Searching for a BNP standard: glycosylated proBNP as a common calibrator enables improved comparability of BNP immunoassays results

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Introduction

B-type natriuretic peptide (BNP) is widely accepted as a useful and cost-effective biomarker of cardiac function. Clinical studies suggest that absolute plasma BNP concentrations measured by various immunoassays differ substantially, complicating interpretation of results and rendering the cut-off concentration method dependent. 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 BNP or peptide standard should be used for calibration of BNP assays, as manufacturers are using different BNP calibrators. Considering this, we suggested that a common calibrator may reduce the degree of between-assay variability of existing commercial BNP immunoassays. Since endogenous proBNP is O-glycosylated, such common BNP calibrator also is expected to be glycosylated.

THE PURPOSE OF OUR STUDY: to compare BNP-related proteins to determine a form that could be used as a common calibrator to improve the comparability of commercial BNP immunoassays.

Materials and Methods

PLASMA SAMPLES: EDTA-plasma samples were obtained 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 (benzamidine 10 mmol/L and AEBSF 5 mmol/L).

BNP IMMUNOASSAYS: BNP concentrations were measured with five commercial BNP assays: 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) (Fig. 1).

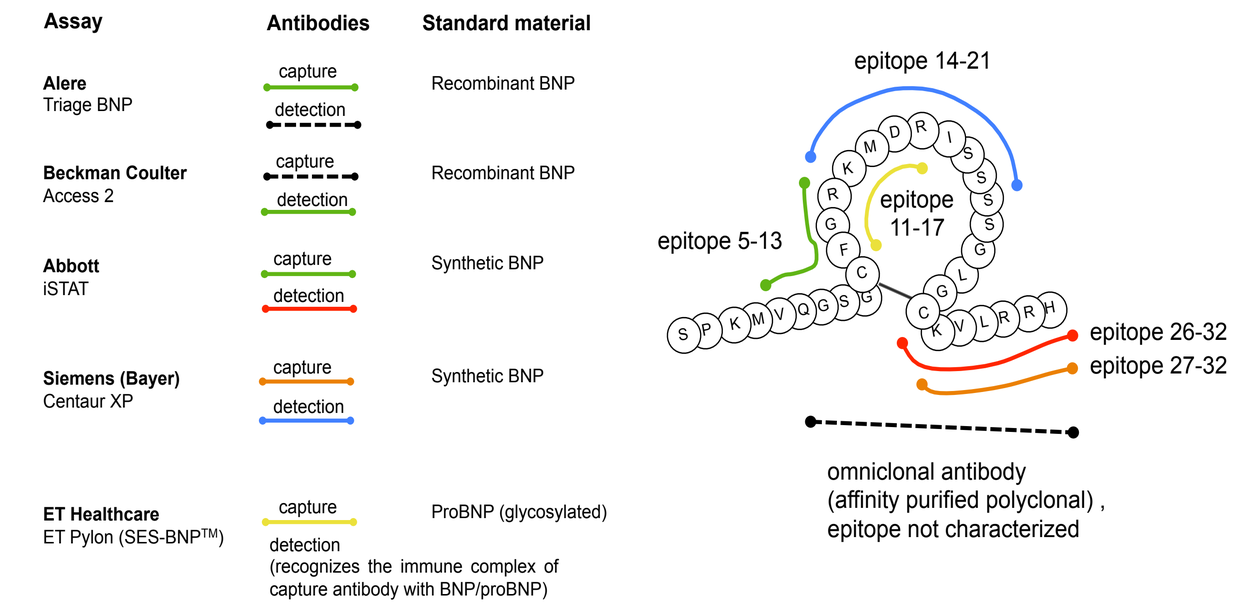

FIGURE 1. **Antibodies and standard materials of BNP immunoassays used in the study (according to the information presented at www.ifcc.org).**

EXTERNAL CALIBRATORS: 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 HEK cells; HyTest) and recombinant glycosylated proBNP (expressed in CHO cells, in-house).

CALCULATION OF THE BNP VALUES WITH EXTERNAL CALIBRATORS: the initial BNP concentrations were obtained with the internal standards provided by a manufacturer. 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.
The degree of equivalence was analyzed by the linear regression relationship between results of every pair of BNP immunoassays for internal calibrators and 6 external calibrators.

Results

We observed that the 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 (Fig. 2).

Absolute BNP concentrations measured in HF plasma samples differed considerably between BNP immunoassays for internal calibrators (up to 3.6-fold differences; range 0.9 to 3.6) and 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 1.0 for almost every pair of assays (Table 1).

Two forms of glycosylated proBNP expressed in HEK and CHO cells were not similar. This apparent discrepancy may be explained by the differences in the level of glycosylation of proBNP expressed in HEK and CHO cells.

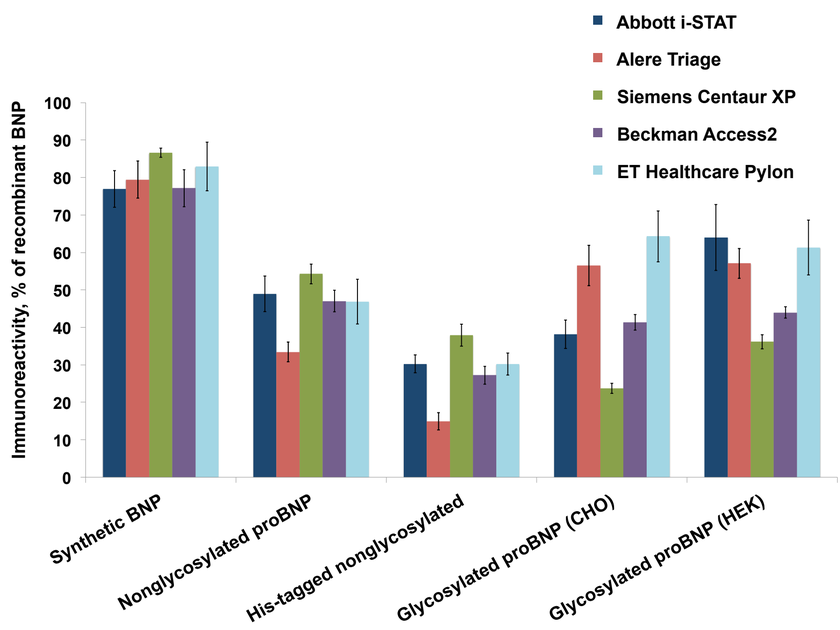

FIGURE 2. **Immunoreactivity of 6 external calibrators measured with 5 commercial BNP assays.** The values of 4 measurements (0.585, 0.195, 0.065, 0.022 nM for each calibrator) were averaged (±SD).

TABLE 1. Agreement between BNP concentrations measured by different BNP assays with different calibrators.

Calibrator / Assays combination	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	0,5238x + 3,5932 R² = 0,9897	0,7934x - 13,446 R² = 0,9886	0,8034x - 4,9589 R² = 0,9893	0,7934x - 13,446 R² = 0,9886	1,6224x + 33,221 R² = 0,9899	0,5462x - 6,3346 R² = 0,9893	**1,007**x - 33,151 R² = 0,9876
Beckman Access2/Abbot i-STAT	0,8063x - 53,328 R² = 0,9808	0.6991x - 37.912 R² = 0.97712	0.635x - 10.066 R² = 0.98383	0,6991x - 37,912 R² = 0,9771	0.9155x - 163.99 R² = 0.97263	0.6544x - 67.62 R² = 0.97805	**1.0416**x - 65.273 R² = 0.97769
Beckman Access2/Alere Triage	1.5334x - 56.349 R² = 0.98349	0.8792x - 25.309 R² = 0.98381	0.7873x - 5.2601 R² = 0.986867	0.6308x - 60.7 R² = 0.98162	0.5626x - 179.32 R² = 0.97656	1.1946x - 58.206 R² = 0.98288	**1.032**x - 29.689 R² = 0.98538
Beckman Access2/Siemens Centaur XP	1.366x - 25.375 R² = 0.99669	1.1755x - 22.718 R² = 0.99586	0.985x - 6.6407 R² = 0.99705	1.1682x - 21.734 R² = 0.99678	1.4711x - 46.968 R² = 0.99669	0.5641x - 21.809 R² = 0.99691	**0.9567**x - 63.68 R² = 0.99398
ET Healthcare Pylon/Abbot i-STAT	0.8237x + 33.157 R² = 0.95886	0.6396x + 43.999 R² = 0.9539	0.8656x + 21.854 R² = 0.95625	0,6991x - 37,912 R² = 0,9771	1.127x - 50.003 R² = 0.96059	0.5434x + 14.709 R² = 0.95895	**0.927**x + 26.061 R² = 0.95885
ET Healthcare Pylon/Alere Triage	1.5457x + 39.002 R² = 0.9361	0.789x + 61.157 R² = 0.92428	1.0565x + 33.313 R² = 0.92941	0.822x + 32.263 R² = 0.93539	0.6833x - 50.564 R² = 0.93883	0.9767x + 30.543 R² = 0.93418	**0.9006**x + 67.279 R² = 0.92915
ET Healthcare Pylon/Beckman Access2	0.999x + 101.23 R² = 0.93486	0.8868x + 87.051 R² = 0.91731	1.3381x + 41.219 R² = 0.93649	0,6991x - 37,912 R² = 0,9771	1.1955x + 184.97 R² = 0.93151	0.8096x + 82.674 R² = 0.93193	**0.8665**x + 96.399 R² = 0.92969
ET Healthcare Pylon/Siemens Centaur XP	1.3686x + 74.041 R² = 0.93725	1.0503x + 64.744 R² = 0.92743	1.32x + 31.873 R² = 0.93651	1.5095x + 88.746 R² = 0.93378	1.7643x + 125.15 R² = 0.93423	0.4579x + 63.812 R² = 0.93375	**0.8369**x + 36.367 R² = 0.94185
Siemens Centaur XP/Abbot i-STAT	0.5914x - 21.409 R² = 0.98794	0.5967x - 13.843 R² = 0.98749	0.6455x - 3.7951 R² = 0.98937	0,5967x - 13,843 R² = 0,9875	0.6244x - 82.068 R² = 0.98247	1.1627x - 83.832 R² = 0.98567	**1.0923**x - 3.6686 R² = 0.99005
Siemens Centaur XP/Alere Triage	1.1235x - 23.071 R² = 0.98838	0.7486x - 2.4625 R² = 0.98975	0.7994x + 1.3667 R² = 0.99007	0.5406x - 33.978 R² = 0.98737	0.3833x - 91.687 R² = 0.98426	2.1198x - 65.667 R² = 0.98797	**1.0783**x + 35.769 R² = 0.99053

Almost 2-fold reduction in mean between-assay CV (%) was observed after recalibration with glycosylated proBNP expressed in HEK cells compared to internal calibrators (14.8% vs. 28.9%) (Fig. 3).

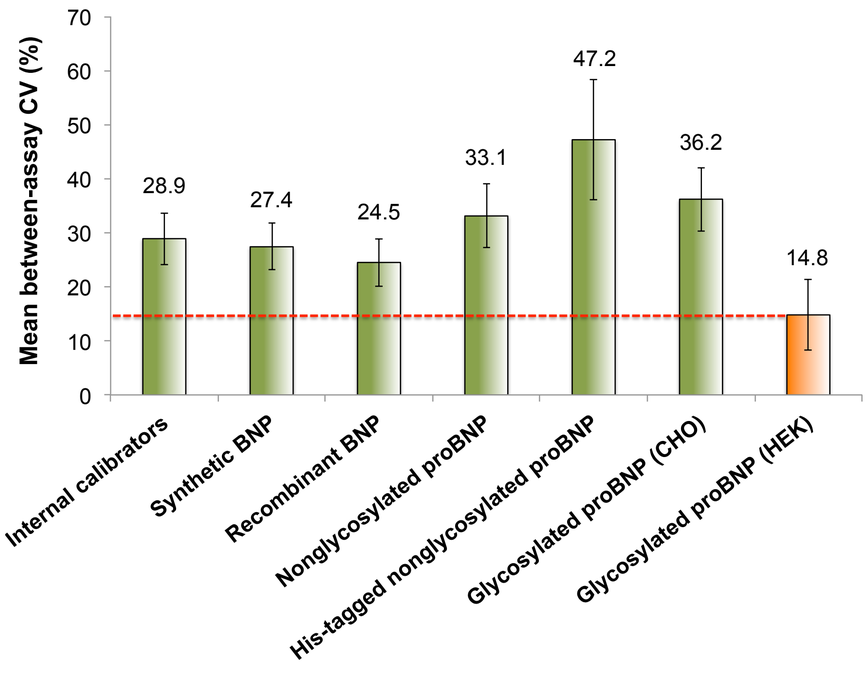

FIGURE 3. **Equivalence of 5 commercial BNP assays calculated as the mean between-assay CV (%) for internal calibrators and 6 external calibrators (±SD).**

Conclusions

1. Recombinant glycosylated proBNP (expressed in HEK cells) 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.

2. This study may be considered as a starting point toward standardization of BNP measurement results. Further studies organized by standardization committees are needed to evaluate the potential of the suggested calibrator to become a reference material.