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RAPID COMMUNICATION

Colorectal cancer screening by non-invasive metabolic biomarker fecal tumor M2-PK

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Abstract

AIM: To evaluate the utility of the innovative fecal tumor M2-Pyruvate kinase (M2-PK) test in our daily clinical routine, as a marker for the pre-selection of patients who should subsequently undergo colonoscopy for the diagnosis or exclusion of colorectal cancer.

METHODS: Fecal tumor M2-PK was measured in stool samples of 96 study participants (33 patients with colorectal cancer, 21 patients with rectal carcinoma and 42 controls) who all underwent total colonoscopy.

RESULTS: In 39 of 42 individuals in the control group, fecal tumor M2-PK was below 4.0 kU/L (93% specificity). Colorectal tumors were accompanied by a highly significant increase ($P < 0.001$) in fecal tumor M2-PK levels (median: colon carcinoma, 23.1 kU/L; rectal carcinoma, 6.9 kU/L; colorectal carcinoma, 14.7 kU/L), which correlated with Duke's staging and T-classification. The overall sensitivity was 78% for colorectal cancer, increasing from 60% for stage T1 to 100% for stage T4 and from 60% for Duke's A to 90% for Duke's D tumors.

CONCLUSION: Fecal tumor M2-PK is an appropriately sensitive tool to pre-select those patients requiring colonoscopy for the further diagnostic confirmation or exclusion of colorectal cancer.

Key words: Tumor M2-Pyruvate kinase; Pyruvate kinase type M2; Colon cancer; Rectal cancer; Adenoma; Feces; Cancer screening

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INTRODUCTION

In Germany, about 70 000 people are diagnosed with colorectal cancer each year[1]. This figure is about 1 million worldwide, with approximately 528 000 deaths from colorectal cancer each year[2]. The gold standard for the early detection of colorectal cancer is colonoscopy. However, the acceptance of this costly and invasive method is low. Only 1.7% of people entitled to colonoscopy under the German national colorectal cancer screening program actually undergo the procedure[3].

In order to increase the participation in colorectal cancer screening programs, an easy, fast and economical initial screening method, with good patient compliance, is absolutely necessary. This allows identification of those patients most likely to have colorectal cancer, who require further investigation by colonoscopy.

The guaiac-based fecal occult blood test (FOBT), which is based on the premise that polyps and cancer bleed more than normal mucosa[4], is currently the most commonly used test for colorectal cancer screening. Guaiac-based FOBTs have been investigated in a number of large studies and shown to reduce mortality by about 15%-33% in screened populations[5,6]. However, they have limited sensitivity. For example, Lieberman *et al*[7] and Koss *et al*[8] found their sensitivity was less than 30% for colorectal cancer and less than 15% for advanced adenomas. Newer immunological FOBTs showed higher sensitivities[9,10] with the advantage of no dietary restrictions. In most studies with immunological FOBTs to date, however, colonoscopy has been performed only in FOBT-positive cases. Non-bleeding colorectal tumors and those not consistently discharging sufficient blood into the gut lumen are not detected by either guaiac or immunological FOBTs.

Recently a new screening test for the early detection of adenomas and colorectal tumors has been described. The tumor M2-Pyruvate kinase (M2-PK) stool test is based on the measurement of a key enzyme involved in tumor metabolism[8,11-14].

Tumor M2-PK is the dimeric form of the glycolytic pyruvate kinase isoenzyme type M2[15]. The enzyme catalyzes the last reaction step within the glycolytic

sequence from phosphoenolpyruvate (PEP) to lactate and is responsible for net ATP production within this pathway. Enzymatic characterization of a wide range of different tumors revealed that tumorigenesis is accompanied by an increase in total pyruvate kinase v-max activities. There is also a shift towards the expression of the pyruvate kinase isoenzyme type M2 (M2-PK) and away from the tissue-specific isoenzymes (L-PK in liver and kidney, M1-PK in muscle and brain and R-PK in erythrocytes)[16-18]. The increased expression of M2-PK is under the control of ras, and the transcription factors SP1 and HIF-1. Ras and HIF-1 are both consistently altered in gastrointestinal tumors[19-22]. M2-PK can occur in a tetrameric form which is characterized by a high affinity to its substrate PEP and in a dimeric form with a low PEP affinity. The tetramer: dimer ratio of M2-PK determines the proportion of glucose carbons used for glycolytic energy production (tetrameric form) or channeled into synthetic processes (dimeric form). In tumor cells, M2-PK is mainly found in the dimeric form (tumor M2-PK) due to direct interaction with various oncoproteins, i.e. $pp60^{v\text{-}src}$ kinase and HPV-16 E7[15,21,23]. Tumor M2-PK is released into the blood, and in the case of adenomas and tumors in the lower gastrointestinal tract also into the stool of patients. An increase in tumor M2-PK in EDTA plasma samples is found in gastrointestinal cancers, as well as a wide range of other tumors such as lung, renal, breast and cervical cancer. The EDTA plasma test is highly suitable for patient monitoring[24-30].

The fecal tumor M2-PK test has been described as a promising new screening tool for adenomas and colorectal cancer[8,11-14]. Therefore, the aim of our study was to evaluate the utility of the tumor M2-PK test in our own daily clinical routine as a marker for the pre-selection of patients requiring subsequent diagnostic colonoscopy.

MATERIALS AND METHODS

Patients

Our study consisted of 96 participants who underwent complete colonoscopy. The control group consisted of 42 healthy individuals (15 male and 27 female; median age: 58 years; range: 25-79 years) without any findings at colonoscopy, who were participating in the national screening colonoscopy program provided by the German health insurance system. All screening colonoscopies were conducted between September 2005 and April 2006 in a primary care gastroenterology and hepatology medical center. Healthy individuals were included in the control group.

The 54 participants with colorectal cancer underwent diagnostic colonoscopy at the Offenbach Municipal Hospital between January 2003 and April 2006. Rectal carcinomas were diagnosed in 21 patients (15 male and 6 female; median age: 70 years; range: 52-84 years). Colonic adenocarcinomas were diagnosed in 33 patients (24 male and 9 female; median age: 70 years; range: 43-84 years).

All participants received a stool sample collection pot and were instructed to collect a single stool sample (naturally produced, walnut sized) one day prior to the laxative administration in preparation for colonoscopy. No special diet was recommended. Paper collecting devices were used to avoid stool contact with water in the toilet bowl. Stool samples were initially stored at room temperature by the participants until the day of colonoscopy. Thereafter, these pre-colonoscopy stool samples were stored at -20℃ at the medical center or hospital until analyzed for tumor M2-PK.

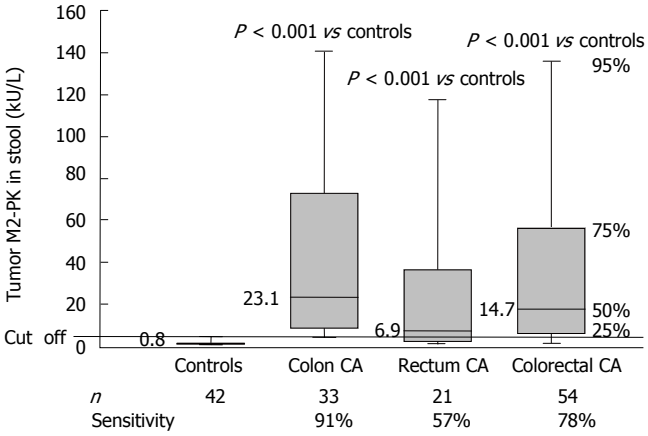

Figure 1 Tumor M2-PK levels in stool samples of healthy control individuals and patients with colon or rectal cancer.

Measurement of fecal tumor M2-PK concentrations

Fecal tumor M2-PK concentrations were determined using a commercially available sandwich ELISA based on two different monoclonal antibodies which specifically recognize the dimeric form of M2-PK (ScheBo® · Biotech AG, Giessen, Germany). A positive test result was defined as > 4.0 kU/L, as indicated by the manufacturer.

Statistical analysis

Since the data were skewed to the right, the statistical analysis was conducted using the Kruskall-Wallis ANOVA test (Statistica, StatSoft® Inc., Tulsa, USA).

RESULTS

This study evaluated 54 patients with colorectal cancer and 42 healthy controls with no indication of gastrointestinal diseases at colonoscopy. In the control group, fecal tumor M2-PK levels were below 4.0 kU/L in 39 of the 42 subjects (median: 0.8 kU/L), resulting in 93% specificity (Figure 1). In two of the three control samples which were above the cut-off value only a slight increase of tumor M2-PK (4.4 kU/L and 5.3 kU/L) was measured.

Colorectal tumors were accompanied by a highly significant increase in fecal tumor M2-PK levels. The median value was 23.1 kU/L for colon carcinoma ($P < 0.001$), 6.9 kU/L for rectal carcinoma ($P < 0.001$) and 14.7 kU/L ($P < 0.001$) for colorectal carcinoma when both groups were combined.

At a cut-off level of 4.0 kU/L, the sensitivity was 91% for colon carcinoma, 57% for rectal carcinoma and 78% when both groups were combined. Both T classification and Duke's staging of the colorectal tumors revealed a

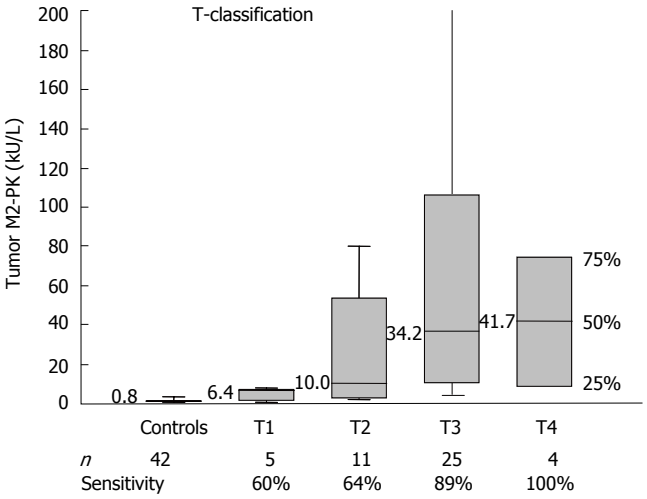

Figure 2 Correlation between fecal tumor M2-PK levels and TNM staging.

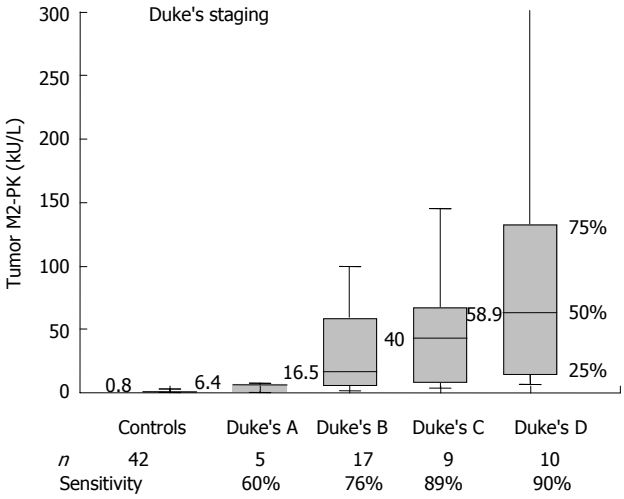

Figure 3 Correlation between fecal tumor M2-PK and Duke's staging.

Table 1 Correlation between fecal tumor M2-PK levels and TNM classification or duke's staging

Classification	*n*	Median (kU/L)	Mean (kU/L)	SE (kU/L)	Range (kU/L)
Controls	42	0.8	1.5	0.4	0.1-17.3
T1	5	6.4	4.5	1.6	0.3-7.7
T2	11	10.0	30.5	10.1	1.5-100
T3	25	34.2	106.3	35.2	1.7-620
T4	4	41.7	41.4	19.2	6.6-76
Duke's A	5	6.4	4.5	1.6	0.3-7.7
Duke's B	17	16.5	63.5	34.7	0.2-604
Duke's C	9	40.0	50.0	17.8	1.9-176
Duke's D	10	58.9	138.5	65.3	4.5-620

strong correlation between fecal tumor M2-PK levels and staging. The sensitivities increased from 60% for stage T1 to 100 % for stage T4 and from 60% for Duke´s A to 90% for Duke's D (Figures 2 and 3; Table 1).

DISCUSSION

Tumor M2-PK is the synonym for the dimeric form of the glycolytic pyruvate kinase isoenzyme type M2[15]. M2-PK is the pyruvate kinase isoenzyme which is characteristic of all proliferating cells and can occur in a tetrameric form as well as a dimeric form. Previous studies describe that tumor M2-PK is released into the stool of patients with adenomas and colorectal tumors and can easily be quantified with a commercially available sandwich ELISA[8,11-14].

In order to evaluate whether tumor M2-PK is a practical tool for the pre-selection of patients with colorectal cancer in our daily routine, we measured fecal tumor M2-PK in a cohort of 96 individuals. All 42 healthy control individuals, 33 patients with colon carcinoma and 21 patients with rectal carcinoma underwent total colonoscopy in order to confirm or exclude colorectal cancer. Our study revealed a highly significant increase ($P < 0.001$) in tumor M2-PK in the stool samples of those patients with colorectal cancer, whereby fecal tumor M2-PK values correlated well with Duke's staging and T-classification (Figures 1-3; Table 1). Even stage T1 or Duke's A showed 60% sensitivity, increasing to 100% in stage T4 and to 90% in Duke's D tumors.

At a cut-off value of 4.0 kU/L, our overall sensitivity for colorectal carcinoma was 78 %. These data correspond well with the results of Hardt *et al*[13] who reported a sensitivity of 78% in 60 colorectal cancer patients, and those of Naumann *et al*[31] who found a sensitivity of 85.2% in a cohort of 27 colorectal cancer patients. A higher sensitivity was reported by McLoughlin *et al*[14] (92% in 25 colorectal cancer patients and 67% in 30 patients with adenomas) and by Koss *et al*[8] (92.3 % in 26 colorectal cancer patients and 60% for adenomas > 1 cm in ten patients).

The most commonly used fecal test in current screening programs is the guaiac-based FOBT[32,33]. Liebermann *et al*[7] and Koss *et al*[8] have reported an overall sensitivity for guaiac-based FOBTs of less than 30% for colorectal cancer and less than 15% for advanced adenomas. Results with newer, immunological FOBTs showed higher sensitivities than guaiac-based FOBTs for colorectal cancers[9,10] but in most studies colonoscopy was performed only in FOBT-positive cases.

The overall sensitivity of tumor M2-PK is increased if a higher proportion of late stage patients are included, but this is also true for FOBTs[10]. Nevertheless, McLoughlin *et al*[14] reported a sensitivity of 67% for adenomas with the fecal tumor M2-PK test. Similarly, Koss *et al*[8] found a sensitivity of 60% for adenomas > 1 cm.

In a head-to-head comparison of fecal tumor M2-PK and the commonly used guaiac-based FOBT, Koss *et al*[8] demonstrated a sensitivity for colorectal cancer of 92.3% for fecal tumor M2-PK and 20% for FOBT. No comparative study of fecal tumor M2-PK and immunochemical FOBTs is currently available.

The high sensitivity of the tumor M2-PK test is due to its ability to detect bleeding and non-bleeding tumors. From a practical viewpoint, the use of a single random formed stool sample for tumor M2-PK analysis, without

requiring dietary restrictions, might be of greater patient convenience compared with the need to collect stool on three consecutive days for the guaiac FOBT.

In our study, the control group consisted of individuals without any signs of gastrointestinal diseases at colonoscopy. The median tumor M2-PK value in this group was 0.8 kU/L. In 39 of 42 subjects, tumor M2-PK levels were below the cut-off value; in two further control samples tumor M2-PK levels were only slightly increased (4.4, 5.3 kU/L). The resulting specificity at a cut off value of 4.0 kU/L is 93%, which is in general accordance with the studies of Hardt *et al*[13], Koss *et al*[8] and McLoughlin *et al*[14] who report specificities between 78% and 98%. Naumann *et al*[31] found increased fecal tumor M2-PK levels in cases of active Crohn's disease and ulcerative colitis in which increased cell proliferation is expected. In addition, patients with inflammatory bowel disease have an increased risk of developing colorectal cancer, probably linked to frequent cycles of damage and regeneration of the colonic mucosa associated with flares of active disease.

Another new approach for pre-selective colorectal cancer screening is the determination of mutated oncogenes and anti-oncogenes[22,34-38]. These tests have the advantage of very high specificities. However, due to high genetic heterogeneity within colorectal cancers, a panel of different targets (k-ras, p53, APC genes, as well as microsatellite instability marker) must be measured in order to reach acceptable sensitivity which makes the test extensive and expensive. Using a 21-target multipanel, sensitivities between 44% and 91% and specificities between 93% and 100% are described[36-38]. Furthermore, to assure the stability of DNA within the stool, samples have to be frozen at -80℃ within 12 h after defecation. Fecal tumor M2-PK is stable for 48 h at room temperature and for up to one year when frozen at -20℃ (manufacturer's data sheet), which makes it practical for routine use. In addition, the tumor M2-PK test could be conducted in virtually all hospital and private diagnostic laboratories because it can either be run manually combined with an ELISA plate reader or automated using existing commercially available equipment.

Overall, our results are in general agreement with previous studies which have demonstrated that fecal tumor M2-PK is an appropriate tool to achieve a sensitive pre-selection by identifying those patients with the greatest need to undergo diagnostic colonoscopy to confirm or exclude colorectal cancer.

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