Clinical Biochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Prognostic significance of active and modified forms of endothelin 1 in patients with heart failure with reduced ejection fraction

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ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 26 November 2014
Accepted 12 December 2014
Available online xxxx

Keywords:
Endothelin
Heart failure
Congestive
Prognosis

ABSTRACT

Objectives: Concentrations of endothelin I (ET1) are elevated in CHF patients and, like other biomarkers that reflect hemodynamic status and cardiac pathophysiology, are prognostic. The Singulex assay (Sgx-ET1) measures the active form of ET1, with a short in vivo half-life and the Brahms assay measures C-terminal endothelin-1 (CT-ET1), a modified (degraded) product with longer half-life. We aimed to determine the prognostic importance of active and modified forms of endothelin 1 (Singulex and Brahms assays) in comparison with other commonly measured biomarkers of inflammation, hemodynamic status and cardiac physiology in CHF.

Design and methods: Plasma biomarkers (Sgx-ET1, CT-ET1, NTproBNP, IL-6, TNFα, cTnI, VEGF, hs-CRP, Galectin-3, ST2) were measured in 134 NYHA class II and III CHF patients with systolic dysfunction. Prognostic importance of biomarkers for hospitalization or death were calculated by both logistic regression and Kaplan–Meier survival analyses.

Results: CT-ET1 (OR = 5.2, 95% CI = 1.7–15.7) and Sgx-ET1 (OR = 2.9, CI = 1.1–7.7) were independent predictors of hospitalization and death and additively predicted events after adjusting for age, sex, and other significant biomarkers. Other biomarkers did not improve the model. Similarly, in Cox regression analysis, only CT-ET1 (HR 3.4, 95% CI = 1.4–8.4), VEGF (2.7, 95% CI = 1.3–5.4), and Sgx-ET1 (HR 2.6, 95% CI = 1.2–5.6) were independently prognostic.

Conclusions: Elevated concentrations of endothelin 1 predict mortality and hospitalizations in HF patients. Endothelin 1 was more prognostic than commonly obtained hemodynamic, inflammatory, and fibrotic biomarkers. Two different assays of endothelin 1 independently and synergistically were prognostic, suggesting either complementary information or extreme prognostic importance.

Introduction

Concentrations of endothelin 1 (ET1), a potent vasoconstrictor produced by endothelial cells, are elevated in heart failure (HF) patients and, like other biomarkers that reflect hemodynamic status and cardiac pathophysiology, have been shown to be prognostic in HF patients [1,2]. However, there are different forms of the peptide that are measured by two available ET1 assays. The Singulex assay (Sgx-ET1) measures the active form of ET1, with a short in vivo half-life. On the other hand, C-terminal endothelin 1 (CT-ET1) is measured by the Brahms assay and is a modified (degraded) product with a longer half-life. The prognostic importance of these forms of ET1 is unknown.

Furthermore, few studies have compared the prognostic importance of ET1 to the multiple biomarkers which are prognostic in HF patients. Natriuretic peptides, markers of inflammation, and indicators of fibrosis all can predict mortality and morbidity. Whether ET1 adds information to that provided by these biomarkers has not been fully characterized.

Therefore, we sought to determine the relative prognostic importance of the active and modified forms of ET1 in comparison with other commonly measured biomarkers of inflammation, hemodynamic status, and cardiac physiology.

Materials and methods

Plasma biomarkers were measured in 134 subjects with ischemic and non-ischemic HF that were recruited as part of the BETRHeart study evaluating the interaction of psychologic and physiologic characteristics of HF patients and their interactions with outcomes. Entry criteria included New York Heart Association (NYHA) classification II or III HF for at least 3 months and left ventricular ejection fraction ≤40%. This study was approved by the Human Volunteers Research committees at the University of Maryland Baltimore and the Uniformed

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http://dx.doi.org/10.1016/j.clinbiochem.2014.12.012
0009-9120/

Table 1
Analytical characteristics of biomarkers.

Biomarker	Limit of detection	Low limit of quantitation	Upper reporting limit	Upper reference range (99th percentile for cTnI)	Precision profile (CV) and comments
IL-6	0.01 pg/mL	0.38 pg/mL	100 pg/mL	7.2 pg/mL	12% at 1.3 pg/mL 12% at 12 pg/mL
TNFα	0.01 pg/mL	0.11 pg/mL	50 pg/mL	4.3 pg/mL	11% at 1.2 pg/mL 7% at 17.8 pg/mL
cTnI	0.09 pg/mL	0.40 pg/mL	250 pg/mL	7.1 pg/mL	11% at 3.0 pg/mL 15% at 60.9 pg/mL
VEGF	1.0 pg/mL	6.0 pg/mL	400 pg/mL	65.8 pg/mL	12% at 22.7 pg/mL 10% at 39.3 pg/mL
Sgx-ET1	0.07 pg/mL	0.20 pg/mL	250 pg/mL	3.7 pg/mL	14% at 1.8 pg/mL 7% at 7.8 pg/mL
CT-ET	0.4 pmol/L	3 pmol/L	500 pmol/L	73.9 pmol/L	<10% at 40–80 pmol/L <6% at >80 pmol/L:
NTproBNP	5 pg/mL	50 pg/mL	35,000 pg/mL	<75 years old < 125 pg/mL >75 years old <450 pg/mL	ICON cutoffs for HF diagnosis: <50 years old: >450 pg/mL 50–75 years old: >900 pg/mL >75 years old: >1800 pg/mL exclusion of acute heart failure (any adult age): <300 pg/mL
ST2	1.31 ng/mL	2.35 ng/mL	200 ng/mL	35 ng/mL*	Storage Condition Stability Room temperature (22–28 °C) 48 h; refrigerated (2–8 °C) 7 days, frozen (−20 °C or -80 °C) at least 18 months
Galectin-3	1.13 ng/mL	1.32 ng/mL	94.8 ng/mL	17.8 ng/mL**	2.0% at 6.1 ng/mL 5.1% at 17.6 ng/mL 7.7% at 20.7 ng/mL 4.2% at 26.3 ng/mL 4.4% at 46.2 ng/mL 8.0% at 72.2 ng/mL

* Higher risk of adverse outcomes when ST2 levels are above this cutoff value.
** Higher risk of adverse outcomes when Galectin-3 levels are above 17.8 ng/mL.

Services University of the Health Sciences. All patients signed an informed consent.

Assays

Serum and plasma samples were assayed for a panel of biomarkers. Blood was drawn in K2 EDTA, heparinized, and plain tubes and immediately spun for 15 min in a refrigerated centrifuge. Serum and plasma were separated and stored at −20 °C until assayed. Research-Use-Only (RUO) assays ET1 (Sgx-ET1), IL-6, TNFα, cardiac specific troponin I (cTnI), and Vascular endothelial growth factor (VEGF) were all measured using high sensitivity single-molecule counting technology (RUO, Erenna® Immunoassay System, Singulex, Inc.) at no charge. This system utilizes paramagnetic microparticles as the solid phase format in combination with single-molecule counting. In the Erenna system, the immunoassay complex formed on the MP surface results in the release of fluorescently labeled detection antibody. The resulting solution is sipped into a 100 μm flow capillary, and photons are counted, via confocal microscopy, as they pass through a 2 μm interrogation space. CT-ET1 was measured on the Brahms Kryptor System (Thermo Fischer); this assay quantifies an inactive c-terminus degradation product of ET1, which has a longer half-life than the parent molecule. NTproBNP and hs-CRP were both determined using the ElecSys 2010 analyzer (Roche Diagnostics, Indianapolis, IN).

Galectin 3 (BG Medicine, Boston, MA) and ST2 (Critical Diagnostics, San Diego, CA) were measured by ELISA in 115 patients. Because of the smaller number of measurements, these biomarkers were not included in the primary analyses. However, they were included in secondary analyses.

The ET-1, cTnI, IL-6, TNFα, and VEGF assays were performed using single-molecule counting technology. All of the assays, except VEGF, have been validated as laboratory developed tests in the Singulex CLIA licensed, CAP-accredited laboratory. The VEGF assay was at a validated development stage. The analytical characteristics are depicted in Table 1.

Since ET-1 is a biologically active small peptide derived from a larger precursor molecule and has a demonstrated short biological half-life in circulation (is rapidly cleared), it was important to understand the physical stability of the molecule in plasma. Sgx-ET1 was measured in 20 freshly drawn (day 0) EDTA plasma samples, which were then stored at 2–8 °C, for 1, 4, 8, and 10 days and tested for ET-1 on the respective day. Sgx-ET1 did not show decay from day 0 values up to day 4. Samples on days 8 and 10 showed diminished concentrations of SgX ET-1. Additionally, 10 plasma samples were tested after being frozen at −80 °C and thawed 0, 1, and 2 times. ET-1 concentrations were stable for up to 2 freeze-thaw cycles. Taken together, these findings demonstrate that ET-1 is reasonably stable in plasma for analytical testing.

Statistical analysis

The prognostic importance of the biomarkers for hospitalization or death were calculated by logistic regression, Cox's regression, and Kaplan–Meier survival analyses.

Table 2
Biomarkers dichotomized using the empirical distribution function.

Biomarker	Cut point	Total CV at Cut point
IL-6	15 pg/mL	13%
TNFα	5.3 pg/mL	9%
VEGF	36.7 pg/mL	10%
CT-ET1	80 pmol/L	6%
Sgx-ET1	4.4 pg/mL	11%
cTnI	9.1 pg/mL	8%
NTproBNP	1000 pg/mL	2%
CRP	4.2 mg/L	3%

Please cite this article as: Gottlieb SS, et al, Prognostic significance of active and modified forms of endothelin 1 in patients with heart failure with reduced ejection fraction, Clin Biochem (2014), http://dx.doi.org/10.1016/j.clinbiochem.2014.12.012