

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Cardiac troponins are now considered the gold standard in the laboratory diagnosis of myocardial infarction. The May issue of the journal *Clinical Chemistry* includes a report on the role of monitoring changes in serum concentrations of sensitive cardiac troponin I in the diagnosis of myocardial infarction.

The lead author in that study is Dr. Fred Apple. Dr. Apple is Professor of Laboratory Medicine in the Department of Laboratory Medicine and Pathology at the University of Minnesota and Medical Director of Clinical Laboratories and the Clinical Chemistry and Toxicology Laboratories at Hennepin County Medical Center.

Dr. Apple, in your opinion, what are some of the most important recent contributions to the study of cardiac biomarkers?

Dr. Fred Apple: I would say the most important contributions of cardiac biomarkers center around the growing experience we have with cardiac troponin, either cardiac troponin I or cardiac troponin T. Manufacturers of these assays are improving the precision at the 99th percentile, which therefore imparts an improved diagnostic accuracy, with increased clinical sensitivity, and also, we are learning, as the analytical characteristics improve, we're seeing improved risk stratification for adverse events.

Host: Tell us the importance of measuring changes or a delta for cardiac troponin concentrations as soon as possible after a patient arrives at the hospital.

Dr. Fred Apple: What we have learned regarding the importance of looking at the delta, or a change over time, between the initial sampling of a patient and then a sampling taken at a second time after presentation, is that we're learning that it's adding diagnostic value to improving, not the clinical sensitivity, but we're seeing improvements in the ability to improve diagnostic specificity and ruling out for myocardial infarction in patients who present with symptoms suggestive of acute coronary syndrome.

And also, we're learning that this change in delta is also imparting improvements in risk stratification of these patients, specifically those that present with acute coronary syndrome.

Keep in mind just for definition, delta is a term that was coined maybe ten years ago, that it's just looking like a delta, a change between one time point and another time point, looking at two concentrations for cardiac troponins that are measured.

Host: Can the 30% delta used in your study be applied to all cardiac troponin assays?

Dr. Fred Apple: No, the 30% delta value that we use in the current study was specifically based on the assay that we utilized in the study, which was an assay manufactured and marketed by Ortho-Clinical Diagnostics, their VITROS ES Assay. The reason I select that specific comment is the importance of understanding that each assay tends to have different analytical characteristics, and it's critically important that the database based on each assay be evaluated statistically on its own.

For the current study that we published using the Ortho Assay, we did an ROC curve and we optimized the delta value, and in our study we looked at a six-hour window, just the way the samples were drawn in the patient care arena at Hennepin County Medical Center, where we enrolled the patients, and we found that the optimal delta concentration percentage was 30%.

We have looked at other assays that are not published in the study, and we found one to be 50% and one to be 90%. So therefore, although there are some guidelines published on expert opinion, in one case and point the NACB guidelines talks about using a 20% delta that should not be uniformly accepted as the delta concentration for all assays as we proved in this specific study.

Host: Are there still some holes in the evidence-based literature regarding delta cardiac troponin measurements, and ideally, what further study should be added?

Dr. Fred Apple: Yes, I think the delta is in its, I would say, early stages of assessment regarding the diagnostic accuracy in approving outcomes-based prediction.

A couple of these have to do with which assay, as we mentioned earlier, the assay diversity. As we know there are over a dozen FDA-cleared assays in the marketplace by different manufacturers. So each assay needs to be assessed based on its own

individual dataset to find out which delta value is optimal.

Second, it's critically important to understand the timing of the specimens. If the first specimen we draw at presentation we will call zero hour, when we start calculating deltas, some studies are based at six hours. There was one study that it showed that was optimal at three hours. That I think what we are going to find as we improve towards improved high sensitive cardiac troponin assays, that timing will become smaller.

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So we need to, as we move forward with improved and better assays, we almost have to reinvent the study each time to investigate if timing plays a key role to improve, as we talked about the diagnostics and risk stratification.

Then also, we cannot lose sight of the fact that each assay has its inherent precision based around what we call the 99th percentile or the upper reference value, as well as each assay may have a slight different biological variation.

So taking into consideration, not just the changes and time between two samplings, but also the assay in precision at that cutoff, the 99th percentile and what the biological variation, will be very important.

Now, secondly, we had just talked about first, the use of this delta in acute coronary syndrome patients, a very important part will be to explore further pathologies that have different mechanisms of injury. These are the pathologies that are not overt ischemic heart disease presentations.

For example, patients who come in renal failure, patients who come in with rhabdomyolysis, some other form of cardiac injury, from a blunt chest trauma, some pulmonary embolism. So I think the one area of research that could really impact in the non-ECS group would be to systematically go through each of these pathologies and see if the delta does play a role in improving specificity, because the one thing we did show that was critical to the current study was the fact that if we combined a initial sample greater than 99th percentile and then invoked the 30% change or delta criteria, we were able to show first, as we look at the outcomes type risk assessment of the rate of adverse events, the

combination of a greater than 99th percentile initial sample and a greater than 30% change, we saw a rate of adverse events of 78%.

If we go back and just take a look, based under initial sample with a normal less than 99th percentile, we saw our adverse rate was 11%. So we saw a sevenfold increase, a relative risk increase of adverse events.

Second, where the delta is a very powerful tool is in the role of clinical specificity. As I talked about, many patients who do come into the hospital have an increased cardiac troponin that is not due to someone being ruled out for heart attack. So therefore, in the case of this Ortho-Clinical Diagnostics' ES assay, at presentation, the clinical specificity was 77%, and the six hour sample, the specificity was 81%.

Now, that means that two out of ten positive results did not have a heart attack, and that's going to be concerning to clinicians, because they're going to say, no, this assay shows a lot of not false-positives, but a lot of positive results that are not indicative of myocardial injury due to a heart attack.

By looking at the delta we were able to improve the clinical specificity to 91%, and that is really on the order where we used to be in the old ROC curve cutoff days.

So improved diagnostic specificity and improved risk outcomes assessment are two areas which need further studies as we look at each individual assay as they are approved and cleared by the FDA in this field.

Host: In what ways may delta calculations impact future guidelines for clinical and laboratory practice?

Dr. Fred Apple: Currently, the guidelines are predicated on the 99th percentile of cardiac troponin, and we use that 99th percentile first to say, any increase above that is indicative of myocardial injury, number one.

Secondly, we use that cutoff to say that in the clinical setting of myocardial ischemia and an increasing cardiac troponin above the 99th percentile, those two criteria are enough to make the call of a diagnosis of myocardial infarction.

So the universal definition of myocardial infarction is predicated on troponin, an increased troponin, and a change, a rising and/or falling cardiac troponin value. So therefore, what we need to think about in the future, as I had mentioned the specificity, even though we are very sensitive in detecting, what we'll need to look for in the future for guidelines is to implement that delta.

So the global task force that was responsible for the universal definition, the biochemical subcommittee of that is now looking at the evidence-based literature and what's out there for any assay, looking at deltas and trying to develop an educational scheme that will be the first step in bringing real evidence into the guidelines versus just an expert opinion.

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Host: What strategies would you suggest for implementing delta measurements in reporting in current clinical and laboratory practice?

Dr. Fred Apple: I think the role of implementing a delta does not really have to wait for guidelines. For example, what our plans are for our institution is, the current assay that we use; we have put a database together and we have real data on our six months, a year worth of patients who have come in to know what our clinical sensitivity is, and we know what our clinical specificity is.

We have gone ahead and calculated what the appropriate delta is, which turns out to be about 60%, which will show that with the delta 60%, and we have a four hour time window, because we have orders at our hospital at zero, four, eight hours, we can actually show again that we improved specificity to about 90% from about 75% clinical specificity, just based on an individual value.

So I would recommend to each institution if they have the power to do this, which they should if they're computerized, they should be able to use their own hospital database, they should be able to do their own calculations, and they should provide to their clinicians, whether it's through a type of white paper or even in their electronic LIS system or electronic health record, publish a little table that shows what their clinical specificity is based on the 99th percentile and build into that what the optimal delta would be.

So if you do have two specimens, show what's either equal to or better as far as discerning the specificity issue. Where this is critical is, it will help educate clinicians not to think that every troponin that's elevated means a heart attack, and not to think that every time they see an elevated troponin, they need to take the patient for example to the cath lab.

So what this will help discern is the acute presenting patient with a real onset of chest pain or equivalent due to onset of acute myocardial infarction with a rising pattern, we're going to see a very high sensitivity and we're going to see a very good specificity.

The specificity part will help discern the patient with the chronic change, where there is increased troponin that it stays static. So if your cutoff is, in this case, for this assay, in our paper is 0.034, if we see 0.06, that stays 0.06, that stays 0.06 over a zero, six, twelve hour window, it indicates injury, but it's not an acute event.

So I think the importance of educating clinicians between an acute changing value or an acute value that shows an increase but does not change will be very instrumental, if we can provide real data from our own hospital database implementing that delta. Note, 30% change, it's a chronic elevation, a rapid changing delta, pointing towards a more acute event. Any therapies in triage and management will most likely be somewhat different for those two sets of patients.

Host: Okay. Well, to your knowledge, are there any biomarkers in development now that will improve or potentially replace cardiac troponin as the primary diagnostic or risk stratification biomarker in patients who have symptoms of acute coronary syndrome?

Dr. Fred Apple: There is a wealth of, I will call them proof of principle type of studies on multiple biomarkers that are attempting to improve risk assessment outcomes of adverse events based on looking at markers, I would say upstream from the markers of necrosis cardiac troponin.

So markers from just systemic inflammation, like CRP, to markers for a plaque unstabilization or destabilization or ischemia, many different biomarkers have been looked at. One for example is myeloperoxidase.

The thought with these is, can they be used together or independently with troponin to improve risk or diagnostics? So number one, there are some multi-marker studies out there that shows cardiac troponin is really the definitive marker for diagnostic accuracy. So there is no other biomarker that assists and/or will replace cardiac troponin, except the next-generation of cardiac troponin, which I will call the high-sensitive cardiac troponin assays.

Although this is the same assay, what we now are moving towards is the ability to measure cardiac troponin at very low concentrations.

For example, the contemporary assays we currently use in the marketplace, and I will base it around the paper we just published, for example, the Ortho Assay uses a cutoff for the lower limit of detection of 12 pg/mL. The newer generation assay, the high sensitive assays that have been published are showing down to 0.2 pg/mL. So essentially an order or two magnitudes lower.

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There was a recent paper published by a group out of Brigham Hospital in Boston that was able to show that acute changes based on one of these four described high-sensitive assays, and the four that have been described have been, company's names, Singulex and Nanosphere, and Beckman has described an assay, and so has Roche for troponin T, those four companies. This assay that was used in the European Heart Journal Study by the Brigham Group showed that acute changes using this high sensitive assay was accompanied by Singulex. They were able to predict inducible myocardial ischemia looking over a window as short as two hours and looking at a delta of 3 pg/mL, and now that 3 pg/mL change is even lower than the current contemporary assay's lower limit of detection.

So I think what we are going to see is not a new marker but an improved cardiac troponin marker, and that's going to even raise the bar for troponin such that for any other biomarker to come into really add substantial risk stratification potential will be very difficult.

What we have seen though is some early data that maybe one of the natriuretic peptides in combination with the contemporary troponin assays do add some value. But the tests and the proof will have to be to

take one of these high-sensitive assays and add another biomarker, whether it's a natriuretic peptide or something else: to see if there is added value in predicting.

The challenges will be, whether as we move towards improved high-sensitive assays, again we're going to have to keep in mind these assays still have to demonstrate quality specifications regarding assay precision, demonstrating day-to-day reproducibility, and again, the concept of how we truly define what a normal range is so we can have a cutoff that will be like a line in the sand to say normal or not normal.

But I think the future is quite bright for cardiac troponin, and I'm not hedging the bet that this will be the biomarker around in the next five to ten years without something else that's going to supplant it.

Host:

Dr. Fred Apple is the Professor of Laboratory Medicine in the Department of Laboratory Medicine and Pathology at the University of Minnesota, and Medical Director of Clinical Laboratories and the Clinical Chemistry and Toxicology Laboratories at Hennepin County Medical Center, and he has been our guest on this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.

Total Duration: 17 Minutes