Brain natriuretic peptide (BNP) is an acknowledged marker of heart failure (HF) and is widely used in clinical practice for HF diagnosis and patient management. Most commercial BNP assays are designed as a sandwich-type immunoassays utilizing two monoclonal antibodies (MAbs) specific to two the different epitopes. At least one of these two antibodies is specific to the ring structure of the BNP molecule, while the other is specific either to the N-terminus or to C-terminus of the peptide. All commercial BNP assays cross-react with the proBNP forms (non-glycosylated and glycosylated), but the rate of cross-reactivity varies among assays.

It was shown recently that HF patients plasma contains a small portion of the full-length BNP form (BNP-32) along with multiple forms truncated from both the N- (BNP-3-32, 4-32, 5-32) and C-termini (BNP-5-31, 1-25, 1-26) (Niederkofer et al., 2008). So it could be expected that BNP measurements by current versions of commercial assays could be affected by proteolytic degradation. Recently we have reported a new type of BNP assay - “Single Epitope Sandwich assay” (SES assay) (Tamm et al., 2008, in which one MAB 24C5 is specific ring fragment of the BNP molecule (epitope 11-17) and the second MAB – Ab-BNP2 recognizes the immunocomplex of MAB 24C5 with BNP (proBNP) only. Thus, only one epitope, located in the relatively stable part of the BNP molecule is needed for BNP measurements in the SES assay. This feature gives a substantial advantage for the SES assay approach over conventional assays because truncated BNP (or proBNP) forms presented in patients’ blood could be also detected by the SES assay. The aim of this study was to compare results of BNP measurements by the SES assay and by the conventional-type Siemens ADVIA Centaur BNP immunosassay in order to verify hypothesis according to which SES assay should detect more BNP in the sample than conventional-type immunosassay.

Introduction

Siemens ADVIA Centaur BNP immunosassay

BNP measurements were performed with the Siemens ADVIA Centaur analyzer according to the manufacturer’s recommendations. In the Siemens assay, the capture Mab is specific to the C-terminus (BC 203, epitope 27-32) and the detection Mab is specific to the ring structure of the BNP molecule (KY-HNP-II, epitope 14-21).

Materials and Methods

Monoclonal antibodies (MAbs) specific to the region 11-17 of human BNP (MAB 24C5) and to the immune complex consisting of MAB 24C5 and BNP molecule (Ab-BNP2) were from HyTest.

Human recombinant proBNP expressed in E. coli was from HyTest. Rec. proBNP was used as calibrator in the SES and Siemens BNP immunosassays.

Plasma samples

EDTA-plasma samples from 94 patients with HF were used for BNP measurements. The diagnosis of HF was based on symptoms of dyspnea, orthopnea, lung rales and leg edema and was confirmed by echocardiography studies and X-ray examination. The left ventricle ejection fraction (measured by the modified Simpson’s method) was measured for only 63 HF patients and was about 34.2 ± 8.3% (mean ± SD).

Patients enrolled in the study: male/female = 33/61, age 72.6 ± 12.7 years (mean ± SD).

Plasma samples were stored at ~70°C not more then for two months until analysis.

Single Epitope Sandwich (SES prototype) immunofluorescent assay

A mixture of equal quantities of biotinylated MAB 24C5 and Eu3+-conjugated MAb Ab-BNP2 (200 ng per well of each antibody) in 50 µL of assay buffer (Wallac PerkinElmer) was incubated in streptavidin-coated plates with a test sample or calibrator for 30 min at room temperature with gentle shaking. After washing, the fluorescence was measured using a Victor 1420 multilabel counter (Wallac PerkinElmer).

Results

We had tested blood samples from 94 HF patients using conventional Siemens assay and SES assay. Siemens BNP assay as well as SES assay are able to detect both forms of the antigen displaying BNP immunoreactivity – BNP and proBNP – in human blood. Both assays were calibrated using recombinant proBNP (E, col). As it follows from Fig. 2 in all samples the SES assay measured more BNP, from 1.2- to 7.2-fold (2±1.0 ± 0.9 [mean ± SD], compared to the Siemens assay. For six patients (6%), BNP concentrations measured by the SES assay differed significantly (3- to 7.2-fold higher) from that, measured by the Siemens assay. BNP concentrations measured in the plasma samples ranged from 116 to 9159 ng/L (median 973 ng/L) and from 65 to 6274 ng/L (median 536 ng/L) as was determined by the SES assay and the Siemens assay, respectively. The difference between the median SES and Siemens BNP values was significant (p<0.001).

Discussion

Possible reasons for observed difference in determined BNP concentrations.

The observation that in the same blood samples Siemens assay detects lower BNP concentrations than the SES assay can be explained by the proteolytic degradation of the C-terminus of BNP/proBNP molecules in circulation. When the C-terminus of the BNP molecule is cleaved by proteases, the Siemens assay that uses a Mab specific to the terminal epitope 27-32 is unable to recognize the resulting endogenous peptide. The only one epitope 11-17 recognized by the SES assay is located on the stable part of the molecule. Being less susceptible to partial proteolytic degradation of the analyte, the SES assay likely detects both intact and terminus-truncated peptide forms in circulation. It is also possible that the lower BNP concentrations detected by the Siemens assay could be partially explained by the fact that the Siemens assay recognizes BNP and proBNP with different efficiency. It has been shown by Liang et al. (2007) that the Siemens assay recognizes glycosylated recombinant proBNP (expressed in a mammalian cell line) with a slightly lower reactivity than BNP. However, this suggestion should be further investigated.

Conclusions

Precise measurements of BNP values are required for the correct diagnosis of patients with the signs and symptoms of HF. The underestimation of BNP/proBNP concentrations due to anate proteolytic degradation may result in the misdiagnosis and/or misclassification of patients.

The SES assay is able to detect BNP molecules partially truncated from the both termini, thus detecting more molecules in the sample than the conventional-type assay.

Further clinical studies are still needed to determine the implications of using the SES assay for the diagnosis, management and outcomes assessment of HF patients.

References


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