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EXPERT
REVIEWS

Tumor M2 pyruvate kinase: a tumor marker and its clinical application in gastrointestinal malignancy

Expert Rev. Mol. Diagn. 8(5), 579–585 (2008)

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Proliferating cells, in particular tumor cells, express a dimeric isoenzyme of pyruvate kinase, termed Tumor M2 pyruvate kinase. In the last few years, much attention has been paid to this novel tumor marker that can be determined in EDTA–plasma and in the feces. It has been used in diagnosis and surveillance of a variety of malignant diseases. As compared with the established tumor markers, Tumor M2-PK in EDTA–plasma proves to have at least equal sensitivity in pancreatic, gastric, esophageal, colorectal and cholangiocellular cancer. In combination with established tumor markers, EDTA–plasma M2-PK is a useful tool in diagnosis and surveillance of gastrointestinal tumors. In colorectal cancer, M2-PK in EDTA–plasma even proves superiority as compared with CEA. Fecal Tumor M2-PK testing resembles a good noninvasive screening parameter for colorectal cancer with a reported sensitivity of 68.8–91.0% and a specificity of 71.9–100%. It is superior to fecal occult blood testing in colorectal cancer screening. Since it is effective, easy to handle and bears rather low costs, fecal Tumor M2-PK testing is recommended for large-scale CRC screening.

Keywords: colorectal carcinoma • gastrointestinal malignancy • tumor M2 pyruvate kinase • tumor marker • tumor screening

In the last few years, much attention has been paid to a novel biomarker, Tumor M2 pyruvate kinase (M2-PK). Tumor M2-PK reflects the metabolic state of tumors and is not linked to a specific cell type or a specific organ. There is good clinical evidence on the use of this parameter in different types of gastrointestinal (GI) malignancy as well as for lung cancer, breast cancer, ovarian cancer, renal cancer, melanoma and other solid tumors.

Tumor M2-PK can be detected in EDTA–plasma and in the feces. In particular, its determination in the feces, used as a screening parameter for colorectal carcinoma (CRC), has recently attracted much attention and is controversially discussed.

The following special report focuses on this novel tumor marker and its clinical application in GI malignancy.

Pathophysiology

The metabolism of tumor cells (and other proliferating cells) is considerably different from normal cell metabolism. Besides other tumor characteristic changes, cancer cells show an upregulation

of glycolysis and glutaminolysis, while gluconeogenesis is reduced. This finding has been observed in all malignant tissues investigated so far [1–3]. An increased rate of glycolysis is important for tumor cells for several reasons: energy can be provided without oxygen consumption and intermediates of glycolysis are used as precursors for the synthesis of cell building material such as nucleotides, amino acids, phospholipids and triglycerides. The regulation of these metabolic changes depends on a shift in the isoenzyme equipment of pyruvate kinase. Tissue-specific isoenzymes, such as L-PK in liver and M1-PK in brain disappear and M2-PK is expressed [1–6]. In contrast to all other pyruvate kinase isoenzymes which always occur in a tetrameric form, M2-PK may occur in a tetrameric form with a high affinity for phosphoenolpyruvate but also in a dimeric form with a low affinity for phosphoenolpyruvate. The tetramer:dimer ratio of M2-PK is controlled by oncoproteins and metabolic intermediates. In malignant cells, the dimeric form is predominant and has been termed Tumor M2-PK [1,3,7,8].

In proliferating cells the tetramer:dimer ratio of M2-PK determines whether the glucose carbons are degraded to lactate under the production of energy or channeled into synthetic processes. In accordance with this fact, the content of the M2-PK protein in malignant tissues was described to be considerably higher than in normal tissues.

Several years ago it was hypothesized that it might be possible to detect elevated levels of this enzyme in the blood or body egesta of patients with malignant diseases.

Application of Tumor M2 PK in EDTA-plasma

Elevated levels of all tumor markers described until now can also be due to nonmalignant conditions, such as chronic inflammation. Tumor M2-PK is a metabolic parameter characteristic for proliferation. It can also be elevated in different acute and chronic inflammatory conditions, due to an increase in leukocyte activity and number. Especially in rheumatic diseases [9,10], diabetic nephropathy [11], chronic heart diseases [12], inflammatory bowel disease (IBD) [13], acute and chronic pancreatitis [14], sepsis and polytrauma [15], elevated Tumor M2-PK levels can be detected that are not due to malignancy. When interpreting test results, these facts should always be kept in mind.

Quite a few tumor markers (CEA, CA 19-9, AFP, SCC, CA 72-4) have already been established in diagnosis and/or surveillance of malignant diseases of the GI tract. These markers are not to be used as screening parameters for malignant diseases due to low specificity (with the exception of AFP in patients with liver cirrhosis). New tumor markers such as Tumor M2-PK must first prove their equality or superiority before they can be adopted as a new option.

Pancreatic carcinoma (EDTA-plasma)

Observations of Oremek *et al.* in 1997 provide the first evidence that Tumor M2-PK in EDTA-plasma might be of clinical utility in detection of GI malignancy. Compared with the standard tumor marker CA 19-9, Tumor M2-PK showed a similar diagnostic sensitivity and specificity (71 vs 68%) for pancreatic carcinoma [16].

These data were further supported by Cerewenka *et al.* in 1999. They were able to show that Tumor M2-PK levels correlate with existence of metastasis. This is not observed for CA 19-9 and CEA [17]. However, there are also reports of other research groups which were able to detect a slightly better performance of CA 19-9 as compared with Tumor M2-PK.

A comparative meta-analysis from 1956 until 2006 recently published by Kumar *et al.* showed comparable sensitivities of CA 19-9 and Tumor M2-PK. The diagnostic sensitivity was shown to be 60% with a specificity of 95% [18].

An interesting option might therefore be the combination of both tumor markers which leads to a significant increase in sensitivity (73/85% up to 96%) without a loss of specificity according to the data of Schulze *et al.* [19].

As is well known for other tumor markers such as CA 19-9, one can also detect elevated levels in benign diseases of the pancreas.

In conclusion, pancreatic cancer Tumor M2-PK and CA 19-9 cannot be suggested for primary screening on their own but can

always be helpful diagnostic tools in combination with, for example, imaging results. A combination of both parameters might even be an interesting option for primary screening.

Gastric carcinoma (EDTA-plasma)

In gastric carcinoma no convincing tumor markers have been established so far. CA 72-4 has been used, its diagnostic sensitivity ranging between 30 and 80% according to tumor stage. CA 19-9 and CEA are used as additional tumor markers with poor sensitivity.

In 2000, we were able to show a superior diagnostic sensitivity of Tumor M2-PK as compared with the established CA 19-9, CEA and CA 72-4 markers [13]. These results were further supported by a retrospective clinical trial by Schulze *et al.* who described a clearly improved diagnostic sensitivity in gastric cancer in the presence of metastasis (M2-PK: 71% vs CA 72-4: 57%, CA 19-9: 54%, CEA: 39%) and in absence of metastasis (63% vs CA 72-4: 25%, CA 19-9: 31%, CEA: 12%) [19]. A prospective clinical trial of Schneider *et al.* could demonstrate equal performance of Tumor M2-PK and CA 72-4 (57.0 vs 60.7%) [20]. However, both markers were clearly superior to CA 19-9 (45.5%) and CEA (23.8%).

Publications from Asiatic research groups where the prevalence of gastric carcinoma is far higher than in Western countries also report similar diagnostic sensitivities. A Korean study of 73 patients with GI malignancy by Kim *et al.* reports a diagnostic sensitivity of 62.2% for Tumor M2-PK [21]. A Chinese study of 54 patients with gastric carcinoma by Zhang *et al.* reports a diagnostic sensitivity of 50.5% as compared with CA 72-4 with 35.4%. The absolute Tumor M2-PK levels correlated with tumor stage and the presence of metastasis [22].

A meta-analysis by Kumar *et al.* finds the sensitivity of Tumor M2-PK for gastric/esophageal carcinoma to be 62.1%, the specificity 89.0%, the positive-predictive value 88.0% and negative-predictive value 64.0% [23].

In conclusion, one has to state that Tumor M2-PK measurement in the plasma and CA 72-4 seem to perform at least equally. However, the combination of both tumor markers might provide a further benefit in gastric cancer. Neither marker should be used as a primary screening parameter for gastric cancer.

Esophageal carcinoma (EDTA-plasma)

The incidence of esophageal carcinoma in Western countries is low compared with other cancer entities. However, some countries, such as China, still report esophageal carcinoma as a common cancer. Most esophageal cancers are squamous cell cancers. Therefore a tumor marker for esophageal cancer is supposed to be SCC. However, this tumor marker is not of clear clinical utility and is only used in surveillance of esophageal cancer. Its sensitivity is reported to be 0–27% (stage I) up to 45–50% (stage IV) [24].

Data for the use of Tumor M2-PK in esophageal cancer are rather scarce. There are studies reporting a diagnostic sensitivity of 55–60% [13,19,20], yet a direct comparison to SCC is not available. Results comparing the performance of Tumor M2-PK with CEA

(M2-PK superior), CA 19-9 (M2-PK superior) and CA 72-4 (equal performance) exist, however, detailed information of histology and tumor stage are missing [19,20].

In conclusion, one cannot give valid data on the use of Tumor M2-PK in esophageal cancer. In the absence of other reliable parameters it might well be considered. Further studies are encouraged and required.

Cholangiocellular carcinoma (EDTA-plasma)

Literature research reveals a diagnostic sensitivity between 55 and 79% for the established tumor marker CA 19-9. The tumor marker CA 72-4 is reported to have a diagnostic sensitivity of 35–52% [25,26]. Only very few data on the use of Tumor M2-PK in cholangiocellular carcinoma are reported to date. Kim *et al.* report a diagnostic sensitivity of 75% [21], Goonetilleke *et al.* find its sensitivity in periampullary cancer to be 66% and its specificity to be 58%. In this study, elevated Tumor M2-PK levels were additionally strongly correlated with the subsequent finding of poorly differentiated cancer and/or metastatic disease and strongly predicted survival [27].

Colorectal carcinoma (EDTA-plasma)

In 2000, our research group was able to show an equal sensitivity of Tumor M2-PK and the established tumor marker CEA for detection of CRC [13]. A retrospective analysis of 163 patients by Schulze *et al.* even demonstrated superiority of Tumor M2-PK as compared with CEA (sensitivity 50 vs 42%). On analyzing the subgroups of CRC with or without metastasis, however, CEA showed a better performance in CRC with metastasis (72 vs 54%) whereas Tumor M2-PK showed a better performance in CRC without the presence of metastasis (48 vs 34%) [19]. According to this study, a combination of both markers seems an interesting option, improving sensitivity up to 67%.

A prospective study of Schneider and Schulze with 250 patients, revealed a sensitivity of 47.8% for M2-PK, 33.6% for CEA and 30.4% for CA 19-9. This study also proved superiority of Tumor M2-PK in the nonmetastatic stage of the disease whereas an equal sensitivity in metastatic stages was achieved [20].

Kim *et al.* were able to show a sensitivity of up to 66.7% for M2-PK and of 86.2% for the combination of M2-PK and CEA [21].

A study of Zhang *et al.* demonstrated a clear correlation between tumor stage according to the Dukes classification and plasma Tumor M2-PK levels. A sensitivity of 68.5% for M2-PK and of 43.1% for CEA was reported [22].

Further evidence is reported from Pinedo *et al.* who demonstrated superiority of M2-PK compared with CEA or CA19-9 (82.1% compared with 64.1% for CEA and 46.2% for CA 19-9) [28].

A meta-analysis by Kumar *et al.* reports the sensitivity, specificity, positive-predictive value and negative-predictive value of Tumor M2-PK to be 57.3, 89, 85.7 and 64.8%, respectively [23].

In conclusion, compared with the established marker CEA, Tumor M2-PK seems superior for the detection of colorectal cancer, especially in a nonmetastatic stage of the disease. A combination of Tumor M2-PK and CEA could further improve diagnostic sensitivity. However, neither marker can be recommended for primary screening.

Summary of the application of M2-PK in EDTA-plasma

As compared with the well-established tumor markers, Tumor M2-PK proves to have at least equal sensitivity in pancreatic, gastric, esophageal, colorectal and cholangiocellular cancer. However, no known GI tumor marker can be used as a standard laboratory screening parameter due to low sensitivity and specificity (exception: AFP in patients with liver cirrhosis). They should only be used in the follow-up of malignant diseases. An interesting option might be the combination of two or more tumor markers in order to improve sensitivity as has been reported recently [29].

In colorectal cancer, Tumor M2-PK proves to be an advancement as compared with CEA and it is of clear clinical utility. It could therefore be used alternatively for surveillance of colorectal tumor patients.

Application of M2-PK in the feces

In 2003, we first proposed to measure this enzyme in the feces to determine GI cancer. Laboratory methods were adapted for the measurement in stool samples and the enzyme could be detected and quantified in the feces of patients with different GI cancer types, mainly in colorectal cancer. In the meantime, several groups have performed studies on the use of fecal Tumor M2-PK as a marker of colorectal cancer.

Screening of colorectal carcinoma

As described earlier, Tumor M2-PK can be determined in the feces by using a tumor-M2-PK-ELISA. The currently established methods for CRC screening include screening colonoscopy (e.g., Germany), sigmoidoscopy and fecal occult blood testing (FOBT). Endoscopic techniques are of limited success due to poor acceptance by the population. Screening by FOBT is currently the only means that has proven a reduction in mortality in large population studies. Furthermore, the diagnostic performance of FOBT has recently been improved by using immunological FOBT assays [30]. Nevertheless, specificity still seems to be rather low as compared with the data published for fecal Tumor M2-PK. Thus, determination of Tumor M2-PK in the feces for CRC screening seems an interesting idea.

To date, there are several retrospective and prospective studies concerning the use of Tumor M2-PK in the feces for CRC screening [31–40]. An overview of the available studies is given in TABLE 1.

In conclusion, all available studies demonstrate a good performance of Tumor M2-PK in CRC screening with an overall sensitivity ranging from 68.8 to 91.0% and an overall specificity ranging from 71.9 to 100%.

Some studies included patients with unspecific GI complaints (indication for colonoscopy) as a control group. It is known that symptomatic patients or patients with known IBDs can show elevated levels of Tumor M2-PK. These patient groups do not represent a normal screening population and are under endoscopic surveillance anyway. They can therefore not be included in control populations in clinical trials. Studies including symptomatic patients might produce misleading results concerning sensitivity, specificity and negative- or positive-predictive value of a marker

Table 1. Currently available studies concerning the use of Tumor M2-PK in feces as a screening tool for colorectal carcinoma.

| Author (year) | Study design | Patients (n) | Reported sensitivity for CRC (%) | Reported specificity for CRC (%) | Ref. |
|---------------------------------|----------------------------------|--------------|-----------------------------------|----------------------------------|------|
| Hardt PD <i>et al.</i> (2003) | Unicenter, retrospective study | 78 | 68.8 (adenoma: 50) | 98.0 (general population) | [31] |
| Hardt PD <i>et al.</i> (2004) | Multicenter, retrospective study | 204 | 73.3 | 78.8 (endoscopy controlled) | [32] |
| Naumann M <i>et al.</i> (2004) | Multicenter, prospective study | 232 | 85.2 (adenoma: 45.8; > 1cm: 61.5) | 65.3 (endoscopy controlled) | [33] |
| Vogel T <i>et al.</i> (2005) | Multicenter, prospective study | 138 | 77.3 (adenoma 47.6) | 71.9 (endoscopy controlled) | [34] |
| Koss K <i>et al.</i> (2005) | Unicenter, prospective study | 45 | 87.5 (adenoma: 37.5; > 1cm: 60.0) | 100.0 (endoscopy controlled) | [35] |
| Tonus C <i>et al.</i> (2006) | Multicenter, retrospective study | 96 | 77.8 | 92.9 (endoscopy controlled) | [36] |
| Shastri YM <i>et al.</i> (2006) | Multicenter, prospective study | 317 | 81.1 (adenoma: 25.8; > 1cm: 20.0) | 71.1 (endoscopy controlled) | [37] |
| Mulder SA <i>et al.</i> (2007) | Unicenter, retrospective study | 181 | 85.0 (adenoma: 28.0) | 90.0 (endoscopy controlled) | [38] |
| Haug U <i>et al.</i> (2007) | Retrospective study | 982 | 85.0 | 79.0 (general population) | [39] |
| Koss K <i>et al.</i> (2008) | Unicenter, retrospective study | 55 | 91.0 (adenoma: 20.0; > 1cm: 60.0) | 92.0 (general population) | [40] |
| Total | | | 68.8–91.0 (adenoma: 20–61) | 71.9–100 | |

CRC: Colorectal carcinoma.

and should therefore be interpreted with caution. This is especially true for the studies of Naumann *et al.* [33] and Shastri *et al.* [37] where symptomatic patients with, for example, inflammatory lesions were included in the supposedly healthy control group.

Studies without this bias (control group resembles patients with colonoscopies without pathological findings) for example, Hardt *et al.* [31,32], Koss *et al.* [35], Tonus *et al.* [36] certainly reflect a more realistic situation.

Some of the studies also evaluated the performance of the fecal Tumor M2-PK in patients with adenomas. The reported sensitivities ranged between 25.8 and 61.5% depending on the size of the adenoma. Therefore, the quantification of Tumor M2-PK in the feces might even be of some use in detecting larger adenomas.

Furthermore, we could demonstrate a clear correlation between Tumor M2-PK levels in the feces and the stage of the tumor according to the Dukes classification [32]. These results were confirmed by Tonus *et al.* and Koss *et al.* who also report a clear correlation between tumor stage and M2-PK level in the feces [36,40].

Additionally, it was noted by Koss *et al.* that Tumor M2-PK level reduction is associated with successful surgical intervention [35,40].

At the present time, the FOBT is most commonly used as a noninvasive screening parameter for CRC. However, FOBT seems to be clearly inferior with a reported sensitivity of 40% for CRC and less than 20% for larger adenomas [41,42]. Despite the improvement of diagnostic performance of FOBT obtained

by immunological methods (immunological FOBT), one major limitation of FOBT remains the fact that many carcinomas do not bleed at all or bleed only intermittently [43,44]. Limitations of FOBT are also shown in many clinical trials (Denmark, UK, USA) [45–49]. In these studies FOBT proved a reduction of approximately 18–33% in CRC mortality over a follow-up period of 10–18 years. Unfortunately, there has never been a randomized clinical trial comparing the performance of FOBT and fecal Tumor M2-PK in colorectal cancer screening. However, it can be supposed that fecal Tumor M2-PK detection must be superior to FOBT as far as we know from the currently published data on sensitivity and specificity.

Another available noninvasive screening method for CRC is testing for genetic alterations such as, for example, K-ras, p53, APC, MSI, 'long DNA', etc. In particular, multitarget genetic testing seems to be promising with a reported sensitivity of 63–100% [50]. However, the major shortcomings of this screening tool are its high costs, time-consuming and tedious sample preparation and the very limited handling and shipping time of the feces.

Summary of the use of Tumor M2-PK in the feces

Tumor M2-PK is becoming established as a good noninvasive screening option for colorectal cancer with a reported sensitivity between 68.8 and 91.0% and an overall specificity ranging from 71.9 to 100%.

This test might even be used to detect larger adenomas, yet its sensitivity for adenomas is lower (20–61%, depending on size) than for CRC.

It should not be used for CRC screening in patients with IBD since false-positive results can be expected in up to 90% of cases. However, patients with IBD are subject to endoscopic surveillance anyway. Therefore, Tumor M2-PK elevation in IBD patients is not a limitation for the use of this marker in general population screening.

The use of Tumor M2-PK in the feces can be recommended for large-scale population screening. According to the present data, the use of Tumor M2-PK seems superior to FOBT.

Expert commentary

Today, colonoscopy seems to be the most sensitive and specific method for colorectal cancer screening. However, although this opportunity has been introduced in the German health system since 2002, the acceptance of this screening method by the general population is very poor (<10% participation). Therefore, we are in urgent need for other screening strategies.

Concerning the present studies on Tumor M2-PK we recommend using Tumor M2-PK in the feces as a screening test for CRC in the general population. Patients with a positive Tumor M2-PK test should then undergo a diligent colonoscopy.

With respect to handling, effectiveness and costs, fecal Tumor M2-PK seems superior to other noninvasive screening tests.

Fecal Tumor M2-PK is superior to the present FOBT concerning effectiveness. While Guaiak-based FOBT is clearly less expensive than fecal Tumor M2-PK, the immunological-based FOBT costs are comparable. Testing for genetic alterations in the feces is interesting, yet clearly inferior to fecal Tumor M2-PK concerning handling and its costs. Fecal Tumor M2-PK seems to be a good possibility for large-scale population screening. Long-term prospective studies comparing fecal Tumor M2-PK, FOBT and genetic testing are still lacking and are awaited impatiently.

Determination of M2-PK in the plasma in the surveillance of pancreatic, cholangiocellular, esophageal, colorectal or gastric carcinoma seems at least equal to the established tumor markers. However, combinations of Tumor M2-PK in the plasma with other established tumor markers will further improve diagnostic sensitivity and is certainly an interesting option for the future.

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Plasma M2-PK for CRC surveillance seems slightly superior to CEA yet, as described earlier, a combination of both markers might be an interesting option to increase sensitivity in the future.

Five-year view

According to the present data we suggest and expect the fecal Tumor M2-PK test to be adopted for preselection of colonoscopy candidates in large-scale CRC screening since it is effective, easy to handle, noninvasive and rather cheap compared with other suggested screening parameters (e.g., genetic testing). However, genetic testing of tumor-specific alteration is also emerging and might be an interesting concurrent method. To date, the limitations of this method are its high costs and the very limited handling time of the feces.

Plasma M2-PK might be valuable in the surveillance of CRC as well as in other GI tumors in combination with the established tumor markers.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Proliferating cells, in particular tumor cells, are characterized by a high amount of the dimeric form of the pyruvate kinase isoenzyme type M2 (Tumor M2-PK).
- Tumor M2-PK can be determined in EDTA–plasma and feces.
- In combination with established tumor markers, EDTA–plasma Tumor M2-PK is a useful tool in diagnosis and/or surveillance of gastrointestinal tumors.
- Fecal Tumor M2-PK testing is a good noninvasive screening parameter for colorectal cancer with a reported sensitivity of 68.8–91.0% and specificity of 71.9–100%.
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