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EDITORIAL COMMENT

Pathobiology of Troponin Elevations*

Do Elevations Occur With Myocardial Ischemia as Well as Necrosis?

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Cardiac troponins are components of the contractual apparatus in cardiac myocytes and are expressed exclusively in the heart. A number of nonischemic conditions including myocarditis, pulmonary embolism, acute and chronic heart failure, and sepsis may be associated with elevated troponin levels (1,2), although they may include supply–demand imbalance and thus at least some element of ischemia. Elevation of troponins with these conditions is associated with worse prognosis than the prognosis for patients without troponin elevations, and the prognosis is usually worse than that for patients with troponin elevation with acute coronary syndromes (ACS) (3).

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There has been a mantra that elevation of troponin levels, no matter what the etiology, is always caused by myocyte necrosis (4). This is supported by histological staining for troponin in animal studies showing troponin T and I release from cells was only associated with necrotic tissue (5). Yet, we know that there are clinical situations where release of troponins may occur without necrosis.

The introduction of more sensitive assays for troponins means improved analytical sensitivity but decreased specificity (6,7). Low, but detectable, troponin levels are measurable in normal people (8,9) with significant biological variability (10).

Early Releasable Troponin Pool

Approximately 5% to 8% of troponin I and T is unbound in the cytosol (11) or as part of an early releasable pool (12). This unbound pool of troponin is released first, regardless of the cause of the type of myocyte injury (Table 1). It would be expected that if there is release from this pool that the troponin would be released quickly and that blood levels would fall with rapid washout. The half-life of troponin T and troponin I in the blood is about 2 h (13). Rapid rise and fall within 24 h may therefore be consistent with release of this pool and reversible myocyte damage rather than myocyte necrosis where a time-dependent fall over a longer period (4 to 10 days) would be expected because of gradual degradation of myofibrils and release of the troponin complex (11). The prolonged half-life seen in ACS may be due to continued breakdown of the contractile proteins.

A number of studies have suggested that troponins may be released from cardiac myocytes in situations other than myocyte necrosis without the cell membranes being necrotic. It is not known whether this process is active or passive. It is very common clinically for patients to present with supraventricular tachycardia without either ischemic symptoms or electrocardiographic changes and to have elevated troponin levels. These levels usually rise and fall rapidly, and coronary angiography may be normal (14). Pulmonary embolism may be another setting where there is a troponin release profile that may indicate reversible transmembrane release of troponin from the early releasable pool (15), in addition to some myocyte necrosis.

Troponin Release With Ischemia

In this issue of the *Journal* the study by Turer et al. (16), evaluated 19 patients who underwent diagnostic cardiac catheterization and rapid atrial pacing for 60 to 180 min with measurement of myocardial lactate production and highly sensitive cardiac troponin T (hs-cTnT) from the coronary sinus and the peripheral blood. The assay for troponin T fulfills the international biochemical requirements for a 10% coefficient of variation (CV) below the 99th percentile of a reference population (2,4) and supports the classification of this assay as guideline acceptable (17). The experimental design has been used before and is valid; although it is notable that measurement of lactate elution is relatively insensitive for ischemia. The trial appears to have been well conducted. However, there are several findings and methodological aspects that merit critical attention.

The rationale as to why the lower limit of hs-cTnT was set to 1.5 ng/l and not to 3.0 ng/l (limit of blank) is not clear. This would have increased the chance of finding a significant concentration difference between the patients with coronary artery disease (CAD) and no lactate production, those with CAD and lactate production, and those without CAD and no lactate production. Many hs-cTnT values were below the limit of blank, and most values before and after rapid atrial pacing were below the 99th percentile (13 ng/l).

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Table 1 Pathobiological Classification of Types of Potential Mechanisms Causing Troponin Elevations

Type 1	Myocyte necrosis
Type 2	Apoptosis
Type 3	Normal myocyte turnover
Type 4	Cellular release of proteolytic troponin degradation products
Type 5	Increased cellular wall permeability
Type 6	Formation and release of membranous blebs

hs-cTnT concentrations in coronary sinus blood increased after pacing and tended to be higher in patients with lactate production and CAD. However, levels also increased in patients without lactate production and without CAD, and the absolute changes were similar to those with lactate production and CAD.

Lactate and the transcoronary lactate extraction was measured but is not fully presented in the results. The only statement is that transcoronary lactate extraction was 0.36 mg/dl at rest and 0.25 mg/dl at peak stress with a negative value indicating myocardial ischemia. It would have been nice to see a display of the association between troponin rise and transcoronary lactate extraction with regression analysis to support their conclusion.

The peak value of hs-cTnT did not exceed the upper limit of normal (≥14 pg/ml) in any of the patients who did not develop ischemia on an electrocardiogram or produce lactate. This finding is reassuring in respect to the proposed cut points (≥14 ng/l) and delta change of 50% for the diagnosis of myocardial infarction (7).

The conclusion of the authors that the rise in hs-cTnT levels is due to myocardial ischemia is not consistently supported by the study findings. The authors found a troponin rise even in patients without CAD and without a lactate gradient, which supports the hypothesis that tachycardia alone may account for troponin release. Troponin release may be mediated by tachycardia stimulating stretch-responsive integrins, which are mechanotransducer molecules that link the extracellular matrix to the intracellular cytoskeleton (18). Also, there remains the possibility that 60 min of pacing produced myocyte necrosis of a small number of cells without lactate production as measured in this study. Therefore, the conclusion that ischemia alone caused the increase in hs-cTnT levels is not fully substantiated by the study findings.

In patients undergoing stress testing with nuclear imaging, low levels of troponin elevations were detected with the ultrasensitive Singulex TnI assay (Singulex, Alameda, California), which uses single-molecule detection technology, suggesting the possibility that transient provoked ischemia can produce troponin release in the absence of necrosis (19). The increases in troponin levels were associated with the presence and severity of nuclear perfusion defects. These data are different from a previous study where hs-cTnT did not increase after brief exercise or pharmacologically induced myocardial ischemia (20). Using a novel nanoparticle troponin I assay, 82% of patients presenting with unstable angina, with a negative contemporary troponin I assay, had an elevated nanoparticle troponin I level indicating that myocardial injury was detectable in most patients with ischemia and again raises the possibility that troponin may be released during ischemia without necrosis occurring (21). These studies, however, cannot distinguish between small areas of myocyte necrosis, which may be better detected with more precise assays, versus reversible myocyte injury with troponin release from the early releasable pool through a viable intact cellular mechanism. The precise mechanism of this process is not defined.

Potential Mechanisms of Troponin Release

There are 6 potential major pathobiological mechanisms for troponin elevations (Table 1).

Myocyte necrosis. Myocyte necrosis is the most common mechanism, and under this rubric are ischemic, inflammatory, infiltrative, direct trauma, and toxic causes including sepsis (1).

Apoptosis. A second cause is apoptosis, or programmed cell death. Apoptosis with preserved membrane integrity is associated with activation of caspases that mediate the cleavage of structural proteins that may lead to the release of troponin (22).

Normal myocyte cell turnover. A third type is normal myocyte cell turnover. Study of the integration of carbon-14 into the DNA of myocardial cells, generated by nuclear bomb testing, has shown that cardiac myocytes regenerate (23). There is a decrease from 1% annual turnover at the age of 25 to 0.45% at the age of 75 years with approximately 50% of cells exchanged during a normal life span. Whether such low-grade turnover results in release of troponin to the systemic circulation is unknown.

Cellular release of proteolytic troponin degradation products. Another potential cause of troponin release (type 4), without death of the cell and cellular membrane disruption, is the cellular release of proteolytic troponin degradation products (24,25). Thus, proteolysis to create small fragments could allow these to pass through a cellular membrane with normal membrane integrity. Only 15 min of mild ischemia has been shown to cause development of troponin I degradation products (26).

Increased cellular wall permeability. A fifth type is increased permeability of the cell membrane without cell necrosis. Reversible injury to the cardiac myocyte membrane allowing permeability of troponins from the cytosol may occur due to myocardial stretch, or ischemia. Simulation of stretch-responsive integrins has been shown to result in release of intact troponin from cultured viable cardiomyocytes (18) without an increase in lactate production inferring that release may occur without ischemia or necrosis. Also, in a rat model, increasing pre-load has been shown to be associated with release of troponin I, independent of ischemia (25).

Formation and release of membranous blebs. Active secretion of vesicles (blebs) or membrane expression with shedding has been hypothesized to be a mechanism to enable troponin to be released from cardiac cells. This

mechanism has been described in liver cells, where large molecules can pass from intracellular to extracellular spaces without necrosis occurring, by the formation of membranous blebs during ischemia. These may be released into the circulation without rupture of the plasma membrane. Cultured cardiac myocytes have been shown to develop blebs during anoxia and to release cytosolic enzymes without undergoing necrosis (27), but there is little evidence supporting this concept in man.

There are also likely to be unknown causes of troponin elevations. It is not known as to why sepsis causes the release of troponin from cardiac myocytes, although heat shock proteins and tumor necrosis factor have been implicated (28). It is thought that increased troponin levels with renal failure are not related to decreased renal excretion (28), but rather to toxic products, and supply and demand issues probably also play a role. It is also possible that there are low-grade reparative processes compensating for myocyte loss due to various causes.

If we accept that troponin release occurred with rapid pacing in the current study as a result of ischemia rather than necrosis, then the question arises as to what process caused the release. Whether troponins are released only with necrosis or with ischemia alone, perhaps through a stretch mechanism, should not prevent us using the higher sensitivity troponin assays, which fulfill the international recommendations for performance (17) and have been shown to be related to worse prognosis (29). The challenge is how to best incorporate higher sensitivity troponins into clinical practice.

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