Colorectal cancer detection in an asymptomatic population: fecal immunochemical test for hemoglobin *vs*. fecal M2-type pyruvate kinase

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Abstract

Introduction: Screening programs for colorectal cancer (CRC) are mainly based on a first-line fecal immunochemical test for hemoglobin (FIT). Fecal M2-type pyruvate kinase (M2-PK) has been evaluated in clinical settings showing promising results for early CRC detection. However, the impact of fecal M2-PK assessment on the performance of first-round CRC screening programs is not known. We investigated whether fecal M2-PK alone or in combination with FIT may improve CRC screening efficacy in the general population.

Materials and methods: A total of 1027 asymptomatic subjects (median age 66 [59-74] years; females 504 [49.1%]), identified through the general practitioners' rosters, were invited for the collection of 2 fecal samples for FIT and M2-PK evaluation. Participants with at least positive one fecal test were referred for colonoscopy. Quality indicators for screening performance were calculated and analyzed using Fisher's exact test.

Results: Overall, 572 subjects underwent both FIT and M2-PK assessment (participation rate 55.7%): 93 participants showed positive results for at least one test (positivity rate 16.3%). Only 10 patients were positive for both tests. Attendance rate to colonoscopy was 86.0% and a total of 65 adenomas and 7 cancers were detected. Combined use of FIT and fecal M2-PK permitted the identification of 18 more neoplasm (25%) without improving colonoscopy workload, as deduced by the comparable number needed to scope ($P = 0.402$).

Conclusion: The addition of M2-PK testing to FIT offers the potential to detect additional neoplasms that either do not bleed or only bleed intermittently without reducing participation rate and without increasing endoscopy workload.

Key words: biomarker; colorectal cancer; occult blood; M2-type pyruvate kinase; screening

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Introduction

Colorectal cancer (CRC) is the third most frequent cancer in humans, and is a major health problem in developed countries (1). More than 90% of cases originate from colorectal adenoma following a long, stepwise carcinogenic process (2,3). Thus, the early detection and removal of adenomatous lesions are crucial in preventing mortality and morbidity due to CRC (4). Currently, fecal immunochemical test for hemoglobin (FIT) is considered the standard approach for population-screening programs whereas total colonoscopy represents the gold standard for CRC diagnosis (5). However, both FIT and colonoscopy have several limitations including low sensitivity and specificity for the former, and low acceptance rate and expensiveness for the latter (6). Consequently, there is fervent interest towards finding new strategies and new approaches for CRC screening.

M2-type pyruvate kinase (M2-PK) is an alternative form of the enzyme pyruvate kinase that is expressed during cancer development and plays a central role in controlling the metabolism of cells with high proliferation rate (7). In CRC and adenoma, M2-PK is released into the feces and can be

easily quantified by sandwich enzyme-linked immunosorbent assay (ELISA) (8).

To date, several studies have evaluated fecal M2-PK levels for CRC detection in high-risk or symptomatic populations (6,9). However, only few studies have investigated the performance of fecal M2-PK in CRC screening programs involving potentially healthy subjects (10). Moreover, no data on fecal M2-PK efficacy in the first-round screening programs performed under real practice conditions are available. Therefore, in the present study, we examined whether fecal M2-PK assessment alone or in combination with FIT might improve CRC screening efficacy in order to achieve a more accurate and restrictive selection of screened subjects requiring prompt colonoscopy.

Materials and methods

Subjects

From April 2012 to October 2012, a total of 1027 asymptomatic average-risk subjects (median age 66 [59-74] years; females 504 [49.1%]) drawn from the general practitioners' rosters were invited to join the present prospective, single-center, population-based study in the setting of CRC screening program (Centro di Prevenzione Oncologica [CPO Piemonte], Molinette Hospital, Turin, Italy). The study design is presented in Figure 1. General practitioners were asked to exclude from the invitation subjects who had undergone colonoscopy within the previous 5 years or with a diagnosis of inflammatory bowel disease, taking into account

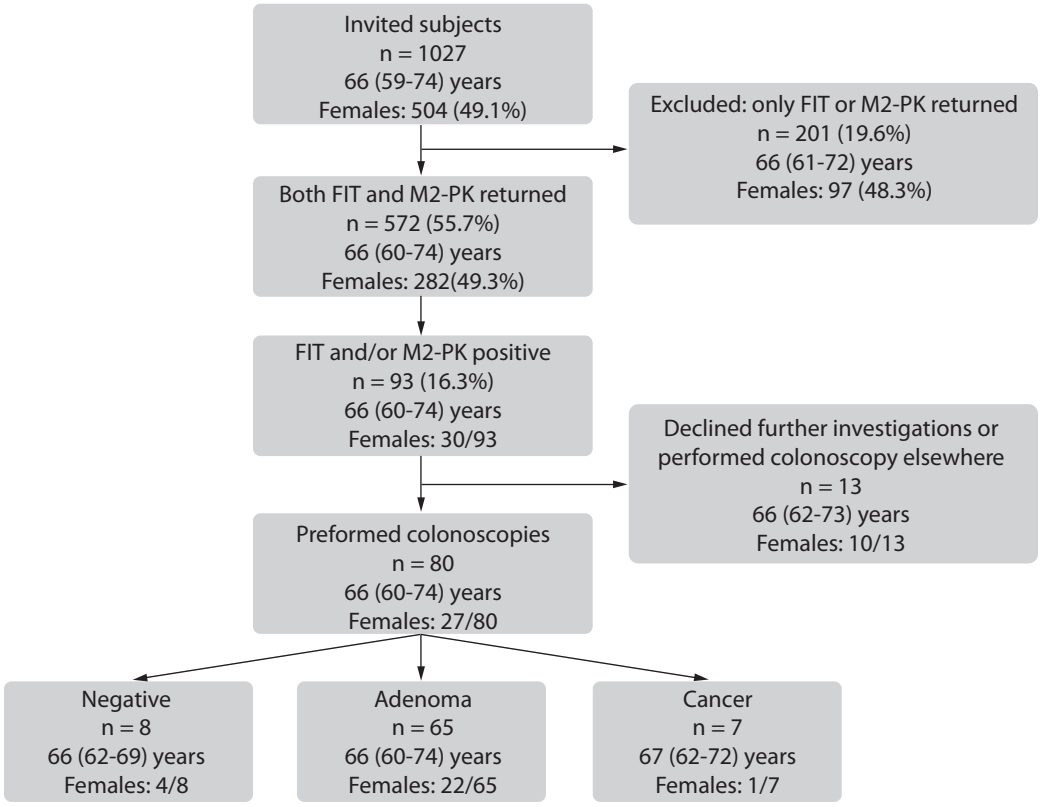

Figure 1. Flow chart of the study.
Of 1027 invited subjects, 201 were excluded from the study because they provided only fecal immunochemical test for hemoglobin (FIT) or M2-type pyruvate kinase (M2-PK) fecal sample, whereas 254 (24.7%) did not respond to the invitation. Overall, 572 out 1027 subjects underwent both FIT and M2-PK assessment (response rate 55.7%). Subjects with at least one positive fecal test were invited to undergo colonoscopy. Among the 93 participants with at least one positive test (positivity rate 16.3%), 13 subjects declined further investigations or performed colonoscopy elsewhere due to the waiting time for endoscopic examination, whereas 80 subjects underwent colonoscopy (86.0% attendance rate). Of these, 8 subjects reported negative results for neoplasm, 65 subjects were detected with an adenoma that was classified as advanced in 19 cases, and 7 colorectal cancers were diagnosed.

the need to avoid including patients with gastrointestinal symptoms such as bloating, abdominal pain and diarrhea. The study was performed according to the Declaration of Helsinki guidelines and was approved by the local Ethics Committee. All subjects gave their written informed consent prior to recruitment.

Methods

Participants were asked to collect two non-watery fecal samples for FIT and M2-PK evaluation. A sampling stool device (Eiken Chemical Co., Tokyo, Japan) and a M2-PK Quick-PrepTM tube (Schebo® Biotech AG, Giessen, Germany) for fecal samples collection were provided with the invitation letter. A leaflet reporting a brief description of the screening procedure and the instruction for stool collection was included. According to manufacturers' instructions, no dietary or medication restrictions were required. Participants were requested to maintain fecal samples refrigerated at +4 °C up to 48 hours until delivery at CPO laboratory. Trained technicians performed the fecal tests. All FITs were carried out with OC-Sensor-Diana (Eiken Chemical Co., Tokyo, Japan) and were considered positive for values ≥ 100 ngHb/mL. According to the Faecal Immunochemical TesTs for Haemoglobin Evaluation Reporting (FITTER) guidelines (11), OC-SENSOR hemoglobin (Hb) concentrations units were converted to micrograms of Hb *per* gram of feces (µgHb/g) multiplying obtained data by a conversion factor of 0.2 (100 ngHb/mL is equal to 20 µgHb/g feces) (12). Measurement of fecal M2-PK was performed in duplicate by using a commercially available kit (Tumor M2-PK™ ELISA Stool Test, Schebo® Biotech AG, Giessen, Germany) with a linear dynamic range of 1-20 units/mL (U/mL) and intra/inter-assay mean coefficient of variance, reported by manufacturer, of 5.3% (range: 3.0-7.9%) and 6.8% (range: 4.4-9.4%), respectively. According to manufacturer instructions, the positive cut-off value was set to 4 U/mL.

Subjects with at least one positive fecal test were invited to undergo colonoscopy. Although uncommon, colonoscopy may lead to complications such as perforations, tears or bleeding. For this reason, subjects attending colonoscopy were instructed on early signs of possible complications including abdominal pain, fever or rectal bleeding. Endoscopic examination was performed within 2 months of FIT and M2-PK assessment. Standard oral bowel preparation with a 4-L polyethylene glycol-electrolyte solution was adopted for colon cleansing (4). Colonoscopy was performed by experienced gastroenterologists of the endoscopy unit of Molinette Hospital. Polyps detected during each procedure were removed and examined by an expert gastrointestinal pathologist who remained blinded to the results of the fecal tests. Histology of polyps and cancers was classified according to the World Health Organization criteria (13). The definition of advanced adenoma included all adenomas with a diameter ≥ 10 mm and/or villous component ≥ 20% and/or high-grade dysplasia, whereas non-advanced adenoma included < 10 mm tubular type polyps and low grade dysplasia. Cancer was defined as carcinoma invading at least the submucosa across the muscolaris mucosa (14).

Statistical analysis

Normality of data distribution was checked by D'Agostino-Pearson test. Age was expressed as median and range. FIT and M2-PK concentrations were expressed as median and interquartile range. Kruskal-Wallis test was used to evaluate differences in FIT and M2-PK concentrations among subgroups, while Fisher's exact test was employed to analyze dichotomous data. The degree of association between two continuous variables was analyzed by Pearson correlation coefficient (r). Positivity rate was defined as the percentage of positive tests among the tested subjects. Adenoma (ADR), advanced adenoma (AADR), neoplasm (NDR), advanced neoplasm (ANDR) and cancer detection rate (CDR) for FIT and M2-PK, alone or in combination, were calculated as the proportion of histologically-proven diagnosis per 100 screened subjects. Positive predictive values (PPVs) were calculated as the number of histologically-proven diagnosis among subjects that underwent colonoscopy. Number needed to scope (NNS) was defined as the number of colonoscopies needed to detect

one advanced neoplasm. A P-value < 0.05 was considered statistically significant. All statistical analyses were performed using MedCalc version 9.2.1.0. (MedCalc Software bvba, Ostend, Belgium).

Results

Overall, 572 out of 1027 subjects underwent both FIT and M2-PK evaluation (response rate 55.7%). At least one test was positive in 93 out of 572 subjects (positivity rate 16.3%). FIT was positive in 70 subject (positivity rate 12.3%; 56 [41-100] µgHb/g) and M2-PK in 33 (positivity rate 5.8%; 8 [5-11] U/mL), whereas both tests were positive in only 10 participants (positivity rate 1.7%; FIT: 113 [36-240] µgHb/g; M2-PK: 11 [5-20] U/mL). The median FIT and M2-PK concentration in the total positive cohort was 37 (21-116) µgHb/g and 1 (1-5) U/mL, respectively. No correlation was found between the two tests in the cohort of 93 subjects (r = -0.07, 95% confidence interval (CI) = -0.27-0.14; P = 0.501).

Among the 93 participants with at least one positive test, 80 subjects underwent colonoscopy (86.0% attendance rate). Detection rates, PPVs, NNS values and correspondent statistical significances are reported in Table 1. Of note is the fact that 18 subjects with M2-PK-positivity-only and with neoplasm revealed at colonoscopy, repeated FIT within one month from the first test before colonoscopy, and 8 out of 18 resulted positive in the second round.

Fecal concentration of FIT and M2-PK in the different diagnostic subgroups is shown in Figure 2. Regarding FIT concentrations, a statistically significant difference was found between subjects with adenoma (38 [29-45] µgHb/g) and those with cancer (240 [134-240] µgHb/g) (P = 0.023). Conversely, M2-PK concentration was significantly different between participants with normal colonoscopy findings (1 [1-1] U/mL) and subjects with both adenoma (1 [1-7] U/mL) and cancer (2 [1-4] U/mL) (P = 0.037 and P = 0.018, respectively). In addition, no

Table 1. Screening indices of FIT and M2-PK, alone or in combination.

Indices	FIT	M2-PK	FIT and M2-PK	P*	P†	P‡
Positive rate	70/572 (12.3%)	33/572 (5.8%)	93/572 (16.3%)	< 0.001	0.063	< 0.001
ADR	48/572 (8.4%)	24/572 (4.2%)	65/572 (11.4%)	0.005	0.113	<0.001
AADR	13/572 (2.3%)	10/572 (1.7%)	19/572 (3.3%)	0.675	0.370	0.131
CDR	6/572 (1.0%)	3/572 (0.5%)	7/572 (1.2%)	0.506	1.000	0.342
NDR	54/572 (9.4%)	27/572 (4.7%)	72/572 (12.6%)	0.003	0.108	< 0.001
ANDR	19/572 (3.3%)	13/572 (3.3%)	26/572 (4.5%)	0.370	0.362	0.049
Cancer PPV, 95%CI	0.097 (0.035-0.211)	0.111 (0.023-0.323)	0.088 (0.035-0.180)	0.860	0.918	0.986
Neoplasm PPV, 95%CI	0.871 (0.654-1.136)	1.000 (0.659-1.455)	0.900 (0.704-1.133)	0.100	0.198	0.604
Advanced neoplasm PPV, 95%CI	0.307 (0.185-0.479)	0.482 (0.256-0.823)	0.325 (0.212-0.476)	0.150	0.857	0.169
Cancer NNS	10	9	11	0.847	0.961	0.715
Advanced neoplasm NNS	3	2	3	0.381	0.402	0.998

P*: FIT *vs*. M2-PK
P†: FIT *vs*. FIT and M2-PK combination
P‡: M2-PK *vs*. FIT and M2-PK combination
All statistical analyses were performed by Fisher's exact test.
AADR - advanced adenoma detection rate; ADR - adenoma detection rate; ANDR - advanced neoplasm detection rate; CDR - cancer detection rate; CI - confidence interval; FIT - fecal immunochemical test for hemoglobin; M2-PK - M2-type pyruvate kinase; NDR - neoplasm detection rate; NNS - number needed to scope; PPV - positive predictive value.

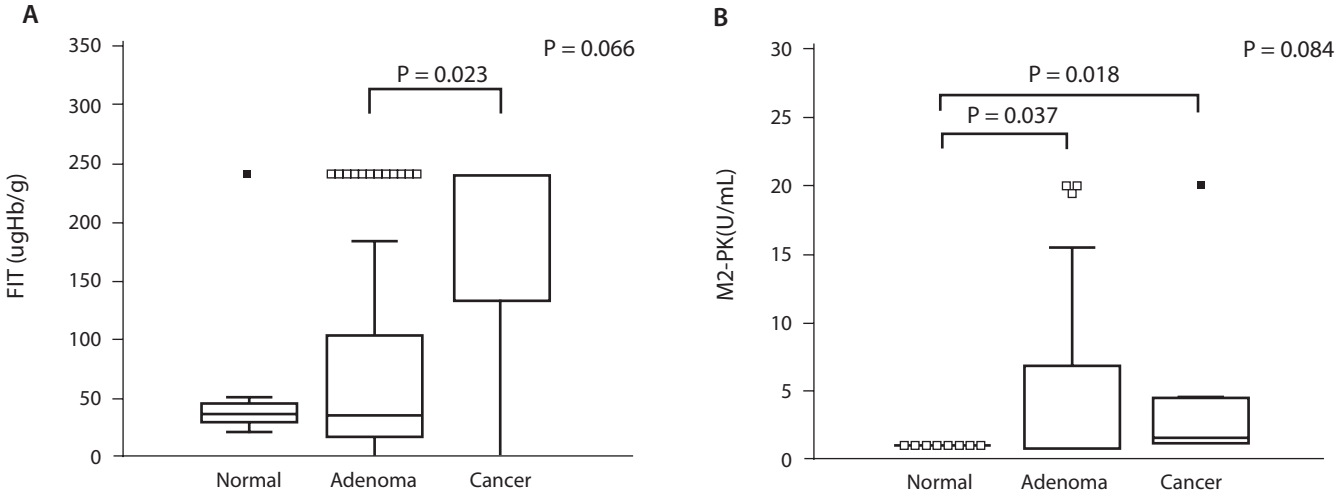

Figure 2. FIT (A) and M2-PK (B) concentrations in the different subgroups of subjects according to colonoscopy findings. All statistical analyses were performed by Kruskal-Wallis test. P values in top right corner refer to FIT (A) and M2-PK (B) median comparison between all three subgroups simultaneously.

differences were found in the concentration of both FIT and M2-PK between non-advanced and advanced adenomas (34 [20-70] µgHb/g *vs*. 32 [13-219] µgHb/g, P = 0.679, and 1 [1-8] U/mL *vs*. 4 [1-7] U/mL, P = 0.077, respectively. All subjects with negative results had M2-PK levels below the assay detection limit (< 1.00 U/mL).

Discussion

To our knowledge, this is the first study investigating fecal M2-PK assessment either in comparison or in combination to FIT in a real practice first-round CRC screening program. Overall positivity rate increased from 12.3% to 16.3% with the addition of M2-PK test to FIT (P = 0.063). In particular, fecal M2-PK assessment permitted the detection of 18 FIT-negative subjects with neoplasm revealed at colonoscopy. In 2006, Shastri and colleagues compared fecal M2-PK to guaiac-based fecal occult blood test in a total of 317 consecutive subjects with various clinical diagnoses. They reported a lower specificity for M2-PK that led to an unacceptable high number of false positive results and in turn reduces colonoscopy appropriateness (15). Subsequently, the authors compared M2-PK with FIT in a cohort made of 640 symptomatic subjects and found that FIT had a significantly higher specificity, PPV, and positive likelihood ratios compared to M2-PK (16). More recently, in a prospective multicenter Italian study, it has been reported that FIT in combination with fecal M2-PK assessment had a high sensitivity (91%) and negative predictive value (97%) for CRC detection encouraging their use in clinical practice for a more appropriate management of colonoscopy waiting lists (17). In addition, Kim and colleagues, in a case-control study, found that immuno-chromatographic M2-PK test was superior to FIT in terms of sensitivity for both CRC and adenomas detection (92.8% *vs*. 47.5% and 69.4% *vs*. 12.1%, respectively) (18). However, previous studies were performed on symptomatic patients recruited in clinical settings. Leen and colleagues performed the first study to assess the performance of fecal M2-PK addition to FIT-based screening program (10). The authors reported a significant improvement in ADR leading to the identification and removal of 70% more polyps. However, the study was performed within a second round of a screening program, and it was not possible to draw any defini-

tive conclusions about the potential role of the combined approach in a first-round screening.

In the present study, from a total of 1027 subjects invited, 572 (55.7%) accepted to participate and provided samples for both FIT and M2-PK. According to the Italian Group for Colorectal Cancer Screening (GISCoR) "Operative report of quality indicators", participation rate was more than acceptable (acceptable > 45%, desirable > 65%) (19), indicating that the addition of fecal M2-PK assessment to the screening protocol did not significantly reduce compliance and, subsequently, screening program performance. Positivity rate was significantly lower for fecal M2-PK compared to FIT (5.8% *vs*. 12.3%, $P < 0.001$) denoting a higher specificity for M2-PK test. However, no differences were found regarding PPVs between the two tests (cancer PPV, $P = 0.860$; neoplasm PPV, $P = 0.100$; and advanced neoplasm PPV, $P = 0.150$). Interestingly, only 10 subjects had both FIT and M2-PK positive. In fact, FIT-positivity is related to bleeding adenomas and tumors whereas M2-PK-positivity is related to the release in feces of a metabolic biomarker characteristic of tumor cells and their precursors (6). The discrepancy among the results may be explained by the different parameters analyzed by these two tests. However, the observed variance supports the usefulness of a combined screening approach based on both FIT and fecal M2-PK evaluation. In fact, the addition of fecal M2-PK led to the identification of 18 neoplasms that resulted negative to FIT. Importantly, the eventual use of the combined approach will not significantly improve colonoscopy workload, as deduced by the comparable cancer and advanced neoplasm NNS ($P = 0.961$ and $P = 0.402$, respectively).

Regarding fecal M2-PK levels, we did not find any significant difference between cancers and adenomas ($P = 0.630$). Conversely, Koss and colleagues previously reported a significant increase in M2-PK concentrations in adenoma-carcinoma sequence progression (20). This discrepancy could be explained by both the low number of CRCs found in our study which may have reduced the statistical power of the analysis, and the different population studied.

One limitation of our study is the absence of endoscopic investigation in all participants, which did not allow to calculate the sensitivity and specificity of FIT and fecal M2-PK for neoplasm detection. However, this research was carried out under real practice condition, and consequently, colonoscopies were not performed in subjects with negative fecal tests results.

In conclusion, fecal M2-PK is not a reliable alternative to FIT for CRC screening but when combined with FIT offers the potential to detect additional adenomas and cancers that either do not bleed or only bleed intermittently without reducing participation rate and without increasing endoscopy workload. Further studies taking into account alternative cut-off values and/or different strategies for priority management of endoscopic examination are necessary. Moreover, a cost-benefit analysis is still required to evaluate the affordability of FIT and fecal M2-PK combined approach in the setting of CRC screening programs.

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Potential conflict of interest

None declared.

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