Characterization of Plasma Endothelin-1 in a Large Community-based Patient Population at Risk for Cardiovascular Disease

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ABSTRACT

Background: Endothelin (ET-1, a 21-amino acid) is a potent physiological vasoconstrictor that plays a pivotal role in vascular dysfunction. Elevated ET-1 levels have been associated with both the occurrence and severity of atherosclerosis and heart failure; however, no reports characterize ET-1 in a population, judging at risk for cardiovascular disease (CVD) using traditional biomarkers (HDL, HbA₁c).

Objective: To determine plasma levels of ET-1 and identify the effect of co-morbidities on its levels in a large cohort of CVD risk study population.

Methods: We previously reported the development of a Single Molecule Counting immunoassay to quantify ET-1 plasma ET levels. This assay was further developed into a lab developed test and offered in a CLIA-certified, CAP-licensed clinical laboratory. In this large and diverse patient population (gender, age, BMI, HDL, HbA₁c, NTproBNP, IL-6, TNF-α, IL-17A, or cTnI), we previously reported the results of a high sensitivity assay targeting the active 21 amino acid ET-1 molecule in a large and diverse population of patients. We are the first to report the results of a high sensitivity assay targeting the active 21 amino acid ET-1 molecule in a large and diverse population of patients.

Results: The concentrations of ET-1 in the CVD risk population are shown in Table 1. ET-1 was quantifiable in >99.9% patients and >5.2% were above the upper limit of quantification (ULOQ). Patient levels were significantly higher in patients > 50 yrs, males, as well as those with CV risk factors of pre-diabetes, diabetes and HDL dyslipidemia. Of note ET-1 was paradoxically higher in patients with elevated results for HDL, HbA₁c, NTproBNP, IL-6, TNF-α, IL-17A, and cTnI, respectively, p(<0.001, 0.01), p<0.0001. The adjusted odds ratios predicting an elevated NTproBNP trended with increasing ET-1 quartiles. Quarters 3 and 4 were significantly higher than the reference quartile. Patients greater than the ET-1 99th%-tile were 5.4 (2.8,10.5) times more likely to have an elevated cTnI after adjustment.

Conclusions: The ET-1 assay was developed and performance characteristics were determined by Singulex. This test has not been validated by a different laboratory. Although there are statistically significant differences in patient ET-1 values based upon age and gender, these differences do not appear to warrant age and gender specific reference ranges.

METHODS

SMC™ ET-1 Quantitative Testing Method

A quantitative fluorescent one-step sandwich immunoassay was developed to measure human ET-1 levels in EDTA plasma samples. Samples, controls, standards, capture reagent, and detection reagent were added to the wells of a 96-well microplate to start the reaction. During incubation, the ET-1 in the specimen bound to capture antibodies biotinylated to microparticles (MP) and to fluorescently-conjugated detection antibodies. After the unbound reagents were removed, detection antibodies labeled with a wash procedure, an elution buffer was added to dissolve biotin-labeled detection antibodies, releasing fluorescent detection antibody into the eluant. The contents of each microparticle well were transferred to a 96-well plate and loaded onto the Envision® System for single molecule counting. The measured amount of fluorescently-labeled detection antibodies is directly proportional to the amount of ET-1 in the sample.

The ET-1 reference population consisted of patients considered to be healthy based on self-questionsnaires. The reference population consisted of 300 healthy patients (50% female) from 5 US states. The ‘at-risk’ and high-risk reference limits were established at the distribution 99th and 99.9th%ile.

HbA₁c, HDL, NTproBNP, SMC IL-6, SMC TNF-α, SMC IL-17A, and SMC cTnI Testing Methods

Hemoglobin A1c was measured on the Roche cobas® 6000 System by a turbidimetric inhibition immunoassay. The measured range was 4.5-6.5% and the clinical cutoff was <5.9%. HDL was directly measured on the Roche cobas® 6000 System by an enzymatic colorimetric reaction. The measurable range was 0.9-1.3 mg/dL, and the clinical cutoff was <40 mg/dL depending on gender. NTproBNP was measured on the Roche cobas® 6000 System by electrochemiluminescence immunoassay. The measured range was 0-10,000 mg/L and the clinical cutoff was >124 mg/L (lower limit of quantification, ULLOQ=upper limit of quantification).

Increased Medium ET-1 Concentrations in Patients Partitions by the Clinical Determinations from Seven CVD Biomarkers

HDL, HbA₁c, NTproBNP, SMC IL-6, SMC TNF-α, SMC IL-17A, and SMC cTnI were measured on the Singulex System. The SMC ET assay was developed and performance characteristics were determined by Singulex. This test has not been validated by a different laboratory. Although there are statistically significant differences in patient ET-1 values based upon age and gender, these differences do not appear to warrant age and gender specific reference ranges.

CONCLUSIONS

ET-1 is measurable in 99.9% of a large community based patient population. Six percent (6%) of the patient population is greater than the 99th%ile reference limit. ET-1 is measurable in 99.9% of a large community based patient population.