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In-Depth Analysis of Molecular Heterogeneity of Circulating N-Terminal pro-BNP: Does Detailed Characterization of Analyte Structure Really Matter for Its Diagnostic Use?

Article:
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Guest: Dr. Alexander Semenov of the biotech company HyTest located in Turku, Finland and the School of Biology, Lomonosov Moscow State University in Russia.

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.

The two B-type natriuretic peptides are produced in the heart in response to increased wall stretch and volume overload. Since their production and secretion increases in the heart with progression of heart failure, they have emerged as useful and cost-effective heart failure biomarkers. Since the discovery of BNPs some 30 years ago, considerable effort has been made to accurately measure BNP and NT-proBNP using immunoassays for reliable heart failure diagnostics. As a result, the measurement of these biomarkers is used as a tool by clinicians worldwide to diagnose acute and chronic heart failure, stratify risk, and monitor response to therapy.

In the September 2020 issue of *Clinical Chemistry*, Herbert Lindner and his colleagues at the Innsbruck Medical University in Austria described 16 previously unknown fragments of NT-proBNP, revealing a more profound degradation of NT-proBNP than previously known. An Editorial appearing in the same issue of *Clinical Chemistry* discusses this in-depth analysis of the molecular heterogeneity of circulating N-terminal proBNP and asks the question, does detailed characterization of analyte structure really matter for its diagnostic use? The author of that Editorial is Alexander Semenov, from the biotech company HyTest located in Turku, Finland, and the School of Biology, Lomonosov Moscow State University in Moscow, Russia. He is our guest in this podcast. So, Dr. Semenov, first of all, what are the clinical applications of B-type natriuretic peptides and NT-proBNP?

Alexander Semenov: Oh well, BNP and NT-proBNP are widely used for diagnostic evaluation of heart failure. Measurements of these biomarkers are globally accepted and used in clinical practice for the diagnosis of acute and chronic heart failure for risk stratification and monitoring response to therapy. In clinical practice, BNP and NT-proBNP detection is done by immunoassays as a standard method. The first BNP and NT-proBNP immunoassays were developed in the mid-90s.

And since that time, a number of assays have become commercially available and were introduced into clinical practice. However, the complexity of circulating BNP related forms and the diversity of assays resulted in relatively poor comparability between different assays and platforms and this complicates the interpretation of results.

Bob Barrett: What aspects of natriuretic peptide's biochemistry as well as nuances in measuring these analytes do you see as most important?

Alexander Semenov: BNP is produced in the heart in response to increased wall stretch and volume overload. Active BNP molecules are released into the circulation along with physiologically inert integral BNP as a result of endoproteolytic cleavage of precursor molecules called proBNP. During its maturation in cardiomyocytes, proBNP undergoes post-translational modifications. This is like oscillation in the N-terminal region of the molecule. BNP is present in the circulation as a mixture of multiple proteolytic forms and an additional level of complexity arises from the presence of glycosylated proBNP as a predominant circulating BNP-immunoreactive form in both heart failure and healthy individuals. In its turn, NT-proBNP is represented by a mixture of glycosylated and non-glycosylated forms. It should be highlighted that commercial assays for BNP and NT-proBNP detect all fragments containing the epitope recognized by utilized antibodies. There is currently no commercially available BNP or NT-proBNP assay that does not cross-react with proBNP due to the presence of common epitopes.

Bob Barrett: Do you think that elucidation of protease mediated forms of BNP has attracted more attention than that of NT-proBNP or proBNP?

Alexander Semenov: Apart from glycosylation, the diversity of circulating NT-proBNP/proBNP forms may arise from the coexistence of forms generated by the activity of proteolytic enzymes in the circulation. Indeed, protease-mediated forms of BNP have attracted more attention than NT-proBNP/proBNP due to the effects of BNP proteolysis on the physiological activity of BNP. For instance, some attempts are currently ongoing to find the BNP form to monitor patient's dynamics and the treatment with certain heart failure drugs like Entresto.

Bob Barrett: How did the results of the study by the Austrian group improve our current understanding of the molecular heterogeneity of the circulating forms of NT-proBNP and proBNP?

Alexander Semenov: The detailed characterization of truncated NT-proBNP or proBNP forms has been lacking until recently. The authors described 16 previously unknown fragments of NT-proBNP

and proBNP. Nine fragments are located in the N-terminal region and seven fragments located in the C-terminal region. The study revealed a more profound degradation of NT-proBNP than previously known.

Bob Barrett: Well doctor, how would you evaluate the methodological approaches used in that paper to characterize the multiple forms of NT-proBNP and proBNP?

Alexander Semenov: The authors applied the cutting-edge quality of mass spectrometry based analysis to characterize fragments of NT-proBNP and I would especially highlight a very elegant approach using O-18 isotope labeling during proteolytic in-solution digestion to validate the specificity of the cleavage by the end of proteinase and this is the first report describing NT-proBNP and proBNP forms in healthy subjects. The authors successfully have overcome the challenging conditions for detection due to very low concentrations in healthy subjects.

Bob Barrett: Are there any limitations to that study that you think should be considered when interpreting the results?

Alexander Semenov: Well, I would just mention a few limitations of this study. First, the authors did not perform separation of proBNP and NT-proBNP prior to mass spectrometric analysis. This could be easily accomplished by means of affinity matrix with BNP specific antibodies and because of this, it's not possible to make any conclusions about any possible differences in N-terminal truncation between NT-proBNP and proBNP. Second, the choice of antibodies for immunoextraction of NT-proBNP doesn't seem to be optimal for the efficient binding of all possible circulating fragments and third, from the clinical perspective, it would be very interesting to see if certain peptides might be more disease-specific than others. However, this issue lies beyond the scope of the study.

Bob Barrett: What are the consequences of those findings for routine diagnostic use of natriuretic peptides in clinical practice?

Alexander Semenov: Well, science is known to be a gradual process of never-ending updates and updates are slowly pushing the boundaries of our knowledge. As has been well exemplified by cardiac troponins, detailed characterization of an analyte structure and metabolism may take decades of research finally resulting in novel assays with improved precision. So, tiny pieces of data generated in numerous studies are absolutely critical for the development of highly sensitive and accurate assays. Obviously, detailed characterization of an analyte structure metabolism is absolutely crucial to understand the reasons for potential discrepancies in the measurements obtained by different assays and platforms.

Bob Barrett: Well finally doctor, do you think that the number as well as the diversity of NT-proBNP assays will increase and should we consider the need for standardization of NT-proBNP assays?

Alexander Semenov: Considering that heart failure is constantly increasing in prevalence, we can expect that the number of NT-proBNP assays will increase to meet the growing demand in heart failure diagnostics and especially the introduction of point of care testing assays may contribute to assay variability. These increases may require that we have to reconsider the need for a common reference material for NT-proBNP assays and this is certainly a very demanding task.

Bob Barrett: That was Dr. Alexander Semenov, project manager in the R&D Department of the biotech company HyTest located in Turku, Finland and also from the School of Biology, Lomonosov Moscow State University in Moscow, Russia. He has been our guest in this podcast on the molecular heterogeneity of circulating and terminal proBNP. His Editorial on that topic appears in the September 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.