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Full-Size Cardiac Troponin I and Its Proteolytic Fragments in Blood of Patients with Acute Myocardial Infarction: Antibody Selection for Assay Development

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Guest: Dr. Ivan Katrukha is a researcher at HyTest Limited in Turku, Finland and at the Department of Biochemistry, School of Biology of Moscow State University.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Cardiac troponins are recognized as the primary biomarkers used in the diagnosis of acute myocardial infarction. These are commonly detected by immunoassays, which offer the wide availability and rapid results needed in clinical care scenarios to rule out myocardial infarction. However, immunodetection methods can be limited when there is degradation of the target analyte, as is the case with cardiac troponin I.

Early studies suggested that the central region, located between amino acid residues 30 and 110, was the most stable part of the molecule. This was a driver for the majority of current diagnostic systems to utilize antibodies with epitopes located within the central region. A down side to this approach is that auto antibodies also bind to this region, potentially causing interference and erroneous results.

As more recent studies have identified additional cardiac troponin I proteolytic fragments, it is important to understand their composition and prevalence in the blood of patients with acute myocardial infarction. Furthermore, there is a need to evaluate whether monoclonal antibodies specific to the regions beyond the central section of the molecule can be used to develop cardiac troponin I assays with improved performance over those currently available.

An original article appearing in the July 2018 issue of *Clinical Chemistry* describes a study investigating these concepts. We are joined for this podcast by the article's first author, Dr. Ivan Katrukha. He is a researcher at HyTest Limited in Turku, Finland, and a researcher at the Department of Biochemistry at the School of Biology of Moscow State University.

Doctor, what was the main idea of doing this study?

Dr. Katrukha: So cardiac troponin I is currently measured in a lot of patients with presumed heart attack by means of immunoanalysis. However, several studies have shown that troponin I is prone to degradation and this is really might influence the result of the analysis. And up to 11 different troponin I fragments were found in blood of patients with myocardial infarction.

In one of our early in vitro studies, we have shown that the middle portion of troponin I that lies approximately between the 30s and 110th amino acid residue is the most stable part of the molecule. And since then, it was suggested that in order to get the most reliable, the most precise, results the diagnostic immunoassay should include monoclonal antibodies that are specific to this central region of troponin I. But later, it was shown that this very central part of troponin I is the most affected by autoantibodies that are present in some patients. And these autoantibodies negatively influence the immunodetection of troponin I.

So after that, our researchers were kind of caught between a rock and a hard place. On the one hand, the degradation of troponin I forced them to use the central part of the molecule for the assay development. But on the other hand, usage of the central part could have led to a false-negative result due to the interference caused by autoantibodies.

And so, the idea of our work was to investigate the degradation of cardiac troponin I more thoroughly. First we wanted to border the fragments of troponin that were present in blood of patients with myocardial infarction. And second, we want to calculate the relative abundance of these fragments and to study the change of this relative abundance in time after the infarction. Based on this information, we wanted to understand if it would be safe to use the antibodies that are specific to some portions of cardiac troponin I other than the central stable region when developing a diagnostic immunoassay.

Bob Barrett: So, what methods did you use during the study?

Dr. Katrukha: We have worked with a series of serum samples of patients with ST-elevation myocardial infarction that underwent stenting or the occluded coronary artery. And each series consisted of five samples. The first one was taken before the revascularization, this was approximately from one to five hours after the onset of chest pain, and four other samples were taken after stenting. And those were taken for up to 36 hours after the onset of chest pain. And we have studied the degradation of cardiac troponin I by two different methods.

First one was Western blotting, and the second was sandwich fluoroimmunoanalysis. To border the fragments, we performed Western blotting of troponin that was immunoprecipitated from the blood samples of patients with heart attacks and we stained this blotting with 15 different monoclonal antibodies that were specific to the different epitopes on the cardiac troponin I molecule. In order to calculate the relative abundance of this fragment, we stained troponin and its fragments with one monoclonal antibody that we knew was able to interact with all detectable fragments. And most importantly, this antibody did it with equal intensity.

To perform pure immunoanalysis, we measured the concentration of cardiac troponin in the samples by two antibody pairs. One pair utilized antibodies that were specific to the terminal portions of the molecule, and the other one utilized antibodies that were specific to the stable central part of the molecule. And then we calculated the ratio of the measured concentration for each patient at each time point. And our idea was that if the terminal regions of troponin I were cleaved off during the study timeframe, we would have seen a considerable decrease in the calculated ratio. If the terminal regions were not cleaved off, the ratio would not decrease over time.

Bob Barrett: So let's get down to it, doctor. What were some of your findings?

Dr. Katrukha: We were able to detect the full-sized troponin I and 11 of its proteolytic fragments. And we also discovered that the set of fragments was the same in all study samples. The smallest detected fragment consisted approximately of amino acids starting from 23rd to 126th. And actually, this concurs well with the most stable region that was described by us previously.

We also quantified the amount of different fragments that showed that approximately 40% of all detected troponin I was presented by the intact troponin; which is even more surprising, more than 80% of all detected troponin comprised the amino acids 23 to 196. Studying the samples that were taken at different times after infarction, we have quite unexpectedly found that first of all, stenting didn't significantly influence the abundance of different cardiac troponin I fragments. And secondly, that the abundance of different fragments did not significantly change during the first 36 hours after infarction. The latter was also confirmed by means of fluoroimmunoanalysis of the samples, where we saw only two-fold falling of the ratio of the concentrations that were measured by terminal and central pairs of monoclonal antibodies.

Bob Barrett: So finally doctor, unpack this a little bit, what does this mean for us?

Dr. Katrukha: Previously, it has been thought that cardiac troponin I is rapidly degraded in the blood flow of patients with myocardial infarction. And this imposed obvious restrictions on the usage of antibodies that were specific to the terminal parts of the molecule. But actually, our study shows that the degradation of cardiac troponin I in blood flow is not as profound as it was thought before. First, the absence of the dynamics in the abundance of different fragments and time indicates that *in vivo* degradation of cardiac troponin I takes place mainly in the necrotic myocardium but not in the blood flow of the patient.

Secondly, the fact that the ratio of cardiac troponin I fragments did not really change after stenting indicates that we have used the correct model and the data we obtained from patients that underwent stenting could be actually extrapolated to patients that are being diagnosed for infarction. The diagnostics obviously happen prior to stenting. Next, the fact that more than 80% of the detected troponin I comprised the epitopes that fall within the amino acid residue from 23rd to 196th.

And that the ratio of the fragments remained almost unchanged during the first 36 hours after infarction shows that immunoassay utilizing monoclonal antibodies specific to these amino acid residues would be able to detect more than 70-90% of troponin I and its fragments in the sample. Not only in the early sample, but in a relatively late sample as well.

Earlier, Dr. Kim Pettersson and his team first showed that monoclonal antibodies that are specific to these regions of cardiac troponin I would be almost insensitive to the presence of autoantibodies. So the selections of the antibodies that are specific to the region starting from 23rd ending with 40th amino acid residues, or to the region that starts from 140th amino acid residues and at approximately 200th amino acid residues, could lead to a reasonable compromise between two major shortcomings of cardiac troponin I assays: negative interferance of the proteolytic degradation of cardiac troponin I and negative interference of troponin I outer antibodies.

Bob Barrett: Dr. Ivan Katrukha is a researcher at HyTest Limited in Turku, Finland, and is a researcher in the Department of Biochemistry at the School of Biology of Moscow State University. He has been our guest in this podcast from *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening.