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Searching for a BNP standard: Glycosylated proBNP as a common calibrator enables improved comparability of commercial BNP immunoassays

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ABSTRACT

Background: Circulating B-type natriuretic peptide (BNP) is widely accepted as a diagnostic and risk assessment biomarker of cardiac function. Studies suggest that there are significant differences in measured concentrations among different commercial BNP immunoassays. The purpose of our study was to compare BNP-related proteins to determine a form that could be used as a common calibrator to improve the comparability of commercial BNP immunoassay results.

Methods: BNP was measured in 40 EDTA-plasma samples from acute and chronic heart failure patients using five commercial BNP assays: Alere Triage, Siemens Centaur XP, Abbott I-STAT, Beckman Access2 and ET Healthcare Pylon. In parallel with internal calibrators from each manufacturer, six preparations containing BNP 1–32 motif a) synthetic BNP, b) recombinant BNP (*E. coli*), c) recombinant nonglycosylated proBNP (*E. coli*), d) recombinant His-tagged (N-terminal) nonglycosylated proBNP (*E. coli*), e) recombinant glycosylated proBNP (HEK cells), and f) recombinant glycosylated proBNP (CHO cells) were also used as external calibrators for each assay.

Results: Using the internal standards provided by manufacturers and for five of six external calibrators, up to 3.6-fold differences (mean 1.9-fold) were observed between BNP immunoassays (mean between-assay CV 24.5–47.2%). A marked reduction of the between-assay variability was achieved, when glycosylated proBNP expressed in HEK cells was used as the common calibrator for all assays (mean between-assay CV 14.8%).

Conclusions: Our data suggest that recombinant glycosylated proBNP could serve as a common calibrator for BNP immunoassays to reduce between-assay variability and achieve better comparability of BNP concentrations of commercial BNP immunoassays.

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1. Introduction

B-type natriuretic peptide (BNP) is a 32 amino acid circulating peptide hormone produced by myocardium [1–3]. BNP is widely accepted as both a clinical useful and cost-effective biomarker for heart failure (HF) diagnosis and therapy monitoring [4–6]. The BNP gene encodes a 134-amino acid preproBNP precursor, which is converted to 108-amino acid proBNP by the cleavage of a 26-amino acid signal peptide. The processing

of proBNP gives rise to two fragments: the N-terminal fragment of proBNP (NT-proBNP, 1–76 aar) and the C-terminal region, biologically active BNP hormone (77–108 aar) [1,7,8].

There is substantial variety of BNP commercial immunoassays on the market. Recent comparative studies show there are marked differences of the measured concentration of BNP obtained on different platforms [9,10]. Plasma BNP concentrations measured by various immunoassays differ substantially, complicating interpretation of results and rendering the cut-off concentration method dependent; especially if patient's specimens are analyzed by 2 different assays. As a consequence, the results of BNP measurements obtained by different assays and platforms can not be compared with reliability. One of the reasons for the lack of equivalence between existing BNP immunoassays may be the absence of a common calibrator. Presently, there is no agreement on which

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BNP or peptide standard should be used for calibration of BNP assays, as manufacturers are using different calibrators. Considering this, we suggested that a common calibrator may reduce the degree of between-assay variability of existing commercial BNP immunoassays.

It has been shown that apart from bioactive BNP, unprocessed proBNP glycosylated in N-terminal part, within 1–76 amino acid region of the molecule, represents a substantial or even major part of BNP immunoreactivity observed in the plasma of HF patients and healthy donors [11–14]. One may speculate that the reference material for BNP immunoassays should be proBNP, the natural and major BNP antigen form found in the circulation. Further, all commercial BNP assays have been shown to cross-react with the proBNP form, although the extent of cross-reactivity varies among assays [15,16]. In the current study we compared 6 BNP-related proteins to determine a form that could be used as a common calibrator to improve the comparability of commercial BNP immunoassays.

2. Materials and methods

2.1. Plasma collection

Following Institutional Review Board approval, EDTA-plasma samples were obtained on consent from 20 acute and 20 chronic heart failure patients at Hennepin County Medical Center, Minneapolis, MN. Plasma collection was performed in the presence of protease inhibitors to prevent proteolytic degradation of BNP [17,18]. Briefly, EDTA vacutainer plastic blood collection tubes were used for plasma collection. After centrifugation, the samples were immediately transferred into storage tubes containing benzamidine (Sigma, final inhibitor concentration 10 mmol/L) and 4-(2-aminomethyl)benzenesulfonyl fluoride hydrochloride (Sigma, final inhibitor concentration 5 mmol/L).

2.2. Sample set preparations

71 samples were prepared for measurements of BNP, in duplicate, by each immunoassay. In addition to the 40 EDTA-plasma samples from HF patients, 30 samples with 6 external calibrators (5 concentrations for each calibrator) diluted in BNP/proBNP/NT-proBNP-free EDTA-plasma (HyTest) were prepared. The following external calibrators were used in the study: synthetic BNP (Bachem), recombinant BNP expressed in *Escherichia coli* (*E. coli*) (Raybiotech), recombinant nonglycosylated proBNP (expressed in *E. coli*; HyTest), His-tagged (N-terminal) recombinant nonglycosylated proBNP (expressed in *E. coli*; Raybiotech), recombinant glycosylated proBNP (expressed in Human Embryonic Kidney (HEK) cells; HyTest) and recombinant glycosylated proBNP (expressed in Chinese Hamster Ovary (CHO) cells). Expression of proBNP in CHO-S cells (Invitrogen) and purification from conditioned medium was performed as described [14,19]. Calibrators were diluted in BNP/proBNP/NT-proBNP-free EDTA-plasma to give rise solutions with 5 different concentrations: 1.17, 0.585, 0.195, 0.065, 0.022 nmol/L, respectively (equal to 4050, 2025, 675, 225, 75 pg/mL for BNP 1–32, respectively). One blank sample in every set didn't contain any BNP/proBNP (BNP/proBNP/NT-proBNP-free plasma). All samples (calibrators and plasma samples) were aliquoted and provided with a set of ready-to-use encoded probes.

2.3. Deglycosylation of recombinant proBNP

ProBNP derived from HEK and CHO cells were incubated with a deglycosylation enzyme cocktail consisting of O-glycanase, sialidase A, β [1–3,4]galactosidase, β [1–4,6]galactosidase and β-*N*-acetylhexosaminidase (ProZyme). Reactions were carried out in 10 mM phosphate buffer, pH = 5.0, at 37 °C, for 16 h and stopped by addition of SDS-PAGE sample buffer, then subjected to Western blotting analysis.

2.4. SDS-PAGE and Western blotting

Proteins were separated by Tris-Tricine SDS-PAGE in 16.5% T, 3% C gel [20]; 1.5 μg of protein per lane was applied. The proteins were afterwards transferred onto nitrocellulose membrane for Western blotting analysis [21]. Antibody 15F11 (anti-NT-proBNP, epitope 13–20, HyTest) was used as primary detection antibody; bands were visualized using 3,3′-diaminobenzidine dihydrochloride.

2.5. BNP measurements

BNP concentrations were measured with five commercial BNP assays exactly according to manufacturers' guidelines using appropriate quality control materials: Alere Triage, Siemens Centaur XP, Abbott I-STAT, Beckman Access2 (performed at Hennepin County Medical Center, Minneapolis, MN) and ET Healthcare Pylon BNP assay (performed at ET Healthcare). Duplicates of each sample were measured within one run. Quality control materials were analyzed for all assays along manufacturers' guidelines, and found accepted, with %CVs < 13% at approximately 100 ng/L for all assays: Alere Triage with CV = 12.5% (at mean 111 ng/L); Siemens Centaur XP with CV = 10.1% (at mean 165 ng/L); Abbott I-STAT with CV = 12.8% (at mean 215 ng/L); Beckman Access 2 with CV = 9.5% (at mean 145 ng/L), ET Healthcare Pylon BNP assay with CV = 10.2% (at mean 100 ng/L).

2.6. Calculation of the BNP values with external calibrators

The initial BNP concentrations were obtained with the internal standards provided by a manufacturer. Concentrations of two repeats were averaged. For subsequent calibration of the assays with the external calibrators the concentrations of BNP obtained for the samples containing external calibrators were used to create calibration curves, which were plotted in logarithmic scale (log-log). The equations obtained with power-law fitting for every calibrator were used to recalculate the BNP concentrations in plasma samples.

2.7. Data analysis

The agreement between BNP concentrations measured by different BNP assays was analyzed for every pair of assays by using Passing-Bablock regression analysis (XLSTAT 2014) [22]. Coefficients of determination (R^2) were calculated with Microsoft Excel for Mac 2011, version 14.1.0. Between-assay CVs were calculated with Prism 5 for Mac OS X, version 5.0a, 2007.

3. Results

BNP immunoassays compared in the present study were selected to represent a variety of antibodies and standards utilized in commercial BNP immunoassays. Characteristics of the antibodies and BNP standards utilized in the assays are summarized in Table 1. BNP concentrations measured in the 40 HF plasma samples differed considerably between the 5 BNP immunoassays. Up to 3.6-fold differences (1.9-fold mean; range 0.9 to 3.6) were observed when using immunoassays and their calibrators provided by manufacturers. As shown in Table 2, the degree of equivalence was analyzed by Passing-Bablock regression analysis between results of every pair of BNP immunoassays and similar results were obtained for five out of six external calibrators. When glycosylated proBNP expressed in HEK cells was utilized as a common external calibrator for all assays, a significant reduction of the between-assay variability was achieved with regression line slopes close to 0.9–1.0 for almost every pair of assays. There was a good linear relationship for all BNP immunoassays and all calibrators, as shown by the coefficient of determination (R^2) values. Almost 2-fold reduction in mean between-assay CV (%) was observed after recalibration with glycosylated proBNP

Table 1
Characteristics of antibodies and standard materials of BNP immunoassays used in the study [6,23,27].

Immunoassay/ Instrument	Epitope recognized by capture antibody	Epitope recognized by detection antibody	Standard material
Alere Triage	5–13	Omniclonal (epitope not characterized)	Recombinant BNP
Siemens Centaur XP	27–32	14–21	Synthetic BNP
Abbott I-STAT	5–13	26–32	Synthetic BNP
Beckman Access2	Omniclonal (epitope not characterized)	5–13	Recombinant BNP
ET Healthcare Pylon	11–17	Recognizes the immune complex of capture antibody with BNP/proBNP	Glycosylated proBNP

expressed in HEK cells compared to internal calibrators (14.8% vs. 28.9%) (Table 3).

As follows from Fig. 1, the 5 different BNP assays were not equal in recognition of BNP, nonglycosylated proBNP and glycosylated proBNP, reflecting the differences in cross-reactivity of commercial BNP immunoassays for different BNP-related forms (BNP compared to proBNP, glycosylated proBNP compared to nonglycosylated proBNP).

SDS-PAGE analysis followed by Western blotting of two forms of glycosylated proBNP expressed in HEK cells and CHO cells revealed the differences in electrophoretic mobility of these proteins. A lower mobility of proBNP expressed in HEK cells compared to proBNP expressed in CHO cells reflects a higher extent of O-glycosylation of this protein, since the treatment with a mix of glycosidases completely diminished the difference between two forms of recombinant proBNP (Fig. 2).

4. Discussion

Numerous manufacturers currently market BNP immunoassays integrated in different platforms. Studies suggest that there are marked differences among the BNP values obtained on different platforms [9, 10]. As a consequence, BNP results are often unique to a certain method or instrument, such that different results from different assays and platforms are poorly comparable. Although the current FDA diagnostic cut-off to exclude acute HF for BNP is set at 100 ng/L, considering the high substantial differences between different BNP immunoassays we suggest that more appropriate medical decisions concentrations should be determined for each individual assay, or that a standard reference material be used in the calibration of all BNP assays.

The main causes of non-harmonized BNP assays are a) different epitope specificities of the capture and detection antibodies used and b) the lack of a common reference material for calibration of BNP assays by manufacturers. Commercial BNP assays are based on different antibodies and standard materials. Almost all BNP immunoassays employ two antibodies specific for two distantly located epitopes of the BNP peptide chain, directed at either for the intact cysteine ring or for the N- or C-terminus of the peptide. The only exception used in the current study was the single-epitope BNP immunoassay (SES-BNP™) implemented in the platform by ET Healthcare [23]. This assay differs from conventional sandwich-type BNP assays in that it utilizes one antibody specific to the relatively stable ring fragment of the BNP molecule (epitope 11–17, capture antibody) which is within the biologically active cysteine ring and a detection antibody which recognizes the immune complex of capture antibody with BNP/proBNP only. Epitope specificity is an internal characteristic of immunoassays, which cannot be influenced externally. However, one may speculate that a common calibrator may help to reduce this variability of the BNP concentrations and achieve a good comparability between BNP concentrations obtained with different assays.

The great heterogeneity of proBNP-derived peptides circulating in human blood can partially explain the differences among the results provided by immunoassay methods considered specific for BNP [16]. Due to such a high heterogeneity and diversity of circulating BNP-related peptides, there is no way to prepare a calibrator, which will be absolutely identical to endogenous BNP. However, considering the prevalence of glycosylated proBNP as a major BNP-immunoreactive form, one might suggest that glycosylated proBNP could serve as a common calibrator and stable standard for BNP immunoassays.

In the present study we observed that BNP concentrations measured in HF plasma samples differed considerably between BNP immunoassays.

Table 2
Agreement between BNP concentrations measured by different BNP assays with different calibrators.
The equations obtained with Passing-Bablock regression analysis ($y = ax + b$) and coefficient of determination (R^2) for every calibrator used to recalculate the BNP values in patients' plasma samples are presented in table cells for every pair of assays.

Assays combination	Calibrator						
	Internal calibrator	Synthetic BNP	Recombinant BNP	Recombinant proBNP nonglyc	Recombinant proBNP nonglyc His-tagged	Recombinant proBNP glyc (CHO cells)	Recombinant proBNP glyc (HEK cells)
Alere Triage/Abbot I-STAT	$0.53x + 4.36$ $R^2 = 0.99$	$0.78x - 5.23$ $R^2 = 0.99$	$0.81x - 1.89$ $R^2 = 0.99$	$1.21x - 5.19$ $R^2 = 0.99$	$1.67x + 24.19$ $R^2 = 0.99$	$0.44x - 2.47$ $R^2 = 0.99$	$0.97x - 15.84$ $R^2 = 0.99$
Beckman Access2/Abbot I-STAT	$0.69x + 3.33$ $R^2 = 0.98$	$0.57x - 1.09$ $R^2 = 0.98$	$0.56x + 5.16$ $R^2 = 0.98$	$0.62x - 4.84$ $R^2 = 0.98$	$0.73x - 17.06$ $R^2 = 0.97$	$0.44x - 3.40$ $R^2 = 0.98$	$0.87x - 3.03$ $R^2 = 0.98$
Beckman Access2/Alere Triage	$1.35x - 22.0$ $R^2 = 0.98$	$0.76x - 6.31$ $R^2 = 0.98$	$0.71x + 2.90$ $R^2 = 0.99$	$0.54x - 22.53$ $R^2 = 0.98$	$0.45x - 64.64$ $R^2 = 0.98$	$0.27x - 31.93$ $R^2 = 0.98$	$0.92x - 3.58$ $R^2 = 0.99$
Beckman Access2/Siemens Centaur XP	$1.29x - 3.41$ $R^2 = 1.00$	$1.07x - 4.29$ $R^2 = 1.00$	$0.94x + 0.41$ $R^2 = 1.00$	$1.10x - 2.85$ $R^2 = 1.00$	$1.37x - 6.82$ $R^2 = 1.00$	$0.84x + 6.89$ $R^2 = 1.00$	$0.83x - 16.43$ $R^2 = 0.99$
ET Healthcare Pylon/Abbot I-STAT	$0.93x + 9.33$ $R^2 = 0.96$	$0.77x + 9.90$ $R^2 = 0.95$	$0.99x - 2.66$ $R^2 = 0.96$	$1.10x - 19.14$ $R^2 = 0.98$	$1.16x - 61.91$ $R^2 = 0.96$	$0.49x - 11.84$ $R^2 = 0.96$	$1.04x - 7.53$ $R^2 = 0.96$
ET Healthcare Pylon/Alere Triage	$1.69x - 15.24$ $R^2 = 0.94$	$0.96x + 11.02$ $R^2 = 0.92$	$1.20x - 0.78$ $R^2 = 0.93$	$0.88x - 19.60$ $R^2 = 0.94$	$0.66x - 84.12$ $R^2 = 0.94$	$0.28x - 18.39$ $R^2 = 0.93$	$1.04x + 8.20$ $R^2 = 0.93$
ET Healthcare Pylon/Beckman Access2	$1.29x - 8.7$ $R^2 = 0.93$	$1.23x + 12.37$ $R^2 = 0.92$	$1.72x - 11.09$ $R^2 = 0.98$	$1.69x - 5.32$ $R^2 = 0.93$	$1.58x - 32.06$ $R^2 = 0.93$	$0.63x + 0.54$ $R^2 = 0.93$	$1.14x - 1.75$ $R^2 = 0.93$
ET Healthcare Pylon/Siemens Centaur XP	$1.65x - 19.09$ $R^2 = 0.94$	$1.37x + 7.19$ $R^2 = 0.93$	$1.57x - 11.41$ $R^2 = 0.94$	$1.86x - 17.95$ $R^2 = 0.93$	$2.12x - 44.51$ $R^2 = 0.93$	$0.88x - 5.04$ $R^2 = 0.93$	$0.97x - 24.38$ $R^2 = 0.94$
Siemens Centaur XP/Abbot I-STAT	$0.56x + 1.82$ $R^2 = 0.99$	$0.56x + 1.07$ $R^2 = 0.99$	$0.63x + 3.44$ $R^2 = 0.99$	$0.58x - 4.65$ $R^2 = 0.99$	$0.54x - 14.16$ $R^2 = 0.98$	$0.85x - 9.92$ $R^2 = 0.99$	$1.07x + 11.24$ $R^2 = 0.99$
Siemens Centaur XP/Alere Triage	$1.06x - 11.11$ $R^2 = 0.99$	$0.72x + 1.41$ $R^2 = 0.99$	$0.78x + 1.88$ $R^2 = 0.99$	$0.50x - 10.35$ $R^2 = 0.99$	$0.34x - 39.18$ $R^2 = 0.98$	$0.53x - 41.10$ $R^2 = 0.99$	$1.09x + 19.76$ $R^2 = 0.99$

Table 3

Equivalence of 5 commercial BNP assays (Alere Triage, Siemens Centaur XP, Abbott I-STAT, Beckman Access2 and ET Healthcare Pylon BNP) calculated as the mean between-assay CV (%) for internal calibrators and 6 external calibrators.

Calibrator	Mean between-assay CV (%) (SD)
Internal calibrators	28.9 (4.8)
Synthetic BNP	27.4 (4.3)
Recombinant BNP	24.5 (4.4)
Nonglycosylated proBNP	33.1 (5.9)
His-tagged nonglycosylated proBNP	47.2 (11.1)
Glycosylated proBNP (CHO)	36.2 (5.9)
Glycosylated proBNP (HEK)	14.8 (6.5)

This confirmed the lack of equivalence of BNP immunoassay measurements observed in previous studies. We assessed 6 candidate BNP-related calibrators for their suitability to reduce between-assay variation. Synthetic and recombinant BNPs were compared with recombinant nonglycosylated proBNPs from 2 different vendors and glycosylated proBNPs expressed in 2 different mammalian cell lines. According to our findings, among 6 tested calibrators, glycosylated proBNP expressed in HEK cells taken as a common calibrator enabled improved comparability of BNP immunoassays concentrations, as confirmed by significant reduction of between-assay CV from 28.9% for internal calibrators to 14.8% for glycosylated proBNP expressed in HEK cells.

Two forms of recombinant proBNP, nonglycosylated (produced in *E. coli*) and glycosylated (produced in mammalian cells, HEK and CHO) were compared in this study. These two forms of proBNP differ in their N-terminal part, which is glycosylated for proBNP expressed in HEK and CHO cells and nonglycosylated for proBNP expressed in *E. coli* [8,14,19]. One may expected that the BNP concentrations obtained after recalculation should be similar for both forms of proBNP, nonglycosylated and glycosylated, since the BNP-part of both molecules is the same (non-modified by glycosidic residues). However, this was not the case. Glycosylated and nonglycosylated proBNPs showed very different results in reduction of between-assay variability and exhibited different immunoreactivity. Additionally, two forms of glycosylated proBNP expressed in HEK and CHO cells were not similar, as one may
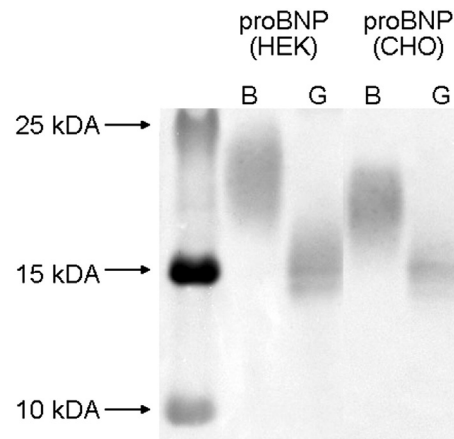

Fig. 2. Western blot analysis of purified proBNP expressed in HEK and CHO cells. Samples of recombinant proBNPs (1.5 μg/lane) were treated with either a deglycosylation cocktail (G) or buffer alone (B) for 16 h. Immunostaining was performed with anti-NT-proBNP antibodies 15F11 (epitope 13–20).

also have expected. This apparent discrepancy may be explained by the influence of O-glycosylation on the recognition of proBNP by antibodies utilized in the assays. The differences in the level of glycosylation of proBNP expressed in HEK and CHO cells follow from the results of SDS-PAGE analysis and have also been previously shown [19]. The extent of glycosylation and the structure of attached glycosidic residues probably interfere with the recognition of proBNP by antibodies, due to the steric hindrance of the glycosidic residues. This may explain the reason why different proBNP forms taken as a common calibrators exhibited different results in harmonization of BNP results.

An important characteristic of a calibrator is stability. Glycosylated proBNP expressed in HEK cells was shown to have a high stability in plasma samples, fulfilling the requirement of robust stability [24–26]. The stability of glycosylation pattern of recombinant proBNP expressed in HEK cells upon storage is additionally confirmed by the observation that the results obtained in the present study are
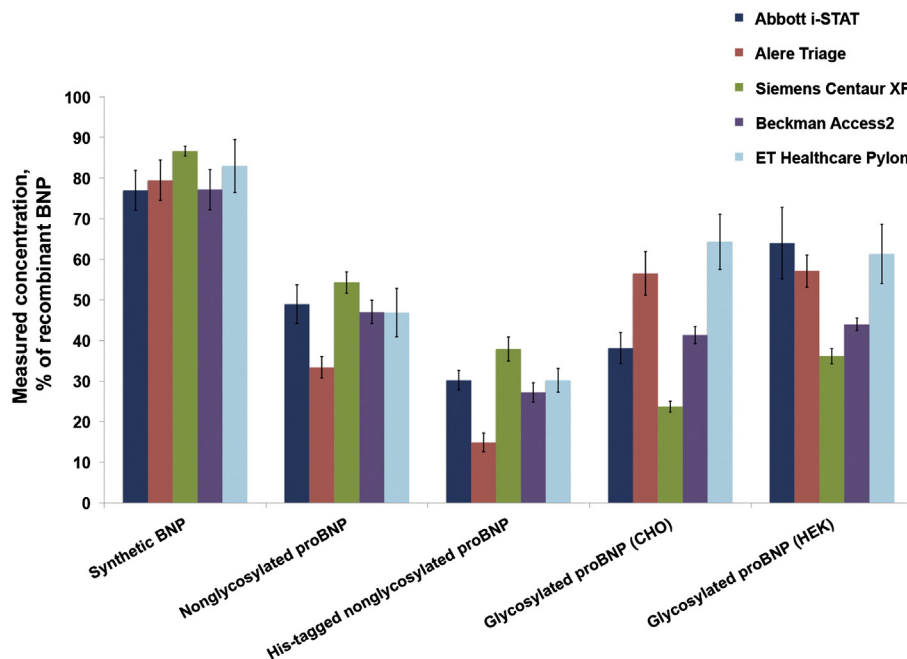

Fig. 1. Relative measured concentrations of human synthetic BNP, recombinant nonglycosylated proBNP, recombinant His-tagged nonglycosylated proBNP, recombinant glycosylated proBNP (HEK cells) and recombinant glycosylated proBNP (CHO cells) measured with Alere Triage, Siemens Centaur XP, Abbott I-STAT, Beckman Access2 and ET Healthcare Pylon BNP assays. The data are expressed as percentage of measured concentration obtained for recombinant BNP. The ratios of values for 4 measurements (0.585, 0.195, 0.065, 0.022 nM for each calibrator) were averaged (±SD).

in a good accordance with those obtained in our preliminary study: the same batch of proBNP which was kept for 1.5 years at − 70 °C was used in both studies.

It should be noted that there are some limitations to the present study. First, only one batch of recombinant proBNP expressed in HEK cells has been tested and no batch-to-batch variation has been evaluated. Given the pronounced effect of the extent of glycosylation on reduction of inter-assay variability, the low variability of the glycosylation pattern of recombinant glycosylated proBNP is expected to be an essential characteristic for a protein suggested as a common calibrator for BNP immunoassays.

The extent of glycosylation of proBNP expressed in HEK cells is reproducible in different preparations of recombinant proBNP, as confirmed by the analysis of recognition by antibodies specific for the regions of proBNP modified by glycosidic residues in a quality control procedure. The lot-to-lot variations do not exceed on average 10%.

Further, one may speculate that the positive effect of glycosylated proBNP expressed in HEK cells is caused not by its glycosylation, but rather by the presence of some contaminations in protein preparation. Although this assumption can not be completely excluded, the effect of glycosylation on proBNP recognition by antibodies has been confirmed in previous studies showing different degree of cross-reactivity of commercial BNP assays to glycosylated and nonglycosylated proBNP [15,16]. Also the different effect on reduction of between-assay variability of proBNPs expressed in HEK and CHO cells with different extent of glycosylation, may indicate that the effect on reduction of between-assay variability observed in the present study is caused by glycosylation.

Presently, there is no primary reference material and no primary reference measurement procedure for BNP measurements. The current study demonstrates that harmonization of commercial BNP immunoassays is technically possible. Among assessed 6 different candidate calibrators for their suitability to reduce between-assay variation, glycosylated proBNP (expressed in HEK cells) taken as a common calibrator enables significantly improved comparability of BNP immunoassays results. These data suggest that glycosylated proBNP expressed in HEK cells has a potential to become a reference material that may allow standardization of BNP measurement results. Future studies need to better define our promising preliminary observations.

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References

[1] T. Sudoh, K. Maekawa, M. Kojima, N. Minamino, K. Kangawa, H. Matsuo, Cloning and sequence analysis of cdna encoding a precursor for human brain natriuretic peptide, Biochem. Biophys. Res. Commun. 159 (1989) 1427–1434.

[2] Y. Kambayashi, K. Nakao, M. Mukoyama, Y. Saito, Y. Ogawa, S. Shiono, et al., Isolation and sequence determination of human brain natriuretic peptide in human atrium, FEBS Lett. 259 (1990) 341–345.

[3] H. Yasue, M. Yoshimura, H. Sumida, K. Kikuta, K. Kugiyama, M. Jougasaki, et al., Localization and mechanism of secretion of b-type natriuretic peptide in comparison with those of a-type natriuretic peptide in normal subjects and patients with heart failure, Circulation 90 (1994) 195–203.

[4] K. Thygesen, J. Mair, C. Mueller, K. Huber, M. Weber, M. Plebani, et al., Recommendations for the use of natriuretic peptides in acute cardiac care: a position statement from the study group on biomarkers in cardiology of the esc working group on acute cardiac care, Eur. Heart J. 33 (2012) 2001–2006.

[5] W.H. Tang, G.S. Francis, D.A. Morrow, L.K. Newby, C.P. Cannon, R.L. Jesse, et al., National academy of clinical biochemistry laboratory medicine practice guidelines: clinical utilization of cardiac biomarker testing in heart failure, Circulation 116 (2007) e99–109.

[6] F.S. Apple, M. Panteghini, J. Ravkilde, J. Mair, A.H. Wu, J. Tate, et al., Quality specifications for b-type natriuretic peptide assays, Clin. Chem. 51 (2005) 486–493.

[7] H. Tateyama, J. Hino, N. Minamino, K. Kangawa, T. Minamino, K. Sakai, et al., Concentrations and molecular forms of human brain natriuretic peptide in plasma, Biochem. Biophys. Res. Commun. 185 (1992) 760–767.

[8] A.G. Semenov, N.N. Tamm, K.R. Seferian, A.B. Postnikov, N.S. Karpova, D.V. Serebryanaya, et al., Processing of pro-b-type natriuretic peptide: furin and corin as candidate convertases, Clin. Chem. 56 (2010) 1166–1176.

[9] C. Prontera, M. Zaninotto, S. Giovannini, G.C. Zucchelli, A. Pilo, L. Sciacovelli, et al., Proficiency testing project for brain natriuretic peptide (bnp) and the n-terminal part of the propeptide of bnp (nt-probnp) immunoassays: the cardioormocheck study, Clin. Chem. Lab. Med. 47 (2009) 762–768.

[10] A. Clerico, M. Zaninotto, C. Prontera, S. Giovannini, R. Ndreu, M. Franzini, et al., State of the art of bnp and nt-probnp immunoassays: the cardioormocheck study, Clin. Chim. Acta 414 (2012) 112–119.

[11] T.G. Yandle, A.M. Richards, A. Gilbert, S. Fisher, S. Holmes, E.A. Espiner, Assay of brain natriuretic peptide (bnp) in human plasma: evidence for high molecular weight bnp as a major plasma component in heart failure, J. Clin. Endocrinol. Metab. 76 (1993) 832–838.

[12] K.R. Seferian, N.N. Tamm, A.G. Semenov, K.S. Mukharyamova, A.A. Tolstaya, E.V. Koshkina, et al., The brain natriuretic peptide (bnp) precursor is the major immunoreactive form of bnp in patients with heart failure, Clin. Chem. 53 (2007) 866–873.

[13] I. Giuliani, F. Rieunier, C. Larue, J.F. Delagneau, C. Granier, B. Pau, et al., Assay for measurement of intact b-type natriuretic peptide prohormone in blood, Clin. Chem. 52 (2006) 1054–1061.

[14] U. Schellenberger, J. O'Rear, A. Guzzetta, R.A. Jue, A.A. Protter, N.S. Pollitt, The precursor to b-type natriuretic peptide is an o-linked glycoprotein, Arch. Biochem. Biophys. 451 (2006) 160–166.

[15] K.N. Luckenbill, R.H. Christenson, A.S. Jaffe, J. Mair, J. Ordonez-Llanos, F. Pagani, et al., Cross-reactivity of bnp, nt-probnp, and probnp in commercial bnp and nt-probnp assays: preliminary observations from the ifcc committee for standardization of markers of cardiac damage, Clin. Chem. 54 (2008) 619–621.

[16] F. Liang, J. O'Rear, U. Schellenberger, L. Tai, M. Lasecki, G.F. Schreiner, et al., Evidence for functional heterogeneity of circulating b-type natriuretic peptide, J. Am. Coll. Cardiol. 49 (2007) 1071–1078.

[17] W.L. Miller, M.A. Phelps, C.M. Wood, U. Schellenberger, A. Van Le, R. Perichon, A.S. Jaffe, Comparison of mass spectrometry and clinical assay measurements of circulating fragments of b-type natriuretic peptide in patients with chronic heart failure, Circ. Heart Fail. 4 (2011) 355–360.

[18] E.E. Niederkofler, U.A. Kiernan, J. O'Rear, S. Menon, S. Saghir, A.A. Protter, et al., Detection of endogenous b-type natriuretic peptide at very low concentrations in patients with heart failure, Circ. Heart Fail. 1 (2008) 258–264.

[19] A.G. Semenov, A.B. Postnikov, N.N. Tamm, K.R. Seferian, N.S. Karpova, M.N. Bloshchitsyna, et al., Processing of pro-brain natriuretic peptide is suppressed by o-glycosylation in the region close to the cleavage site, Clin. Chem. 55 (2009) 489–498.

[20] H. Schagger, G. von Jagow, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa, Anal. Biochem. 166 (1987) 368–379.

[21] H. Towbin, T. Staehelin, J. Gordon, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 4350–4354.

[22] H. Passing, Bablok, A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, part i, J. Clin. Chem. Clin. Biochem. 21 (1983) 709–720.

[23] N.N. Tamm, K.R. Seferian, A.G. Semenov, K.S. Mukharyamova, E.V. Koshkina, M.I. Krasnoselsky, et al., Novel immunoassay for quantification of brain natriuretic peptide and its precursor in human blood, Clin. Chem. 54 (2008) 1511–1518.

[24] A. Katrukha, N. Tamm, A. Semenov, K. Seferian, A. Postnikov, M. Agafonov, et al., Recombinant proform of brain natriuretic peptide (probnp), expressed in eukaryotic cells as a stable standard for bnp immunoassay, Clin. Chem. 53 (2007) A23-A.

[25] J. Jiang, N. Pristera, W. Wang, X. Zhang, Q. Wu, Effect of sialylated o-glycans in pro-brain natriuretic peptide stability, Clin. Chem. 56 (2010) 959–966.

[26] A.G. Semenov, K.R. Seferian, N.N. Tamm, M.M. Artem'eva, A.B. Postnikov, A.V. Bereznikova, et al., Human pro-b-type natriuretic peptide is processed in the circulation in a rat model, Clin. Chem. 57 (2011) 883–890.

[27] A. Clerico, M. Franzini, S. Masotti, C. Prontera, C. Passino, State of the art of immunoassay methods for b-type natriuretic peptides: an update, Crit. Rev. Clin. Lab. Sci. 52 (2015) 56–69.