Highly Accurate and Validated RNA Panels for Early Detection of Colorectal Cancer using Small Amount of Blood Sample

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Background

Despite an extensive screening program for colorectal cancer (CRC) covered by health insurance since 2002 in Germany, only 5.400 patients out of a total of 73.000 patients diagnosed with colorectal cancer in 2008 were detected at screening colonoscopy. Though colonoscopy is highly accurate, acceptance of colonoscopy as a screening tool is limited due its inconvenience and invasive nature.

Stool-based screening (gFOBT, iFOBT) for CRC is the method of choice in many countries. However, participation is only about 40% at time of initiation as in the UK and France. In the US and Germany participation in stool-based screening is rapidly decreasing since four years. It has been shown that a bleeding of 20 ml blood per day into the colon is necessary for the gFOBT to become positive, or 2 ml/d in case of iFOBT.

It holds for all directly tumor derived diagnostic methods (gFOBT, iFOBT, CEA, tumor DNA in stool, methylated DNA in plasma), that sensitivity and specificity are directly dependent on tumor mass or stage of disease, for a comparison of available tests see Table 1.

Table 1: Comparison of available blood-based IVDs for early detection of colorectal cancer in comparison to Haemocult II

Company	Type of Test	# Marker	Sensitivity	Specificity	False Negative Rate[1]
Epigenomics	Methylation	1	62,5%[2]	89%[2]	1 in 233
OncoMethylome Sciences	Methylation	2	56%[3]	91%[3]	1 in 205
GeneNews	RNA	7	72%[4]	70%[4]	1 in 255
gFOBT (Haemocult II)	*Stool*	*1*	*37,1%[5]*	*97,7%[5]*	*1 in 154*

Legend Table 1: [1] Computation based on 10000 patients with a CRC-incidence of 1% | [2] www.epigenomics.com | [3] Louwagie et al. Joint ECCO 15-34th ESMO Multidisciplinary Congress, Berlin 20-24 September 2009, European Journal of Cancer Supplements, Vol. 7, No 3, September 2009, Page 9 | [4] Han M, Liew CT, Zhang HW, Chao S, Zheng R, Yip KT, Song ZY, Li HM, Geng XP, Zhu LX, Lin JJ, Marshall KW, Liew CC. Clin Cancer Res. 2008,14, 455-60. | [5]Allison et al. (1996) N Engl J Med. 334, 155-9

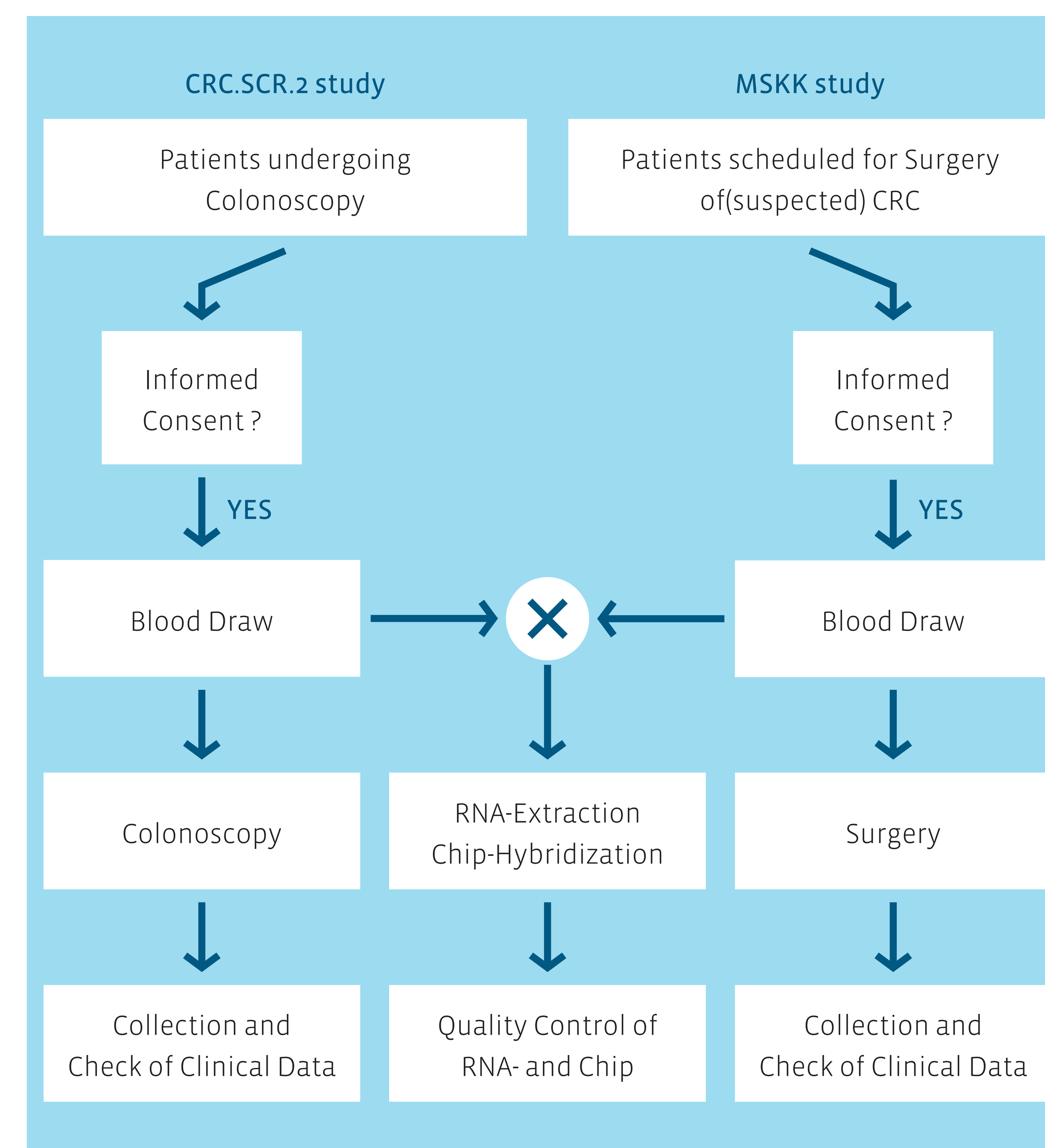
Thus, there remains a high medical need for an accurate, blood-based test that has a specificity (S^-) of at least 0.85 and a sensitivity (S^+) of at least 0.70 for CRC, especially in UICC stage I and II.

Methods

To this end, we initiated two prospective multi-center studies collecting 2.5 ml Pax-RNA blood samples and clinical data from individuals undergoing screening colonoscopy in 13 colonoscopy screening centers and patients admitted to surgery for CRC in 20 primary care hospitals in Germany.

Blood was drawn either prior to colonoscopy or prior to surgery. RNA was isolated, quality was controlled, and hybridized onto Affymetrix U133 2.0 Plus arrays containing 54.000 human gene transcripts. All clinical data were monitored. Prior to RNA extraction and monitoring of clinical data, all samples were randomized. The first 119 Pax-RNA blood samples (55 CRC cases, 64 controls) from this randomization list after all QCs formed the discovery set. The next 343 samples (210 CRC cases, 133 controls) were used as independent validation set.

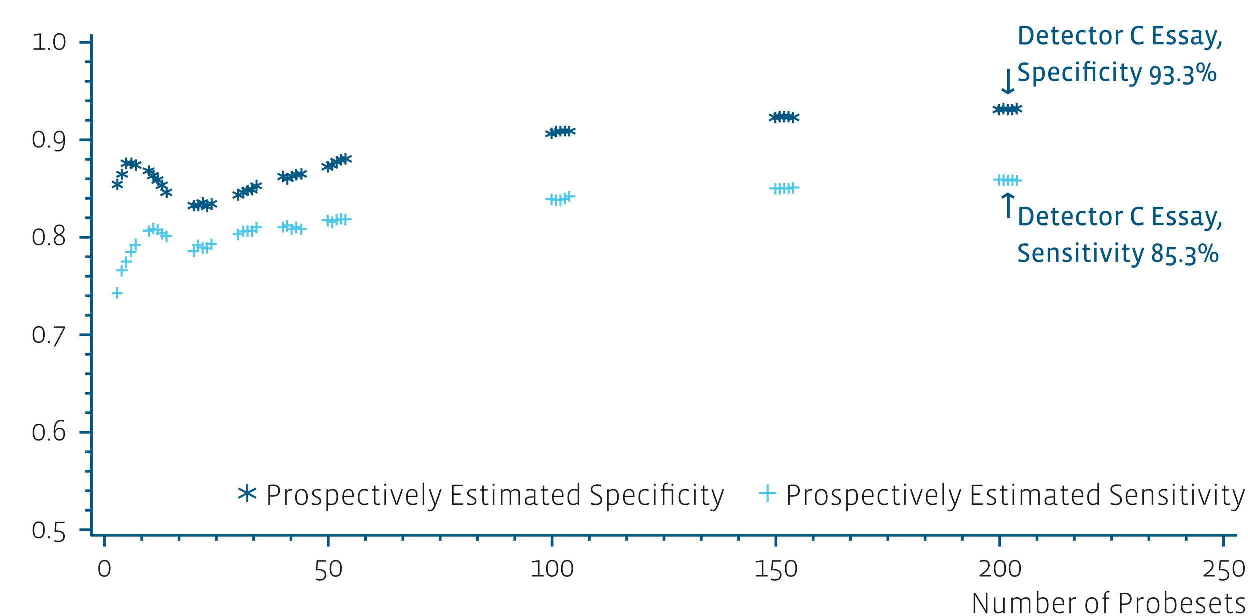
Figure 1: Sample and Data flow
RNA-Extraction and RNA Quality Control
Chip-Hybridization and Chip Quality Control



Results

Using a double nested bootstrap with random forrest and SVM as feature selection methods, we identified a series of potentially useful gene signatures in the discovery set of 119 samples. Two gene signatures, Detector-C and Detector-C+, were chosen for detailed analysis and prospective validation. Detector-C, combining 202 genes and discriminating between cases and controls, has a second order unbiased estimate for S^+ of 0.853 and S^- of 0.933 (Figure 2). Detector-C+ has a second order unbiased estimate for S^+ of 0.869 and for S^- of 0.960.

Figure 2: Prospective Performance Results by Number of Probesets (Genes) in Detector-C



Formulation of Prospective Performance Evaluation Hypothesis Based on Discovery Results of Dtector-C

Given a sensitivity of 85.3% and a specificity of 93.2% obtained from the discovery and given an one-sided α of 2.5% (error of first kind) and a β of 2.5% (error of second kind, or a power or 97.5%), 122 CRC cases and 109 controls are needed to simultaneously show that the sensitivity of the test is > 70% and that the specificity of the test is > 80%.

Results of the prospective performance evaluation are displayed in Table 2.

Table 2: Results of Prospective Performance Evaluation – Overall and by UICC-Stage of Detector-C

	UICC Stage	Freqency	Cumulative Frequency	Result	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Sensitivity	All	189	210	**0.90**	0.851	0.937
Specificity	All	117	133	**0.88**	0.812	0.930
Sensitivity	I	48	54	**0.89**	0.774	0.958
	II	52	58	**0.90**	0.788	0.961
	III	63	70	**0.90**	0.805	0.959
	IV	26	28	**0.93**	0.765	0.991

Multivariate analysis of possible confounding effects showed no significant effect of stage, age, gender, tumor localization, or RNA quality on correct prediction (Table 3).

Table 3: Analysis of Possible Confounding Effects on Correct Classification by Detector-C

Effect	Degrees of Freedom	P-Value
UICC Stage (No CRC (N=133); I (N=54); II (N=58); III (N=70); IV (N=28))	4	0.5187
RNA Integrity (RIN) (< 6.6 (N=88); 6.7 - 7.1 (N=79); 7.2 - 7.5 (N=80); > 7.6 (N=96))	3	0.5639
Gender (Male (N=166); Female (N=177))	1	0.5096
Study (Controls (N=132); Cases (N=211))	1	0.2943
Tumor Localization (No CRC (N=133); Colon (N=144); Rectum (N=56))	2	0.3511
Age Classes (< 55 (N=22); 55-<65 (N=120); 65-<75 (N=120); >75 years (N=81))	3	0.2813

Even low grade and high grade intraepithelial neoplasia was dected by Dector C. (Table 4).

Table 4: Secondary Result: Classification of Patients with Intraepithelial Neoplasia

Classified as	Observed	Observed
	Low Grade IEN (LIEN)	High Grade IEN (HIEN)
No CRC	17	4
CRC	5	8
Σ	22	12
False Positive (?)	5/22 ~ 22,73%	8/12 ~ 66,67%

Conclusions

Colorectal cancer can be detected with high sensitivity and high specificity using a novel blood-based IVD test Detector-C. Detector-C measures the host response of white blood cells to tumor lesions and is highly suited for screening of CRC due its easy of use, non-invasiveness and its high sensitivity for early-stage CRC/neoplastic lesions.

The test is extremely safe: only 1 in 872 tested individuals will be false negative (based on CRC incidence of 1%). The false negative rate of Detector C blood test is four times lower than the false negative rates of other blood tests based on methylation of free tumor DNA in the plasma. In comparison with Haemocult II (gFGOBT) Detector C's false negative rate is seven times lower. The test also showed a sensitivity of 66% for high grade intraepithelial neoplasia.

The high specificity ensures that available endoscopy resources will not be exhausted by large scale use of this blood-based test. The high sensitivity ensures that 90% of patients with undetected CRC will be identified.