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Faecal dimeric M2 pyruvate kinase in colorectal cancer and polyps correlates with tumour staging and surgical intervention

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Abstract

Objective & Method A dimeric form of pyruvate kinase isoenzyme (tumour M2-PK) is predominantly found in highly proliferating cells. Sandwich ELISA with monoclonal antibodies against dimeric (tumour) M2-PK was used to measure faecal tumour M2-PK in; 13 controls, 10 patients with colonic polyps and 32 patients with colorectal cancer.

Results Levels of faecal tumour M2-PK were higher in patients with colorectal cancer (median 11.72 U/ml; range 0.9–146.95 U/ml, $P = 0.0001$) and polyps greater than 10 mm (median 2.54 U/ml; range 0.9–29.46 U/ml, $P = 0.041$) when compared with controls (median 1.75 U/ml; range 0.9–3.41 U/ml). Furthermore, levels were higher in stages Duke's B ($P = 0.013$) and Duke's C ($P = 0.43$) than in Duke's A. Six months postsurgery faecal tumour M2-PK levels fell significantly to 3.46 U/ml (range 1.03–9.05 U/ml, $P = 0.001$). The sensitivity of a positive faecal tumour M2-PK test, defined as a level above 3.33 U/ml, was 91% for colorectal cancer, 60% for >10 mm and 20% for <10 mm polyps, with a specificity of 92%.

Conclusion Faecal tumour M2-PK is a highly sensitive marker for colorectal cancer and larger polyps. It also correlates with more advanced stages of colorectal cancer and its reduction is associated with successful surgical intervention.

Keywords Faecal, M2-PK, pyruvate kinase, colorectal cancer, polyps, screening

Introduction

It has been known for over 70 years that many tumours consume glucose rapidly and produce a large amount of lactic acid [1,2]. A glycolytic phenotype of invasive human cancers gives a powerful growth advantage, as malignant lesions grow progressively further from their blood supply in hypoxic conditions [3,4]. Overproduction of hypoxia inducible factor (HIF-1), which regulates glucose metabolism and transport, may play an important role as well as direct interaction of glycolytic enzymes with certain oncoproteins [5–7]. Upregulation and a shift in glycolytic enzymes may occur at the RNA and protein level, as well as the level of enzyme activity [8–10]. During neoplastic transformation the tissue-specific pyruvate kinase isoenzymes (such as L-PK in liver and kidney; M1-PK in skeletal muscles and brain) are replaced by M2-PK isoenzyme [11]. Furthermore, the tetrameric form of M2-PK is shifted to less active dimeric form by direct interaction with oncoproteins such as HPV-16, E7 and $pp60^{v-src}$ [6,7,12].

A wide range of tumours, including many gastrointestinal cancers, such as oesophageal, gastric, pancreatic and colorectal carcinomas, have been shown to overexpress tumour M2-PK [13–16]. Tumour M2-PK has not only been detected in histological specimens but also in blood and stool samples. An enzyme-linked immunoabsorbant assay (ELISA) test with antibodies against tumour M2-PK has been used in pilot studies in screening for colorectal cancer [17,18]. An antigen epitope of tumour M2-PK remains stable in faeces at room temperature for 2 days and for up to 1 year at −20°C, giving a practical advantage over other recently described faecal tests requiring fresh stool samples [19,20].

Our aim was to assess faecal tumour M2-PK levels along the adenoma-carcinoma sequence and to evaluate its levels in different stages of colorectal carcinoma. The

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sensitivity and specificity of faecal tumour M2-PK was compared with a guaiac-based faecal occult blood test (gFOBT). We also evaluated faecal tumour M2-PK pre and postsurgery.

Method

Patients and samples

Approval was obtained from The Shropshire Research Ethics Committee (03/36/RSH) prior to commencing this study. All 55 patients were reviewed or admitted to the Royal Shrewsbury Hospital with a history of microcytic anaemia and underwent colonoscopy and oesophago-gastro-duodenoscopy (OGD). All colonic biopsies were evaluated by two independent histopathologists.

All 13 patients included in our control group (six men, seven women; median age 69 years; range 20–85 years) had a normal OGD and colonoscopy. These patients were not an average-risk population.

Ten patients were diagnosed with tubulovillous or tubular adenoma colonic polyps (median age 64 years, range 50–84 years; seven male, three female patients). Five patients had smaller than 10 mm polyps and five patients had greater than 10 mm polyps. High-grade dysplasia was found in only one patient.

Out of the 32 patients with primary colorectal cancer in our study, 29 patients were already known to have colorectal cancer and were awaiting surgery. The remaining three patients had a new diagnosis of colorectal cancer at first colonoscopy. The median age of patients with colorectal cancer was 66 years (range 46–86 years) with a male to female ratio of 3:1. All patients underwent surgical resection of colorectal cancer, which was classified according to Duke's staging yielding stage A ($n = 3$), stage B ($n = 17$) and stage C ($n = 12$). All patients were followed-up for 12 months. No deaths were observed during that period.

Six months after surgery, 19 out of 32 patients with colorectal cancer provided a second stool sample for faecal tumour M2-PK. A further 13 patients withdrew from our study after surgery.

All stool samples were collected within 2 days a stored at −20°C for up to 6 months prior to analysis.

Measurement of faecal tumour M2-PK level

Tumour M2-PK was measured with a commercially available sandwich ELISA (ScheBo® Biotech AG, Giessen, Germany) with monoclonal antibodies against dimeric M2-PK. A random stool specimen was extracted with the Quick-Prep tube, diluted and analysed as described by the manufacturer [21].

Statistical analysis

Statistical significance was determined by the Kruskall–Wallis, Mann–Whitney test and Student's *t*-tests. *P*-values less than 0.05 were interpreted as statistically significant.

Results

The median faecal tumour M2-PK level in the control group was 1.75 U/ml and ranged from 0.9 U/ml to 3.41 U/ml. The upper limit of normal range was optimized at 3.33 U/ml to obtain a desired specificity of greater than 90%.

The median faecal tumour M2-PK level in patients with colonic polyps was 2.54 U/ml and ranged between 0.9–29.46 U/ml. There was no significant difference in levels between patients with polyps smaller than 10 mm (median 1.2 U/ml, range 0.9–6.8 U/ml, $P = 0.96$) and healthy controls. Only patients with polyps larger than 10 mm had a significantly higher level (median 5.32 U/ml, range 2.99–29.46 U/ml) than the controls ($P = 0.041$).

Patients with colorectal adenocarcinoma had the highest median faecal tumour M2-PK level at 11.72 U/ml (range 0.9–146.95 U/ml) (*vs* control group, $P = 0.0001$; *vs* patients with polyps, $P = 0.02$) (Fig. 1). It was observed that faecal tumour M2-PK levels were higher in Duke's C (median 13.26 U/ml, range 2.01–111.75 U/ml, $P = 0.043$) and Duke's B (median 12.33 U/ml, range 1.71–146.95 U/ml, $P = 0.013$) than in Duke's A (median 3.81 U/ml, range 0.90–3.87 U/ml). There was no significant difference in levels between stages Duke's B and C. (Fig. 2). No association was found between tumour size and faecal tumour M2-PK levels. Although we could only speculate as why there is no such association, it is likely that cancer metabolism,

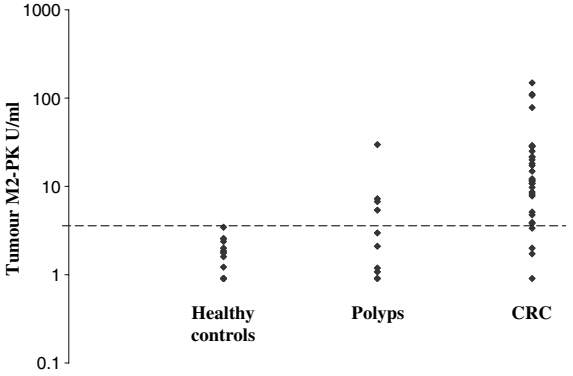

Figure 1 Faecal tumour M2-PK (U/ml) in healthy controls, patients with colorectal polyps and colorectal cancer (CRC) expressed on logarithmic scale. The dashed line at 3.33 U/ml represents the cut-off level for abnormal values.

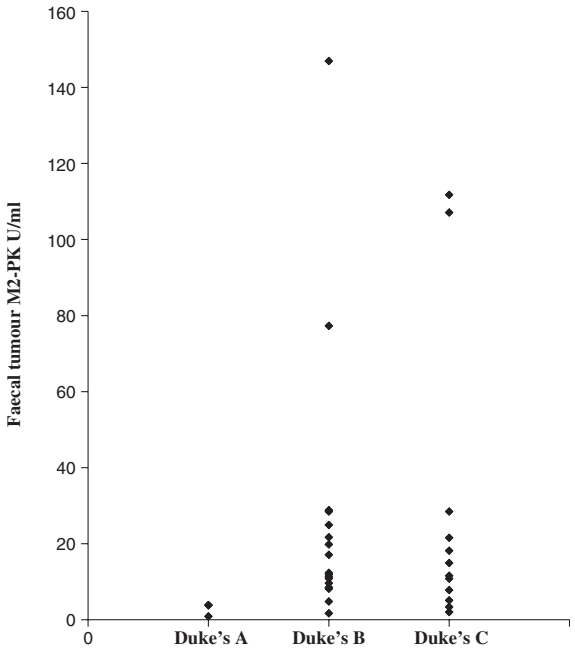

Figure 2 Faecal tumour M2-PK (U/ml) in patients with colorectal cancer staged according to Duke's A, B and C classification.

growth and invasiveness regulated by multiple factors e.g. tissue hypoxia, HIF-1 and oncoproteins may have more significant effect on tumour M2-PK production rather than the size of malignant tumour. Those factors play no or only partial role in the metabolism of polyps.

We found no association between faecal tumour M2-PK level and patients' age, sex or co-morbidities (including diabetes mellitus, heart failure and rheumatoid arthritis).

It was observed that 6 months postsurgery the median faecal tumour M2-PK level fell significantly from 10.87 U/ml (range 1.71–111.75 U/ml) to 3.46 U/ml (range 1.03–9.05 U/ml) ($P = 0.001$). Six month post-surgery faecal tumour M2-PK levels were above normal level in seven out of 19 patients. One patient suffered from local cancer recurrence, three patients had remaining colorectal polyps; two patients had rectal inflammation with abscess formation and one patient had normal follow-up colonoscopy.

Faecal tumour M2-PK sensitivity for polyps greater than 10 mm was 60% (95% CI: 23–88%) and 20% (95% CI: 4–62%) for polyps smaller than 10 mm. The sensitivity of faecal tumour M2-PK for colorectal cancer was 91% (95% CI: 76–97%) with specificity of 92% (95% CI: 67–99%). In patients with larger polyps the likelihood ratio of a positive result (LR+) of faecal tumour M2-PK was 7.8 (95% CI: 1.4–47.3) and the likelihood ratio of a negative result (LR−) was 0.43 (95% CI: 0.13–0.89). In patients with smaller polyps LR+ was 2.6 (95% CI: 0.3–21.6) and LR− was 0.87 (95% CI: 0.40–1.28). LR+ of faecal tumour M2-PK in patients with colorectal cancer was 11.8 (95% CI: 2.7–66.2) and LR- was 0.10 (95% CI: 0.03–0.27).

Increasing the cut-off level of faecal tumour M2-PK to 4.0 U/ml resulted in a lower sensitivity of 81% (95% CI: 65–91%) for colorectal cancer, unchanged sensitivity for polyps and an increased specificity of 100% (95% CI: 77–100%).

In comparison, the sensitivity of gFOBT for colorectal cancer (based on single stool sample but performed in duplicate) was 21% (95% CI: 9–40.5%), 20% for larger than 10 mm polyps (95% CI: 4–62%) and 0% for smaller than 10 mm polyps (95% CI: 0–43%). The specificity of gFOBT in our study was 100% (95% CI: 76–100%).

Discussion

Two-step colorectal cancer screening requires a simple, inexpensive preliminary test (the first stage) to select those individuals who are most likely to benefit from more accurate but invasive second step investigation such as colonoscopy.

In the present study, we evaluated a newly developed ELISA test for faecal tumour M2-PK, a dimeric form of pyruvate kinase isoenzyme, which is commonly overexpressed by tumour cells. We demonstrated that levels of faecal tumour M2-PK increased as the adenoma-carcinoma sequence progressed. In patients with dysplastic polyps, faecal tumour M2-PK levels were higher in those with larger polyps when compared to those with smaller polyps or healthy controls. In patients with colorectal cancer faecal tumour M2-PK level were higher in stages Duke's B and C than Duke's A.

We observed that following successful surgery levels of faecal tumour M2-PK fell to the normal range. It remained elevated in patients with tumour recurrence, in those with remaining colonic polyps or complicated colonic abscesses. Therefore faecal tumour M2-PK may be used in monitoring patients postsurgery to select those who require further colonoscopic investigations. Plasma tumour M2-PK is reported to be more sensitive in non-metastatic colorectal, gastric and oesophageal cancers than serum carcinoembryonic antigen CEA [22,23]. Therefore, in the postsurgical management of patients a combination of faecal and plasma tumour M2-PK tests may be more sensitive in detecting recurrence of colorectal cancer than the faecal test alone or serum CEA alone.

The sensitivity of faecal tumour M2-PK for smaller polyps was 20% and for larger polyps 60% with specificity of 92%. Faecal tumour M2-PK has shown

high sensitivity (91%) and specificity (92%) for colorectal cancer and larger than 10 mm colorectal polyps (sensitivity 60%). Hardt *et al.* [17] reported sensitivity of 73% and specificity of 78% of faecal tumour M2-PK for colorectal cancer. The reduced sensitivity found in their study may be partially explained by their higher faecal tumour M2-PK cut-off level of 4 U/ml. Re-calculated sensitivity of faecal tumour M2-PK for colorectal cancer in our study with increased cut off level to 4 U/ml (as recommended by manufacturer) is lower at 81.25% (95% CI: 65–91%) with specificity of 100% (95% CI: 77–100%). However, two patients in our study with Duke's A colorectal cancer will not be detected if the cut off level is increased to 4 U/ml. More studies are therefore required to evaluate the role of faecal tumour M2-PK in detecting patients with Duke's A colorectal cancer. The sensitivity for larger than 10 mm polyps with the increased cut off level to 4 U/ml is unchanged in our study (60%).

Positive results of faecal tumour M2-PK may be founding in other gastrointestinal tract tumours but also in patients with colonic inflammation as observed in two patients with rectal abscesses and patients with ulcerative colitis (data not included).

The gFOBT has been shown to reduce disease specific mortality in population based randomized trials [24–26]. It is an easy and practical test to perform and has a low cost of about £5900 per life-year saved [27]. At the same time it has limited sensitivity (24–50%) and about half of all colonoscopies carried out on the basis of a positive test result show no evidence of neoplasia [25,28].

The M2-PK test is more expensive than standard gFBOT. The M2-PK kit costs approximately £8.00 per person and we estimate that with analysis the costs will be approximately £13.50 plus VAT compared with an FOBT, which costs approximately £5.00 per person. Nevertheless if the markedly increased sensitivity of the M2-PK is confirmed in screening studies and results in more early colorectal cancer detection, this test is likely to be cost-effective in terms of incremental cost/life year saved compared with gFOBT.

The study design has some limitations. A case–control design was chosen which could overestimate the accuracy of the test compared with cohort studies in average risk individuals [29]. Furthermore, the test would ideally detect colorectal cancer at an early stage, however, there were only a few patients with Duke's A colorectal cancer in our study. We therefore cannot determine whether the accuracy of M2-PK is similar for early and late stage colorectal cancer. The M2-PK test however is a promising non-invasive test and warrants further study in the general population. Development of improved screening methods should go hand in hand with better chemoprevention for colorectal cancer to achieve an ultimate goal of reduced mortality [30].

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What is current knowledge?

- Tumour M2-PK is a dimeric form of pyruvate kinase isoenzyme, which is commonly overexpressed by tumour cells.
- It gives a powerful metabolic advantage to tumour cell.
- Tumour M2-PK may be founding in all gastrointestinal tumours.

What is new here?

- Level of faecal tumour M2-PK increases as the adenoma-carcinoma sequence progresses.
- Faecal tumour M2-PK is highly sensitive and specific to colorectal cancer and larger polyps.
- Faecal tumour M2-PK can be used in screening for colorectal cancer and monitoring patients postsurgery.

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