Clin. Lab. 2019;65:XXX-XXX

ORIGINAL ARTICLE

Combined Plasma MicroRNA and Fecal Occult Blood Tests in Early Detection of Colorectal Cancer

Xiaoya Luo, Yongdong Wu, Ming Ji, Shutian Zhang

Department of Gastroenterology, Beijing Friendship Hospital, Capital Medical University. National Clinical Research Center for Digestive Diseases, Beijing Digestive Disease Center, Beijing Key Laboratory for Precancerous Lesion of Digestive Diseases, Beijing, China

SUMMARY

Background: **Colorectal cancer (CRC) is one of the most common malignancies and a major cause of cancer-related death worldwide. Fecal occult blood tests (FOBT) are non-invasive colorectal cancer screening tests. In recent years plasma microRNAs (miRNAs) have shown great potential in early non-invasive cancer detection.**
Methods: **FOBT (immunochemical) and a panel of 12 plasma miRNAs were tested in two independent groups: 57 CRC patients and 125 neoplasm free controls, in addition to 58 advanced adenoma patients and 67 neoplasm free controls. miRNA levels were assessed by quantitative real-time polymerase chain reaction (qRT-PCR).**
Results: **Plasma levels of 7 miRNAs (miR-18a, miR-20a, miR-21, miR-92a, miR-133a, miR-143, miR-145) differed significantly between CRC patients and neoplasm free controls. miRNA plasma levels did not differ between advanced adenoma patients and controls. For 7 dysregulated miRNAs in CRC patients, AUCs ranged from 0.585 to 0.632 for CRC detection, in comparison to an AUC of 0.857 for iFOBT. The combination of miR-133a and iFOBT achieved a higher AUC (0.894) than iFOBT alone. At 97.8% specificity, miRNAs showed much lower sensitivities than iFOBT, but the miRNA panel and iFOBT in combination detected CRC with a higher sensitivity than iFOBT alone.**
Conclusions: **The diagnostic performance of miRNAs was poorer than iFOBT. Nevertheless, plasma miRNA profiles offer an innovative non-invasive approach for early CRC detection. The potential advantage of combining plasma miRNA profiles with iFOBT needs to be further studied in a larger cohort of patients.**
(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2018.180926)

Correspondence:
Shutian Zhang
95 Yong'an Road
Beijing, 100050
China
Phone: +86 1063139842
Email: zhangst@ccmu.edu.cn

Manuscript accepted October 9, 2018

KEY WORDS

microRNA, plasma, fecal occult blood tests (FOBT), colorectal cancer, adenomas, early detection

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related death worldwide [1,2]. Due to its slow development from premalignant lesions and high survival rates at earlier stages, early detection and treatments are particularly promising tools to reduce the burden of this malignancy [3], and screening is predicted to have the potential to reduce colorectal cancer deaths by 60% [4].
Current clinical options for CRC screening in the average-risk population include stool tests for occult blood

or exfoliated DNA, and clinical examinations including flexible sigmoidoscopy and colonoscopy [5,6]. The guaiac-based fecal occult blood test (gFOBT) and immunochemical FOBT (iFOBT) are non-invasive, simple to perform, and relatively cheap tests, and thus are the most commonly used tests for CRC screening [7]. Randomized controlled trials (RCT) have reported that sensitivity of iFOBTs for CRC detection ranged from 20% to 85% [5,8,9] and significant reductions in CRC mortality were observed [10,11]. However, their sensitivity is limited, especially for the detection of CRC precursors [12-14].

Recently miRNA epigenetic regulation has been highlighted as one of the pivotal regulatory mechanisms in tumor development and progression [15,16] and recent findings indicated that circulating miRNAs are useful non-invasive biomarkers for the detection of CRC [17]. However, only a few studies have assessed the capacity of plasma miRNAs alone or in combination with iFOBT to predict colon cancer. The aim of this study was to determine the diagnostic performance of selected miRNAs alone or in combination with FOBTs for CRC detection.

MATERIALS AND METHODS

Study population

In total, 307 samples were collected: 57 patients with CRC confirmed by pathohistological diagnosis and 125 healthy controls, and another group of 58 patients with advanced adenoma confirmed by pathohistological diagnosis and 67 healthy controls. Control patients were randomly selected and matched with respect to age and gender.

Patients with colorectal cancer, advanced colorectal adenomas, and colorectal neoplasm free controls were randomly selected from participants who underwent colonoscopy screening at Beijing Friendship Hospital between October 2012 and May 2017. Blood and stool samples of participants were taken about one week prior to screening colonoscopy. The histology was confirmed by two experienced pathologists and staged according to the tumor-node-metastasis (TNM) staging system of the International Union Against Cancer. Patients with a known history of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, any other tumors, and obvious inflammatory diseases were excluded.

This study was approved by the ethics committees of the Medical Boards of Beijing Friendship Hospital. Written informed consent was obtained from each participant.

Sample collection and laboratory measurements

All participants collected a stool sample from one bowel movement within a small container (20 mL) we had provided and kept it in a provided plastic bag in the freezer. Blood samples were collected in EDTA tubes prior to colonoscopy. Blood and stool samples were transported to the central laboratory via a cool chain. Blood samples were centrifuged at 2,123 x g for 10 minutes, and the supernatant was transferred into new tubes and stored at -80°C until further use.

A quantitative iFOBT (One-Step Fecal Occult Blood (FOB) Diagnostic Kit, Suzhou Hailu Biotech Co., Ltd, China) was performed. The cutoff value for iFOBT positivity given by the manufacturer is 0.2 µg/mL.

Total RNA containing small RNAs was extracted from 300 µL of plasma using Trizol LS reagent (Invitrogen, Carlsbad, CA, USA) followed by the miRNA easy Mini Kit (Qiagen, Hilden, Germany). RNA was then eluted in water and immediately stored at -80°C. Expression of 12 miRNAs highlighted in previous studies (miR-18a, miR-20a, miR-21, miR-29a, miR-92a, miR-106b, miR-133a, miR-143, miR-145, miR-181b, miR-342-3p, and miR-532-3p) [18,19] was measured using TaqMan MicroRNA Assays (Applied Biosystems) according to the manufacturer's protocol. In brief, for each sample qRT-PCR was performed in triplicate using a Light Cycler 480 Real-time PCR system (Roche Applied Science, Germany). Expression of miRNAs in CRC/advanced adenoma patients was calculated relative to the neoplasm free controls using the comparative cycle threshold (Ct) method. The average Ct value of the internal control miR-16 for every sample was subtracted from the Ct value for each respective miRNA reaction, yielding the ΔCt value [20,21]. All stool and blood tests were performed blinded with respect to diagnosis.

Statistical analysis

miRNA expression levels were compared between CRC/adenoma patients and neoplasm-free controls using the Wilcoxon-Mann-Whitney-Test. All tests were two-sided and p-values of 0.05 or less were considered to indicate statistical significance.

Logistic regression analyses were employed to assess the relationship between miRNA, combined miRNAs/miRNAs and iFOBT with colorectal neoplasms. The optimal multiple logistic regression model was based on a backward variable selection procedure in which the Akaike's information criterion (AIC) was applied [22]. Receiver operating characteristic (ROC) curves were constructed and the area under the ROC curves (AUC) were calculated both from unadjusted (apparent) and adjusted ("optimism-corrected") estimates of sensitivity and specificity to assess discrimination of the prediction model between patients with and without colorectal neoplasms. The .632+ bootstrap method (with 1,000 replicates) was used to adjust for overfitting of the apparent misclassification error and over-estimation of sensitivity and specificity by the unadjusted estimates [23,24]. Confidence intervals (95%) for the adjusted and unadjusted estimates were obtained using ordinary bootstrap analyses (with 1,000 replicates).

Markers exhibiting better discriminative power at 96.4% specificity were selected and with the same marker cutoff points we assessed the diagnostic perfor-

mance of all combinations of these markers. In combinations, samples with at least one marker above the cutoff were classified as positive. Further analyses of differences in test performance between CRC patients at different cancer stages and locations were performed. SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria) were used to conduct analyses. The R-packages "Daim" and "boot" were employed to perform bootstrap analyses [25,26].

RESULTS

Characteristic of study population

In total, 307 plasma and stool samples from CRC patients, advanced adenoma patients and neoplasm free controls were included in this study. Patients' characteristics are shown in Table 1.

Evaluation of individual markers

In our study, levels of miR-18a, miR-20a, miR-21, miR-92a, miR-133a, miR-143, and miR-145 expression were found to be significantly higher in the plasma of CRC patients than in that of neoplasm free controls (all $p < 0.05$, Figure 1), while miRNA expression levels were not observed to differ between advanced adenoma patients and neoplasm free controls.

ROC curves for CRC/advanced adenoma detection with miRNAs and iFOBT are presented in Figure 2A and 2B (adjusted estimates) and the AUCs are presented in Table 2. MiR-18a showed the largest adjusted AUC 0.610 (95% CI: 0.501 - 0.723) for CRC detection among the 12 investigated miRNAs. In addition, iFOBT showed the largest adjusted AUC 0.863 (95% CI: 0.752 - 0.928) for CRC detection and 0.612 (95% CI: 0.423 - 0.711) for advanced adenoma, among all tests.

Evaluation of combinations of markers for CRC detection

In this study, we aimed to examine whether a combination of markers (miRNAs and iFOBT) can detect CRC more sensitively and/or specifically than a single marker. For this purpose, a backward variable selection procedure was applied in the multiple logistic regression. The adjusted AUC of 6 selected miRNAs (miR-20a, miR-29a, miR-92a, miR-106b, miR-181b, and miR-342-3p) was 0.683 (95% CI: 0.576 - 0.783) in comparison to 0.621 (95% CI: 0.592 - 0.789) for all 12 tested miRNAs. The adjusted AUC of selected markers - iFOBT and miR-133a was 0.897 (95% CI: 0.815 - 0.936) in comparison to 0.839 (95% CI: 0.799 - 0.918) for all markers combined (Table 2 and Figure 2A). Next, we assessed whether any combination of markers achieved greater sensitivity. For this purpose, we determined a cutoff point for each marker yielding 96.4% sensitivity. In our study, 8 miRNAs (miR-18a, miR-20a, miR-21, miR-92a, miR-106b, miR-143, miR-145, and miR-181b) exhibited sensitivity above this cutoff. Next, we assessed the sensitivities and specificities of all combinations of these 8 miRNAs (247 different combinations including at least 2 miRNAs). In our study two combinations showed the best results: the combination of miR-21 and miR-92a, miR-106b and miR-143 achieved a sensitivity of 26.7% (95% CI: 13.3% - 41.4%) at a specificity of 88.3% (95% CI: 80.5% - 93.2%) while the combination of miR-21, miR-92a, miR-106b, miR-143, and miR-181b achieved a sensitivity of 26.9% (95% CI: 13.8% - 43.6%) at a specificity of 85.9% (95% CI: 76.1% - 91.0%).

Furthermore, we assessed the sensitivities and specificities of all miRNAs' combinations together with iFOBT. The best results were achieved by the combination of miR-143 and iFOBT, which reached a sensitivity of 78.9% (95% CI: 62.8% - 89.9%) at a specificity of 93.1% (95% CI: 86.5% - 96.3%), and the combination of miR-106b, miR-145, and iFOBT which reached a sensitivity of 81.2% (95% CI: 61.2% - 93.1%) at a specificity of 93.4% (95% CI: 84.3% - 93.8%, Table 3). Next, we examined the sensitivity for CRC detection stratified by stage and location at cutoffs yielding 97.8% specificity for iFOBT and miRNAs. Under these conditions iFOBT still achieved much higher sensitivity than miRNAs. However, the combination of 12 miRNAs and iFOBT improved the sensitivity of CRC detection. Furthermore, the sensitivity of iFOBT combined with miR-21, miR-92a, miR-106b, miR-143, and miR-181b and the combination of miR-106b, miR-145, and iFOBT were found to be significantly related to CRC stage and location ($p < 0.05$). Moreso, a combination of 12 miRNAs and iFOBT, was found to be a more sensitive predictor of tumors at late stages ($p < 0.05$). The sensitivity of the combined 12 miRNAs was significantly higher for proximal colon cancer than distal colon and rectal cancer (Table 4).

Evaluation of combinations of markers for advanced adenoma detection

Having identified combinations of markers that were sensitive and specific markers, we then constructed ROC curves and calculated the AUCs for discrimination between advanced adenoma patients and neoplasm free controls. The adjusted AUC for 12 miRNAs was 0.516 (95% CI: 0.436 - 0.742), and the adjusted AUC of the miR-20a, miR-29a, miR-92a, miR-106b, miR-181b, and miR-342-3p was 0.538 (95% CI: 0.438 - 0.713).

Next, iFOBT achieved an adjusted AUC of 0.635 (95% CI: 0.488 - 0.732), while the adjusted AUC when all miRNAs were included was 0.614 (95% CI: 0.558 - 0.828), and when only miR-133a was added it was 0.620 (95% CI: 0.512 - 0.745) (Figure 2B). The sensitivities of different advanced adenoma detection tests are presented in Table 3.

Table 1. Characteristics of the study population.

	Colorectal cancer patients	Healthy controls	Advanced colorectal adenoma patients	Healthy controls
	n (%)	n (%)	n (%)	n (%)
Gender				
Male	30 (52.6)	67 (53.6)	35 (60.3)	40 (59.7)
Female	27 (47.4)	58 (46.4)	23 (39.7)	27 (40.3)
Age				
Mean	68.5	66.8	63.3	62.1
≤ 65 years	18 (31.6)	38 (30.4)	28 (48.3)	38 (56.7)
> 65 years	39 (68.4)	87 (69.6)	30 (51.7)	29 (43.3)
Stage				
I	14 (24.6)			
II	18 (31.6)			
II	19 (33.3)			
III	5 (8.8)			
Not specified	1 (1.75)			
Location				
Proximal colon	18 (31.6)		28 (48.3)	
Distal colon and rectum	39 (68.4)		30 (51.7)	
Total	57	125	58	67

Table 2. iFOBT and miRNA AUC value for CRC detection.

Test	AUC (95% CI)						0.632+ bootstrap adjusted AUC (95% CI)					
iFOBT	0.891	(	0.794	-	0.939	)	0.863	(	0.752	-	0.928	)
miR-18a	0.597	(	0.568	-	0.742	)	0.610	(	0.501	-	0.723	)
miR-20a	0.609	(	0.553	-	0.721	)	0.597	(	0.434	-	0.686	)
miR-21	0.651	(	0.562	-	0.727	)	0.612	(	0.515	-	0.710	)
miR-29a	0.586	(	0.505	-	0.681	)	0.521	(	0.472	-	0.652	)
miR-92a	0.603	(	0.538	-	0.730	)	0.627	(	0.473	-	0.733	)
miR-106b	0.507	(	0.485	-	0.666	)	0.581	(	0.416	-	0.640	)
miR-133a	0.662	(	0.565	-	0.733	)	0.601	(	0.471	-	0.703	)
miR-143	0.591	(	0.534	-	0.718	)	0.607	(	0.480	-	0.691	)
miR-145	0.609	(	0.523	-	0.700	)	0.565	(	0.485	-	0.659	)
miR-181b	0.616	(	0.461	-	0.633	)	0.403	(	0.428	-	0.538	)
miR-342-3p	0.513	(	0.451	-	0.633	)	0.492	(	0.421	-	0.549	)
miR-532-3p	0.501	(	0.429	-	0.616	)	0.499	(	0.417	-	0.596	)
12 miRNAs	0.708	(	0.662	-	0.821	)	0.621	(	0.592	-	0.789	)
6 miRNAs * †	0.772	(	0.655	-	0.816	)	0.683	(	0.576	-	0.783	)
iFOBT+ 12 miRNAs	0.932	(	0.871	-	0.975	)	0.839	(	0.799	-	0.918	)
iFOBT+ miR-133a †	0.912	(	0.854	-	0.961	)	0.897	(	0.815	-	0.936	)

Abbreviations: iFOBT - immunochemical fecal occult blood test, AUC - area under receiver operating characteristics curves, CRC - colorectal cancer.
† The optimal multiple logistic regression model was based on a backward variable selection procedure in which the Akaike's information criterion (AIC) was applied.
*** miR-20a, miR-29a, miR-92a, miR-106b, miR-181b, miR-342-3p.**

Table 3. Diagnostic performances of miRNA, iFOBT, and different combinations of markers: sensitivity and specificity for advanced adenoma and colorectal cancer detection.

Test	Colorectal cancer (57 vs. 125)		Advanced adenoma (58 vs. 67)	
	Sensitivity% (95% CI)	Specificity% (95% CI)	Sensitivity% (95% CI)	Specificity% (95% CI)
iFOBT	69.3 (52.5 - 81.3)	96.6 (91.8 - 98.8)	26 (14 - 39.6)	97.2 (86.3 - 99.5)
miR-18a	2.7 (0.1 - 11.3)		11.8 (3.5 - 19.1)	
miR-20a	5.1 (0.5 - 14.5)		13.2 (4.7 - 25.3)	
miR-21	8.8 (2.4 - 20.4)		7.2 (1.3 - 17.2)	
miR-29a	0.3 (0 - 8.6)		5.1 (0.5 - 14.2)	
miR-92a	7.1 (1.3 - 17.5)		17.2 (7.5 - 30.2)	
miR-106b	7.5 (1.3 - 17.5)		11.7 (3.5 - 19.1)	
miR-133a	5.1 (0.5 - 14.5)		4.9 (0.5 - 14.2)	
miR-143	11.5 (3.6 - 23.1)		11.3 (3.5 - 19.1)	
miR-145	9.7 (2.4 - 20.4)		11.6 (3.5 - 19.1)	
miR-181b	5.3 (0.5 - 14.5)		11.7 (3.5 - 19.1)	
miR-342-3p	0.6 (0 - 8.6)		6.1 (0.5 - 14.2)	
miR-532-3p	3.2 (0.1 - 11.3)		5.3 (0.5 - 14.2)	
miR-21, 92a, 106b, and 143	26.7 (13.3 - 41.4)	88.3 (80.5 - 93.2)	17.6 (7.5 - 30.2)	69.1 (53.3 - 80.5)
miR-21, 92a, 106b, 143, and 181b	26.9 (13.8 - 43.6)	85.9 (76.1 - 91.0)	11.3 (3.5 - 19.1)	86.1 (68.6 - 91.4)
miR-143 and iFOBT	78.9 (62.8 - 89.9)	93.1 (86.5 - 96.3)	42.9 (29.5 - 58.8)	78.1 (61.8 - 87.0)
miR-106b, miR-145, and iFOBT	81.2 (61.2 - 93.1)	93.4 (84.3 - 93.8)	37.7 (24.0 - 52.7)	91.5 (80.8 - 97.8)

Abbreviations: iFOBT - immunochemical fecal occult blood test, CRC - colorectal cancer.

Table 4. Sensitivities of iFOBT and miRNAs at given levels of specificity for the detection of advanced adenoma and colorectal cancer.

	Number	Sensitivity at cutoff point yield 97.8% specificity % (95% CI)				
		iFOBT	12 miRNAs	miR-21, 92a, 106b, 143 and 181b	iFOBT+12 miRNAs	iFOBT+miR-145+miR-106b
Advanced adenoma	58	30.1 (18.7 - 46.3)	16.9 (9.0 - 32.6)	17.6 (7.5 - 30.2)	24.1 (13.6 - 39.6)	30.1 (16.6 - 44.1)
CRC	57	65.1 (50.7 - 79.1)	11.9 (4.8 - 25.7)	16.8 (7.7 - 30.8)	75.9 (62.0 - 87.7)	61.6 (52.9 - 80.9)
CRC stage TNM I + II	32	58.1 (38.8 - 77.6) [a]	20.9 (8.6 - 42.3)	19.6 (6.3 - 38.1) [a]	61.6 (46.0 - 83.5) [a]	61.8 (42.4 - 80.6) [a]
CRC stage TNM III + IV	24	75.1 (48.8 - 91.0) [a]	20.5 (6.1 - 45.6)	15.1 (3.2 - 29.8) [a]	85.1 (60.4 - 96.6) [a]	77.9 (54.4 - 94.0) [a]
CRC location						
Proximal colon	18	50.9 (25.1 - 80.8) [a]	31.7 (9.1 - 61.4) [a]	31.2 (9.1 - 61.4) [a]	68.8 (38.6 - 90.9)	52.1 (25.1 - 80.8) [a]
Distal colon and rectum	39	68.6 (51.3 - 84.4) [a]	14.9 (5.1 - 31.9) [a]	16.1 (5.1 - 31.9) [a]	68.6 (51.3 - 84.4)	73.4 (54.5 - 86.7) [a]

Abbreviations: CRC - colorectal cancer, iFOBT - immunochemical fecal occult blood test.
[a] $p < 0.05$ by chi-square test.

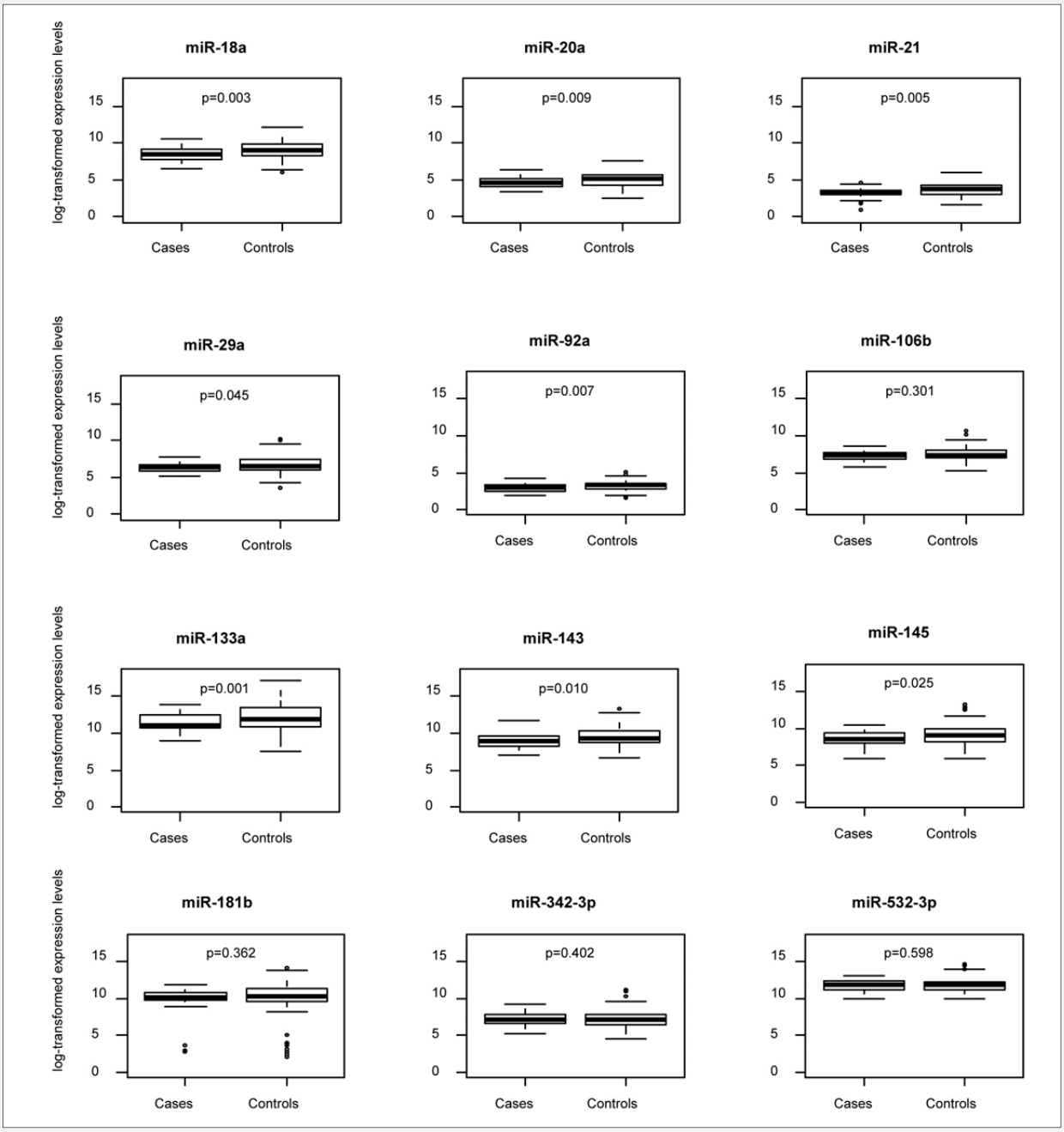

Figure 1. Expression levels of selected 12 miRNAs in 57 CRC patients and 125 neoplasms-free controls.

DISCUSSION

In recent years, miRNAs have been identified as key regulators of development and progression in many different cancers. Several tissue-based studies have shown that miRNAs can predict tumor tissue of origin, patient outcome, as well as the response to anti-tumor therapy [27,28]. However, the predictive role of specific circulating miRNAs in cancer remains to be well characterized. Since miRNAs are quite small, relatively stable, and can easily be detected by PCR, which is widely available in most laboratories, they represent good candidates for a new class of biomarkers.

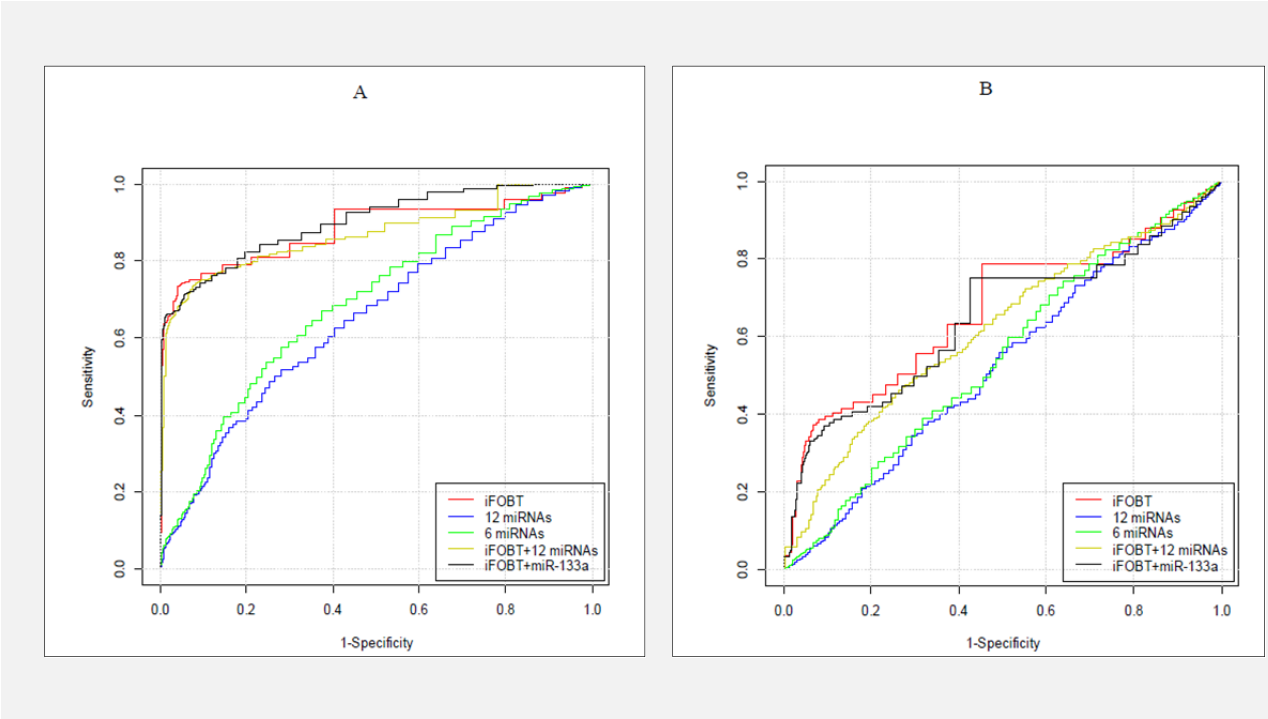

Figure 2. Area under the receiver operating characteristics curve for iFOBT and miRNAs for the detection of colorectal cancer (A) and colorectal advanced adenoma (B).

Early detection of colorectal cancer, or better yet adenomas, remains the best preventive measure to reduce CRC mortality. In this study, we aimed to compare the usefulness of plasma miRNA signatures and FOBTs for the detection of advanced adenomas and CRCs. Based on the results of our previous studies we selected 12 miRNAs for this study (five miRNAs identified to be differentially expressed from our own research (miR-29a, -106b, -133a, -342-3p, -532-3p) and seven miRNAs reported to be differentially expressed in the literature (miR-18a, -20a, -21, -92a, -143, -145, -181b)) [18,19]. The levels of these miRNAs have been reported to be significantly higher in the plasma of CRC patients than in neoplasm free controls. This is not surprising since miR-18a, miR-20a, and miR-92a belong to miR17-92 cluster which is often amplified in colorectal cancer [29]. Moreover, serum levels of miR-21 and miR-29a, the latter of which was not altered in our study, were reported to be higher in precancerous lesions and early stages of colorectal cancer than in healthy controls. In the mentioned study, AUC values for serum miR-21, miR-29a, miR-125b, and their combined score were reported to be 0.706, 0.741, 0.806, and 0.827, respectively [30]. Brunet Vega et al. reported that miR-18a and miR-29a levels were also higher in the sera of stage III CRC patients than in healthy controls. Furthermore, plasma levels of miR-18a were found to be higher in CRC patients, distinguishing CRC patients from the healthy controls (AUC 0.804) [31].

Liu et al. reported that miR-21 and miR-92a serum levels were higher in patients with advanced adenomas and CRC than in controls. For miR-21 it was 0.802 (for CRC) and 0.709 (for advanced adenomas), while for miR-92a an AUC of 0.786 (for CRC) and 0.701 (for advanced adenomas) was reported. In combination, both miRNAs had an AUC of 0.847 for discriminating CRCs and 0.722 for discriminating advanced adenomas [32]. As we observed, Chen et al. also reported that miR-20a serum levels were higher in CRC patients [33]. A recent meta-analysis of the diagnostic value of miR-29a reported a sensitivity of 0.59 and specificity of 0.89 with an AUC of 0.9128 and 0.8453 [34]. Recently, Zekri et al. reported that serum levels of miR-17, miR-19a, miR-20a, and miR-223 were potential diagnostic biomarkers of CRC, while among the other expression profiles examined, expression of miR-18a, miR-21, and miR-92a were not associated with CRC.

In our study, the diagnostic performance of 12 examined miRNAs was found to be inferior to that of FOBTs for the detection of advanced adenomas and CRC. Indeed, in our study the sensitivities of 12 miRNAs were found to be less than 15% for CRC detection, in comparison to 69.3% for iFOBT. Furthermore, the sensitivities of these miRNAs for advanced adenoma detection were also lower than that observed for iFOBT. Nevertheless, the combination of miR-20a, miR-29a, miR-92a, miR-106b, miR-181b, and miR-342-3p exhibited higher sensitivity for CRC/advanced adenoma detec-

tion. In addition, ROC curve analyses demonstrated a slight improvement of this combination of miRNAs when compared to iFOBT for CRC detection; however, this was not the case for the detection of advanced adenomas.

Huang et al. studied 37 advanced adenoma patients, 100 CRC patients, and 59 healthy controls and observed that miR-29a and miR-92a achieved 83% sensitivity and 84.7% specificity as a cutoff point for CRC detection, while 73% sensitivity and 79.7% specificity was reported as a cutoff point for advanced adenoma [35]. Furthermore, Ng reported that miR-92a achieved 89% sensitivity and 70% specificity at a cutoff point for CRC detection based on 90 CRC patients and 50 healthy controls [36].

Our findings compare favorably with alternative blood markers for the detection of CRC such as C-reactive protein (CRP), serum CD26 (sCD26), complement C3a anaphylatoxin and tissue inhibitor of metalloproteinase 1 (TIMP-1) and CEA [37-39]. Although individual miRNAs were not accurate diagnostic tools for CRC/advanced adenoma detection, the combination of miR-21, miR-92a, miR-106b, miR-143, and miR-181b detected CRC at a sensitivity of 16.8% at a cutoff point yielding 97.8% specificity. ROC curve analyses confirmed a modest benefit of combining miRNAs with iFOBT for the detection of CRC. Thus, combining multiple tests represents a promising approach for further research. Nevertheless, the effectiveness of a diagnostic test depends on its diagnostic and economic performance, as well as the extent of the compliance.

The combined signature of several differentially expressed miRNAs is probably a more informative and reliable marker of CRC in patients than any individual marker. The advantage of our study is that all included participants underwent colonoscopy, which minimizes potential misclassification of tumor patients into the control group. Furthermore, direct comparison and a combination of different tests was feasible in our study, since all tests were applied to the same set of individuals. Finally, bootstrap techniques were applied to correct for overestimation. However, the proportion of early stage (stage I and II) CRC patients in our study was lower than the proportion in a true screening setting, which may have led to overestimation of the overall sensitivity. Another limitation of this study is that patients with non-advanced adenoma and hyperplastic polyps might be present in the neoplasm free control group, which would decrease test specificity at given cutoff points. Besides, miR-16 was used as an internal control in this study, as it has been in many other miRNA studies, but concerns have recently been raised due to its inconsistent expression in sera [40].

CONCLUSION

Taken together, our results indicate that 7 miRNAs were dysregulated in the plasma of CRC patients when compared with neoplasm free controls. However, the diagnostic performance of these miRNAs was inferior to that of iFOBT for CRC or advanced adenoma detection. Nevertheless, the combination of plasma miRNAs with iFOBT slightly improved CRC detection. Although the plasma miRNAs investigated in our study do not appear to represent an optimal alternative for non-invasive CRC screening, their performance could be improved by addition of further promising markers. Additionally, beyond those assessed in this study, many more miRNAs deserve further attention and research.

Financial Support:
The research was supported by the National Key Technologies R&D Program (2015BAI13B09).

Declaration of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

References:

1. Ferlay J, Soerjomataram I, Ervik M, et al. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v 1.0 IARC CancerBase No. 11. International Agency for Research on Cancer, Lyon. France 2013. http://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012

2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90 (PMID: 25651787).

3. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017 Apr;66:683-91 (PMID: 26818619).

4. He J, Efron JE. Screening for colorectal cancer. Adv Surg. 2011; 45:31-44 (PMID: 21954677).

5. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the us multi-society task force on colorectal cancer, and the American college of radiology. CA Cancer J Clin. 2008 May-Jun;58(3):130-60 (PMID: 18322143).

6. Lieberman DA. Clinical practice. Screening for colorectal cancer. N Engl J Med. 2009;361:1179-87 (PMID: 19759380).

7. Song LL, Li YM. Current noninvasive tests for colorectal cancer screening: An overview of colorectal cancer screening tests. World J Gastrointest Oncol. 2016;8:793-800 (PMID: 27895817).

8. Whitlock EP, Lin JS, Liles E, Beil TL, Fu R. Screening for colorectal cancer: A targeted, updated systematic review for the U.S. Preventive services task force. Ann Intern Med. 2008;149:638-58 (PMID: 18838718).

9. Senore C, Ederle A, Benazzato L, et al. Offering people a choice for colorectal cancer screening. Gut. 2013;62:735-40 (PMID: 22442162).

10. Libby G, Brewster DH, McClements PL, et al. The impact of population-based faecal occult blood test screening on colorectal cancer mortality: A matched cohort study. Br J Cancer. 2012;107:255-9 (PMID: 22735907).

11. Pox C. Colon cancer screening: Which non-invasive filter tests? Dig Dis. 2011;29 Suppl 1:56-9 (PMID: 22104755).

12. Levi Z, Rozen P, Hazazi R, et al. A quantitative immunochemical fecal occult blood test for colorectal neoplasia. Ann Intern Med. 2007;146:244-55 (PMID: 17310048).

13. Lieberman DA, Weiss DG; Veterans Affairs Cooperative Study Group 380. One-time screening for colorectal cancer with combined fecal occult-blood testing and examination of the distal colon. N Engl J Med. 2001;345:555-60 (PMID: 11529208).

14. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. N Engl J Med. 2004;351:2704-14 (PMID: 15616205).

15. Frixa T, Donzelli S, Blandino G. Oncogenic microRNAs: Key players in malignant transformation. Cancers (Basel). 2015;7:2466-85 (PMID: 26694467).

16. Chan SH, Wang LH. Regulation of cancer metastasis by microRNAs. J Biomed Sci. 2015 Jan 23;22:9 (PMID: 25614041).

17. Clancy C, Joyce MR, Kerin MJ. The use of circulating micrornas as diagnostic biomarkers in colorectal cancer. Cancer Biomarkers. 2015;15:103-13 (PMID: 25547322).

18. Luo X, Stock C, Burwinkel B, Brenner H. Identification and evaluation of plasma micrornas for early detection of colorectal cancer. PLOS One. 2013;8:e62880 (PMID: 23690963).

19. Luo X, Burwinkel B, Tao S, Brenner H. MicroRNA signatures: Novel biomarker for colorectal cancer? Cancer Epidemiol Biomarkers Prev. 2011;20:1272-86 (PMID: 21551242).

20. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative c(t) method. Nat Protoc. 2008;3:1101-8 (PMID: 18546601).

21. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 2008;105:10513-8 (PMID: 18663219).

22. Akaike H. Statistical predictor identification. Ann Inst Math Stat. 1970;22:203-17. https://link.springer.com/article/10.1007%2FBF02506337

23. Adler W, Lausen B. Bootstrap estimated true and false positive rates and roc curve. Comput Stat Data An. 2009;53:718-29. https://www.sciencedirect.com/science/article/pii/S016794730800460X?via%3Dihub

24. Efron B, Tibshirani R. Improvements on cross-validation: The .632+ bootstrap method. J Amer Statistical Assoc. 1997;92:548-60. https://www.tandfonline.com/doi/abs/10.1080/01621459.1997.10474007

25. Canty A, Ripley B: Boot: Bootstrap r (s-plus) functions. R package version 1.3-18. Available from http://cran.R-project.Org/package=boot (accessed: 30 Jul 2017).

26. Potapov S, Adler W. Daim: Diagnostic accuracy of classification models. R package version 1.1.0. Available from: https://cran.r-project.org/web/packages/Daim/ (Accessed: 13 Sep 2013).

27. Liu HT, Gao P. The roles of microRNAs related with progression and metastasis in human cancers. Tumour Biol. 2016:1-15 (PMID: 27714675).

28. Fiorucci G, Chiantore MV, Mangino G, Percario ZA, Affabris E, Romeo G. Cancer regulator microRNA: Potential relevance in diagnosis, prognosis and treatment of cancer. Curr Med Chem. 2012;19:461-74 (PMID: 22204349).

29. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, et al. Mir-17-92 cluster is associated with 13q gain and c-myc expression during colorectal adenoma to adenocarcinoma progression. Br J Cancer. 2009;101:707-14 (PMID:19672269).

30. Yamada A, Horimatsu T, Okugawa Y, et al. Serum mir-21, mir-29a, and mir-125b are promising biomarkers for the early detection of colorectal neoplasia. Clin Cancer Res. 2015;21:4234-42 (PMID: 26038573).

31. Brunet Vega A, Pericay C, Moya I, et al. MicroRNA expression profile in stage III colorectal cancer: Circulating miR-18a and miR-29a as promising biomarkers. Oncol Rep. 2013;30:320-6 (PMID: 23673725).

32. Liu GH, Zhou ZG, Chen R, et al. Serum mir-21 and mir-92a as biomarkers in the diagnosis and prognosis of colorectal cancer. Tumor Biol. 2013;34:2175-81 (PMID: 23625654).

33. Chen WY, Zhao XJ, Yu ZF, et al. The potential of plasma miRNAs for diagnosis and risk estimation of colorectal cancer. Int J Clin Exp Pathol. 2015;8:7092-101 (PMID: 26261602).

34. Zhi ML, Liu ZJ, Yi XY, Zhang LJ, Bao YX. Diagnostic performance of microrna-29a for colorectal cancer: A meta-analysis. Genet Mol Res. 2015;14:18018-25 (PMID: 26782449).

35. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer. 2010;127(1):118-26 (PMID: 19876917).

36. Ng EK, Chong WW, Jin H, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009;58(10):1375-81 (PMID: 19201770).

37. Tao S, Haug U, Kuhn K, Brenner H. Comparison and combination of blood-based inflammatory markers with faecal occult blood tests for non-invasive colorectal cancer screening. Br J Cancer. 2012;106:1424-30 (PMID: 22454079).

38. Shimwell NJ, Wei W, Wilson S, et al. Assessment of novel combinations of biomarkers for the detection of colorectal cancer. Cancer Biomark. 2010;7:123-32 (PMID: 21263188).

39. Nielsen HJ, Brunner N, Jorgensen LN, et al. Plasma timp-1 and cea in detection of primary colorectal cancer: A prospective, population based study of 4,509 high-risk individuals. Scand J Gastroenterol. 2011;46:60-9 (PMID: 207999110).

40. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One. 2009;4:e6229 (PMID: 19597549).