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This is the podcast from *Clinical Chemistry*. I am Bob Barrett. In the early 1990s, *Clinical Chemistry* reported on the development of monoclonal antibodies for an assay for measurement of cardiac troponin-I and the preliminary results evaluating application to the detection of myocardial damage.

Since its publication, the paper by Dr. Jack Ladenson and his colleagues has been cited nearly 300 times. The March issue of *Clinical Chemistry* included a citation classic article by Dr. Ladenson that followed almost three decades of troponin-I assay developments, which has led to high-sensitivity assays and to troponin in the blood being one of the requirements for the definition of myocardial infarction.

Dr. Ladenson is currently the Oree M. Carroll and Lillian B. Ladenson Professor of Clinical Chemistry at Washington University School of Medicine in St. Louis, and he is our guest in this podcast.

In the late '70s, some people may have thought that researching CK-MB, or creatine kinase-MB, monoclonal antibody assay, was not practical because of the technical difficulties involved. So tell us Dr. Ladenson what were some of the challenges you and your colleagues faced when developing the assay?

Dr. Jack Ladenson:

Well, actually there was a lot of interest in assay for CK-MB, and as I recall, a company, HyperTech, which was later bought by Beckman, had some type of point-of-care assay based upon an antibody to the M subunit and an antibody to the B subunit, but I am not sure it got particularly popular. However, CK-MB in the early 80's did have a number of challenges.

First was strain of mice to be immunized to create the monoclonal antibody. Almost, everyone used the BALB/c strain, but we had very poor success with it, and it turned out that a different strain called the "A/J strain" was the one that ultimately gave the strongest response to some of the antibodies that actually eventually got used in the assay.

The other major challenge was the screening assay to see if the mice had produced antibody, because at that time, the way one screened was by "coding" the antigen, just basically putting a solution of the antigen on a plastic well of an ELISA plate.

After you cleared the solution, you washed it with a solution that had blocking agents that would block on the attached slides on the plastic because this was the fairly non-specific

reaction between a protein and components on the plastic of the well.

After that, you would then add an antisera to the species you are raising the antibody in. So if it was a monoclonal mice, you might add an anti-mouse rabbit antibody which would have a label usually an enzyme on it, and that's what you would detect.

The difficulty with this is that when you put the antigen directly on the plastic, it often assumes a different conformation that has in its native solution form. And for reasons I don't fully understand the animals can actually make antibodies to this altered conformational form. And those types of antibodies will not bind into the substance when it's in solution like blood. They will only bind in when it's on plastic or if it's been through the process to make a Western Blot.

We actually in the lab used to call those non-sense antibodies, and we had a tremendous trouble with this until we changed the standard approach, and we modified the screening Assay. So we were able to get, be looking at the antigen in its more native form, and then we started to get antibodies that did react with the native protein.

And I firmly believe that part of our success is that we were really not trained in Immunology, but we were trained in Analytical Diagnostics, and one of the team colleague, Dave Dietzler, was a really renowned entomologist, and he kind of understood the enzymes and CK-MB was an enzyme, and we actually today, in our work, used not inbred mice like the strains I mentioned, but totally outbred mice, I think they are called Swiss Websters, to get a broader spectrum of antibody response because as you create these inbred strains, it limits the way they present the antigen.

Host: So how exactly did this assay differ from what was already in use? What was its benefit to patients and clinicians?

Dr. Jack Ladenson: CK, creatine kinase, has three forms: MM, a MB, and a BB, and MB form is enriched in the heart and that was the logic behind using it as a test. These three forms have slightly different charges so we could use electrophoresis to separate the proteins on the basis of charge, and then we would overlay the paper electrophoresis or cellulose acetate, whatever medium, with a visualization agent, something that would react with creatine kinase or any color.

The process took two to three hours, and initially it was okay, but in the early '80s they started to be very specific therapy for myocardial infarction, and the purpose of the therapy was to break up the clot, which was the cause of

the infarction, the blockage of blood flow, and initially it was done by drugs, first Streptokinase, and then tPA.

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And once these drugs started to get into clinical trials, and I think the first was approved in early 1980s, there now is a premium for having a very safe assay to use these drugs as soon as possible. So, now we in the laboratory had a ballpark three-hour assay, and the clinicians had a drug that they were told to have it used within six hours. And it really got to be kind of an interesting relationship between the lab and the clinicians to stay mildly.

We eventually I think got to seven routines, starting around electrophoresis. So, all in all we have a test that worked, but it's the therapeutic changes that made its timing to be really inappropriate. So, the big advantage of antibodies they test, I think eventually there were a number of companies that came out, but almost all of them were in the 10 to 15 minute range, a tremendous advance from the roughly three hours.

Host: What about your exploration of cardiac troponin I for the detection of myocardial damage?

Dr. Jack Ladenson: Once we did have this quantitative assay for CK-MB, we really could see that there was much more CK-MB in blood and even in some tissues, and tissues particularly skeletal muscle than it was originally thought. So if somebody had a runner or somebody exercises, they could actually would be turning over skeletal muscle, and some of those numbers were quite high. But even people without that degree of exercise, it turned out that they had significant amounts of CK-MB, which were being derived from skeletal muscle and not cardiac muscle.

Part of the reason that became more apparent is with the electrophoresis procedure, we used to have to often dilute the samples to be able to run among these electrophoretic gels, and we often won't see small amounts of CK-MB. But now with direct quantitation, we did see them. So we started to look mainly by literature search at that time for possible substances that might have more specificity. And in the literature, there were two that looked promising: one was a substance called myosin-light-chain, and the other troponin I.

Now it turns out that myosin-light-chain, as it got further explored by others has the exact same structure in cardiac and skeletal muscle, and the assay for it that had been reported after it was looked at more closely by Hugo Katus, it turned out to be an assay for troponin T.

So, on troponin I, a group led by Cummings at Cambridge, England had in the late 80's did some nice work showing that it looked like it had some promise for detecting heart damage, and that's why we actually simultaneously started the study, both proteins than in centered on troponin I.

Host: Now the development of the troponin I Assays has led to high-sensitivity Assays. In your opinion, are these high-sensitivities Assays experiencing widespread use today?

Dr. Jack Ladenson: Well, high-sensitivity is the relative term, which really has no true meaning, because there is always something higher than high. Once the specificity of the troponins was established, then it made sense to increase the singlet, noise of the assay to get more sensitivity. And now terms, such as "high-sensitivity," and I'm sure someone will start using "higher-sensitivity" soon, have come about. For the most part they've been widely used.

But in the latest versions, if the current data hold up, then it appears there might be a spread of different values of troponin I even in otherwise healthy individuals in essence of normal range, whereas the most common assays in use, one doesn't detect troponin I unless there has been some cardiac damage. So if this indeed bears out, then one would really have to understand a lot more in few respects. It was thought that the heart did not turnover that essentially myocytes were not under routine turnover, which if there is a spread of values in blood would imply it is.

I think there is a whole bunch of understanding about why and how that occurs if we're going to use assays that are really taking us down very, very low. So we can make sure we have true pathology behind the presence of finding these proteins that are typically only inside the heart cell and not just from this turnover which we do not understand, because we did not know it was there.

Host: Over the decades, we've seen a pattern of new markers replacing older ones, transaminases replaced by CK, CK replaced by CK-MB, CK-MB replaced by the troponins. Do you believe the current refinements of troponin measurements will continue or will an entirely new marker replace the troponins in the near future?

Dr. Jack Ladenson: I don't know. Obviously, there has been a move towards more specificity and with it some sensitivity, but what it would require is some type of a substance that would have at least the same sensitivity and specificity as troponin, which might get to blood much more rapidly.

One of the difficulties in interpreting these tests is, when there is a heart attack, there is a blockage of blood flow on the arterial side, so there is poor blood flow in the damaged area. Now one has these proteins or other substances coming through the inside of a cell, we now have a blocked blood flow.

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And most of it actually gets into the main blood system through the lymphatic system, and it's kind of like trying to drive from Washington, DC, to Boston using side-streets and not the inner state.

So if there is going to be an improvement, I think if it has to be some kind of a substance which could be much more rapidly detected following the start of an MI, and I think it might be difficult to find such a substance, but one never says never.

There is another area of investigation, which I think is intriguing is people are starting to look at a marker for ischemia of the heart cell. In other words where it's just enlarged which is thought to be the earlier processes developing heart disease.

I think if a marker of that type could be found then I think it would be very valuable because cardiologists have a number of very good tools for stress test, angiography, and things of this nature that could follow up a screening test that would say that we were looking for it.

I kind of look at say something that would be 6 to 12 months before a possible MI, it's being a potentially very valuable test. We have very good ones that can give predictive value of 20 years later that most people I know don't usually worry about 20 years but they will worry like heck about a few months.

Host: With that in mind, what are you currently working on?

Dr. Jack Ladenson: Well, the heart is kind of getting busy, so I have shifted over to looking at markers of brain injury. We have some promising data but we're running into—one always finds new issues when you are doing this type of work, and issues such as substances having across the blood-brain barrier to get the blood rather slow flow as spinal fluid, and the sampling points relative to the time of the injury and actually try, and now this won't get the sampling point closer to the time of injury. These are all factors we're trying to sort out now.

Host: Dr. Jack Ladenson is the Oree M. Carroll and Lillian B. Ladenson Professor of Clinical Chemistry at Washington University School of Medicine in St. Louis, and he has been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.

Total Duration: 12 Minutes.