To T or Not to T, That Is the Question

The quest for a test that is absolutely disease- and tissue-specific could be considered the Holy Grail of the clinical chemist, dreamt of but seldom achieved. Initial reports on measurement of the cardiac troponins, cardiac troponin T (cTnT) and cardiac troponin I (cTnI), for diagnosis of myocardial infarction suggested that these markers would become the “gold standard,” replacing all other existing tests. Questions have been raised as to the specificity of cTnT for cardiac damage in patients with extreme rhabdomyolysis, renal failure, polymyositis, and muscular dystrophy. This problem is addressed by the papers of Bodor et al. [1] and Müller-Bardorff et al. [2] in this issue of Clinical Chemistry. This is therefore a reasonable time to review what we know at the basic science level and how this relates to both papers and current clinical practice.

Cardiac and skeletal muscle cells are closely related but arise from different embryonic lineages and express distinct gene sets when terminally differentiated. During embryonic development, both muscle types cross-express several genes. There are three troponin T genes, corresponding to slow skeletal, fast skeletal, and cardiac troponin. During early embryonic development, the cTnT gene is activated and transcribed at relatively low levels in both cardiac and skeletal muscles until mid-fetal development, when expression is divergently regulated. In cardiac cells, transcription of the cTnT gene is sharply up-regulated, whereas in the skeletal cells it is repressed [3–5]. Similarly, three isoforms of troponin I exist, also the products of three separate genes: fast skeletal muscle, slow skeletal muscle, and cardiac muscle troponin I. During fetal development, slow skeletal muscle troponin I is the predominant isoform in the heart. After birth, the slow skeletal isoform is lost such that, by 9 months of postnatal development, the cardiac isoform is the only detectable isoform [6, 7].

Multiple isoforms of cTnT with different amino acid sequences have been described, arising from alternative splicings of mRNA. The nomenclature used for these isoforms varies slightly between authors [8–11]. The sequences corresponding to the different isoforms have been characterized [9, 11, 12], as have the sequences for fast skeletal [13] and slow skeletal troponin T [14]. The protein isoform expressed in embryonic cardiac and skeletal muscle cells is unique to this developmental stage [5]. Multiple isoforms of cTnT are expressed in the fetal heart. In the adult heart, a single isoform of cTnT is present, referred to as cTnT2 [9] or cTnT3 [10]. In fetal skeletal muscle, the adult cTnT isoform is detectable but at a very low amount. A second isoform, corresponding to a fetal cardiac isoform, is also detectable. This isoform is present in relatively greater abundance than the adult cTnT isoform. In both cases, these isoforms are present in low abundance relative to the skeletal isoforms [7, 10]. No isoforms of cTnT can be detected in normal adult skeletal muscle, either by Western blot [10] or by reverse transcriptase polymerase chain reaction (RT-PCR) [9]. Reexpression of a fetal isoform of cTnT in cardiac failure has been described by some [9–11] but not all [12] workers. Theoretically, because cTnT isoforms are expressed in fetal skeletal muscle during development and then suppressed, whereas cTnI is expressed only in the adult heart, cTnT might be reexpressed in damaged or regenerating skeletal muscle [10], but cTnI is unlikely to be found in noncardiac muscle. Reexpression, therefore, might result in detectable cTnT in the serum.

The data presented by Bodor and Müller-Bardorff must now be analyzed against these findings. Bodor et al. [1] approached the questions of specificity by investigating tissue immunohistochemistry. Their previously published studies on cTnI [15] convincingly demonstrated that this isoform is not present in normal or pathological skeletal muscle from adults, findings entirely compatible with the molecular biology described above. In their studies of cTnI, they used the same antibody in the immunohistochemistry as was used in the serum assay. The current study [1] does not; instead, the antibody used is directed at residues 3–15 of the N-terminal sequence of cardiac troponin T. This sequence is found in all of the isoforms of cTnT so far described, including fetal isoforms [9]. The study of Müller-Bardorff et al. [2] uses a more conventional approach. The anti-cTnT monoclonal antibodies used in the original and reformulated assay of Müller-Bardorff et al. recognize a sequence specific for cTnT but not found near the N-terminus. The two antibodies in the reformulated assay recognize epitopes 6 amino acid residues apart. In evaluating the data presented, these differences must be kept in mind.

The Western blot data for normal skeletal muscle presented by Bodor et al. is entirely compatible with the molecular biology data summarized above. The lack of cross-reaction of skeletal muscle from psoas, diaphragm, and quadriceps presented by Müller-Bardorff et al. is similarly consistent. The measurement of tissue extracts in a system not optimized for such analyses may account for the positive values seen, as may the possibility of cross-reaction; it is difficult to be certain that these are not complicating factors. The immunohistochemical data are more interesting. The majority of skeletal muscle studied by Bodor et al. did not show staining, but ~20% of the muscle fibers from the diaphragm did. The finding of cTnT in normal skeletal muscle is not consistent with any of the previously cited studies or with the Western blot data presented by Bodor et al. I can suggest three possible explanations for their findings. The antibody used may cross-react with skeletal troponin T. Indeed, the published sequences for fast skeletal troponin T, slow skeletal troponin T, and cTnT show a complete match for a pentapeptide within residues 3–15 of cTnT (the epitope recognized by the antibody used). Some cross-reaction might occur because of the polyclonal nature of the detection antibody used.
Either of these might yield some low-intensity staining. Although the authors [1] demonstrated by competition assay that they could block the binding of antibody by using recombinant cTnT, they did not demonstrate that skeletal troponin T did not interfere. A third, and much more intriguing, possibility exists: Because fetal and adult isoforms of cTnT share a common N-terminal sequence, including residues 3–15, expression of any cardiac isoform in skeletal muscle will be recognized. The dominant isoform of cTnT expressed in fetal skeletal muscle is not the adult isoform but instead resembles that reexpressed in heart failure [9–11]. Any fetal isoform present might therefore be identified by the immunohistochemical technique. The finding of immunostained tissue seems to be confined to the diaphragm. However, it is possible that fetal cTnT isoform is present in some of these skeletal muscle fibers. If so, the amounts appear to be very low and to not cross-react in the reformulated assay of Müller-Bardorff et al.

This information must now be put into its clinical context. At the analytical cutoff values quoted for the current commercially available assays, cTnT and cTnI are not detected in serum from normal reference groups. Studies on exertional rhabdomyolysis have not found cTnT in serum, despite evidence of skeletal muscle trauma and release of creatine kinase [16–18]. Only when a large-scale release of skeletal troponin T occurs with nonspecific binding to the assay tube wall will a problem appear, because of detection of this isoform by the second antibody [19]. This has been resolved by the reformulation of the assay. The study by Müller-Bardorff et al. shows that small increases found in the first-generation ELISA were abolished in the second-generation test. We may therefore conclude that, even if cTnT is present in normal skeletal muscle, it does not pose a problem in routine clinical practice.

The data for muscular dystrophy are more suggestive. Reexpression of cTnT would be most likely in this group. The immunohistochemistry data show clear staining of some but not all fibers, although the staining is less intense than that seen in fetal skeletal muscle or fetal cardiac muscle. Again, which one of the isoforms is being expressed is unknown; the data are compatible with a fetal or an adult isoform of cTnT. The Western blot data show multiple bands but the positions of the bands differ from that of the dominant cardiac isoform. Müller-Bardorff et al. show a decrease in the cTnT detected with the reformulated assay. Perhaps the first-generation ELISA shows cross-reaction with a reexpressed isoform or a skeletal isoform. Until histochemistry and Western blot analysis are performed with an antibody specific for the dominant adult isoform of cTnT—ideally, the antibodies used by Müller-Bardorff et al.—the questions raised by these studies remain unresolved.

The differential diagnosis of cardiac damage in patients with muscular dystrophy is interesting but is not a frequent problem in routine clinical practice. Much of the discussion on the relative merits of cTnT and cTnI comes from studies in patients with renal failure. One of the problems is that early reports used high cutoff values for cTnI [19]. Reformulation of the cTnT ELISA reduces but does not abolish the number of patients with renal failure in whom cTnT is detectable. Conversely, cTnI clearly is also detectable in some patients with renal dysfunction [20]. The relative proportion of patients with detectable cTnT exceeds that of cTnI in all reports [21–24] and in my experience. The question of the origin of the excess of detectable cTnT in this group remains unexplained. Does the greater proportion of patients with renal dysfunction in whom cTnT is detected rather than cTnI mean that cTnT is more sensitive to minor cardiac damage in this patient group? The possibility of reexpression remains to be definitively confirmed or excluded. However, a single, properly timed measurement of cTnT or cTnI will be sufficient only to exclude rather than confirm cardiac damage. A positive result will require sequential measurements. The clinical significance of increases of cTnT and cTnI in patients with renal failure is unknown and will require outcome studies.

It is important to maintain a sense of perspective. The role of cardiac troponins is to detect myocardial injury in patients in whom acute coronary syndromes are suspected. Unlike with other markers, investigators have made a direct attempt to link measurement with outcomes. The ability of cTnT to predict short- and long-term prognosis has been unequivocally demonstrated in several studies in patients with unstable angina [25–28] and myocardial infarction, both with and without ECG changes on admission [29, 30], and there is a developing body of literature (although not as extensive) for cTnI, at least for short-term prognosis [31, 32]. Use of either marker is recommended by the American Heart Association [33]. Direct comparison of cTnT and cTnI has demonstrated either a similar prognostic efficiency [34] or superior prognostic efficiency for cTnT [35], depending on methodology. The suggestion has been made that cTnT and cTnI may recognize different subpopulations in unstable angina patients [36] and that cTnT may be more sensitive to minor degrees of damage [37]. The improved version of the cTnT ELISA seems to have overcome the problems of the first-generation assay in the presence of extensive muscle damage, leaving both cTnT and cTnI superior to CK-MB for differential diagnosis in patients with acute chest pain with or without skeletal muscle trauma.

Is there reexpression of a fetal (or adult) isoform of cTnT that gives rise to false-positive values in certain patient populations? If so, is this of clinical diagnostic and prognostic significance? Both papers address these questions. It appears possible that cTnT is reexpressed in certain patient groups. This may cause diagnostic difficulty or may provide a convenient marker for a generalized myopathy that includes a cardiomyopathy. Neither paper provides complete answers. One cannot categorically claim superiority of one troponin over another, and we may indeed find that cTnT and cTnI measurements are not exactly equivalent. The current data suggest that more