Measurements of cardiac troponin concentrations represent, according to the jointly released guidelines from the European Society of Cardiology (ESC)/American College of Cardiology (ACC) in 2000, the new “gold-standard biochemical method” in the diagnosis of acute myocardial infarction (AMI), and provide useful prognostic information in patients with acute coronary syndromes (ACS). In 2007, the same organizations in collaboration with the American Heart Association (AHA) and the World Health Federation (WHF) have published “Universal definition of myocardial infarction”, confirming and expanding the diagnostic role of cardiac troponins in the management of ACS patients as well as in those undergoing percutaneous and surgical revascularization procedures. The prognostic role of these marker proteins in clinical conditions other than ACS has also been increasingly recognized and addressed.

There are, however, several limitations of the currently available cardiac troponin assays that need to be considered in the interpretation of the test results. With regard to the analytical performance of the troponin tests, it is recommended that the 99th percentile reference value determined in a reference population should be used as the cut-off point for diagnosing AMI. Furthermore, to enhance the reliability of the test performance, this 99th percentile reference value should have a coefficient of variation (CV) of 10 percent or less. Unfortunately, nearly all of the current troponin assays do not fulfill this requirement. Other important limitations are the fact that several troponin tests, even with the new assay generation, show analytical interferences in the presence of heterophile antibodies and by using some anticoagulants.

The clinical limitation of the troponins lies in the low diagnostic sensitivity in the early hours after onset of symptoms, since they are released into the circulation after 3-4 hours following infarction. In cases of an early presentation of the patients, it is recommended to use an earlier marker, e.g. myoglobin, which can be detected to be increased in the blood as early as 1 hour after myocardial necrosis. On the other hand, the relatively low analytical sensitivity of the current troponin assays and high imprecision at low-range troponin concentrations do not allow for reliable biochemical detection of the presence of myocardial ischemia in patients with stable angina pectoris or congestive heart failure, which in turn may have prognostic significance.

With the advent of the new generation of troponin assays with a high analytical sensitivity, improvements in analytical performance and enhancement in diagnostic and prognostic values are to be anticipated. The present mini-review summarizes and presents recent information obtained from literature searches for the terms “high-sensitivity, ultrasensitive, troponins” in electronic databases, and discusses the analytical performance and clinical significance of the new high-sensitivity cardiac troponins. The emerging role of cardiac troponin testing in veterinary medicine and preclinical research will also be addressed.

Analytical performance
Several factors have been reported to affect the analytical performances of the conventional troponin assays, which have limited diagnostic and prognostic values. These include the imprecision at low-range concentrations, analytical interference by heterophile antibodies, rheumatoid factor (RF) or human anti-animal antibodies, as well as by using certain anticoagulants.

Analytical precision
Most, if not all, of the current troponin assays show high imprecision at low-range concentrations or at the reported cut-off concentrations. According to the new recommendation from the ESC/ACC/AHA/WHF, the discrimination limit for diagnosing AMI should be the troponin concentration at the 99th percentile value determined in a reference population, and the value...
should have a CV of 10% or less. This can be translated into a 99th percentile: 10% CV ratio of ≥ 1. As shown in Table 1, the analytical performance of all conventional cTnT or cTnI assays did not meet this criterion. Due to this analytical imprecision of the assays, it has been recommended by some authors to use troponin levels at the 10% or 20% CV instead of the 99th percentile for cut-off concentrations for diagnosis of AMI.6,7

With introduction of the new generations of troponin assays, improvements in the analytical sensitivity were reported. Most of these assays fulfill the recommended imprecision criterion (Table 1). For example, the new high-sensitive cTnT assay (Roche Diagnostics) shows a 10% CV at the concentration of 0.006 ng/ml and a CV of around 8% at the 99th percentile value of 0.012 ng/ml. Similar results were obtained with ultrasensitive cTnI reported by other manufacturers. In this context, it should be born in mind that the data given by the manufacturers may not be congruent with those published in research studies.6 Thus, more information is needed from future investigations on different populations.

Analytical interference

Most of the troponin assays employ 2-site (sandwich) immunoassay with 2 monoclonal or polyclonal antibodies directed at different epitopes of the troponin molecules, and thus are susceptible to produce false-positive results in the presence of heterophile antibodies, RF or human anti-animal antibodies. These antibodies are specific for the Fc portion of the assay species immunoglobulin and may crosslink the 2 assay antibodies in the absence of the intended analyte.3 In the past, there have been several reports of analytical interferences by heterophile antibodies or anti-animal antibodies, which were found almost exclusively in the cTnI immunoassay.3,8 False positive cTnT results were also found in the presence of RF in some immunoassays.9,10 To reduce or eliminate the effect of any heterophile antibody present in the sample, non-specific antibodies are generally added to the assay kits of the new assay systems, thereby improving the analytical performance.3,11

The effect of anticoagulant has been shown to affect the troponin detection. Gerhardt et al.12 were among the first who demonstrated reduced concentrations of both cTnT and cTnI in lithium heparin plasma when compared with serum. Their results were later confirmed by a study of Septh et al.13 who also reported a decrease in both cTnT and cTnI in heparin plasma samples, even with the addition of protamine and heparinase to reverse the heparin effect. This may have an impact on the interpretation of test results in the low-range concentrations or, more importantly, in the concentrations near the discrimination or cut-off levels. Nevertheless, as has been recently reported by Dominici et al.14 and Wang et al.15, the problem of anticoagulant interference seems to have been solved with the new troponin assay generations.

Clinical relevance

One of the problems related to the diagnosis of AMI in patients presenting early to the emergency department is the low clinical sensitivity of the conventional troponin assays. This delay in diagnosis may affect the decision making in triaging and treatment of the patients. In addition, due to high imprecision of the current troponin immunoassays in determining concentrations in the very low levels, they do not provide reliable methods for risk stratification and prognosis in patients with unstable coronary disease or other non-ACS-related clinical conditions. Furthermore, although the current assays show improved sensitivity in detecting myocardial injury in conditions other than ACS, they cannot distinguish reversible from irreversible myocardial damage. Therefore, it is to be anticipated that the novel ultrasensitive troponin assays may be able to solve some of the problems mentioned above.

Diagnostic sensitivity and early detection

At present, all of the commercially available cTnT or cTnI immunoassays show a low time-sensitivity in detecting myocardial injury. Due to the distribution characteristic in the myocytes, the free troponins in the cytosolic pool (3-7% of the total content) are released into the circulation first, followed by the bound form of troponins in the myofilament. However, both cTnT and cTnI are usually found to be increased above the cut-off concentration after 3-4 hours following the onset of symptoms, and thus achieving the optimum sensitivity of more than 95% when 9 hours have passed. For comparison, the same sensitivity is reached by myoglobin some 3 hours earlier. It is, therefore, recommended that for patients admitted within 4 hours after the onset of chest pain, the myoglobin level is to

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Analyzer</th>
<th>Troponin</th>
<th>LLD</th>
<th>Troponin 99th PCT concentration (ng/ml)</th>
<th>10% CV</th>
<th>ROC</th>
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<tr>
<td>Roche</td>
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<td>-</td>
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</tr>
<tr>
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<td>0.044</td>
<td>0.014</td>
<td>-</td>
</tr>
</tbody>
</table>

All data per manufacturer
LLD = lower limit of detection (analytical sensitivity), PCT = percentile, CV = coefficient of variation, ROC = receiver operation characteristics.
be measured first, followed by determination of the more cardiac specific troponin 3-6 hours later for confirmation.16

Promising results which have indicated the troponins as early markers of myocardial necrosis, thus obviating the need for myoglobin measurements, are derived from 3 recent studies utilizing the new ultra-sensitive cTnI assays. Melanson et al.17 compared conventional cTnI (ADVIA Centaur analyzer, Siemens Medical Solutions) with the new Tnl-Ultra from the same manufacturer in a study performed on 103 patients who initially had a negative cTnI result followed by a positive cTnI result in subsequent specimens. It was found that cTnl-Ultra was positive before conventional cTnI in 64% of the patients. Since the ESC/ACC guidelines recommended serial cardiac troponin measurements, and the physician usually waited at least 6 hours to obtain a subsequent troponin level, it was estimated that the cTNl-ultra might allow the diagnosis of ACS to be made on an average of 9.5 hours earlier. These results were confirmed by a more recent study of the same authors who reported that the cTnI-ultra, with a cut-off concentration of 0.04 ng/ml, could detect myocardial injury earlier than conventional cTnI (cut-off 0.10 ng/ml).18 They concluded that the more sensitive cardiac troponin assay allow the time window for serial measurements to be narrowed and, thus, will allow for more rapid interpretation and triage of patients with suspected ACS.

In a prospective study of 371 consecutive patients presenting with symptoms suggesting ACS, Apple et al.19 reported clinical sensitivities of a cTnI-Ultra assay (ADVIA Centaur, Siemens) on the presenting blood specimen (5 hours after symptom onset) and follow-up samples (6 to 24 hours later) to be 74% and 94%, respectively. These results demonstrate a substantial improvement in diagnostic performance compared to the conventional troponin assays, which usually showed diagnostic sensitivities of only 3% to 33% and between 50% and 92% in the corresponding time periods, respectively. Nevertheless, although the new assay showed a higher sensitivity (90%) than the conventional Dade Behring assay (86%) at 6 to 12 hours, the specificity was comparatively lower (83% for Siemens vs. 92% for Dade Behring). The data indicate that utilization of high-sensitivity troponin assay and the 99th percentile cut-off will not only improve sensitivity of a test, but at the same time lead to increased false positive rates of conditions other than ACS due to decreased assay specificity. Therefore, it is imperative that the new assays should always be used and interpreted in conjunction with clinical symptoms and ECG results.

In this context, it should be noted that the troponin results mentioned above were derived from determinations on cTnI immunoassay from one manufacturer. Thus, it remains to be seen, as to whether these promising results can be confirmed by new ultra-sensitive cTnI assays from other manufacturers, or from the high-sensitivity cTnT assay.

Risk stratification and prognostication

In addition to their high sensitivity and specificity for detecting myocardial injury, both cTnT and cTnI have emerged as powerful predictors of future adverse cardiac events, and were used for risk assessment in patients with a clinical syndrome consistent with ACS.20 Even minor elevations of cardiac troponins in the low-range concentrations were able to provide useful prognostic information in patients with stable and unstable coronary heart disease.21

To our knowledge, there have been thus far two studies assessing the prognostic value of ultrasensitive troponin; one in the general population and the other in patients with ACS. In a recent risk stratification study from Sweden, Zethelius et al.22 reported greater risk of adverse events in 70-year-old men with measurable, but normal values compared to those with cTnI (second-generation Beckman assay) values less than the limit of detection. Similar findings were observed in a prospective study by Apple et al.19 who showed increasing cardiac event rates with increasing cTnI (Tnl-ultra, Siemens) levels: 2.8% versus 11.1%, 24.1% and 55.1% with levels of < 0.006 ng/ml, between 0.006 and 0.04 ng/ml, > 0.04 - 0.10 ng/ml and > 0.10 ng/ml, respectively. After adjustment for established risk factors, the calculated relative risks for the increasing cTnI cut-off concentrations were 3.9, 8.9 and 25, respectively. The authors concluded that the Tnl-Ultra assay is an independent predictor of adverse events at any measurable cTnI in ACS patients.

Levels of cardiac troponins have also been used as prognostic indicators in patients with clinical conditions other than ACS.23 These include congestive heart failure,24 pulmonary embolism,25 stroke26 and sepsis.27 In a recent study on patients with stable chronic heart failure,28 a significantly higher percentage of detectable concentrations of high-sensitivity cTnT (≥ 0.001 ng/ml) compared with the conventional cTnT (≥ 0.01 ng/ml) was found (92% versus 10.4%). Patients with elevated cTnT or with high-sensitivity cTnT above the median (0.012 ng/ml) had more severe heart failure as assessed by the New York Heart Association (NYHA) functional class. More importantly, circulating concentrations of highly sensitive troponin T showed an incremental prognostic accuracy in the presence of B-type natriuretic peptide (BNP), at present the best biomarker of heart failure.

Although there was thus far no study investigating the prognostic value of high-sensitivity troponins in other non-ACS patients, it is to be expected that the new troponin assays with improved analytical performance will be able to provide reliable prognostic information and, thus, optimal care of patients with myocardial injury of non-atherothrombotic etiology. In addition, high-sensitivity troponins may allow for better risk stratification in the group of patients with chronic stable angina, in whom the current assays do not detect elevated troponin levels.

Application in veterinary medicine and pre-clinical research

In the past decade, there was an increasing interest in utilizing cardiac biomarkers, especially troponins, in veterinary medicine. Due to preservation of the troponin molecular structure across phyla,29 cardiac troponin testing, initially developed for human use, can be applied for clinical studies and research in a wide range of mammalian species including rats, cats, dogs, pigs, sheep, cattle and horses (for review see references 30 and 31). The test was also used to define sex-related difference of cardiac markers in avian species.32

Two research fields in clinical physiology and
pathology are of special veterinary interest. The first lies in the long debated question, as to whether the troponins are able to differentiate myocardial ischemia from necrosis, reflecting reversible and irreversible damage, respectively. In this context, it has been shown that a certain number of healthy athletes exhibited increased cardiac troponins above the 99th percentile reference limit, but below the cut-off concentration of MI, after strenuous endurance exercise (marathon running, road cycling, triathlon). Similar results were obtained from several animal studies. Of particular importance was the observation that troponin elevation occurred even after an exercise event of shorter duration and lower intensity. These results were interpreted as physiological adaptation of the myocardium in response to physical stress. However, since most of the conventional troponin assays do not provide reliable and accurate measurements in the very-low-range concentration, they cannot be used to distinguish reversible from irreversible myocardial injury.

To address the issue, Kurz et al.7 serially measured high sensitivity cTnT (Roche diagnostics) and evaluated perfusion defects using single-photon emission computed tomography (SPECT) in patients undergoing dynamic stress testing with bicycle exercise or pharmacologic stress test with dipyridamol. It was found that cTnT concentrations did not differ significantly among the groups with fixed or reversible perfusion defect compared with the no perfusion defect group. The authors could not, however, exclude the possibility that longer or more severe myocardial ischemia could have caused troponin release.

Theoretically, both cTnT and cTnI with a molecular weight of 37 and 24 kDa, respectively, may be too large to traverse across viable cell membranes during myocardial ischemia. However, several studies have shown that troponins can undergo in situ degradation into smaller fragments during prolonged periods of myocardial ischemia, and these fragments appear in the blood of patients with ACS within the first hour after symptom onset, well before detection by conventional troponin assays. Thus, it is possible that the troponins are released and can be detected in the circulation in the presence of reversible injury. Further studies utilizing ultrasensitive cardiac troponins, especially cTnI, performed in human as well as in animal models are required to clarify the situation.

Another topic of research interest is the preclinical drug-safety. Numerous studies have clearly demonstrated that cTnI and cTnT represent sensitive and specific biomarkers of drug-induced myocardial damage in laboratory animals. The US "National Toxicology Program (NTP)" convened a workshop in 2006 with the aim of identifying biomarkers that could be used in toxicology studies with animals including rodents. For this purpose, the NTP recommended a troponin assay for routine use as a screening tool for cardiac damage, as well as for evaluation of subclinical myocardial injury. Utilization of a high-sensitivity troponin assay which provides reliable analytical performance will improve and enhance the ability to detect early cardiac events, and to understand the disease process associated with toxic cardiac injury. Furthermore, developments of the new tests should determine whether minimal increases below a threshold concentration of the troponins might reflect reversible myocardial damage.

In this regard, promising results were obtained from a recent study using an ultrasensitive cross-species cTnI assay (Erenna immunoassay system, Singulex). It was demonstrated that rats receiving subcutaneous injections of isoproterenol hydrochloride exhibited marked increases followed by decreases in cTnI concentrations. The magnitude of increases was higher in the rats given the 8.0 mg/kg dose compared to the 0.5 mg/kg dose. Histopathologic findings which showed scattered areas of myocardial necrosis in the hearts of animals administrated with 8 mg/kg of the drug were more severe than those with 0.5 mg/kg of isoproterenol. The authors concluded that the ultrasensitive cTnI assay used provides a reliable method for assessing potential cardiotoxicity in toxicology studies.

CONCLUSION

The high analytical imprecision of the conventional troponin assays in the low-range concentrations has led to uncertainty and confusion in the interpretation of test results. With the introduction of the ultrasensitive assay systems with improved analytical performances, more reliable diagnostic and prognostic information provided by the new troponin tests can be anticipated. Preliminary results from recent clinical trials have shown that the high-sensitivity troponin assays detect myocardial injury earlier than the conventional assays and substantially reduce the diagnostic time window, thereby obviating the need for using early markers of myocardial necrosis, e.g. myoglobin. The prognostic value of the new tests in ACS and non-ACS-related clinical conditions may also be enhanced. Other promising uses lie in their application in preclinical and toxicologic studies using animal models. In addition, determination of the novel ultrasensitive troponins may help in defining the mechanism of a disease process and, thus, may lead to better understanding of the pathophysiology of cardiac diseases.

Nevertheless, much more information from clinical trials is needed before introduction of the new tests into clinical practice.

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