

Host: This is the *Clinical Chemistry* Podcast. I am Bob Barrett. Since the 1950s, with the discovery of aminotransferase as the first biomarker of myocardial infarct, medical laboratories have had a defining role in diagnosis of heart disease. Enzymes, and more recently other markers, such as the troponins, have been included in many diagnostic guidelines.

In the last two years or so high sensitivity troponin assays have been available. Is this an advance or merely marketing hype?

Dr. Allan Jaffe is a cardiologist who has spent his career working in the area of cardiac biomarkers. Dr. Jaffe sits on editorial boards of many prestigious journals, in both cardiology and laboratory medicine, and has been a member of most committees in these areas that formulate diagnostic guidelines.

He is presently Professor of Medicine and Chair of Core Clinical Laboratory Services at the Mayo Clinic in Rochester, Minnesota.

Dr. Jaffe, I am sure you know about the new high-sensitivity troponin assays.

Dr. Allan Jaffe: Yes, fortunately I have had a chance to work with some of them, and I am glad to discuss them with you. I just need to say that I will try and need the focus on those things that are in the public domain or have been either presented, either in abstract or paper form, because there are certain things that when you work with companies you simply can't review at an early stage of development.

Host: Now, are you an advocate for the diagnostic use of the new high-sensitivity troponin assays?

Dr. Allan Jaffe: Like in general, I am. I think that whenever you have assays that will increase precision, increase sensitivity, you have the potential to start to diagnose a far larger number of individuals who are at risk and who have heart disease, and also to do this with a greater rapidity.

The challenge is to understand both the analytic problems that this high sensitivity assays are going to bring to us, and also to understand how to use them by doing appropriate clinical studies. So, I think if we do our due diligence, it will turn out these high-sensitivity assays are going to be of great utility. If we don't, they will cause confusion.

Host: But aren't all present-day troponin assays high sensitivity?

Dr. Allan Jaffe: Well, really not, and I think this is where there is some confusion in the field. The first generation assays were really very, very insensitive, and many companies didn't put out second or third generation assays very readily. So, when these second and third generation assays came out, clinicians said, my goodness, these are very much more sensitive, and point of fact they were, and the names that the companies used often were misleading.

So, for example, the second-generation Bayer assay was called the Ultra, and it was thought to be ultra sensitive. From my perspective, that assay simply fits with a lot of the other very sensitive and very good high sensitivity troponin assays that we are using in a contemporary fashion today.

The reason why I think it's important to understand that is because the data that we have from other studies that validated how to use the values in an intelligent way to help patients still obtain, as we get the higher sensitivity assays, the next generation, which is what I would monitor high sensitivity, then we are going to have to do additional analytic and clinical validation. With the assays today though, they are more sensitive than they used to be, do not fulfill that rubric in my estimation.

Host: Well, then, if most modern assays are not high sensitivity, how should high sensitivity be defined?

Dr. Allan Jaffe: Well, it's an interesting question, and it's a little bit of a problem. The reality is that you can't take numbers from one assay and extrapolate them to another, because these assays are set up differently and what is called "calibrated" differently so that a given number with one doesn't correlate with a given number for another. So, we have been struggling with how to define the next generation of assays.

I think one of the consensus that is starting to develop is that it will be done by looking at the number of normals that one can define as having a value.

So, for example, presently most of the assays do not define values for most of the normal population. The values are undetectable or below the level where you can really trust the accuracy of the assay.

So, I think the way we are going to define these high sensitivity assays is by saying, they have to detect 50% or 70% or even 100% of normal individuals and provide a value and a baseline from which one can work.

Host: Are there assays out there that do that?

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Dr. Allan Jaffe: Well, there do appear to be so. One that is published in *Clinical Chemistry* is from a group called Singulex, which suggest that they can measure values in all normal individuals.

The same implication, although I am not sure that a publication has validated this, comes from a group called Nanosphere. Last year at the American Association for Clinical Chemistry meeting there were abstract presentations from both Roche, with a high-sensitivity troponin T assay and Beckman, with a high-sensitivity I assay. Both of which suggested that again, they could measure values in all normals.

Which I might add parenthetically is sort of interesting, because many of us have been sort of concerned and thought perhaps that one should not be able to measure anything from troponin since its indicative of a cardiac cell injury, and it's sort of inelegant to think that there is even a very low concentration, but it appears to be that that's the case.

Host: How low a concentration can these assays go?

Dr. Allan Jaffe: Well, again, it depends upon the assay that you look at. At present, one looks, often the assays are in the picogram or a say 20 or 30 or 40 picograms as a value. With the Singulex assay for example, as published, it's down in the low picogram level, one to say ten. The same thing with the Roche and the Beckman assays. The Nanosphere assay actually is reported that they are able to measure down into the femtogram level, even more sensitive.

Host: So it seem you are proposing that these new high-sensitivity assays replace the older so-called first and second-generation tests. Are these high sensitivity assays ready for widespread use today?

Dr. Allan Jaffe: No, I don't think so, and I think we have to be very careful before we turn them loose on the clinical population. The first thing to understand is that when you get this level of exquisite sensitivity, one has to be sure that all of the things that are analytically important, such as making sure what you are really measuring.

I have posed the question as to whether or not you could be measuring at these very low levels, some sort of cross-reacting interference substance, and not really troponin. We did a small study with the Singulex antibodies looking at that by substituting a nonspecific antibody in the capture

position and seeing if we detected anything with our tagged antibody.

Unfortunately, we didn't detect very much. It was only one patient where we detected something, and that wouldn't have moved that patient from a normal to an abnormal status.

Nonetheless, this is an important issue in terms of making sure we are really detecting troponin, the same thing. All of the studies suggesting that troponins have total specificity for the heart were done with much less sensitive assays and methods than we have today.

I am not sure that we should suspect that there will be problems with specificity, but very much we must redo these sorts of studies to confirm that.

Similarly, interfering substances like hemolysis, which tends to lower the values in some assays and raise them in others, need to be explored specifically, because with these sensitive assays, a tiny bit of an interference, let's say, lowered value, could move it to a normal range, similarly a small amount to a high range. So we have got to be terribly careful analytically.

In addition, we need to do clinical studies. At present, as I indicated above, with the present troponin assays if someone comes in with, for example, chest discomfort, and one thinks they may be having, what we call acute coronary syndrome, or most of the time what ends up being diagnosed as a non-ST MI, myocardial infarction, we know that those patients benefit from the aggressive use of anticoagulants like heparin, from platelet inhibitors like 2b/3a, and from going to the cath lab for an early invasive strategy.

As we begin to use these new high-sensitivity assays, there may come a time when we are detecting such small amounts of injury that that may longer be the case. So, we have got to go back and do the clinical studies, not just of these patients who present acutely, but about patients who might have more chronic elevations as well.

Host: So, what's your estimate of when these will be ready for general use?

Dr. Allan Jaffe: I think the analytic part, once it's clear that these studies must be done—and I think they are hard studies to do so there may be some resistance to doing them—can be done fairly quickly. I would say within a year.

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I think the longer problem is going to be trying to figure out clinical use. For example, most of the clinical validation of these assays and how to use them have come from very large clinical trials, maybe 10,000 and 20,000 patients. These old assays were used in those trials. Maybe there are some samples that remain to test these new high-sensitivity assays, but I am not sure that's the case. If so, one may need to find the next set of clinical trials in this area to get sufficient numbers of patients to really confirm what the right strategies are.

In addition, we have ended up finding that as we have gotten more and more sensitive, a fair number of chronic elevations of troponin, which are indicative of underlying cardiovascular disease, and at some point in time, these chronic elevations and these acute elevations are going to overlap more substantially than they do today. We need to develop the strategies with data about how we are going to deal with that, otherwise, we are going to have tremendous difficulty knowing which patients require acute care in the hospital and which patients can be sent home.

So, I think that's the big challenge. I think that will take much longer than the year it will take to do the analytics. It partially depends on how aggressive companies who are promulgating these assays are willing to be in terms of trying to find these answers.

Host: Well, let's look ahead, once validated, how do you see these assays helping physicians in making a diagnosis?

Dr. Allan Jaffe: Well, because the assays will be more sensitive, and there will be also—by definition, in order to have a sensitive assay, you need a high degree of precision. So that it will be much easier to see when an elevation is present, much easier to see if the pattern of elevation is rising. So that these assays will allow much earlier diagnosis.

There are a variety of examples used with going from less sensitive to more sensitive assays. The group from the Brigham and Women's Hospital, Mallinson and David Morrow have shown that when they went from the first-generation Bayer assay to the second-generation assay that patients who did not have elevated troponins for eight or twelve hours, now the vast majority had elevations right at the time of admission.

David Morrow has presented data, at least verbally, suggesting that the Nanosphere assay also markedly facilitates the rapidity with which one can make a positive diagnosis.

One of the questions I have that I think we need to focus on a little bit more, however, is how rapidly one can then make the rule-out diagnosis. I have some concerns about that because there may be some patients in whom the anatomy is such that it still will take three or six hours to really be sure that an event is not occurring.

Finally, we will be able to start to see elevations that are more chronic in patients perhaps who develop LVH, who are starting to have an early signs for a cardiomyopathy. There is elegant data from the group in Milan suggesting, even with the present iteration of assays that we can start to monitor drug toxicity, such as with Adriamycin. The group has gone further to show that one can develop therapeutic approaches to those elevations and therefore reduce the frequency of chronic heart failure.

So, I think we will see more of those elevations, and we will be able to start to monitor things like drug toxicity. There is an elegant paper suggesting carbon monoxide poisoning can be diagnosed, and that there is maybe an important role there.

So, I think we are going to be able to find a large number of additional disease entities if we are smart and work on it. The diagnosis of which will be facilitated by these high-sensitivity assays.

Host: Any emerging technology is going to involve some risk. What problems will these assays pose?

Dr. Allan Jaffe: Well, I mean the first thing, and I mentioned it above is that we have got to be sure that we eliminate false-positives and false-negatives by having these assays be much more analytically pristine. If we don't, clinicians are going to see false-positive elevations that they can't understand in people who look normal, and in point in fact may be, and false-negative elevations, and they are going to send patients home who are going to do badly.

So, the first thing we need to do is to make sure the analytics are absolutely solid. Then we are going to have to develop the criteria that are necessary to make these work.

So, for example, Dr. Wu, using the Singulex assays has suggested that if one adds biologic variability to the equation of analytic variability, that may be to know that a pattern is rising may take, my recollection serves me correct, a change of 47%.

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So, we have to start developing these operational guidelines of how we use troponin to decide when an elevation is present. If we don't, clinicians are going to have difficulty figuring out whether or not one number, which goes up modestly, is enough to meet that criteria, and that's going to cause confusion. So we are going to need to work that through.

Finally, we are going to see a lot of elevations that we can't explain. This has been the history of troponin elevations all the way through. Just recently, several groups, including my own, have published data that suggest that there are a substantial number of individuals, mostly women, who present with chest pain, elevated troponins, seem to have normal coronary arteries, but when one does magnetic resonance imaging, they have a signal suggesting that they have acute myocardial infarction.

This fits data in women suggesting they have more endothelial dysfunction. They have more plaque erosion. But we had to learn that because early on clinicians who saw patients who had normal coronary arteries and who otherwise looked like they were having myocardial infarction, thought that the biochemical test was falsely positive. In point of fact, we now know that that's a true positive.

Similarly, we learned that when we were looking for these patients that a huge number, actually 50% of individuals who present with chest pain, elevated troponins, often ECG changes, and who have normal coronary arteries, when one does magnetic resonance imaging have myocarditis. This was something we didn't know, and I think we underestimated the frequency of myocarditis, because we didn't have the tools; neither troponin nor MR guidance was available until recently.

Now we have learned that some of these elevation troponins supposed to being false-positives are due to myocarditis, and myocarditis is becoming a much more important entity, both acutely and chronically.

We are starting even to see in a paper published just last week that myocarditis can masquerade as other disease entities, such as arrhythmogenic right ventricular dysplasia. So, as we get down in the noise, there is going to be a large number of low-level elevations of troponin, and people are going to say, what are these due to and we won't know. We are going to need to take some time to figure out how we diagnose these patients so that the test actually add value

to patient care, rather than confusing the clinicians who are doing them.

Host: Well in the end, are you optimistic that the advantages are going to outweigh any disadvantages?

Dr. Allan Jaffe: Very much so. I think that if we do our due diligence. So, we make sure the assays are good, and we make sure that we do the clinical research that is necessary, that this will be a major advance that will help the field.

If we rush these new assays too quickly, however, I think we have a chance to confuse the field and end up hurting it. And already there are problems that clinicians face every day, with the present iteration of sensitive assays, that will be magnified with these new assays if we don't go slow enough and generate the data that we need to make these work well.

Host: Dr. Jaffe, thank you so much for being with us today.

Dr. Allan Jaffe: It's my pleasure.

Host: Dr. Allan Jaffe is a cardiologist who has spent his career working in the area of cardiac biomarkers. He is presently Professor of Medicine and Chair of Core Clinical Laboratory Services at the Mayo Clinic in Rochester, Minnesota.

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