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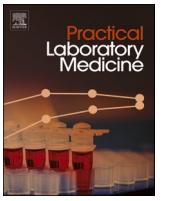

Evaluation of creatine kinase (CK)-MB to total CK ratio as a diagnostic biomarker for primary tumors and metastasis screening

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ABSTRACT

Objective: Creatine kinase (CK) and its myocardial band isoenzyme (CK-MB) were considered important diagnostic indicators for identifying suspected acute myocardial infarction. However, the serum level of CK-MB is frequently exaggerated in some pathological states without cardiogenic damage, like cancer. Sometimes, the CK-MB level is even greater than the total CK. This study intended to investigate the association between malignancy and an abnormally high ratio of CK-MB to total CK (CK-MB/CK) and to assess the diagnostic relevance of this ratio as a biomarker for cancer.

Methods: Patients hospitalized between September 2019 and September 2022 at Shandong Provincial Qianfoshan Hospital (Jinan, Shandong, China) with serum CK-MB activity greater than total CK activity (CK-MB/CK $>$ 1.0) were recruited as research subjects. Then the demographic and clinical characteristics of these patients were systemically analyzed. The correlation between clinical characteristics (such as cancer types, tumor locations, and tumor metastasis) and laboratory test results (such as serum CK-MB activity, total CK activity, and the CK-MB/CK ratio) was also investigated.

Results: We found that over 44% of the patients with CK-MB/CK $>$ 1.0 were diagnosed with malignancies, and the CK-MB/CK ratio in malignancies patients was significantly higher than in non-malignancies patients. The increase of CK-MB/CK ratio was most obvious in patients with colorectal carcinoma and prostatic carcinoma. Additionally, extremely elevated CK-MB/CK ratios were observed in individuals with metastatic neoplasms, especially in those who suffered from numerous sites of metastasis.

Conclusions: The serum CK-MB/CK ratio can be utilized as a readily accessible supplement diagnostic biomarker in screening for primary and metastatic cancers.

1. Introduction

Creatine kinase (CK, EC 2.7.3.2) is an enzyme that is expressed in myocardial and skeletal muscle cells, as well as in the brain, small intestine and lung, and it is composed of a dimer consisting of M and B subunits. Three typical isoenzymes of CK have been identified in

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the cytoplasm of various tissues and organs, namely CK-MM, the hybrid CK-MB, and CK-BB, which differ in their subunit compositions. CK-MM is the predominant isoenzyme, and it is primarily located in the skeletal muscle and myocardium [1]. CK-MB is mostly present in cardiac muscle and contributes to approximately 25–46% of the total CK activity in the myocardium, with minor amounts found in skeletal muscle [2,3]. CK-BB is mainly expressed in the brain tissue, as well as the prostate, bladder, uterine smooth muscle, and gastrointestinal tract [3]. However, CK-BB is typically present in very low levels in the serum of healthy individuals [4]. Besides these three types of cytoplasmic CK isoenzymes, a fourth CK isoenzyme has been identified in the mitochondria of cells, known as mitochondrial CK [5].

The level of CK-MB in the serum will be rapidly increased in response to the damage to either cardiogenic or non-cardiogenic tissue, with the release of large amounts of muscle enzymes into the bloodstream [6]. Therefore, a marked elevation of CK-MB levels in serum or plasma is a crucial diagnostic indicator of coronary syndromes, especially acute myocardial infarction (AMI) [6,7]. The activity of the total CK and its isoenzymes MB can be quantified by the automatic biochemical analyzer with rate and immune-inhibition methods, respectively. The immune-inhibition assay employs a specific antibody against CK-M subunit to selectively suppress its activity while keeping the activity of CK-B unaltered and quantifiable [1]. As a result, the activity of CK-MB in serum can be estimated by doubling the activity of the CK-B isoform. As an isoenzyme of CK, theoretically, the activity of CK-MB should not be higher than that of total CK. In general, CK-MB comprises less than 5% of total CK in the serum of healthy individuals [8]. In patients with myocardial infarction, the proportion of CK-MB activity to total CK activity can increase from 6% to 25% but generally does not exceed 30%. However, in some cases, the CK-MB activity can be significantly overestimated in patients with conditions such as malignancies, brain injury, severe shock syndrome, and hypothermia. In these pathological states, the results of immune-inhibition methods may be compromised by the presence of markedly elevated CK-BB, macro creatine kinase (macro-CK), or mitochondrial CK (Mit-CK) in the serum of patients. These factors can lead to falsely elevated CK-MB activity levels and may interfere with accurate diagnosis [4,9,10]. An overestimation of CK-MB activity has been observed in the serum of patients with neoplasms, rather than myocardial infarction [11, 12], leading to abnormally high ratios of CK-MB to total CK (CK-MB/CK $>$ 1.0). Nevertheless, there have been few reports on the systemic analysis of the demographic and clinical characteristics of patients with a falsely elevated ratio of CK-MB/CK. In this study, we performed a systematic retrospective analysis of patients with a falsely elevated CK-MB activity, and we calculated individual CK-MB/CK ratios for each of these patients. In addition, we further assessed the association between this ratio and malignancies to investigate the potential diagnostic or predictive value of serum CK-MB/CK ratio in patients with primary or metastatic malignancies.

2. Materials and methods

2.1. Subjects of the retrospective study

This retrospective observational study analyzed patients admitted to the Shandong Provincial Qianfoshan Hospital (Jinan, Shandong, China) between September 2019 and September 2022. In this study, we assessed a total of 30,363 patients with available data on serum CK-MB activity and total CK activity. For each patient, we calculated the CK-MB/CK ratio individually. The inclusion criteria for this study were: 1) patients with serum CK-MB activity higher than serum total CK activity; 2) patients with clear and complete demographic and clinical data. Exclusion criteria were: Subjects with hemolysed specimens.

2.2. Ethical approval

This retrospective analysis study was reviewed and approved by the ethical committee of The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital in Jinan, China (No. 2023S355).

2.3. Laboratory and clinical data

The laboratory measurements of CK and CK-MB activity were conducted using the Cobas 8000 modular analyzer series (Roche, United States). The serum CK-MB activity was measured by the immunosuppression method [13], and the activity of the serum total CK was measured by the rate method. If a patient had multiple available laboratory data for CK-MB and total CK activity, the initial assay value for CK-MB and CK would be selected for the calculation of the CK-MB/CK ratio. All patients' demographic data, including age and sex, were collected. The clinical data, containing the primary diagnosis, medical history, tumor location, tumor metastatic status, image study and pathological evidence of malignancies, as well as patient outcomes, were obtained from the Electronic Medical Records System (EMRS). According to a report by Thomas L et al., the reference interval for serum CK-MB activity were $<$25 U/L, while the reference interval for total serum CK activity were $<$170 U/L for females, and $<$190 U/L for males [14].

2.4. Statistical analysis

The data in this study, including serum CK-MB activity, total CK activity, and CK-MB/CK ratio were presented as median (interquartile range; IQR). The age of the patients and the CK-MB/CK ratio were presented as mean $\pm$ standard deviation (SD). To determine the significance of the differences in serum CK-MB activity, total CK activity and CK-MB/CK ratio between groups, the Mann–Whitney *U* test was employed. The association found in the bivariate analysis ($P < 0.05$) was validated using the chi-square test and Fisher's test. The statistical analysis was performed with SPSS statistical software (version 19.0; SPSS Inc., Chicago, USA) and GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). A *P* value less than 0.05 was considered statistically significant. The receiver operator curve

(ROC) analysis of the CK-MB/CK ratio for malignancies were performed using serum samples from 520 patients with malignancies (311 men, 209 women; mean age 46.11 ± 26.68 years, range of 3 months–91 years) and 637 non-malignancy patients (304 men, 333 women; mean age 41.71 ± 31.70 years, range of 2 days–96 years) as control. Besides, ROC analysis of the CK-MB/CK ratio for metastasis was performed using serum samples from 153 solid tumors metastasis patients and 215 primary tumors patients as control. The calculation of cut-off values was performed using the Youden Index, which can be defined as max [sensitivity + specificity – 1] and ranges between 0 and 1. AUC: area under the curve.

3. Results

3.1. Demographic and laboratory characteristics of the patients with CK-MB/CK > 1.0

In this study, 1157 patients with a CK-MB/CK ratio greater than 1.0 (CK-MB/CK > 1.0) were enrolled. The gender distribution of patients with a CK-MB/CK > 1.0 was shown in Table 1. Among these patients, males accounted for 53% (615/1157) and females for 47% (542/1157). The median serum CK-MB activity and the total CK activity in male patients were 50 IU/L (interquartile range [IQR] of 28–111 IU/L) and 40 IU/L (IQR of 23–83 IU/L), respectively. In female patients, the corresponding values were 50 IU/L (IQR of 26–112 IU/L) and 43 IU/L (IQR of 21–87 IU/L) for serum CK-MB activity and total CK activity, respectively. There was no statistically significant difference in either serum CK-MB activity or total CK activity between male and female patients ($P = 0.845$ and 0.916, respectively). Among patients with CK-MB/CK ratios greater than 1.0, 78% (481/615) of males and 77% (416/542) of females had serum CK-MB activity values above the standard reference range. Furthermore, only one male patient and two female patients had CK-MB values below the reference range, respectively. The median value of CK-MB/CK ratio in male patients was 1.21 (IQR of 1.11–1.39), while corresponding value in female patients was 1.22 (IQR of 1.09–1.40), and the difference between the two groups was not statistically significant ($P = 0.819$).

To investigate any potential age-related differences, we also analyzed the age distribution of the patients with serum CK-MB/CK ratios greater than 1.0. The retrospective analysis revealed that the mean age of these 1157 participants enrolled in this study was 43.71 ± 29.58 years, with an age range of 2 days–96 years and a median of 54 years. Subsequently, all enrolled patients were divided into five age groups with a 20-year interval. As shown in Fig. 1, patients with CK-MB/CK > 1.0 were predominantly found in children and adolescents aged 0–19 years, with a particularly high proportion among those aged 0–12 years, accounting for 96% of patients in this age group. Patients aged 0–19 years accounted for the largest proportion (32%) among all age groups, followed by those aged 60–79 years, while those aged 20–39 years represented the smallest proportion (5%) of the total patient population.

To investigate the laboratory characteristics of enrolled patients, we performed further analyses of serum CK-MB activity, total CK activity, and the CK-MB/CK ratio across different age groups (Table 2). The statistical analyses revealed significant differences in serum CK-MB activity and total CK activity across different age groups ($F = 5.711$, $P = 0.0001$ for serum CK-MB activity, and $F = 6.364$, $P < 0.001$ for total CK activity). However, there were no significant differences in the ratio of CK-MB/CK in different age groups.

3.2. Features of disease spectrum in patients with CK-MB/CK > 1.0

We further analyzed the types of disease present in the patients with CK-MB/CK > 1.0. Among these 1157 enrolled patients, 520 (45%) were diagnosed with malignancies and 637 (55%) were diagnosed with non-malignancies. The disease distribution of patients with CK-MB/CK > 1.0 is presented in Fig. 2. Among the patients diagnosed with malignancies, almost one-third of them (n = 152, 29%) were found to have hematological malignancies, with the majority being children and adolescents aged 0–18 years (n = 132, 83%). Among patients with CK-MB/CK > 1.0, the second and third most common malignancies were lung cancer (n = 93, 18%) and hepatobiliary malignancies (n = 80, 15%), followed by gastric carcinoma (n = 50, 10%), colorectal carcinoma (n = 32, 6%), rectal carcinoma (n = 24, 5%), breast carcinoma (n = 21, 4%), gynecologic cancer (n = 14, 3%), esophageal carcinoma (n = 13, 3%), pancreatic carcinoma (n = 10, 2%), urologic neoplasms (n = 8, 2%), and prostatic carcinoma (n = 6, 1%), among others. In contrast, respiratory infections (n = 149, 23%), gastrointestinal disorders (n = 80, 13%), cardiovascular disease (CVD) (n = 68, 11%), hepatopathy (n = 58, 9%), and aplastic anemia (n = 55, 7%) were the five most prevalent non-malignant diseases among individuals with CK-MB/CK > 1.0.

3.3. Comparison of the demographic and laboratory characteristics between the malignancy patients and non-malignancy patients

The statistical analysis revealed that the average age of the patients diagnosed with malignancy was 46.11 ± 26.68 years, which was considerably higher than the average age of patients without malignancy (41.71 ± 31.70 years) ($P = 0.041$). The malignancy group consisted of 311 males (60%) and 209 females (40%), while the non-malignancy group consisted of 304 males (48%) and 333

Table 1
Gender distribution and laboratory characteristics of patients with CK-MB/CK > 1.0.

Gender	n	CK-MB activity (IU/L)	Total CK activity (IU/L)	CK-MB/CK ratio
Male	615	50 (28–111)	40 (23–83)	1.21 (1.11–1.39)
Female	542	50 (26–112)	43 (21–87)	1.22 (1.09–1.40)

The data for serum CK-MB, total CK activity values and the CK-MB/CK ratio were presented as median (interquartile range; IQR).

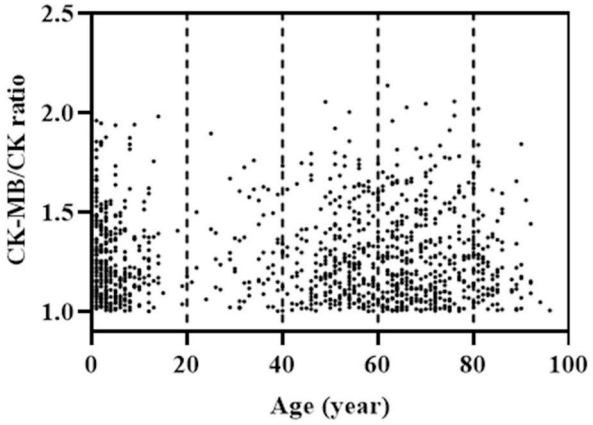

Fig. 1. The age distribution of the patients with serum CK-MB/CK > 1.0.

Table 2
Age distribution and laboratory characteristics of patients with a CK-MB/CK > 1.0.

Age (year)	n	CK-MB (IU/L)	CK (IU/L)	CK-MB/CK
≤19	371	33 (21–70)	28 (17–55)	1.22 (1.11–1.39)
20～39	60	56 (29–161)	43 (26–135)	1.23 (1.12–1.43)
40～59	260	70 (36–120)	58 (31–97)	1.22 (1.10–1.41)
60～79	362	62 (33–149)	52 (28–111)	1.21 (1.09–1.43)
≥80	104	39 (21–124)	30 (18–88)	1.19 (1.10–1.35)

The data of serum CK-MB, total CK activity values and the CK-MB/CK ratio were presented as median (IQR).

females (52%). Significant differences were observed in the proportion of male and female patients between the malignancy and non-malignancy groups ($\chi^2 = 16.789$, $P < 0.001$). The median serum CK-MB activity in patients with malignancies was 53 IU/L (IQR of 27–112 IU/L), which was not significantly different from that of the non-malignancy group (69 IU/L, IQR of 35–137 IU/L, $P > 0.05$). There was no significant difference in the median serum total CK activity between the two groups of patients (42 IU/L, IQR of 22–84 IU/L, vs. 40 IU/L, IQR of 22–86 IU/L). In contrast, the CK-MB/CK ratio was significantly higher in patients with malignancies than those without malignancies [1.26 (IQR of 1.12–2.14) *vs* 1.19 (IQR of 1.09–1.36), $P < 0.001$] (Table 3).

In addition, the diagnostic efficiency of the CK-MB/CK ratio for malignancies was assessed by ROC curve analysis (Fig. 3). The AUC was 0.570 (95% confidence interval [CI]: 0.537–0.604). The cut-off value of the CK-MB/CK ratio was 1.238. The sensitivity of CK-MB/CK ratio was 0.591 (95% CI: 0.552–0.628), and the specificity of CK-MB/CK ratio was 0.541 (95% CI: 0.498–0.583).

Further, the age distribution of the patients with CK-MB/CK > 1.0 in malignancy and non-malignancy groups is shown in Table 4. The majority of children and adolescents (0–19 years), young patients (20–39 years), and elderly patients over 80 years old with CK-MB/CK > 1.0 were diagnosed with non-malignant conditions. Infectious diseases, such as respiratory infections, were most prevalent among pediatric and adolescent patients (0–19 years) and elderly patients over 80 years old (data not shown). Conversely, the number of patients diagnosed with malignancies was slightly higher among the middle-aged group (40–59 years) and the elderly group (60–79 years) compared to those diagnosed with non-malignant conditions. There were significant differences in the incidence of malignancies and non-malignancies conditions observed across different age groups ($\chi^2 = 43.92$, $P < 0.001$).

We conducted a detailed analysis of the laboratory characteristics of patients diagnosed with different types of malignancies. Our findings showed that the medium values of serum CK-MB activity and total CK activity of the patients with lung cancer, hepatobiliary malignancies, gastric carcinoma, colorectal carcinoma, rectal carcinoma, breast carcinoma, esophageal carcinoma, urologic neoplasms, prostatic carcinoma were higher than the medium value of the whole malignancies group (detailed information was shown in Table 5). On the other hand, the medium values of CK-MB activity and total CK activity in patients with hematological malignancies, gynecologic cancer, and pancreatic carcinoma were lower than those of the whole malignancies group (Table 5). Notably, most of the patients with hematological malignancies were infants and children. Furthermore, the median values of CK-MB/CK ratio of patients with gastric carcinoma [1.27 (IQR of 1.07–1.49)], colorectal carcinoma [1.50 (IQR of 1.28–1.63)], rectal carcinoma [1.29 (IQR of 1.12–1.48)], breast carcinoma [1.34 (IQR of 1.08–1.62)], urologic neoplasms [1.28 (IQR of 1.25–1.37)] and prostatic carcinoma [1.52 (IQR of 1.24–1.84)] was higher than that of the whole malignancies group [1.26 (IQR of 1.12–2.14)]. Of these, the CK-MB/CK ratio of patients with colorectal carcinoma and prostatic carcinoma was significantly higher than that of the whole malignancies group ($P < 0.001$ and $P < 0.05$, respectively).

We conducted a retrospective analysis of clinical data from patients diagnosed with solid tumors and found that 153 of them had secondary neoplasms or metastases. We compared the serum CK-MB activity, total CK activity, and CK-MB/CK ratio of patients with secondary neoplasms or metastases to those of patients with only primary tumors. The results, shown in Table 6, indicate that the medium values of serum CK-MB activity and total CK activity were significantly higher in patients with metastases than in those with

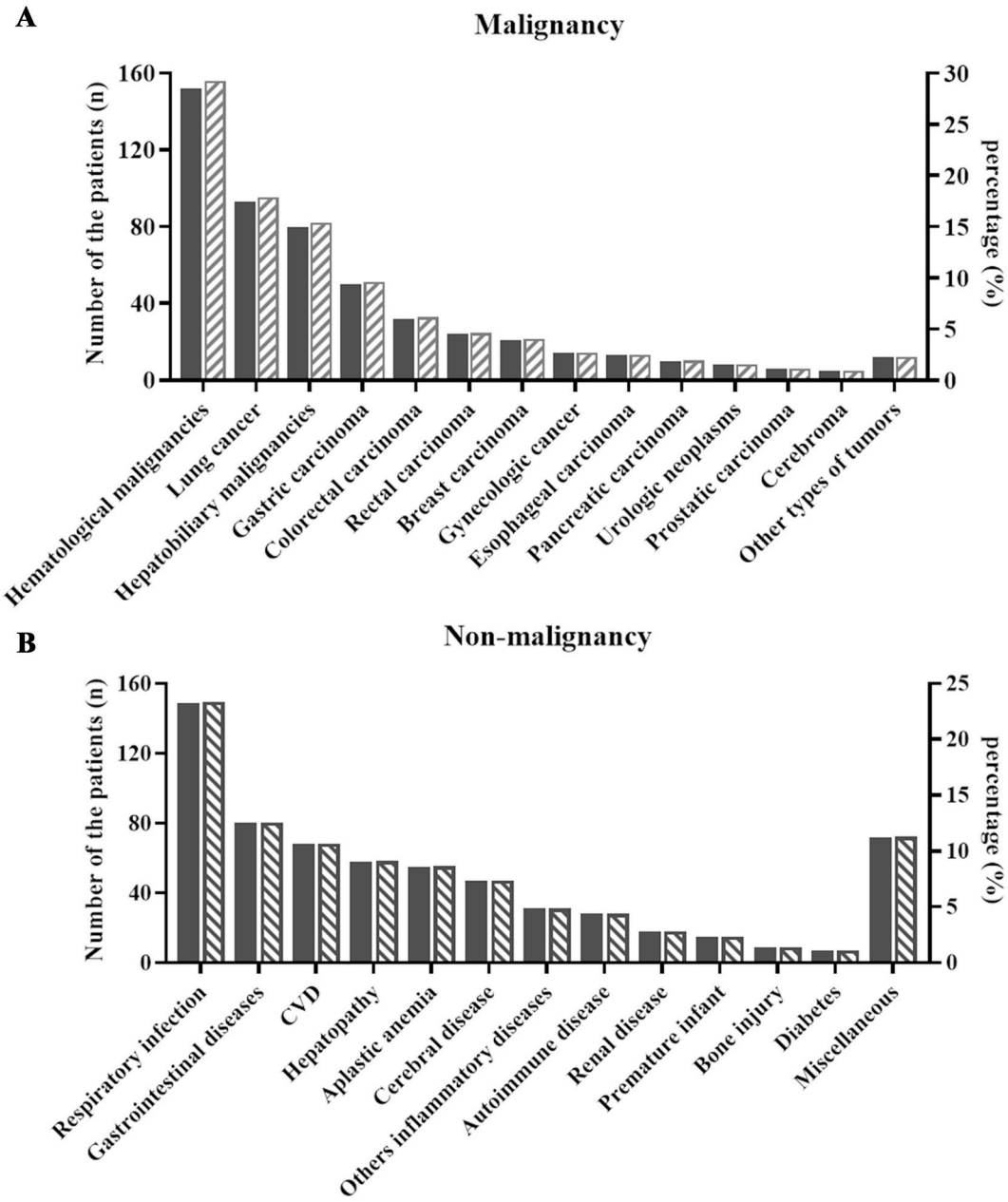

Fig. 2. The distribution of disease types in patients with CK-MB/CK > 1.0. (A) The detail distribution of tumor types in the malignancy patients with CK-MB/CK > 1.0. (B) The detail distribution of disease types in the non-malignancy patients with CK-MB/CK > 1.0. The gray columns represent the number of patients, and the diagonal columns represent the proportion of the patients. CVD, cardiovascular disease.

primary neoplasms (116 IU/L with IQR of 63–223 IU/L *vs* 62 IU/L with IQR of 34–100 IU/L for serum CK-MB activity, $P < 0.0001$; 90 IU/L with IQR of 54–152 IU/L *vs* 51 IU/L with IQR of 28–81 IU/L for total CK activity, $P < 0.0001$). The CK-MB/CK ratio was also significantly higher in patients with metastatic malignancies compared to those with primary neoplasms [1.36 (IQR of 1.19–1.60) *vs* 1.20 (IQR of 1.10–1.38), $P < 0.0001$)]. In addition, we examined the association between the degree of metastasis (solitary metastasis or multiple metastases) and the CK-related laboratory characteristic, as shown in Table 6. Notably, the CK-MB/CK ratio was significantly higher in patients with multiple metastases than in those with solitary metastasis [1.41 (IQR of 1.20–1.63) *vs* 1.31 (IQR of 1.16–1.50), $P = 0.035$]. However, no significant difference was observed in the serum CK-MB activity or total CK activity between these two groups (127 IU/L with IQR of 64–237 IU/L *vs* 100 IU/L with IQR of 58–176 IU/L for serum CK-MB activity, $P = 0.141$; 95 IU/L with IQR of 54–165 IU/L *vs* 75 IU/L with IQR of 50–123 IU/L for total CK activity, $P = 0.253$).

Furthermore, the diagnostic efficiency of the CK-MB/CK ratio for metastatic malignancies was also assessed by ROC curve analysis (Fig. 4). The AUC was 0.668 (95% CI: 0.611–0.725). The cut-off value of the CK-MB/CK ratio was 1.316. The pooled sensitivity of CK-

Table 3
Demographic and laboratory characteristics of patients with serum CK-MB/CK > 1.0 in malignancy and non-malignancy groups.

Characteristic/	Malignancy	Non-malignancy
Patients' number	520 (45%)	637 (55%)
Age (year)	46.11 ± 26.68*	41.71 ± 31.70
Gender (male/female)[a,b]	311/209***	304/333
CK-MB (IU/L)	53 (27–112)	49 (27–112)
CK (IU/L)	42 (22–84)	40 (22–86)
CK-MB/CK ratio	1.26 (1.12–2.14)***	1.19 (1.09–1.36)

The data of serum CK-MB, total CK activity values and the CK-MB/CK ratio were presented as median (IQR). The data of age was presented as mean ± SD.
*$P < 0.05$, ***$P < 0.001$.
[a] : 0 cells (0%) have an expected count of less than 5.
[b] : The minimum expected count is 243.60.

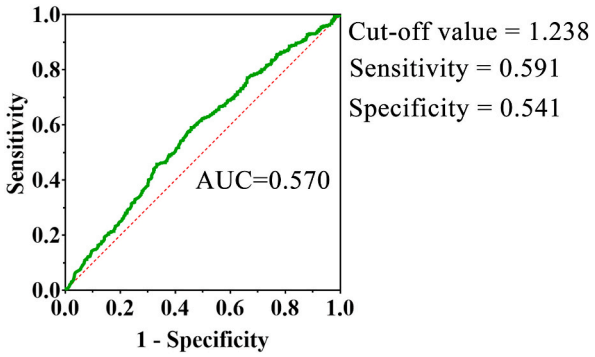

Fig. 3. The diagnostic performance of CK-MB/CK ratio in malignancy, shown by ROC curve. ROC curves were constructed by plotting the sensitivity and 1 - specificity for the confirmed malignancy patients group (n = 520) and the non-malignancy patients group (n = 637).

Table 4
Age distribution of patients with CK-MB/CK > 1.0 in malignancy and non-malignancy groups.

Age (year)	Disease[a,b]		Total (n)
	Malignancy (n)	Non-malignancy (n)	
≤19	139	232	371
20～39	22	38	60
40～59	149	111	260
60～79	182	180	362
≥80	28	76	104
Total	520	637	1157

[a] : 0 cells (0%) have an expected count of less than 5.
[b] : The minimum expected count is 26.97.

MB/CK ratio was 0.582 (95% CI: 0.503–0.657), and the pooled specificity of CK-MB/CK ratio was 0.684 (95% CI: 0.619–0.742).

4. Discussion

In this study, we observed that more than 44% of the patients with CK-MB/CK > 1.0 were diagnosed with malignancies. Notably, here we found that the CK-MB/CK ratio in patients with malignancies was significantly higher than that of non-malignancy patients. And the patients with secondary neoplasms or metastases had significantly higher serum CK-MB activity, total CK activity, and CK-MB/CK ratio than those with primary neoplasms. Furthermore, the CK-MB/CK ratio was significantly higher in patients with multiple metastases than in those with solitary site metastasis. This finding was not previously described, but a previous study reported similar conclusions, where the CK-MB/CK ratio was markedly higher in advanced-stage malignancy and liver metastasis [15]. For the first time, we evaluated the diagnostic efficiency of the CK-MB/CK ratio for malignancies and metastatic malignancies by performing the ROC curve analyses. The AUCs of the CK-MB/CK ratio for patients with malignancies and were 0.570 (95% CI: 0.537–0.604) and 0.668 (95% CI: 0.611 to 0.725), respectively. These results demonstrated that CK-MB/CK ratio is more effective in discriminating between metastases and primary tumors than in screening malignancies, which indicates its potential role as a screening biomarker for

Table 5
The serum CK-MB activity, total CK activity, and CK-MB/CK ratio of patients in various malignancies.

Malignancy types	CK-MB activity (IU/L)[a]	Total CK activity (IU/L)[a]	CK-MB/CK ratio[b]
Hematological malignancies	24 (18–35)	20 (14–27)	1.24 (1.10–1.39)
Lung cancer	83 (40–172)	63 (33–120)	1.27 (1.12–1.44)
Hepatobiliary malignancies	63 (35–102)	50 (29–86)	1.20 (1.11–1.37)
Gastric carcinoma	97 (58–170)	75 (52–129)	1.27 (1.07–1.49)
Colorectal carcinoma	116 (63–181)	72 (39–126)	1.50 (1.28–1.63)****
Rectal carcinoma	98 (64–208)	81 (55–153)	1.29 (1.12–1.48)
Breast carcinoma	111 (72–217)	87 (59–161)	1.34 (1.08–1.62)
Gynecologic cancer	38 (22–56)	34 (19–49)	1.16 (1.05–1.34)
Esophageal carcinoma	69 (29–98)	52 (26–91)	1.18 (1.02–1.30)
Pancreatic carcinoma	41 (20–89)	30 (17–59)	1.25 (1.16–1.53)
Urologic neoplasms	98 (38–127)	79 (28–97)	1.28 (1.25–1.37)
Prostatic carcinoma	141 (71–1386)	99 (54–709)	1.52 (1.24–1.84)*
Other neoplasms	57 (28–93)	53 (24–72)	1.26 (1.12–1.44)

*$P < 0.05$, ****$P < 0.0001$.
[a] The data of serum CK-MB, total CK values and the CK-MB/CK ratio were presented as median (IQR).
[b] The individual CK-MB/CK ratio of patients with different types of neoplasms was compared with that of the whole malignancies group.

Table 6
The serum CK-MB activity, total CK activity and CK-MB/CK ratio of patients with primary malignancies or metastases.

Characteristic	CK-MB activity (IU/L)	Total CK activity (IU/L)	CK-MB/CK ratio
Primary or metastasis			
Primary neoplasms	62 (34–100)	51 (28–81)	1.20 (1.10–1.38)
Metastases	116 (63–223) ****	90 (54–152) ****	1.36 (1.19–1.60) ****
Metastatic lesions			
Solitary metastasis	100 (58–176)	75 (50–123)	1.31 (1.16–1.50)
Multiple metastases	127 (64–237)	95 (54–165)	1.41 (1.20–1.63)*

The data of serum CK-MB, total CK values and the CK-MB/CK ratio were presented as median (IQR). IU/L, international units/liter. *$P < 0.05$, ****$P < 0.0001$.

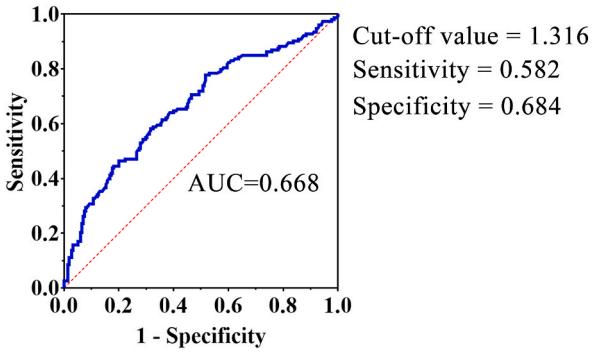

Fig. 4. The diagnostic performance of CK-MB/CK ratio in metastatic malignancies, shown by ROC curve. ROC curves were constructed by plotting the sensitivity and 1 - specificity for the confirmed metastatic malignancies patients group (n = 153) and the primary tumors patients with group (n = 215).

metastases. For patients with an abnormally elevated CK-MB/CK ratio, especially up to 1.3 or even more, but without myocardial injury, it is importance for clinicians and laboratory physicians to pay early attention to the possible occurrence of neoplastic diseases or metastases and conduct rational treatment.

As mentioned earlier, the presence of variant CK isoenzymes, such as CK-BB, mitochondrial CK and macro-CK, can cause falsely elevated CK-MB levels in certain pathological conditions. Numerous studies have suggested that the presence of these CK isoenzymes has prognostic implications in non-cardiologic disorders, including malignancy. Several reports have shown that serum CK-BB levels are elevated in patients with various types of cancer, including giant cell tumors of bone, prostatic cancer, hepatocellular carcinoma, breast cancer, prostate cancer, lung cancer, as well as colorectal cancer [4,16–20]. Furthermore, elevated CK-BB levels are correlated with tumor metastasis. For example, breast cancer patients with high CK-BB have a higher risk of relapse or death than those with low CK-BB levels [21]. Additionally, it has been reported that colorectal cancer cells that highly express CK-BB promote malignant metastasis by enhancing extracellular phosphocreatine levels from exogenous precursors [22]. Another CK isoenzyme, mitochondrial

CK, is central to cellular energetics and has also been found to be overexpressed in several tumors, associated with poor prognosis [5]. A previous study demonstrated that increased serum mitochondrial CK activity, which is correlated with the stage of liver fibrosis and hepatocellular damage, is an independent risk factor for hepatocarcinogenesis in patients with chronic hepatitis C [23]. Additionally, recent research has shown that high expression of mitochondrial CK1 in non-small cell lung cancer (NSCLC) tissues is significantly associated with poor progression of NSCLC [24].

Macro-CK, an atypical CK enzyme complex, can be a neglected cause of falsely increased serum CK-MB activity during routine laboratory assays as it is indistinguishable from normal CK or CK isoenzymes [25]. Two types of macro-CK have been identified in human serum. Macro-CK type 1 is constituted by the combination of CK-BB and immunoglobulin through antigen-antibody reactions. While early studies have found large variations in the incidence of macro-CK type 1 among different disease types [26,27], later investigations indicated that macro-CK type 1 was commonly present in patients who develop cardiovascular or autoimmune processes [28,29]. Macro-CK type 2, a complex composed of mitochondrial-derived CK polymers, was reported to be closely related to malignant proliferation or severe liver damage, such as cirrhosis, and was regarded as a tumor biomarker [29,30]. Previous studies have reported the presence of macro-CK type 2 in patients with prostatic carcinoma, and a recent study has described its occurrence in a prostate cancer patient with hepatic metastasis [31,32].

Cancer is often considered a disease of aging, with incidence increasing dramatically with age [33]. It has been reported that cancer is the leading cause of death for men and women aged 60–79 years, with a slightly higher incidence in men than in women [34]. In our study, we found that patients with malignancies were mainly concentrated in the 60–79 years group, followed by the 40–59 age group. Infants, children, adolescents (aged 0–19 years) were more likely to suffer from non-malignancy diseases, especially infectious diseases. Previous study reported that due to the immature formation of the blood-brain barrier in infants and young children, viruses are more likely to cross the barrier and invade brain tissue, resulting in CK-BB release and a false increase in CK-MB activity [35]. The pediatric reference ranges for serum CK-MB activity are unavailable in the literature. However, it was reported that, reference intervals for the mass of CK-MB are far higher in neonates and infants up to three months old (0–4.8 μg/L). And the reference ranges for this biomarkers decrease rapidly with increasing age (0–1.9 μg/L during the age of 3–6 months, and 0–1.7 μg/L during the age of 7 months–18 years) [36]. Therefore, we speculated that the false increase in serum CK-MB activity in enrolled children in this study might be also caused by the presence of high levels of serum CK-BB or macro-CK in physiological or pathological conditions.

About ten million people are diagnosed with cancer each year worldwide, and it is one of the most complex and aggressive diseases with a high relapse rate and metastasis rate, leading to significant morbidity and mortality [37]. Therefore, there is a need for effective, precise, and easily available biomarkers for early diagnosis and cancer risk evaluation. However, the widespread use of novel tumor markers is often limited in clinical laboratories, especially in the developing area, due to their high cost and requirement for advanced detection platforms. The CK-MB/CK ratio, calculated from serum CK-MB and total CK activity data, is a readily available indicator in clinical laboratories for screening primary malignancies and metastases. In this study, high CK-MB/CK ratios were observed in both patients with primary malignancies and metastases, particularly those with multiple site metastases. However, the underlying connection between the falsely elevated CK-MB activity and metastatic neoplasms remains unclear. Further investigation into the role of variant CK isoenzymes in the pathogenesis and the progression of cancer could help guide risk assessment and treatment strategies.

5. Limitations

The present study demonstrated the diagnostic value of serum CK-MB/CK ratio for screening primary malignancies and metastasis. However, several limitations also existed in this study. Firstly, it is a retrospective study that only enrolled data from one clinical laboratory. Secondly, although patients with non-malignancy diseases were analyzed separately, the conditions of malignancy patients with other comorbidities were not taken into consideration in this study. However, it is important to note that some comorbidities commonly experienced by cancer patients, such as pulmonary infection, myocardial injury, or severe shock syndrome, may also lead to a significant elevation of CK-MB activity. Furthermore, the serum CK-MB activity was measured by the immunosuppression method in this study, which could not distinguish the variant CK isoenzymes that could result in the false elevation of CK-MB activity. Therefore, the underlying factor giving rise to the significantly false increase of CK-MB activity in the metastatic neoplasms patients compare to primary malignancy patients is still unknown. Further studies should combine the CK isoenzyme electrophoresis and CK-MB mass measurement to distinguish the CK isoenzyme pattern and conform the actual CK-MB level in the sera of malignancies patients with various metastatic lesions.

6. Conclusions

This present retrospective study provides a comprehensive analysis of the biological and clinical characteristics of patients with a CK-MB/CK ratio greater than 1.0. It was found that patients with malignancies had statistically higher serum CK-MB/CK ratios than those with non-malignant diseases. Particularly, patients with metastatic neoplasms, especially those with multiple site metastases, had markedly elevated CK-MB/CK ratios. These findings establish the potential diagnostic value of serum CK-MB/CK ratio for screening primary malignancies and metastasis. In summary, the serum CK-MB/CK ratio can be utilized as a readily accessible supplement diagnostic biomarker in screening for primary and metastatic cancers.

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Contributorship

LY researched the literature, analyzed and interpreted the data, and wrote the first draft of the manuscript. CY interpreted the data in collaboration with LY and revised the manuscript. SB, LJ, HR, FS, ZF and CX were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. WY and ZX conceived and designed the project, supervised the study and contributed to critically revising the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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