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EDITORIAL COMMENT

Testing the Wrong Hypothesis: The Failure to Recognize the Limitations of Troponin Assays*

Allan S. Jaffe, MD, FACC
Rochester, Minnesota

The study by Khan et al. (1) in this issue of the *Journal* seeks to determine whether elevated levels of cardiac troponin I (cTnI) have prognostic significance in "asymptomatic patients with chronic renal failure" being treated with chronic hemodialysis. Unfortunately, because of the limitations of the assay for troponin and the cut values chosen, the study fails to evaluate this question and serves rather as an example of an increasing common problem (i.e., attempting to use troponin assays to make distinctions that are beyond the analytic capabilities of the assay). Studies with these sorts of problems, which are proliferating, have the potential to confuse clinicians about how to use troponin measurements.

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The question that the study was designed to answer is an important one. It is now clear as indicated by the investigators that frequent elevations of troponin occur in patients with renal failure. Although most of the previous studies have suggested that these elevations have adverse prognostic significance in this population, most studies have included a heterogeneous group of patients, including many with coronary artery disease (CAD). Because CAD is the most common cause of morbidity and mortality in patients with renal failure (2), many of the elevations, as in patients with ischemic heart disease but without renal failure, likely will have prognostic significance (3). However, it is not at all clear that all elevations are related to CAD in this group of patients. There have been reports that elevations in troponin may be related to the presence of left ventricular hypertrophy (LVH) in association with markers of abnormal coronary vasomotion (4), and marked changes in ventricular volume and thus afterload (i.e., wall stress), which occur frequently in patients with renal failure, have been suggested to be the cause of troponin elevations in patients with congestive heart failure (5).

Furthermore, there are substantial perturbations in protein synthesis associated with the abnormal metabolic milieu of renal failure (6). Thus, there likely are elevations in troponin that may not be due to coronary heart disease, and there is legitimate question about whether these elevations also impart an adverse prognosis. From first principles it is unlikely these abnormalities impart a positive prognosis; however, the extent to which they may be transient and be responsive to therapy, and the magnitude of their effect and the time course during which an effect may be manifest, are all unclear.

What can be said with greater reassurance is that from the data available, the troponin being detected comes from the heart. In the subset of patients with renal failure included in the study by Ooi et al. (7) (20 of the 78 patients), elevations of troponin were almost always associated with evidence of cardiac injury. In addition, multiple studies have attempted to find evidence of troponin expression in organs other than the heart in patients with renal failure, and all have thus far confirmed the fact that neither cTnI nor T values are elevated for this reason (8–11). From this perspective, the question Khan et al. (1) want to answer is an important issue related to whether elevations of cTnI not likely due to CAD but likely due to other more subtle myocardial injurious processes can result in an adverse prognosis over time.

The investigators attempt to exclude all patients with possible ischemic heart disease by eliminating all of those with any history suggestive of it or with chest discomfort or electrocardiographic findings suggestive of ischemia. It may be that they also excluded many but clearly not all of the patients with volume overload or LVH as well. This concern could have been obviated by providing more information about the patients who were enrolled and at least some information about the group from which they were chosen.

The investigators then measured one cTnI value in the patients and separated the group into those with putative elevations and those without. It is unclear whether the clinicians caring for the patients were or were not aware of the cTnI values or whether any actions were taken in these patients by those clinicians. Indeed, we know very little at all about the management of these patients over time.

These patients were then followed for a two-year period. The primary end point of the study was death or hospital admission. Why the investigators elected not to use myocardial infarction as an end point is unclear. Indeed, elevations of troponin substantially over the baseline value with a pattern characteristic of acute cardiac injury could easily have been used to confirm the diagnosis. One wonders, was there a difference in this diagnosis? Similarly, it is unclear whether the frequency of a percutaneous coronary intervention, which can be done as an outpatient, was similar between the groups. It would have been helpful for the investigators to have reassured us in the Results section about these issues.

Nonetheless, even addressing these issues would not have saved the study; this is because of the problems related to

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From the the Mayo Clinic and Graduate Medical School, Rochester, Minnesota. Dr. Jaffe is a consultant for Dade Behring and receives research support from Dade.

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the cut points they chose to use and the analytical characteristics of the assay they employed. It should be appreciated that the assay chosen was a first-generation assay, with all of the difficulties of first-generation assays. This is clear from the literature (12). Unfortunately, at times investigators wish to lower the cut points for detection using these first-generation assays when second- and third-generation assays that are more sensitive become available. At times that is done without recognizing the analytical problems that exist. It is the lack of appreciation of the analytical problems of the assay and cut points used in this study that casts substantial doubt on the credibility of the results reported. The analytical problems associated with the approach used include the following:

1. As with all assays, there is substantial analytical variability at the lower limit of detection. Being cognizant of this fact is why so many manufacturers suggested higher values for diagnosis despite the fact that it was clear that lower values would likely be of significance. For this assay, the within-run assay variability was reported as <20% for a value of 0.046 ng/ml (12). Thus, it was likely greater at 0.03 ng/ml, and the variability between runs is invariably much greater than the within-run value. Hence, it is likely that the variability of the assay used for this study was in the range of 30% to 50%. Taking 50% as an example, which would not be unexpected given issues such as lot-to-lot variability, a value of 0.03 ng/ml could be as low as 0.015 ng/ml or as high as 0.045 ng/ml. Accordingly, the mean values between the group called *elevated* and the one called *normal* probably overlap very substantially and are no doubt similar statistically. From the standard deviations provided, it appears that there were very few values that were substantially different from each other once this high degree of variability was taken into account. Unfortunately, the raw values are not provided, but it would not take too many misclassifications to undercut the validity of the study given the modest number of patients involved. This problem could have been diminished to some extent had all samples been done in duplicate. Even if lower values for variability are used, the principle is, nonetheless, the same although the magnitude of the effect might be lesser.
2. All assays can have problems related to heterophilic anti-mouse antibodies. There has been some suggestion that the Beckman assay may be more prone than others. If the frequency is as suggested for the average assay to be 1% or 2%, one of two of the elevations, if analytically real, could have been due to this problem (13).
3. This assay and several other first-generation assays have been known to have elevations attributable to fibrin interference. Indeed, all elevations with this assay for study purposes need to be confirmed after recentrifugation to reduce the importance of this problem (M. Panteghini, personal communication, 2001). The information from Beckman in the package insert emphasizes this problem and how to reduce its impact.
4. The assay in question is presently being removed from the market not solely to replace it with a second-generation assay but because it is now clear that the epitopes for the antibodies used for detection in this assay are cleaved from the carboxy terminal both in myocardium and in blood. Thus, if increases depend on accumulation over time, they could be missed compared to other assays due the degradation of the epitopes needed for detection (14). This could be still more of an important consideration if cTnI is additionally cleared by dialysis. Although the investigators quote studies suggesting that cTnI is not cleared during dialysis, others suggest it is (15). It may be that the details of dialysis (dialysis pore size, flow rate and adjunctive therapy) and the specific assay used may be key factors in explaining such discrepancies.
5. It is known that there is substantial variability with this assay if samples are not measured immediately, owing to the degradation of the epitopes involved with detection and the fibrin problems mentioned above. Several examples of this have been presented (M. Panteghini, personal communication, 2001). The investigators (1) do not mention whether the assays were done immediately after samples were obtained and spun. If not, this would introduce still another source of error.

If one considers these issues conjointly, it appears very likely that the groups were not separated adequately for the purposes of risk stratification. It is likely that some patients with elevations were missed due to the high degree of variability and cleavage of the epitopes of interest; also, some elevations were spurious. In a study of this size, the misclassification of even small numbers of patients could badly skew the results. This lack of ability to separate the groups raises substantial questions about whether the study really addressed the question it posed.

Unfortunately, given these considerations, it appears that the investigators failed to answer the question they posed about cTnI elevations in asymptomatic patients with renal failure. However, they have confirmed what clinicians see and struggle with every day—that is, the assays they believe they are supposed to rely on do not work in the way that the experts suggest they should. This is a major problem that many of us are very concerned about. It has been a consistent problem in the cardiologic literature where very few investigators tend to pay attention to these issues.

Until and unless cardiologists and laboratory researchers join together to promulgate clear standards, these problems will persist. That is why many of us have championed the need for consistent standards for cut points and for imprecision. The European Society of Cardiology/American College of Cardiology (ESC/ACC) (16,17) and the International Federation of Clinical Chemistry and Laboratory

Medicine (IFCC) (18) have recommended that the cut points used for decision making be associated with no more than 10% imprecision. Manufacturers are beginning to understand this and to develop the techniques to meet this challenge. Dr. Fred Apple and I, with the support of the chairs of the ESC/ACC conference on the redefinition of myocardial infarction, have written to the Food and Drug Administration (FDA) under their good guidance program, suggesting that the FDA request and publish the information needed to make these decisions for all the troponin assays they evaluate. In addition, assiduous quality control is necessary in all aspects of the laboratory efforts in this area to detect false positives and to consult on the pros and cons of various assays. It is essential that cardiologists realize that all assays cannot be used at highly sensitive levels and give satisfactory answers.

This report (1) is not the last to test the assay involved and find it wanting. We need to make sure that we distinguish between studies that do that from those that test the principle in which we are interested. The issue of whether troponin elevations are of importance in patients with renal failure on dialysis who have no evidence of ischemic heart disease is still one worthy of continuing research, but only with assays and cut points that allow that principle to be tested.

Reprint requests and correspondence: Dr. Allan S. Jaffe, Cardiovascular Division and the Department of Laboratory Medicine and Pathology, Mayo Clinic and Graduate Medical School, West 16-Cardiovascular Diseases, 200 First Street SW, Rochester, Minnesota 55905.

REFERENCES

1. Khan IA, Wattanasuwan N, Mehta NJ, et al. Prognostic value of serum cardiac troponin I in ambulatory patients with chronic renal failure undergoing long-term hemodialysis: a two-year outcome analysis. J Am Coll Cardiol 2001;38:991–8.
2. U.S. Renal Data System: USRDS 1998 Annual Report. Chapter VI. Causes of death. Bethesda, MD: The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1998.
3. Ottani F, Galvani M, Nicolini FA, et al. Elevated cardiac troponin levels and the risk of adverse outcome in patients with acute coronary syndromes. Am Heart J 2000;140:917–27.
4. Lowbeer C, Ottosson-Seeberger A, Gustafsson SA, Norrman R, Hulting J, Gutierrez A. Increased cardiac troponin T and endothelin-1 concentrations in dialysis patients may indicate heart disease. Nephrol Dial Transplant 1999;14:1948–55.
5. Missov E, Calzolari C, Pau B. Circulating cardiac troponin I in severe congestive heart failure. Circulation 1997;96:2953–8.
6. Jaffe AS, Ritter C, Meltzer V, Harter H, Roberts R. Unmasking artifactual increases in creatine kinase isoenzymes in patients with renal failure. J Lab Clin Med 1984;104:193–202.
7. Ooi DS, Isotalo PA, Veinot JP. Correlation of antemortem serum creatine kinase, creatine kinase-MB, troponin I, and troponin T with cardiac pathology. Clin Chem 2000;46:338–44.
8. Davis GK, Labugger R, Van Eyk JE, Apple FS. Cardiac troponin T is not detected in Western blots of diseased renal tissue. Clin Chem 2001;47:782–3.
9. Bodor GS, Porterfield D, Voss EM, Smith S, Apple FS. Cardiac troponin-I is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue. Clin Chem 1995;41:1710–5.
10. Ricchiuti V, Voss EM, Ney A, Odland M, Anderson PAW, Apple FS. Cardiac troponin T isoforms expressed in renal diseased skeletal muscle will not cause false-positive results by the second-generation cardiac troponin T assay by Boehringer Mannheim. Clin Chem 1998;44:1919–24.
11. Haller C, Zehelein J, Remppis A, Müller-Bardorff M, Katus HA. Cardiac troponin T in patients with end-stage renal disease: absence of expression in truncal skeletal muscle. Clin Chem 1998;44:930–8.
12. Christenson RH, Apple FS, Morgan DL, et al. Cardiac troponin I measurement with the ACCESS® immunoassay system: analytical and clinical performance characteristics. Clin Chem 1998;44:52–60.
13. Fitzmaurice TF, Brown C, Rifai N, Wu AHB, Yeo KTJ. False-positive increases in cardiac troponin I with heterophilic antibodies. Clin Chem 1998;44:2212–4.
14. Katrukha AG, Bereznikova AV, Filatov VL, et al. Degradation of cardiac troponin I: implication for reliable immunodetection. Clin Chem 1998;44:2433–40.
15. Wayand D, Baum H, Schatzle G, Scharf J, Neumeier D. Cardiac troponin T and I in end-stage renal failure. Clin Chem 2000;46:1345–50.
16. Antman E, Bassand JP, Klein W, et al. 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.
17. Jaffe AS, Ravkilde J, Roberts R, et al. It's time for a change to a troponin standard. Circulation 2000;102:1216–20.
18. International Federation of Clinical and Laboratory Medicine (IFCC). Quality specification for cardiac troponin assays. Clin Chem Lab Med 2001;39:174–8.