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# Different Susceptibility of B-Type Natriuretic Peptide (BNP) and BNP Precursor (proBNP) to Cleavage by Neprilysin: The N-Terminal Part Does Matter

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**Guest:** Dr. Alexander Semenov is Project Manager of Research and Development at the biotech company HyTest in Turku, Finland.

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

Well, the B stands for brain. The B-type Natriuretic Peptide, or BNP, is a circulating peptide hormone involved in maintaining cardiorenal homeostasis. BNP is synthesized in a form of a precursor called proBNP, which consists of 108 amino acids.

Increased production of proBNP is associated with cardiac pathologies caused by pressure or volume overload. Both products of proBNP processing, BNP and the n-terminal peptide of proBNP, are currently established as biomarkers of heart failure. It is thought that BNP clearances at least partially associated with cleavage by Neprilysin, a ubiquitous protease that is especially abundant in the kidney.

In the clinical laboratory, BNP concentrations are most often determined by means of immunoassays. Because conventional BNP immunoassays cross-react with proBNP, BNP measurements actually reflect the total concentration of both immunoreactive forms, the bio-active BNP and the intact pro-hormone proBNP. Since there may be an influence of Neprilysin inhibition on BNP measurements in patient plasma samples, it is important to know whether proBNP, the major form of BNP immunoreactivity is as susceptible to Neprilysin as BNP is.

The April 2016 issue of *Clinical Chemistry* published a paper comparing the susceptibility of both BNP and proBNP to proteolysis by Neprilysin. Dr. Alexander Semenov is the Senior Author of that paper and he joins us in this podcast. He is the Project Manager of Research and Development at the biotech company HyTest, in Turku, Finland.

Dr. Semenov, tell us about the current interest in protease Neprilysin.

Dr. Semenov: Well, Neprilysin was actually unknown by the cardiologist society until the release in 2014 of the new heart failure drugs called LCZ696. The brand name is Entresto. LCZ was

 

developed by Novartis and recently approved by the FDA. This new heart failure drug is made up two components, sacubitril which is a Neprilysin inhibitor and the other one valsartan, which is angiotensin receptor inhibitor. So, the question is why was Neprilysin chosen as a therapeutic target.

Neprilysin is an ubiquitous protease which is specially abundant in the kidney. This protease is responsible for the degradation of various important vasodialactic peptides, also including natriuretic peptide. Thus, the idea is to augment endogenous level of natriuretic peptide by inhibiting Neprilysin, and because Neprilysin is a therapeutic target, all this highly promising drugs, it explains the current growing interest to Neprilysin itself.

Bob Barrett: What can you tell us about how it affects the natriuretic peptide system?

Dr. Semenov: Yeah. The action of this drug is twofold, it augments endogenous concentrations of natriuretic peptides and inhibition and degradation by Neprilysin; and secondly it causes vasodilation while inhibition of the renin-angiotensin-aldosterone system. So the LCZ is expected to augment circulating concentrations of natriuretic peptide by inhibition of the degradation by Neprilysin.

Bob Barrett: Is Neprilysin involved in processing of precursors of natriuretic peptide?

Dr. Semenov: Actually, quite an interesting point. It's known, natriuretic peptides are synthesized in the form of prohormones which have to be clipped to produce biologic compounds. Both protease precursors are mediated by specific enzyme. For example, the BNP precursor is clipped by protease furin, and ANP precursor is clipped by protease corin. We have been studying the mechanisms of protease processing for quite a while since these mechanisms are important understanding of these mechanisms, help to understand the mechanism of heart failure development and also to better understand the result of natriuretic peptide measurements.

Bob Barrett: So, doctor, it seems that this new heart failure medicine LCZ696, or Entresto, has raised some important questions regarding the use of brain natriuretic peptide and N-terminal prohormone brain natriuretic peptide as heart failure biomarkers along with the Entresto therapy. Can you comment on this?

Dr. Semenov: Well, those are the tools. There are several important issues that are again in the use of BNP and NT-proBNP as heart failure biomarkers that has been raised by the release of these new heart failure drugs. It's thought that BNP

 

clearance is at least partially associated with the cleavage by Neprilysin and since LCZ is a Neprilysin inhibitor, it's expected to increase the circulating concentrations of BNP and consequently make BNP measurements ambiguous misleading from a diagnostic point of view.

Neprilysin is thought to degrade BNP and have no effect on the breakdown of NT-proBNP. One may expect that patients who are treated with LCZ will have higher plasma BNP levels due to the inhibition of Neprilysin activity. Conversely, in NT-proBNP. Levels are expected to be not influenced by Neprilysin inhibition. However, I should say that this suggestion is based on a very simple model of biochemistry of natriuretic peptide. This model does not consider the presence of proBNP or prohormone NT-BNP in the circulation, and as we know proBNP is often a major BNP immunoreactive form.

Bob Barrett: Do you agree with the commonly raised suspicion that a wider clinical use of this new heart failure drug may diminish the clinical utility of B-type natriuretic peptide testing?

Dr. Semenov: Well, the answer to the question is clearly not obvious. First of all, we should keep in mind that the conflict biochemistry (00:06:06) heart failure forms. As the BNP levels are augmented by LCZ, one might suggest that the measurements of BNP should not be used along with LCZ therapy and instead NT-proBNP should be used to follow the therapy. I would say that the two competing views, it should be first that so far the augmentation of BNP level on patients on the LCZ therapy was observed only with the one commercial BNP assay, and it follows from our present study, the different epitopes of BNP have different sensitivity to the cleavage by Neprilysin.

Consequently, different BNP assays may give different results. The effects of Neprilysin inhibition on the level of BNP and NT-proBNP should be studied in future clinical trials using several immunoassays based on different antibodies to give a definite answer to these questions.

Bob Barrett: According to recent studies, high levels of circulating brain natriuretic peptide may inhibit Neprilysin. Given this, do you think that LCZ696 may have different effects in patients with low and high levels of circulating BNP?

Dr. Semenov: Right. The data obtained in the recent study of people of (00:07:29) suggest that elevated BNP levels may impede the activity of circulating Neprilysin. So it means that BNP may act as an endogenous inhibitor of Neprilysin. From a clinical perspective, this means that LCZ may have a different effect in patients with low and high levels of circulating BNP due to the different activity of Neprilysin.

Consequently, we may suggest that the BNP measurements can be used to understand at what BNP levels that LCZ therapy should be applied.

Bob Barrett: Well finally, doctor, do you agree with the suggestion that either both biomarkers, brain natriuretic peptide or N-terminal prohormone brain natriuretic peptide, or their ratio, should be applied along with the Entresto therapy to fully utilize the diagnostic and prognostic value of these biomarkers?

Dr. Semenov: Personally, I think that presently it's too early to make any final conclusions whether the BNP or the NT-proBNP should be preferred as biomarker to use along with LCZ therapy. Currently, we have limited data and definitely more clinical data are needed to answer these questions. In future clinical trials, this would be studying the effect of Neprilysin inhibition on the level of BNP and NT-proBNP using several immunoassays based on different antibodies.

The effect is expected to be assay dependent. This data should shed some light on the subject and help clinicians to decide whether BNP or NT-proBNP alone or maybe both biomarkers should be used in order to fully understand the heart failure status of patients and finally choose the appropriate treatment strategy. For example, an increased level of BNP may serve as a readout of an adequate LCZ dosing while in NT-proBNP levels might reflect the effects of drug on the function of the heart.

Bob Barrett: That was Dr. Alexander Semenov. He is a Project Manager of Research and Development at the biotech company HyTest in Turku, Finland. He has been our guest in this podcast from *Clinical Chemistry* on BNP, Pro-BNP and Neprilysin. His paper on that topic appeared on the April 2016 issue of *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening!