant recurrences (27.0%, p=0.002), but the proportion with a potentially curative outcome was similar between the groups (80% and 60%, p=0.329)

3. **Quantitative threshold criterion for Colvera:** The area under the ROC curve was 0.819 (0.744-0.894, p<0.001). The optimal cut-off was 11.8pg/sample.

4. **Test accuracy for CEA and Colvera** is shown in Table 2.
- The methylated ctDNA test was significantly more sensitive (66.0%) for recurrence detection than CEA (31.9%).
- The methylated ctDNA test was significantly better than CEA at detecting recurrence whether cases were of stage II, III or IV at diagnosis (data not shown).
- Applying a threshold for Colvera (>11.8pg/sample) significantly improved the specificity from 90.4% to 97.9% (p=0.023) compared to qualitative determination (any signal = positive). Reporting Colvera in qualitative or quantitative mode had no effect on sensitivity.
- Positive predictive values (PPV) did not significantly differ between the two blood tests: Colvera (cut-off, >11.8pg/sample; 94.3%) and CEA (>5ng/mL, 83.3%, p=0.262) but the negative predictive value (NPV) was significantly greater for Colvera (84.4% vs. 71.7%, p<0.0001).

Table 2. Summary performance statistics for CEA and ctDNA testing

Summary Statistic	CEA	ctDNA (BCAT1/IKZF1)	
Cut-off	≥5ng/mL	>0pg/sample	≥11.8pg/sample
N	131	144	144
No. Positive cases (%)	18 (13.7)	42 (29.2)	35 (24.3)
No. recurrence	47	50	50
Sensitivity, % (95%CI)	31.9 (19-47)	66.0 (51.2-78.8)	66.0 (51.2-78.8)
Specificity, % (95%CI)	96.4 (91.4-99.0)	90.4 (84.7-94.7)	97.9 (93.2-99.6)
Likelihood ratio (+)	8.9 (2.6-38.0)	6.9 (3.6-13.9)	31.0 (8.4-183.8)
Likelihood ratio (-)	0.7 (0.6-0.8)	0.4 (0.3-0.5)	0.3 (0.3-0.5)
Odds Ratio	12.7 (3.4-46.7)	18.2 (7.4-45.2)	89.3 (19.6-407.5)
PPV, %	83.3 (59.7-95.5)	78.6 (65.8-88.1)	94.3 (81.6-99.0)
NPV, %	71.7 (67.9-73.6)	83.3 (78.1-87.2)	84.4 (80.3-85.9)

Reference: 1. Symonds et al. CTG 2016

Table 3. Test Concordance | Recurrence

N=47: Recurrence		CEA (≥5ng/mL) +	CEA (≥5ng/mL) -
Methylated ctDNA (≥11.8pg/sample)	+	14	18
	-	1	14
McNemar's p value		0.000076	

Table 4. Test Concordance | No Recurrence

N=84: No Recurrence		CEA (≥5ng/mL) +	CEA (≥5ng/mL) -
Methylated ctDNA (≥11.8pg/sample)	+	0	2
	-	3	79
McNemar's p value		>0.9999	

5. **Test Concordance:** Limiting the analysis to paired samples (n=131), the sensitivity of Colvera for recurrence (cut-off, 11.8pg/sample) was 68.1%; this was significantly higher than the sensitivity of CEA (31.9%, cut-off, 5ng/mL, p=0.00076), Table 3
- **Of the 47 cases with recurrence** in whom both tests were performed, 14 (29.8%) were positive by both tests, whereas the ctDNA test detected an additional 18 (38%) cases that were CEA negative (p<0.001). Only one recurrence case (2.1%) was CEA positive only, Table 3.
- **In cases with no recurrence**, no case was positive for both tests. Two patients (2.4%) were ctDNA positive only and three (3.6%) were CEA positive only (p>0.9999), Table 4. In the 34-72 months of follow-up since a positive test, no evidence of recurrence had been recorded in any of these patients.

6. **Odds Ratio**: ctDNA positive patients were 89.3 more likely to have a recurrence confirmed compared to a negative ctDNA result (p<0.001). A positive CEA returned a odds ratio of 12.7. When adjusting for other predictors of recurrence (incl. stage at primary diagnosis), a ctDNA positive results were 155.7 times more likely to have a recurrence confirmed compared to a negative ctDNA (p<0.001), while a positive CEA test returned an odds ratio of 2.5 (p = 0.407).

7. **Recurrence amenable to surgery:** Despite the observational nature of the study (i.e. radiology was not triggered by a positive ctDNA result), the ctDNA test was positive in 60% (12/20) of patients with recurrence amenable to curative whereas 4/20 (20%) were positive for CEA. The ctDNA test was positive up to 11.6 months before confirmation of recurrence while no CEA test was positive more than 6 months before recurrence confirmation.

CONCLUSIONS

These findings indicate that the methylated ctDNA assay is more sensitive for detecting CRC recurrence and positive up to five months earlier than the CEA test.

Importantly, methylated ctDNA was detected in 60% of cases amenable to surgery with curative intent versus 20% for CEA. Using an 11.8pg/sample cut-off, the methylated ctDNA test was 98% specific with a PPV of 94%.