



# Specificity of B-type natriuretic peptide assays: Knockin' on the assay door

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Comment on: Saenger AK, Rodriguez-Fraga O, Ler R, *et al.* Specificity of B-Type Natriuretic Peptide Assays: Cross-Reactivity with Different BNP, NT-proBNP, and proBNP Peptides. *Clin Chem* 2017;63:351-8.

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The journey to reach international method standardization is often long and difficult, especially for proteins and polypeptides that usually are quantified using immunological methods. An important part of this road is to gain detailed information on the specificity of individual assays. In this issue, Saenger *et al.* (1) presents information on the specificity of nine frequently used assays for B-type natriuretic peptides. This provides important information towards an international standardization of natriuretic peptides determinations.

Human brain natriuretic peptide (BNP) and the N-terminal fragment of the BNP precursor (NT-proBNP) are globally endorsed as heart failure (HF) biomarkers. HF is the most common diagnosis among elderly inpatients and affects a large number of patients. HF is expensive with average HF admission costs of \$7,000–13,000 (2) and the disease is associated with a significant mortality (3). The death rates within 1 month are approximately 10% to 15% (4). The number of HF patients is expected to grow as an effect of the increased life expectancy. Thus, the number of BNP and NT-proBNP assays will continue to increase. Most laboratories use decision limits to rule out heart failure (5,6). These decision limits are not assay specific but general recommendations which emphasizes the need for a good co-calibration of methods.

In the article, Saenger *et al.* (1) show that BNP or NT-proBNP results are not transferable between the current existing immunoassays owing to their differences regarding cross-reactivity and ability to detect various glycosylated forms. The study also clearly shows that there is no cross-

reactivity between NT-proBNP in BNP assays, that the NT-proBNP assays do not detect BNP peptides or glycosylated proBNP-derived peptides and that the proBNP assays studied are highly specific for proBNP peptides. The study proves that if we shall be able to standardize assays for natriuretic peptides we need to increase our knowledge on the processing of these peptides *in vivo* and we also need to know more about the assays that we are using. There is usually very limited information from the manufacturers regarding the glycosylation of the immunogens used for producing the antibodies in the different assays or the glycosylation of the calibrators used. An antibody raised against non-glycosylated NT-proBNP will most likely react differently to non-glycosylated calibrators and controls than to a glycosylated patient sample. This may cause assay problems similar to what we previously observed with cystatin C where the patient results could change by 50% without affecting the results of the control samples (7). This just further emphasizes the importance of studies on assay specificity and cross-reactivity.

In Sweden, we currently see the introduction of dedicated out-patient units for heart failure within the hospital and also in primary care. I also expect that we will soon see the introduction of point of care tests (POCT) for NT-proBNP and BNP at these units as the possibility to have the results at the time of consultation will most likely improve patient flow and patient compliance. Centralized measurements for natriuretic peptides either mean that the patient has to come in advance for blood sampling or the doctor has to inform the patient after the consultation,

usually by phone. HF is mainly seen in elderly patients that often have problems coming to the hospital for blood samplings and may have hearing problems that makes telephone consultations difficult. This increases the interest for having POCT assays for natriuretic peptides. The study by Saenger *et al.* (1) also included instruments intended for POCT testing of BNP and NT-proBNP. The introduction of POCT instruments means that the patients will be tested in parallel with the POCT instrument and the centralized method. It is necessary that the instruments provide the same type of results especially when the methods are used for treatment monitoring that includes repeated testing. In our county we have previously decided that it is not practical to mix NT-proBNP and BNP assays as we considered the risk of confusing the two types of assay results too great. The current article also shows that there are problems when using two NT-proBNP or two BNP assays in parallel. Previously I thought that much of the assay differences were due to the lack of international reference materials and that this problem would be largely solved with the introduction of new reference materials. The current article shows that the calibration differences may not be that easy to solve.

The National Institute of Standards and Technology (NIST) has been working together with the International Federation of Clinical Chemistry (IFCC) Committee for the Standardization of Markers of Cardiac Damage to develop reference measurement procedures and reference standards procedures for BNP and NT-proBNP. Initially it was considered by many as a rather straightforward project as BNP and NT-proBNP are small polypeptides that could be readily defined. As shown by the article by Saenger *et al.* BNP and NT-proBNP is not that simple to detect and define. Both molecules are present in Proforms and the peptides may be glycosylated to a varying degree (8), the analytes may be present in multimeric forms (9) and the antibodies used could cross-react with peptides with similar tertiary structures. Because of these problems, we still lack an international reference material for BNP and NT-proBNP. The work to develop reference materials and procedures is ongoing and work such as the work of Saenger *et al.* (1) are important steps to reach an international standardization of BNP and NT-proBNP assays. Because of this, it appears that the path towards developing a complete reference measurement system and uniform method calibrations for BNP and NT-proBNP might not be as short as we have hoped for.

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