Enzymatic Estimation of Infarct Size
Thrombolysis Induced Its Demise:
Will It Now Rekindle Its Renaissance?

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Infarct size was central to the major thrust of
cardiac research in the 1970s and as such
occupied center stage as the culprit to be
conquered by interventions designed to cardioprotect
and limit myocardial damage (infarct size). Myocar-
dial infarction, with its consequent necrosis, was the
leading cause of death and came under intense attack
with infarct size posing as the surrogate villain.
Investigators were unified in their attack on infarct
size, namely, that its course must be charted, the
extent of damage quantified, and ultimately therapy
designed for its elimination. Studies demonstrated
that the evolution of myocardial infarction is a
dynamic process and that its rate of evolution, as well
as the ultimate extent of damage, could be favorably
modified.1,2 Quantitative histologic estimates of
infarct size were regarded as the only gold standard
but obviously were not possible in patients surviving
acute myocardial infarction. Attempts to quantitate
infarct size attracted many investigators from multi-
ple disciplines using a variety of techniques. Enzym-
ic estimates of infarct size, derived from esti-
mates of the total creatine kinase (CK) released into
plasma, correlated closely with histologic estimates of
infarct size in the animal.3-5 Development of a
quantitative assay for CK-MB6 paved the way for
estimation of infarct size in patients with complica-
tions such as cardiac failure and shock,6 eliminating
the potential for contamination from CK-MM
released from tissues other than the heart. The
accuracy of the technique was ultimately confirmed
in humans in a multicentered, randomized study in
which quantitative histologic estimates of infarct size
were performed on the hearts of patients who died
and were compared with enzymatic estimates that
had been performed by an independent laboratory.7
The results were analyzed by an independent statis-
tical core, and the two estimates exhibited a very close
correlation (r=0.89), which confirmed the results of
other such studies. Estimates of infarct size based on

CK-MB, performed by a variety of investigators
throughout the world, were shown to correlate closely
with prognostic indices such as mortality,8 cardiac
failure,9 arrhythmias,10 and ventricular function.11
Enzymatic estimates of infarct size were used rou-
tinely in clinical trials to assess a variety of interven-
tions designed to salvage ischemic myocardium.

An enzymatic estimate of infarct size is only valid
as a surrogate end point if the intervention to be
assessed does not in itself alter the parameters on
which the estimation is based.12 Because only a
proportion of the CK activity depleted from the
myocardium as a result of infarction is recovered in
the plasma, a critical parameter of enzymatic esti-
mates is the ratio of CK released into the plasma to
that depleted from the myocardium. In studies by
Vatner et al,13 early reperfusion was associated with
enzymatic estimates of infarct size that were larger
than histologic estimates, suggesting increased wash-
out of CK, namely, an alteration in the ratio. Because
the change in the ratio might vary as the time from
onset to reperfusion varies, the technique did not
appear appropriate to assess reperfusion. In recog-
nition of this perturbation by thrombolytic therap-
y, most clinical studies assessing thrombolysis have used
mortality or ventricular function as end points rather
than infarct size. This brings us to the article in this
issue of Circulation by Hermens et al,14 which, if
correct, has the potential to resurrect enzymatic estimates
as valid estimates of infarct size during reper-
fusion. A brief review of the method used to calculate
CK release in appreciating the claims of the present
investigators over that of preceding investigators may
be helpful. Coronary occlusion, followed by sacrifice of
the animal at 48-72 hours, showed the CK activity
depleted from the myocardium correlated closely and
consistently with histologic estimates of infarct size.
However, to estimate myocardial CK depletion in vivo
from plasma CK activity required the estimation of
several other parameters.12 The change in plasma CK
enzyme (E) activity for any interval of time (dt) depends
on the rate of release of CK into the plasma
f(t) and its rate of disappearance (k_d) during that same
interval, which may be formulated as follows:

\[ \frac{dE}{dt} = f(t) + k_d E. \]
The $k_a$ was determined from the rate of clearance of bolus injections of the purified CK in animals and from the downslope of the plasma CK curve in humans and found to be constant. The rate of change of enzyme activity ($dE/dt$) in the plasma is determined simply by measuring serially the plasma CK activity, which in humans was found to be sufficient if assessed every 4–6 hours. $dE/dt$ and $k_a$ are known and $f(t)$ can be found; these known factors combined with the total time over which CK is released will allow the calculation of the total amount of CK released into the plasma.

To calculate the total CK released, however, the volume in which it is distributed must be known. If plasma (5% of body weight) is used as the volume, the amount presumed to be released would be much less than if 10% of the body weight were used as the volume, despite no change in our directly measured parameters, namely, $dE/dt$, $f(t)$, and $k_a$, or the derived value for infarct size. On comparing CK released into the plasma with that depleted from the myocardium measured directly after sacrificing the animal, it was found that by using plasma volume, only about 15% of the CK could be accounted for in the plasma, whereas by using two-compartment analysis, about 30% of the CK depleted from the myocardium could be accounted for. In converting to grams of myocardium destroyed (infarct size), the release ratio was taken into account and, as indicated earlier, values obtained (CK-gram-equivalents) correlated closely with histologic estimates in animals and in humans. This also indicated that the release ratio was relatively constant, at least in the situation of sustained coronary occlusion. The disparity between CK released into the circulation and CK and depleted from the myocardium was assumed in part to be due to proteolysis of the enzyme in the ischemic or necrotic myocardium. Quantitative estimation of the volume of the extravascular compartment must be based on certain mathematical assumptions that are virtually impossible to verify precisely. For example, it must be assumed that all of the CK leaving the vascular space returns without any loss, which is unlikely. In humans, with the onset of thrombolytic therapy and early reperfusion, it was observed that maximal plasma CK levels were obtained much earlier; it was assumed to be due to more rapid washout and, thus, a greater amount of CK had escaped proteolysis. Until a new formulation could be found and properly validated, enzymatic estimation of infarct size to assess thrombolytic therapy appeared elusive.

In the present study by Hermens et al., myocardial infarction was induced in the dog ($n=12$) by sustained coronary occlusion. The animal was killed, and myocardial CK and hydroxybuterate dehydrogenase (HBD) depletion was calculated and compared with the amount of CK and HBD released into the circulation. The remainder of this editorial comment will concentrate on CK enzyme activity because, in humans, HBD remains elevated in the plasma for 10–14 days, is less specific than CK-MB for myocardium, and has not been used routinely in humans. To the amazement of most of us, the authors conclude that all of the CK enzyme activity missing from the myocardium, as a result of the infarction, can be accounted for in the circulation. This is in marked contrast to the 30% accountable by the previous formulation; but, most importantly, if all of the CK is released into the circulation, it would sanctify the use of enzymatic estimates as an end point for assessing thrombolytic therapy. If all of the CK is released, despite sustained coronary occlusion, it would simply be released faster with early reperfusion. It could not come at a more opportune time because mortality is around 5% in patients with myocardial infarction who received thrombolytic therapy, making death a very insensitive end point, particularly because future studies will be comparing the efficacy of one thrombolytic agent with another rather than with a placebo group. What have the investigators discovered or innovated? Have they verified their estimations? Can the formulation now be accepted and the business of evaluating therapeutic efficacy of thrombolytic agent on infarct size in small clinical trials be a priority?

The investigators have made no fundamental changes in the original formulation—just minor modifications that they claim are valid and have a major quantitative effect. These changes are as follows: 1) myocardial CK depletion was calculated in the area of infarction as opposed to the whole ventricle, 2) the myocardium underwent homogenization and sonification to extract myocardial CK, 3) myocardial CK activity was expressed per gram dry weight rather than per gram wet weight and 4) the elimination rate for CK determined by the present investigators is 0.51/hour, which is almost twice that used by previous investigators. In determining myocardial CK activity, biopsies are obtained in the normal posterior wall from which is calculated the CK activity per gram of normal myocardium, and this multiplied by the weight of the total ventricle determines the CK activity expected to be present. The whole ventricle is then homogenized and the CK activity measured directly, and the difference between it and that expected represents CK depletion.

The investigators claim in their hands that the myocardial CK activity per gram throughout the myocardium varies by 6–7%. This is very similar to the variation consistently observed in several laboratories over the years. The investigators, however, conclude that this is marked variation and, without giving reason, conclude that it is likely to be greater after infarction, which surely will be in the area of necrosis but that, of course, is what is attempting to be quantified. There is no reason why activity in the normal myocardium is going to vary any more than it would under normal circumstances. The investigators, however, conclude that because of this variation, they measured the CK depleted from the area of myocardium that encompasses the infarction rather than measuring the CK activity of the
whole heart; they believed that this would decrease the potential for variation. While perhaps logical, the concept of analyzing CK data from only a portion of the heart with the hope that the scatter will be reduced does not appear to be appropriate when the real interest is in the total myocardial CK depleted. Nevertheless, this is not a major concern because the variation is minimal and its quantitative significance is probably trivial. In the original data on myocardial CK depletion, many different attempts were made, including sonification to extract the most CK possible from the damaged myocardium; sonification was not found to be helpful and, thus, the present claim would need to be confirmed. It seems unlikely that up to 20% more CK remaining in the myocardium may be accounted for. They also claim that only small aliquots must be homogenized.

The investigator should be congratulated on expressing the CK per dry weight because it does provide a reliable denominator that is the same for normal, ischemic, and necrotic tissue and really eliminates the error due to dilution from swelling. One would not expect the swelling to introduce such a large error (20%), but in the approach taken by the investigators of analyzing only the damaged area, the swelling assumes a much larger percentage. In previous investigations in which the whole infarct was analyzed and the infarction was of average size, swelling probably accounted for only 3–5%. Even with massive infarction (20–40% of the ventricle), based on the dilution factor in the present study it would probably be in the range of only 8–10%. Nevertheless, reducing the common denominator to dry weight attenuates the error of any variability in the dilution factor.

The fourth modification is major and clearly the one that accounts for the major disparity between the results of this and previous studies. In our initial studies, the average CK disappearance rate (k_d) derived in the dog was that of 0.28/hr; using plasma volume as the CK distribution space (5% of body weight), about 15% of the CK depleted from the myocardium in the circulation could be accounted for. However, if one used a two-compartment analysis with a second extravascular space, about 30% of CK could be recovered. We later showed that the two-compartment analysis was probably the more appropriate method to analyze the clearance of the CK; however, because it was difficult to quantify the extravascular compartment and the estimates of infarct size did not change, it became of only relative importance and, thus, was more of theoretical than practical significance. Hermes et al claimed the clearance of CK (FCR), taking into account the CK disappearance rate k_d and the total distribution space, would account for about 70% of the CK in the circulation as opposed to 15% or 20%: “If the values of k_d used in some studies, i.e., k_d=0.09 h⁻¹ and k_d=0.11h⁻¹ are replaced by the value of FCRCK= 0.36h⁻¹, recoveries of about 20%, as found in these studies, are changed to about 70%, i.e., close to the value found in the present study without correction for edema (Table 2).” The elimination of CK, which includes the metabolic turnover and the extravascular value that the investigators claim accounts for up to 70% of the difference between their value for CK release and that of previous investigators, are, of course, theoretical values and more difficult to prove. In this regard, I think the theoretical value is arbitrary and some independent proof is required.

The question at this time then is whether the experimental data provided by these studies claiming that all of the CK depleted from the myocardium can be accounted for in the circulation is correct. If indeed this is correct, estimates of infarct size by the formulation used over the years or by the present modifications will be the same because the elimination rate and the release ratio are constant. The previous serious concerns,13,19 namely, only 15–30% of the CK is recovered and so any variation would markedly affect the estimates, would be unfounded because recovery is 100%. To some extent, the investigators have perhaps enlightened us on what has intuitively been for me bothersome, namely, if the released ratio is only 15–30%, why have the estimates been so reliable over the years for prognosis and as an end point for a variety of interventions. What about thrombolytic therapy: Is the same amount of CK simply released more rapidly? I would categorically state that we can not accept enzymatic estimates of infarct size to assess thrombolytic therapy until it has been confirmed in the experimental setting, which, in my opinion, is a major shortcoming of this study. The major factor accounting for the lost CK in this study by Hermens et al is the elimination rate. This is a theoretical value that can be properly validated only by reperfusion. Experiments are clearly needed to show that early reperfusion will not change the total CK recovered in the plasma, and it will not exceed 100% of that depleted from the myocardium. Since the original formulation by Sheh et al, many modifications have been added to improve this technique; clearly, this would be part of the finale to establish its reliability and make it more applicable in the more recent clinical setting of thrombolysis. Hermens and his colleagues have halted the disappearance of enzymatic estimation of infarct size into antiquity and in fact are likely to initiate a much needed renaissance just as mortality with thrombolytic therapy has decreased 5–7%. The need for a technique that will quantify the extent of myocardial damage with adequate sensitivity and specificity could not be more welcome. Thus, one may well ask, did the initial premature demise of enzymatic estimates of infarct size by thrombolytic therapy become the impetus to make it the end point to assess thrombolytic therapy? Infarct size may, thus, again move to center stage, interestingly enough in part because the villain (death) for which infarct size was the surrogate, has been partially conquered (at least statistically), and this time infarct size could become clinically and biologically the true center player.
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References


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