

# Tumour M2-pyruvate kinase: a gastrointestinal cancer marker

Yogesh Kumar, Niteen Tapuria, Naveed Kirmani and Brian R. Davidson

**Background** Gastrointestinal cancer tumour markers are valuable in the detection of recurrence following resection or in monitoring response to chemotherapy. CEA, CA19-9, CA-50 and CA72-4 are currently available but are nonspecific and have a low sensitivity. 'Tumour M2-pyruvate kinase' was described by Eigenbrodt around 1985. In cancers the active tetrameric form of the M2 isoenzyme of pyruvate kinase converted to an inactive dimeric form by direct interaction with oncoproteins to channel glucose carbons into DNA synthesis. This review summarizes the current knowledge of this unique tumour marker with regard to its biochemistry, assay and potential use as a diagnostic and screening tool in gastrointestinal cancer.

**Methods** A literature search was conducted for entries from 1980 to 2005 using PubMed and NeLH databases using tumour M2-pyruvate kinase, faecal tumour M2-pyruvate kinase, tumour metabolism, tumour markers and carcinoembryonic antigen as keywords. A total of 56 references relevant to tumour M2-pyruvate kinase were retrieved. Eighteen references were clinical studies involving plasma/faecal tumour M2-pyruvate kinase and gastrointestinal cancer. The remaining 38 references were clinical/nonclinical trials and reviews on tumour metabolism and plasma/faecal tumour M2-pyruvate kinase assay. Seven of the 18 clinical studies involved faecal M2-pyruvate kinase. Three of the 11 plasma tumour M2-pyruvate kinase studies were non-English language and were excluded. The sensitivity, specificity, positive predictive and negative predictive value for plasma/serum tumour M2-pyruvate kinase in the detection of gastrointestinal cancer was determined for each of the remaining eight studies. Data for gastrointestinal cancer M2-pyruvate kinase were compared with other gastrointestinal cancer markers. Data from three of the eight studies using a diagnostic cut-off value of 15 U/ml for

ethylenediaminetetraacetic acid (EDTA) plasma tumour M2-pyruvate kinase were analysed together as a small meta-analysis.

**Results** At a diagnostic cut-off value of 15 U/ml for tumour M2-pyruvate kinase in EDTA plasma the sensitivity, specificity, positive predictive and negative predictive value was 57.3, 89, 85.7 and 64.8%, respectively, for colorectal cancers, 62.1, 89, 88 and 64%, respectively, for gastric/oesophageal cancers and 72.5, 89, 58 and 94%, respectively, for pancreatic cancers. As a faecal marker for colorectal cancers, faecal tumour M2-pyruvate kinase has a sensitivity of 73–92% at a cut-off value of 4 U/ml as against 50% sensitivity for Guaiac faecal test.

**Conclusion** Circulating tumour M2-pyruvate kinase is more commonly elevated in oesophageal, gastric and colorectal cancer patients than conventional tumour markers. Faecal M2-pyruvate kinase is a sensitive marker of colorectal cancer. The clinical role of tumour M2-pyruvate kinase in gastrointestinal cancer management should be investigated in large-scale clinical trials.

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## Introduction

Gastrointestinal (GI) cancer is one of the commonest causes of cancer death in Europe [1,2]. In the UK, colorectal cancer accounts for 12% of all cancers. It is the second most common cancer among women after breast cancer and the third most common in men after lung and prostate cancer [3]. Stomach and pancreatic cancer account for 3% of all reported cases of cancer [3]. The high mortality of GI cancers may relate to their advanced stage at diagnosis and early detection is an important way of reducing cancer mortality. Current tumour markers have a low sensitivity for detecting cancer and their role is

limited to detecting recurrence after surgery or monitoring response to treatment. Even the most commonly used GI tumour marker, carcinoembryonic antigen (CEA), has been repeatedly questioned regarding its clinical usefulness [4,5].

Tumour M2-pyruvate kinase (PK), the inactive dimeric form of the M2 isoenzyme of PK (a glycolytic pathway enzyme), was first described in 1985 by Eigenbrodt as a tumour characteristic metabolic marker [6–8]. Initial studies in patients with cancers of the lung, pancreas, liver, kidney and breast showed increased activity of PK

type M2 in blood as well as cancer tissues and its role is emerging in the management of GI cancers [9–14]. It can be measured in both blood and faeces. The review aims to provide a critical review of the current literature on tumour M2-PK as a marker of GI cancer.

## Methods

A literature search was conducted for the period from 1980 to 2005 using PubMed and NeLH databases using the following keywords: tumour M2-pyruvate kinase, faecal tumour M2-pyruvate kinase, tumour metabolism, tumour markers and carcinoembryonic antigen. A total of 56 references relevant to tumour M2-PK were retrieved. Thirty-eight references were reviews, book chapters and bibliographic links from the reviews on tumour M2-PK biochemistry, assay and measurement [6–11,13–44]. Eighteen references were the clinical trials involving circulating/faecal tumour M2-PK and GI cancer [12,45–61]. Seven of these 18 clinical studies were related to faecal tumour M2-PK in GI cancer [49,53–58]. Of the remaining 11 studies for plasma/serum tumour M2-PK, three studies were in non-English language and have been excluded [59–61]. Full papers on eight studies with serum/plasma tumour M2-PK and GI cancer were reviewed [12,45–48,50–52]. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for tumour M2-PK for individual GI cancer types and in comparison with other cancer markers. Three [46,48,50] of the eight studies, which all used the same diagnostic cut-off value of 15 U/ml for EDTA plasma tumour M2-PK, were used for a small meta-analysis. Only one full published English language paper [55] on faecal tumour M2-PK is available. The rest of the data on faecal tumour M2-PK was obtained from two clinical trials [49,58], two published abstracts [53,54] and two German studies with English abstract [56,57].

## Current gastrointestinal tumour markers – roles and limitations

### Carcinoembryonic antigen

This glycoprotein has a structural similarity to the adhesion proteins, intercellular adhesion molecule (ICAM)-1 and ICAM-2 [62,63], suggesting a role in cancer invasion and dissemination [64,65]. It can be measured in the serum and its clinical use has been investigated in GI cancers. It is less frequently elevated in early stage (Duke's A and B) colon cancers, the stages at which early detection is most likely to result in curative surgery. In a study by Wang *et al.* [66] the proportion of patients with increased serum CEA concentration (> 5 ng/ml) in Duke's A and Duke's B stage disease were 25 and 39%, respectively, compared with 71% in Duke's C stage. As pointed out by Fletcher [67,68], sensitivity in symptomatic individuals is likely to be higher than in the asymptomatic individuals, because the

former group is likely to have advanced disease. Serum CEA can also be increased in other forms of cancer and in multiple benign disorders [68]. A high preoperative serum CEA level is associated with a poor outcome in colorectal cancer [66,69–74]. Unfortunately, no clinical benefit has been demonstrated by the use of adjuvant chemotherapy based solely on increased preoperative CEA concentration [5]. Elevated CEA levels following bowel cancer resection is also correlated with an adverse outcome [5]. In a landmark study Moertel *et al.* [75] demonstrated that CEA monitoring following bowel cancer resection had a 59% sensitivity rate for recurrence, but with a 16% false-positive rate. In a randomized prospective study, Ohlsson *et al.* [76] showed no difference in 5-year survival rate or cancer-specific survival rates between an intensive CEA-based follow-up and a group with no follow-up. Recent meta-analyses of randomized trials suggest that intensive CEA, computed tomography scan and colonoscopy-based post-operative surveillance improves 5-year survival rates by approximately 10% compared with less intensive follow-up [77–79]. Current guidelines by the National Institute for Clinical Excellence, therefore, recommend the measurement of CEA along with serial imaging following colorectal cancer resection [80].

### Carbohydrate antigen 19-9 (CA19-9)

This is an oligosaccharide related to the Lewis A blood group substance [4]. It has been proposed as a sensitive marker for pancreatic, gastric and hepatobiliary malignancies [81]. CA19-9 is elevated in nearly 80% of advanced pancreatic cancer patients. The false-positive rates, however, are also high at 20–30% in benign hepatobiliary and pancreatic diseases [82]. Other benign conditions associated with elevated CA19-9 levels include pneumonia, pleural effusion, renal failure and systemic lupus erythematosus (SLE) [81]. Recent reviews and multicentre studies [83,84] have questioned the clinical significance of elevated levels of CA19-9. Confident discrimination between benign and malignant disease cannot be made on the basis of a solitary elevated CA19-9 (> 33 U/ml) measurement [84]. Elevated levels are associated with advanced disease at presentation and with disease progression during follow-up [83]. The clinical role of the tumour markers CEA and CA19-9 in GI cancer diagnosis and management is limited and new serological markers are required.

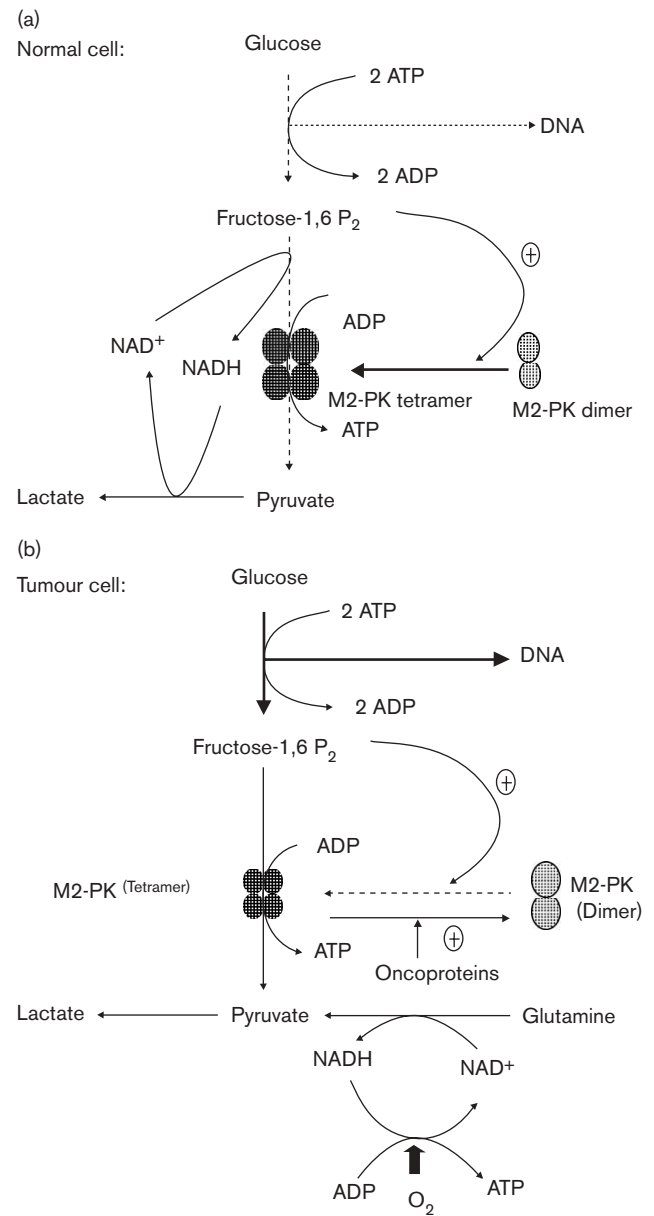
### Tumour metabolome

The term tumour metabolome (in analogy to tumour genome and tumour proteome) was coined by Mazurek and Eigenbrodt in 2001 for the metabolic characteristics of tumour cells [19,20,24]. In differentiated cells glucose is mainly converted to pyruvate via glycolysis and thereafter to CO<sub>2</sub> and water or lactate depending on oxygen supply.

The final reaction of glycolysis is catalysed by the highly regulated enzyme, PK. This enzyme mediates the transfer of high energy phosphate of phosphoenolpyruvate (PEP) to generate ATP and pyruvate in differentiated cells. PK has different isoenzymes. L-PK is present in tissues with gluconeogenesis such as the liver and kidney, R-PK is present in erythrocytes and M1-PK is found in tissues requiring large amounts of energy such as the brain and muscle [6,7,13,25,41]. M2-PK is present in all proliferating cells such as embryonic and adult stem cells, but especially in tumour cells. M2-PK can occur in a highly active tetrameric form with high affinity for its substrate PEP and in an inactive dimeric form with a low affinity to PEP [6–8,18–20,24,26,41]. The tetrameric form is associated with other glycolytic enzymes within the so-called glycolytic enzyme complex which leads to a very effective conversion of glucose to lactate [7,13,18,25,41]. In tumour cells the dimeric form is always predominant and has therefore been labelled as tumour M2-PK [7,18,24,26,37] (Fig. 1). The dimeric form switches to the tetrameric form with high levels of fructose 1,6 bi-phosphates in tumour cells [18]. During tumourogenesis, different tissues with totally different basic metabolism, for example, liver and brain, shift to the same metabolic phenotype [18]. The common result is increased glycolysis, glutaminolysis, expansion of phosphometabolites and a shift of metabolism to the synthesis of nucleic acids, amino acids and phospholipids [6–8,18–20,26,29,38,41,85]. Energy production is facilitated by an alternative pathway called glutaminolysis [28] (degradation of the amino acid glutamine to lactate), which depends on an adequate oxygen supply and high NAD(P) levels [6,18,21,41]. In the absence of oxygen, M2-PK is reactivated to the tetrameric form by high bi-phosphate levels and glutaminolysis is inhibited, thereby switching glucose metabolism to energy production. Thus, M2-PK may act as a sensor of the tumour metabolome allowing the tumour cells to adapt to varying oxygen and nutrient supply. Although tumour cells are able to compensate for nutrient starvation for a while, if NAD(P) levels are low then both glycolysis and glutaminolysis are inhibited and tumour apoptosis occurs [18]. A similar mechanism for tumour cell apoptosis is induced by chemotherapeutic drugs in which decreased NAD(P) levels result in the inability of tumour cells to recycle NAD.

M2-PK is a target of different oncoproteins with totally different physiological mechanisms such as the pp60v-src kinase [30] and HPV-16 E7 [22]. The pp60v-src kinase phosphorylates M2-PK in tyrosine. The E7 oncoprotein of the human papilloma virus type 16 directly binds to M2-PK. Thus the tetrameric form of M2-PK is dissociated to the dimeric form during transformation of normal cells to oncoprotein-expressing cells [18,20,22,29,30,41].

Fig. 1



M2-pyruvate kinase (M2-PK) in normal proliferating cells and cancer cells. The tetrameric form is predominantly present in normal cells while the dimeric form is predominant in cancer cells. Reproduced from [18].

### Quantification of tumour M2-pyruvate kinase Blood

Tumour M2-PK can be detected by a highly sensitive enzyme-linked immunosorbent assay (ELISA), which allows the quantitative measurement of tumour M2-PK in EDTA-plasma samples. The test is based on two monoclonal antibodies, which specifically react with tumour M2-PK and do not cross-react with the other isoforms of PK (types L, R and M1) [31,32,86]. Tumour

M2-PK is adsorbed onto microtitre wells coated with a specific monoclonal antibody. It is quantified after incubation with a biotinylated second monoclonal antibody and with streptavidine–peroxidase conjugate [86]. The mean intra-assay coefficient of variance (CV) is 3.5% and the mean interassay coefficient of variance is 5.3% [33,34]. A reference concentration of  $\leq 15.0$  U/ml in EDTA plasma corresponds to a specificity of 90% for a control group of patients without cancer ( $n = 393$ ) [34] (Fig. 2). A study involving 695 healthy controls showed a specificity of 95% at a diagnostic cut-off value of 17.5 U/ml in EDTA-plasma samples [17]. The tumour M2-PK concentration in these healthy individuals ranged from 2 to 30 U/ml with a median value of 6 U/ml. Tumour M2-PK concentrations have been shown to be affected by haemolysis of blood samples (median value: 50.5 U/ml), icterus (median value: 39.1 U/ml) and lipaemia (median value: 30.8 U/ml). A correlation with the severity of these conditions, however, has not been reported [17].

### Stool

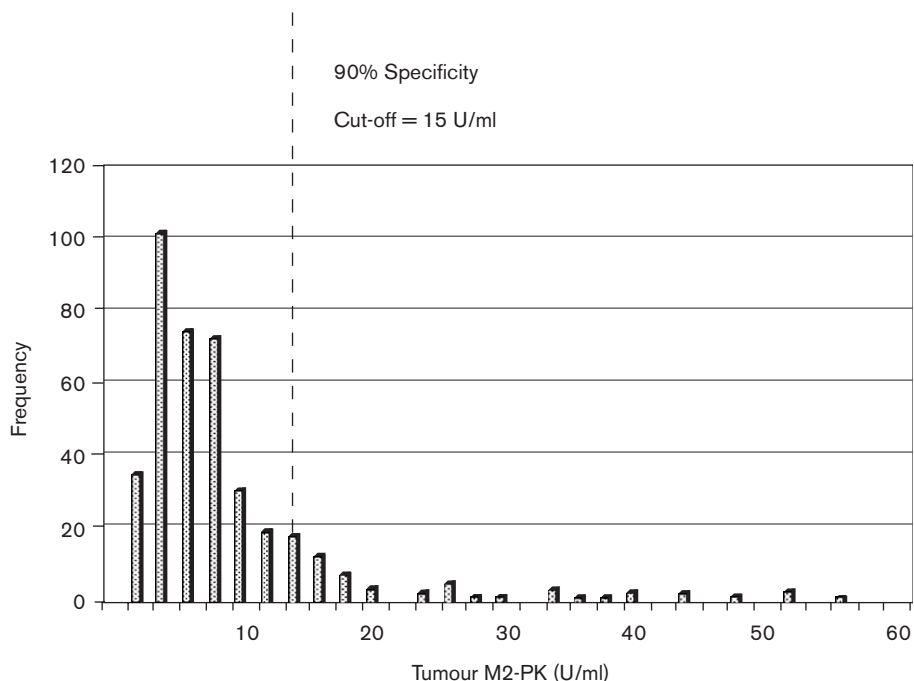
Tumour M2-PK can be measured in stool by a similar ELISA technique using the same monoclonal antibody as used in the serum/plasma assay. A reference concentration of 4 U/ml corresponds to a specificity of 83% for a control group of individuals aged 50 to 89 years [44]. The intra-assay mean CV was 7.9% and the interassay mean CV was 7.3% [44].

## Factors affecting tumour M2-pyruvate kinase in plasma

### Benign diseases

Tumour M2-PK levels in EDTA plasma have been found to be elevated in bacterial infection as opposed to severe sepsis and polytrauma [35]. Other benign conditions reported to have tumour M2-PK elevations include: rheumatic diseases [16], diabetic nephropathy [15], chronic cardiac failure [36], inflammatory bowel disease [48] and acute and chronic pancreatitis [45]. Plasma tumour M2-PK, at cut-off value of 25 U/ml is elevated in 39% of patients with diabetic nephropathy [15]. In chronic cardiac failure (CCF), the median tumour M2-PK level in plasma of patients with NYHA grade-2 disease was 24 U/ml, with grade-3 disease was 30 U/ml and with grade-4 disease was 46 U/ml. The diagnostic cut-off value for CCF was 5 U/ml. How often it was elevated in both controls and patients with CCF was not mentioned in this study [36]. The mechanism suggested for the rise in plasma tumour M2-PK value in patients with heart disease was the increased glycolysis to meet the metabolic demand related to the increased ventilation and neurohormonal activation, for example, seen in CCF. An alternative explanation was that the increased bilirubin and triglycerides levels commonly observed in CCF patients had caused analytical interference with the tumour M2-PK assay. These postulations were not investigated although the authors had ruled out the

Fig. 2



Distribution of tumour M2-PK (M2-pyruvate kinase) levels in 393 noncancer controls with 90% of subjects had tumour M2-PK levels below 15 U/ml (data obtained by personal communication with ScheBo Biotech, Giessen, Germany).

impaired renal function seen in CCF as a cause of elevated plasma tumour M2-PK levels [36]. Oehler *et al.* [35] studied the expression of PK type M2 in neutrophils of polytrauma patients. Using Western blotting for identifying M2-PK expression, they noticed strong expression of M2-PK in 62% of polytrauma patients as compared with none of the healthy volunteers. Oremek *et al.* [16] showed elevated levels of plasma tumour M2-PK (diagnostic cut-off 17.5 U/ml) in different types of rheumatic diseases. It was elevated in 82% of rheumatoid arthritis patients, 82% of seronegative spondyloarthritis patients and 63% of patients with collagen disorders. The overall median value of plasma tumour M2-PK in rheumatic diseases was 26 U/ml. Plasma tumour M2-PK (diagnostic cut-off 15 U/ml) was elevated in 68% of patients with inflammatory bowel disease with a median value of 12 U/ml [48], 68% of patients with acute pancreatitis with a median value of 22 U/ml and 67% of patients with chronic pancreatitis with a median value of 11 U/ml (cut-off 8.9 U/ml) [45]. These levels were significantly higher than the median levels in respective controls. The cause of this rise in tumour M2-PK value with benign disease has not been elucidated in any of these studies. The mechanism suggested is an increased glycolysis to meet the metabolic demand related to the stress of trauma and inflammatory reaction [45]. No correlation between plasma tumour M2-PK levels and the severity activity index or C-reactive protein levels was found in these inflammatory conditions [48]. Cross-reactivity of monoclonal antibodies with the tetrameric form of M2-PK cannot explain these results as the two monoclonal antibodies used in these studies are highly specific to the dimeric form. The level of tumour M2-PK in EDTA plasma should therefore be interpreted with caution in patients with these benign conditions.

#### **Tumour M2-pyruvate kinase levels and tumour stage**

As with most tumour markers, the concentration of tumour M2-PK tends to increase with disease stage. Zhang *et al.* [46] showed an increase in plasma tumour M2-PK levels with increasing tumour stage in gastric [compared with tumour node metastasis (TNM) stage], colorectal (compared with Duke's stage) and pancreatic cancers (compared with TNM stage) [45]. The level of tumour M2-PK in patients with pancreatic cancer ( $n = 60$ ) differed significantly between those with stage I–II disease and those with distant metastasis (stage IV). Among non-GI cancers the association between tumour M2-PK levels and disease stage has also been found [24,37,40]. In lung tumours the sensitivity of tumour M2-PK was observed to be 28% in stage I, increasing progressively to 73% in stage IV. A similar correlation is seen in renal cancer staging (Robson staging) with serum/EDTA plasma tumour M2-PK increasing in sensitivity from 60% in stages I and II to 100% in stage IV. Faecal levels of tumour M2-PK showed a strong correlation with TNM and Duke's staging in colorectal cancer [87]. Faecal

tumour M2-PK has a higher sensitivity than plasma tumour M2-PK in determining cancer stage in colorectal cancer [50,87].

#### **Sample stability for the tumour assay**

The level of tumour M2-PK in blood can be influenced by the mechanical stress of shaking the sample, the type of anticoagulant (EDTA, heparin, citrate), duration before the blood sample is centrifuged and the temperature at which the centrifuged sample is stored. Hugo *et al.* [33] observed a high reproducibility of tumour M2-PK levels in EDTA plasma but not with serum or citrated/heparinized plasma blood samples from 10 healthy volunteers. Shaking or leaving the samples at room temperature for several hours before centrifugation led to a 2–3-fold increase of tumour M2-PK in serum and heparin-plasma samples. In contrast, the quantification in EDTA plasma and citrate plasma was absolutely stable after 24 h [33]. Lymphocytes were found to be a potential source for the increased concentration in serum and citrate plasma [33]. After centrifugation the EDTA-plasma sample is stable for 3 days at 4°C or for up to 1 year at –20°C [56,57]. No known factors are found that can interfere with the faecal tumour M2-PK levels. Excessive dilution of stool can lower the faecal M2-PK level. Therefore, a formed stool sample should always be analysed. Undiluted stool extracts can be stored at 4–8°C for 1 day or up to 4 weeks at –20°C without losing their stability [44].

#### **Tumour pathology**

There have been no GI cancer studies so far correlating M2-PK levels with the tumour size, grade and histological type. In renal cell carcinoma (RCC) patients ( $n = 40$ ), a significant correlation was found between serum tumour M2-PK and RCC grade (50% in G1-RCC, 70% in G2-RCC and 86% in G3-RCC) [37]. No correlation was found between serum tumour M2-PK levels and histological type or tumour diameter. Similarly in lung cancer neither plasma tumour M2-PK nor immunohistochemical staining showed significant correlation with the histological type or differentiation of cancer but the concentration of tumour M2-PK in EDTA plasma correlated well with tumour staging [10,43].

#### **Tumour M2-pyruvate kinase: role as a gastrointestinal cancer marker**

##### **Faecal M2-pyruvate kinase in screening for gastrointestinal cancer**

Following the completion of a pilot project based on centres in Scotland (Fife, Tayside and Grampian) and England (Coventry and Warwickshire) in which around 120 000 patients aged 50–69 years old were enrolled [88], the UK Department of Health announced the introduction of national colorectal cancer screening, which will begin to be 'rolled out' from 2006 in England for men and women aged 60–69 and from March 2007 in Scotland for

those aged 50–74 years [89]. Under these programmes patients will be offered a guaiac faecal occult blood (FOB) test every 2 years, with positive FOB test results being further investigated by diagnostic colonoscopy. A similar approach is also currently being assessed in Australia [90]. Randomized trials of screening by FOB test have been shown to reduce the disease-specific mortality by 15–18% although screening for cancer remains controversial owing to the large number of false-positive results [88,91,92]. The data from the Nottingham study showed a positive predictive value of only 12% (false-positive rate 88%) for colorectal cancer in individuals who underwent subsequent colonoscopy after FOB test [91]. Sigmoidoscopy, colonoscopy or combinations are the other current practices of searching for and removing adenomatous polyps to prevent colorectal cancer [93], but they are limited by poor patient compliance, complications and cost effectiveness [94,95]. Therefore newer screening tools for colorectal cancer are under evaluation and may take their place in future guidelines. Hardt *et al.* [49,58] showed that tumour M2-PK can be detected in the faeces of GI cancer patients. Symptomatic patients undergoing colonoscopy for various reasons had faecal tumour M2-PK measured. The faecal level of tumour M2-PK was higher in patients with histology proven colorectal cancers as compared with controls (non cancer patients). The sensitivity of faecal tumour M2-PK at a cut-off value of 4 U/ml was 73% with a specificity of 78%. The false-positive rate was 15%. This low false-positive rate, however, should be viewed with caution when comparing it with the high false-positive rate for Haemoccult faecal blood test used in the Nottingham study and the Danish trial which were based on a large asymptomatic population [91,92]. Faecal tumour M2-PK levels were higher with more advanced disease. The sensitivity increased from 57% in case of T1 cancer, 78% in T4 and 90% in patients with distant metastasis [55]. Two recent studies also showed a high sensitivity (92%) of faecal tumour M2-PK for detecting colorectal cancer [53,54]. Using a cut-off of 3.33 U/ml,

Koss *et al.* [53] found a specificity of 92%. At a cut-off of 4 U/ml McLoughlin *et al.* [54] found a similarly high sensitivity of 95%. These studies also looked at the sensitivity for the detection of polyps, finding a sensitivity of 63% for adenoma [54], 63% for polyps > 1 cm [53] and 25% for polyps < 1 cm [53]. One study has compared faecal tumour M2-PK with a guaiac and an immunological FOB test [57]. Sensitivity of the guaiac FOB test was only 27% for colorectal cancer and 10% for polyps, whereas it was 77 and 48%, respectively, for faecal tumour M2-PK and 91 and 19%, respectively, for the immunological FOB test. Specificity was 89, 72 and 94%, respectively. Small meta-analyses of studies with faecal tumour M2-PK reported an overall sensitivity of 77.9% for the detection of colorectal cancer and specificity ranging from 74.3 to 83.3%. Overall sensitivity for adenomatous polyps was 45.9%, increasing to 61.1% for those > 1 cm [56]. No randomized trial has been found to be comparing faecal M2-PK with FOB test or colonoscopy as a screening tool in terms of efficacy, cost effectiveness, feasibility and reducing the cancer-related mortality.

#### Plasma M2-pyruvate kinase in detection of different gastrointestinal cancers

In this review we analysed the data of eight clinical studies related to tumour M2-PK and GI cancer [12,45–48,50–52]. The diagnostic cut-off values for tumour M2-PK used in these studies ranged from 8.9 to 28 U/ml. Three studies [46,48,50] used the same cut-off value of 15 U/ml for tumour M2-PK in EDTA plasma and were chosen for meta-analysis of histologically proven GI cancers.

#### Oesophageal cancer (Table 1)

Three studies (one prospective and two retrospective) were found related to histologically proven oesophageal cancer [47,48,50]. One study combined data for gastric and oesophageal cancer [48]. The plasma tumour M2-PK concentration in oesophageal cancer ranged from 3.2 to 397 U/ml with a mean value of 42 U/ml. The controls

**Table 1 Studies comparing the tumour markers: tumour M2-PK, CEA, CA19-9 and CA72-4 in oesophageal cancers**

Reference	Study detail	Tumour marker (cut-off value)	Sensitivity %	PPV%	NPV%
[51]	Retrospective study <i>n</i> = 87 Controls = 141 Specificity = 89% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	59	76	77
		CA72-4 (4 U/l)	12	38.4	62
		CEA (5 µg/l)	15	45	63
		CA19-9 (25 U/l)	43	70	71
[48]	Prospective study <i>n</i> = 86 Controls = 76 Specificity = 95% <sup>a</sup>	Plasma tumour M2-PK (19.8 U/ml)	55.8	92	65
		CA72-4 (3.2 U/l)	53.5	92	64
		CA19-9 (23 U/l)	27.9	85.7	54
		CEA (8.3 µg/l)	14.5	75	49
[49]	Retrospective study <i>n</i> = 20 <sup>b</sup> Controls = 60 Specificity = 90% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	60	66.6	87
		CEA (3 µg/l)	25	50	78
		CA19-9 (37 U/l)	33	53.8	80

CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value; M2-PK, M2-pyruvate kinase.

<sup>a</sup>The specificity of CEA, CA72-4 and CA19-9 was not stipulated in these studies.

<sup>b</sup>Oesophageal/gastric cancers.

used in these studies were nonmalignant disease patients. The mean control value was 9.3 U/ml. The diagnostic cut-off value of 15 U/ml (published cut-off) was used in two of the studies [48,50] with a specificity of 89%, whereas the other study [47] used 19.8 U/ml cut-off value with a specificity of 95%. When data from the two oesophageal cancer studies with the same diagnostic cut-off level for plasma tumour M2-PK are analysed, 107 patients with 201 controls have been evaluated with an overall sensitivity of 59%, specificity of 89%, PPV of 74% and NPV of 80%. The overall sensitivity, PPV and NPV of plasma tumour M2-PK was higher as compared with those of CEA (14–25%, 45–75% and 49–78%, respectively), CA72-4 (12–53%, 38–92% and 62–64%, respectively) and CA19-9 (28–43%, 54–86% and 54–80%, respectively). The range represents the lowest and the highest value for these tumour markers in the three studies. Because of different cut-off values the data from the individual studies could not be combined. The specificity of CEA, CA72-4 and CA19-9 was not clearly stated in these studies.

**Gastric cancer (Table 2)**

Five studies (two prospective and three retrospective) were reviewed with data relevant to histology proven gastric cancer and tumour M2-PK [12,46–48,50]. One study combined data for gastric and oesophageal cancers [48]. Serum tumour M2-PK measurement rather than EDTA-plasma concentration was measured in one study [12]. Tumour M2-PK levels in gastric cancer ranged from 2 to 965 U/ml with mean value of 43 U/ml. The controls used in these studies were mainly healthy donors. The mean control value of tumour M2-PK was 9.3 U/ml. The diagnostic cut-off value for tumour M2-PK in plasma was

15 U/ml in three of the studies, 19.8 U/ml in one study [47] and 22 U/ml in another [12] with specificity ranging from 89 to 95%. When data from the three gastric cancer studies with the same diagnostic cut-off level for plasma tumour M2-PK are analysed, 211 patients with 221 controls have been evaluated giving an overall sensitivity of 64%, specificity of 89%, PPV of 85% and NPV of 72%. The sensitivity, PPV and NPV of CA72-4 (35–91, 14–95 and 34–100%, respectively) are superior to CEA (24–38, 6–80, and 44–99%, respectively) and CA19-9 (33–49, 3.8–93, and 52–99%, respectively). The efficacy of tumour M2-PK (57–67, 10–94 and 44–99%, respectively) was comparable. The range of values is the least and the best value for sensitivity, PPV, and NPV for these tumour markers in the five studies. Owing to different cut-off values, the data from the individual studies could not be combined. The specificity of CEA, CA72-4 and CA19-9 in these studies was again not stipulated. Low sensitivity and PPV was found in one study [12] which used serum tumour M2-PK rather than EDTA plasma and a high diagnostic cut-off. The cut-off values of CEA, CA19-9 and CA72-4 in this study were historical.

**Pancreatic cancer (Table 3)**

Seven studies (six prospective and one retrospective) analysed tumour M2-PK in histologically proven pancreatic cancer [12,45,47,48,50–52]. One study used serum tumour M2-PK measurement [12]. The plasma/serum levels of tumour M2-PK in pancreatic cancer patients ranged from 0.1 to 195 U/ml with median level of 33 U/ml which was significantly higher than the median level in controls (9 U/ml). The controls used in some of the studies were healthy blood donors [12,48,51], whereas other studies used noncancer individuals as controls

**Table 2 Studies comparing the tumour markers: TuM2-PK, CEA, CA19-9, CA72-4 and CA50 in gastric cancers**

Reference	Study detail	Tumour marker (cut-off value)	Sensitivity %	PPV%	NPV%
[13]	Prospective study n = 12 Controls = 666 Specificity = 90% <sup>a</sup>	Serum tumour M2-PK (22 U/ml)	58	9.6	99
		CA72-4 (4 U/l)	91	14.3	100
		CA19-9 (65 U/l)	49.5	8.3	99
		CEA (10 µg/l)	38	6.4	99
		CA50 (50 U/l)	47.2	8.2	99
[51]	Retrospective n = 137 Controls = 141 Specificity = 89% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	67	84	74
		CA72-4 (4 U/l)	41	76.8	62
		CEA (5 µg/l)	26	70	55
		CA19-9 (25 U/l)	45	79	62
[48]	Prospective study n = 122 Controls = 76 Specificity = 95% <sup>a</sup>	Plasma tumour M2-PK (19.8 U/ml)	57	94	58
		CEA (8.3 µg/l)	23.8	80	44
		CA72-4 (3.2 U/l)	60.7	95	60
		CA19-9 (23 U/l)	45.5	93	52
[49]	Prospective study n = 20 <sup>b</sup> Controls = 60 Specificity = 90% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	60	66.6	87
		CEA (3 µg/l)	25	50	78
		CA19-9 (37 U/l)	33	52	80
[47]	Prospective study n = 54 Controls = 20 Specificity = 90% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	57	94	44
		CA72-4 (4 U/l)	35.3	90	34

CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value; M2-PK, M2-pyruvate kinase.

<sup>a</sup>The specificity of CEA, CA72-4 and CA19-9 was not stipulated in these studies.

<sup>b</sup>Oesophageal/gastric cancer.

**Table 3 Studies comparing the tumour markers: tumour M2-PK, CEA, CA19-9, CA72-4 and CA50 in pancreatic cancers**

Reference	Study detail	Tumour marker (cut-off value)	Specificity %	Sensitivity %	PPV%	NPV%
[52]	Prospective study n=38 Controls=128	Plasma tumour M2-PK (28 U/ml)	90 <sup>a</sup>	79	60	93
		CEA (5 µg/l)		22	38	79
		CA19-9 (37 U/l)		65	66	90
[13]	Prospective study n=64 Controls=666	Serum tumour M2-PK (22 U/ml)	90 <sup>a</sup>	71	40.5	97
		CA19-9 (65 U/l)		68.5	40	97
		CEA (10 µg/l)		37	27	94
		CA50 (50 U/l)		63.4	37.7	96
[51]	Retrospective n=26 Controls=141	Plasma tumour M2-PK (15 U/ml)	89 <sup>a</sup>	73	54	95
		CA72-4 (4 U/l)		43	41	89
		CEA (5 µg/l)		42	41	89
		CA19-9 (25 U/l)		85	58	97
[48]	Prospective study n=24 Controls=76	Plasma tumour M2-PK (19.8 U/ml)	95 <sup>a</sup>	72.9	81	91
		CEA (8.3 µg/l)		33.3	66	82
		CA19-9 (23 U/l)		28	86	54
[49]	Prospective study n=14 Controls=60	Plasma tumour M2-PK (15 U/ml)	90 <sup>a</sup>	71.4	62.5	93
		CEA (3 µg/l)		46.2	50	87
		CA19-9 (37 U/l)		83	78.5	95
[46]	Prospective study n=60 Controls=205 <sup>b</sup>	Plasma tumour M2-PK (8.9 U/ml)	41	85	32	90
		CA19-9 (60 U/l)	81	75	56	92
[53]	Prospective study n=77 Controls=69	Plasma tumour M2-PK (27 U/ml)	60	66	64	61
		CA19-9 (38.5 U/l)	73	71	74	69

CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value; M2-PK, M2-pyruvate kinase.

<sup>a</sup>The specificity of CEA, CA72-4 and CA19-9 was not stipulated in these studies.

<sup>b</sup>Benign pancreatic disease (acute or chronic pancreatitis), cystic neoplasms, neuroendocrine tumours of pancreas, other abdominal malignancies and healthy controls.

[12,45,46,50,52]. The diagnostic cut-off value for tumour M2-PK was between 8.9 and 28 U/ml. The diagnostic cut-off value for tumour M2-PK in plasma was 15 U/ml in two studies [48,50]. When data from the pancreatic cancer studies using the same diagnostic cut-off level of 15 U/ml for plasma tumour M2-PK are analysed, 40 patients with 201 controls have been evaluated giving a sensitivity of 72%, specificity of 89%, PPV of 58% and NPV of 94%. The overall sensitivity, specificity, PPV and NPV for tumour M2-PK was (66–85%, 41–95%, 32–62% and 61–97%, respectively. This was comparable with those of CA19-9 (28–85%, 73–95%, 40–86% and 54–97%, respectively). The range of values is the lowest and the highest value for sensitivity, PPV and NPV for these tumour markers in all the seven studies. Owing to different cut-off values the data from the individual studies could not be combined. The specificity of CEA and CA19-9 was not clarified in five studies [12,47,48,50,51]. The low specificity of tumour M2-PK level in one study may be due to use of patients with acute pancreatitis, chronic pancreatitis, cystic tumours and neuroendocrine tumours of pancreas, various benign digestive disorders and other abdominal malignancies as controls [45]. The low cut-off value (8.9 U/ml) used in this study may also contribute to the low specificity.

#### **Colorectal cancer (Table 4)**

Four studies (two prospective and two retrospective) evaluated tumour M2-PK and colorectal cancer patients [46–48,50]. The level of plasma tumour M2-PK in colorectal cancer patients was in the range of 2–986 U/ml with a mean value of 44 U/ml. The controls used in these studies were either healthy blood donors or patients with nonmalignant disease. The mean value of

tumour M2-PK in controls was 9.6 U/ml. The diagnostic cut-off used in these studies was either 15 or 19.8 U/ml in EDTA plasma. Three studies used 15 U/ml cut-off level [46,48,50] and included 251 patients with colorectal cancer and 221 controls with a sensitivity of 57%, specificity of 89%, PPV of 86% and NPV of 65%. The overall specificity of tumour M2-PK ranged from 89 to 95% with sensitivity, PPV and NPV (48–76%, 81–97% and 35–87%, respectively. Tumour M2-PK was better compared with CEA (sensitivity 34–71%, PPV 80–95% and NPV 30–84%) and CA19-9 (sensitivity 27–55%, PPV 50–95% and NPV 29–77%). The range of values is the least and the best value for sensitivity, PPV and NPV for these tumour markers in the four studies. The specificity of CEA and CA19-9 was not clarified in all four studies.

#### **Combining tumour M2-pyruvate kinase with other gastrointestinal markers (Table 5)**

Combining tumour M2-PK with the conventional tumour markers increases its diagnostic efficacy, as shown in three studies [45,50,52]. In oesophageal cancer combining tumour M2-PK with CEA increases the sensitivity, PPV and NPV from 59, 76 and 77%, respectively, to 65, 78 and 80%, respectively. In gastric cancer it increased from 67, 84 and 74%, respectively, to 82, 87 and 97%, respectively, when tumour M2-PK was combined with CA72-4. Similarly, in pancreatic cancer an increase in sensitivity, PPV and NPV was seen from 73, 54 and 95%, respectively, to 96, 61 and 99%, respectively, when tumour M2-PK was combined with CA19-9. In colorectal cancer combining tumour M2-PK with CEA increases the sensitivity, PPV and NPV from 50, 83 and 60%, respectively, to 67, 87 and 70%, respectively.

**Table 4 Studies comparing the tumour markers: tumour M2-PK, CEA and CA19-9 in colorectal cancer**

Reference	Study detail	Tumour marker (cut-off value)	Sensitivity %	PPV%	NPV%
[51]	Retrospective <i>n</i> = 163 Controls = 141 Specificity = 89% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	50	83.5	60
		CEA (5 µg/l)	42	81	57
		CA19-9 (25 U/l)	27	50	46
[48]	Prospective study <i>n</i> = 250 Controls = 76 Specificity = 95% <sup>a</sup>	Plasma tumour M2-PK (19.8 U/ml)	47.8	96.7	35
		CEA (8.3 µg/l)	33.6	95	30
		CA19-9 (23 U/l)	30.4	95	29
[49]	Prospective study <i>n</i> = 34 Controls = 60 Specificity = 90% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	76.5	81	87
		CEA (3 µg/l)	71	80	84
		CA19-9 (37 U/l)	55.2	75	77
[47]	Prospective study <i>n</i> = 54 Controls = 20 Specificity = 90% <sup>a</sup>	Plasma tumour M2-PK (15 U/ml)	68.5	95	51
		CEA (3 µg/l)	43.12	92	37

CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value; M2-PK, M2-pyruvate kinase.

<sup>a</sup>The specificity of CEA, CA72-4 and CA19-9 was not stipulated in these studies.

**Table 5 Combining tumour M2-PK with other GI cancer markers**

Cancer type	Study reference	Tumour marker (cut-off value)	Specificity %	Sensitivity %	PPV%	NPV%
Oesophageal cancer	[51]	Tumour M2-PK + CA19-9	89	65	78	80
		Tumour M2-PK (15 U/ml)	89	59	76	77
		CA19-9 (25 U/l)	89	43	70	71
Gastric cancer	[51]	Tumour M2-PK + CA72-4	89	82	87	97
		Tumour M2-PK (15 U/ml)	89	67	84	74
		CA72-4 (4 U/l)	89	41	77	62
Pancreatic cancer	[51]	Tumour M2-PK + CA19-9	89	96	61	99
		Tumour M2-PK (15 U/ml)	89	73	54	95
		CA19-9 (25 U/l)	89	85	58	97
	[46]	Tumour M2-PK + CA19-9	38	97	64	99
		Tumour M2-PK (8.9 U/ml)	41	85	32	90
		CA19-9 (60 U/l)	81	75	56	92
	[53]	Tumour M2-PK + CA19-9	60	77	68	69
		Tumour M2-PK (27 U/ml)	60	66	64	61
		CA19-9 (38.5 U/l)	73	71	74	69
Colorectal cancer	[51]	Tumour M2-PK + CEA	89	67	87	70
		Tumour M2-PK (15 U/ml)	89	50	83	60
		CEA (5 µg/l)	89	42	81	57

GI, gastrointestinal; M2-PK, M2-pyruvate kinase; NPV, negative predictive value; PPV, positive predictive value.

### Plasma tumour M2-pyruvate kinase levels in post-treatment surveillance

Only one study has been found assessing tumour M2-PK levels and the response to therapy as far as GI cancers are concerned. Ventrucci *et al.* [45] showed a rise in plasma tumour M2-PK levels shortly (within 2 weeks) after pancreaticoduodenectomy for pancreatic cancers. This immediate postoperative rise was attributed to accelerated glycolysis due to healing [35]. Only one study has been found so far monitoring the serum tumour M2-PK levels after the resection of cancer. In this study, with only six patients followed after renal cell carcinoma resection, tumour M2-PK normalized 11 weeks after surgery and showed rising levels 2 months before computed tomography detected recurrence [37]. In studies with advanced breast and lung cancer patients tumour M2-PK levels in plasma decreased within 4 weeks after the start of palliative chemotherapy and rose again with disease progression [9,39]. In another study with

lung cancer patients, plasma tumour M2-PK concentration reflected the course of the disease and correlated well with tumour progression or remission following treatment [43].

### Summary and conclusion

Tumour M2-PK can be quantified in blood with a specificity of 90–95% at a diagnostic cut-off value of 15–17.5 U/ml and in stool with a specificity of 83–95% at a cut-off value of 3.33–4 U/ml. The stability of tumour M2-PK is best in EDTA plasma for 24 h at room temperature and is not influenced by any mechanical stress. The quantification in blood/stool is by highly sensitive ELISA using two monoclonal antibodies specific to tumour M2-PK. It can be elevated in benign conditions including chronic cardiac failure, diabetic nephropathy, rheumatic diseases, inflammatory bowel disease and pancreatitis. The inclusion of these benign

conditions as noncancer controls can result in false-positive rates ranging from 38 to 82%. Studies in gastric, colorectal and pancreatic cancer show a good correlation between plasma/faecal tumour M2-PK and disease stage [45,46,50,55]. Although no prospective data are available on plasma tumour M2-PK, faecal tumour M2-PK has sensitivity of 64% to detect early stage (T1, T2) colorectal cancers. As a screening tool for bowel cancer, the overall sensitivity of faecal tumour M2-PK is 73% with false-positive rate of 15% in symptomatic individuals. It has not yet been validated in a large scale screening of an asymptomatic population. In our meta-analysis tumour M2-PK showed good diagnostic accuracy for oesophageal/gastric and colorectal cancers with PPV of 86–88%. Recently tumour M2-PK has been established as an important marker of transformed and highly proliferating cells during progression of the metaplasia–dysplasia–adenocarcinoma sequence in Barrett’s oesophagus [42]. The diagnostic accuracy of tumour M2-PK was better than CEA and CA19-9 in oesophageal and colorectal cancer and was comparable to CA72-4 in gastric cancer and CA19-9 in pancreatic cancer. A combination of these tumour markers increases their diagnostic strength, especially in pancreatic cancers. The current literature on tumour M2-PK and GI cancer is limited, but would justify further investigation of this novel cancer marker. Faecal tumour M2-PK has a potential role in bowel cancer screening. A proper screening trial is required on an asymptomatic population comparing faecal tumour M2-PK with the Haemoccult test and validating its efficacy, cost effectiveness, feasibility and impact on cancer specific mortality. Limited information exists as yet on the utility of tumour M2-PK as a prognostic marker, as a marker of malignant transformation or in assessing tumour recurrence or response to treatment. Large multicentre trials are, therefore, needed to define its clinical role.

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