Troponin measurements during drug development—considerations for monitoring and management of potential cardiotoxicity: An educational collaboration among the Cardiac Safety Research Consortium, the Duke Clinical Research Institute, and the US Food and Drug Administration

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Drug-induced cardiac toxicity is a recognized challenge in development and implementation of pharmacotherapy. Appropriate biomarkers are needed to detect these abnormalities early in development and to manage the risk of potentially cardiotoxic drugs or biologic agents. Circulating cardiac troponin (cTn) is the most widely used biomarker for detection of myocardial injury. Although most commonly used to detect myonecrosis in the setting of ischemia, cTns are also elevated with other acute and chronic disease processes, including heart failure, renal failure, sepsis, pulmonary embolic disease, and many others. High-sensitivity assays for both cTnI and cTnT are now available that achieve acceptable imprecision (coefficient of variation <10%) at the 99th percentile of a normal reference population. Even more sensitive assays are being developed that detect cTn in ranges that are near the level of normal cellular turnover (apoptosis). These properties of cTn and the continuing evolution of highly sensitive assays position cTn as a potentially uniquely informative marker for early detection of cardiac toxicity. This article summarizes collaborative discussions among key stakeholders in the Cardiac Safety Research Consortium about the use of cTn monitoring in drug development. (Am Heart J 2011;162:64-73.)

Drug-induced cardiac toxicity is a recognized challenge in development and implementation of pharmacotherapy. Cardiotoxicity may take many forms, including direct myocardial injury, arrhythmias, valvular lesions, and ischemic events, as well as secondary effects on the heart that result from blood pressure changes or neurohormonal effects. To help detect these abnormalities early in development and to manage the risk of potentially cardiotoxic drugs or biologic agents, appropriate biomarkers are needed.

Circulating cardiac troponin (cTn) is the most widely used biomarker for the detection of myocardial injury. Troponins T, I, and C are integral components of the contractile apparatus in striated muscle (cardiac and skeletal). Importantly, cardiac-specific isoforms of TnI and TnT have been leveraged to develop immunoassays that are both sensitive and highly specific for a cardiac source of Tn release. In the setting of clinical symptoms consistent with an ischemic etiology, cTn is the criterion standard for diagnosis of myocardial infarction (MI) and has been demonstrated to correlate with clinical outcome.[1-3] Cardiac troponins may also be elevated in blood specimens from patients with other acute and chronic disease processes including heart failure, renal failure, sepsis, pulmonary embolic disease, and many other medical conditions, and distinguish worse prognosis among these individuals as well.[4-14]

Because of this, the application of cTn monitoring has expanded beyond its original validation as a marker for diagnosis and prognosis in patients with suspected acute coronary syndrome to preclinical testing and to evaluation of nonischemic cardiac events. High-sensitivity assays for both cTnI and cTnT are now available that achieve acceptable imprecision (coefficient of variation [CV] ≤10%) at the 99th percentile of a normal reference population.[15,16] Ultrasensitive assays are being developed that detect cTn in ranges that are near the level of normal cellular turnover (apoptosis). These properties of cTn and the continuing evolution of highly sensitive assays position cTn as a potentially uniquely informative marker for early detection of cardiac toxicity.

Based on the Food and Drug Administration's Critical Path Initiative goals, the Cardiac Safety Research Consortium (CSRC) was developed to foster collaborations among academicians, industry participants, and regulators that address cardiac safety issues relating to development of new medicines (www.cardiac-safety.org). A CSRC subgroup was established to foster stakeholder discussion about cTn monitoring in drug development. This article summarizes discussions within the CSRC and will focus on what is known, not known, and controversial regarding the use of cTn in drug and biologic development.

Preclinical characterization of cardiac toxicity with cTn

Preclinical hazard assessment for drug- or biologic-associated myocardial injury is usually identified by histopathologic evaluation of heart samples from animal models in repeat-dose general toxicology studies. The morphology, progression, and distribution of cardiac lesions are important features that can provide information regarding pathogenesis. These studies also provide an opportunity to identify translational biomarkers (eg, serum cTn) and characterize their appropriate use for safety monitoring as a part of clinical development.[17-20] Serum cTnI and cTnT are sensitive and specific markers of active/ongoing ischemic and nonischemic cardiomyocellular injury in a variety of animal safety models, including the rat, mouse, dog, and monkey. Recognition and characterization of basal levels of cTn in target populations (animals or human) are important for interpreting whether changes in serum levels associated with drug administration represent safety signals within those populations.

Recent gains in the sensitivity of cTn assays now make it possible to measure circulating levels of troponin in otherwise healthy animals and to detect relatively small increases from baseline that may not be associated with morphologic lesions in the heart.[19-23]

In this regard, cTn testing may increase the sensitivity of safety studies in which hematoxylin and eosin staining has become routine rather than the preferred phosphotungstic acid hematoxylin stain. Interpretation of these findings is not straightforward, however. If the changes are maintained and progressive, other measurements including the use of biomarkers such as N-terminal pro–B-type natriuretic peptide and physiologic measurements of cardiac performance in conscious unrestrained electronically monitored animals can be of value. These small and often not repeatable increases may represent minimal focal cardiomyocellular injury associated with either spontaneous disease processes (eg, progressive cardiomyopathy of rodents, vasculopathy of beagles, background inflammation in wild-caught primates, etc)[24,25] or stresses of handling and confinement, although no data currently exist to confirm these latter associations. The extent of injury reported by these low-magnitude cTn increases is not expected to be associated with cardiac dysfunction. However, because increases in serum cTn "report" cardiomyocellular injury regardless of cause, it is important to put any increases seen in safety studies in the context of all of the data generated in a study to understand drug-relatedness of those increases. Thus, the decision to implement monitoring in subsequent clinical studies will depend on the overall preclinical cardiac evaluation.

Significant cTn elevations are generally considered markers of cardiomyocellular necrosis (ie, irreversible injury). Whether it is a "soluble cytoplasmic pool" or an "early release pool" of cTn within cardiomyocytes (<5%), the potential for measurable increases in serum to occur with increased membrane permeability due to reversible cell injury has also been considered (but difficult to confirm experimentally). Thus, until such data are available, the current interpretation of any significant cTn elevation is that it implies irreversible myocyte damage. Drugs that induce acute and short-duration cardiomyocellular injury (eg, isoproterenol) have been associated with transient (24-48 hours) increases in cTn with magnitudes that reflect the severity of injury.[26,27] Drugs that induce chronic and/or progressive cardiomyocellular injury are more likely to cause small but persistent increases in cTn reflecting the duration of ongoing cardiomyocyte injury and differences in pathogenesis of the lesion (eg, doxorubicin). The size of the window of opportunity for detecting increases in cTn that report active cardiomyocellular injury is often directly related to the pathogenesis and magnitude of the lesion. The magnitude and duration of the preclinical cTn elevations should be paramount in the consideration of potential risk to patients and, thus, whether to use cTn as a biomarker for monitoring potential cardiotoxicity during clinical trials.

Routine preclinical in vivo studies in rodent and nonrodent species generate robust data sets that include clinical observations, gross necropsy, clinical pathology,

and light microscopic evaluation of a broad spectrum of tissues. In addition, dedicated investigational studies that include nontraditional end points such as cardiac electrophysiology and contractility, blood pressure, and cardiac genomics may be indicated following detection of cardiac injury in preclinical species using primary study end points. Relevant information derived from preclinical evaluations includes time to onset, dose-response curve, exposure effect level, rate of progression, and reversibility of the lesions as well as associated elevations in cTn and other cardiac biomarkers (eg, B-type natriuretic peptide in dogs).

Although most extensively studied as a marker of early ischemic cardiomyocyte necrosis, preclinical and clinical experience with cTn demonstrates that it has wider utility for "reporting" cellular injury of varied pathogenesis.[28] Whereas reversibility cannot be considered a likely event at the cellular level (ie, myocardial necrosis is not reversible), the sensitivity of cTn is such that with ample monitoring, injury can be minimal (ie, clinically inconsequential) when detected. Furthermore, with understanding of pathogenesis, early recognition of injury detected by cTn monitoring may offer the opportunity to limit exposure and cardiac injury. However, such utility will be dependent upon being able to discriminate minimal events from potentially more threatening events.

Although cTn is becoming more widely used for preclinical detection of cardiotoxicity, several shortcomings exist:

1. There is no clear consensus on what preclinical signals would require cTn monitoring in the clinical setting or how these particular signals should be monitored. The totality of the data must be considered, including the apparent margin of safety to the human dose.
2. There is no direct or obvious way using current technologies (eg, cardiac imaging/functional studies) to confirm that small cTn elevations associated with microscopic injury in preclinical studies occur in human subjects.
3. Most cTn assays have been developed for human, not animal, studies. However, several current automated assays that have demonstrated adequate cross-reactivity for use in animals have been characterized by Apple et al.[19]

To summarize, cTn monitoring during clinical studies should be considered when there is a preclinical signal for drug-induced cardiomyocyte injury or the potential to exacerbate preexisting disease in target populations. There are clear caveats and unknowns in the use of cTn with respect to translating preclinical findings and their implications for clinical drug development. Clinical cTn monitoring should also be considered when there is ischemic or nonischemic preclinical cardiomyocyte injury.

As more preclinical and translational information is gathered, further validation of the utility of cTn for drug-induced cardiac toxicity will be established.

Translation of cTn to the clinical setting

Cardiac troponins I and T are both more sensitive and more specific than creatine kinase–MB for the detection of myocardial injury in the clinical setting. Current-generation cTn assays are nearly 100% cardiac specific, and sensitivity continues to increase with newer-generation assays. With this increasing sensitivity, assay precision is also improving such that newer assays can achieve ≤10% CV at the 99th percentile.

The higher sensitivity of newer cTn assays creates a paradox in clinical development. It provides opportunity for early detection of potentially progressive injury when changes in other assessment modalities may not be present (eg, echocardiography, electrocardiogram [ECG], standard microscopy); alternatively, ultrasensitive cTn assays under development may reveal elevations that represent normal variability in the target population with no clear evidence of drug-induced disease. Whereas older cTn assays revealed abnormalities when significant irreversible myocardial damage was present, newer ultrasensitive testing may reflect subclinical damage in apparently healthy patients or normal variations in the circulating cTn pool.[29] Interpretation of relatively small increases in cTnI measured by ultrasensitive assays will require collection, sharing, and interpretation of data from preclinical safety studies over several species with a range of pharmacologic agents.

With the rapid proliferation of high-sensitivity assays, some have called for scoring systems to allow comparisons of performance characteristics across assays and allow clinically useful classification of assays. In a scoring system proposed by Apple,[30] assays would be designated as guideline acceptable, clinically usable, or not acceptable based on their CV percentage at the 99th percentile (≤10%, >10 to ≤20%, >20%, respectively). Furthermore, assays would be scored based on the sensitivity of the assay according to the percentage of individuals who had a detectable level of cTn less than the 99th percentile. Understanding each cTn assay's operational characteristics is particularly important in selecting which one to use and what would be the most appropriate cutoff for monitoring potential drug-induced cardiotoxicity.[31] Ideally, given the rapid evolution in troponin assay development, creation, and maintenance of a central, "living library" of troponin assays in which this information could be archived as a reference tool for clinicians, researchers and drug developers would be an invaluable resource. In addition, knowledge of potential interferents and preanalytical and analytical characteristics of the selected assay would also be critical when interpreting the data. Examples of possible technical causes for false-positive

cTn include fibrin clot in serum of incompletely clotted specimens (coagulopathy or anticoagulant therapy), heterophile antibodies, human anti-animal antibodies, rheumatoid factor and autoantibodies (commercially available heterophile blocking reagents can be used to rule out a false positive for this reason), interference from other endogenous components in the blood such as bilirubin and hemoglobin, immune complex formation, microparticles in the specimen, high concentration of alkaline phosphatase, and analyzer malfunction.[32]

Cardiac troponins have been validated extensively for diagnosis and prognosis in ischemic heart disease. Elevated cTn greater than the 99th percentile of a normal reference population with a characteristic rise and/or fall is the criterion standard for the definition of MI in a clinical context consistent with ischemia (characteristic symptoms, electrocardiographic changes, imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities).[1] In this setting, cTn status is highly prognostically relevant[3,33]; and for several acute therapies for ischemic heart disease (low–molecular-weight heparins, glycoprotein IIb/IIIa inhibitors, early invasive strategy), cTn elevation defines a group that has enhanced benefit from treatment.[34-37] Following from this relationship, cTns are often applied for the evaluation of cardiac damage in nonischemic settings in clinical drug/biologic development as well as in clinical practice. However, despite their impressive analytical sensitivity and specificity for myocardial injury, cTn assays are not specific for the *cause* of the myocardial injury. Table I displays a listing of well-recognized clinical etiologies of elevated cTn other than acute ischemic heart disease.

This lack of etiological specificity and high assay sensitivity makes it critical to understand the circumstances under which cTn testing is being used to appropriately interpret the results of the testing, either clinically or statistically. According to the Bayes theorem, when the pretest probability that an individual has the disease of interest is low (or the prevalence of the disease of interest in the population is low), the likelihood of a positive test result reflecting the disease of interest is expected to be low; and the positive predictive value is similarly compromised. Thus, if cardiotoxicity from an agent in development is low in the population studied, the positive predictive value of a positive test result in that population may be low because of the high sensitivity but low mechanistic specificity of cTn assays. On the other hand, even without mechanistic specificity, as a specific marker of myocardial cell lesion, cTn assay abnormalities may be useful as an early marker of toxicity of a new molecular entity.

Another important question that arises is whether a test developed and validated in ischemic disease and that has been shown to have prognostic relevance in other disease states should be used for evaluation of nonischemic cardiotoxicity resulting from administration of

Table I. Nonischemic heart disease conditions associated with cTn elevation in clinical practice

Clinical condition

Trauma (contusion, ablation, pacing, ICD firings including atrial defibrillators, cardioversion, endomyocardial biopsy, cardiac surgery, after interventional closure of ASDs)
Congestive heart failure (acute and chronic)
Aortic valve disease and HOCM (with significant LVH)
Hypertension
Hypotension (often with arrhythmias)
Postoperative noncardiac surgery patients who appear uncomplicated
Renal failure
Critically ill patients (especially with diabetes, respiratory failure, gastrointestinal bleeding, sepsis)
Drug toxicity (eg, Adriamycin, 5-FU, Herceptin, snake venoms, carbon monoxide poisoning)
Hypothyroidism
Abnormalities in coronary vasomotion (including coronary vasospasm)
Apical ballooning syndrome
Inflammatory diseases (eg, myocarditis, pericarditis)
Infection (parvovirus B19, Kawasaki disease, smallpox vaccination, myocardial extension of bacterial endocarditis)
Post-PCI patients who appear to be uncomplicated
Pulmonary embolism
Severe pulmonary hypertension
Sepsis
Burns (especially if TBSA >30%)
Infiltrative diseases (amyloidosis, hemochromatosis, sarcoidosis, and scleroderma)
Acute neurological disease (stroke, intracerebral and subarachnoid bleeds)
Rhabdomyolysis with cardiac injury
Transplant vasculopathy
Vital exhaustion
Strenuous exercise

ICD, implantable cardioverter defibrillator; *ASD*, atrial septal defect; *HOCM*, hypertrophic obstructive cardiomyopathy; *LVH*, left ventricular hypertrophy; *5-FU*, 5-fluorouracil; *PCI*, percutaneous coronary intervention; *TBSA*, total body surface area. Adapted from Jaffe et al.[38]

drugs/biologic agents. This seems intuitively reasonable despite a lack of actual validation with prognostic data. However, it remains unclear whether small cTn elevations associated with nonischemic cardiotoxicity would result in an adverse prognosis similar to that seen with ischemic heart disease; and currently, none of the available cTn assays is indicated for detection of cardiac pathology in the non–acute coronary syndrome (ACS) drug development setting.

How to handle low-level but abnormal nonischemic cTn elevations during drug development remains a critical question to answer, as a metric of risk tolerance for both the medication and the population. It has not been established whether there are situations in which drug-induced cTn elevations can occur without an association with clinical sequelae. For example, are there situations with investigational products in drug development analogous to elite athletes who have cTn elevations after prolonged vigorous activities? Cardiac troponin elevations in this situation would not necessarily be expected to adversely influence prognosis.

This may become more apparent with the development of newer ultrasensitive cTn assays that demonstrate potentially "normal" measurable cTn levels in non-pathologic states. In addition, it is unclear if cTn would be useful to detect pathologic changes such as cardiac hypertrophy or nonfibrotic damage in the absence of cell destruction. In keeping with good scientific practice, adequate numbers of placebo-treated individuals are required to appropriately interpret the cTn findings across a population of patients in a clinical development program. This may not be feasible in certain populations in which placebo control cannot be used, for example, certain metastatic cancers, and will be a limitation in early clinical studies when only a small number of subjects have been exposed to the new molecular entity. Anthracycline cardiotoxicity has been associated with elevations of cTn in a number of studies and may serve as a good model of how a test developed to detect myocardial necrosis in the setting of coronary ischemia is useful for the detection of drug-induced cardiomyopathy.[39]

Monitoring of cTn during drug development

Once the decision is made to evaluate for cardiotoxicity with cTn from either preclinical or clinical studies, several considerations should be made:

1. Which subjects should be included in clinical trials, and what would be the expected cTn variability in those with no active (or background) treatment?
2. What level of cTn elevation would require dose modification or discontinuation?
3. Which assay should be used?
4. How frequently should the serum samples be analyzed?
5. What is the appropriate follow-up of individuals demonstrating cTn elevations?
6. How do cTn elevations affect the benefit:risk balance of the compound in development?

It is recognized that a single algorithm cannot be generated for all of the potential scenarios. As always, consensus across stakeholders regarding assay, monitoring strategy, and prospective plans for data interpretation should ideally be reached early in the study design process.

Entry criteria

In general, for clinical trials in non-ACS populations, it might be reasonable to exclude subjects if their baseline cTn levels were >99th percentile for the assays (an assay with 10% CV at the 99th percentile is preferred). However, this approach may not be appropriate for all studies; and issues like the study objectives and the intended study population should be considered (eg,

healthy volunteers vs treatments in oncology patients where tolerance for cTn elevation pre- and postdose may be different). In addition, such an approach may be less critical for large well-controlled studies and could even have unintended consequences including the problem of regression to the mean if all cTn-positive subjects were excluded.

Once randomized, the threshold for relevant cTn elevation postrandomization must be considered. Typically, when evaluating acute ischemic events in the setting of clinical symptoms consistent with ischemia, the 99th percentile is considered appropriate. When applying the assay to asymptomatic individuals in which ischemia is not being evaluated, if the assay does not achieve ≤10% CV at the 99th percentile, the 10% CV value may more reliably represent the actual level indicative of potential cardiac pathology. However, the choice of 99th percentile and/or 10% CV level should be considered in light of the population under study, the magnitude and duration of the cTn elevation, the performance characteristics of the assay, and the strength of the preclinical findings.

As discussed previously, it should be understood that certain "high-risk" populations for cardiovascular disease (eg, patients with angina or end-stage renal disease) or patients at risk of cardiac dysfunction due to underlying disease (eg, sepsis) may have baseline elevations in cTn levels. Therefore, the criteria for study entry or threshold for concern may need to be modified to appropriately reflect the target population under study. Similarly, the interpretation of changes in cTn levels during the course of treatment may be more complex.

Dose modification and discontinuation criteria

The frequency of cTn monitoring will typically be determined by the type and extent of cardiotoxicity seen in previous preclinical and clinical studies. In addition, it must consider the type of subjects being studied (symptomatic or asymptomatic; healthy or diseased), the type of development program (ie, indication), the stage in development, the characteristics of the experimental treatment, and the alternative therapeutic options available. In general, it is assumed that any symptomatic cardiac or potential cardiac toxicity would be immediately evaluated regardless of the timing of the previous cTn sampling. In asymptomatic healthy volunteers and patients, a more pragmatic approach could be taken. For example, if there were minor reversible abnormalities seen in preclinical studies at large exposure margins over what is administered to humans, cTn may be evaluated at "routine" time intervals such as during regularly scheduled safety monitoring follow-ups. On the other hand, if there were evidence of significant

myocardial damage in preclinical models at exposures that were close to anticipated human exposures (eg, a compound intended to be tested to treat refractory metastatic cancer), then cTn levels may be needed with higher frequency. It may be reasonable to consider potential benefits and limitations of obtaining concomitant evaluations of pharmacokinetics with cTn testing. However, in cardiotoxicity, cTn elevations may be better associated with accumulation and concentrations at steady state than Cmax. However, in the case of ischemic events, multiple rises and falls may occur and be more closely related to Cmax. In addition, when there is consideration of evaluating cTn levels and the expectation of clinical cardiotoxicity, it may be reasonable to obtain cardiac imaging (eg, an echocardiogram) at baseline in all of the individuals participating in the study. In this way, if there is a slight cTn elevation, serial comparison with the baseline imaging may help to evaluate whether or not an abnormality is new or preexisting, for example, to differentiate between a drug effect and other underlying abnormalities that could otherwise be misinterpreted as drug related. Therefore, collection of baseline samples and images improves the interpretation within extensive cardiac safety monitoring programs.

Subjects with confirmed postdose cTn elevations who reach a particular threshold (eg, >10% CV or 99th percentile if that is the predetermined criteria for the study) would be referred to their physicians for clinical evaluation and management. Serial cTn levels may be part of recommended follow-up. Alternatively, collecting parallel samples to run in another laboratory using a higher sensitivity and/or more precise assay may be useful to better discern a potential analytical false-positive result.

If the patient were asymptomatic, a second value could be obtained; and if it remained greater than the 10% CV level (or 99th percentile), a number of options could result depending on the drug, risk:benefit balance, and clinical scenario. For example, if the overall benefit were modest, the study subject could be withdrawn from the study or referred to a cardiologist for evaluation and decision on continuing or stopping therapy dependent upon the results of further testing. If the second value were within the reference range, the patient could be considered for continued participation in the study with close follow-up of symptoms, ECGs, and further cTn measurements as clinically indicated. Essential in safety evaluation and risk management is to consider factors that may affect cTn levels and potential benefit:risk profile of the compound; these include types of subjects (human volunteers vs patients), clinical signs and symptoms, underlying disease (eg, compound to treat refractory metastatic cancer vs an antibiotic with no new efficacy), comedications, and comorbidities.

As with any investigation in human subjects, patient care and protection are the first priorities. If the subject were symptomatic and/or the cTn level approached the threshold for MI according to the local laboratory parameters, the subject might be withdrawn from the study drug and either hospitalized or urgently referred to a cardiologist for appropriate medical care. Situations may arise in which high-risk patient populations, patients with extremely poor short-term prognosis, or patients receiving medications with known adverse cardiac effects can continue the investigational product with additional intensive monitoring. One such example could be patients being treated with anthracyclines in addition to an investigational agent. Incorporation of prespecified criteria into the protocol for absolute discontinuation (single high value) as well as duration/persistence of low-level values could assist with patient and study management.

Different discontinuation criteria may be proposed on a compound-specific basis, considering the preclinical signal, target population, and concomitant cardiotoxic medications. In addition, when monitoring for cardiotoxicity, it is good practice to preengage a cardiologist at the local site who is familiar with the investigational agent and the protocol. Finally, for later-phase drug development, the use of an independent Data and Safety Monitoring Board (DSMB) is essential. The role of a DSMB in early, first-in-man testing is less clear, but could also be considered.

A variety of nonchemical tools may be used to assess cardiac toxicity in human subjects who have cTn elevations. Electrocardiograms, arrhythmia monitoring, as well as noninvasive cardiac imaging studies such as echocardiograms, nuclear imaging, or cardiac magnetic resonance imaging may be useful. Which tools are applicable or even required may be specified by the protocol to ensure the consistency of patient evaluation in the setting of cTn elevation beyond specified thresholds.

Consistent with best practice in treating patients, cardiac events should be confirmed by multiple tests before intervention. It is recognized that small cardiac lesions may not be detected with current imaging technology; but electrocardiography, repeat cTn measurements, and ejection fraction and wall motion evaluations may represent the best available integration of modalities for detection of significant drug-induced cardiotoxicity with existing technology. Serial assessments may also be helpful to define the physiological consequences of potential lesions and to better determine what abnormalities are reversible after drug discontinuation versus what abnormalities are not reversible.

Assay options (central vs local laboratories)

Because of the potential lag time in reporting cTn if a central laboratory is used as well as higher study costs, especially for multicenter international studies,

local cTn testing may be used as the first assay of choice to direct clinical decision making regarding the study participant; a central laboratory assay may be reserved for confirmation or for poststudy analysis. In this case, samples could be split, with an aliquot retained at a central repository for later analysis. The frequency of nonroutine sampling at the time of a clinical problem should be guided by the level of clinical concern. Given the variability in performance characteristics among assays and the diversity of assays used across clinical/investigative sites, to interpret the results of cTn testing when performed locally, consideration should be given to collecting the details of the assay used for each cTn test performed locally (eg, manufacturer, type of assay [I or T], 99th percentile, 10% CV level, clinical decision limit in use locally). It may be prudent to report both absolute values and the percentage elevation or fold change from baseline. Good investigational practice dictates that samples should be retained for core laboratory assays using a single high-quality assay for future use in resolving any questions raised by local assessments and/or if further information were needed. The use of a central laboratory as appropriate for safety laboratories and the use of a clinical end points classification committee for blinded determination of clinical events could be considered as a part of the general clinical investigational plan.

Current-generation and new high-sensitivity assays can also detect subclinical injury that may not be detected with ECGs or cardiac imaging. Awareness of this fact could have major implications for clinical care as well as in drug development programs. Appropriate application and interpretation of assay results in a proactive fashion are needed to ensure that minor variability in troponin levels are not misinterpreted as drug-induced cardiotoxicity. Thus, the decision to continue dosing in this setting would need to be made with consideration of the potential benefit versus risks related to the compound under study. In addition, waiting until the cTn returns to baseline does not necessarily mean that the lesion or pathologic event has regressed or normalized. Without definitive information on management of these situations, investigators and sponsors may need to base their clinical decisions on the short- and long-term preclinical studies as a potential guide to evaluate cardiac responses to the investigational drug.

Real-time (low latency) versus retrospective monitoring

Real-time (low latency) reporting of the results of cTn measurements is important in some studies so that threshold values of concern can be reported to the investigator for the immediate management of the study subject. Retrospective monitoring describes batched analysis of cTn (timing based on duration of assay viability with storage), which is not performed for real-time management of study subjects but for the determination of population trends. Use of real-time testing versus retrospective monitoring depends on many factors including the drug being tested, the preclinical findings, the severity of the underlying disease being treated, the likelihood that the disease itself may result in cTn elevation (eg, sepsis/septic shock), and the baseline clinical characteristics of the patients being studied. In addition, the practical realities of conducting clinical trials, particularly large, multisite, multinational efforts, must be considered in the decision to use real-time versus retrospective monitoring, just as is the case for the decision to use a core laboratory.

Where there is a regulatory mandate or sponsor expectation, real-time monitoring, usually via a local laboratory, is encouraged because this will provide data needed for acute management of subjects in clinical studies. It is suggested that, with "real-time" monitoring, the investigators be immediately notified of all abnormal cTn values so that appropriate evaluation and follow-up of the study subjects can take place in a timely, clinically appropriate fashion and, if indicated, study drug treatment can be stopped before there are extensive or irreversible changes. In addition, when protocol-specified workup and evaluation of cTn abnormalities are instituted, this will allow optimal and consistent patient management—especially because the majority of cases may not involve cardiologists as investigators.

There are certain situations in which retrospective analysis without real-time monitoring can be instituted, including studies that are exploratory in nature. In these situations, arrangements for notification of patients and their physicians may need to be made for reporting and workup of any observed abnormalities. This may be performed as part of the protocol itself or an ad hoc analysis of serum samples if a safety signal is detected. These decisions must be made on a study-by-study basis with the plan for monitoring and response to results laid out in advance of study initiation.

Real-time management and thresholds

Action thresholds based on cTn concentrations may differ in certain situations such as when an investigational product is administered in patients already exposed to cardiotoxic drugs (eg, anthracyclines).

Known cardiotoxic drugs are likely to confound the interpretation of cTn used as a biomarker for the safety assessment of a new drug. In such settings, a change from baseline rather than or in addition to an analytical cutoff may be considered. Alternatively, there may be situations in which an investigational compound is being studied in a population that exhibits a high prevalence of cTn

elevation. For example, patients with acute exacerbation of chronic obstructive lung disease, patients with acute pulmonary embolism, and critically ill patients in the intensive care unit frequently have cTn elevations due to their underlying disease. Highly trained athletes after extreme, prolonged exertion may also have cTn elevations.[5-10,14,38] In these situations, alternative "actionable" thresholds would have to be considered on a compound-by-compound or study-by-study basis to optimally differentiate a drug effect from that of the comorbid disease or other confounding factor. Importantly, the collection of cTn data in these types of studies could be used to improve our knowledge on expected cTn changes in these populations and then guide future monitoring and response to results of testing.

Cardiac troponin elevations in an asymptomatic individual should not automatically require an ischemia workup or necessitate cessation of study drug; rather, a workup should be directed to identifying a specific pathology defined by preclinical testing or clinical class effects. As mentioned previously, a cardiovascular specialist familiar with the drug, preengaged at an investigational site, would help ensure appropriate cardiac testing. Cardiac troponin elevation in this setting may be evaluated with ECGs, telemetry, and noninvasive cardiac imaging as opposed to an invasive coronary angiographic approach for compounds not demonstrating ischemic cardiac effects. In addition, DSMB input may be helpful in developing appropriate compound-specific inclusion, discontinuation, or dose-modification thresholds. The DSMB oversight may be structured to fixed periods of enrollment/follow-up or to virtually real-time oversight functionality, depending on the needs of the study design, the compound, and the subjects being enrolled.

Evaluation of clinical cardiovascular events when there is a preclinical or clinical cardiac signal

In settings in which there has been evidence of cardiac toxicity in preclinical or early clinical testing, careful assessment of cardiovascular end points is warranted in later-phase human testing. Which end points are assessed may be dictated by the preclinical observations or early clinical signals and may include ischemic coronary events (MI, unstable angina, cardiovascular mortality) or clinical heart failure end points. Close follow-up with comprehensive details of these events will allow for post hoc adjudication, if needed, to better understand if there is a causal relationship between the investigational product and the cardiac event. Ideally, the occurrence of clinical events would not only be reported by investigators but objectively adjudicated by a blinded clinical events classification committee adhering to standardized event

definitions. For MI, definition should be guided by the recommendations of the universal definition of MI and reported according to type as recommended[1]; but in each case, all cTn (or creatine kinase–MB) data, inclusive of measured levels in the samples, assay manufacturer, 99th percentile, and 10% CV level, should be provided so that the data can be independently evaluated. Where possible, for MI definition, collection of serum that can be frozen for later core laboratory assessment of cTn using a single assay may be reasonable. Study reporting should include whether there is an imbalance in adjudicated clinical events between treatment and control groups and whether or not the clinical events detected correlated with preclinical signals observed in animals or early clinical studies.

Future directions

1. Collect data to establish further information on the epidemiology of cTn elevations in various populations, including intra- and interindividual variability in "healthy volunteers" over a wide range of ages (particularly with newer high-sensitivity or ultra-sensitive assays), the expected background rates of cTn elevation due to disease state, and rates of cTn elevation that are expected in response to existing treatments for various illnesses. Such a registry or warehouse could also be used to determine the prognostic relevance of cTn elevation within and across various disease entities and treatments, In particular, randomized clinical trials with a placebo arm provide a unique opportunity to explore these questions.

2. Set up a "cTn warehouse" to collect information from across industry. This information could then be used to better understand the behavior of various cTn assays in healthy volunteers as well as specific target populations. In establishing such a warehouse, a set of "essential minimum" clinical data about subjects and treatments along with assay data would need to be established along with the development of a simple data collection tool.

3. Collect data on all patients enrolled in clinical trials and existing registries/databases, including Centers for Medicare & Medicaid Services (CMS), both to understand the variability of cTn within and across sites and subjects and by treatment and as exploratory end points in clinical trials. When MI data are collected as a primary end point, data relating to the timing of an event are instructive in understanding the safety profile. Wherever possible, correlations of cTn serial levels and serial comparisons of cardiac images would be helpful to better understand the significance of more modest cTn changes and cardiac toxicity recognizable in human subjects.

4. Encourage collection of serum or plasma in a standardized fashion that can be stored for future analyses as indicated.

5. Engage clinical chemistry community in establishing a "living" database of assay characteristics and performance metrics. Such an analytical resource might consolidate information of sample stability/longevity; serve to develop parameters for frequency of sampling, particularly with high-sensitivity assays, for early detection of cardiotoxicity; determine whether sex- or age-specific differences in assay interpretation are needed; and establish generalizable reference ranges for populations with low probability of cardiovascular disease.

Conclusion

This document describes a consistent and a practical approach to cTn testing in various phases of drug development and provides supporting rationale for and limitation of the use of cTn as a biomarker for detecting potential cardiotoxicity in clinical studies. It is not intended to be proscriptive, but rather to provide a framework within which to optimize subject safety in clinical studies, while improving the evaluation of cardiotoxic liabilities of new investigational drugs.

In general, cardiomyopathic lesions observed in preclinical studies should trigger more intensive clinical monitoring, which in most instances will result in determination of cTn levels as well as other cardiac evaluations (eg, imaging) in clinical development. It is recognized that although there are limited data on the expected variability of cTn in these settings, it is clearly the most sensitive and specific serum cardiac biomarker to date. Appropriate inclusion, discontinuation, and dose modification should be determined by the cTn assay characteristics as well as the target patient population under study and the balance of risks and benefits of continuing experimental treatment. When adequate, placebo data should be collected to ensure appropriate comparisons between treated and control patients. In addition, epidemiologic data on cTn distributions in various populations should be augmented and could be used to define expected safety margins. When significant cTn elevations are detected in asymptomatic individuals, careful evaluation should be undertaken to ensure immediate subject safety and follow-up. It would be expected that most symptomatic individuals would be permanently discontinued from the investigational product. Ultimately, the decision to proceed with or terminate a development program will need to be made in the context of the frequency and degree of cTn abnormalities and the overall benefit:risk profile of the product. As further data are collected on cTn measurements in these non-ACS settings, enhanced understanding and ultimately more refined use of this biomarker can be expected for clinical development programs.

Disclosures

Disclaimer: The views expressed in this article reflect the opinions of the authors only and do not necessarily represent regulations and policies of the Food and Drug Administration, the Agency for Healthcare Research and Quality, the Department of Health and Human Services, or the authors' affiliated organizations.

References

1. Thygesen K, Alpert JS, White HD, et al. Universal definition of myocardial infarction. J Am Coll Cardiol 2007;50:2173-95.
2. Baubuin L, Jaffee AS. Troponin; the biomarker of choice for the detection of cardiac injury. CMAJ 2005;173:1191-202.
3. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342-9.
4. Kociol RD, Pang PS, Gheorghiade M, et al. Troponin elevation in heart failure prevalence, mechanisms, and clinical implications. J Am Coll Cardiol 2010;56:1071-8.
5. Mehta NJ, Jani K, Khan IA. Clinical usefulness and prognostic value of elevated cardiac troponin I levels in acute pulmonary embolism. Am Heart J 2003;145:821-5.
6. King DA, Codish S, Novack V, et al. The role of cardiac troponin I as a prognosticator in critically ill medical patients: a prospective observational cohort. Crit Care 2005;9:R390-5.
7. Guest TM, Ramanathan AV, Tuteur PG, et al. Myocardial injury in critically ill patients. A frequently unrecognized complication. JAMA 1995;273:1945-9.
8. Lim W, Queshmag I, Cook DJ, et al. Elevated troponin and myocardial infarction in the intensive care unit: a prospective study. Crit Care 2005;9:634-5.
9. Abroug F, Quanes-Besbes L, Nciri N, et al. Left heart dysfunction and severe exacerbation of COPD: diagnostic performance of cardiac biomarkers. Am J Respir Crit Care Med 2006;174:940-6.
10. Kelley WE, Januzzi JL, Christenson RH. Increases of cardiac troponin in conditions other than acute coronary syndrome and heart failure. Clin Chem 2009;55:2098-112.
11. Saenger AK, Jaffee AS. The use of biomarkers for the evaluation of treatment of patients with acute coronary syndromes. Med Clin North Am 2007;91:657-81.
12. De Gennaro L, Burnetti ND, Cuculo A, et al. Increased troponin levels in non-ischemic cardiac conditions and non-cardiac diseases. J Interv Cardiol 2008;21:129-39.

13. Roongsritong C, Warraich I, Bradley C. Common causes of troponin elevations in the absence of acute myocardial infarction: incidence and clinical significance. Chest 2004;125:1877-84.

14. Tulloh L, Robinson D, Patel A, et al. Raised troponin T and echocardiographic abnormalities after prolonged strenuous exercise—the Australian Ironman Triathlon. Br J Sports Med 2006; 40:605-9.

15. Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined—a consensus document of the joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69.

16. Wu AH, Jaffe AS. The clinical need for high-sensitivity cardiac troponin assays for acute coronary syndromes and the role for serial testing. Am Heart J 2008;155:208-14.

17. Berridge BR, Pettit S, Walker DB, et al. A translational approach to detecting drug-induced cardiac injury with cardiac troponins: consensus and recommendations from the Cardiac Troponins Biomarker Working Group of the Health and Environmental Sciences Institute. Am Heart J 2009;158:21-9.

18. ICH M3(R2) Nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals EMEA, CPMP/ICH/286/95. 2009. Available at http://www.ich.org/LOB/media/MEDIA5544.pdf.

19. Apple FS, Murakami MM, Ler R, et al, for the Technical Committee of Biomarkers Working Group on Cardiac Troponins. Analytical characteristics of commercial cardiac troponin I and T immunoassays in serum from rats, dogs, and monkeys with induced acute myocardial injury. Clin Chem 2008;54:1982-9.

20. Gaze DC, Collinson PO. Cardiac troponins as biomarkers of drug- and toxin-induced cardiac toxicity and cardioprotection. Expert Opin Drug Metab Toxicol 2005;1:715-25.

21. Schultze AE, Carpenter KH, Wians FH, et al. Longitudinal studies of cardiac troponin I concentrations in serum from male Sprague Dawley rats: baseline reference ranges and effects of handling and placebo dosing on biologic variability. Toxicol Pathol 2009;37: 754-60.

22. Milaelian I, Coluccio D, Hiraler GM, et al. Assessment of the toxicity of hydralazine in the rat using an ultrasensitive flow-based cardiac troponin I immunoassay. Toxicol Pathol 2009;37:878-84.

23. York M, Scudamore C, Brady S, et al. Characterization of troponin responses in isoproterenol induced cardiac injury in the Hanover Wister rat. Toxicol Pathol 2007;35:606-17.

24. Drevon-Gaillot E, Perron-Lepage MF, Clément C, et al. Review of background findings in cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins. Exp Toxicol Path 2006;58: 77-88.

25. Buchanan JW. Prevalence of cardiovascular disorders. In: Fox PR, Sisson D, Moise NS, editors. Textbook of canine and feline cardiology. 2nd ed. Philadelphia: WB Saunders; 1999. p. 457-70.

26. Zhang J, Knapton A, Lipshultz SE, et al. Isoproterenol-induced cardiotoxicity in Sprague-Dawley rats: correlation of reversible and irreversible myocardial injury with release of cardiac troponin T and roles of iNOS in myocardial injury. Toxicol Pathol 2008;36: 277-8.

27. Brady S, York M, Scudamore C, et al. Cardiac troponin I in isoproterenol-induced cardiac injury in the Hanover Wistar rat: studies on low dose levels and routes of administration. Toxicol Pathol 2010;38:287-91.

28. O'Brien PJ. Blood cardiac troponin in toxic myocardial injury: archetype of a translational safety biomarker. Expert Rev Mol Diagn 2006;6:685-702.

29. de Lemos JA, Drazner MH, Omland T, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA 2010;304:2503-12.

30. Apple FS. A new season for cardiac troponin assays: it's time to keep a scorecard. Clin Chem 2009;55:1303-6.

31. Apple FS, Jesse RL, Newby LK, et al. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage laboratory medicine practice guidelines: analytical issues for biochemical markers of acute coronary syndromes. Clin Chem 2007;53:547-51.

32. Tate JR. Troponin revisited 2008: assay performance. Clin Chem Lab Med 2008;46:1489-500.

33. Heidenreich PA, Alloggiamento T, Melsop K, et al. The prognostic value of troponin in patients with non–ST elevation acute coronary syndromes: a meta-analysis. J Am Coll Cardiol 2001;38:478-85.

34. Morrow DA, Antman EM, Tanasijevic M, et al. Cardiac troponin I for stratification of early outcomes and the efficacy of enoxaparin in unstable angina: a TIMI-11B substudy. J Am Coll Cardiol 2000;36: 1812-7.

35. Morrow DA, Cannon CP, Rifai N, et al, for the TACTICS-TIMI 18 Investigators. Ability of minor elevations of troponins I and T to predict benefit from an early invasive strategy in patients with unstable angina and non–ST elevation myocardial infarction: results from a randomized trial. JAMA 2001;286:2405-12.

36. Newby LK, Ohman EM, Christenson RH, et al, for the PARAGON B Investigators. Benefit of glycoprotein IIb/IIIa inhibition in patients with acute coronary syndromes and troponin T–positive status: the PARAGON B Troponin T Substudy. Circulation 2001;103:2891-6.

37. Iijima R, Ndrepepa G, Mehilli J, et al. Troponin level and efficacy of abciximab in patients with acute coronary syndromes undergoing early intervention after clopidogrel pretreatment. Clin Res Cardiol 2008;97:160-8.

38. Jaffe AS, Babuin L, Apple FS. Biomarkers in acute cardiac disease: the present and the future. J Am Coll Cardiol 2006;48:1-11.

39. Auner HW, Tinchon C, Linkesch W, et al. Prolonged monitoring of troponin T for the detection of anthracycline cardiotoxicity in adults with hematologic malignancies. Ann Hematol 2003;82: 218-22.