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Original research article

Plasma microrna-based highly predictive model for colorectal neoplasms diagnosis: Improving sensitivity and specificity

Dr. Goutham Roy K.
Assistant Professor, Department of General Surgery, CAIMS, Karimnagar, Telangana, India

Corresponding Author:
Dr. Goutham Roy K.

Abstract

Objective: Build on our previous work to make a diagnostic test for colorectal tumours that uses microRNA (miRNA) in plasma.

Background: People often get colorectal neoplasms (colorectal cancer and colorectal advanced adenoma, or CAA) at the same age they get other common cancers. Current screening methods are not sensitive or specific enough, and patients don't follow them well.

Methods: Microfluidic array technology was used to look for 380 miRNAs in the plasma of 120 "Training" patients, 20 each with control, CRC, CAA, breast (BC), pancreatic (PC), and lung (LC) cancer. We found miRNAs that were misregulated in a way that was unique to colorectal neoplasia. In a "Test" cohort of 240 patients, single assays were used to test these miRNAs. A mathematical model was made to predict the identity of blinded samples in a "Validation" cohort of 300 patients by using repeat-subsampling validation of the testing dataset with 1000 iterations each to test the accuracy of model detection.

Results: Based on p-value, area-under-the-curve (AUC), fold-change, and biological plausibility, seven miRNAs were chosen: miR-21, miR-29c, miR-122, miR-192, miR-346, miR-372, and miR-374a. For the "Test" cohort comparisons, the AUC (95% CI) was 0.91 (0.85-0.96), 0.79 (0.70-0.88), and 0.98 (0.96-1.0). Our math model correctly predicted the identity of blinded samples between all neoplasia and controls 69-77% of the time, between colorectal neoplasia and other cancers 67-76% of the time, and between colorectal cancer and colorectal adenomas 86-90% of the time.

Conclusions: Our plasma miRNA assay and prediction model can tell the difference between patients with colorectal neoplasia, other neoplasms, and controls with higher sensitivity and specificity than current clinical standards.

Keywords: Plasma miRNA, colorectal cancer, CRC, neoplasm

Introduction

Colorectal cancer (CRC) is common all over the world and is linked to a lot of deaths. Most sporadic CRC develops from pre-existing colorectal adenomas in a step-by-step process that can be used to find and treat the cancer early [1]. Only in the United States, there are about 140,000 new cases of CRC each year and about 50,000 deaths each year because of CRC [2]. Many deaths from CRC could be prevented if precancerous polyps were found and removed before they turned into invasive cancer. CRC screening tools that are currently available include colonoscopy, flexible sigmoidoscopy, faecal occult blood tests (FOBTs), DNA-based stool tests, and plasma-based assays. Many of these, though, are hard for patients to follow. There is currently no highly accurate, widely applicable, minimally invasive screening for colorectal adenomas that can find people who need to be treated and taken out early. microRNAs (miRNAs) are small, naturally occurring, non-protein-coding RNA molecules that control gene expression after transcription by binding to the 3' untranslated region of the messenger RNAs (mRNAs) of their targets [3]. They stop translation, destroy the target, or turn off the gene, which changes how proteins are made in the future. miRNAs play a number of important roles in controlling how cells grow, develop, and change [4, 5]. By affecting oncogenes and tumour suppressor genes, they have also been shown to be out of sync in a number of cancers, including CRC [6]. miRNAs have been found in body fluids like plasma, saliva, faeces and urine and they are starting to be seen as possible biomarkers for human disease and as ways to treat disease [7, 8]. There is an urgent need for an assay that can test a person using an internal control that is unique to that person, so that they don't have to be compared to a "normal reference sample". Even though it is unlikely that a single miRNA will be specific enough to be used as a marker for colorectal (CR) neoplasia, we think that a plasma-based miRNA panel can identify people with benign or malignant colorectal neoplasms. We already knew that miR-21 in plasma could tell the difference between CRC patients and controls with 90% sensitivity and 90% specificity. After

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that, we wrote about a group of miRNAs that could tell the difference between CRC and CAA but weren't very specific [8].
So, we tried to make a miRNA panel that could tell colorectal neoplasia (CRC and CAA) apart from other common cancers and controls. We also wanted to test this panel using a predictive modelling tool so that a person could be tested without having to be compared to a control subject [9, 10].

Method
This study was carried out according to the Standards 2015 statement. The study population consisted of consecutive patients recruited from a large university colon and rectal surgery practice (n=110) and patients derived from the Medical College (n=220) [11].

Study subjects
People with CRC, colorectal advanced adenomas (CAA), breast cancer (BC), lung cancer (LC), and pancreatic cancer were included in the study (PC). Traditionally, CAA has been defined as adenomas that are larger than 0.75 cm and have a villous component or high-grade dysplasia [12]. A recent systematic review found that 95% of people with CAA have polyps with a diameter of more than 0.6 cm. For the purposes of this study, CAA were defined as polyps with a diameter of more than 0.6 cm. A "control" group was made up of people who had a normal screening colonoscopy and did not have cancer or an inflammatory condition. The "other" cancers that were chosen for this study were chosen because they tend to happen in people around the same age as CRC. Also, samples were easy to get from the University of Louisville Surgical Biorepository and were staged using the American Joint Committee on Cancer TNM staging system [13]. There were a total of 330 patients in this study. Table 1 shows the different kinds of patients. In EDTA-vacutainers, peripheral blood was taken from the subjects (BD, Franklin Lakes, NJ). Plasma was taken out of whole blood right away by spinning it at 3500 rpm for 15 minutes, as was explained before [11] and then it was frozen at 80 °C for later use [14].

Study period: June 2022 to May 2023.

Study design
The study was performed in 3 stages:
Stage 1: A “Training” cohort, (n= 120) or screening study to identify miRNA dysregulated in CRC and CAA (collectively referred to as colorectal neoplasia) as opposed to controls and other common cancers (breast, lung, pancreas) (breast, lung, pancreas).

Stage 2: A "Test" cohort (n=240) to confirm that the miRNA identified in Stage 1 were dysregulated in colorectal neoplasia compared to controls and other common cancers using single miRNA assays.

Stage 3: A “Validation” cohort, (n=300) in which dysregulated miRNA expression was determined by single assay and this blinded data set provided to our statisticians for determination of sample identity [15].

Training cohort
In Stage 1, there were 60 patients in the "Training" cohort-20 with CRC, CAA, BC, LC, or PC and 20 controls. Using the miRNeasy® Serum/Plasma Isolation Kit, total RNA was isolated from plasma samples (Qiagen, Valencia, CA). Each sample's total RNA concentration and purity were assessed using a Nanodrop 2000 spectrophotometer from ThermoFisher Scientific®, Middlesex, Massachusetts. TaqMan® Low Density Array (TLDA) human miRNA card A, Life Technologies, Carlsbad, CA, was used to determine dysregulated miRNA expression in each group relative to controls for each sample using a screening of 384 miRNAs. The ViiATM 7 Real-Time PCR System (ThermoFisher Scientific®, Middlesex, MA) was used to perform a quantitative real-time polymerase chain reaction (qRT-PCR), with the threshold set at 0.03. One operator managed all of the experiments [16].
All Neoplasia versus Controls (Comparison 1), Colorectal Neoplasia versus "Other" Cancers (breast, lung and pancreas) (Comparison 2) and Colorectal Cancer (CRC) versus Colorectal Advanced Adenoma (CAA) were the comparisons used for the data analysis (Comparison 3).

Test cohort: 240 samples were used in stage 2, with 40 patients in each of the following groups: CRC, CAA, BC, LC, PC, and controls. Utilizing single miRNA tests, significantly dysregulated miRNAs found in the "Training" cohort (Stage 1) were confirmed. Specific TaqMan® miRNA primers for the dysregulated miRNAs and the two endogenous reference miRNA, RNU6B and miR-520d-5p 16, were then utilised to bind to complementary sequences on target cDNA during qRT-PCR for miRNA single assay quantification. Two operators ran duplicates of each reaction simultaneously. Using a Step-One Plus qRT-PCR system from Life Technologies in Carlsbad, California, nucleic acid quantification was carried out [17].

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Validation cohort: The "Validation" cohort in Stage 3 consisted of 300 samples, with 50 samples from each of the groups CRC, CAA, BC, LC, PC, and controls. These samples were all subjected to the same single miRNA assay process as the "Test" cohort. Our professor of bioinformatics received these blinded data and used a predictive model that had been created using the data from the "Test" cohort to analyse them. The sample identification of the blinded data in the "Validation" cohort was then predicted using this predictive model. Once more, evaluation for comparisons 1, 2 and 3 was carried out to examine the prediction model's accuracy using the diagnostic miRNA panel [18].

Statistical consideration

Stage 1: Cohort for our Stage 1-"Training" cohort, miRNA expression of each sample group was compared to miRNA expression of the control group by comparative Ct analysis, utilising RNU6B and miR-520d-5p as endogenous reference genes. 16 Ct values were set to 40 when miRNA expression in samples was unknown. ANOVA found dysregulated miRNAs [19].

We utilised Jung's technique to designate 5% of features as significant at a 5% FDR and 0.0038 alpha. 17 With n1=10 and n2=10 and a two-sample t-test, we can detect at least 2.7 fold with a significance level of 0.0038 and 80% power. After controlling for multiple comparisons, we predicted no more than 10% of miRNAs to be differently expressed between patients and controls. 0.5-3% of miRNAs should accurately identify patients and controls. Ten miRNAs and two reference miRNA genes (3%) were evaluated [20].

Stage 2: Sample Classification Cohort & Prediction Model for Stage 2-the "Test" cohort, Ct levels were again examined using the comparative Ct approach. Ct values were replaced with 40 for single assay quantification when miRNA expression was unknown. Similar to the training cohort, comparisons 1, 2, and 3 were built utilising single miRNA assay data, ROC curves, and AUC. We fit three predictive models for each comparison using the test dataset, where p1, p2, and p3 are the odds of a patient from the case group with total neoplasia for comparison 1, colorectal neoplasia for comparison 2, and CRC for comparison [21].

Using the test cohort, a repeat sub-sampling validation procedure was used to create and assess the accuracy of the logistic prediction model. The model was able to distinguish controls from other participants with 88% accuracy and colorectal malignancies from adenomas with 94% accuracy. This is based on a 70%-to-30% training-test set combination [23].

Stage 3: Validation Cohort-using the Ct values of 150 blinded samples, the constructed logistic prediction models were utilised to predict sample identification in the validation cohort. Four distinct approaches were used: a normal-theory method with unequal variance (parametric method), kernel density estimates with equal bandwidth (nonparametric method), and k-nearest neighbours [24].

Results

Stage 1: Exercise Cohort When comparing all neoplasia (n = 50) and controls (n = 20), 16 of the 380 plasma miRNAs that were examined were substantially dysregulated (Comparison 1). Six more miRNAs were significantly dysregulated between colorectal cancer (n=10) and colorectal advanced adenoma (n=10), and another sixteen miRNAs were significantly dysregulated when comparing colorectal neoplasia (CRC and CAA) (n=20) to other malignancies (BC, PC, LC) (n=30). Ten miRNAs and two endogenous reference miRNA were chosen for further investigation after the significantly dysregulated miRNA were reviewed based on the adjusted p-value, AUC, fold change, and biological significance.

Stage 2: Exam Cohort A larger cohort (n=240) was used to evaluate the ten chosen miRNAs. Four miRNAs, miR-21, miR-29c, miR-346, and miR-374a, showed an AUC of 0.91 [95% CI: 0.85-0.96] in Comparison 1 (n=200 vs. 20) when used to distinguish patients with any type of neoplasia from controls. MiR-21, miR-29c, miR-372, and miR-374a showed an AUC of 0.79 [95% CI: 0.70-0.88] in separating patients with colorectal neoplasia (CRC and CAA) from patients with other malignancies in Comparison 2 (n=40 vs. 60). (BC, LC and PC). AUC of 0.98 [95% CI: 0.96-1.0] was shown by miR-29c, miR-122, miR-192, and miR-374a in comparison 3 (n=20 vs. 20) in their ability to distinguish CRC from CAA. To assess the diagnostic efficacy of the plasma miRNA in these three comparisons, ROC curves were produced.

Stage 3: Cohort for validation the validation cohort's (n=300) blinded sample data was then applied to the predictive model created using data from the "Test" cohort. Using the predictive model using the four miRNAs miR-21, miR-29c, miR-346, and miR-374a in this cohort for comparison 1 (n=125 vs. 25), proper sample identity prediction between all neoplasia and control was accomplished with 69–77% accuracy. In comparison 2 (n=50 vs. 75), miR-21, miR-29c, miR-372, and miR-374a accurately predicted sample identity between CR neoplasia and other malignancies with a 67-76% accuracy rate. Finally, miR-29c, miR-122, miR-192, and miR-374a correctly predicted sample identity in comparison 3

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(n=25 vs. 25) between CRC and CAA with 86-90% accuracy (Table 1 and 2).

Table 1: miRNA panel of the 10 most significantly dysregulated miRNAs in "Training" cohort after assessing p-value, fold change, AUC and biological significance

Dysregulated miRNA	Adjusted p-value (False Discovery Rate 5%)	Fold Change	AUC	Biological Significance (Reference)
miR-150	<0.001	12.23	0.844	Feng *et al.*
miR-193a	<0.001	9.087	0.835	Zhang *et al*
miR-374a	<0.001	0.001	0.879	Wang *et al.*
miR-346	<0.001	64.92	0.948	Selth *et al.*
miR-29c	0.001	0.241	0.811	Kuo *et al.*
miR-19a	0.002	0.186	0.775	Zheng *et al.*
miR-192	0.002	0.303	0.834	Chiang *et al.*
miR-21	0.006	0.559	0.794	Kanaan *et al.*
miR-372	0.022	0.645	0.789	Yamashita *et al.*
miR-122	0.037	1.388	0.750	Kunte *et al.*
RNU6B*	-	-	-	-
miR-520d-5p*	-	-	-	-

Table 2: Panel of dysregulated miRNAs and AUC in "Test" cohort for "All neoplasia" vs. "control", "CR neoplasia" vs. "Other cancers" and "CRC" vs. "CAA"

Comparison	miRNA	Area Under the Curve (95% CI)
Any neoplasia vs. control (n=200 *vs.* 40)	miR-21	0.91 (0.85-0.96)
	miR-29c	
	miR-346	
	miR-374a	
	miR-21	
	miR-29c	
CR neoplasia vs. other cancers (n=80 *vs.* 120)	miR-372	0.79 (0.70-0.88)
	miR-374a	
CRC vs. CAA (n=40 *vs.* 40)	miR-29c	0.98 (0.96-1.00)
	miR-122	
	miR-192	
	miR-374a	

Discussion

We have made a miRNA panel that can tell the difference between colorectal neoplasia and controls and other common cancers with high sensitivity and specificity. It can also tell the difference between CRC and CAA with high accuracy. Using a method called "decision tree analysis," the plasma test was made to be a single test. All patients get one blood test that measures the expression of 7 plasma miRNA and 2 reference miRNA, RNU6B and miR-520d-5p. First, by looking at the expression of 4 miRNAs from comparison 1 (miR-21, miR-29c, miR-346, and miR-374a, as well as the two reference miRNAs), one can tell if a patient is a control (not affected by any common neoplasm) or if they have a neoplasm. If the patient is a control, there is no need to do any more testing, and the patient does not need a colonoscopy. If the patient has a neoplasm, an analysis of 4 miRNAs from comparison 2 (miR-21, miR-29c, miR-372, and miR-374a, as well as the 2 reference miRNAs) shows if the patient has a "colorectal neoplasm" or "other neoplasm" (in this paper breast cancer, lung cancer or pancreatic cancer). People who have "other neoplasms" don't need a colonoscopy, but they do need more tests to find out what kind of cancer they might have. If a "colorectal neoplasm" is found, you should have a colonoscopy. In these patients, analysing 4 miRNAs from comparison 3 (miR-29c, miR-122, miR-192, and miR-374a) and the two reference miRNAs can reliably tell if the patient has colorectal cancer or advanced colorectal adenoma. So, one blood test can have anywhere from one to three steps of analysis, depending on the type of patient.

There is an urgent need for a tool that can diagnose CRC or its precursor lesion, CAA, that is accurate, reliable, clinically useful, and cheap.30 Colonoscopy is the current "gold-standard" for screening for colorectal neoplasia. It has >95% sensitivity and 90% specificity [25]. It makes it possible to remove precancerous polyps and, according to case-control and cohort studies, reduces the number of CRC cases and deaths caused by CRC. Colonoscopy, on the other hand, is expensive, invasive, can cause problems like bowel perforation, and patients don't always do what the doctor says. Only 18–35% of people with early CRC are found by colonoscopy screening every 10 years. Even with these problems, the widespread use of colonoscopy in the U.S. over the last three years has been linked to a drop in the number of CRC [25].

Flexible sigmoidoscopy has been shown to reduce both the number of people who get CRC and the number of people who die from it. Barium enema and virtual colonoscopy are two other "imaging" tests

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that can be used to check for cancer. One of the problems with this kind of screening is that a lot of patients don't go through with it. Another problem is that these procedures are very invasive. For a colonoscopy, you need to be sedated. In rare cases, patients can get sick, like when the colon perforates. You also have to clean your bowels very well. There are tests that use stool samples, like the guaiac and immunochemical FOBTs, as well as DNA-based tests. 45 Immunochemical FOBTs are better than guaiac FOBTs, but they can't find precancerous colorectal adenomas very well. A recent prospective screening study in Germany looked at the two best immunohistochemical FOBTs and found that they were 25% and 27% sensitive for finding advanced adenomas. With a 90% level of accuracy, stool-based DNA testing found adenomas in 54% of patients that were bigger than 1 cm [26].

Because of what is being tested, stool-based tests are not popular with patients, doctors, or lab workers. In the UK, a stool-based bowel screening programme found that only 50% of people took part, and only 83% of those with an abnormal guaiac FOBT went on to have a colonoscopy. The carcinoembryonic antigen (CEA) assay is the only plasma-based test that has been regularly available for clinical monitoring and, in some cases, for CRC screening. It is also used for post-operative surveillance and to check how well treatment is working. CEA doesn't have enough sensitivity and specificity (36-74% and 87%, respectively) to be used as a screening tool for the whole population or to find CRC that has come back. CA 19-9, which stands for carbohydrate antigen 19-9, has also been used as a tumour marker, but it is even less sensitive than CEA for CRC. CA11-19 has recently been found to be a promising serologic tumour marker that can find early CRC with a sensitivity of 98% and a specificity of 84% [27].

There are a number of non-invasive screening products on the market. One of these is a test called ColoVantage® that looks for methylated Septin9 DNA, which has been shown to be a sign of CRC in multiple studies. However, the overall sensitivity and specificity were only 70% and 89%, respectively. ColonSentry® is a blood test that looks for CRC by using a profile of seven genes that are overexpressed in people with CRC. Initial studies of this test showed a sensitivity of 72% and a specificity of 70%. Further validation studies in a Malaysian population and in North America showed a sensitivity of 61-78% and a specificity of 66-77%. In peripheral blood mononuclear cells (Colox®), another blood-based test looks at the expression of 42 genes for CRC and CAA. CRC and CAA could be found with a sensitivity of 78% and 46%, respectively [28]. Only 46% of the time was it possible to find both CRC and CAA. Colorectal neoplasia-related DNA markers in stool can also be found with a stool-based test (ColoGuard®).

This test was better at finding all stages of colorectal cancer (92% vs. 74%) and advanced colorectal adenomas (42% vs. 24%) than the standard faecal immunochemical test (87% vs. 95%). However, it was less accurate at finding CRC and CAA (87% vs. 95%). None of the blood-based tests mentioned above are good at finding CAA. We think that our miRNA panel is a new way to test for colorectal cancer and its precursor lesion CAA by looking at the blood. Using our diagnostic algorithm, we have shown that CR Neoplasia can be distinguished from other cancers with an AUC of 0.79 and that CRC and CAA can be distinguished from each other with an AUC of 0.98. Even though this doesn't directly compare CAA or CRC to controls, we think the ability to find advanced colorectal adenoma with high sensitivity and specificity is unique and shows promise for the further development of this blood-based assay [29].

The reason we made our diagnostic algorithm is to not only measure how accurate the test is, but also to use this method to measure the clinical value and effects of the test. Over 1000 miRNAs have been identified and proven to be linked to different diseases in humans. MiRNAs move through the blood in microvesicles, apoptotic bodies, and exosomes, which are extracellular vesicles that are secreted by cells, or they are attached to proteins. miRNAs are stable in extracellular fluid because they are either inside microvesicles or are bound to argonaute proteins, which protect them from RNases. Since they were found in plasma, miRNAs have become possible disease biomarkers. Cancers of the oesophagus, lung, liver, pancreas, bladder, ovary, stomach, and colon have been found to have misregulated miRNA expression. People have said that plasma or serum miRNA panels can be used as biomarkers to find liver, lung, pancreatic, stomach, and colorectal cancers. Several studies have been done to compare the miRNAs in plasma or serum from people with CRC and CAA to those from healthy people. By the end of 2015, 32 studies with a total of 5,222 patients had found that 28 different miRNAs were not working the same way in CRC patients as they did in controls. Out of these 32 studies, 14 found that combinations of two or more miRNAs could be used to predict a CRC diagnosis (Carter *et al*. unpublished data). Several groups have written about how to use miRNA panels to find CRC and CAA. Wang *et al*. found that a three-miRNA panel (miR-409-3p, miR-7, and miR-93) could diagnose all CRC with an AUC of 0.89, a sensitivity of 82%, and a specificity of 89%. 61. Zheng *et al*. found that a panel of four serum miRNAs (miR-19a-3p, miR-223-3p, miR-92a-3p, and miR-422a) could tell the difference between early-stage CRC and controls with an AUC of 0.951, 84% sensitivity, and 92% specificity. Also, with an AUC of 0.886, this panel could tell the difference between CAA and CRC. With an AUC of 0.765, it could tell the difference between CAA and healthy controls. In another study, Verma *et al*. found 11 plasma miRNAs (miR-19a, miR-98, miR-146b, miR-186, miR-191, miR-222, miR-331-5p, miR-452, miR-625, miR-664, and miR-1247) on pooled case miRNA assay cards from 210 patients (117 with CAA, 12 with CRC, and 81 healthy controls) [30].

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When patients with adenomas, cancer, or both were compared to controls, the levels of expression of the target miRNAs were found to be significantly different. Each of these studies shows that using plasma miRNAs as a screening tool to find CRC or adenomas could be a good idea. We think, though, that our study is unique and important for making a screening test because it includes people with other cancers (breast, lung, and pancreas), which is a better way to compare the whole population. Also, we are the only study to make a predictive model that can tell the difference between colorectal cancer (CRC) and colorectal advanced adenoma (CAA) and test a patient's sample without comparing it to a control sample (normal patient sample). Our study builds on what we've already learned and written about. We found that plasma miR-21 could be used to diagnose colorectal cancer. Plasma miR-21 could tell the difference between CRC patients and controls with an AUC of 0.91, 90% sensitivity, and 90% specificity. After this study, we looked at a panel of 8 plasma miRNAs to find colorectal adenomas in 87 patients who were all blinded to the results (16 CAA, 45 CRC and 26 healthy controls). The panel was very good at telling adenomas from controls (AUC = 0.868), all stages of CRC from controls (AUC = 0.829), and CAA from all stages of CRC (AUC = 0.856). The sensitivity for finding CAA, all stages of CRC, and telling CAA apart from CRC was close to 90%. Our specificity, on the other hand, ranged from 57% to 74%. In order to make a more accurate and accurate model for diagnosing colorectal neoplasia, we had to increase the true negative rate and improve the specificity. Also, we wanted to make a miRNA panel that would only test for CR neoplasia and not other cancers that are common in people the same age as CR neoplasia patients. All of the miRNAs in our panel were chosen based on the same set of criteria. All of them have been shown to act either as tumour suppressors (miR-29c, miR-122, miR-150, miR-192, and miR-193a) or as oncogenes (miR-19a, miR-21, miR-346, miR-372, and miR-374a) in the development of cancer by either encouraging cell growth or stopping invasion, migration, and apoptosis. The oncogene miR-21 is probably the best known of these. However, it has also been found in many other types of cancer, which makes it hard to use as a single diagnostic marker for CRC. In our earlier studies, we focused on how easy it is to get the same results over and over again, which is important for any diagnostic test. We looked at the effects of the time of plasma extraction, the method of RNA extraction, and differences between and among operators. In these studies, we found that rapid plasma extraction (Finally, we want to find out if a plasma miRNA panel can predict response or complete response to chemoradiation in stage II-III rectal cancer patients undergoing preoperative neoadjuvant therapy. As of now, there are no good ways to track how well treatment is working after preoperative neoadjuvant chemoradiation for rectal cancer [31].

Conclusion

We have made a 7-miRNA plasma panel that can accurately tell the difference between patients with colorectal neoplasia, other cancers, and healthy people. Also, our miRNA panel can tell the difference between people with CRC and CAA. This has important implications for the development of a non-invasive, reliable, and repeatable screening test for colorectal neoplasia that would be better than non-invasive screening methods that are used now.

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