EVALUATION OF THE ANALYTICAL PERFORMANCE OF A NEW IMMUNOENZYMOMETRIC ASSAY FOR BRAIN NATRIURETIC PEPTIDE

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AIM OF THE STUDY

- Aim of this study is to evaluate the analytical characteristics of the novel immunoassay method ST AIA-PACK (TOSOH) for BNP determination. For this purpose we examined plasma samples obtained from healthy subjects and patients with heart failure, as well as control samples from the external quality assessment (EQA) program CardioOrmoCheck.
- Furthermore we compared this method with the most used BNP methods (Beckman-Coulter, Siemens, Abbott).

MATERIALS AND METHODS

- The ST AIA-PACK BNP method (TOSOH CORPORATION, Tokio, Japan), is a sandwich immunoenzymometric assay.
- The BNP assay was performed in our laboratory using the AIA-2000 platform using plasma-EDTA samples. BNP levels were also measured using the TRIAGE-Biosite method (Beckman-Coulter) with the UniCell DxI 800 platform (Beckman-Coulter, Inc. Fullerton, USA).
- Healthy people (n=126; 72 males, age: 49±12 years) were recruited from the laboratory staff, blood donors, or voluntary subjects, included in screening programs for preventive medicine (MHELP study, Montignoso, Massa, Italy). The presence of cardiac or systemic diseases was excluded in all subjects by history, accurate clinical examination, ECG, cardiac imaging, and laboratory tests; all subjects denied the use of drugs for at least two weeks before the sample collection. The informed consent was obtained by all subjects and patients enrolled in the study.
- Patients with heart failure (HF, n=77) were admitted to the clinical wards and intensive coronary unit of the Fondazione Toscana G. Monasterio, Pisa, Italy. These patients were classified according to the New York Heart Association (NYHA) classification: NYHA I, II (n= 31; 19 males; age: 55±10 years), NYHA III, IV (n=46; 25 males; age: 74±11 years).
- Standard statistical analyses were carried by Stat-View 5.0.1 program (1992-98, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA).

RESULTS

1) EVALUATION OF ANALYTICAL SENSITIVITY AND REPRODUCIBILITY

1.1 The limits of blank (LOB) and detection (LOD) were determined according to the CLSI EP17-A protocol:

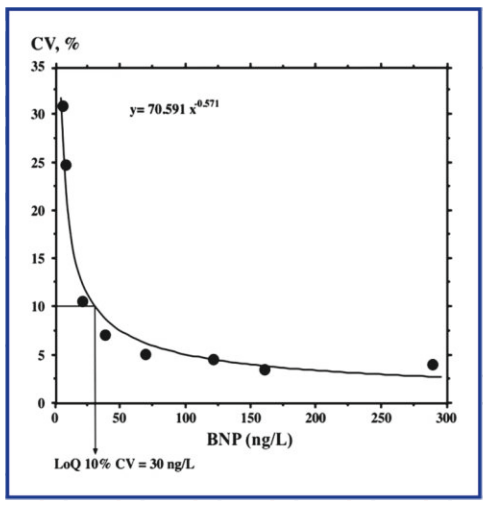
LoB = 2.6 ng/L LoD = 5.4 ng/L

LOB = meanblank + 1.645 SDblank; Blank = calibrator 1 (BNP = 0 ng/L)
LOD = LOB + 1.645 SD; SD = standard deviation of a plasma pool (BNP 3.68 ± 3.16 ng/L, n= 60)

Evaluation of assay reproducibility according to the CLSI EP5-A2 protocol

Plasma EDTA	N	Mean BNP (ng/L)	Within-run CV (%)	Total CV (%)
A	20	8.70	11.25	19.36
B	20	21.72	4.27	9.27
C	20	38.72	2.56	4.95

1.2 IMPRECISION PROFILE

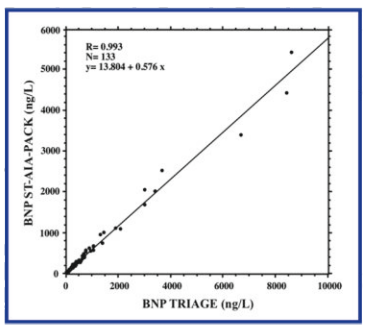


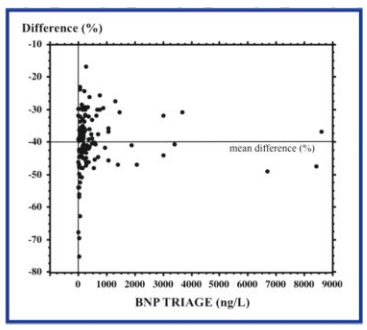
The between-runs imprecision profile was performed by repeatedly measuring, in 20 different runs, 8 plasma-EDTA pools obtained from healthy subjects and patients with heart failure.

LoQ at 20% CV = 9 ng/L
LoQ at 10% CV = 30 ng/L

2) COMPARISON BETWEEN METHODS

2.1 BNP ST AIA-PACK (TOSOH) VS. BNP TRIAGE (BECKMAN-COULTER)

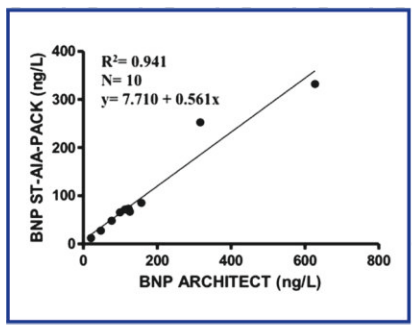


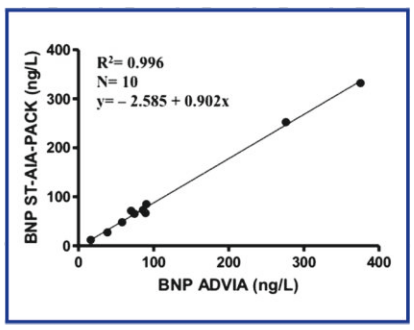


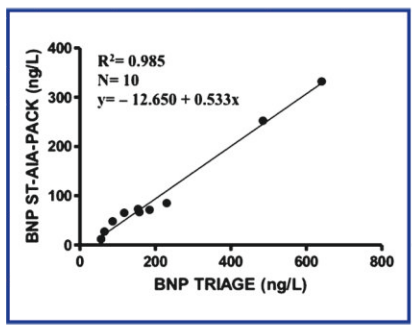
A very close linear relationship was found when comparing BNP values obtained measuring 133 EDTA-plasma samples of healthy subjects and cardiac patients, both with the TOSOH and Triage (Beckman-Coulter) method. However the TOSOH method showed a significant negative bias (i.e., underestimation) of BNP values compared to Beckman-Coulter of 40.0% ± 8.8% (mean ± SD, $p < 0.0001$).

There is not a significant correlation between the percentage difference and the BNP concentration ($R = 0.009$, $p = 0.9178$), while there is a close correlation between the absolute difference of the values obtained with the two methods and the BNP concentration ($R = 0.987$, $p < 0.0001$). These data show that the absolute difference increases with the concentration, instead the percentage difference is constant at each measured BNP value.

2.2 BNP ST AIA-PACK (TOSOH) VS. EQA CARDIOORMOCHECK 2012



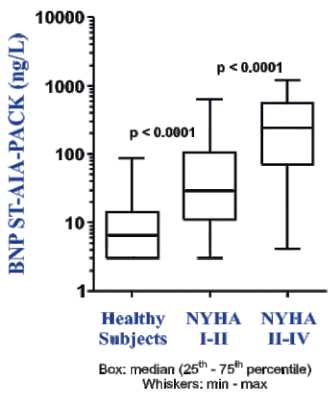




The samples of the CardioOrmoCheck, distributed in the EQA program of 2012, were analysed by BNP ST-AIA-PACK thus allowing a comparison with the most used BNP methods in Italy (Triage Beckman-Coulter, Architect Abbott and ADVIA Siemens).

Data showed a close correlation between BNP Tosoh and the other three methods. Tosoh method showed a good agreement with ADVIA while it underestimated BNP values in comparison with Triage and Architect methods.

3) CLINICAL RESULTS



We measured the plasma concentration of BNP with BNP ST-AIA-PACK in:
- Healthy subjects (n=126)
- HF patients, functional class NYHA I and II (n=31)
- HF patients, functional class NYHA III-IV (n=46)

ROC curve analysis showed the following results:
- AUC: 91.1 % (95%CI: 86.7% - 95.5%), $p < 0.001$
- Optimal cut-off value: 21.5 ng/L (Spec.: 88,8%, Sens.: 80.5%)

CONCLUSIONS

- The BNP ST-AIA-PACK has a good reproducibility and analytic sensitivity as the most used automated methods in commercial available.
- The Tosoh method for BNP closely correlate with other methods (Triage, Architect, ADVIA) and allows a correct clinical classification of HF patients.
- BNP TOSOH showed a good agreement with ADVIA, while it understimated values in comparison with Triage method and likely with Architect; these differences might be due to the different antibodies used.