Original article

The faecal tumour M2-PK screening test for invasive & pre-invasive colorectal cancer: estimated specificity & results as a function of age for a study population of 4854 volunteers

Carolin Tonus, Gero Neupert, Kai Witzel

Faecal tumour M2-Pyruvate kinase levels were measured in a large, unselected, asymptomatic screening population to determine its specificity and to demonstrate the distribution in different age groups. Faecal tumour M2-Pyruvate kinase levels were measured with a sandwich ELISA in stool samples of 4854 screened volunteers. The levels were below the cutoff value of 4.0 kU/L in 4425 of the population. This means that 91.2% of all the people we screened had faecal tumour M2-Pruvate kinase levels within the normal, non-pathological range. The percentage of individuals with elevated tumour M2-Pruvate kinase concentrations increased from 6.9% in the age group of 30–39 years to 16.0% in the age group of 70–79 years. The estimated specificity range of the test, using prevalence data and our study results was 0.93-0.96. These results underline the usefulness of the tumour M2-Pruvate kinase stool test as a non-invasive, easy, fast, and economical method for colorectal cancer screening.

Key words: tumour M2-Pyruvate kinase, pyruvate kinase type M2, colorectal cancer, adenoma, polyps, cancer screening, bowel cancer

Introduction

Every year approximately 1 million people worldwide are diagnosed with colorectal cancer and nearly 529,000 people die from this neoplasm [1]. Due to slow development of the disease, colorectal cancer screening is particularly effective. Therefore, regular screening examinations can significantly reduce cancer morbidity and mortality. Several guidelines for colorectal screening in different countries have been published, with recommendations including various methods, such as the faecal occult blood test, sigmoidoscopy, double-contrast barium enema, or colonoscopy [2-5].

Although colonoscopy is the *gold standard* for the early detection of colorectal cancer and its precancerous lesions, it does have some drawbacks. The procedure is invasive, expensive, associated with the highest rates of complications [6] and has low acceptance by the general population. For example, only 1.7% of those people entitled to colonoscopy under the German National Colorectal Cancer Screening Programme actually undergo the procedure [7]. In contrast to colonoscopy, the faecal occult blood tests (FOBTs) are widely accepted and practicable [3-5]. They are based on the premise that polyps and cancers bleed more than normal mucosa [8].

Consequently, non-bleeding colorectal tumours or polyps and those not consistently discharging sufficient blood into the intestinal lumen are not detected by either guaiac or immunological FOBTs. Therefore, the sensitivities of these tests are limited. For the guaiac-based FOBT, sensitivities of less than 30% for colorectal cancer and less than 15% for advanced adenomas have been described by Lieberman et al [9] and by Koss et al [10]. In addition, there is a need for both dietary and medication restrictions because certain foods (such as red meat, and some raw foods and vegetables), vitamin C or aspirin may lead to false-positive results. This has a negative impact on the specificity of guaiac-based FOBTs [11]. However, despite these limitations, guaiac-based FOBTs have been shown to reduce mortality in the range of 15-33% in screened populations [12, 13]. Even though the disadvantage of dietary restriction has been overcome by the development of more expensive immunological FOBTs, the problems of detecting intermittently bleeding cancers and precancerous polyps still remain. In order to increase participation in colorectal cancer screening programmes, a non-invasive, easy, fast, and economical screening method is needed. Further it should not be dependent upon the presence of occult blood and should have good patient compliance, high sensitivity and high specificity. The tumour M2-PK stool test is therefore of interest, as it is a screening test for the detection of adenomas and colorectal tumours. It is based on the measurement of a key enzyme involved in tumour metabolism [10, 14-21] and is independent of the

Herz-Jesu-Hospital
Surgical Clinic
Fulda
Germany

presence of occult blood. Tumour M2-PK is the dimeric form of the glycolytic pyruvate kinase isoenzyme type M2 [22, 23]. This enzyme catalyses the last reaction step within the glycolytic sequence, from phosphoenolpyruvate to lactate, and is responsible for net ATP production within this pathway. Enzymatic characterisation of a wide range of different tumours revealed that tumourigenesis is accompanied by an increase in total pyruvate kinase v-max activity. There is also a shift towards the expression of the pyruvate kinase isoenzyme type M2 (M2-PK) and away from the tissue-specific isoenzymes (L-PK in liver and kidney, M1-PK in muscle and brain and R-PK in erythrocytes) [24-27].

The increased expression of M2-PK is under the control of ras, and the transcription factors SP1 and HIF-1. Ras and HIF-1 are both consistently altered in gastrointestinal tumours [28-31]. M2-PK can occur in a tetrameric form which is characterised by a high affinity to its substrate phosphoenolpyruvate (PEP) and in a dimeric form with a low PEP affinity. The tetramer:dimer ratio of M2-PK determines the proportion of glucose carbons used for glycolytic energy production (tetrameric form) or channelled into synthetic processes (dimeric form). In tumour cells, M2-PK is mainly found in the dimeric form (tumour M2-PK) due to direct interaction with various oncoproteins, such as pp60v-src kinase and HPV-16 E7 [22, 30, 32]. In patients with adenomas or colorectal cancer, Tumour M2-PK is released into the blood and the stool. An increase in tumour M2-PK in EDTA-plasma samples is found in gastrointestinal cancers, as well as in a wide range of other tumours such as lung, renal, breast and cervical cancer. The EDTA-plasma test is highly suitable for patient monitoring [33-40]. The usefulness of the faecal tumour M2-PK test as a screening tool with a good sensitivity and specificity for colorectal cancer or adenomas has been widely demonstrated [10, 14-20]. Depending on the study, the specificity reported is between 0.71 and 0.98

for colorectal cancer [10, 16-20, 41-44]. The number of participants included in the different studies are in the range of 55-982. Control groups vary from study to study and comprise symptomatic and/or asymptomatic patients who (**a**) underwent colonoscopy [16-18, 42] or colonoscopy and oesophago-gastro-duodenoscopy [10] without any pathological findings; (**b**) individuals presenting for investigation of colonic symptoms, or with a clinical suspicion of colorectal cancer or inflammatory bowel diseases, or for colorectal screening, who all underwent colonoscopy [43]; or (**c**) older persons who participated in a large-scale population based cohort study which aimed to evaluate new approaches to the prevention, early detection and therapy of chronic diseases and who did not undergo colonoscopy [44]. Until now there is only limited information on the specificity of the tumour M2-PK stool test in a large cohort being screened for colorectal cancer. Therefore, the aim of our study was to describe the specificity of the faecal tumour M2-PK test in a large, unselected, asymptomatic screening population and to report the distribution of faecal tumour M2-PK levels in different age groups.

Materials & methods

Patients

We recruited 4854 volunteers for our study in the age range of 19-94 years, **Figure 1,** of whom 2461 (50.7%) were male and 2393 (49.3%) were female. All volunteers were participating in an employer-based colorectal cancer screening programme from March 2005 to March 2006. No special diet was recommended.

Stool samples

All participants received a Quick-Prep™ dosing device (ScheBo® • Biotech AG, Giessen, Germany) and were instructed to collect a single naturally produced stool sample.
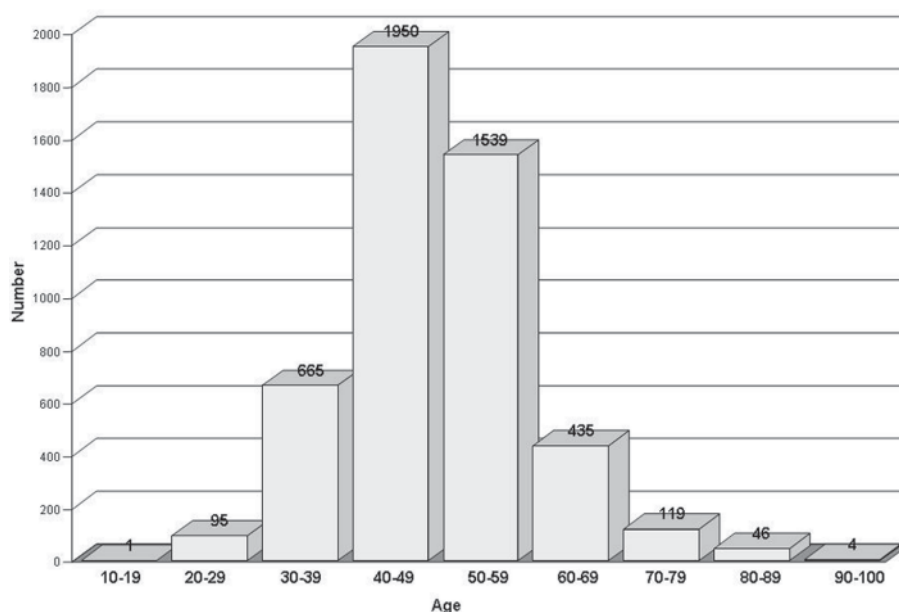
Figure 1. Age distribution of the screening volunteers

Paper collecting devices were used to avoid stool contact with water in the toilet bowl. Stool samples were stored for up to 48 hours at room temperature.

Measurement of faecal tumour M2-PK concentrations

After extraction and homogenisation of the stool samples, faecal tumour M2-PK concentrations were determined using a commercially available sandwich ELISA based on two different monoclonal antibodies which specifically recognise the dimeric form of M2-PK (ScheBo® • Biotech AG, Giessen, Germany). A positive test result was defined as > 4 kU/L, as indicated by the manufacturer. All analyses were carried out in a single laboratory under standardised conditions.

Estimates of specificity

Specificity was computed using the standard 4x4 format, **Table I**, where a = true positives, b = false positives, c = false negatives, d = true negatives. Specificity is the probability of a negative test in patients without the disease and is calculated by d/(b + d). Sensitivity is the probability of a positive test result in people with the disease and is calculated as a/(a + c). The only data available to our study from the 4854 volunteers were the total positive test results (a + b) and the total negative test results (c + d). Although individuals with an elevated faecal tumour M2-PK level were advised to undergo colonoscopy, the reason for the lack of some patient's data was the fact that the decision on whether to accept this advice had to be left solely to the patient. Concerning these volunteers it was also not possible to insist on them reporting back colonoscopy findings to the study organiser. In order to obtain estimates for the positive test result individual cell values a and b, estimates were used of the prevalence of colorectal cancer [45] of 50 per 100,000 persons and of the prevalence of pre-invasive conditions associated with

**Table I. Data grouping for the computation of specificity**

Test result	Disease status		Totals
	Present	Absent	
Positive	a	b	(a + b)
Negative	c	d	(c + d)
Totals	(a + c)	(b + d)	(a + b + c + d)

colorectal cancer [46] of 330 per 100,000 persons. The estimated total prevalence used to compute the sensitivity was thus assumed to be 380 per 100,000 persons. However, since this was an estimate we also calculated the sensitivity for a prevalence range of 200-500 per 100,000 persons. For the negative test result cells we had to assume that c = 0, i.e., all negative test results were associated with an absent disease status, because no subsequent follow-up from the volunteers was available: as mentioned above. Because of this our results for specificity must be regarded as *estimates* only.

Results

Distribution of M2-PK levels

Faecal tumour M2-PK levels were below the cut-off value of 4.0 kU/L in 4425/4854 participants, which implies that 91% of the population screened had normal range nonpathological faecal tumour M2-PK levels. The tumour M2-PK concentrations were below 2 kU/L in 3537/4854 persons (73%) whereas concentrations in the range of 2-4 kU/L were observed in 888/4854 (18%).
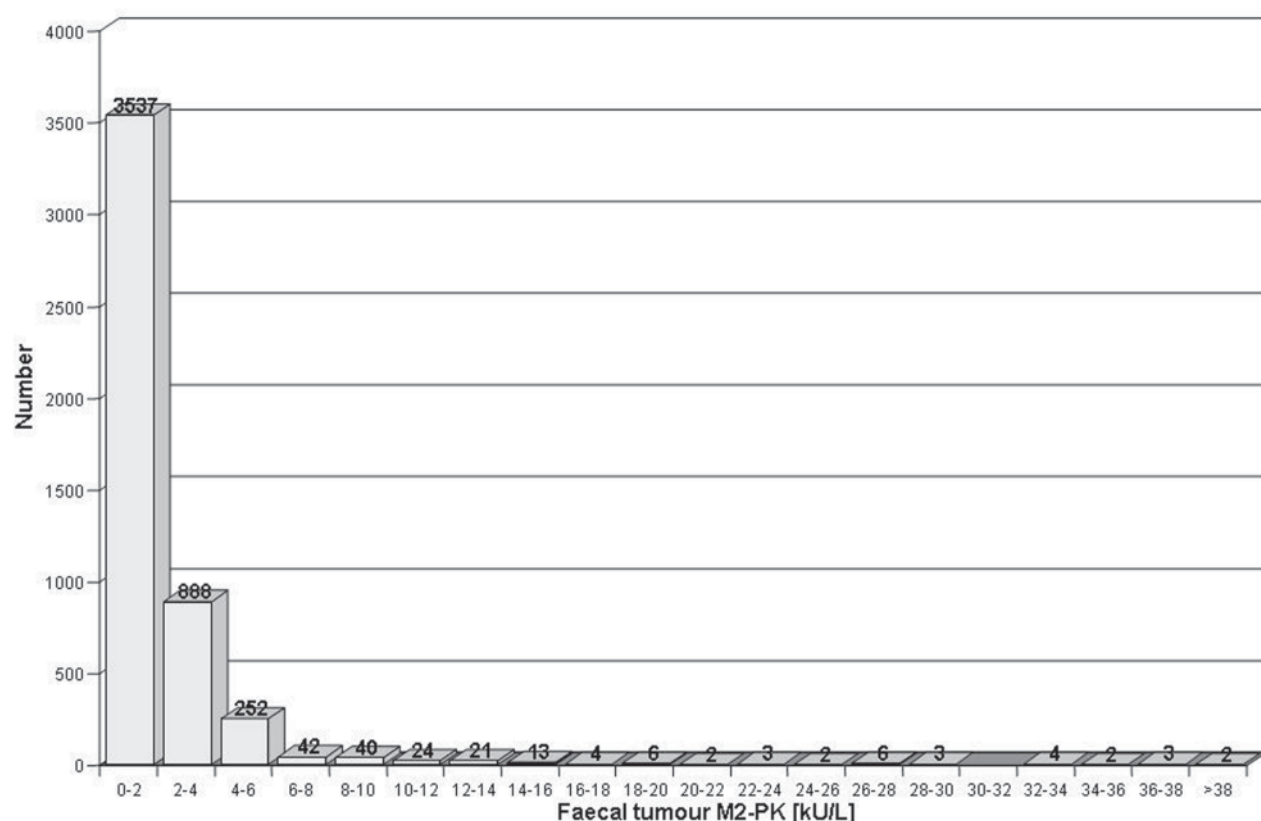
Figure 2. Number of volunteers & measured faecal tumour M2-PK concentrations

Table II. Faecal tumour M2-PK concentrations (kU/L) by age and sex

Statistic	Females		Males	
	Age (years)	Tumour M2-PK	Age (years)	Tumour M2-PK
Median	48.00	0.71	48.00	0.79
Mean	48.88	1.56	48.79	1.72
Std Deviation	10.22	3.40	10.02	3.20
Std Error	0.21	0.07	0.20	0.06
95% CI	0.41	0.14	0.40	0.13
99% CI	0.54	0.18	0.52	0.17
Cohort size	---	2393	---	2461

Faecal tumour M2-PK levels (> 4 kU/L) were found in 429/4854 (9%) and in 252/4854 (5%) tumour M2-PK concentrations were in the range of 4–6 U/L. Elevated tumour M2-PK concentrations of > 6 kU/L (up to a maximum of 85.5 kU/L) were detected in the stool samples of 177/4854 (4%), **Figure 2**.

M 2 - P K   l e v e l s   &   a g e

The mean faecal tumour M2-PK level of all 4854 participants was 1.6 kU/L, with a range of < 2-85.5 kU/L, a standard deviation of $\pm$ 3.3 kU/L, and with a median of 0.7 kU/L, see **Table II** for the results by sex. The male and female groups showed no significant differences in age or faecal tumour M2-PK levels. The percentage of tumour M2-PK positive test results as a function of 10-year age groups are given in **Figure 3**. A highly significant difference ($P < 0.001$) was found between the tumour M2-PK level for participants aged 20-49 years (median M2-PK of 0.66, mean 1.49, SD $\pm$ 2.93) and 50-79 years (median M2-PK of 0.086, mean 1.82, SD $\pm$ 3.72), **Figure 4**.

Estimates of specificity

Table III shows our results for specificity in the range of 0.93-0.96.

Table III. Specificity estimates calculated where d = 4425

Estimated prevalence per 100,000 persons	Table I cell values for 429 positive test results			Estimate of specificity
	a	b	(a + b)	d/(b + d)
2.0	97	332	429	0.93
3.8	184	245	429	0.95
5.0	243	186	429	0.96

Discussion

Tumour M2-PK is the synonym for the dimeric form of the glycolytic pyruvate kinase isoenzyme type M2 [22,23]. M2-PK is the pyruvate kinase isoenzyme
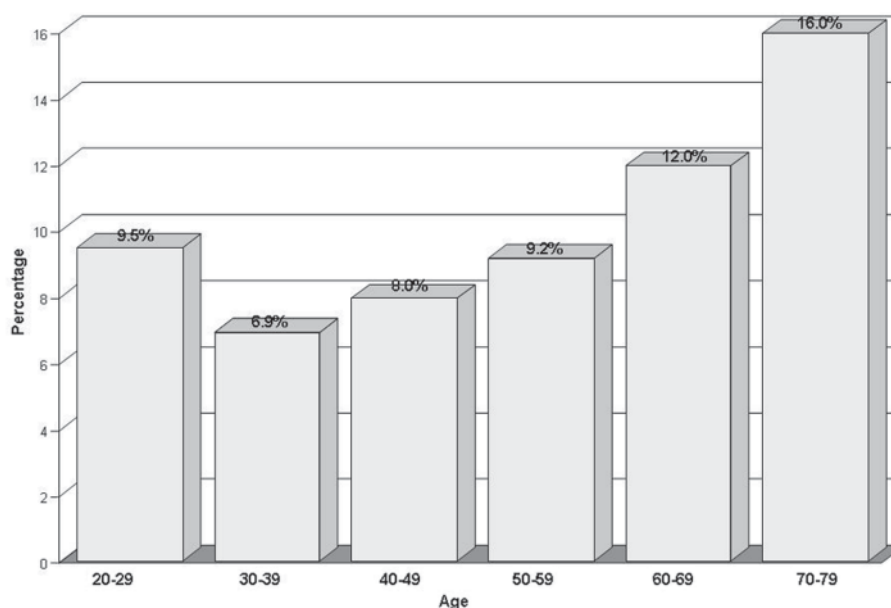
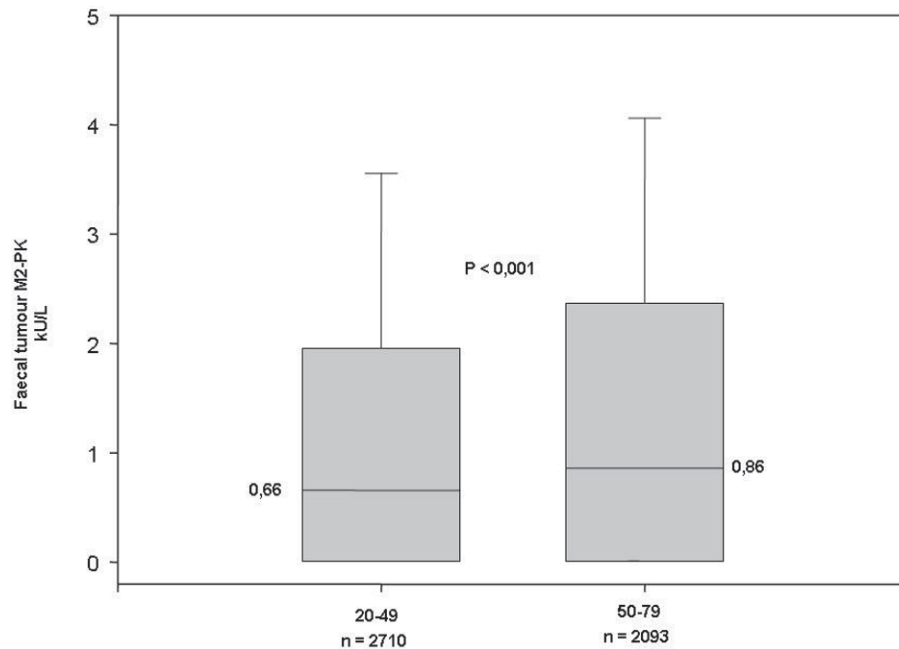**Figure 3.** Percentage of tumour M2-PK positive volunteers as a function of age group

Figure 4. Faecal tumour M2-PK levels of volunteers for two age groups 20-49 & 50-79 years

which is characteristic of all proliferating cells and can occur in a tetrameric form as well as in a dimeric form. Previous studies have described tumour M2-PK as being released into the stool of patients with adenomas and colorectal tumours, which can easily be quantified with a commercially available sandwich ELISA [10, 14-20].

The usefulness of the faecal tumour M2-PK test as a screening tool with good sensitivity and specificity for colorectal cancer or adenomas has already been demonstrated [10, 14-20]. The specificity has been reported in the range of 0.71-0.98 for colorectal cancer [10, 16-20, 41-44], but the number of participants in the different studies are in the range of 55-982 which is far lower than our own study of 4854 participants. Our results for specificity of 0.93-0.96 are close to the maximum of the already published range of 0.71-0.98. It is not ideal that follow-up reports of all of our 4854 cases postcoloscopy could not be obtained, but that is not surprising with such a large number of persons, particularly when they are volunteers. Nevertheless, our results underline the usefulness of the tumour M2-Pruvate kinase stool test as a non-invasive, easy, fast, economical method for colorectal cancer screening. In addition, the M2-PK levels as a function of age provide a more accurate set of data than any previous publication.

Carolin Tonus Dr. med
Herz-Jesu-Hospital
Surgical Clinic
Buttlarstr. 74
36039 Fulda
Germany
e-mail: c.tonus@herz-jesu-krankenhaus.de

References

1. Parkin DM, Bray F, Ferlay J et al. Global cancer statistics 2002. *CA Cancer J Clin* 2005; 55: 74-108

2. Smith RA, Cokkinides V, Eyre HJ. Cancer screening in the United States, 2007: a review of current guidelines, practices, and prospects. *CA Cancer J Clin* 2007; 57: 90-104.

3. Schmiegel W, Pox C, Adler G et al. Deutschen Gesellschaft für Verdauungs – und Stoffwechselkrankheiten; Deutschen Krebshilfe; Deutschen Krebsgesellschaft; Deutschen Gesellschaft für Hämatologie und Onkologie; Deutschen Gesellschaft fur Innere Medizin; Deutschen Gesellschaft für Koloproktologie; Deutschen Gesellschaft für Pathologie; Deutschen Gesellschaft für Radioonkologie; Deutschen Gesellschaft für Viszeralchirurgie; Deutschen Röntgengesellschaft; Deutschen vereinten Gesellschaft für klinische Chemie und Laboratoriumsmedizin e.V. [S3-guideline conference „Colorectal Cancer" 2004] *Dtsch Med Wochenschr* 2005 ; 130 Suppl 1: S5-53. Review. German.

4. Rhodes JM. Colorectal cancer screening in the UK: Joint Position Statement by the British Society of Gastroenterology, The Royal College of Physicians & The Association of Coloproctology of Great Britain and Ireland. *Gut* 2000; 46: 746-8.

5. Australian Cancer Network Colorectal Cancer Guidelines Revision Committee. *Guidelines for the Prevention, Early Detection and Management of Colorectal Cancer*. Sydney: The Cancer Council Australia & Australian Cancer Network, 2005.

6. Waye JD, Kahn O, Auerbach ME. Complications of colonoscopy and flexible sigmoidoscopy. *Gastrointest Endosc Clin N Am* 1996; 6: 343-7.

7. Darmkrebs – wenig Resonanz auf Angebot zur Früherkennung. *Ärztezeitung*. 27.10.2004.

8. Macrae FA, St.John DJB. Relationship between patterns of bleeding and hemoccult sensitivity in patients with colorectal cancers or adenomas. *Gastroenterology* 1982; 82: 891-8.

9. Lieberman DA, Harford WV, Ahnen DJ et al. One-time screening for colorectal cancer with combined fecal occult-blood testing and examination of the distal colon. *N Engl J Med* 2001; 345: 555-60.

10. Koss K, Maxton D, Jankowski JA. Faecal dimeric M2 pyruvate kinase in colorectal cancer and polyps correlates with tumour staging and surgical intervention. *Colorectal Dis* 2008; 10: 244-8.

11. Ransohoff DF, Lang CA. Screening for colorectal cancer with the fecal occult blood test: a background paper. American College of Physicians. *Ann Intern Med* 1997; 126: 811-22.

12. Hardcastle JD, Chamberlain JO, Robinson MHE et al. Randomised controlled trial of faecal occult blood screening for colorectal cancer. *Lancet* 1996; 348: 1472-7.

13. Mandel J, Church TR, Ederer F, Bond JH. Effectiveness of biennial screening for fecal occult blood. *J Natl Cancer Inst* 1999; 91: 434-7.

14. Ewald N, Toepler M, Akinci A et al. Pyruvatkinase M2 (Tumor M2-PK) im Stuhl als Screeningparameter für kolorektale Neoplasien. Eine

Übersicht über bisher publizierte Daten. *Z Gastroenterol* 2005; 43: 1313-7.

15. Hardt PD, Toepler M, Ngoumou B et al. Measurement of fecal pyruvate kinase type M2 (Tumor M2-PK) concentrations in patients with gastric cancer, colorectal cancer, colorectal adenomas and controls. *Anticancer Res* 2003; 23(2A): 851-3.

16. Hardt PD, Mazurek S, Toepler M et al. Faecal tumour M2 pyruvate kinase: a new, sensitive screening tool for colorectal cancer. *Br J Cancer* 2004; 91: 980-4.

17. McLoughlin R, Shiel E, Sebastian S et al. Tumor M2-PK, a novel screening tool for colorectal cancer. In: Poster Abstracts & Trade Exhibition Book: NCRI Cancer Conference; 2005 Oct 2-5; Birmingham, United Kingdom. London: Callisto, 2005: 202.

18. Tonus C, Neupert G, Sellinger M. Colorectal cancer screening by noninvasive metabolic biomarker fecal tumor M2-PK. *World J Gastroenterol.* 2006; 12: 7007-11.

19. Ewald N, Schaller M, Bayer M et al. Fecal pyruvate kinase-M2 (tumor M2-PK) measurement: a new screening concept for colorectal cancer. *Anticancer Res* 2007; 27: 1949-52.

20. Kumar Y, Tapuria N, Kirmani N et al. Tumour M2-pyruvate kinase: a gastrointestinal cancer marker. *Eur J Gastroenterol Hepatol* 2007; 19: 265-76, review.

21. Hathurusinghe HR, Goonetilleke KS, Siriwardena AK. Current status of tumor M2 pyruvate kinase (tumor M2-PK) as a biomarker of gastrointestinal malignancy. *Ann Surg Oncol* 2007; 14: 2714-20, review.

22. Eigenbrodt E, Reinacher M, Scheefers-Borchel U et al. Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. *Crit Rev Oncogenesis* 1992; 3: 91-115.

23. Christofk HR, Van der Heiden MG, Wu N et al. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature 2008*; 452: 181-6.

24. Reinacher M, Eigenbrodt E. Immunohistological demonstration of the same type of pyruvate kinase isoenzyme (M2-PK) in tumors of chicken and rat. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1981; 37: 79-88.

25. Board M, Humm S, Newsholme EA. Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and tricarboxylic acid cycle in normal, neoplastic and suppressed cells. *Biochem J* 1990; 265: 503-9.

26. Hacker HJ, Steinberg P, Bannasch P. Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. *Carcinogenesis* 1998; 19: 99-107.

27. Christofk HR, Van der Heiden MG et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 2008; 452: 230-3.

28. Discher DJ, Bishopric NH, Wu X et al. Hypoxia regulates β-enolase and pyruvate kinase promoters by modulating Sp1/Sp3 binding to conserved GC element. *J Biol Chem* 1998; 273: 26087-93.

29. Semenza GL. Hypoxia-inducible factor 1: master regulator of O2 homeostasis. *Curr Opin Genet Dev* 1998; 8: 588-94.

30. Mazurek S, Zwerschke W, Jansen-Durr P et al. Metabolic cooperation between different oncogenes during cell transformation: interaction between activated ras and HPV-16 E7. *Oncogene* 2001; 20: 6891-8.

31. Nishikawa T, Maemura K, Hirata I et al. A simple method of detecting K-ras point mutations in stool samples for colorectal cancer screening using one-step polymerase chain reaction/restriction fragment length polymorphism analysis. *Clin Chim Acta* 2002; 318: 107-12.

32. Mazurek S, Boschek B, Hugo F et al. Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* 2005; 15: 300-8.

33. Oremek GM, Teigelkamp S, Kramer W et al. The Pyruvate Kinase Isoenzyme Tumor M2 (Tu M2-PK) as a Tumor Marker for Renal Carcinoma. *Anticancer Res* 1999; 19: 2599-602.

34. Wechsel HW, Petri E, Bichler KH et al. Marker for renal cell carcinoma (RCC); the dimeric form of pyruvate kinase type M2 (Tu M2-PK). *Anticancer Res* 1999; 19: 2583-90.

35. Lueftner D, Mesterharm J, Akrivakis C et al. Tumor Type M2 Pyruvate Kinase expression in advanced breast cancer. *Anticancer Res* 2000; 20: 5077-82.

36. Schneider J, Morr H, Velcovsky HG et al. Quantitative detection of Tumor M2-pyruvate kinase in plasma of patients with lung cancer in comparison to other lung diseases. *Cancer Detect Prev* 2000; 24: 531-5.

37. Kaura B, Bagga R, Patel FD. Evaluation of the Pyruvate Kinase isoenzyme tumor (Tu M2-PK) as a tumor marker for cervical carcinoma. *J Obstet Gynaecol Res* 2004; 30: 193-6.

38. Ventrucci M, Cipolla A, Racchini C et al. Tumor M2-pyruvate kinase, a new metabolic marker for pancreatic cancer. *Dig Dis Sci* 2004; 49: 1149-55.

39. Zhang B, Chen JY, Chen DD et al. Tumor type M2 pyruvate kinase expression in gastric cancer, colorectal cancer and controls. *World J Gastroenterol* 2004; 10: 1643-6.

40. Goonetilleke KS, Mason JM, Siriwardana P et al. Diagnostic and prognostic value of plasma tumor M2 pyruvate kinase in periampullary cancer: evidence for a novel biological marker of adverse prognosis. *Pancreas* 2007, 34: 318-24.

41. Naumann M, Schaum B, Oremek GM et al. Faecal pyruvate kinase type M2--a valid screening parameter for colorectal cancer? Preliminary results from a multicenter comparative study. *Dtsch Med Wochenschr* 2004; 129: 1806-7.

42. Vogel T, Driemel C, Hauser A et al. Comparison of different stool tests for the detection of cancer of the colon. *Dtsch Med Wochenschr* 2005; 130: 872-7.

43. Shastri YM, Naumann M, Oremek GM et al. Prospective multicenter evaluation of fecal tumor pyruvate kinase type M2 (M2-PK) as a screening biomarker for colorectal neoplasia. *Int J Cancer* 2006; 119: 2651-6.

44. Haug U, Rothenbacher D, Wente MN et al. Tumour M2-PK as a stool marker for colorectal cancer: comparative analysis in a large sample of unselected older adults vs colorectal cancer patients. *Br J Cancer* 2007; 96: 1329-34.

45. Segnan N, Senore C, Andreoni B et al. Baseline findings of the Italian multicenter randomized controlled trial of "once-only sigmoidoscopy" – SCORE. *J Natl Cancer Inst* 2002; 94: 1743-72.

46. Liu HH, Wu MC, Peng Y, Wu MS. Prevalence of advanced colonic polyps in asymptomatic Chinese. *World J Gastroenterol* 2005; 11: 4731-4.