Clinical Chemistry 64:11
1617–1625 (2018)

Endothelin-1 Measurement in Patients Undergoing Diagnostic Coronary Angiography—Results from the Catheter Sampled Blood Archive in Cardiovascular Diseases (CASABLANCA) Study

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BACKGROUND: Endothelin-1 (ET-1) is a vasoconstrictor produced by vascular endothelial cells and may play a role in risk for development of coronary artery disease (CAD) and heart failure (HF). In a cohort of 1084 patients referred for coronary angiography, we investigated cross-sectional associations between ET-1 concentrations and prevalent CAD, as well as value of ET-1 for prognostication of future cardiovascular events.

METHODS: Associations between ET-1 and presence/severity of CAD were assessed. Patients were followed for a median of 4 years for outcomes including incident HF, myocardial infarction (MI), cardiovascular mortality, and all-cause mortality.

RESULTS: The median concentration of ET-1 was 2.57 ng/L. Patients with ET-1 concentrations above the median were more likely to have higher risk clinical features. Among those without prevalent MI at presentation, ET-1 concentrations were not associated with presence or severity of CAD. In adjusted Cox proportional hazards analyses, log-transformed ET-1 concentrations predicted incident HF [hazard ratio (HR) = 1.51 per increase in log-SD; 95% CI, 1.06–2.15; $P = 0.02$] and all-cause mortality (HR = 1.61 per increase in log-SD; 95% CI, 1.03–2.53; $P = 0.04$). Concentrations of ET-1 above the median were associated with shorter time to incident HF, MI, cardiovascular mortality, all-cause mortality, and the composite of incident HF/MI/cardiovascular mortality (all log-rank $P < 0.001$).

CONCLUSIONS: Despite epidemiologic links to CAD, we found no cross-sectional association between biologically active ET-1 and prevalent coronary atherosclerosis in an at-risk population referred for coronary angiography. Increased ET-1 concentrations independently predict incident HF and death and are associated with more near-term cardiovascular events.

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The endothelium produces numerous biologically active substances that play an integral role in the regulation of inflammation, cell growth, thrombosis, and vascular tone. Dysregulation of this process may lead to vascular endothelial dysfunction, 1 of the earliest findings in the development of cardiovascular disease *(1)*. Hickey and colleagues were the first to characterize a 21-amino acid-long peptide vascular smooth muscle vasoconstrictor produced by cultured endothelial cells in 1985 *(2)*, subsequently named endothelin. Since then, 3 distinct isoforms of endothelin have been identified: endothelin-1 (ET-1)[7], ET-2, and ET-3, with ET-1 being the most abundant and best described. ET-1 is produced by a variety of cells in the cardiovascular, urinary, nervous, immune, skin, and respiratory systems *(3)*.

ET-1 is a biologically active small peptide derived from a larger precursor molecule with a short biological half-life in circulation because of its rapid clearance. Mature ET-1 is a 21-amino acid peptide formed via a 39-amino acid intermediate, Big ET-1; this latter peptide is processed by a family of ET-converting enzymes and other enzymes such as chymases, metalloproteinases, and endopeptidases *(1)*. The different forms of the 21-amino acid-long peptide are measured by 2 commercially available ET-1 assays. One measures the active form of ET-1, which has a short in vivo half-life and low variability. A C-terminal ET-1 assay is also

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Received January 10, 2018; accepted July 30, 2018.
Previously published online at DOI: 10.1373/clinchem.2017.286385
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[7] Nonstandard abbreviations: ET, endothelin; CAD, coronary artery disease; HF, heart failure; MI, myocardial infarction; CASABLANCA, Catheter Sampled Blood Archive in Cardiovascular Diseases; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro B-type natriuretic peptide; hs-cTnI, high-sensitivity cardiac troponin I; OR, odds ratio; HR, hazard ratio.

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available, which measures a stable degradation product with a longer half-life *(4)*. There are 2 ET receptor subtypes, ET_A and ET_B, that mediate the action of ET. ET_A receptors promote vasoconstriction, cellular growth, and inflammation, and ET_B receptors produce opposing actions of vasodilation, inhibition of cellular growth and inflammation, and increase in sodium excretion *(5)*.

Through its biologic effects, concentrations of ET-1 may play a central role in the pathogenesis of cardiovascular disease, including both coronary artery disease (CAD) and heart failure (HF). Recently, Gupta and colleagues described a potential role of genetic variation in the 6p24 locus that distally regulates *EDN1*[8] expression and is associated with risk of 5 vascular diseases, including CAD *(6)*. A single noncoding variant in the locus was associated with *EDN1* expression in cultured endothelial cells, circulating ET-1 levels, and vascular disease risk. Although this finding implicates ET-1 in the development of CAD, the role of ET-1 as a biomarker for CAD progression remains unclear, with occasionally conflicting results from studies examining its importance *(7)*.

Beyond these findings, circulating ET-1 has been shown to be a prognostic marker in patients with HF and acute myocardial infarction (MI) *(8, 9)* and may predict risk for incident pulmonary hypertension, mortality, and HF in community-based subjects *(10)*. Less is known about the prognostic meaning of ET-1 in a more representative population of patients with cardiovascular disease, such as those referred for coronary angiography; such a population represents a group of higher risk patients that might be specifically targeted for therapeutic interventions to reduce their risk. Accordingly, using resources of the Catheter Sampled Blood Archive in Cardiovascular Diseases (CASABLANCA) study, we sought to investigate the meaning of ET-1 concentrations in a population of patients undergoing coronary angiography. We hypothesized ET-1 concentrations to be linked to prevalent CAD and thereby would further prognosticate future cardiovascular events.

Methods

All study procedures were approved by the Partners Healthcare Institutional Review Board and carried out in accordance with the Declaration of Helsinki.

STUDY DESIGN AND PARTICIPANTS

The design of the CASABLANCA study has been detailed previously *(11)*. Briefly, 1251 patients undergoing coronary and/or peripheral angiography with or without intervention between 2008 and 2011 were prospectively enrolled at the Massachusetts General Hospital in Boston, MA. Patients were referred for angiography for various acute and nonacute indications. Of the 1251 patients enrolled, we focused this analysis on 1084 patients undergoing coronary angiography.

DATA ACQUISITION

After informed consent was obtained, detailed clinical and historical variables were recorded using a standardized case report form at the time of the angiographic procedure. This case report form included >100 clinical variables acquired at the time of study entry and results of coronary angiography. Angiographic results were based on visual interpretation by the operator and verified via the catheterization report.

FOLLOW-UP

The median follow-up period was 4 years, with a maximum follow-up period of 6 years. Follow-up was complete for all patients. Processes for identification and adjudication of clinical end points were as previously described *(11)* and included review of medical records; follow-up phone calls with patients and/or managing physicians were performed by physicians blinded to biomarker concentrations. The Social Security Death Index and/or postings of death announcements were used to confirm vital status. A detailed definition of end points for CASABLANCA was previously published *(11)*.

Specific to this analysis, incident HF was defined as signs and symptoms of HF in a patient without a previous diagnosis of chronic HF, as well as at least 1 of the following: (*a*) initiation or increase in dosage of diuretic or (*b*) radiographic evidence for pulmonary congestion or (*c*) structural heart disease with documentation of left ventricular ejection fraction <40% or (*d*) diastolic dysfunction or (*e*) B-type natriuretic peptides (BNPs) ≥400 ng/L or N-terminal pro B-type natriuretic peptide (NT-proBNP) according to age: <50 years, ≥450 ng/L; 50 to 75 years, ≥900 ng/L; >75 years, ≥1800 ng/L *(12)*.

Incident MI was defined using the criteria proposed by the Global Task Force for the Universal Definition of MI to include types 1 through 5 *(13)*. Presence of CAD was categorized into any stenosis severity, ≥50% stenosis in any vessel, and ≥70% stenosis in any vessel. Death was classified into cardiovascular and noncardiovascular causes; in cases when the cause of death was unknown or unwitnessed, a cardiac cause was assumed.

BIOMARKER TESTING

A total of 15 mL of blood was obtained immediately before the angiographic procedure through a centrally placed vascular access sheath. The blood was immediately centrifuged for 15 min, and serum and plasma were aliquoted on ice and frozen at −80 °C until biomarker

[8] Human Gene: *EDN1*, endothelin 1.

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measurement. The samples for this study were analyzed after the first freeze–thaw cycle for baseline biomarker values only.

Measurement of ET-1 was performed using a laboratory-developed test in a CLIA-licensed, College of American Pathologists–accredited laboratory (Singulex, Alameda, CA) that detects the 21-amino acid active form of the peptide. Analyses were performed on an Erenna platform. This system uses magnetic microparticles as a solid phase, in combination with confocal microscopy-based single-molecule counting of labeled detection antibodies passing through an interrogation space. The reporting range of this assay is from 0.18 ng/L to 250 ng/L. Assay sensitivity (limit of detection) was calculated to be 0.07 ng/L, and the assay lower limit of quantification determined from the precision profile was found to be 0.175 ng/L at 20% CV and 1.31 ng/L at 10% CV. The reference interval for this assay is 0.8 to 2.7 ng/L (90% of a healthy population). In this analysis, the assay had intraassay and interassay imprecision of 5% and 11% at 3.2 ng/L, respectively, whereas at 9.1 ng/L, the corresponding imprecision was 6% and 10%, respectively.

High-sensitivity cardiac troponin I (hs-cTnI) was measured on the Erenna platform (Singulex); this highly sensitive assay has a limit of detection of 0.5 ng/L and a 99th percentile reference limit of 6 ng/L in apparently healthy individuals. Suppression of tumorigenicity (ST2) measurement was performed using the Presage ST2 method (Critical Diagnostics) with intraassay and interassay imprecision of 5.2% and 9% at 6 ng/mL, respectively. NT-proBNP, myeloperoxidase, and cystatin (Siemens) were measured on a Vista platform.

STATISTICAL ANALYSIS

Baseline characteristics between those with ET-1 concentrations below and above the median were compared. Dichotomous variables were compared using χ^2 or Fisher exact tests, whereas continuous variables were compared using the *t*-test or Kruskal–Wallis test. Univariate linear regression models and univariate logistic regression models were used to identify predictors of log-transformed ET-1 concentrations and ET-1 concentrations dichotomized by the mean, respectively (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue11).

For the CAD analysis, univariate logistic regression analysis with clinical and laboratory variables including log-transformed ET-1 concentration was used to identify predictors of CAD as defined by the previously described categories. We then included the covariates significant at an α level of 0.10 into stepwise logistic regression models (using an α level of 0.10 for both entry and retain) with log-transformed ET-1, age, and sex forced into the model. A similar analysis was performed with ET-1 concentration dichotomized at the median. We excluded 90 patients presenting with MI from the CAD analysis to avoid confounding. Similarly, age- and sex-adjusted Cox proportional hazard regression including log-transformed ET-1 was used to predict each of incident HF, incident MI, cardiovascular mortality, and all-cause mortality. We then included the covariates significant at an α level of 0.10 into stepwise Cox proportional hazard models (using an α level of 0.10 for both entry and retain) to develop a prediction model. Similar analyses were performed with ET-1 concentration dichotomized at the median. Kaplan–Meier survival curves were constructed using median ET-1. Incident HF, cardiovascular mortality, all-cause mortality, and combined outcome models were additionally adjusted for history of HF and previous MI.

The clinical and biomarker covariates included heart rate, systolic blood pressure, history of atrial fibrillation/flutter, hypertension, previous documented history of CAD, HF, chronic obstructive pulmonary disease, diabetes, and chronic kidney disease; medications including angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β blockers, aldosterone antagonists, loop diuretics, nitrates, and calcium channel blockers; and biomarkers including myeloperoxidase (Siemens), hs-cTnI (Singulex), NT-proBNP (Siemens), cystatin C (Siemens), soluble (s)ST2 (Critical Diagnostics), and estimated glomerular filtration rate.

In all statistical analyses, a 2-tailed *P* value of <0.05 was considered statistically significant. All analyses were performed using SAS version 9.4.

Results

BASELINE CHARACTERISTICS

The median concentration of ET-1 was 2.57 ng/L. Patients with an ET-1 concentration above the median (n = 550) tended to be older and had a history of smoking, atrial fibrillation/flutter, hypertension, HF, peripheral artery disease, chronic obstructive pulmonary disease, diabetes, and chronic kidney disease. A higher proportion of patients with an ET-1 concentration above the median were taking angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, loop diuretics, aspirin, and warfarin. Patients with ET-1 concentration above the median had worse left ventricular ejection fraction ($P < 0.001$). They also had lower estimated glomerular filtration rate and tended to be more anemic. They had higher concentrations of hs-cTnI, NT-proBNP, cystatin C, and ST2 (all *P* values significant) (Table 1).

ET-1 CONCENTRATIONS AND CAD

For analyses of ET-1 and CAD presence/extent, we excluded 90 (8.3%) patients with prevalent acute MI to avoid confounding. In this cohort of patients without MI, ET-1 concentrations were not associated with presence or severity

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Table 1. Baseline characteristics of patients with ET-1 above vs below median concentration.

Characteristics	ET-1 < median (n = 534)	ET-1 ≥ median (n = 550)	*P* value
Demographic			
Age (mean ± SD)	64.9 ± 11.1	68.5 ± 11.5	<0.001
Male sex	73.6%	68.9%	0.09
White	92.3%	94.2%	0.22
Medical history			
Smoker	11.5%	15.7%	0.05
Atrial fibrillation/flutter	12.4%	26.0%	<0.001
Hypertension	68.5%	77.8%	<0.001
CAD	51.9%	50.0%	0.54
Previous MI	22.1%	25.8%	0.15
HF	11.6%	29.5%	<0.001
Peripheral artery disease	13.3%	22.6%	<0.001
COPD[a]	14.0%	20.8%	0.004
Diabetes type I/type II	22.3%	29.1%	0.01
CVA[b]/TIA[c]	9.6%	10.2%	0.73
CKD[d]	7.3%	16.7%	<0.001
Renal replacement therapy	1.1%	4.9%	<0.001
Previous angioplasty	10.7%	9.6%	0.57
Previous stent	30.9%	24.6%	0.02
Previous CABG[e]	19.5%	19.3%	0.93
Medications			
ACEi[f]/ARB[g]	48.5%	59.3%	<0.001
β blocker	71.9%	69.6%	0.40
Aldosterone antagonist	3.6%	4.8%	0.32
Loop diuretics	11.1%	31.5%	<0.001
Nitrates	19.3%	19.6%	0.89
CCB[h]	21.7%	26.5%	0.07
Statin	73.4%	69.6%	0.17
Aspirin	82.5%	69.7%	<0.001
Warfarin	8.2%	22.3%	<0.001
Clopidogrel	27.0%	18.1%	<0.001
Previous echo test			
LVEF[i], %[j]			
Mean ± SD	59.2 ± 12.6	52.5 ± 17.6	<0.001
Angiography results			
≥30% coronary stenosis in ≥2 vessels	62.5%	59.6%	0.33
≥30% coronary stenosis in ≥3 vessels	44.1%	44.8%	0.81
≥50% coronary stenosis in ≥2 vessels	49.3%	47.7%	0.59
≥50% coronary stenosis in ≥3 vessels	28.8%	28.9%	0.98
≥70% coronary stenosis in ≥2 vessels	37.7%	35.7%	0.48
≥70% coronary stenosis in ≥3 vessels	19.1%	16.3%	0.23

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Table 1. Baseline characteristics of patients with ET-1 above vs below median concentration. *(Continued from page 1620)*

Characteristics	ET-1 < median (n = 534)	ET-1 ≥ median (n = 550)	*P* value
Lab measures			
Sodium, mmol/L			
Mean ± SD	139 ± 3	139 ± 3	0.14
Blood urea nitrogen, mg/dL			
Mean ± SD	19 ± 8.2	22 ± 11	<0.001
Creatinine, mg/dL			
Mean ± SD	1.2 ± 0.8	1.4 ± 1.2	<0.001
CKD-EPI eGFR[k], mL/min/1.73 m^2			
Mean ± SD	98 ± 23	81 ± 30	<0.001
Hemoglobin, g/dL			
Mean ± SD	13.6 ± 1.6	12.9 ± 1.7	<0.001
Baseline biomarkers			
MPO[l], pmol/L			
Median (Q1, Q3)	406 (309, 564)	434.0 (324, 609)	0.05
hs-cTnI, ng/L			
Median (Q1, Q3)	3 (2, 8)	7 (3, 19)	<0.001
NT-proBNP, ng/L			
Median (Q1, Q3)	165 (71, 455)	805 (251, 2230)	<0.001
Cystatin C, mg/L			
Median (Q1, Q3)	0.7 (0.7, 0.9)	0.9 (0.7, 1.1)	<0.001
sST2[m], ng/mL			
Median (Q1, Q3)	35 (26, 44)	38 (28, 55)	0.006

[a] Chronic obstructive pulmonary disease.
[b] Cerebrovascular accident.
[c] Transient ischemic attack.
[d] Chronic kidney disease.
[e] Coronary artery bypass graft.
[f] Angiotensin-converting enzyme inhibitor.
[g] Angiotensin receptor blocker.
[h] Calcium channel blocker.
[i] Left ventricular ejection fraction.
[j] Any echocardiogram performed in the 6 months before study enrollment was included; 231 patients with ET-1 < median had an echocardiogram and 315 with ET-1 > median had an echocardiogram.
[k] Estimated glomerular filtration rate.
[l] Myeloperoxidase.
[m] sST2, soluble ST2.

of CAD. In logistic regression models adjusted for clinical variables and prognostic biomarkers in those patients without presenting MI, log-transformed ET-1 concentration did not predict presence of any CAD [odds ratio (OR) = 0.54; 95% CI, 0.31–0.96; $P = 0.04$] or presence of either ≥50% or ≥70% stenosis in any vessel (OR = 0.70; 95% CI, 0.44–1.12; $P = 0.14$ and OR = 0.59; 95% CI, 0.39–0.91; $P = 0.02$, respectively). Similarly, ET-1 concentration above the median did not predict presence of any CAD (OR = 0.90; 95% CI, 0.60–1.35; $P = 0.61$) or ≥50% or ≥70% stenosis in any vessel (OR = 0.94; 95% CI, 0.67–1.32; $P = 0.73$ and OR = 0.79; 95% CI, 0.57–1.08; $P = 0.13$, respectively). Similarly, with log-transformation of all biomarkers used in the regression models, ET-1 was not predictive of any CAD (see Table 2 in the online Data Supplement).

ET-1 CONCENTRATIONS AND INCIDENT HF, INCIDENT MI, CARDIOVASCULAR MORTALITY, AND ALL-CAUSE MORTALITY

Details regarding the number of events can be found in Table 2. In Cox regression models adjusted for clinical variables and other prognostic biomarkers, continuously modeled log-transformed ET-1 concentration was predictive of incident HF [hazard ratio (HR) = 1.51 per

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Table 2. Event rates throughout study.

Events	Number of events
MI	15.1% (164/1084)
HF	23.6% (256/1084)
CV[a] death	11.5% (125/1084)
All-cause death	15.2% (165/1084)
MI/CV death	22.1% (239/1084)
MI/All-cause death	24.3% (263/1084)
HF/MI/CV death	34.7% (376/1084)
HF/MI/All-cause death	36.3% (393/1084)

[a] Cardiovascular.

increase in log-SD; 95% CI, 1.06–2.15; $P = 0.02$], as well as all-cause mortality (HR = 1.61 per increase in log-SD; 95% CI, 1.03–2.53; $P = 0.04$). In contrast, log-transformed ET-1 showed no clear association with incident MI (HR = 1.05 per increase in log-SD; 95% CI, 0.67–1.64; $P = 0.85$). In large part owing to relatively low event rates, log-ET-1 concentrations did not significantly predict cardiovascular death (HR = 1.49 per increase in log-SD; 95% CI, 0.88–2.53; $P = 0.14$), although the HR for this outcome was relatively consistent with all-cause death (see Table 3 in the online Data Supplement). Lastly, log-transformed ET-1 showed a trend toward independently predicting the composite of incident HF/MI/cardiovascular death (HR = 1.29 per increase in log-SD; 95% CI, 0.96–1.73; $P = 0.09$). In cumulative hazard analyses, ET-1 above the median was associated with shorter time to incident HF, MI, cardiovascular mortality, all-cause mortality, and the composite of incident HF/MI/cardiovascular mortality (Figs. 1–4; see also Fig. 1 in the online Data Supplement).

ET-1 CONCENTRATIONS AND MI SUBTYPE

When we considered incident type I and type I MIs separately, we found ET-1 concentrations predictive of type II MI but not of type I MI. There were 164 incident MIs: 33 type I and 112 type II. This remained true for both log-transformed ET-1 concentrations (HR = 1.76, CI = 1.05–2.97, $P = 0.03$ and HR = 1.64, CI = 0.68–3.98, $P = 0.28$, respectively) and ET-1 concentrations above the median (HR = 1.91, CI = 1.20–3.04, $P = 0.006$ and HR = 1.58, CI = 0.76–3.27, $P = 0.22$, respectively).

SEX DIFFERENCES

In considering the sex differences seen in other studies with respect to the prognostic ability of ET-1, ET-1 remained prognostic of incident HF, incident MI, cardiovascular death, all-cause mortality, and the composite end point of HF/MI/cardiovascular death in men. However, in women, associations between ET-1 and outcomes were attenuated, with the biomarker predicting only cardiovascular death (HR = 2.80, CI = 1.18–6.62, $P = 0.02$) and lesser associations with other outcome measures. It remained that ET-1 concentrations were not predictive of prevalent CAD in both men and women (see Table 4 in the online Data Supplement).

Discussion

In an at-risk population of patients undergoing coronary angiography for various acute and nonacute indications, we found increases in ET-1 concentrations were associated with prevalent comorbidities associated with risk in cardiovascular disease and worse left ventricular function, and ET-1 was accompanied by higher concentrations of several other prognostic biomarkers. Although previous work has implicated ET-1 as potentially a par-

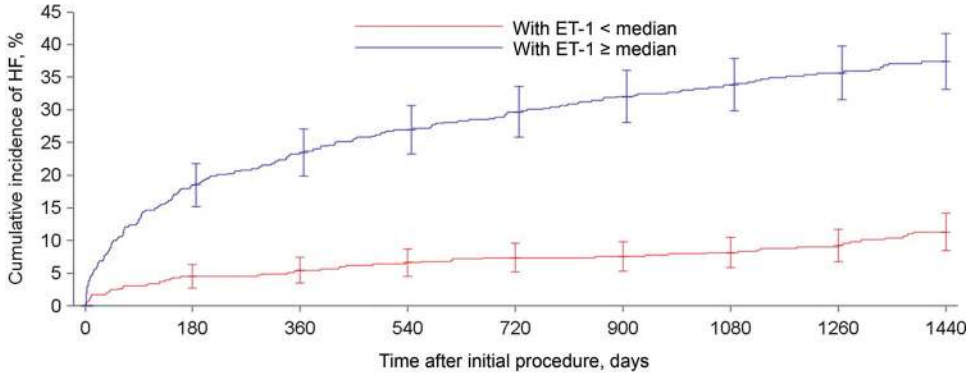

Fig. 1. Cumulative hazard curves for incident HF dichotomized by median ET-1 concentration.

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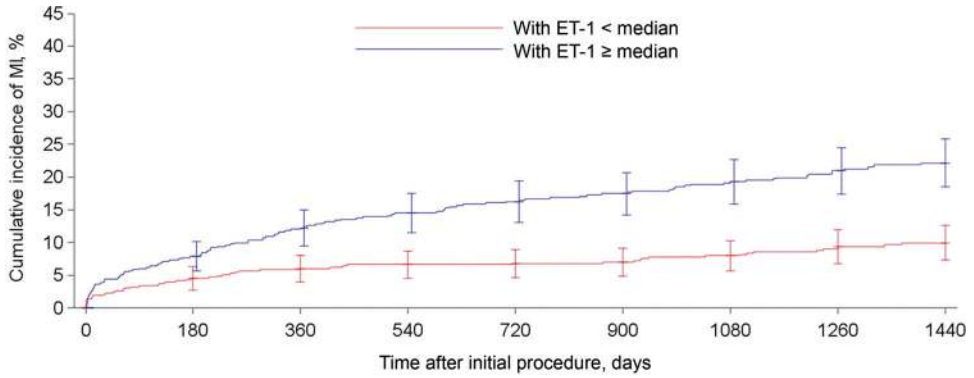

Fig. 2. Cumulative hazard curves for incident MI dichotomized by median ET-1 concentration.

ticipant in the development of several vascular disease states, including CAD, we could not show cross-sectional measurement of biologically active ET-1 predicted presence or severity of coronary atherosclerosis at coronary angiography. However, increased ET-1 concentrations measured with this assay predicted incident HF and all-cause death even when adjusted for other prognostic biomarkers. When constraining models to either men or women, we found log-transformed ET-1 concentrations remained prognostic across all outcomes in men, whereas in women ET-1 predicted only cardiovascular death, with attenuated associations in other events. Interestingly, when we examined type I and type II MIs separately, ET-1 concentrations predicted type II but not type I MIs.

In our subjects, increased ET-1 was associated with greater prevalence of hypertension and peripheral arterial disease; however, in adjusted analyses we could not show ET-1 predicted cross-sectional presence or extent of CAD. This may be explained by the fact the assay used to measure ET-1 in this study measures biologically active concentrations of the peptide *(4)*. Concentrations of biologically active ET-1 tend to rise and fall acutely with acuity of disease, which may explain why our results were better at predicting acute manifestations of disease (including incident HF and incident MI) but not for prediction of more indolently progressive disease (i.e., CAD). In other studies, Big ET-1 concentrations were measured. Big ET-1 is the precursor protein and thought to be more stable in circulation. In a recent study by Schooling and colleagues *(14)*, Mendelian randomization was used to link 3 genetic variants of ET-1 to ischemic heart disease, but it was not related to diabetes or lipids. It is possible that circulating ET-1 concentrations are associated with MI risk, but the variability is too high to show association with CAD.

Of note, the association of ET-1 with adverse outcome is independent of other biomarkers. When we looked at type I and type II MIs separately, we found that ET-1 concentrations predicted type II but not type I MIs,

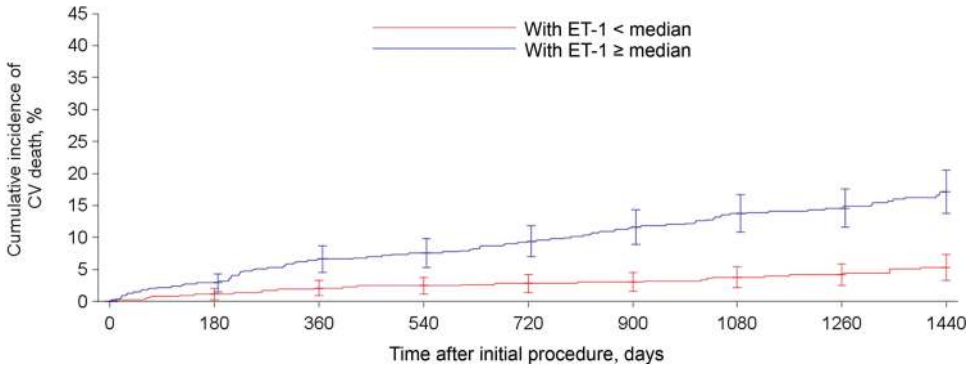

Fig. 3. Cumulative hazard curves for cardiovascular death dichotomized by median ET-1 concentration.
CV, cardiovascular.

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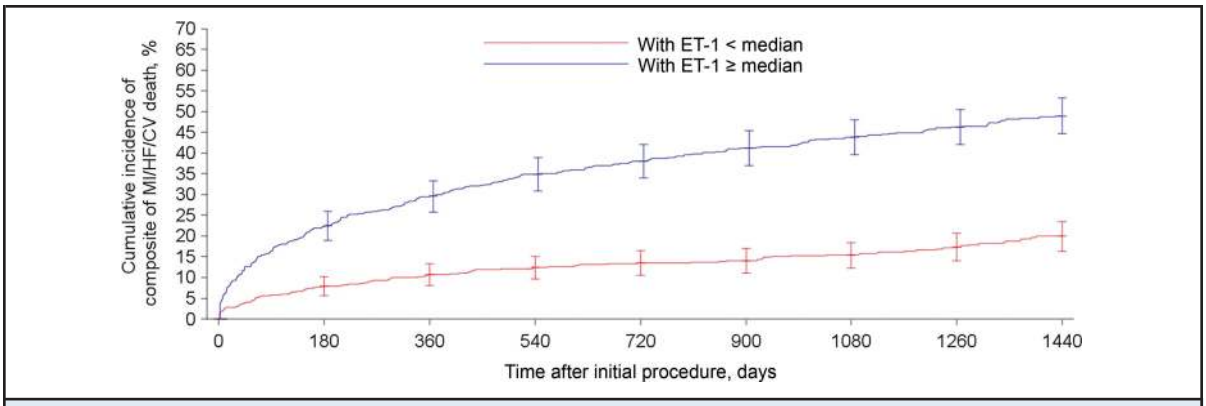

Fig. 4. Cumulative hazard curves for composite of incident MI/HF/cardiovascular death dichotomized by median ET-1 concentration.

Higher ET-1 concentration was associated higher risk of each event (all log-rank *P* values < 0.001). CV, cardiovascular.

which may indicate that our patient cohort included sicker patients with more comorbidities such as HF, in whom type II MIs are more common. Refining risk for incident cardiovascular disease is important to better treat patients at risk for such events. Although ET-1 has been associated with risk for future cardiovascular events in previous cross-sectional analyses, most of these earlier assessments did not adjust for contemporary biomarkers or examined different populations, such as those studied in CASABLANCA. Our analysis, which features multiple contemporary biomarkers with strong prognostic associations, demonstrates that ET-1 may predict incident HF and death despite careful adjustment for NT-proBNP, hs-cTnI, and sST2. Although, admittedly, our patient population is unique in that inherently they are a higher risk patient population referred for coronary angiography, these findings are of importance.

ET-1 is produced by the endothelium, vascular smooth muscle cells, and cardiomyocytes under conditions of inflammation, neurohormonal activation, hypoxia, and vascular stress *(15)*. Several biological factors regulate its release, including adrenalin, thrombin, angiotensin II, vasopressin, interleukins, and tumor necrosis factor, which all stimulate its release, and nitric oxide, prostacyclin, and heparin, which inhibit its release *(3)*. ET-1 may play an important role in the pathogenesis of cardiovascular diseases through several processes, including vasoconstriction, proinflammatory actions, proliferative effects, and stimulation of free radical formation. Indeed, Gupta and colleagues recently implicated a single-nucleotide polymorphism as a distal regulator of the *endothelin 1* gene, suggesting this noncoding variant might influence risk for several vascular diseases, including CAD, migraine, arterial hypertension, vascular dissection, and fibromuscular dysplasia *(6)*. Clinically, Daka and colleagues found an increased risk of incident CAD events in women free of prevalent CAD with increasing ET-1 concentrations (HR = 1.51; 95% CI, 1.1–2.1; $P = 0.015$) *(7)*, whereas in another study of 510 patients by Qing and colleagues, ET-1 concentrations were an independent predictor or coronary artery calcification as measured by computed tomography angiography with an area under the ROC curve of 0.83 (95% CI, 0.79–0.87; $P < 0.001$) *(16)*. More recently, in a study of 216 patients, ET-1 was independently associated with coronary artery ectasia *(17)*, suggesting that ET-1 not only predicts atherosclerotic-based disease but also structural arterial disease.

A major advantage of our cohort is its detailed characterization and our experience working with this group of patients; however, limitations to our study exist. The CASABLANCA cohort was predominantly male, white, and representative of patients in a tertiary care referral center. Additionally, because the patients in our study were referred for coronary and/or peripheral angiography, the pretest probability for development of cardiovascular events was higher. How one might best respond to an increased ET-1 concentration remains unclear. Identification of a biomarker as being a risk mediator may not be sufficient to justify its use; fashioning an actionable response to the result of risk biomarkers is a substantial challenge. In the case of ET-1, its measurement might best identify patients eligible for targeted efforts to either reduce ET-1 release or block the peptide at its receptor level. Although ET-1 receptor antagonists did reduce risk in patients with HF *(18)*, use of these agents specifically in those with increased ET-1 has not been examined. Such "precision" approaches for cardiovascular care deserve consideration.

In conclusion, in a typical at-risk population undergoing coronary angiography for various acute and non-

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acute indications, biologically active ET-1 concentrations did not cross-sectionally predict presence/severity of CAD as hypothesized. However, concentrations of ET-1 predicted major cardiovascular events such as HF and death. Future studies should consider how patients with increased concentrations of biomarkers such as ET-1 might benefit from interventions to mitigate future risk.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: H.K. Gaggin, Roche Diagnostic, Amgen, Ortho Clinical; J.L. Januzzi, Jr., Roche, Abbott, Critical Diagnostics.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: Singulex.
Expert Testimony: None declared.
Patents: None declared.
Other Remuneration: H.K. Gaggin, research payments for clinical end-point committees for EchoSense and Radiometer.

Role of Sponsor: The funding organizations played a direct role in the review and interpretation of data. The funding organizations played no role in the design of study, choice of enrolled patients, preparation of manuscript, or final approval of manuscript.

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