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Non-invasive detection of colorectal tumours by the combined application of molecular diagnosis and the faecal occult blood test

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Abstract

The treatment of early-stage tumours decreases the overall mortality of colorectal tumour patients. In this retrospective study we determined the sensitivity and the specificity of the faecal occult blood test (FOBT) and the molecular diagnosis (MD). We analysed 57 stool samples from patients with colorectal carcinomas for the presence of occult blood using a standard FOBT and for alterations in the three different tumour relevant markers APC, BAT26 and L-DNA. Stool samples from 44 control donors were analysed to determine the specificity of the applied methods. Twenty-nine (51%; 95% confidence interval (CI): 38–63%) stool samples of the cancer patients gave positive FOBT results. Thirty-seven (65%; CI: 52–76%) samples showed alterations in at least one DNA marker. Sixteen (28%) samples were positive only in the FOBT, and 24 (42%) samples showed a positive result exclusively in MD. The combined application of both methods resulted in a sensitivity of 93% (CI: 83–97%) and an overall specificity of 89% (CI: 76–95%). The combined application of FOBT and MD resulted in an overall sensitivity, which could not be achieved by any of the methods alone and which is in the range of invasive diagnostic methods.
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Keywords: Non-invasive colorectal cancer diagnosis; Faecal DNA analysis; Molecular diagnosis; Faecal occult blood test

1. Introduction

The diagnosis and the therapy of early-stage tumours have the potential to decrease morbidity and mortality of colorectal cancer patients. Invasive methods like sigmoidoscopy, colonoscopy and

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roentgenography are valuable tools for the specific and sensitive detection and the exact localization of a colorectal tumour (reviewed in [1]). These techniques are unsuitable for broad screening programs mainly because of their low general acceptance [2]. The most common screening method is the faecal occult blood test (FOBT). Randomised trials revealed the clinical effectiveness in reduction of mortality through the implementation of the FOBT [3–5].

Sidransky et al. introduced the analysis of faecal DNA for tumour specific alterations as a novel non-invasive diagnostic strategy [6]. Until now several studies have proven the usefulness and the feasibility of faecal DNA analysis (reviewed in [7]). Recent multi-target studies achieved sensitivities between 62 and 91% and specificities up to 98%, respectively [8–10]. A few studies compared the outcomes of the molecular diagnosis (MD), which is the detection of tumour relevant alterations in faecal DNA, with these of the FOBT. The finding that 9 out of 11 asymptomatic adenoma patients were identified by the analysis of five different DNA markers, whereas none was detected by FOBT, indicated a higher diagnostic sensitivity of the MD [9]. The data of a large prospective study, which is close to completion, will allow assessing the two methods and their combined application (annual meetings of the American College of Gastroenterology (ACG), Baltimore 2003 and of the American Gastroenterology Association (AGA), New Orleans 2004). We intended to judge in this retrospective study whether the analysis of three faecal DNA markers has the potential to complement or even to replace the FOBT and whether the combined application of the two methods might increase the overall diagnostic reliability.

2. Materials and methods

2.1. Study population and sample collection

All patients and control donors provided their informed oral and written consent to provide samples. This study was approved by the responsible ethical committees at the Klinik für Chirurgie Marienhospital (Herne) and at the Berufsgenossenschaftliche Kliniken Bergmannsheil (Bochum). None of the patients and none of the control donors had familial adenomatous polyposis coli or hereditary non-polyposis colorectal cancer. Patients were diagnosed with colorectal tumours at the Berufsgenossenschaftliche Kliniken Bergmannsheil, the Marienhospital Herne or the Katholisches Krankenhaus Dortmund-West between 1998 and 2003. All tumours were diagnosed and located by imaging methods like colonoscopy and roentgenography. At least seven days after colonoscopy and between 2 and 4 days before surgical removal of the tumour 2 samples were taken from regular stools, immediately frozen at −20 °C and transferred to −80 °C within 24 h. The surgically removed tumours were classified according to the AJCC/TNM system (American Joint Committee on Cancer (AJCC) stages I through IV; Table 1) [11]. Because of personnel alternations in the clinical departments in the course of the study, we could not reproduce the staging information for 12 tumours, the age and the gender of 8 patients and the location for 3 tumours at the time of the analysis of the faecal samples. Control stool samples were from 24 female and 20 male donors, who volunteered to participate. The age group of control donors was 24–78 years (mean age 49). Control donors did not show any symptoms or signs of colorectal diseases as determined in personal interviews.

2.2. FOBT

Two stool samples from each cancer patient and one sample from each control donor were analysed for the presence of occult blood by a standard guaiac test using commercial reagents and standard protocols (Care Diagnostica). Each FOBT was performed twice. A blue colour reaction within 120 s after the addition of the hydrogen peroxide solution was rated as a positive result. In case the double FOBT analysis of one stool sample or of two stool samples from one patient gave discrepant results, the sample was rated positive.

2.3. MD

Faecal DNA from all samples was purified using a method, which was optimised for faecal specimen [12]. An essential step of this method is the matrix-based elimination of PCR inhibiting and DNA damaging compounds (Stool Mini Kit, Qiagen).

Table 1
Patient and tumor data of all samples and results obtained by the analysis of stool samples from cancer patients by FOBT and by MD

Patient	Gender/age	AJCC/TNM	FOBT	MD positive markers
79	M/70	n.a.*	neg	neg
80	M/58	II/T3N0MxG2	pos	APC
83	M/67	II/T3N0MxG2	neg	APC, L-DNA
84	M/85	II/T3N0MxG2	neg	BAT26
87	n.a.	n.a.	neg	APC
88	F/76	II/T3N0MxG2	pos	neg
89	n.a.	n.a.	neg	L-DNA
90	F/62	I/T2N0MxG2	neg	L-DNA
92	F/53	n.a.	pos	neg
93	n.a.	n.a.	pos	APC
95	F/68	I/T2N0MxG2	pos	APC
96	n.a.	n.a.	neg	APC, L-DNA
121	M/51	I/T2N0MxG2	neg	L-DNA
136	M/61	I/T2N0MxG2	neg	APC
138	M/65	I/T2N0MxG2	pos	neg
139	M/74	II/T3N0MxG2	neg	neg
140	F/73	II/T3N0MxG2	pos	neg
141	n.a.	n.a.	pos	APC
172	F/75	II/T3N0MxG2	neg	APC
173	M/73	II/T3N0MxG2	neg	APC
174	F/79	IV/T3N2M1hepG2	pos	APC, L-DNA
175	F/75	II/T3N0MxG3	pos	neg
176	M/63	IV/T3N1M1hepG3	neg	APC
179	M/68	III/T3N1MxG2	neg	APC
180	F/65	II/T4N0MxG2	neg	APC
181	F/92	III/T4N1M0G2	pos	neg
182	M/74	I/T2N0MxG2	neg	APC
183	M/60	II/T3N0MxG2	neg	L-DNA
184	M/65	IV/T3N2M1hepG2	neg	L-DNA
185	M/75	I/T2N0MxG2	pos	BAT26
186	M/47	III/T3N1MxG2	neg	L-DNA
187	M/73	II/T3N0MxG2	pos	neg
188	M/65	IV/T3N1M1hepG2	neg	neg
189	M/76	III/T3N2MxG2	neg	APC, L-DNA
190	M/69	I/T2N0MxG2	pos	BAT26, L-DNA
191	M/76	III/T3N1MxG2	pos	L-DNA
192	M/89	II/T3N0MxG2	pos	L-DNA
193	F/64	I/T2N0MxG2	neg	BAT26, L-DNA
251	M/55	IV/T3N2M1G2	pos	L-DNA
252	M/74	II/T3N0MxG2	pos	neg
253	F/83	III/T3N1MxG2	neg	L-DNA
254	M/66	II/T4N0MxG2	pos	BAT26, L-DNA
255	M/62	III/T3N2MxG2	pos	neg
257	n.a.	n.a.	neg	APC
274	F/70	I/T2N0MxG3	pos	neg
275	n.a.	n.a.	pos	APC, L-DNA
276	n.a.	n.a.	neg	L-DNA
278	M/86	n.a.	neg	L-DNA
283	M/82	III/T3N1MxG2	pos	APC, L-DNA
284	F/72	n.a.	neg	L-DNA
285	M/81	II/T3NxMxG2	neg	neg
286	M/64	IV/T3NxM1hepG2	pos	neg

(*continued on next page*)

Table 1 (*continued*)

Patient	Gender/age	AJCC/TNM	FOBT	MD positive markers
287	M/92	III/T3N1MxG3	pos	neg
289	M/71	II/T4NxMxG3	pos	neg
290	M/66	IV/T4N0M1	pos	neg
292	M/80	I/T1N0MxG2R0	pos	neg
294	F/83	III/T3N2MxG2	pos	neg

n.a., data not available

The quality of the purified DNA was validated by agarose gel electrophoresis. One tenth of the DNA obtained from one stool sample was used in a capturing step to enrich the human DNA from the DNA of non-human origin [13]. The overall yield of the capturing could be increased by the restriction digestion of the DNA with the enzyme DraI. To fish the APC gene out of the pool of genomic DNA, 10 pmol biotin labelled primer APC1 (5′-TTAAAATATGCCACAGATATTCCT TCA TCACAGAAACAGT-3′) was added to 100 μl DraI digested stool DNA. After denaturing and re-annealing 10 μl streptavidin coupled magnetic beads (Dynal) were added and the mixture was incubated over night at room temperature. After washing, the beads coupled DNA was completely used as template in a first PCR. The APC gene region from codon 1069 through codon 1984 including the mutation cluster region was analysed by PCR and by PTT of two overlapping fragments. The detailed experimental conditions of the following PTT have been published [13] (Hauss and Müller, The protein truncation test in mutation detection and molecular diagnosis, in: Methods in Molecular Biology, edited by Guido Grandi, The Humana Press, in press). Briefly, the primers of the first PCR coded the HA-tag, and the primers of the second PCR coded the Kozak motif and the T7 promoter sequence, which are necessary for the following PTT. The first PCR was carried out at the following conditions: 95 °C (5 min)//[94 °C (30 s)/56 °C (30 s)/72 °C (90 s)] 20×//[94 °C (30 s)/58 °C (30 s)/72 °C (90 s)] 20×//72 °C (10 min) and under the use of the following primers: APC fragment I: 5′-CGCCATGTACCCCTACGACGTGCCCGACTACGCCTTCCAACCACATTTTGGACAGCAG-3′ and 5′-CCGTCATTTTTCTGCCTCTTTCTCTTGGTT-3′; APC fragment II: 5′-CGCCATGTACCCCTACGACGTGCCCGACTACGCCGATGTGGAATTAAGAATAATGCCT-3′ and 5′-CCGTCATTTTCTTTATTGTTGTTTTCTTGGTC-3′. One fifth of the volume of the first PCR was used in a second PCR at the conditions: 95 °C (5 min)//[94 °C (30 s)/60 °C (30 s)/72 °C (90 s)] 40×//72 °C (10 min) and using the following primers, which anneal within the products of the first PCR: APC fragment I: 5′-ATCCTAATACGACTCACTATAGGGAGCCACCATGTACCCCTACGACGTG-3′ and 5′-CCGTCATTTTTCTGCCTCTTTCTCTTGGTT-3′; APC fragment II: 5′-ATCCTAATACGACTCACTATAGGGAGCCACCATGTACCCCTACGACGTG-3′ and 5′-CCGTCATTTTCTTTATTGTTGTTTTCTTGGTC-3′. Three of the control samples gave no reproducible results in the PCR reactions. All other faecal DNA samples from control donors and all samples from cancer patients gave robust PCR products. DNA purified from two stool samples from each cancer patient and from one sample from each control donor was analysed for the presence of alterations in 3 different markers. Each positive result was confirmed in a repeated analysis. All samples showing signals, which were different to the wild-type controls, were further examined. For this the PCR fragments were cloned in a T/A cloning vector and the cloned gene fragments were sequenced by conventional methods. The analyses of all positive samples were repeated by starting with the purified DNA in order to limit the number of false positive results. The analysis of alterations in the microsatellite marker BAT26 and the detection of the relative amount of non-apoptotic L-DNA were performed according to published protocols [9,14]. All results are based on the analysis of at least two independently amplified PCR products. The primer sequences for L-DNA analysis were kindly provided by A.P. Shuber and D.A. Ahlquist. In case of discrepant MD results between the two different samples from one patient, DNA was extracted from a new faecal aliquot of the same patient and the analysis was repeated.

Table 2
Localization of tumours relative to the splenic flexure of the colon and number of the corresponding stool samples diagnosed as positive by FOBT or by MD

Site	*N*	FOBT	MD	Combined use
Proximal	27	16	14	25
Distal	27	11	21	26
n.a.	3	2	2	2
Total	57	29	37	53

N, number of samples; n.a., at the time of analysis the information of the tumour localization was not available.

2.4. Statistical evaluation

Ninety-five percent confidence intervals were determined based on the exact binomial distribution. The Fisher exact test was used for comparison of the test results between patients with different tumour stages. To compare the single application of FOBT or MD with the combined application of both methods the McNemar's test was used.

3. Results

The aim of this study was to determine the sensitivity and the specificity of the two non-invasive diagnostic methods, FOBT and MD, which is the analysis of faecal DNA for alterations in the three different markers APC, BAT26 and L-DNA. We analysed stool samples from 57 colorectal cancer patients and from 44 control donors. Twenty-seven tumours were located proximal and 27 tumours were located distal to the splenic flexure of the colon (Table 2). Twenty-eight patients had tumours, which did not spread to proximal or distant lymph nodes (stage I or II) at the time of staging diagnosis. Lymphatic spread was identified in 17 patients (stage III or IV) (Tables 1 and 3).

Twenty-nine stool samples (51%; 95% confidence interval (CI): 38–63%) showed a positive FOBT test (FOBT pos). Thirty-seven faecal DNA samples (65%; CI: 52–76%) were MD positive (MD pos) demonstrating an alteration in at least one of the three tested DNA markers (Tables 1 and 3). The MD positives include nineteen samples (33%) with truncated APC protein fragments in the PTT analysis (Fig. 1). We sequenced these positive samples and found mutations, which result in stop codons between codons 1254 and 1472 (Table 4). The sizes of the predicted translation products of PCR fragments carrying these stop codons correlate with the approximate sizes of the translation products, which were detected in the PTT (Fig. 1). The number of APC mutations detected in the samples was in the range of the numbers

Table 3
Summarized results of the analysis of the stool samples from cancer patients and sensitivity of MD and FOBT

AJCC stage	n.a.	I	II	III	IV	Total
N	12	11	17	10	7	57
Mean age (range)	70.3 (53–86)	67.2 (51–80)	72.7 (47–92)	76.1 (47–92)	65.3 (55–79)	70.9 (47–92)
Gender (male/female)	2/2	7/4	12/5	7/3	6/1	34/15
n.a.	8	0	0	0	0	8
FOBT pos	4	6	9	6	4	29 (51%; 38–63%)
MD pos	10	8	9	6	4	37 (65%; 52–76%)
APC	6	3	5	3	2	19 (33%)
BAT26	0	3	2	0	0	5 (9%)
L-DNA	6	4	4	5	3	22 (39%)
MD pos or FOBT pos	11	11	15	10	6	53 (93%; 83–97%)
MD(APC or L-DNA) pos or FOBT pos	11	11	14	10	6	52 (91%; 81–96%)
MD pos and FOBT neg	7	5	6	4	2	24 (42%; 30–55%)
FOBT pos and MD neg	1	3	6	4	2	16 (28%; 18–41%)

The results, the relative sensitivities and the corresponding 95% confidence intervals are given for the FOBT, the MD and the combined application of the two methods. A sample was rated MD positive (MD pos), when one molecular marker was tested positive in the PTT, in the BAT26 or in the L-DNA analysis. n.a., AJCC staging of the corresponding tumours or age and gender were not available at the time of analysis.

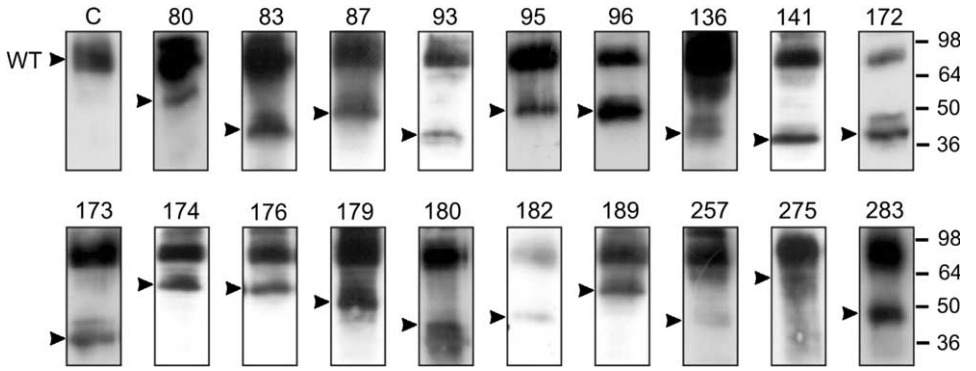


Fig. 1. Positive PTT results of the APC gene analysis. Arrowheads point to bands, which represent the wild-type full-length protein (WT) or smaller proteins, which are truncated due to mutations. On the right side the sizes of marker fragments are indicated in kilodaltons. C, non-mutated control.

described in other studies [9,15]. Ahlquist et al. (2000) detected APC mutations in 24% faecal DNA samples of cancer patients. Traverso et al. (2002) found APC mutations in 63% DNA samples of patients with T3N0M0 tumours. We detected BAT26 patterns different to the wild-type control in 5 (9%) samples (Fig. 2). Twenty-two (39%) samples showed high levels of L-DNA amplification products (Fig. 3). Thirty-five stool samples (61% of all samples or 95% of MD positive samples) showed alterations in the APC gene or in the relative L-DNA level. This high number reflects the high proportion of colorectal tumours, which harbour an APC gene mutation and a dysfunction in the regulation of apoptosis (Tables 1 and 3).

Forty-two percent of the samples (CI: 30–55%) were positive in at least one of the MD markers, but gave negative FOBT results. Twenty-eight percent of the samples (CI: 18–41%) were positive exclusively in the FOBT, but not in the molecular test. When the results of both methods were combined, 53 (93%; CI: 83–97%) samples were positive in FOBT or in molecular testing. The combined application of the two methods together leads to a significantly higher sensitivity than the application of any of the methods alone ($P < 0.01$). When FOBT was combined with a limited MD, which is the analysis of the two molecular markers APC and L-DNA, a detection rate of 91% (CI: 81–96%) was achieved. The sensitivity of this combined application is higher than the sensitivity of the analysis by limited MD or by FOBT alone ($P < 0.01$).

In one control sample we found occult blood by FOBT resulting in an overall FOBT specificity of 98% (CI: 88–100%) (Table 5). In another control sample we detected a truncated APC protein fragment by PTT. The following sequence analysis revealed a C to T transition at nucleotide position 4117 leading to

Table 4
Sequence alterations in APC detected by sequencing of MD positive samples

Sample	Mutation at sequence position	Resulting stop at codon
80	C4278del	1472
83	C4117T	1367
87	G4153T	1379
93	G4075T	1353
95	C4366T	1450
96	C4366T	1450
136	G3943T	1309
141	A3835T	1273
172	G3943T	1309
173	C3777del	1254
174	4409–4412del	1472
176	A4330del	1472
179	G4152^G4153insA	1414
180	C4117T	1367
182	C4117T	1367
189	T4310^G4311insGCCACCAA	1472
257	C4117T	1367
275	A4382del	1472
283	C4055A	1346

Nucleotide changes, the nucleotide sequence positions and the resulting stop codons are listed for the detected APC mutations. All faecal DNA samples were screened by PTT for alterations in APC. Positive samples were sequenced.

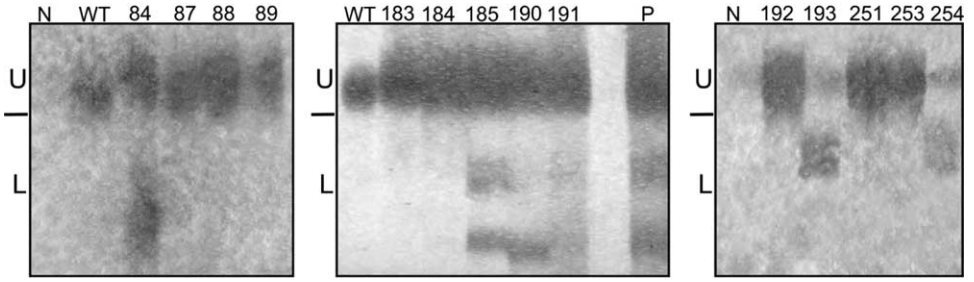


Fig. 2. Polyacrylamide gels for the analysis of alterations in the BAT26 microsatellite patterns. All BAT26 positive samples and 9 representing negative samples are shown. The upper (U) region of the gel contains PCR products representing the wild-type full-length products. Bands in the lower (L) regions of the gels indicate deletions in the BAT26 microsatellite. N, negative control without any DNA template; WT, wild-type control from normal tissue; P, positive control from tumour tissue containing the mutant BAT26.

a stop signal in the corresponding codon 1367 (not shown). Both control donors showed no signs of malignancies or polyps according to colonoscopy analysis. Three samples from the control group did not give reproducible PCR results. In the final statistical calculation of the overall specificity these control samples were rated positive (Table 5). Thus the specificity of MD was 91% (CI: 79–96%), and the overall specificity of the combined application of FOBT and MD was 89% (CI: 76–95%).

4. Discussion

Tumour specific mutations in faecal DNA are supposed to be sensitive and specific markers for the non-invasive diagnosis of colorectal cancer (reviewed in [7,16]). In this study the sensitivity of the retrospective diagnosis of colorectal carcinoma by the analysis of the three different molecular markers APC, BAT26 and L-DNA was 65%. The sensitivities described by others for the analysis of five different markers reach from 64 to 90% [9,10]. The differences between the sensitivities might be due to variations in sample quality, in the applied analytical methods and in the laboratory-specific handling of the samples. The high potential of the MD became obvious, when we compared the MD outcomes with those of the most common non-invasive screening test, the FOBT. The MD showed a 14% higher detection rate than the FOBT, and 42% of all samples were negative in the FOBT, but still detectable by MD. Nevertheless, our data also confirm the value of the FOBT. Transferring the data to a putative random non-invasive screen 28 out of 100 cancer patients would be detected only by FOBT but not by MD. The sensitivity of MD can be even further increased up to 93% by the additional application of FOBT. While the combination of the FOBT and MD leads to a significant increase in sensitivity, it leads only to a marginal decrease in specificity (Table 5).

There was no significant correlation of the FOBT or MD results with the gender, the age or the tumour stages. This finding is in line with the results of others [9]. Furthermore, we did not find a significant correlation of the positive markers with the location

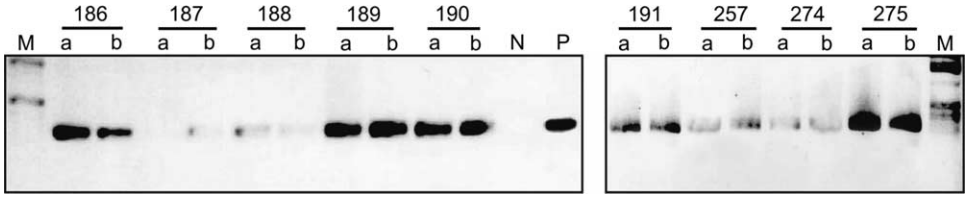


Fig. 3. Agarose gels for the analysis of the amplification of L-DNA. The two gels show the analysis of PCR of KRAS-L-DNA from 9 representing stool DNA samples, which were amplified in duplicates (a, b). M, Marker; N, negative control without any DNA; P, positive control.

Table 5
The numbers of positive samples and Summary of results

	Cancer patients	Control donors	Sensitivity (%)	Specificity (%)
FOBT pos	29	1	51 (38–63)	98 (88–100)
MD pos	37	4	65 (52–76)	91 (79–96)
MD pos or FOBT pos	53	5	93 (83–97)	89 (76–95)
MD(APC or L-DNA) pos or FOBT pos	52	5	91 (81–96)	89 (76–95)

The relative sensitivities, specificities and the corresponding 95% confidence intervals of the MD, the FOBT and of the combined application of the two methods are given. The MD positive samples of the control donors include three samples, which could not be analysed.

of the tumours (Table 2). The findings of others, who described indications for a correlation between tumour location and BAT26 and L-DNA markers, have to be confirmed by the analysis of a high number of samples [9].

In order to determine the specificity we analysed the samples of randomly chosen control donors, who showed no obvious symptoms of colorectal diseases. This strategy might have led to an overestimated specificity in comparison to an authentic screening, which includes both non-symptomatic and symptomatic cancer patients and healthy donors. Because the analysed MD markers APC and BAT26 correlate directly to tumour development and do not necessarily correlate with the onset of symptoms, the analysis of these two markers should not distort the overall specificity. We are still aware that the FOBT outcomes might have led to the overestimation of the overall specificity. A subsequent study, which will include samples from non-symptomatic as well as samples from symptomatic individuals, will clarify this aspect.

This is one of the first studies, which evaluate and directly compare the potential of the two non-invasive methods MD and FOBT in the diagnosis of colorectal carcinomas. The combined application of the two methods together led to a significantly higher sensitivity than the application of any of the methods alone. Regarding the low cost and the little technical expense of the FOBT, we suggest that MD and FOBT should complement rather than replace each other. The sensitivity of the combined application of MD and FOBT is in the range reported for invasive methods. This leads to the question whether both methods should be used together in broad diagnostic screening programs. We are aware that our retrospective data do not allow any conclusion on the potential of this approach in diagnostic screening. Prospective comparative studies, which include a very high number of individuals, have to prove the value of the combined application of MD and FOBT. Preliminary results of a large study were presented in abstract form (ACG and AGA annual meetings). The final results of this study will allow further conclusions about the effectiveness of the two methods and their combined application.

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References

[1] C.J. Kahi, D.K. Rex, Current and future trends in colorectal cancer screening, Cancer Metastasis Rev. 23 (2004) 137–144.
[2] A.L. Frazier, G.A. Colditz, C.S. Fuchs, K.M. Kuntz, Cost-effectiveness of screening for colorectal cancer in the general population, J. Am. Med Assoc. 284 (2000) 1954–1961.
[3] J.S. Mandel, J.H. Bond, T.R. Church, D.C. Snover, G.M. Bradley, L.M. Schuman, F. Ederer, Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study, N. Engl. J. Med. 328 (1993) 1365–1371.
[4] J.D. Hardcastle, J.O. Chamberlain, M.H. Robinson, S.M. Moss, S.S. Amar, T.W. Balfour, et al., Randomised controlled trial of faecal-occult-blood screening for colorectal cancer, Lancet 348 (1996) 1472–1477.

[5] O. Kronborg, C. Fenger, J. Olsen, O.D. Jorgensen, O. Sondergaard, Randomised study of screening for colorectal cancer with faecal-occult- blood test, Lancet 348 (1996) 1467–1471.
[6] D. Sidransky, T. Tokino, S.R. Hamilton, K.W. Kinzler, B. Levin, P. Frost, B. Vogelstein, Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors, Science 256 (1992) 102–105.
[7] K.S. Tagore, T.R. Levin, M.J. Lawson, The evolution to stool DNA testing for colorectal cancer, Aliment. Pharmacol. Ther. 19 (2004) 1225–1233.
[8] D. Calistri, C. Rengucci, R. Bocchini, L. Saragoni, W. Zoli, D. Amadori, Fecal multiple molecular tests to detect colorectal cancer in stool, Clin. Gastroenterol. Hepatol. 1 (2003) 377–383.
[9] D.A. Ahlquist, J.E. Skoletsky, K.A. Boynton, J.J. Harrington, D.W. Mahoney, W.E. Pierceall, et al., Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel, Gastroenterology 119 (2000) 1219–1227.
[10] K.S. Tagore, M.J. Lawson, J.A. Yucaitis, R. Gage, T. Orr, A.P. Shuber, M.E. Ross, Sensitivity and specificity of a stool DNA multitarget assay panel for the detection of advanced colorectal neoplasia, Clin. Colorectal. Cancer 3 (2003) 47–53.
[11] F.L. Greene, D.L. Page, I.D. Fleming, A. Fritz, C.M. Balch, D.G. Haller, M. Morrow,, sixth ed. AJCC Cancer Staging Manual, XIV, 2002.
[12] R. Deuter, S. Pietsch, S. Hertel, O. Muller, A method for preparation of fecal DNA suitable for PCR, Nucleic Acids Res. 23 (1995) 3800–3801.
[13] S. Kahmann, P. Herter, C. Kuhnen, K.M. Muller, G. Muhr, D. Martin, et al., A non-radioactive protein truncation test for the sensitive detection of all stop and frameshift mutations, Hum. Mutat. 19 (2002) 165–172.
[14] G. Traverso, A. Shuber, L. Olsson, B. Levin, C. Johnson, S.R. Hamilton, et al., Detection of proximal colorectal cancers through analysis of faecal DNA, Lancet 359 (2002) 403–404.
[15] G. Traverso, A. Shuber, B. Levin, C. Johnson, L. Olsson, D.J. Schoetz, et al., Detection of APC mutations in fecal DNA from patients with colorectal tumors, N. Engl. J. Med. 346 (2002) 311–320.
[16] D.A. Ahlquist, A.P. Shuber, Stool screening for colorectal cancer: evolution from occult blood to molecular markers, Clin. Chim. Acta 315 (2002) 157–168.