



NON-INVASIVE SCREENING METHODS FOR EARLY DETECTION OF COLORECTAL CANCER

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ABSTRACT:

Introduction: Colorectal cancer (CC) is the third most common cause of cancer worldwide. Common symptoms of CC (change in bowel physiology, rectal bleeding, and weight loss) present late in the course of the disease and have low positive predictive value (3-4%). The most important prognostic factor is the stage of the disease at diagnosis; therefore, screening for CC is important for people ≥ 60 years of age.

Objective: To determine the importance of faecal calprotectin (FCP) and M2-pyruvate kinase (M2-PK) values for the early diagnosis of CC with different localization.

Material and Methods: A total of 90 participants were prospectively studied, divided into three groups as follows: 30 patients with colorectal carcinoma (target group), 30 patients with chronic organic disease of the gastrointestinal tract, and 30 patients with irritable bowel syndrome (control groups). All subjects were admitted or visited the University Hospital "Dr G. Stranski" – Pleven. The values of FCP and M2-PK were examined in all of them.

Results: There is a significantly different degree of correlation between the values of FCP and M2-PK – in 85% of patients with colorectal carcinoma they are elevated, and in 50% of patients with irritable bowel syndrome, compared between the two groups and compared to participants in the control group.

Conclusions: The present study shows that both faecal M2-PK and calprotectin levels can contribute to the early, noninvasive detection of colorectal cancer. These markers may serve as useful screening tools, especially in asymptomatic individuals, and help prioritize patients for further endoscopic investigation.

Keywords: colorectal cancer, M2-pyruvate kinase, fecal calprotectin, non-invasive methods,

INTRODUCTION

Colorectal cancer is the third most common cause of cancer worldwide, and among the population in developed countries, approximately 5% will be diagnosed with the disease during their lifetime. The incidence of colorectal cancer is strongly related to age, and in developing countries the median age at diagnosis is 68 years for men and 73 years for women [1, 2]. Most cases of colorectal cancer are sporadic and progress gradually over time through the adenoma-carcinoma sequence [3]. Common symptoms of colorectal cancer (change in bowel physiology, rectal bleeding, and weight loss) present late in the course of the disease. However, these symptoms are non-specific and therefore have a rather low positive predictive value (3-4%), and are therefore not sufficiently informative to make a diagnosis based on them alone. However, the most important prognostic factor is the stage of the disease at diagnosis, and colorectal cancer screening programs have been established in many countries for individuals over 60 years of age [4]. The gold standard for colorectal cancer diagnosis is colonoscopy, but the disadvantages of this method are that it is invasive, resource-intensive, and expensive [2, 4, 5, 6]. Instead, the screening method for colorectal cancer used in most countries is the faecal haemoglobin (F-Hb) test, followed by instrumental examination of the colon in subjects with a positive test. The accuracy of the F-Hb test depends on the method used and the cut-off value for defining a positive test. Faecal immunochemical tests (FITs) perform better than the F-Hb test, having a specificity of 94% for detecting colorectal cancer, but only a moderate sensitivity (74%) and failing to detect precancerous conditions (polyps and adenomas). The low sensitivity of FIT for detecting early stages of colorectal carcinoma highlights the need to find new biomarkers that could improve the detection rate in colorectal carcinoma screening [7, 8, 9].

The primary **objective** of this paper is to determine the significance of faecal calprotectin and M2-pyruvate kinase values for early diagnosis of colorectal cancer of different localization.

MATERIALS AND METHODS

A total of 90 participants were prospectively studied, divided into three groups as follows: 30 patients with colorectal carcinoma, 30 patients with established chronic organic diseases of the gastrointestinal tract, and 30 patients with irritable bowel syndrome – two control groups. All subjects included in the project were patients with symptoms of the gastrointestinal tract or who visited the University Hospital “Dr G. Stranski” EAD – Pleven for reasons unrelated to treatment (e.g. prophylactic examinations, control examinations). The values of faecal calprotectin (FCP) and M2-pyruvate kinase were examined in all of them. In this study, we adhered to ethical guidelines and obtained ethical approval from the Ethical Commission of Medical University – Pleven. Prior to participation, written informed consent was obtained from all participants after providing them with comprehensive information about the research objectives, procedures, and potential risks or benefits involved. We ensured that participants had a clear understanding of their rights and responsibilities as research participants. The data were entered and processed with the statistical packages IBM SPSS Statistics 21.0 and MS Excel v. 2010. Descriptive statistical methods were used and $p < 0.05$ has been considered as significant.

RESULTS

Laboratory results of the patients with colorectal cancer – target group ($N_1 = 30$) have been shown on Table 1.

Table 1. Descriptive statistics of CRC patients – target group ($N_1 = 30$)

Variable	n	Mean	SD	Median	F statistic	p	Significance
Fecal calprotectin	30	589.81	267.79	559.95	106.8622	0.000000	***
M2-PK	29	52.3	17.7	52.7	214.8067	0.000000	***

*** $p < 0.0001$; ** $p < 0.01$; SD – standard deviation

Results of laboratory parameters in patients with Crohn's disease and ulcerative colitis – control group 1 ($N_2 = 30$) have been shown on Table 2.

Fecal calprotectin – the values ranged from 89.7 to 210.5 mg/kg (166.93 ± 29.25). There were 25 patients with borderline values (25/30; 83.33%), with elevated values – five patients (5/30; 16.67%) ($p > 0.05$). In men, fecal calprotectin ranged from 118.9 to 210.5 mg/L (mean 168.47 ± 28.49). There were 17 patients with borderline values (17/21; 80.95%), with elevated values – four patients (4/21; 19.05%). In women, the indicator ranged from 89.7 to 203.7 mg/L (mean 163.32 ± 32.42). There were eight women with

Fecal calprotectin (Reference values: 0-50 U/mL – negative result; 50-200 U/mL – borderline result; >200 U/mL – presence of inflammation of the gastrointestinal tract) – the values of the indicator are in the range from 233.5 to 1305.4 mg/kg (589.81 ± 267.79). All patients in the group had elevated values (100%) ($p < 0.0001$). Regarding the stage of the disease, the highest average value of the indicator was in stage 1 (638.48 ± 204.21), followed by stage 2 (542.33 ± 285.31), but without a significant difference ($p > 0.05$). Regarding the localization of CRC, the highest fecal calprotectin is in carcinoma of the colon transversum (665.67 ± 183.96), followed by the rectum (614.01 ± 234.57), sigmoid colon (601.11 ± 324.46), colon descendens (519.8 ± 185.97) and colon ascendens (447.67 ± 199.57), but the differences are not significant ($p > 0.05$).

Fecal M2-pyruvate kinase (Reference values: <4 U/mL) – the values are in the range from 19.7 to 90.2 U/mL (52.30 ± 17.70). All patients in the group have elevated values (100%) ($p < 0.0001$). Regarding the stage of the disease, the highest mean value of the indicator is in stage 2 (55.98 ± 17.49), followed by stage 1 (45.18 ± 17.63), but without a significant difference ($p > 0.05$). Regarding the localization of CRC, the highest M2-pyruvate kinase is in carcinoma of the colon transversum (64.50 ± 30.97), followed by colon ascendens (59.3 ± 15.77), sigmoid colon (56.55 ± 16.72), colon descendens (40.8 ± 1.84) and rectum (40.01 ± 15.09), but the differences are not significant ($p > 0.05$).

borderline values (8/9; 88.89%), with elevated fecal calprotectin there was one woman (1/9; 11.11%). There were no significant gender differences ($p > 0.05$).

Fecal M2-pyruvate kinase – from 2.7 to 12.1 U/mL (5.99 ± 2.51). There were 21 patients (21/30; 70.0%) with elevated values of the indicator ($p < 0.05$). In men, fecal M2-pyruvate kinase ranged from 2.7 to 12.1 U/mL (5.86 ± 2.60), with thirteen patients (13/21; 61.90%) with elevated values of the indicator. In women, the indicator ranged from 3.8 to 11.5 U/mL (6.31 ± 2.43), with eight patients with elevated values (8/9; 88.89%). There were no significant gender differences ($p > 0.05$).

Table 2. Descriptive statistics of patients with Crohn's disease and ulcerative colitis – control group 1 ($N_2 = 30$)

Variable	n	Mean	SD	Median
Fecal calprotectin	30	166.93	29.25	166.8
M2-PK	30	5.99	2.51	5.8

Laboratory parameters in patients with irritable bowel syndrome – control group 2 (N₃ = 30) have been shown on Table 3.

Fecal calprotectin – from 12.5 to 47.1 mg/kg (25.38 ± 8.26). All patients in the group had normal values (30/30; 100%) (p > 0.05). In men, fecal calprotectin ranged from 12.5 to 41.5 mg/L (mean 25.27 ± 8.37). All men had fecal calprotectin in the reference range. In women, the indicator ranged from 17.6 to 47.1 mg/L (mean 25.52 ± 8.46). All women in the group had normal fecal

calprotectin. There are no significant gender differences (p > 0.05).

Fecal M2-pyruvate kinase – from 1.0 to 4.7 U/mL (2.95 ± 0.90). There are 6 patients with increased values (6/30; 20.0%) (p > 0.05). In men, fecal M2-pyruvate kinase is in the range from 1.0 to 4.2 U/mL (2.81 ± 0.91), with three patients with increased indicator (3/17; 17.65%). In women – from 1.7 to 4.7 U/mL (3.15 ± 0.88), with three patients with increased values (3/13; 23.08%). There are no significant gender differences (p > 0.05).

Table 3. Descriptive statistics of patients with irritable bowel syndrome – control group 2 (N₃=30)

Variable	n	Mean	SD	Median
Fecal calprotectin	30	25.38	8.26	23.35
M2-PK	30	2.95	0.9	2.95

DISCUSSION

Having discussed the above-mentioned fecal occult blood tests, we decided to shift the screening narrative from looking only for microscopic blood traces to detecting the metabolic and inflammatory tumor microenvironment. Inflammation of the colon can lead to carcinogenesis. The presence of an inflammatory microenvironment is a key hallmark of cancer. In addition, inflammatory responses at the local and systemic levels play an important role in disease progression and survival in colorectal cancer [10]. Current methods for assessing local inflammation rely on tissue sampling, which is not suitable for population screening [6, 11, 12].

We decided to start from key stages in carcinogenesis and select biomarkers that encompass different biological pathways of early colorectal oncogenesis:

Metabolic switch (M2-PK): M2-PK is a key dimeric glycolytic enzyme overexpressed by proliferating tumor cells. It reflects altered tumor metabolism (aerobic glycolysis – the so-called “Warburg effect”), not bleeding. M2-PK allows the panel to detect non-bleeding adenomas and early-stage tumors that standard fecal immunochemical tests (FIT) often miss.

Local inflammatory response (fecal calprotectin): Calprotectin is a neutrophil-derived protein that indicates inflammation of the intestinal mucosa [5, 13]. Although highly sensitive to colorectal changes, it lacks specificity, as it is also elevated in inflammatory bowel disease (IBD). We discussed that combining it with M2-PK would help differentiate malignant inflammation from benign organic intestinal diseases.

Fecal calprotectin (FCP) is a sensitive marker of colonic inflammation, primarily used in the clinical evaluation of inflammatory bowel disease. It is used to differentiate between organic and functional disorders of the colon. It is a calcium-binding protein and is found primarily in the cytoplasm of neutrophils and the membrane of monocytes. FCP is released on neutrophils upon cell death or injury and is thought to have regulatory roles on components of the inflammatory process, including

other myeloid cells (e.g., CD11b+ cells) [5, 13]. FCP enters the intestinal lumen by migration and is resistant to enzymatic degradation and can therefore be readily detected in body materials such as feces. Elevations in FCP occur in a wide variety of gastrointestinal conditions, including colitis and malignancy, and although it is a sensitive measure of inflammation, it is not specific for any condition. However, given the importance of inflammation in the development and progression of cancer, the presence of elevated FCP may provide additional assessment of a patient’s risk of colorectal neoplasia and progression [14, 15, 16, 17]. FCP is resistant to enzymatic degradation during transit through the gastrointestinal tract and remains stable for up to 7 days at room temperature. It is also easily measurable. The final result can be available after 15 minutes of processing. A value < 50 µg/g is considered normal [13].

Pyruvate kinase isoenzyme type M2 (M2-PK) is an isoenzyme of pyruvate kinase, a key enzyme within glycolysis, controlling the metabolism of cells with a high rate of proliferation, such as tumor cells. It detects specific changes in intestinal cells in polyps and colorectal carcinoma, as well as patients with acute or chronic intestinal inflammation (e.g. ulcerative colitis, Crohn’s disease). This isoenzyme catalyzes the conversion of phosphoenolpyruvate to adenosine triphosphate, producing pyruvate. Depending on the metabolic functions of the tissues, different isoenzymes of pyruvate kinase are prominent. The isoenzyme M2-pyruvate kinase (M2-PK) can occur in a highly active tetrameric form, as well as in a dimeric form with low activity [18, 19].

The dimeric form is almost inactive and favors the channeling of glucose carbons into synthetic processes, such as nucleic acid, amino acid and fatty acid synthesis. The tetrameric form is highly active and favors the energy-reducing conversion of phosphoenolpyruvate to pyruvate and lactate. In tumor cells, M2-PK is mainly found in a dimeric form and is therefore called “tumor M2-PK”. M2-PK dimerization is induced by interaction with various oncoproteins, including pp60v-src-kinase, oncogenic

fibroblast growth factor 1, and human papillomavirus 16 E7. The dimeric form of M2-PK is released from tumors into the blood and can be quantified by sandwich enzyme-linked immunosorbent assay (ELISA; ScheBo Biotech AG, Giessen, Germany). There have been about 40 published studies on M2-PK concentrations in blood since 1997. They have shown a significant increase in M2-PK and a correlation with the staging of the following tumors: melanoma, thyroid, breast, lung, kidney, esophageal, gastric, pancreatic, colorectal, ovarian, cervical and renal cell carcinoma. Long-term determination of M2-PK in EDTA plasma is used as a follow-up tool to monitor failure, relapse or success during therapy. In colorectal carcinoma and adenoma, M2-PK is released in the feces.

M2-PK is essential for CRC tumor growth by regulating cell proliferation and migration of colon cancer cells. Higher amounts of this isomer are found in advanced tumor stages and are associated with lymphatic metastasis in patients with colorectal cancer. Tumor M2-PK is detected in the feces of patients with colorectal carcinoma and colorectal polyps. The amount of M2-PK in feces can be quantified by enzyme-linked immunosorbent assay (ELISA), and thus fecal M2-PK has been proposed as a tool for the detection of colorectal carcinoma [19, 20, 21].

The rapid fecal M2-PK test showed sensitivity, specificity, positive predictive value, and negative predictive value of 58.8%, 85.5%, 11.2%, and 98.5%, respectively, in the detection of colorectal adenocarcinoma. For

the detection of colorectal adenoma, this test gave sensitivity, specificity, positive predictive value, and negative predictive value of 27.3%, 86.3%, 27.0%, and 86.5%, respectively [22].

The summary of our results shows the following constellations: 1) in the group with colorectal carcinoma – high M2-PK, moderate FCP; 2) in the group with chronic inflammatory diseases – elevated FCP, moderate to low M2-PK, and 3) in the group with irritable bowel syndrome – both biomarkers are within reference range.

CONCLUSIONS

Our study confirms that there are clinicomorphological and laboratory correlations with different localization of CRC along the colon and they should be taken into account in the assessment of the treatment strategy of the individual patient, as well as in the prognosis for 5-year survival after complex surgical and oncological treatment. The lack of mandatory screening leaves many patients with early stage CRC undetected. This predisposes to slow development and progression of the disease stage, until it manifests itself with some of its complications. This indicates the need for a national strategy regarding mandatory screening for CRC.

Acknowledgements:

The present study was supported by funds provided by the Medical University-Pleven (Scientific Project No. D2/ 2024), for which we express our gratitude.

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Please cite this article as: Petrova D, Stoykov D, Gorcheva Z. Non-invasive Screening Methods for Early Detection of Colorectal Cancer. *J of IMAB*. 2026 Jul-Sep;32(3):7076-7080. [Crossref - <https://doi.org/10.5272/jimab.2026323.7076>]

Received: 28/01/2026; Published online: 03/09/2026



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