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

Semenov A.1, Tamm N.J., Apple F.J., Schulz K.2, Love S.3, Feygina E.3, Katrukha A.1,3
1 - HyTest Ltd., Turku, Finland; 2 - Department of Laboratory Medicine and Pathology, Hennepin County Medical Center and University of Minnesota, Minnesota, USA; 3-School of Biology, Moscow State University, Moscow, Russia

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 AEBFS 5 mmol/L).

BNP IMMUNOASSAYS: BNP concentrations were measured with five commercial BNP immunoassays (Table 1). Siemens 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).

External Calibrators:

Synthetic proBNP (expressed in HEK cells) was utilized as a common external calibrator. 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.

Calculation of the BNP Values with External Calibrators

Glycosylated proBNP (HEK) expressed in HEK cells compared to Synthetic BNP (produced in CHO cells).

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

Conclusion

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.

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).

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).

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 calibrant BNP (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.

HyTest Ltd. Intelligate 1, 6th floor, Joukahaisenkatu 6, FI-20520 Turku, FINLAND | Tel. +358 2 512 0900, Fax +358 2 512 0909, e-mail: hytest@hytest.fi | www.hytest.fi