

## EDITORIAL COMMENT

# Another Unanswerable Question\*



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There are many questions that challenge us in society. Is there a God? Why do we need numbers? What does time mean? How biomarkers are released from myocardial cells is one of those issues.

For years, there has been the suggestion that proteins can be extruded from myocardial cells in the absence of cell death. This goes back to rat experiments in which lactate dehydrogenase (LD) was thought to be released in response to exercise (1). LD is a large protein, but because cardiac necrosis was not observed, it was suggested that LD was released in the absence of cell death. Similar reports occurred when increases in creatine kinase (CK) activity were observed after brief periods of ischemia thought to be insufficient to induce cardiomyocyte death (2). As biomarkers became more involved in diagnostics, this became an even more important issue. It is clear in skeletal muscle that severe exertion causes death to skeletal myocytes. It is thought that the cells are replaced by pluripotent stem cells that reside in the muscle, which leads to reparative processes (3). However, this was for years an uncomfortable idea for cardiomyocytes because it was not clear that there were pluripotent cells in the heart capable of repairing or replacing cardiomyocytes. Thus, if cells were dying, there was concern that we would run out of cells. For that

reason, a variety of alternative mechanisms to explain biomarker release in normal individuals and in patients with very brief episodes of ischemia were developed, including normal turnover of myocardial cells, apoptosis, cellular release of cardiac troponin (cTn) degradation products, increased cellular wall permeability, and formation and release from membranous blebs (4). The data to support these hypotheses, however, with the exception of apoptosis, are not robust, and cTnI and cTnT are larger proteins (23.5 kDa and 33.5 kDa for intact chains, respectively). In fact, in an experimental study, Ishikawa et al. (5) used release of mitochondrial CK to support the concept that cell death occurred whenever CK was released. We now know that reparative cells do exist in the heart so that the concept that cardiomyocytes may be dying is a less daunting thought (6). In fact, the field of regenerative medicine is an attempt to harness these reparative processes to repair cardiac damage. Nonetheless, the controversy continues.

As it became clear that all individuals had some level of cTn measurable in their blood, the discomfort in regard to why this might occur again surfaced (4). Because troponins are specific for the heart, it could not be suggested, as with other biomarkers, that the signal came from tissue other than the heart. In addition, most individuals do not manifest cardiac disease. If so, why should they have cTn in their blood? Recently, higher sensitivity cardiac troponin assays have begun to unmask new physiology, making this an issue again. It is known, for example, that even normal individuals increase circulating cTn values in response to dobutamine (7), with rapid atrial pacing (8), or during stress testing (9). Even more potentially concerning are marathon runners or athletes who with extreme exertion elaborate substantial quantities of troponin with values above the 99th percentile upper reference limit with a rising and/or falling pattern: the sine qua non of acute myocardial infarction (4). Does this occur because the

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cells die, or are there some other mechanisms that cause these elevations? At present, the possibility that cell death could be the reason why we all have small amounts of troponin in our blood and why they increase with physiological stimuli is at least a possibility.

This issue has been tackled in the past by the group from Buffalo in experimental studies showing that short-term increases in pre-load proteolyse and release troponin in the absence of myocardial ischemia (10). In the isolated perfused heart, release appears to be due to apoptotic cell death as part of a calpain-mediated process. This may be an important mechanism for troponin release in patients with heart failure and those who are on dialysis, where short-term changes in stretch are relatively common.

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However, whether this mechanism can explain any of the increases in patients who have coronary disease, or the physiological increases discussed in the preceding text, has been unclear. Now, in a new investigation published in *JACC: Basic to Translational Science* from the same group, Canty et al. (11) have explored the issue of brief periods of cardiac ischemia in a pig model. The mid-left anterior descending coronary artery was occluded for 10 min, which was thought to be an insult that would not cause myocardial necrosis. This was done in order to investigate whether or not troponin release was present. Using an assay for porcine troponin, these investigators documented substantial increases in response to 10 min of occlusion and subsequent reperfusion over a period of 24 h. Troponin increases were not immediate as one might think would be the case if there was an early releasable pool that could be mobilized in response to nonlethal injury. This pool was originally called the “cytosolic” pool, which is a misnomer since it is not clear that there is immunohistochemical localization of this pool in the cytosol. Nonetheless, it does appear that this pool is more loosely bound, and it is thought that initial troponin release probably occurs from this pool. However, whether or not cardiomyocytes have died in association with this release is unclear. In this experiment, the investigators interrogated the tissue looking for a mechanism for the release. As expected, no necrosis was found histologically or with triphenyltetrazolium chloride staining. Instead, apoptotic cell death documented by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining and increases in caspase activation. Although these processes are hard to quantitate, this appeared to be a predominate mechanism. The

findings cannot exclude the possibility that other mechanisms also participated in the troponin elevations, but those were not documented. There has been speculation that if one could characterize the troponin fragments in more detail that this understanding might be facilitated (12), but this concept is simply an idea at present.

Multiple questions abound as they do from novel research reports. For now, it appears that the increases in cTn seen with brief periods of ischemia are indicative of cell death due to apoptosis. Is this because of acute myocardial stretch that occurred in the left anterior descending coronary artery territory in this model? It would be of interest to know whether or not inhibition of calpain-mediated stretch would mitigate these effects. If so, this might lead to important ideas about therapeutic interventions. A stretch-mediated mechanism for troponin release could also be relevant to individuals with heart failure either directly due to increases in pre-load or due to subendocardial ischemia. In heart failure patients, increases in cTn appear related to left ventricular (LV) end-diastolic pressure and thus subendocardial wall stress with an attendant reduction in subendocardial perfusion and increased oxygen demand fed by increased LV distension (13). These mechanisms may have similar relevance be they due to stretch or ischemia in patients with end-stage renal disease.

Are they also the reasons why normal subjects have measurable values for cTn, why physiological increases can occur, and why even marked increases may not lead to an adverse prognosis (4)? Perhaps in response to the physiological stressors of daily life, we all lose small numbers of myocytes due to apoptosis. Those with comorbidities such as LV hypertrophy, diabetes, or coronary artery disease, which are known to increase cTn values (14) may accentuate the process. If indeed small levels of troponin that are released in part due to what may be normal physiology in response to rapid atrial pacing, exercise, and dobutamine are due to apoptotic cell death, it may well be that one could, over time, develop protective mechanisms to obviate that. On the other hand, because it is unclear that such elevations have adverse clinical significance, perhaps any disease that occurs in these individuals is due to inadequate reparative processes or repetitive insults or to an underlying nonphysiological process. As with normal skeletal muscle, perhaps these minor elevations are rapidly dealt with in the absence of continuing pathology. However, it may well be that certain individuals, for example, those who have an abnormal substrate such as a propensity to

arrhythmogenic right ventricular dysplasia or those with coronary artery disease and repetitive insults, may evoke abnormal reparative processes that explain disease progression in those diseases.

These questions are likely to persist because the experimental designs to answer all of these questions are problematic, and indeed, although this study shows that the elevations associated with brief ischemic in this model are due to apoptotic cell death, other mechanisms could still exist. After all, absence

of proof is not proof of absence. Accordingly, we will hear about this issue again and again and again. It may well be one of those imponderables that our children years from now will also say is another one of those issues that will vex us forever.

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