Performance of Today's Cardiac Troponin Assays and Tomorrow's

International scientific bodies currently recommend the use of cardiac troponins for the detection of myocardial necrosis *(1, 2)*. Some troponin assays, however, are inadequately appraised before their introduction in clinical use *(3)*. The Committee on Standardization of Markers of Cardiac Damage (C-SMCD) of the IFCC proposed quality specifications for cardiac troponin assays with the objectives to help assay manufacturers and clinical laboratories and to urge the scientific community to select and design research projects on the major issues in troponin determination *(4)*. The study by Uettwiller-Geiger et al. *(5)* published in this month's issue of *Clinical Chemistry* represents a good example that fulfills these suggestions.

The first aspect that merits consideration is the evaluation of antibody specificity in troponin immunoassays. The issue of epitope location is important for cardiac troponin I (cTnI) assays because the amino- and carboxyl-terminal parts of the molecule are susceptible to proteolysis and this degradation may be related to the degree of tissue ischemia *(6)*. Intact cTnI and up to 11 modified products have been detected in the sera of patients with acute myocardial infarction (MI) *(6)*. cTnI is released predominately as a binary complexed form with troponin C (IC), although a minor amount of free cTnI is also found in the bloodstream *(7)*. Moreover, cTnI is released in both oxidized and reduced forms, the oxidation being the result of intramolecular disulfide formation by two cysteine residues *(8)*. Finally, cTnI can also be phosphorylated *(8)*.

Studies of clinical troponin assays must examine the specificities of antibody pairs used in the assays. Preferably the antibodies should recognize only the epitopes that are located in the stable part of the molecule and are not affected by IC or troponin I, T, and C (ITC) complex formation and other "in vivo" modifications. Quite properly, Uettwiller-Geiger et al. *(5)* examine the immunoreactivity of the evaluated immunoassay toward the major cTnI forms present in the bloodstream, i.e., the free protein, its binary and ternary complexes, and the phosphorylated and dephosphorylated forms, and demonstrate that the assay has an equimolar response to these forms.

The second issue is calibration. Lacking an international reference material for cTnI, manufacturers currently prepare their own calibration material, so that different purification procedures and types of calibration antigens (native or recombinant, free or complexed protein) are used. Together with different antibody specificities, this is the most important source of the well-known disagreements among commercially available cTnI assays *(9)*. The C-SMCD recommends calibration of cTnI assays against the material representing the natural and major form of the antigen in blood after tissue release, i.e., the complexed form *(4)*. The Committee for cTnI Standardization of the AACC targeted three candidate reference materials that were complexes of troponin C, I, and T *(10)*. It is hoped that the use of one of these as a reference material will allow reasonable harmonization among cTnI measurement systems.

Only harmonization is possible at present, but true standardization should be the goal. Standardization and traceability of measurements require a complete reference measurement system, including purified troponin complex as primary reference material, a matrixed (serum-based) secondary reference material, and a reference method that can be used to assign a cTnI value to the secondary reference material and to evaluate the analytical performance of the field methods *(11)*.

The most important benefit of the standardization programs will be the availability of common reference and decision limits for different cTnI assays. Until adequate cTnI standardization is possible, reference values and clinical thresholds need to be determined separately for each assay and platform. According to the document on MI redefinition *(2)*, the diagnostics manufacturers now need to provide the 99th reference limit of the specific assays, based on information published in the peer-reviewed literature. Such studies are few. The article by Uettwiller-Geiger et al. *(5)* shows the collaboration among laboratorians, clinicians, and the assay manufacturer that is necessary to define limits for clinical decisions.

Current immunometric techniques provide a wide analytical (dynamic) range, and in most clinical situations, sample dilution is rarely required. However, if required, dilution of samples should not be carried out unless fully investigated first, as dilutions may not be parallel to the calibration curve. Although parallelism appears to be a logical prerequisite to use, linearity of response has not always been demonstrated before the release of assays. In the multicenter study, the collaborative group adequately evaluated assay linearity and found, indeed, no significant bias when samples were diluted *(5)*.

Analytical imprecision also is not uniform among troponin assays *(12)*. The C-SMCD recommends a total imprecision (expressed as CV) of $<10\%$ at the MI decision limit *(1, 4)*. A failure to reach this goal could increase the risk of clinically misleading results. The demand for very precise troponin assays undoubtedly presents a difficult challenge *(13)*. Nevertheless, comparison of different generation assays performed on the same instruments clearly shows that there has been substantial improvement in the precision offered by the newer assays, and this improvement has been considered by manufacturers as a main goal in the design of new assays *(5, 14)*.

Nonspecificity for troponin assays also can be the result of analytic interference *(15)*. False-positive results may occur because of interferences with the antigen-antibody reaction in various immunoassays. Interferences from rheumatoid factors or human anti-mouse antibodies, the so-called "heterophilic antibodies" (HAs), which can mimic troponin by linking the capture and detector antibodies, have been reported. These false-positive results

can lead to unwarranted and potentially dangerous procedures *(16)*.

The lack of interference of HAs in an assay system should be carefully documented by measuring samples containing high concentrations of rheumatoid factor and samples with human anti-mouse antibodies in conjunction with treatment of the sample with agents to obviate interference from HAs *(4)*. Using this approach, Uettwiller-Geiger et al. *(5)* have shown the possibility of heterophile interference in the evaluated cTnI assay. The method gave increased cTnI results (six times the upper reference limit) in one sample containing HAs, with significantly decreased values after treatment with HA-blocking tubes.

The in vitro stability of cTnI appears to be method dependent, creating a need for data for each commercially available assay *(4)*. Uettwiller-Geiger et al. *(5)* appropriately studied the effect of storage on apparent marker concentration (although only for 3 days and only at −20 °C).

The use of plasma instead of serum samples for cardiac troponin determinations can be very useful because it eliminates the extra time needed for clotting, thereby reducing the overall preanalytical time *(1)*. However, there can be significant differences between serum and plasma concentrations of troponins, at least in some analytical systems. Binding of heparin to cardiac troponins may reduce immunoreactivity to various degrees, depending on the assay epitopes and the heparin concentration in sample tubes. On the other hand, EDTA splits the calcium-dependent ITC and IC troponin complexes, thus decreasing the measured concentrations in troponin assays that preferentially measure these molecular forms. Consequently, the use of anticoagulants should be studied and validated thoroughly before it can be recommended for practical use *(4)*. Specifications on the use of a certain anticoagulant must be based on comparisons among different kinds of samples (e.g., lithium heparin plasma vs serum) collected simultaneously at early and late phases after MI onset *(17)*. Using this approach, Uettwiller-Geiger et al. *(5)* showed that values in serum and EDTA plasma were 4% and 14% lower than in matched lithium heparin samples, although they do not indicate the variability among samples.

Powerful tests, such as cardiac troponins, on which critical decisions will rest need highly reliable methods. More peer-reviewed studies are generally needed before new assays are implemented in hospital-based laboratories. Studies must not only provide clinical information, but also evaluate analytical and preanalytical sources of variability. Uettwiller-Geiger et al. *(5)* should be congratulated on their efforts to provide an excellent example of such a study for the field.

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