RESEARCH ARTICLE

Creatine Kinase (CK)-MB-to-Total-CK Ratio: a Laboratory Indicator for Primary Cancer Screening

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Abstract

Background: For the determination of creatine kinase (CK)-MB, the immunoinhibition method is utilized most commonly. However, the estimated CK-MB activity may be influenced by the presence of CK isoenzymes in some conditions like cancer. Thus, a CK-MB-to-total-CK ratio more than 1.0 could be found in such a situation. The study aimed to explore the relationship of cancer to high CK-MB-to-total-CK ratio. Materials and Methods: From January 2011 to December 2014, laboratory data on all CK-MB and total CK test requests were extracted at Far Eastern Memorial Hospital (88,415 requests). Patients with a CK-MB-to-total-CK ratio more than 1.0 were registered in this study. Clinical data including tumor location, tumor TNM stage and metastatic status were also collected. Results: A total of 846 patients were identified with a CK-MB-to-total-CK ratio more than 1.0. Of these, 339 (40.1%) were diagnosed with malignancies. The mean CK-MB-to-total-CK ratio was significantly higher in malignancy than in non-malignancy (1.35±0.28 *vs* 1.25±0.23, $p<0.001$) groups. The most frequent malignancy with a CK-MB-to-total-CK ratio more than 1.0 was colorectal cancer (1.42±0.28, 16.5%, n=56), followed by lung cancer (1.38±0.24, 15.9%, n=54) and hepatocellular carcinoma (14.5%, n=49). Higher CK-MB-to-total-CK ratios in hematological malignancies (1.44±0.41)were also noted. Additionally, the CK-MB-to-total-CK ratio was markedly higher in advanced stage malignancy than in early stage (1.37±0.26 vs. 1.29±0.31, $p=0.014$) and significantly higher in liver metastasis than in non-liver metastasis (1.48±0.30 vs. 1.30±0.21, $p<0.001$). Conclusions: The CK-MB-to-total-CK ratio is an easily available indicator and could be clinically utilized as a primary screening tool for cancer. Higher ratio of CK-MB-to-total-CK was specifically associated with certain malignancies, like colorectal cancer, lung cancer and hepatocellular carcinoma, as well as some cancer-associated status factors such as advanced stage and liver metastasis.

Keywords: Creatine kinase - cancer - tumor biomarker - CK-MB-to-total-CK ratio

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Introduction

Creatine kinase (CK), consisting of two cytosolic subunits either from brain (B) or muscle (M), appeared as three isoforms including CK-BB, CK-MB and CK-MM. Of these, CK-MB was known as one of the specific indicators for evaluating myocardial tissue injury. For most clinical laboratories, serial determination of CK-MB was frequently utilized to help the differential diagnosis of myocardial infarction. Since the most CK distribution in the serum was CK-MM (96-100%) and CK-MB (0-6%), and CK-BB was almost undetectable in the sera of healthy subjects, this feature of serum CK distribution was applied in the laboratory measurement of CK-MB. For the determination of CK-MB, CK-M subunit was immunoinhibited by using the specific antibody, and the CK-B activity was measured, equivalently reflecting the serum concentration of CK-MB. However, CK-MB could be overestimated in a variety of diseases, such as malignancies, severe shock syndrome, brain injury, hypothermia, Reye's syndrome, and so on. In these conditions, the estimated value of CK-MB could be even higher than total CK activity. This aberrancy could be explained by the unexpected presence of CK-BB or macro-CK, the complex composed of CK-BB and immunoglobulin (type 1) or another CK isoenzymes derived from mitochondria (type 2)(Wu et al., 1982).

Earlier case reports had described that false increase of CK-MB, even higher than whole CK, was observed in the sera of patients with neoplasms but not myocardial infarction (Gries et al., 1997; Jap et al., 2000). Previous studies also demonstrated the phenomenon was associated with increased activity of CK-BB (Huddleston et al., 2005; Ishikawa et al., 2005). Based on the analysis employing the technologies of microarray and quantitative proteomics, it was suggested that CK-BB could therefore

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serve as a potential biomarker for early diagnosis of certain malignancies (Huddleston et al., 2005; Zeng et al., 2012). Besides, recent reports indicated that increased mitochondrial CK activity was associated with serious illnesses such as tumors as well (Ruiz Ginés et al., 2006). Nevertheless, no systemic review or clinical application regarding the spuriously increased activity of CK-MB caused by other CK isoenzymes in malignancies was reported.

Utilizing the characteristics of spurious enhancement of CK-MB even higher than total CK in neoplasms, we calculated the CK-MB-to-total-CK ratio individually and evaluate the association of this ratio with malignancies. To our best knowledge, the present study first time declared the relationship of CK-MB-to-total-CK ratio and neoplasms as well as established the potential role of CK-MB-to-total-CK ratio in the primary cancer screening.

Materials and Methods

We conducted a retrospective study between January 2010 and December 2014. During this period, all patients who visited Far Eastern Memorial Hospital (FEMH), New Taipei city, Taiwan, and had available data of CK-MB and total CK, were eligible for study enrollment. The laboratory data of CK-MB and total CK were acquired by the clinical biochemistry laboratory information management system (Technidata, TD-synergy). The CK-MB-to-total-CK ratio was calculated individually and a value of 1.0 and less was excluded. One value of the CK-MB-to-total-CK ratio would be randomly selected if a patient had multiple laboratory data of CK-MB and total CK, and thus the CK-MB-to-total-CK ratio. The clinical data, including the primary diagnosis or medical history of cancers, tumor location, TNM stage, image study and pathological evidence of malignancies, tumor metastatic status, management of cancers as well as the outcome of patients, were obtained via the electronic medical chart review.

In statistical analysis, all data were presented as mean±standard deviation (SD) or median (interquartile range; IQR). Data were stored and analyzed by the performance of SPSS (version 19.0; SPSS Inc., Chicago, USA) statistical software. Comparison between two clinical variables was analyzed by Mann-Whitney U test. A p value less than 0.05 was considered to be statistically significant.

Results

A total of 88,415 requests of CK-MB and total CK data were obtained from January 2010 to December 2014, and 846 patients with the value of CK-MB-to-total-CK ratio more than 1.0 were registered in this study. Of these, 339 (40.1%) patients were found to have established diagnosis of malignancies. Additionally, 18 patients with the value of CK-MB-to-total-CK ratio more than 1.0 were diagnosed with second primary neoplasms. Table 1 revealed the demographics and laboratory characteristics of patients with the CK-MB-to-total-CK ratio of 1.0 and more. The median of estimated CK-MB activity in patients with malignancies was 48 international units /liter (IU/L) with IQR of 29 to 95 IU/L, and markedly lower than those without (the median of 69 IU/L with IQR of 35 to 137 IU/L, p<0.001). Instead, the CK-MB-to-total-CK ratio in patients with malignancies was significantly higher than those without (1.35±0.28 *vs* 1.25±0.23, p<0.001). Figure 1 showed the distribution of cancer characteristics in patients with CK-MB-to-total-CK ratio of 1.0 and more. The most frequent malignancy with this ratio more than 1.0 was colorectal cancer (n=56, 16.5%), followed by lung cancer (n=54, 15.9%), hepatocellular carcinoma (n=49, 14.5%), breast cancer (n=39, 11.5%), urological malignancies (n=34, 10.0%), upper aerodigestive malignancies (n=31, 9.1%), hematological malignancies (n=28, 8.3%), gynecological malignancies (n=19, 5.6%), gastric cancer (n=14, 4.1%), pancreatic cancer (n=11, 3.2%), cholangiocarcinoma (n=9, 2.7%), and so on.

Table 2 revealed the estimated CK-MB activity and CK-MB-to-total-CK ratio in various neoplasms. The median values of breast cancer (123 IU/L with IQR of 56 to 253 IU/L), colorectal cancer (88 IU/L with IQR of 52 to 215 IU/L), urological malignancies (80 IU/L with IQR of 35 to 206 IU/L), lung cancer (70 IU/L with IQR of 33 to 171 IU/L), hepatocellular carcinoma (66 IU/L with IQR of 44 to 96 IU/L), gastric cancer (55 IU/L with IQR of 29 to 105 IU/L) and second primary malignancies (63 IU/L with IQR of 34 to 129 IU/L) were higher than the generalized median value in cancer. Furthermore, the individual CK-MB-to-total-CK ratio of hematological

Table 1. The Demographics and Laboratory Characteristics of Patients with the CK-MB-to-total-CK Ratio of 1.0 and More

Variables	Non-malignancy	Malignancy
Age (year)	70.5±15.6	68.4±12.9
Gender (Male/Female)	207/300	185/154
Estimated CK-MB (IU/L)	69 (35-137)	48 (29-95)*
CK-MB-to-total CK ratio	1.25±0.23	1.35±0.28*

Data were presented as mean±standard deviation (SD) or median (interquartile range; IQR). IU/L, international units/liter. *p<0.05, compared with the non-malignancy group

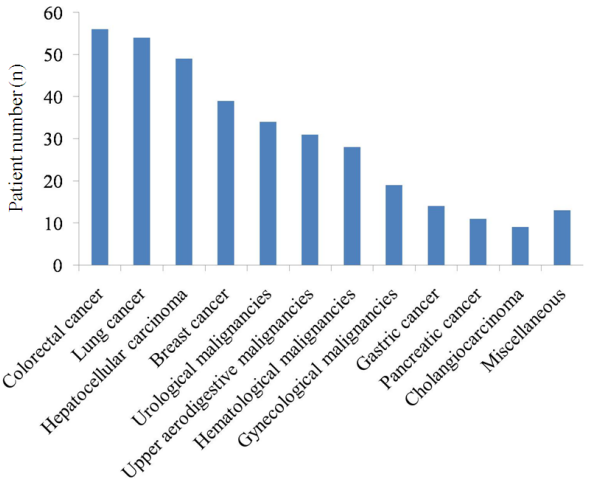

Figure 1. The Distribution of Cancer Characteristics in Patients with CK-MB-to-total-CK Ratio of 1.0 and More

malignancies (1.44±0.41), colorectal cancer (1.42±0.28) and lung cancer (1.38±0.24) was higher than the ratio of whole neoplasms.

Table 3 showed the relationship of cancer-associated states, including cancer stage and solid organ metastasis in advanced stage cancer, to the estimated CK-MB activity and CK-MB-to-total-CK ratio. The CK-MB-to-total-CK ratio in patients with stage III/IV malignancies was significantly higher than those with stage I/II malignancies (1.37±0.26 *vs* 1.29±0.31, p=0.014). There was no statistical difference of the estimated CK-MB activity between the groups in advanced and early stages (the median of 71 IU/L with IQR of 37 to 165 IU/L *vs* the median of 62 IU/L with IQR of 42 to 113 IU/L, p=0.435). Additionally, both the estimated CK-MB activity and CK-MB-to-total-CK ratio in the metastasis to liver were significantly higher than other types of metastasis (the median of 124 IU/L with IQR of 73 to 290 IU/L *vs* the median of 58 IU/L with IQR of 29 to 136 IU/L, p<0.001; 1.48±0.30 *vs* 1.30±0.21, p<0.001, respectively). Patients with bone metastasis had a significantly higher estimated CK-MB activity than those with non-bone metastasis (the median of 124 IU/L with IQR of 58 to 281 IU/L *vs* the median of 70 IU/L with IQR of 36 to 140 IU/L, p=0.012), but had a significantly lower CK-MB-to-total-CK ratio (1.32±0.24 *vs* 1.41±0.28, p=0.023). Besides, a significantly lower CK-MB-to-total-CK ratio was observed in the lung metastasis than in non-lung metastasis (1.33±0.25 *vs* 1.42±0.27, p=0.023), but no difference of the estimated CK-MB activity was found (the median of 72 IU/L with IQR of 42 to 172 IU/L *vs* the median of 85 IU/L with IQR of 33 to 208 IU/L, p=0.888). There was no statistical difference of the estimated CK-MB activity and CK-MB-to-total-CK ratio between the brain and non-brain metastasis.

Table 2. The Estimated CK-MB Activity and CK-MB-to-total-CK Ratio in Various Neoplasms

Variables	Estimated CK-MB (IU/L)	CK-MB-to-total CK ratio
Colorectal cancer	88 (52-215)	1.42±0.28
Lung cancer	70 (33-171)	1.38±0.24
Hepatocellular carcinoma	66 (44-96)	1.30±0.27
Breast cancer	123 (56-253)	1.34±0.23
Urological malignancies	80 (35-206)	1.32±0.25
Upper aerodigestive malignancies	40 (33-65)	1.33±0.31
Hematological malignancies	48 (22-110)	1.44±0.41
Gynecological malignancies	33 (25-70)	1.30±0.23
Gastric cancer	55 (29-105)	1.33±0.25
Pancreatic cancer	40 (24-92)	1.31±0.17
Cholangiocarcinoma	43 (29-73)	1.19±0.09
Miscellaneous	49 (31-131)	1.27±0.21
Second primary cancer	63 (34-129)	1.32±0.14

Data were presented as mean±standard deviation (SD) or median (interquartile range; IQR). IU/L, international units/liter

Table 3. The Relationship of Cancer-associated States to the Estimated CK-MB Activity and CK-MB-to-total-CK Ratio

Variables	Estimated CK-MB (IU/L)	CK-MB-to-total CK ratio
Stage		
I/II	62 (42-113)	1.29±0.31
III/IV	71 (37-165)	1.37±0.26*
Solid organ metastasis		
Brain		
Brain metastasis	56 (35-168)	1.37±0.23
Non-brain metastasis	84 (37-188)	1.37±0.27
Lung		
Lung metastasis	72 (42-172)	1.33±0.25
Non-lung metastasis	85 (33-208)	1.42±0.27*
Liver		
Liver metastasis	124 (73-290)	1.48±0.30
Non-liver metastasis	58 (29-136)*	1.30±0.21*
Bone		
Bone metastasis	124 (58-281)	1.32±0.24
Non-bone metastasis	70 (36-140)*	1.41±0.28*

Data were presented as mean±standard deviation (SD) or median (interquartile range; IQR). IU/L, international units/liter. *p<0.05, compared with the stage I/II cancer group or the specific organ metastasis group

Discussion

Our main finding suggested that approximately 40% of patients with the CK-MB-to-total-CK ratio more than 1.0 were diagnosed with malignancies, of which the most frequent was colorectal cancer, followed by lung cancer and hepatocellular carcinoma. Also, the overestimated CK-MB activity was frequently associated with breast cancer, colorectal cancer, urological malignancies, lung cancer, hepatocellular carcinoma, gastric cancer and second primary malignancies. Meanwhile, the high CK-MB-to-total-CK ratio was frequently accompanied with hematological malignancies, colorectal cancer and lung cancer. The CK-MB-to-total-CK ratio in malignancies with advanced stage was significantly higher than those with early stage and was remarkably higher in the liver metastasis than in non-liver metastasis. To the best of our knowledge, the present study first time elucidated the screening role of CK-MB-to-total-CK ratio in perspective of various cancers.

The prevalence of cancer has been increasing globally; therefore, it is important to establish effective biomarkers for monitoring the cancer risk. However, novel tumor markers are usually cost-ineffective at the initial beginning, resulting in unpopular application in clinical laboratories, especially in the developing area. Hence, easily available biomarkers that help risk evaluation and diagnosis of cancer would be useful and applicable in clinical practice (Baldane et al., 2015). The CK-MB-to-total-CK ratio, based on the data of CK-MB and total CK activity that were commonly used in evaluation of myocardial injury, was such a widely available indicator that could be applied for primary cancer screening in clinical laboratories.

Accumulating evidence indicated that the estimated activity of CK-MB could be falsely increased in patients with neoplasms rather than myocardial disease (Jap et al., 2000; Z'Graggen et al., 2000). The spuriously high expression CK-MB could be explained by the interference of CK-BB or maro-CK, as the immunoinhibition method was used for the CK-MB activity determination (Vrbica et al., 1997). This aberrancy of overestimated CK-MB expression in patients without obvious thoracic symptoms

may confuse physicians, leading to inappropriate diagnosis and unnecessary examination and management. Here, we conversely take advantage of the characteristics of overestimated CK-MB expression and high CK-MB-to-total-CK ratio in patients with potential neoplasms to evaluate the efficacy of CK-MB-to-total-CK ratio as a laboratory indicator for primary cancer screening.

According to the literature review, the elevation of CK-BB activity had been observed in various malignancies, including lung, breast, liver, colon, and prostate cancers (Zarghami et al., 1996; Joseph et al., 1997; Meffert et al., 2005). It was believed that the enhanced CK-BB activity could originated from the release of enzyme due to tumor necrosis (Joseph et al., 1997). In tumorigenesis, increased localization of CK-BB to nuclear matrix was observed in colon cancer (Balasubramani M et al., 2006). Besides, unmethylation of the CK-BB promotor and therefore higher CK-BB mRNA expression in hematological cancer cell lines was identified (Ishikawa J et al., 2005). It was also reported that CK-BB contributed to increasing the number of cancer cell in pre- and mitotic phases, but was not associated with the cell proliferation (Mooney SM et al., 2011). Furthermore, it was recently reported that higher CK-BB activity was associated with human liver metastasis (Loo JM et al., 2015), which was partially compatible with and illustrate our results. Interestingly, the discrepancies that significantly higher estimated CK-MB activity but lower CK-MB-to-total-CK ratio in the bone metastasis than in non-bone metastasis and significantly lower CK-MB-to-total-CK ratio in the lung metastasis than in non-lung metastasis but without difference of the estimated CK-MB activity were also observed. This could be explained by the increase of total CK activity according to the comorbidities of patients, but the underlined mechanism remained unknown and could be worth further research.

It was reported that increased mitochondrial CK was found in some cases of hepatocellular carcinoma (Meffert et al., 2005; Soroida et al., 2012). It was also observed that patients with liver cancer had significant reduced survival time prior to cancer-related treatment, suggesting that high expression of mitochondrial CK could be associated with a poor prognosis in hepatocellular carcinoma (Uranbileg et al., 2014). Besides, it was indicated that increase of mitochondrial CK activity denoted a cumulating risk for hepatocarcinogenesis in patients who had chronic hepatitis C (Enooku et al., 2014). There was one recent study indicating that overexpression of mitochondrial CK was markedly associated with shorter progression-free and overall survival times in patients with breast cancer (Qian et al., 2012). Otherwise, few studies regarding the macro-CK and malignancies except hepatocellular carcinoma were reported.

The prevalence of second primary cancer has been increasing globally (Tsai et al., 2014; Chang et al., 2015). Both the overestimated CK-MB activity and high CK-MB-to-total-CK ratio in second primary cancer were observed in our study. However, the underlying association between the false high activity of CK-MB and second primary neoplasms remains unknown. Further study regarding the role of CK-BB and macro-CK in pathogenesis of second primary cancer could be conducted for risk evaluation and therapeutic strategy.

There are several limitations to our study. Our study was a retrospective study underwent in a single institution. Also, the clinical conditions that could lead to a high CK-MB-to-total-CK ratio rather than neoplasms, including myocardial diseases, brain injury or severe shock syndrome, were not excluded in the register entry of our study because the cancer patients usually had other comorbidities. The comorbidities could also cause enhancement of total CPK level, which may partially explain why the markedly higher estimated CK-MB activity but significantly lower CK-MB-to-total-CK ratio was observed in the bone metastasis than non-bone metastasis. Additionally, the CK-MB levels estimated by the immunoinhibition method were not further clarified to be specific CK isoenzymes, which make it difficult to elucidate the unexpected result that the estimated CK-MB activity in non-malignancy was markedly higher than malignancy. Furthermore, since the blood specimen obtained for determination of CK-MB and total CK activities was collected mostly when patients encountered such emergent and life-threatening situation, it was difficult to evaluate the relationship of CK-MB-to-total-CK ratio and long-term survival rate in the cancer patients.

In conclusion, the CK-MB-to-total-CK ratio is an easily available indicator that could be utilized for primary cancer screening in clinical laboratories. Higher ratio of CK-MB-to-total-CK was associated with certain malignancies, like colorectal cancer, lung cancer and hepatocellular carcinoma, as well as some cancer-associated status such as cancer in advance stage and metastasis to liver.

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