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# Detection of Neprilysin-Derived BNP Fragments in the Circulation: Possible Insights for Targeted Neprilysin Inhibition Therapy for Heart Failure

**Article:**
Evgeniya E Feygina, et al.
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**Guest:** Dr. Evgeniya Feygina is a researcher in the R&D department of the biotech company HyTest located 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 am Bob Barrett.

B-type natriuretic peptides, or BNP, and N-terminal proBNP, or NT-proBNP, are peptides produced in the heart in response to increased wall stretch and volume overload. Their production and secretion increases in the heart with the progression of heart failure and they have emerged as useful heart failure biomarkers. Since the discovery of BNPs in the 1980s, much effort has been made to precisely determine the BNP and NT-proBNP levels via immunoassays for reliable heart failure diagnostics.

Entresto™ is a new heart failure therapy that includes sacubitril as one of its components. Sacubitril is a specific inhibitor of neprilysin. This is a zinc-dependent metalloproteinase that cleaves various peptides including BNP. In fact, augmentation of circulating BNP due to neprilysin inhibition is considered as a possible mechanism of Entresto's positive effects.

A paper appearing in the October 2019 issue of *Clinical Chemistry* examines the circulating products of BNP proteolysis by neprilysin and how they might reflect impact on the metabolism of active BNP. The lead author of that report is Evgeniya Feygina who works as a researcher in the R&D department of the biotech company HyTest in Turku, Finland. Dr. Feygina is our guest in this podcast.

So, doctor, lately a lot of attention has been driven to the role of natriuretic peptides and heart failure pathology. What are the relations between natriuretic peptides, neprilysin, and heart failure management?

Dr. Feygina: Well, natriuretic peptides, NP and BNP, are well-established heart failure biomarkers, and BNP particularly is a gold standard marker for heart failure diagnostics. On the other hand, both NP and BNP, they have physiologically active molecules in their hormones. So, normally as well as in heart failure, NP and BNP promotes, naturalizes, sterilizes

vasodilation and in order to reduce myocardial loads which is good for the failing heart.

Neprilysin is a protease that cleaves active NT and BNP into inactive fragment and prevents their positive effects. So, a lot of efforts were made to therapeutically inhibit neprilysin and thus prevent natriuretic peptide degradation. And a few years ago, Novartis pharma introduced a new drug, Entresto and it was the first-in-class ARNi drug. ARNi stands for Angiotensin Receptor-Neprilysin Inhibitor and as it comes from the TITE. So, it contains specific neprilysin inhibitor called sacubitril. Entresto was shown to be very effective in the reducing heart failure complications. And one the possible mechanisms suggested is that ARNi inhibits neprilysin and facilitates the positive natriuretic peptides activity.

However, now scientists do believe that ARNi is not equally good for every patient, and there are some side effects, sometimes the drug titration is complicated as well. So, if we could pick the right patients who could benefit the most from ARNi therapy, it would be really useful for clinical practice.

So, what patients are the right patients? If we think about the BNP circulation as of a swimming pool, the huge pool was the incoming and outcoming pipes as from the problems, that's crucial mass. We can imagine that the BNP pool is filled through the incoming pipe which is processing and this pool is depleted, so there is outcoming pipes and these are renal clearance, the subdividing proteolytic cleavage. And neprilysin is just one of these outcoming pipes.

So, if we stay inside this picture, neprilysin inhibition is a valve on this outcoming neprilysin pipe. The most benefit from neprilysin inhibition would come from the O4 dose with the fast BNP production and high neprilysin activity. In this case, neprilysin inhibition would make a change. So, we need to distinguish the patients with effective natriuretic peptide production and high neprilysin activity as well.

Bob Barrett: Measuring natriuretic peptide production and neprilysin activity at the same times seems to be challenging. Are there any established methods?

Dr. Feygina: Currently, there are fluorogenic substrates for neprilysin activity measurement in the sample and they're commercially available. But there are actually two problems with this approach. First thing is these substrates tend to lack specificity unfortunately, which is crucial in our case. And another issue is related to neprilysin biochemistry. This

protease exists in two forms. One is transmembrane, tissue bone form and another is soluble, it is present in circulation.

So, as neprilysin is distributed between tissue and blood stream, we need to measure the inherent neprilysin activity and we cannot do that with the fluorogenic substrates and measuring in the sample. And as I said before, apart from neprilysin activity, we need to estimate the natriuretic peptide production level, and how can we do these two things at the same time? I think we suggest the solution: let's measure the circulating products of NP and BNP proteolysis by neprilysin. While, indeed these neprilysin-derived fragments may reflect both parameters, the level of natriuretic peptide production and the level of the degradation.

Bob Barrett: So, considering all the complicated system of neprilysin forms and natriuretic peptides, tell us what is your hypothesis?

Dr. Feygina: The sites of natriuretic peptides proteolysis by neprilysin were described before and for BNP they're the most susceptible ones between amino acids 4 and 5 and the less susceptible one between residue 17 and 19. So, when BNP is cleaved, the new epitopes emerge and we call them neo-epitopes. And for the sites mentioned, they are neo5, neo17 and neo18.

And as I said before, the sites are known, but the BNP forms containing these neo-epitopes and so-called neo-forms. They were never described before. So, we developed the specific BNP-neo17 immunoassay which is based on the antibodies specifically recognizing the neo17 epitope and this study detects only the BNP fragments that are cleaved at 17, 18 neprilysin site, and it does not contract with the BNP molecule.

Our first thing to do was to analyze BNP cleavage by neprilysin in vitro with or without sacubitril or the neprilysin inhibitor, and thus we measured BNP-neo17 formation in vitro. And then we used a rat model to study BNP-neo17 in vivo. We injected human BNP into the bloodstream of rats that were pre-treated with sacubitril or with saline orally and then we collected red plasma samples at several time points after the injection and analyzed the BNP-neo17 formation. And the last thing we did, we measured BNP-neo17 in human plasma in heart failure patient samples.

Bob Barrett: Were your results in accordance with the previous knowledge, and what were the novel findings?

Dr. Feygina: As we expected, we found that BNP-neo17 is generated both in vitro and in vivo, and this process is blocked by sacubitril.

So here, we show for the first time the existence of this new BNP form and we provide evidence that BNP-neo17 formation is neprilysin dependent and we will also successfully detected BNP-neo17 in heart failure plasma samples which is also very new.

Bob Barrett: Okay doctor, finally let's look ahead, your novel BNP form, BNP-neo17, what future perspective does it open?

Dr. Feygina: We believed that neprilysin derived BNP fragments and namely BNP-neo17 might reflect the neprilysin contribution in BNP metabolism. So, if we speculate, BNP-neo17 is a candidate biomarker to predict how effective the neprilysin inhibition would work as a therapy. So, if were able to use BNP-neo17 for prior to treatment, patient's discrimination, it would be another valuable step towards the personalized medicine in cardiovascular field. And we also can imagine that BNP-neo17 would work for heart failure monitoring as well, alone or in combination with other biomarkers. For example, with total BNP or with NT-proBNP and we do believe that BNP-neo17 might provide new information on how natriuretic activities are restored by ARNi which would be relevant fundamentally as well as for the clinical practice.

Bob Barrett: That was Dr. Evgeniya Feygina from the R&D department of the biotech company HyTest located in Turku, Finland. She has been our guest in this podcast on the interrelationships between new heart failure therapies and the marker BNP. Her paper on that topic appears in the October 2019 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.