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VIEWPOINT

Ingenuous Interpretation of Elevated Blood Levels of Macromolecular Markers of Myocardial Injury: A Recipe for Confusion

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Several assumptions about elevations of macromolecular markers of myocardial injury in blood require critical consideration. The dichotomy of modest, persistent elevations of troponins I and T as prognostic factors in patients with unstable angina and absent elevations of isoenzymes of creatine kinase is presently unexplained. Factors influencing the appearance of macromolecular markers of myocardial injury in blood are considered, including the need to estimate baseline values, to consider elevations as deviations from baseline rather than simply points within a distribution of baseline values in normal subjects, to recognize operative biochemical and physiologic determinants of marker release from injured myocytes and washout and to take into account the influence of apoptosis. Elucidation and consideration of mechanisms underlying the appearance of specific macromolecular markers in blood appear likely to improve diagnosis and explain the prognostic power of the troponins in patients with unstable angina. Detection of proteolytic breakdown products of troponins in blood is likely to explain the modest, persistent elevations seen in some patients with unstable angina and their prognostic implications. (J Am Coll Cardiol 2000;35:1355–8) © 2000 by the American College of Cardiology

Karmen (then a medical student), Wroblewski and LaDue (1) revolutionized cardiology when they showed that elevated activities in blood of enzymes from necrotic myocardium establish the diagnosis of acute myocardial infarction (AMI). Almost 50 years later, diverse macromolecular markers are commonplace cornerstones of diagnosis. However, subtle quantitative considerations remain inadequately appreciated, and conundrums persist. One is the prognostic power of increased concentrations in blood of the thin-filament cardiac regulatory proteins troponins I and T (TnI and TnT) in patients with unstable angina in predicting subsequent coronary events (2,3). Conventional wisdom does not adequately explain their proven predictive power, and interpretations are clouded by a reflex to resort to comparing macromolecular markers in terms of which is "best."

In addition to diagnostic difficulties resulting from occasional laboratory errors and inadequate timing or flawed acquisition of blood samples, contributions to blood levels of macromolecular markers from unrecognized noncardiac sources can compromise interpretation. Several fallacies may also obscure accurate diagnosis.

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DISCUSSION

Fallacies reflecting ignorance of baseline values. The results of assays of transaminases, lactate dehydrogenase (LDH), creatine kinase (CK), isoenzymes of these enzymes, and TnI and TnT are generally reported as normal or abnormal with respect to a range indicative of the distribution of values in a normal population. However, a five-fold increase in activity in blood of the myocardial band (MB) isoenzyme of CK (from 1 to 5 IU/L in a hypothetical instance) is more indicative of AMI than is recognition of an "abnormal" value of 9 IU/L in a subject whose baseline value is 9, even though 9 IU/L may be present under physiologic conditions in only a small number of subjects. What is being inferred is the amount of enzyme released from the heart that results in a given increase in activity in blood (4). The inference cannot be made without knowledge of or assumption of a baseline value in a given individual subject. Hence the need for diagnosis based on serial determinations and estimation of a time-activity curve. Myocardial infarction (MI) cannot be excluded by a single "normal" value nor established by a single "abnormal, elevated" value.

Fallacies reflecting unrecognized determinants of release ratios and clearance of specific macromolecular markers. Factors determining the rate of appearance in blood of a macromolecular marker released from myocardium include molecular weight, charge, binding to intra- and extracellular

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Abbreviations and Acronyms

AMI	= acute myocardial infarction
CK	= creatine kinase
HBD	= hydroxybutyric acid dehydrogenase
LDH	= lactate dehydrogenase
MB	= myocardial band
MI	= myocardial infarction
TnC	= troponin C
TnI	= troponin I
TnT	= troponin T

components, degradation intracellularly and in the lymph and interstitial fluid as well as in the blood itself, and clearance via the kidney, liver or reticuloendothelial system (5). Even if the intramyocardial concentrations of two macromolecular markers are identical, time to occurrence in blood of a detectable increase over baseline, specific values attained, time to occurrence of peak values, persistence of elevations above "normal," and the entire shape of the area under the time–activity or time–concentration curve of each can differ markedly. Accurate diagnostic and prognostic interpretations require knowledge of determinants of the behavior of the macromolecular marker being utilized.

Fallacies reflecting flawed assumptions about the nature of cardiac cell death. Myocardium subjected to lethal ischemia undergoes either necrosis or apoptosis. Both occur in any given infarct (6). Macromolecules are readily released from myocytes undergoing necrosis but not from those undergoing apoptosis. As noted recently in a comprehensive review (6) "apoptosis coexists with necrosis in every AMI." Thus, time–activity and time–concentration curves in blood will depend on the proportion of myocytes within a given infarct undergoing each type of cell death. As James has pointed out "it must be reasonably assumed that these dead (apoptotic) myocytes do not release . . . intracellular enzymes" (6). This may explain the rare and confusing false negative results in patients with ultimately proven infarction as judged from electrocardiographic, echocardiographic, and angiographic data. Another potential cause of such false negative occurrences is complete lack of washout (as discussed later) due to complete occlusion of an infarct-related artery and absence of collateral flow.

Fallacies reflecting assumptions regarding the source of macromolecular markers appearing in blood. After the onset of MI the amount of CK appearing in blood is directly related to the number of myocytes that become necrotic (7,8). The amount recoverable from blood has been thought by many to be less than the total initially residing in necrotic cells because of degradation in lymph and other compartments. In an elegant study, Hermens et al. (9) have found that complete recovery of hydroxybutyric acid dehydrogenase (HBD) and CK lost from myocytes can be calculated. However, the result depends on assumed disappearance constants (derived from multicompartmental modeling) for both HBD and CK. The fact that complete recovery is calculable for both enzymes raises another problem, namely, accounting for the likely impact of red cells on accumulation of HBD but not CK in blood in a hemorrhagic infarct. In fact, the amount of HBD (also called LDH-1 and heat-stable LDH) in red blood cells in a hemorrhagic infarct often substantially exceeds the amount present in myocardial cells undergoing necrosis. Because red blood cells are rich in HBD, that is, the "myocardial" LDH isoenzyme, a hemorrhagic infarct will elaborate much more HBD, and a disproportionate amount of HBD compared with CK, than will a bland infarct. Comparable peak activities of HBD in blood do not imply comparable infarct size in the two circumstances.

Fallacies reflecting inadequate consideration of lack of washout. Given the same amount of myocardial macromolecular marker released into the extracellular fluid, the peak concentration in blood will depend on the rapidity of washout from the heart. Thus, in a patient in whom the infarct-related artery is spontaneously, pharmacologically, or mechanically recanalized, the time of occurrence of peak will be abbreviated, and the peak concentration in blood will be augmented, often markedly, compared with what would have occurred in the absence of restoration of patency. Valid prognostic inferences based on absolute values must be predicated on knowledge of the setting and determinations of macromolecular marker release in a given patient and on analysis of time-activity curves, rather than on peak values alone (10).

Potentially spurious assumptions regarding the nature of the troponin/CK dichotomy and factors underlying release of each. In the case of typical Q-wave infarction, characteristic time-activity of CK and time–concentration curves of TnI and TnT are evident. Though the two types of curves differ markedly from each other, the relationship between them is consistent (11). The diagnostic sensitivity and specificity provided by each are virtually the same. These phenomena are consistent with a commonality of determinants of release of both types of macromolecular markers into blood from myocytes undergoing cell death. However, in patients with unstable angina without infarction, CK values do not increase but TnI, TnT or both may exceed "the upper limit of normal." Furthermore, minimal elevations generally persist for hours or days. The conventional wisdom, often not rendered explicitly, is that smoldering, low-level necrosis is ongoing in such patients and that increases in TnI and TnT therefore reflect "increased diagnostic sensitivity" of the troponins compared with that of CK. Moreover, it is believed that the smoldering necrosis is a determinant of an increased likelihood of a subsequent acute coronary event. Although the prognostic implications of such elevations of TnI and TnT are unequivocal and remarkable, it is not clear why. At least several possibilities merit serious consideration.

In the context of the principles outlined earlier, it does not appear likely that the mini-elevations of troponin truly reflect smoldering necrosis secondary to ischemia. Insulted myocytes recover, succumb, or hibernate. None of these states is known to be associated with persistent "leaky" sarcolemma or persistent elaboration of macromolecules with release into blood. It is also difficult to envision any such leaking as giving rise to sufficient release of troponin to sustain modest elevations in the face of ongoing, albeit slow, clearance. Can we envision other testable hypotheses delineating mechanisms that could account for modest, sustained elevations of TnI and TnT and their prognostic import?

The assumption that "cardiac" troponins are released exclusively from cardiac myocytes could possibly be violated. In patients with unstable angina the nature of the coronary vascular disease per se may differ when the likelihood of a subsequent coronary event is high. For example, more extensive inflammation may be present, and this could account for expression of troponin in cells other than cardiac myocytes, such as vascular smooth muscle cells or cellular constituents in vessels in the heart. Slow and persistent release of the anomalously synthesized troponin could explain the persistent elevation of its concentration in blood. Assessment of putative sites of TnI and TnT elaboration by immunohistochemical and in situ hybridization techniques could illuminate this possibility.

Alternatively, clearance of troponins synthesized in myocardium and released slowly (there is some normal turnover) might be diminished in those patients with unstable angina in whom the likelihood of a subsequent coronary event is high. If so, "baseline" concentrations in blood would rise, accounting for the persistent "elevations" indicative of a poor prognosis. The assessment of renal and hepatic function as well as other factors potentially affecting clearance of TnI and TnT and the use of immunoassay to detect unrecognized degradation products could shed light on this hypothesis.

What is perhaps the most likely explanation for the modest, persistent elevations of the blood concentrations of TnI and TnT is relatively straightforward. The immunoassays used to characterize concentrations in blood of these macromolecular markers detect not only TnI and TnT, but also proteolytic degradation products of each, as well as covalently bound complexes of TnI-troponin C (TnC) and TnT-TnC (12). Indeed, in association with myocardial ischemia and infarction very little of what is identified in blood as TnI with the use of conventional immunoassay comprises free, unaltered protein (13,14).

In contrast, it appears that large amounts of unaltered TnT are depleted from the heart (15). McDonough et al. (14) recently characterized the time course of appearance in myocardium and release into the coronary effluent of TnI and its proteolytic breakdown products and covalently bound complexes in association with mild and severe ischemia followed by reperfusion in laboratory animals. Troponin T and its breakdown products were studied as well, but in less detail. Very small amounts of breakdown products of TnI are present in blood under physiologic conditions. Proteolysis products increase markedly with ischemia followed by reperfusion. With progressively more severe ischemia, the proportion of shorter chain length TnI products increases. The calcium-activated protease, calpain, may play a pivotal role in the degradation of the thin filament (14,16,17), thereby potentially mediating impairment of contractile function in stunned myocardium as well (17). It is known that the extent of proteolysis of intracellular myocardial contractile and cytoskeletal proteins increases when the structural integrity of the myofilaments is altered, and that proteolytic breakdown products may egress more rapidly from cells compared with their parent structural proteins; this is why myosin light chains appear in blood after MI. The fact that both TnI and TnT can be detected in blood in normal subjects in very small amounts is consistent with low level, physiologic egress of either these macromolecules or their proteolytic breakdown products and covalently bound complexes, or both. If high risk patients with unstable angina are also subject to repeated, subclinical bouts of ischemia and myocardial stunning with activation of calpain, loss of myofilament integrity and egress of proteolytic breakdown products may ensue. It is worthy of note that in the McDonough et al. (14) study the duration of mild ischemia sufficient to cause release of TnI degradation products was only 15 min, recognized to be an interval too brief to induce cell death.

Alternatively, if increased proteolysis of myofilament proteins reflects heightened intensity of inflammatory processes in the heart, coronary vessels, or the organism as a whole such as those seen in patients with type 2 diabetes, elaboration and leakage of breakdown products into blood might well be increased in a sustained fashion. In either case, detection of proteolytic breakdown products by conventional immunoassay may directly reflect the stability, or lack thereof, of the underlying coronary artery disease and, accordingly, the propensity for future acute coronary events.

This possibility can be tested readily by simply characterizing TnI, possibly TnT, and the relative concentrations of specific degradation products in blood samples from patients with acute myocardial infarction, unstable angina with a good prognosis, and unstable angina with an adverse subsequent outcome. The distribution of breakdown products should reflect the extent of proteolysis and the ratio of myocardium undergoing frank necrosis to myocardium subjected to repeated stunning or heightened inflammation, or both.

Regardless of the explanation ultimately established, it is appropriate to stop assuming that the powerful prognostic impact of modest and sustained increases in concentrations of troponins in blood implies that TnI, TnT, or both are more sensitive markers compared with other macromolecular markers of myocardial necrosis. In fact, persistent, modest elevations of apparent concentration of TnI and TnT in blood may reflect important underlying pathogenic

processes contributing to an adverse prognosis in patients with unstable angina that constitute attractive targets for therapeutic intervention.

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