Clinical Biochemistry 45 (2012) 171–174

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Case Report

Measurement of NT-proBNP with LOCI® technology in heart failure patients

D. Gruson [a,b,*], Sylvie A. Ahn [c], Thibault Lepoutre [b], Michel F. Rousseau [c]

[a] Pôle de recherche en Endocrinologie, Diabète et Nutrition, Institut de Recherche Expérimentale et Clinique, Cliniques Universitaires St-Luc and Université Catholique de Louvain, Brussels, Belgium
[b] Department of laboratory medicine, Cliniques Universitaires Saint Luc, Brussels, Belgium
[c] Division of Cardiology, Cliniques Universitaires St-Luc, Brussels, Belgium

ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 22 July 2011
Accepted 22 July 2011
Available online 6 August 2011

Keywords:
LOCI
NT-proBNP
Heart failure
Severity

ABSTRACT

Objectives: The aim of our study was to determine NT-proBNP concentrations in heart failure (HF) patients with a luminescent oxygen channeling immunoassay (LOCI®).

Design and methods: Seventy HF patients were enrolled. NT-proBNP levels were measured with LOCI® method and compared to a reference NT-proBNP assay.

Results: LOCI® NT-proBNP levels were significantly correlated with the reference NT-proBNP assay and were related to HF severity.

Conclusions: LOCI® assay demonstrates performances close to the comparative assay for NT-proBNP testing and allows a significant reduction of the time of analysis.

Background

Brain type natriuretic peptide (BNP) and amino terminal pro-brain natriuretic peptide (NT-proBNP), both derived from the same precursor proBNP, are released from the cardiac ventricles in response to myocardial stretch [1]. BNP and NT-proBNP represent valuable markers of acute and chronic heart failure (HF) [2,3]. Both markers are significantly increased in cases of left ventricular dysfunction and are recognized as strong predictors of morbidity and mortality in patients with heart failure or coronary disease [2,4–8].

Several assays for natriuretic peptides testing are available for routine use [9]. More recently, homogeneous immunoassays have been developed for cardiac markers [10,11]. The analytical performances of such a homogeneous immunoassay for NT-proBNP testing were evaluated as satisfactory [10].

Therefore, the aim of the present study was to measure NT-proBNP levels in HF patients with LOCI®, luminescent oxygen channeling immunoassay, and with a comparative method and to investigate the relationship with the disease severity, established on the basis of NYHA functional classes, the left ventricular ejection fraction and well-established biomarkers of HF worsening.

Abbreviations: BNP, B-type natriuretic peptide; Big-ET1, Big endothelin1; RIA, Radioimmunoassay; CI, Confident interval; HF, Heart failure; LOCI®, luminescent oxygen channeling immunoassay; NT-proBNP, N-terminal-pro B-type natriuretic peptide; NT-proANP, N-terminal-pro atrial natriuretic peptide; NYHA, New York Heart Association.

* Corresponding author at: Pôle de recherche en Endocrinologie, Diabète et Nutrition, Université catholique de Louvain, Tour Claude Bernard, 54 Avenue Hippocrate, B-1200 Brussels, Belgium. Fax: +32 2 7645418.
E-mail address: damien.gruson@uclouvain.be (D. Gruson).

Materials and methods

Study population

Seventy chronic HF patients and one hundred twenty healthy sex-matched controls were included in the study. The patients were stable and suffered from heart failure since a mean period of 16 months. The patients were recruited in the division of cardiology at the Cliniques Universitaires St-Luc, an academic hospital of Brussels, Belgium. HF was established by an independent experienced cardiologist (M.F.R.) blinded to all other measurements on the basis of clinical signs (pulmonary congestion, jugular venous distension, S3, peripheral edema), chest radiography, echocardiography and/or radionuclide/contrast angiography. The left ventricular ejection fraction (LVEF) was determined by contrast or isotopic ventriculography. Healthy volunteers had no history of hypertension, diabetes mellitus, or ischemic heart disease and were taking no medical therapy. The study protocol was approved by the institutional review board and all patients gave informed consent.

Laboratory investigations

For each patient, 15 mL of blood was collected from antecubital vein into tubes containing EDTA and serum tubes. After centrifugation within 1 h, plasma and serum were carefully separated and stored at −80 °C until assayed. LOCI® NT-proBNP assay was ran on the Dimension® Vista™ 1500 analyzer (Siemens Diagnostics). LOCI® technology utilizes paired synthetic beads, chemibead and sensibead, in a homogeneous immunoassay method with chemiluminescent detection [10]. The limit of detection for the LOCI® NT-proBNP assay was determined by performing 2 separate runs of 10 replicates of a

0009-9120/$ – see front matter
doi:10.1016/j.clinbiochem.2011.07.012

10 g/L bovine serum albumin solution (BSA, Sigma Aldrich, St-Louis, MO, USA). The linearity of the LOCI® NT-proBNP assay was assessed by performing serial dilutions of high values samples with the BSA solution. The samples from the HF patients were also used to evaluate the time needed to obtain the results on analyzers' screens with the LOCI® assay in comparison to the NT-proBNP comparative assay. The circulating levels of other biomarkers related to HF worsening, such as B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP), N-terminal pro-atrial natriuretic peptide $_{68-98}$ (NT-proANP) and Big endothelin-1 (Big ET-1), the inactive precursor of endothelin-1, were also determined. NT-proANP and Big ET-1 levels were measured using specific home-made radioimmunoassay [12]. NT-proBNP and BNP were measured respectively with the Elecsys® 2010 analyzer (Roche Diagnostics) and the DxI® BNP (Beckman Coulter, Alere reagents). All automated assays were performed according to manufacturer's specifications.

Statistical analysis

Statistical analysis and determination of the reference interval were performed using the MedCalc 7.2.1.0 package (Medcalc Software, Mariakerke, Belgium).Log transformation of the variables was performed when the distribution was not normal. Differences between control and CHF groups were assessed with one way analysis of variance with Student–Newman–Keuls test for all pairwise comparisons. Pearson correlation coefficients were determined. A p-value of 0.05 was considered as significant.

Results

Study population characteristics

The healthy volunteers group consists in 92 men and 28 women and the mean age for this group was 56 years (range: 37–64 years). For the HF patients, the mean age was 67 years, (range: 35–79 years; 55 men and 15 women) and the mean LVEF was 23 ± 7%. Twenty eight HF patients were classified as New York Heart Association (NYHA) classes I–II, 26 as NYHA class III and 6 as NYHA class IV. Eighty percent of the HF patients received beta-blockers, 100% angiotensin converting enzyme inhibitors, 86% diuretics, and 78% aldosterone-antagonists. The etiology of HF was dilated cardiomyopathy in 14 patients and ischemic disease in 56 patients.

Comparison of the LOCI® NT-proBNP method with a reference NT-proBNPassay

The limit of detection LOCI® NT-proBNP assay was 5 pg/mL. The mean recovery of the serial dilutions (2, 4, 8 and 16) of two high values samples (194,800 and 26,916 pg/mL) was 104, 98, 96 and 103%. The regression analysis of these data indicates acceptable linearity for the assay between 5 and 30,130 pg/mL. The NT-proBNP concentration ranges tested in HF patients were between 100 to 31,166 pg/mL and 112 to 33,020 pg/mL for the LOCI® and for the Elecsys® assays, respectively. In HF patients, LOCI® NT-proBNP correlates positively with the comparative NT-proBNP assay ($r = 0.998$, 95% CI: 0.998–0.999; $p < 0.0001$). The inter-rater agreement statistic (kappa coefficient) between the two NT-proBNP assays was 0.94, indicating a very good agreement between these assays. The Passing and Bablok regression analysis between the two NT-proBNP assays showed a slope of 0.95 (95% CI: 0.92 to 0.96) and an intercept of 58.1 (95% CI: 24.5 to 135.4) and no significant deviation of linearity (Fig. 1A). Bland and Altman plot of the values obtained with the two NT-proBNP assays revealed a mean difference of −2.6 pg/mL but a trend for slightly lower values for the LOCI® assay for NT-proBNP results above 10,000 pg/mL (Fig. 1B). The time to obtain the first results on analyzer screen reduced with a mean of 8 min for LOCI® in comparison to the conventional assay.

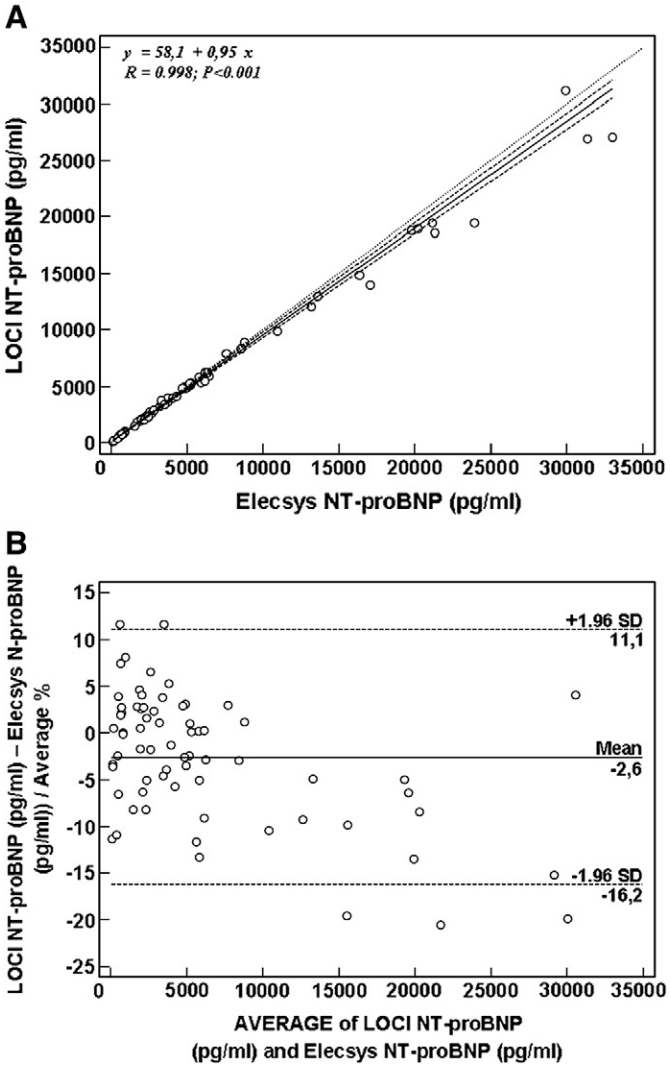

Fig. 1. Method comparison between the two NT-proBNP assays. (A) shows the Passing and Bablok regression plot and (B) the Bland and Altman plot.

Association between LOCI® NT-proBNP levels and HF severity

The range of NT-proBNP concentrations observed for samples from healthy control subjects with the LOCI® assay was between 5 and 169 pg/mL with a median concentration of 32.0 pg/mL. The calculated upper limit of the 95th reference interval was 133 pg/mL. Women had higher LOCI® NT-proBNP levels than men (48.7 vs 25.4 ng/mL). The LOCI® NT-proBNP concentrations were significantly higher in HF patients than in healthy volunteers [Geometric Mean (pg/mL) with 95% CI: 3027 (2204–4157) vs 30.8 (27.0–35.2), respectively; $p < 0.0001$; $n = 70$ vs 120]. LOCI® NT-proBNP were significantly and positively correlated with age ($r = 0.33$, 95% CI: 0.10–0.53; $p = 0.006$) and creatinin ($r = 0.42$, 95% CI: 0.19–0.61; $p = 0.0007$) in HF patients, two established confounders for NT-proBNP testing [13,14]. Moreover, as observed for the comparative NT-proBNP assay (Fig. 2A), LOCI® NT-proBNP levels were significantly related to the severity of HF evaluated according to the NYHA classification (Fig. 2B). Indeed, LOCI® NT-proBNP geometric mean in NYHA I–II, NYHA III and NYHA IV patients were 1226, 4674 and 11,936 pg/mL, respectively ($p < 0.0001$). The association between NT-proBNP levels, measured