Cardiac troponins (cTn) have high sensitivity and specificity for detecting myocardial necrosis, and have enhanced the diagnosis and management of acute coronary syndrome. The European Society of Cardiology/American College of Cardiology (ESC/ACC) committee for the redefinition of myocardial infarction (MI) recommended that those patients with an elevated serum level of cTn resulting from myocardial ischemia be diagnosed as having acute MI.1 Moreover, it has been shown that the lowest detectable levels of cTnI in blood are associated with increased morbidity and mortality.2-5 Despite its clear usefulness, some problems still exist with cTn analysis and interpretation, specifically at the lower limits of elevation.6 Several million patients seek emergency care as a result of chest pain, but only about 10% are subsequently confirmed to “have acute MI.”

STRUCTURE AND FUNCTION
Troponin complexes are distributed at approximately 400-Å intervals along actin filaments of the cardiac myofibril (Figure 1).7 Each troponin complex is composed of 1 molecule each of troponin C (cTnC), troponin I (cTnI), and troponin T (cTnT). The complex binds calcium ions, and this interaction causes the actin-myosin interaction to be altered and allows muscle contraction.
and troponin T (cTnT). Actin-myosin interactions are regulated by calcium ions binding to cTnC. The calcium binding signal is transduced by releasing the thin filament from inhibition associated with cTnI binding to actin, and by altering the interaction of cTnT with tropomyosin.

Although troponin is present in skeletal muscle, the amino acid sequences of cTnT and cTnI isoforms (but not cTnC) are unique to cardiac muscle, allowing separation of skeletal from cardiac troponins via immunologic techniques. It was thought initially that cTnT is present in small amounts in skeletal muscle during human fetal development and is reexpressed in diseases that involve skeletal muscle regeneration, such as Duchenne muscular dystrophy.

**RELEASE AND CLEARANCE OF CTN AND CONTROVERSIES REGARDING ITS USE AS A BIOMARKER FOR MYOCARDIAL NECROSIS**

Whereas most cTn is incorporated in the troponin complex, 3% to 8% is present in a free form dissolved in cytoplasm. After cardiac cell injury, free cytoplasmic cTn is immediately released, followed by a slow continuous release of the myofibril-bound proteins as a binary or ternary complex of cTnI-cTnC-cTnT. Some differences exist between the utilization of cTnI and T as biomarkers (Table 1). Effective and reliable detection of cTn requires that assays contain antibodies for both free and complexed cTn isoforms. The ratio of total to free cTn in the serum varies during the course of an MI and may differ among patients. Following release, troponins are rapidly degraded into various products. It has been thought that as the central epitopes of cTn molecule are bound to cTnC, it becomes “undetectable” to immunoassay early after release although it may be detectable 5 to 7 days after injury. Many degradation products exist; one study, using Western blot analysis, detected intact cTn and a spectrum of 11 modified products in the serum of patients after acute MI.

Clearance of cTn has not been fully explored. Although it has been suggested that minute amounts of cTn degradation products are present under physiological conditions, some investigators report that no detectable cTn should be present. The etiology and relevance of cTn detection in healthy persons remains unclear. Moreover, the ability of the current technology to differentiate background noise from minute levels present under physiological conditions has been questioned.

**DETERMINANTS OF CTN RELEASE**

Although cTn release traditionally is considered to indicate myocardial necrosis, several other pathologic conditions associated with cTn release are debated. Many studies suggest that cTnI or its fragments may act as a marker of myocardial stretch or...
strain, as occurs in heart failure or pulmonary embolism. In a study of patients with nonischemic heart failure and normal coronary arteries, cTnI correlated with levels of the left ventricular wall strain marker, B-type natriuretic peptide (BNP). Myocardial strain or excessive wall tension, with its attendant myocardial cell damage, may result in degradation of cTn with subsequent release of degradation products and antigenic components of cTn. Hamwi et al determined left ventricular mass index and cTnI levels in 74 patients referred for echocardiography. Slight elevation in cTnI was strongly associated with increased left ventricular mass in patients without clinical evidence of active MI.

Modest elevations of cTnI in patients with unstable angina without elevations of creatine kinase-MB fraction (CK-MB) is another source of controversy. Though possibly representative of superior sensitivity of cTnI, some investigators believe elevation may be due to release of free cTnI from the myocyte membrane secondary to short ischemic episodes that result in temporary increases in membrane permeability without release of the complex form of the cTnI or CK-MB. As ischemia resolves, membrane integrity is restored and cTnI release ceases.

Some investigators indicate that apoptosis may cause cTnI release, but evidence is conflicting. Apoptosis is present in many diseases including heart failure and ischemic heart disease. It also was hypothesized that cTn elevation reflects increased cTnI degradation products, an interval too brief to induce cell death and CK-MB release. McDonough et al found that 15 minutes of mild ischemia was sufficient to cause release of cTnI degradation products, an interval too brief to induce cell death and CK-MB release.

In summary, controversy exists as to whether variabilities of pathologic processes such as severe ischemia, apoptosis, increased inflammatory status, and stunned myocardium also could result in cTnI release.

**Patterns of Clearance of cTnI**

Two patterns of cTn release and clearance are recognized. The first is the classical pattern in which cTnI increases 3 to 4 hours after MI and peaks at 10 to 24 hours. Levels remain elevated for 4 to 10 days. This pattern often is associated with a rapid rise and fall in CK-MB and myoglobin levels. Duration of cTnI elevation has been reported to be shorter in non-ST vs ST-segment elevation MI. This may reflect sustained release of cTnI from necrotic myofibrils in ST-segment elevation MI rather than any real prolongation of half-life. Thus, biomarker release as well as clearance rates may be related to the extent of infarction, reperfusion, and to the extent of degradation of combined forms of cTn.

The second pattern of cTnI release has a lower peak and much shorter duration, lasting hours or days. It is hypothesized that this pattern is associated with brief ischemic episodes or reversible cardiac damage resulting in increased permeability of the myocardial membrane and leakage of cytosolic troponin without necrosis or release of the bound troponin complex.

**Analytic Factors Affecting the Validity of the Test**

Several analytic factors result in difficulties and controversies in the interpretation of elevated cTnI levels, such as differences between assays, assay imprecision, and choice of cutoff limits.

**Variation in Commercially Available cTnI Assays**

There is only one manufacturer for the cTnT assay, since it is patent protected. There have been 3 generations of this assay and a fourth generation is being tested for analytic and clinical performance. However, there are approximately 20 cTnI assay types employing different reagents, methods of calibration, and clinical performance characteristics. Manufacturers have used monoclonal antibodies raised against different epitopes of cTnI, and there is no standardization between the cTnI assays. Diagnostic assays for cTnI have resulted in up to 20-fold differences in measured values. These discrepancies may result not only from individual assay differences, but also from the release of numerous cTnI degradation products.

**Controversy Regarding the Upper Limits of Normal**

This term (see Definitions of Parameters on page 432) refers to the 97.5th percentile (mean +2 SD) or the 99th percentile (mean +3 SD) of a normal reference population. The ACC / ESC committee recommended using the 99th percentile of a reference control group. However, there is no standard in selecting the reference population. Systematic screening via physical examination for cardiovascular disease in this reference population is rare, and screening with echocardiography or stress testing is almost nonexistent. For each assay, normal values are based on different groups. Potential bias in selecting a reference population is a recognized problem and is thought to be a major factor in the discrepancy of implications of elevated serum levels of cTnI in large clinical trials vs clinical setting studies. Moreover, for some assays, the upper limit of normal is below the lower limits of assay detection. In addition, biologic variability within the same individual has not been calculated for cTnI, but is about 10% for CK-MB and myoglobin.

Recent evidence also demonstrates the existence of age-, sex-, and race-dependent differences in the 99th percentile.
percentile cTn reference values, which suggests a need for cutoff values specific to these variables.21,22

**Assay Imprecision**

Precision usually is measured by the coefficient of variability (CV) (see Definitions of Parameters on page 432). CV differs for the same reagent at any set of given values. CV is reported by manufacturers and usually obtained by analyzing samples in the same assay run. ESC/ACC guidelines recommend a CV of <10% at the 99th percentile of the reference range for the diagnosis of MI. Although it is generally believed that no cTn assay is able to achieve this precision,23,24 newer cTn assays may actually approach this sensitivity.25 Recently, Panteghini et al compared imprecision profiles for most commercially available cTn assays. No cTn assay achieved CV <10% at the 99th percentile reference limit defined by the manufacturer.46,47 Moreover, 7 assays were not able to measure cTn levels in a pool of serum that contained the lowest limits of cTn. Six assays achieved CV <10% at concentrations that were approximately twofold higher than the 99th percentile in a reference population.

CV measured by the manufacturer differs from real-world measurements, which are run on different machines, at different times, and with different reagents.48 In the Tactics/TIMI (Thrombolysis in Myocardial Infarction)-18 trial, the lowest detectable value of 0.01 µg/L was the most prognostic of increased morbidity and mortality.49 However, when other investigators utilized the same assay in routine clinical settings, they found that 8% of patients would have been assigned to another risk group due to assay imprecision.44

**Problems Regarding the Cutoff Level for Myocardial Infarction or for the Receiver Operating Characteristic Curve as Defined by the Manufacturer**

The receiver operating characteristic (ROC) curve is the value manufacturers suggest for the diagnosis of MI, and is frequently determined through ROC curve analysis. The ROC curve is based on a statistical theory using a presumed "gold" standard.50 Although the ROC curve can be an effective tool, it has flaws. The number of subjects used to plot the graph is not shown and, as the sample size decreases, the ROC plots become increasingly jagged, making decision making imprecise.51 In large clinical trials, the number of patients with acute coronary syndrome and elevated cTn levels is high. False positives are thus diluted into a much larger group of true positives, and for this reason the characteristics of the ROC curve improve. In routine clinical settings there is a more diverse group of patients, some of whom may be at low risk. Inclusion of this population may diminish the diagnostic and prognostic applicability of cutoff values.24,42-45 Some manufacturers plot the ROC curve using CK-MB as the "gold" standard, although CK-MB is less than ideal and its use minimizes the sensitivity of cTn measurements.42

**Controversy Regarding the Cutoff Level for Abnormal cTn in Individual Laboratories**

In 1999, the National Academy of Clinical Biochemists suggested 2 cutoff levels for cTn: a low abnormal value (suggesting minor myocardial injury or unstable angina) and a higher value (suggesting acute MI).44 This approach is still advocated by some investigators.46 In 2000 The ESC/ACC recommended use of the 99th percentile of a reference control group with an acceptable imprecision (CV defined as <10%) as a cutoff limit for diagnosis of MI.47 Because no assay achieves this precision, some investigators recommended using the lowest value at which a CV of 10% can be achieved.23,37,45,49,50

Some experts suggest different cutoff limits depending on the purpose of measuring cTn. If the purpose is only risk stratification of patients with cardiac disease, consideration should be given to lowering the cutoff limit below the 10% CV value. However, these low cTn cutoff levels are not appropriate for the diagnosis of acute MI in patients with chest pain and with a low prevalence of the disease.52,53 Figure 2 illustrates the possible cutoff levels that may be encountered in any assay. For some assays, the lowest level of detection may be higher than the upper limit of normal. The level of a CV of 10% may be below or above the cutoff level of MI as determined by the manufacturer. The 99th percentile and 10% CV levels may be different in clinical settings as compared with research settings.

Recently, Lin et al studied the effect of varying the cutoff limit for cTn on prevalence of MI among 1719 admissions to an urban hospital for suspected acute coronary syndrome.54 The prevalence of cTnI-positive MI patients was about 13.5% if the ROC cutoff was used, 17% if the 10% CV cutoff was used, or 25% if the 99th percentile cutoff was used. This suggests an 85% increase in the diagnosis of cTnI-positive MI between the ROC and the 99th percentile cutoff limits, with 69% of this increase from cases that are cTnI-positive/CK-MB-negative.55 Similarly, Kontos et al found that simply changing the cutoff limit for diagnosis of MI in patients admitted to an inner-city tertiary care center would double the prevalence of acute MI, increase resource utilization, and potentially increase the use of invasive procedures.56

Confusion regarding cutoff limits is not limited to the clinical setting but also affects interpretation of published studies of cardiovascular outcomes.57 Conclusions differ significantly due to the use of different cTn assays at different cutoff values.29-33 The research implications of changing to a cTn cutoff limit are significant, given the frequent use of MI as a "hard" outcome event.

**Clinical Issues Affecting Interpretation**

Clinical issues affecting interpretation of the cTn assay include false-positive and true-positive results caused by diseases other than acute MI.
Figure 2. Possible Cutoff Values for Any Individual Assay

Definitions of Parameters

Lower limit of detection or the minimal detection limit: This is the lowest level detectable that differs from 0. It is calculated as the mean ±2 SD of 20 replicates of the 0 calibrator. It is a measure of noise intrinsic in the measurement system. The lower limit of detection helps to define sensitivity, which widely varies between different assays. If the lower limit of detection is very low and the assay precision is good, the assay will be highly sensitive. The International Federation for Clinical Chemistry has developed pools for testing the sensitivity and precision of all cTn assays, allowing comparisons of sensitivity and precision between assays at the low values often used as cutoffs.1

Upper limit of normal or upper limit of reference range: These terms are used interchangeably and refer to the 97.5th percentile (mean ±2 SD) or the 99th percentile of a presumably normal reference population.

Precision: The ability of a given method to produce the same results on replicate samples. The samples are measured at least 20 times and the standard deviations of the measurements are determined. Operationally, precision is defined as the coefficient of variability.

Coefficient of variability: The standard deviation over the sample mean. It is usually multiplied by 100 and reported as a percentage.

Receiver operating characteristic (ROC) curve: A statistical tool to maximize clinical sensitivity and specificity as compared with a gold standard.6 Curves of true-negative vs false-negative results are used to select an operating point for the instrument that will provide an optimum tradeoff between false-positive and false-negative results. The ROC graph is a plot of all sensitivity/specificity pairs resulting from continuously varying the decision threshold over the entire range of results observed.

FALSE-POSITIVE RESULTS

Several reports indicate that cTnI assay components cross-react with rheumatoid factor, heterophil antibody, fibrin clots, microparticles, bilirubin, or products of hemolysis.14-16,56-62 When suspected, testing using a different type of assay may overcome this source of error. Excess fibrin is a well-documented source of falsely elevated cTnI serum levels.66 Incompletely clotted specimens, specifically in patients receiving anticoagulation, contribute to this problem.6,56,64

Interference of heterophilic antibodies with cTnI assays varies from 17% to 40%.64 Heterophilic antibodies may be present due to use of mouse monoclonal antibodies,69 exposure to microbial antigens,66,67 exposure to foreign animal proteins,68 and autoimmune diseases such as rheumatoid arthritis.69 To minimize this problem, manufacturers suggest adding a heterophilic antibody-blocking agent, although its efficacy is questioned.64 To circumvent the false-positive effect of rheumatoid factor, which can be found in 5% of healthy individuals, a rheumatoid factor-blocking agent may be added.70 Manufacturers continue to improve assays to eliminate or minimize cross-reactivity with other proteins.56

TRUE-POSITIVE RESULTS CAUSED BY DISEASES OTHER THAN ACUTE MYOCARDIAL INFARCTION

Table 2 lists common causes of serum cTn elevation occurring with conditions other than acute MI. Some causes deserve special discussion.

Minimal elevation of cTn in acute coronary syndrome: Cutoff values of <0.01 mg/L (lowest detectable level) using third-generation cTnI assays have both prognostic and diagnostic significance.7 Similar results have been reported for cTnI, where lower cutoff limits have prognostic significance.8 However, these findings may not be valid in patients at lower risk for MI, such as those undergoing emergency department (ED) evaluation who may have low peak cTn concentrations.50 Kontos et al reported that a large number of patients with low cTnI levels (peaks higher than the lowest detectable limit, but lower than the optimal diagnostic value) presenting to an ED did not have cardiac events.6 Many ED patients with chest pain have atypical presentations, whereas few have diagnostic electrocardiographic changes.10 In these patients, elevated cTnI often becomes a primary criterion used to diagnose acute MI.10 Because spurious elevations of cTn are frequently encountered,11,12 serial measurements should be utilized rather than a single measurement.10

On the other hand, a recent study of patients with suspected acute coronary syndrome but no critical epicardial coronary disease found that troponin-positive patients with or without angiographic coronary artery disease had higher C-reactive protein (CRP) and BNP levels compared with troponin-negative patients. The rate of death or reinfarction at 6 months was 3.1% in troponin-positive patients with no angiographic disease, but 0 in troponin-negative patients with no angiographic dis-
Sepsis: Several reports indicated that 31% to 80% of septic patients have elevated cTn but no evidence of significant coronary artery disease. Possible causes for these elevations include bacterial myocarditis, cytokine-mediated inflammation of the myocardium; production of myocardial depressant substances; myocardial ischemia due to elevated oxygen consumption; prolonged hypotension; or use of inotropic medications. It is unclear whether sepsis from any specific pathogen is more likely to be associated with cTn elevation.

Acute pericarditis and pulmonary embolism: Acute pericarditis may elicit troponin elevation presumably by injuring the epicardium adjacent the inflamed visceral pericardium. Right ventricular strain from a sudden increase in pulmonary artery resistance is believed to be the cause of cTn release in pulmonary embolism.

Heart failure: Heart failure is associated with elevated cTn levels and correlates with clinical and functional status. In patients with stable heart failure, cTn levels are not significantly different among patients with vs without ischemia. The mechanism of cTn release in heart failure remains unclear but may be related to myocardial strain or ongoing degradation of contractile proteins and cellular injury. Increased levels of neurohormonal stimulation, oxidative stress, and cytokines are known to promote cardiac cell death, and may also cause cTn elevation in heart failure.

Renal failure: cTnT is more commonly elevated than cTn in patients with renal failure. Elevation of cTnT serum levels occurs in 4% to 17% of patients with renal failure, especially those on hemodialysis. However, not all hemodialysis patients have elevated cTnT levels and there may be a decrease following dialysis. In one study using first-generation assays, elevated cTnT was reported in up to 71% of patients with renal failure but only in 17% when a more specific second-generation assay was used. Though elevations of cTnT in this patient population (especially asymptomatic patients) may not be related to ischemic heart disease, elevations are predictive of adverse outcome. Recently, Ditis al found cTnT fragments ranging in size from 8 to 25 kDa in an entire group of hemodialysis patients. Further, increasing cTnT concentration correlated with the duration of dialysis, which suggested an accumulation of cTnT fragments throughout the course of renal failure and dialysis.

### Table 2. Nonischemic Causes of Elevated Serum Troponin

<table>
<thead>
<tr>
<th>Myocardial Damage Secondary to Cardiac Disease</th>
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<tbody>
<tr>
<td>Acute heart failure [23,37,52]</td>
</tr>
<tr>
<td>Myocarditis [23]</td>
</tr>
<tr>
<td>Pericarditis [37,71]</td>
</tr>
<tr>
<td>Pulmonary embolism [37,74]</td>
</tr>
<tr>
<td>Severe hypotension [4,53]</td>
</tr>
<tr>
<td>Cardiac amyloidosis, sarcoidosis, and glycogen storage diseases [23,37,42,71]</td>
</tr>
<tr>
<td>Persistent tachycardia [23]</td>
</tr>
<tr>
<td>Atrial fibrillation in transplant patients [12]</td>
</tr>
<tr>
<td>Cardiotoxic drugs: chemotherapy, alcohol [23]</td>
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<tr>
<th>Myocardial Damage Secondary to Cardiac Trauma</th>
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<tbody>
<tr>
<td>Percutaneous coronary intervention and cardiac surgery [23,52]</td>
</tr>
<tr>
<td>Direct cardiac trauma and contusion [12,23]</td>
</tr>
<tr>
<td>Radiofrequency ablation [12,23]</td>
</tr>
<tr>
<td>Cardioversion and defibrillation [12,23]</td>
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<tr>
<th>Myocardial Damage Secondary to Systemic Abnormality</th>
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<tbody>
<tr>
<td>Surge in catecholamine causing myocardial damage such as in central nervous system disorder, subarachnoid hemorrhage, cocaine abuse, or pheochromocytoma [23,43]</td>
</tr>
<tr>
<td>Sepsis and septic shock [23,37,42,75]</td>
</tr>
<tr>
<td>Some insect envenomation [12]</td>
</tr>
<tr>
<td>Ultraendurance exercise (marathon, triathlon) [46,76]</td>
</tr>
<tr>
<td>Renal failure [12,51]</td>
</tr>
<tr>
<td>Vasculitis leading to coronary vasculitis [46]</td>
</tr>
</tbody>
</table>

**False Positive Due to Assay Cross-Reactivity**

- cTnI: heterophil antibodies, rheumatoid factor, fibrin clots, microparticles, bilirubin, or hemolysis, hemoglobinopathy and transfusion hemosiderosis [23,37,36,42,44]
- cTnT: Duchenne muscular dystrophy and other regenerating muscle diseases (early-generation assay)

### Analytic Problems

- Variation in commercially available assays for cTn
- Definition of the upper limit of normal
- Definition of the cutoff level for myocardial infarction or cutoff value for the ROC curve as defined by the manufacturers
- Variation in precision of assays
- Definition of the cutoff level for abnormal serum level of cTn in individual laboratories
- Uncertainty of the predictive ability of different assays

cTn = cardiac troponin; ROC = receiver operating characteristic.
CONCLUSION

Although cTn is highly sensitive and specific for myocardial necrosis and has diagnostic and prognostic applicability, myocardial necrosis does not equate to acute MI, and elevated cTn may be caused by factors other than ischemic heart disease. Clinical assessment of patients remains essential. The laboratory is an assistant to—not a replacement for—informed clinical decision making. Clinicians and investigators should be familiar with the details of assays used at their institutions, including the upper limit of normal, the lower level of detection, the cutoff for MI, and the value at which the assay meets the CV of <10%.

REFERENCES

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