New Estimation Method of Total Creatine Phosphokinase Release in Early Stage in Acute Myocardial Infarction

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Abstract In this paper, we introduce a new estimation method for total creatine phosphokinase release based on a physiological model of the serum creatine phosphokinase (CPK) activity change that can be used to estimate the total CPK release accurately, as early as possible, without frequent blood sampling. The physiological model and a new calculation method were applied to the serum CPK activity change of patients who suffered acute myocardial infarction (AMI). According to the results, the physiological model showed good agreement with the serum CPK activity change and the estimated value of the total CPK release also agreed well with that calculated using the conventional technique with clinically acceptable accuracy.

Keywords CPK Activity Change, Acute Myocardial Infarction, Physiological Model, Logistic Function

1. Introduction

Acute myocardial infarction (AMI) is a common occurrence worldwide. Clinically, it is not only important to diagnose AMI, but to also rapidly and accurately estimate the size of the infarction, one of the factors used to predict the prognosis of AMI patients. Indeed, it is critical to determine the cardiac infarct size as early as possible [1] because size correlates closely with mortality and prognostic indexes such as cardiac failure, arrhythmias, and ventricular function.

Clinical tests for biochemical markers are used to estimate myocardial infarct size in AMI patients[2,3]. Of the various tests that are employed, creatine phosphokinase (CPK) is the most conventionally used biomarker. Analysis of change in CPK activity is important for estimating myocardial infarct size. CPK is present in muscle cells such as smooth muscle cells and skeletal muscle cells, including cardiac muscle cells [4]. When these cardiac muscle or smooth muscle cells are injured, CPK is released from the muscle cells into the serum after a delay, resulting in a transient serum CPK activity elevation. Afterwards, serum CPK activity decreases at an exponential rate [5]. The total CPK release from cardiac muscle reflects the volume of cardiac muscle that suffers cardiac infarction at AMI. Total CPK release has correlated closely with infarct size in animal studies [5]. Hence, total CPK release is currently used as the standard biomarker for determining the size of myocardial infarcts. If the total CPK release from the cardiac muscle into serum can be calculated, the myocardial infarct size can be estimated. However, there is a large individual variation in the serum CPK activity change, because not only the total CPK release, but also the rate of CPK appearance from the cardiac muscle and the rate of disappearance of serum CPK activity, depend on the patient [6]. Therefore, it is important to calculate the total CPK released based on the change in serum CPK activity when estimating infarct size.

Notwithstanding, other biomarkers, such as serum troponin, have recently been used to estimate infarct size [7]. However, in the clinic, the correlation of infarct size with troponin levels is weak. In addition, a direct relationship between these new biomarkers and infarct size has not been demonstrated by the few related experimental studies to date. Serum troponin levels increase from 10 hours after the onset of AMI and are maintained at high concentrations after 7 to 10 days. Recently, attempts have been made to estimate myocardial infarct size via the measurement of troponin at a single time-point. However, data obtained four days after AMI onset cannot be used to estimate infarct size early [8]. Measurement of myoglobin is also problematic as serum levels of myoglobin fluctuate too rapidly. Thus, if patients do not present at the hospital immediately after the onset of AMI, the peak myoglobin concentration will have passed, rendering it difficult to accurately estimate the infarct size [3]. Based on these characteristics of other biomarkers, CPK is still standard and reliable biomarker to estimate infarct size.

Accordingly, many previous trials have modeled the change in serum CPK activity. Shell et al. measured the serum CPK activity at constant intervals, applied a model of the serum CPK activity change and estimated the total CPK...
release accurately, on the assumption that there is no CPK release in a serum CPK activity decrease region [5]. Regarding the serum CPK activity change, there are many models expressing the physiological change as an analytical function. For example, a model expressed by the difference in two exponential functions [9], a model using a gamma function [10], a model using a Log-normal function which is a normal distribution curve in the logarithm axis [11,12] and a model using an optimized function [13]. For these models, the evaluated results of which model function is the most expressive reached different conclusions [12,13]. Moreover, there are other models expressing the CPK appearance change as an analytical function, and obtaining the serum CPK activity using the solution of a differential equation [14,15]. In recent research, the myocardial infarct size is quantified using such a serum CPK activity change model because these models were partially evaluated clinically [16]. Infarct sizes can also be estimated using artificial neural network algorithms[17]. However, these analytical functions do not show the physiological phenomena, and also the error with the measured value is relatively large.

The total CPK release can be calculated by using the serum CPK activity change based on these analytical models using the individual parameter of the disappearance rate of serum CPK activity [6,18]. However, because blood sampling at constant intervals (every 2 ~ 4 hours for 5 days) is necessary, using this method imposes a heavy burden not only on the patient, but also on the medical institution.

We have already introduced a physiological model of the serum CPK activity change expressed by an analytical function that was a product of an exponential and a sigmoid function[19]. By using this function, an analytical solution of the serum CPK activity change could be obtained, and total CPK release was easily obtained from the numerical integration. The results led to the following conclusions: the change in serum CPK activity can be expressed using a physiological model and it is possible to estimate the total CPK release with clinically sufficient accuracy deduced from the physiological model.

In this model, although constant-interval measurements of serum CPK activity are not necessary to determine the model parameter of serum CPK activity change, it is clear that the burden on the patient and medical institution will not be reduced because frequent blood sampling cannot be avoided. As mentioned previously, it is optimal to estimate the infarct size in patients with AMI as early as possible in a clinical setting[1]. Therefore, a method of estimating the total CPK release earlier without frequent blood sampling is needed.

In this study, we introduce a new calculation method using a physiological model of the serum CPK activity change that can be used to estimate the total CPK release accurately, as early as possible, without frequent blood sampling. This new calculation method was applied to the serum CPK activity change of patients who suffered AMI, and the physiological model was evaluated by comparing the model value and measured values. In addition, the total CPK release was calculated using the new method and the difference in the value obtained with that estimated using a conventional technique was evaluated.

2. Physiological Model

2.1. Serum CPK Activity Change in Cases of AMI

When cells such as cardiac muscle and smooth muscle cells suffer ischemia for over 30 minutes, irreversible damage to the cell membrane begins, and intracellular enzymes are released to extracellular regions (interstitial fluid) because selective membrane functions are destroyed. The blood flow to the cardiac muscle cells is obstructed due to blockade of the coronary artery when cardiac infarction occurs (hereinafter called onset), and CPK that is present in the cardiac muscle cells is released to the interstitial fluid. However, the serum CPK activity hardly increases at this time because about 4 hours are necessary for CPK appearance in the coronary artery from the interstitial fluid, since the blood flow to the cardiac muscle cells has stopped at this time. Afterwards, the serum CPK activity hardly increases at this time because about 4 hours are necessary for CPK appearance in the coronary artery from the interstitial fluid, since the blood flow to the cardiac muscle cells has stopped at this time. Afterwards, the serum CPK activity shows a transitory high value because the blood flow of the coronary artery is restarted by reperfusion treatment, and then the serum CPK activity decreases exponentially [6]. It is necessary to consider the appearance of CPK from muscles other than the cardiac muscle cells, and to include the CPK appearance from non-cardiac muscle cells as the baseline in the model because in vivo serum CPK activity decreases not mono-exponentially, but double-exponentially [18].

Figure 1 shows the schematic flow of CPK transfer. This figure also includes the baseline of CPK appearance from non-cardiac muscle cells.

![Figure 1. Schematic flow of CPK transfer](image)
According to the consideration in this study, it was assumed that the CPK appearance from non-cardiac muscle cells was a constant baseline value, and the model was corrected for this baseline effect. The model assumes that the change in serum CPK activity $E(t)$ can be expressed using the change in the CPK appearance function $f(t)$ and disappearance rate of serum CPK activity $k_d$ using the following differential equation \[ \frac{dE(t)}{dt} = f(t) + B - k_d E(t) \] (1)

where $B$ is the constant baseline of CPK appearance from non-cardiac muscle cells, and $b=B/k_d$ is the steady-state value of the serum CPK activity derived from the baseline of CPK appearance. Consequently, the CPK release from cardiac muscle cells can be calculated according to the change in serum CPK activity from the presumed CPK disappearance rate, using the following transformed differential equation.

\[ f(t) = \frac{dE(t)}{dt} + k_d (E(t) - b) \] (2)

Clinically, continuous measurement of serum CPK activity: $E(t)$ is impossible. Therefore, the CPK appearance function: $f(t)$ must be derived from the difference formula of (2).

### 2.2. CPK Release Model Using a Sigmoid Function

With respect to the release of CPK from the myocardial interstitial fluid to the serum, an expression using a sigmoid function seems to be appropriate, since it is regarded as a change resulting from diffusion phenomena. Therefore, the following sigmoid function that begins from 0, and converges to 1 at infinity after the runoff rate is gradually increased, is used.

\[ s(t) = \frac{1 - e^{-Kt}}{1 + e^{-K(t-T)}} \] (3)

where $T$ denotes the time delay and $K$ denotes the largest slope of the curve, because a differential function of sigmoid function $s'(t)$ has the maximum value: $K(1 + e^{-K})/4$ at $t=T$. The inverse of $K$ also expresses the time constant which rises from 0 to 1.

The general solution of differential equation (2) on serum CPK activity $E(t)$ becomes an exponential function. Hence, serum CPK activity change is expressed by an analytical function including the product of an exponential function and the sigmoid function as follows:

\[ E(t) = Ae^{-k_d(t-T)} \left( \frac{1 - e^{-Kt}}{1 + e^{-K(t-T)}} \right) + b \] (4)

Consequently, this function of serum CPK activity change has both characteristics of the sigmoid function and exponential function that attenuate in time constant $k_d$.

By using this function (4) substitution to (2), an analytical solution of the CPK appearance function $f(t)$ can be obtained as follows:

\[ f(t) = Ae^{-k_d(t-T)} \left( K \left( \frac{1 + e^{Kt}}{1 + e^{-K(t-T)}} \right) \right) \] (5)

This function becomes a single-peaked double sigmoid curve which resembles the normal distribution and which has a peak value at $t=T$ in the case of $K>>k_d$.

Figure 2 shows the model function of serum CPK activity and CPK appearance. The parameters $A$, $T$ and $K$ mainly express the condition of the CPK appearance function $f(t)$, and the parameters $k_d$ and $b$ mostly express the condition of the serum CPK activity change $E(t)$.

![Figure 2. Model function of serum CPK activity: $E(t)$ and CPK appearance: $f(t)$.](image)

### 2.3. Estimation of Total CPK Release

#### 2.3.1. Conventional Method

The total CPK release can be calculated by integrating the $f(t)$ change without obtaining an analytical solution of $f(t)$ as follows.

\[ \int f(t) \, dt = E(t) + k_d \int (E(t) - b) \, dt \] (6)

In order to perform this numerical integration accurately, it is necessary to measure serum CPK activity at a fixed and frequent time interval. For example, it might be necessary to perform the measurement at least every 4 hours; preferably 2 hours during the 2 days after onset and every 4 hours during the 5 days after onset.

In contrast, using the analytical function of CPK release $f(t)$, the total CPK release can obtain a sufficiently accurate value by doing a numerical integration of (6).

#### 2.3.2. New calculation method

The integral of (6) can be expressed in the next simple approximation, when $K>>k_d$ is assumed.

\[ \int f(t) \, dt \approx A \] (7)

Integral approximation $A$ is estimated using $E_{p}$: peak value of $E(t)$, $t_p$: the time of peak value of $E(t)$, $T$: the time of peak value of $f(t)$, $k_d$ and $K$ as follows:
When trying to calculate \( A \) as early as possible, it is difficult to estimate the accurate value of \( k_d \) of individual patients because the insufficient data has been obtained at the time the peak value is obtained. Therefore, we used a fixed value of \( \sum \bar{f} \) instead of the \( k_d \) of individual patient to estimate \( A \).

The inverse of parameter \( K \) expresses the time constant of a rising sigmoid curve. Therefore, \( K \) is approximated in \( \alpha T \) with the constant value \( \alpha \).

Finally, the estimated value of \( T \) can be determined because \( f(t) \) has a peak value at \( t=T \) in the case of \( K>>k_d \). However, frequent CPK measurements are necessary to determine the peak value of \( f(t) \) accurately, and it is difficult to apply in clinical cases. Therefore, time \( t_h \) at half maximum of the peak value of \( E(t) \) should be used as a substitution value of \( T \).

Under these conditions, (8) can be expressed in the next approximation formula.

\[
\int f(t) dt = E_m e^{\sum (t_m-(1-\alpha) t_h)}
\]  

In this equation, the parameters \( E_m, t_m \) and \( t_h \) can be determined if the serum CPK activity \( E(t) \) reaches the peak value and the parameters \( k_d \) and \( \alpha \) are fixed values. Thus, the total CPK release can be estimated earlier.

### 3. Method

#### 3.1. Method of Clinical Measurements

##### 3.1.1. Patients

To analyze the serial changes in the level of CPK from the onset of the infarct, we studied 18 consecutive patients who were admitted within 12 hours of the onset of their first anterior-wall AMI and underwent reperfusion therapy involving coronary angioplasty with or without subsequent coronary stenting. Our procedures were in compliance with the rules of the Helsinki Declaration, informed consent was obtained, and the study was approved by our institutional ethics committee for human research [20]. The patients enrolled in this study were 14 men and 4 women ranging in age from 51 to 81 years (mean ± SD, 66 ± 9 years). Reperfusion without flow delay was successfully established in all 18 patients. Consequently, this study did not include any patients with failed reperfusion. The interval from the onset of AMI to hospital admission ranged from 1.5 to 12 hours (mean 3.6 hours). Electrocardiographic or enzymatic evidence of reocclusion was not observed in any patient we studied. The other details regarding the patients’ conditions were described in a previous report [21].

##### 3.1.2. Measurement of CPK

Blood samples for the CPK assay were obtained immediately on each patient’s admission and every 4 hours thereafter for 48 hours, and then once a day. Serum CPK activity was measured with a modification of the Rosalski method [22].

#### 3.2. Method of Calculation

##### 3.2.1. Conventional Method

The serum CPK activity change was estimated using the mono-exponential curve at 10–20 hours after onset, and the CPK disappearance rate, \( k_d \), was estimated using the least-squares method of the model. The region which did not agree with the exponential curve was excluded after an adequate time for each patient. The total CPK release was obtained from the numerical summation of (6).

##### 3.2.2. Calculation Method Using a Physiological Model

The model parameter of serum CPK activity change was determined by using the function of (4), using the least-squares method of the measured value and the model. In most patients, the parameter was obtained using all measured values. However, some measured values were excluded because there were some irregular values from cases very soon after onset or reocclusion 2 or 3 days after the reperfusion therapy.

The total CPK releases were obtained from the numerical integration of (6) with the analytical function (5).

##### 3.2.3. Estimation of Total CPK Release Using the New Calculation Method

The total CPK releases were obtained from the approximation formula shown in (9) with the averaged value of \( k_d \) from the physiological model parameter. The value \( \alpha \) was determined so that the average of the estimate error may become 0.

#### 3.3. Statistical Analysis

The relationship between the measured value and model value of peak value \( E_m \) and peak time \( t_m \), and the relationship between peak time of model function \( T \) and estimated value \( t_h \) from the measured value were analyzed by the least-squares method. The \( r \) values were derived from Pearson’s correlation coefficient. Values of \( p<0.01 \) were considered significant. The comparison of the total CPK release between the conventional method and the peak value of serum CPK activity calculated value by the physiological model using numerical integration and calculated value by the new estimation method, respectively, were analyzed by the same method.

The calculation of relative error \( =\{(\text{Reference value})-(\text{Measured value})\}/(\text{Reference value})\) was expressed as mean ± SD.

### 4. Results
4.1. The Evaluation of the CPK Release Model

Examples of serum CPK activity, CPK release and cumulative CPK release are shown in Fig. 3. The symbols show the measured values and estimated values obtained using (6). The curves show the values calculated using the physiological model. The convergence value of the cumulative CPK release becomes the value of total CPK release. In Fig. 4, examples of the time courses for other patients, slow CPK release in Fig 4A and delay reperfusion in Fig 4B, are shown.

Figure 3. Symbol of serum CPK activity, CPK release and cumulative CPK release which are estimated by the equation of Shell.[5] Gray line, bold line and dashed-dotted line show serum CPK activity, CPK release and cumulative CPK release calculated by the physiological model.

A Example of slow CPK release.

B Example of the delay reperfusion.

Figure 4. The variety in CPK release. The symbols and the curves the same as Fig. 3.

Figure 5. Relationship between measured value and model value of A: peak value \( E_m \), B: peak time \( t_m \), and C: relationship between peak time of model function \( f(t) \): \( T \) and estimated value \( t_h \) from measured value.
The results of the comparison proved the following, (i) the constructed physiological model can approximate the serum CPK activity change well, (ii) the total CPK release calculated from the model of the serum CPK activity change agreed well with the measured value.

4.2. Evaluation of the Measured Value of $E_m$, $T_m$ and $T_h$

Figure 5 shows the relationship between the measured value and model value of Fig5A peak value $E_m$, Fig5B peak time $t_m$ and Fig5C the relationship between peak time $T$ of model function $f(t)$ and the estimated value $t_h$ from measured value.

The results were as follows:

i. Measured peak value $E_m$ correlates with the model peak value of $E_m$ significantly, although the measured value is smaller than the model peak value at about 8%. Therefore, the measured maximum value $E_m$ every 4 hours can be used as substitution of the model peak value.

ii. Although the measured value has a margin of error, the measured peak time $t_m$ correlates with the model peak time of $t_m$ significantly. Therefore, the measured maximum time $t_m$ can be used as a substitution of the model peak time.

iii. Measured half peak time of $f(t)$: $t_h$ correlates with the calculated peak time of $f(t)$: $T$ significantly, although the measured value is smaller than the model value at about 13%. Therefore, the measured half peak time of $t_h$ can be used as a substitution of the model peak time $T$.

According to these results, we tried to estimate the total CPK release using the measured value of $E_m$, $t_m$ and $t_h$.

4.3. Evaluation of the New Calculation Method for Total CPK Release

Figure 6 shows the comparison of the total CPK release between the conventional method and 6A: the peak value of serum CPK activity $E(t)$, 6B: calculated value by the physiological model using numerical integration and 6C: the calculated value by the new estimation method. The upper sides of the figures show comparison results of the reference value and the value compared with, while the lower sides show relative error calculated by $\{(\text{Reference value}) - (\text{Measured value})\}/(\text{Reference value})$ (%).

As shown in Fig 6A, when the peak value of serum CPK activity was used as a substitution of the value of total CPK release, the peak value of serum CPK activity was underestimated by around 25%. On the other hand, when the calculated value by the physiological model using numerical integration was used, the total CPK release was estimated with good accuracy and the relative error was within 2.6% of SD. Furthermore, when the calculated value by the new estimation method was used, the total CPK release could be estimated, and the relative error (around SD: 6.4%) was larger than using numerical integration (SD: 2.6%). For the calculation with the new estimation method, 0.0452 (1/h) was used as the fixed value of $k_d$ and 0.167 was used as the $\alpha$ value for when the average of the relative error becomes 0.
The results proved the following:

i. Using the new estimation method, it is possible to estimate the total CPK release early during the serum CPK activity taking the maximum value with sufficient clinical accuracy.

ii. Using numerical integration of model function, the calculation value of total CPK release can be obtained more accurately, when the blood sampling is increased for sufficient time.

5. Discussion

5.1. Evaluation of the Serum CPK Activity Change Model

In past research, many models expressed the CPK change as an analytical function, especially serum CPK activity change or CPK appearance form cardiac cells. However, such analytical functions do not show the real bio-phenomena in vivo, physiologically. On the other hand, our physiological model of the serum CPK activity change can be described by an exponential function derived by a general solution of a differential equation naturally and expressed by a sigmoid function which describes evanescent CPK appearance from cardiac cells in AMI reperfusion treatment. This result coincides with previous research which mentions that the serum CPK activity functionally changes as a sigmoid curve [23]. Furthermore, from the preliminary calculation, the model estimation error of our physiological model was smaller than other analytical models. Under these considerations, the constructed physiological model can express the serum CPK activity change better than other analytical models, and the total CPK release calculated from the model of the serum CPK activity change agreed well with the conventional calculation method.

5.2. Meaning of the Model Parameters of Serum CPK Activity Change and CPK Measurement Frequency

Figure 2 also shows the meaning of the model parameters of the function of serum CPK activity and CPK appearance.

The parameter $T$ expresses the time delay from onset to maximum CPK appearance, which coincides with the time from onset to reperfusion treatment clinically. Good reperfusion treatment recovers the blood flow effectively, thus CPK appears from the interstitial fluid of myocardial cells in a short time. As a result, the maximum value of $f(t)$ increases and parameter $K$ which correlates with the duration of $f(t)$ rising, decreases. Consequently, the effectiveness of reperfusion treatment can presumably form the value of $T$ or $K$. Here, CPK appeared from the interstitial fluid in serum in around 6 to 12 hours when the blood flow was well recovered. By contrast, CPK appeared around 20 hours in the case shown in Fig 4A. This indicates that there was a problem in the recovery of coronary blood
flow by reperfusion treatment in this case. In addition, it seems sufficient to measure serum CPK activity about 6 times, or at most 8 times, because total CPK release could be estimated by using the value of $E_{\text{max}}$ and $t_{\text{m}}$ which were measured in every 4 hours.

The condition of the serum CPK activity change $E(t)$ mostly expressed the parameters $k_d$ and $b$, which depend on the individual patient. However, the serum CPK activity in this region showed a mild monotonic decrease without reperfusion of coronary blood flow. It is effective to measure the CPK activity every day for 4 or 5 days for the accurate parameter estimation and estimation of total CPK release.

5.3. Estimation of Total CPK Release

The results show the constructed physiological model can approximate the serum CPK activity change well and it is possible to estimate the total CPK release early with sufficient accuracy clinically using the new estimation method.

In this model, constant-interval measurements of serum CPK activity are not necessary to determine the model parameter of serum CPK activity change, and it is possible to estimate the total CPK release using the model parameter value. Therefore, it can be expected that the burden on the patient and medical institution will be reduced because blood sampling during the night can be avoided and wide variations in blood sampling time intervals are acceptable.

To estimate the total CPK release as early as possible with sufficient clinical accuracy, it is enough to measure serum CPK activity with a constant interval from onset up to taking the maximum value. In the present study, measurements were done mostly 4 or 5 times (94%). It is a clinically significant outcome that the total CPK release can be estimated even if few measurements of serum CPK activity are made.

Additionally, in order to improve the estimation accuracy of total CPK release, it is possible to determine the individual parameters $k_d$ and $b$ with continuous daily measurement. For this improvement in accuracy, we only need 4 or 5 blood samplings and the enzymatic estimated volume of AMI can be obtained with good accuracy.

In the other enzymes released from cardiac cells in AMI like CPK-Mb, it is possible to use same method shown in this paper because these enzymes have the same physiological characteristics as CPK [24,25].

However, when the disappearance rate of the serum enzymatic activity is rapid, it is anticipated that the estimation error of the method will increase, since the $K>>k_d$ assumption cannot stand. Moreover, when there is considerable individual variability in the disappearance rate, the difference between the average disappearance rate and individual disappearance rate increases. Therefore, it is necessary to evaluate the estimation error as in this study for each enzyme.

Although the number of subjects enrolled in this study is small, the accuracy of the infarct size estimation is very high. Furthermore, since the constructed model is a physiological model, we believe that the conclusion is sound despite the limited number of subjects. With the clinical application of this model in the future, we may be able to obtain more accurate diagnostic values for the parameter of total CPK release.

6. Conclusion

A new calculation method for total creatine phosphokinase release improved from a physiological model of the serum creatine phosphokinase (CPK) activity change was constructed and model parameters were determined from the measured values of serum CPK activity in vivo.

The results led to the following conclusions:

i. Total CPK release can be estimated with clinically sufficient accuracy as early as possible without frequent blood sampling using the new calculation method.

ii. For this estimation of total CPK release, it is sufficient only to measure the serum CPK activity every 4 hours during the activity changes from the increase to the decrease.

iii. It is possible to estimate the total CPK release more accurately using the numerical integration of the physiological model with daily blood sampling for 4 or 5 days.

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