Review Article

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The implication of molecular markers in the early stage diagnosis of colorectal cancers and precancerous lesions

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Abstract: Mortality can be significantly reduced if noninvasive molecular markers that are effective in the diagnosis of both early colorectal cancers and precancerous lesions are used in screening tests. In this study, our aim is to review the studies conducted with molecular markers obtained noninvasively for diagnosis in early-stage colorectal cancer and precancerous lesions and to reveal the most efficient and cost-effective ones. In our study, it has been shown by analyzing noninvasive molecular markers used in the diagnosis of early-stage colorectal cancers and precancerous lesions, that high rates of effective diagnosis can be obtained after given screening processes, even if these are relatively less effective. In particular, miR-21 in faeces and plasma has been found to be the most efficient and cost-effective biomarker. In order to reduce mortality in colorectal cancers, screening tests should be performed with molecular markers that are effective in early-stage colorectal cancers. However, novel biomarkers are also needed to detect both early colorectal cancers and precancerous lesions. When miR-21 analysis in stool and plasma is widely used as a screening test for early-stage colorectal cancer and precancerous lesions, early diagnosis rates can be significantly increased and mortality rates reduced.

Keywords: colon cancer; cost-effectiveness; early diagnosis; miRNA-21; molecular markers; precancerous lesions.

Introduction

The importance of early diagnosis of colorectal cancers

Colorectal cancers (CRCs) still constitute 9.4% of cancer deaths worldwide [1]. According to the data reported in the same study, 1.9 million new CRCs and related 935,000 deaths occurred in 2020. Especially in developed countries, CRC incidence increases every year due to harmful lifestyles such as eating unhealthy foods, obesity, smoking, and alcohol, and the age of disease onset decreases.

According to current research, 60–70% of CRCs worldwide are diagnosed late as Stage IV–V. Although the 5-year survival is 92% for Stage I, this has been reported to be only 12% for Stage IV. The proportion of patients undergoing colostomy is higher in late-stage CRCs, and therefore hundreds of thousands of people subsequently lead an uncomfortable later life with a colostomy.

In a study conducted in the US, it was reported that colostomy was performed in 21% of 2,630 CRC cases operated between 2015 and 2018, and the length of hospital stay and comorbidity rates of these patients were statistically higher than the cases without colostomy [2]. Considering that the cost of colostomy in the US varies between 20,000 and 60,000 USD and the monthly cost of the colostomy bags is 200 USD on average, the cost advantage of early diagnosis in CRCs can readily be understood [3].

In some cancer types, such as pancreatic cancer, even in cases diagnosed early (Stage I diagnosis rate: 10%), mortality rates are high (5-year survival at Stage I, 32%) [4]. On the other hand, mortality rates are much lower in some very common cancer types such as CRC, prostate cancer, breast cancer, and thyroid cancer when diagnosed early [1]. For this reason, screening tests and early diagnosis in such cancer cases are more important than in other cases.

Today, molecular markers (MMs) are involved in the diagnosis, prognosis, and treatment of diseases in every field of medicine with incredible speed. Developments in molecular medicine eventually reduce mortality rates,
especially in cancer, and the life expectancy of human beings is getting longer with early diagnosis and personalized treatment [5–8].

The role of MMs in the early diagnosis of CRCs

Colonoscopy is currently the gold standard in the diagnosis of CRCs. Although colonoscopy is highly effective, it is invasive and can be difficult to perform (requiring several days of bowel preparation prior to the procedure), expensive, and can lead to some complications.

The efficacy of non-invasive tests such as fecal occult blood tests and measurement of biomarkers such as CEA and CA-19 in serum is important. These biomarkers occur at low levels in early-stage CRCs, and serum levels generally increase in advanced stages.

Kupper et al. performed fecal occult blood tests with immunochromate and Guaiac methods in an advanced age group of 1,500 people. They were able to obtain positive results (CRC or adenoma) at a rate of 9.8% with the immunochromate method and only 2.6% with the Guaiac method [9]. A study by Luo et al. on 67 patients at TNM Stages I and II found high levels of CEA in 5 (7.4%) and CA-19 in 3 (5.9%) plasma samples [10].

In many studies on the molecular changes occurring in CRCs, it has been shown that CRCs show molecular heterogeneity, genetic and epigenetic changes [11], with BRAF V600E and KRAS mutations [12–14].

Satorres et al. showed that three different processes play a role in the molecular mechanisms of serrated polyps, including changes in the mitogen-activated protein kinase (MAPK) pathway, production of the CpG island methylation phenotype, and microsatellite instability generation [15]. Wu et al. studied ‘hub’ genes, key mRNAs and potential molecular mechanisms of CRCs. Thus, eight biomarkers (CAD, ITGA2, E2F3, BCL2, PRKACB, IGF1, SGK1, NR3C1) and two key RNAs (hsa-miR-552 and hsa-miR-3a) were detected [16]. In another study by Zhu et al., they showed that miR-146b-5p in CRCs had an oncogenic effect by targeting PDHB [17].

Aim and methods

In this study, our aim is to evaluate the results obtained in studies with noninvasive MMs that are functional only in early CRC and precancerous lesions, and to determine the most efficient and cost-effective MMs. Our ultimate aim is to improve the rate of early diagnosis in CRCs and to contribute to the reduction of mortality rates.

For this purpose, we searched many databases and included studies with noninvasive MMs that are effective only in early CRCs and precancerous lesions.

We did not include studies using invasive molecular diagnostic methods that are effective in advanced CRCs.

In addition, using “binomial probability distribution formula” we investigated whether high sensitivity rates of MMs that are effective in precancerous lesions can be obtained after a certain screening process, even if the efficacy rates are low.

Studies with noninvasive molecular markers in early-stage CRCs and precancerous lesions

In many review studies, it has been reported that early CRC and adenomas can be diagnosed with high sensitivity and specificity rates by means of liquid biopsy, DNA methylation analysis in plasma, faeces and urine, and these methods can be used as a screening test [18–21].

In a systematic analysis of molecular diagnostic markers in CRCs by Nikoloau et al., MMs were divided into four groups (nucleic acids, cytokines, antibodies and proteins), and their diagnostic potential for detecting early- and late-CRCs were systematically analyzed [22]. The results suggested that the most effective MM in CRCs at all stages was septin (SEPT 9) methylated DNA in serum, and the most effective MMs for early-CRCs were SDC-SIGN, SDC-SIGNR, SEPT 9 and SDC two methylated DNA in serum [22].

Another systematic analysis by Mahasnes et al. reported that the most effective MMs in early-CRCs were SEPT 9 in stool and serum, TFP12 in stool and SDC2 in serum [23].

Mai et al., on the other hand, investigated the expression of piRNA 54265, a cirRNA, in serum in the early diagnosis of CRCs [24]. The study was conducted as a prospective case control analysis of 209 healthy controls, 725 CRC cases, 1,303 other digestive system cancers, and 192 benign colorectal tumors. Early-stage (Stage I and II) cases were, 57; intraepithelial neoplasia (HIN), 208; colorectal polyps (CPs), 81. The median levels of piR-54265 in serum were compared in 111 cases of CRC, 209 healthy controls and 1,303 other digestive system cancer cases. A statistically significant difference was found between the median piR-54265 levels of CRC Stage I and HIN patients and the control group. In addition, after curative resection in all 46 CRC patients, piR-54265 expressions were significantly decreased but increased upon relapse. Serum piR-54265 expressions in 101 CRC patients were compared...
with CEA, CA19-9, CA125 and methylated SEPTIN 9. Compared with CEA (in 13.0% of cases), CA19-9 (13.0%), CA125 (15.2%) and methylated SEPTIN 9 (61.4%) the levels were higher for priR-54265 (85.1%) [24].

In a study by Gai et al., it was reported that early diagnosis of CRC can be made at low cost by quantitative analysis of tissue-specific methylated DNAs in plasma [25].

In another study by Wu et al., 11 panels of MMs and CRC-specific cell-free DNA (cfDNA) methylation analyses were performed in two independent groups, training and validation, from 489 plasma samples (108 adenomas, 248 CRC cases and 133 healthy control subjects). They obtained AUC values of 0.85 in advanced adenomas, 0.77 in non-advanced adenomas, 0.90 in CRC TNM stage I cases, and 0.90 in CRC + Advanced adenomas [26].

In a study by Tam et al., it was reported that long non-coding RNAs (lncRNAs) together with miRNAs are important elements in CRCs as in many cancer types [27]. These molecules can trigger tumor formation when homeostatic imbalances occur in lncRNA/miRNA cancer regulatory mechanisms due to biochemical and physiological changes in the cell [27].

In a review study conducted by Frankovilla et al., it was shown that the expression of miR92a-3p, miR-17-5p, miR-196b-35p and miR-21 in serum in early stage CRCs were increased [25–28]. In another review by Poudavoud et al., it was shown that miR-196 could be a good and effective noninvasive MM for the early diagnosis of CRCs [29].

In a review study, Desmond et al. researched the effects of extracellular vesicle miRNAs (EV-miRNA) in the diagnosis of early-stage CRCs [30]. It was thus shown that EV-miRNAs were dysregulated in the circulation of early-stage CRC cases compared to healthy control groups [30].

Zhang et al. performed bioinformatics analysis by including 388 microRNA sequencing data in 371 CRC cases. Using four data sets, namely GSE112264, GSE113486, GSE113740 and GSE124158, the AUC values of miR 451a in the diagnosis of CRC were determined as: 0.912, 0.894, 0.796, 0.922, respectively, and it was reported that miR-451 could be used as a screening test [31]. In another review, Xiao et al. showed that early diagnosis of CRCs could be largely possible by analyzing miRNA-20a levels in faeces and serum with noninvasive methods [32]. In another study by Xu et al., the possible role of the lncRNAs (SNHG11, ZFAS1, LINCO6909, LINCO0654) in the diagnosis of precancerous and early stage CRCs was investigated [33]. The study was designed in 4 phases: They screened for CRC-related lncRNAs in phase 1. The expressions of 14 lncRNAs selected in phase 1 were analyzed in the plasmas of 24 CRC patients, 13 adenomas and 16 healthy controls in phase 2, and validation was performed in phase 3 [33]. Four panel expression analyses were performed in preoperative and postoperative plasma samples of 29 CRC cases operated on in the 4th phase (supplementary phase). According to the results obtained in the study, 4-panel expressions were found to be significantly higher in precancerous and early stage CRCs than in healthy controls [33]. In addition, circulating cell-free SNHG11 plasma values were found to be significantly higher in precancerous lesions compared to the control group.

In a study conducted by Jiang et al. on 70 early-stage CRC (TNM Stage I-II), 70 adenoma cases and 70 healthy control groups, lncRNA nuclear factor-kB interacting (NKILA) increased in serum in early-stage CRCs compared to healthy controls with an AUC of 0.839, sensitivity of 82.9% and specificity of 72.9% [34]. The patients were followed for an average of 40.4 months. Quantitative polymerase chain reaction (qPCR) method was used for serum analyses. In addition, NKILA expression was found to be significantly lower in early-stage (Stage I and II) tumor tissues, compared to normal control group and adenoma tissues. Furthermore, a statistically significant correlation was found between the increase in serum NKILA level and the decrease in NKILA expression in tumor tissue. Preoperative NKILA serum level decreased significantly after the operation [34]. Liu et al. analyzed the serum levels of miR-182 and miR-20a in 40 early-stage (Stage I) CRC cases and 40 healthy control groups [35]. ROC analyses gave the following values for miR-182 (AUC=0.929; cut-off value=3.165); and for miR-20a (AUC=0.801, cut-off value =1.355); for their combination: AUC=0.905 and cut-off value=2.255 [35]. In ROC analyses performed on 50 adenomas in validation, 50 early stage (Stage I) CRC, 50 healthy control groups, the following values were obtained for miR-182 (AUC=0.891), for miR-20a (AUC=0.736) and their combination (AUC= 0.831). The sensitivity and specificity values for miR-182 in early CRC were 78% and 91%, respectively [35]. In a study by Uratani et al., serum levels of 20 cell-free and exosomal miRNAs were analyzed in 47 healthy control cases and 26 high-risk adenomas [36]. According to the results obtained, the total serum levels of miR-21, miR-29a, miR-92a were found to be significantly higher in high-risk adenoma cases compared to healthy controls. In addition, the numbers and sizes of the adenomas were found to significantly parallel the serum levels. However, only the exosomal miR-21 serum level was observed to increase in direct proportion to the number and size of the adenomas. The sensitivities and specificities were 72.0%, 65.4%, 69.8%, and 66.0%, 78.7% 80.0%, respectively.

While the AUC values in small adenomas were 0.755, 0.676 and 0.747, the AUC values in large adenomas were
higher – 0.886, 0.851 and 0.839, respectively [36]. In a study by Liu et al., the exosomal lncRNA CRNDE-h levels were compared in the serum of 80 adenoma cases and healthy controls, and the AUC value was found to be 0.737 for the adenomas [37].

What should be the most appropriate molecular marker for early diagnosis of CRCs and precancerous lesions?

a. **It should be non-invasive and easy to apply:** Invasive and physically hard to apply tests such as endoscopy and tissue biopsy in screening tests are repulsive for people and are not performed by most people in screening and check-up programs. For this reason, the screening tests that should be used in diseases such as CRC where early diagnosis is important should be non-invasive and easy to apply.

b. **Should be effective in precancerous lesions and early CRCs:** Since mortality rates are high in CRC cases diagnosed in late stages, it is important that MMs to be used in screening tests are also effective in early-stage CRCs and precancerous lesions. Carcinogenesis in CRCs occurs in four stages: initiation, promotion, progression, and metastasis. Each stage takes place over a period of about 10 years [38]. Therefore, although the effectiveness of noninvasive molecular diagnostic methods in adenoma precancerous lesions is relatively low compared to CRCs, the probability of detection until the progression stage in annual screening programs is much higher than noninvasive methods that are effective only in CRCs.

For example, in a noninvasive molecular diagnosis method with 50% sensitivity in the diagnosis of adenoma, although the probability of detecting adenoma in the first year in annual check-up programs is 50%, when adapted to the binomial probability distribution formula, the probability of detecting the tumor will increase in repeated screening programs [39].

Binomial probability distribution formula: 
\[ P(x) = \binom{n}{x} p^x (1-p)^{n-x} \]
where \(n\) = number of trials, \(x\) = discrete random variable and \(p\) = probability of success in a single trial [39]. The probability will increase to 75% in the second year and up to 87.5% in the third year. The malignant transformation process of adenomatous polyps is approximately 0.25% per year [40]. Therefore, in CRC screening tests, it is more rational to use MMs that are effective in adenomas as well as early CRCs.

c. **MM tests should have high sensitivity and specificity:** The higher the sensitivity and specificity of MM tests to be used in the early diagnosis of adenomas and CRCs, the higher the rate of detection of the disease in screening tests. Therefore, the sensitivity and specificity of MM tests to be used in CRC screening programs should be as high as possible.

d. **MM test should be cost-effective:** Expensive diagnostic methods such as colonoscopy or panel MM tests, which will be widely used in early stage CRC and adenomas, are not preferred by people with low economic status and cannot be included in nationwide screening programs. Therefore, the noninvasive diagnostic method to be used in CRC screening tests should be cost-effective.

Data analysis

The results of the analyses of data obtained from the studies included here are shown in (Table 1).

For the miR-21 analysis in feces and plasma in adenomas, the ranges of values for sensitivity, specificity and AUC were as follows, respectively: 76.7–85.1%, 62.7–81.1%, and 0.769–0.838, respectively. In early period CRCs, the ranges were 39.2–86.1% (sensitivity), 72.9–81.0% (specificity) and 0.783–0.829 (AUC) [36, 41–43] (Table 1).

In studies on methylated SEPT9 in early stage CRC and adenomas, although a positive prognostic rate of up to 100% was obtained in early stage CRCs, a maximum Positivity rate of 27.4% was obtained in adenomas; however, most of the studies did not have a control group so no validation could be performed [45–48] (Table 1).

According to the results we obtained in our study, the highest AUC values in adenomas (AUC=0.920) and early stage period CRCs (AUC=0.950) were obtained with a six-panel miRNA in serum [55] (Table 1). With single miRNA tests in plasma, the highest AUC value of (0.929) in TNM Stage I (40 CRC cases) was obtained for miR-182. However, miR-182 analysis could not be performed in adenomas (Table 1) [35]. In studies on piR-54265 in serum, AUC values of 0.886 and 0.699 were obtained in early-stage CRC and HIN, respectively (Table 1) [29]. In studies performed with miR-92a analysis in serum, 0.781 AUC values were obtained in early CRCs and 0.749 AUCs in adenomas (Table 1) [50, 53]. In two studies performed on adenomas with the plasma miR-29a test, AUC values of 0.769 and 0.676 were obtained (Table 1) [36, 50]. In studies conducted with fecal
Table 1: Efficacy rates in diagnostic studies conducted in recent years in noninvasive MMs, precancerous lesions and early-stage CRCs.

| Author           | Ref. no. | Non-invasive method                | Sample no. | Pathology | Stage         | Efficiency rate |
|------------------|----------|------------------------------------|------------|-----------|---------------|-----------------|
| Toiyama et al.   | [41]     | Training in serum miR-21           | 43         | Adenoma   | –             | AUC: 0.803       |
|                  |          |                                     |            |           |               | Sensitivity: 76.8%| Speci-
| Validation       |          |                                     | 43         | Adenoma   | –             | AUC: 0.838       |
|                  |          |                                     |            |           |               | Sensitivity: 81.1%| Speci-
| Liu et al.       | [42]     | In feces miRNA-21                  | 120        | Adenoma   | –             | AUC: 0.769       |
|                  |          |                                     |            |           |               | Sensitivity: 85.1%| Speci-
|                  |          | In feces miRNA-146a                | 120        | Adenoma   | –             | AUC: 0.698       |
|                  |          |                                     |            |           |               | Sensitivity: 77.5%| Speci-
|                  |          | In feces miRNA-21 + miRNA146a      | 120        | Adenoma   | –             | AUC: 0.761       |
|                  |          |                                     |            |           |               | Sensitivity: 78.1%| Speci-
| Bestaminejad et al. | [43] | In feces miRNA-21                  | 40         | CRC       | TNM I + II    | AUC: 0.829       |
|                  |          |                                     |            |           |               | Sensitivity: 86.05%| Speci-
|                  |          | In serum miRNA-21                  | 40         | CRC       | TNM I + II    | AUC: 0.783       |
|                  |          |                                     |            |           |               | Sensitivity: 86.05%| Speci-
| Uratani et al.   | [36]     | In serum miRNA-21                  | 26         | Adenoma   | –             | AUC: 0.755       |
|                  |          |                                     |            |           |               | Sensitivity: 73.1%| Speci-
|                  |          | In serum miRNA-29a                 | 26         | Adenoma   | –             | AUC: 0.676       |
|                  |          |                                     |            |           |               | Sensitivity: 72.0%| Speci-
|                  |          | In serum miRNA-92a                 | 26         | Adenoma   | –             | AUC: 0.747       |
|                  |          |                                     |            |           |               | Sensitivity: 65.4%| Speci-
|                  |          | Exosomal miRNA-21                  | 26         | Adenoma   | –             | AUC: 0.778       |
|                  |          |                                     |            |           |               | Sensitivity: 69.8%| Speci-
| Tsukamoto et al. | [44]     | In plasm miRNA-21                  | 51         | CRC       | TNM I         | Positivity rate: | 39.2%          |
|                  |          |                                     | 110        | CRC       | TNM II        | Positivity rate: | 43.6%          |
| Toth et al.      | [45]     | In plasm methylated SEPT9           | 25         | CRC       | TNM I         | Positivity rate: | 84.0%          |
|                  |          |                                     | 14         | CRC       | TNM II        | Positivity rate: | 100%           |
| Warren et al.    | [46]     | In plasm methylated SEPT9           | 7          | CRC       | TNM I         | Positivity rate: | 71.4%          |
|                  |          |                                     | 31         | CRC       | TNM II        | Positivity rate: | 90.3%          |
Table 1: (continued)

| Author          | Ref. no. | Non-invasive method         | Sample no. | Pathology       | Stage           | Efficiency rate |
|-----------------|----------|------------------------------|------------|-----------------|-----------------|-----------------|
| Jin et al.      | [47]     | In plasm methylated SEPT9    | 169        | Polyp           | –               | Positivity rate: 20.7% |
|                 |          |                              | 84         | Adenoma         | –               | Positivity rate: 27.4% |
|                 |          |                              | 18         | CRC             | TNM I           | Positivity rate: 66.7% |
|                 |          |                              | 23         | CRC             | TNM II          | Positivity rate: 82.6% |
| Johnson et al.  | [48]     | In plasm methylated SEPT9    | 26         | CRC             | TNM I           | Positivity rate: 61.5% |
|                 |          |                              | 20         | CRC             | TNM II          | Positivity rate: 80.0% |
| Aherne et al.   | [49]     | In serum miR-34a             | 23         | CRC             | TNM I + II      | AUC: 0.798       |
|                 |          |                              | 23         | CRC             | TNM I + II      | AUC: 0.524       |
|                 |          |                              | 23         | CRC             | TNM I + II      | AUC: 0.574       |
|                 |          |                              | 20         | Polyp           | –               | AUC: 0.610       |
|                 |          |                              | 20         | Polyp           | –               | AUC: 0.640       |
|                 |          |                              | 20         | Polyp           | –               | AUC: 0.600       |
|                 |          |                              | 20         | Adenoma         | –               | AUC: 0.720       |
|                 |          |                              | 20         | Adenoma         | –               | AUC: 0.660       |
| Mai et al.      | [29]     | In serum piR-54265           | 57         | CRC             | TNM I           | AUC: 0.771       |
|                 |          |                              | 208        | CRC             | TNM II          | AUC: 0.886       |
|                 |          |                              | 81         | HIN (High-Grade Intraepithelial) Neoplasia | AUC: 0.669 |
| Huang et al.    | [50]     | In plasm miR-29a             | 37         | Advanced adenoma | –               | AUC: 0.768       |
|                 |          |                              | 37         | Advanced adenoma | –               | AUC: 0.749       |
|                 |          |                              | 37         | Advanced adenoma | –               | AUC: 0.773       |
| Fang et al.     | [51]     | In plasm miR-24              | 54         | CRC             | TNM I + II      | AUC: 0.822;      |
|                 |          |                              | 54         | CRC             | TNM I + II      | Sensitivity: 77.7%|
|                 |          |                              | 54         | CRC             | TNM I + II      | AUC: 0.897;      |
|                 |          |                              |           |                 |                 | Sensitivity: 90.7%|
|                 |          |                              |           |                 |                 | AUC: 0.839;      |
|                 |          |                              |           |                 |                 | Sensitivity: 88.8%|
| Author                  | Ref. no. | Non-invasive method                                                                 | Sample no. | Pathology | Stage       | Efficiency rate |
|-------------------------|----------|-------------------------------------------------------------------------------------|------------|-----------|-------------|-----------------|
| Herreos-Villanueva et al. | [55]     | In serum: miRNA-18a, miRNA-19a, miRNA-19b, miRNA-29a, miRNA-156, miRNA-335          | 101        | Advanced adenoma | TNM I + II | AUC: 0.920; Sensitivity: 91.7%; Specificity: 69.0% |
| Xie et al.              | [54]     | In serum exosomal circ-PNN                                                           | 27         | CRC       | TNM I + II | AUC: 0.854; Sensitivity: 91.7%; Specificity: 69.0% |
| Elshafei et al.         | [53]     | In serum miR-92a                                                                    | 32         | CRC       | TNM I + II | AUC: 0.781; Sensitivity: 85.0%; Specificity: 54.0% |
|                         |          | In serum miR-37s                                                                    | 32         | CRC       | TNM I + II | AUC: 0.719; Sensitivity: 85.0%; Specificity: 54.0% |
|                         |          | In serum miR-760                                                                    | 32         | CRC       | TNM I + II | AUC: 0.875; Sensitivity: 85.0%; Specificity: 54.0% |
| Vychytilova-Faltejikova | [52]     | In serum miR-142-5p; miR-23a-5p; miR-27a-3p; miR-376c-3p                           | 40         | CRC       | TNM I + II | AUC: 0.877; Sensitivity: 81.0%; Specificity: 81.0% |
| Jiang et al.            | [34]     | In serum NKILA                                                                      | 70         | CRC       | TNM I + II | AUC: 0.839; Sensitivity: 82.9%; Specificity: 72.9% |
| Liu et al.              | [35]     | Training in plasm miR-182                                                          | 40         | CRC       | TNM I      | AUC: 0.929 |
|                         |          | Training in plasm miR-20a                                                           | 40         | CRC       | TNM I      | AUC: 0.801 |
|                         |          | Training in plasm miR-182 + miR-20a                                                 | 40         | CRC       | TNM I      | AUC: 0.905 |
|                         |          | Validation in plasm miR-182                                                         | 50         | CRC       | TNM I      | AUC: 0.891 |
|                         |          | Validation in plasm miR-20a                                                          | 50         | CRC       | TNM I      | AUC: 0.736 |
|                         |          | Validation in plasm miR-182 + miR-20a                                               | 50         | CRC       | TNM I      | AUC: 0.831 |
| Xu et al.               | [33]     | In plasm ZFAS1                                                                      | 40         | CRC       | TNM I + II | AUC: 0.800; Sensitivity: 97.5%; Specificity: 50.0% |
|                         |          | In plasm SNHGII                                                                     | 40         | CRC       | TNM I + II | AUC: 0.888; Sensitivity: 76.5%; Specificity: 97.0% |
|                         |          | In plasm LINCOO909                                                                  | 40         | CRC       | TNM I + II | AUC: 0.896; Sensitivity: 83.5%; Specificity: 80.0% |
|                         |          | In plasm LINCOO654                                                                  | 40         | CRC       | TNM I + II | AUC: 0.746; Sensitivity: 74.5%; Specificity: 66.0% |
|                         |          | In plasm panel of above parameters                                                  | 40         | CRC       | TNM I + II | AUC: 0.935; Sensitivity: 81.0%; Specificity: 92.5% |
| Author      | Ref. no. | Non-invasive method                                      | Sample no. | Pathology | Stage       | Efficiency rate |
|-------------|----------|---------------------------------------------------------|------------|-----------|-------------|-----------------|
| Liu et al.  | [56]     | In serum LncRNA CRNDE-h                                 | 80         | Adenoma   | –           | AUC: 0.737      |
| Wu et al.   | [57]     | Erythrocyte specific miRNA (fecal occult blood test)    | 16         | CRC       | TNM I + II | Sensitivity: 67.0%; Specificity: 56.0% |
|             |          |                                                         | 31         | Adenoma   | –           | Sensitivity: 23.0%; Specificity: 13.0% |
| Wu et al.   | [58]     | In feces miRNA-192a                                      | 57         | Polyp     | –           | Sensitivity: 56.1%; Specificity: 73.3% |
| Wu et al.   | [26]     | 11 CRC-specific DNA methylation molecular markers       | 108        | Adenoma   | –           | AUC: 0.920      |
|             |          |                                                         | 40         | Nonadvanced adenoma | – | AUC: 0.770      |
|             |          |                                                         | 68         | Advanced adenoma | – | AUC: 0.850      |
|             |          |                                                         | 66         | CRC       | TNM I       | AUC: 0.900      |
|             |          |                                                         | 248        | CRC       | –           | AUC: 0.910      |
|             |          |                                                         | 248 + 68   | CRC + advanced adenoma | – | AUC: 0.900      |
| Ahlquist et al. | [49]     | Panel in feces: DNA methylation, BMP3, NDrg4-TFP12, KRAS | 30         | CRC       | TNM I + II | Sensitivity: 86%; Specificity: 93% |
|             |          |                                                         | 22         | Adenoma   | –           | Sensitivity: 82%; Specificity: 75% |
| Wang et al. | [59]     | Panel in plasm: miR-7, miR-93, miR-409-3p                | 26         | CRC       | Dukes A+B   | AUC: 0.809      |
|             |          |                                                         | Validation | 11        | CRC       | Dukes A+B       | AUC: 0.892      |
|             |          |                                                         |            |           |             | Sensitivity: 82%; Specificity: 89% |
| Wu et al.   | [60]     | In feces miR-135b                                       | 110        | Adenoma   | –           | Sensitivity: 61.0%; Specificity: 44.0 |
|             |          |                                                         | 59         | Advanced adenoma | – | Sensitivity: 73.0%; Specificity: 46.0% |
|             |          |                                                         | 24         | CRC       | TNM I + II | AUC: 0.868      |
|            |          |                                                         |            |           |             | AUC: 0.722      |
| Kanaan et al. | [61]     | Panel in serum: miR-15b, miR-17, miR-142-3p, miR-195, miR-331,  | 16         | Adenoma   | TNM I + II | AUC: 0.410      |
|             |          | miR-532, miR-532-3p, miR-652                             | 15         | CRC       | –           | Sensitivity: 53.0%; Specificity: 82.0 |
| Yau et al.  | [62]     | In feces: miR-20a + miR-92a                              | 106        | CRC       | TNM I + II | AUC: 0.770      |
|             |          |                                                         | 199        | Adenoma   | –           | Sensitivity: 57.0%; Specificity: 84.0 |
miR-192a, sensitivity and specificity values were 71.6 and 73.3%, respectively, in early CRC cases (n=88); in polyps (n=57), these values were 56.1 and 73.3% (Table 1) [58]. In a study on serum NKILA (70 early CRC cases), the following values were obtained: AUC=0.839, sensitivity=82.9% and specificity=72.9% (Table 1) [34]. In a study performed on exosomal circ-PNN in serum in early CRC, the values were: AUC=0.854; sensitivity=91.7%; and specificity=69.0% (Table 1) [54]. The AUC value was found to be 0.737 in the study performed on 80 adenoma cases with the lncRNA CRNDE-h test in serum (Table 1) [56]. In the analyses of fecal miR-135b in 110 adenomas smaller than 1 cm, 59 adenomas and 24 early CRC cases, the sensitivity values were: 61, 73, 67%, and the specificity values were 61%, 46%, 50%, respectively (Table 1) [60]. For miR-760 analysis in serum, an AUC value of 0.875 was obtained from 32 early-stage CRC cases (Table 1) [53].

The efficacy rates obtained with noninvasive MMs, which have been effective in both CRCs and precancerous lesions in recent years, are also shown (Table 2).

In overall conclusion, we have formulated and shown, for the first time, that high sensitivity can be obtained after a certain period of time when noninvasive MMs which are effective in the diagnosis of precancerous lesions are used in screening tests for the early diagnosis of CRCs. This seems the case even when their absolute sensitivity rates are relatively low.

**Discussion**

In review study, Danese et al. considered which MM(s) should be included in clinical practice for initial, early diagnosis of CRCs by focusing on (i) methods such as immunochemical fecal occult blood test (IFOBT); (ii) detection of circulating tumor cells and cancer-related nucleic acids by liquid biopsy; (iii) methylation status of SEPT9, BCAT1/IKZF1; and (iv) changes in the levels of some microRNAs in plasma and serum were evaluated [63]. It was reported that with the IFOBT screening test performed every year on 46,000 people in the 50–80 age group in the US, the CRC mortality decreased by 30% in the 30-year period; the mortality could be reduced by 40% with endoscopic screening performed on 88,000 people every year for 22 years in the US [63]. The study of Danese et al. revealed that these screening tests, which were reported to be performed in large groups, could significantly reduce CRC mortality. However, although the test costs are low, the sensitivity of IFOBT in the early diagnosis of CRC is significantly limited [9]. On the other hand, colonoscopy is an invasive, difficult and non-cost-effective diagnostic method [29]. For this reason, it is not possible to use both methods as a common screening test and in annual check-up programs.

As can be seen in the analyses of the results obtained in the studies covered here, the number of noninvasive MMs that are effective in early stage CRC and precancerous lesions is quite low (Table 1). In particular, the number of cost-effective noninvasive individual MMs that are effective in both early stage CRC and precancerous lesions is much more limited (Table 2). Our results suggest that plasma and feces miR-21 can serve as a cost-effective MM. Such a test would appear to be applicable to both adenomas and early CRCs (Table 2).

Since faecal or serum miR-21 tests are efficient, cost-effective and easy to apply noninvasively as diagnostic methods for detecting adenomas and early CRCs, most people can easily include this method in their check-up programs. Furthermore, the method can be widely used in national screening programs. Thus, the diagnosis of CRC will most likely be made until the stage of progression. Similarly, the serum PSA, which is used as a screening test for prostate cancer, has been very effective in early diagnosis and mortality rates have been significantly reduced worldwide [64]. In a review by Hibner et al. on the diagnostic potential of miRNAs in CRCs, it was found that the expression of miRNA-21 and miRNA-29a increased in faeces and serum in early-stage CRCs, and their fecal sensitivity and specificity were higher [65]. It was also reported that when analyzed as a panel instead of a single miRNAs, the efficiency of CRCs diagnosis increased; unfortunately, however, the cost of the diagnostic tests also increased [65].
Table 2: Noninvasive cost-effective molecular markers in both early stage CRCs and precancerous lesions.

| Author          | Ref. no. | Non-invasive method          | Sample no. | Pathology     | Efficiency rate         |
|-----------------|----------|-----------------------------|------------|---------------|-------------------------|
| Toiyama et al.  | [41]     | Training in serum miR-21     | 43         | Adenoma       | AUC: 0.803; Sensitivity: 76.8%; Specificity: 81.1% |
|                 |          | Validation                   | 43         | Adenoma       | AUC: 0.838; Sensitivity: 81.1%; Specificity: 76.7% |
| Bestaminejad et al. | [43] | In feces miRNA-21            | 40         | CRC           | AUC: 0.829; Sensitivity: 86.05%; Specificity: 81.08% |
|                 |          | In serum miRNA-21            | 40         | CRC           | AUC: 0.783; Sensitivity: 86.05%; Specificity: 72.9% |
| Uratani et al.  | [36]     | In serum miRNA-21            | 26         | Adenoma       | AUC: 0.755; Sensitivity: 73.1%; Specificity: 68.1% |
|                 |          | In serum miRNA-29a           | 26         | Adenoma       | AUC: 0.676; Sensitivity: 72.0%; Specificity: 66.0% |
|                 |          | In serum miRNA-92a           | 26         | Adenoma       | AUC: 0.747; Sensitivity: 65.4%; Specificity: 78.7% |
| Tsukamoto et al.| [44]     | In plasm miRNA-21            | 51         | CRC           | Positivity rate: 39.2%   |
|                 |          |                             | 110        | CRC           | Positivity rate: 43.6%   |
| Toth et al.     | [45]     | In plasm methylated SEPT9    | 25         | CRC           | Positivity rate: 84.0%   |
|                 |          |                             | 14         | CRC           | Positivity rate: 100%    |
| Warren et al.   | [46]     | In plasm methylated SEPT9    | 7          | CRC           | Positivity rate: 71.4%   |
| Jin et al.      | [47]     | In plasm methylated SEPT9    | 169        | Polyp         | Positivity rate: 20.7%   |
|                 |          |                             | 84         | Adenoma       | Positivity rate: 27.4%   |
|                 |          |                             | 18         | CRC           | Positivity rate: 66.7%   |
|                 |          |                             | 23         | CRC           | Positivity rate: 82.6%   |
| Johnson et al.  | [48]     | In plasm methylated SEPT9    | 26         | CRC           | Positivity rate: 61.5%   |
|                 |          |                             | 20         | CRC           | Positivity rate: 80.0%   |
| Huang et al.    | [50]     | In plasm miR-29a             | 37         | Advanced adenoma | AUC: 0.768; Sensitivity: 62.7%; Specificity: 84.7% |
|                 |          | In plasm miR-92a             | 37         | Advanced adenoma | AUC: 0.749; Sensitivity: 64.9%; Specificity: 81.4% |
|                 |          | In plasm miR-29a + miR91a    | 37         | Advanced adenoma | AUC: 0.773; Sensitivity: 73.0%; Specificity: 79.7% |
| Wu et al.       | [58]     | In feces miRNA-192a          | 57         | Polyp         | Sensitivity: 56.1%; Specificity: 73.3% |
|                 |          |                             | 88         | CRC           | Sensitivity: 71.6%; Specificity: 73.3% |

According to the results obtained in our study, although high sensitivity and specificity values were obtained in diagnostic tests performed with many panel MMs (Table 1), these would be difficult to implement extensively in screening tests and check-up programs because of high cost.

Although a DNA methylation test was performed by Bach for the early diagnosis of CRCs, 75% positivity rate in stage I and 62.5% in stage II was obtained, the number of cases used in the study was very low (Table 1) [21].

For plasma miR-29a, two studies on adenomas obtained AUC values of 0.769 and 0.676 [50]. However, the total number of cases involved in these studies was very low and there was no study on early CRC (Table 1). Although high sensitivity (91.7%) and AUC (0.854) values were obtained in a study performed on early CRCs with exosomal circ-PNN in serum, no study was conducted in adenomas and the number of cases was quite low (Table 1). Sensitivity values of 61, 73, and 67% were obtained with fecal miR-135b for polyps, adenomas, and early-stage CRC cases, respectively; the specificity was as low as 44%, 46%, and 50%, respectively (Table 1). A higher AUC value of 0.875 was obtained for serum miR-760 from 32 early-stage CRC, no similar study has been performed on adenomas and the total number of cases was small.

According to the results obtained here, miR-21 in feces and plasma was found to be effective, including costwise, in the diagnosis of both adenomas and early CRCs (Table 1). miR-21 analyses in serum or faeces would be practical due to the ease of obtaining samples, the simplicity of the test methods, and the results can be obtained in a short time.
The most important potential limitation of the analysis of miR-21 in plasma and faeces is its increased expression in many types of cancer, not only in CRC and precancerous lesions.

In addition, there are no data yet from large populations with these tests. Despite this, with the large amount of research currently being done on this topic, it is likely that much more effective, cost-effective non-invasive MMs will emerge in the near future.

However, considering the high number of deaths due to late diagnosed CRCs, we think that comprehensive screening programs can be initiated with stool and serum miR-21 tests.

**Conclusions**

In order to reduce mortality in CRCs, screening tests should be performed not only with MMs that are effective for detecting early-stage disease but also using MMs that are effective in both early CRCs and precancerous lesions.

The results of our study show that the number of noninvasive MMs that are reported to be effective in precancerous lesions and early-stage CRCs, have high sensitivity and specificity, are cost-effective, and have been tested in a large number of cases is not high (Table 2).

For this reason, we think that early diagnosis rates should be increased and mortality rates should be reduced by including by incorporating miR-21 analysis in faeces or plasma into annual check-up and national screening programs, especially for high-risk risk groups.

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