Atherosclerosis 238 (2015) 113–118

Contents lists available at ScienceDirect

Atherosclerosis

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

Plasma stromal cell-derived factor 1α/CXCL12 level predicts long-term adverse cardiovascular outcomes in patients with coronary artery disease

Nima Ghasemzadeh [a], Abdul Wahab Hritani [a], Christine De Staercke [b], Danny J. Eapen [a], Emir Veledar [c, d], Hatem Al Kassem [a], Mohamed Khayata [a], A.Maziar Zafari [a, e], Laurence Sperling [a], Craig Hooper [b], Viola Vaccarino [a, d], Kreton Mavromatis [a, e], Arshed A. Quyyumi [a, *]

[a] Emory University School of Medicine, Atlanta, GA, USA
[b] Center for Disease Control and Prevention, Atlanta, GA, USA
[c] Department of Biostatistics, Florida International University, Miami, FL, USA
[d] Department of Epidemiology, Rollins School of Public Health, Atlanta, GA, USA
[e] Atlanta Veterans Affairs Medical Center, Decatur, GA, USA

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form
16 October 2014
Accepted 16 October 2014
Available online 29 October 2014

Keywords:
Stromal cell-derived factor1α
CXCL12
Coronary artery disease
Cardiovascular outcomes

ABSTRACT

Objective: Stromal derived factor-1α/CXCL12 is a chemoattractant responsible for homing of progenitor cells to ischemic tissues. We aimed to investigate the association of plasma CXCL12 with long-term cardiovascular outcomes in patients with coronary artery disease (CAD). **Methods**: 785 patients aged: 63 ± 12 undergoing coronary angiography were independently enrolled into discovery ($N = 186$) and replication ($N = 599$) cohorts. Baseline levels of plasma CXCL12 were measured using Quantikine CXCL12 ELISA assay (R&D systems). Patients were followed for cardiovascular death and/or myocardial infarction (MI) for a mean of 2.6 yrs. Cox proportional hazard was used to determine independent predictors of cardiovascular death/MI. **Results**: The incidence of cardiovascular death/MI was 13% ($N = 99$). High CXCL12 level based on best discriminatory threshold derived from the ROC analysis predicted risk of cardiovascular death/MI (HR = 4.81, $p = 1 \times 10^{-6}$) independent of traditional risk factors in the pooled cohort. Addition of CXCL12 to a baseline model was associated with a significant improvement in c-statistic (AUC: 0.67–0.73, $p = 0.03$). Addition of CXCL12 was associated with correct risk reclassification of 40% of events and 10.5% of non-events. Similarly for the outcome of cardiovascular death, the addition of the CXCL12 to the baseline model was associated with correct reclassification of 20.7% of events and 9% of non-events. These results were replicated in two independent cohorts. **Conclusion**: Plasma CXCL12 level is a strong independent predictor of adverse cardiovascular outcomes in patients with CAD and improves risk reclassification.

1. Introduction

Stromal cell-derived factor-1α also known as CXCL12 is a chemokine that plays a key role in recruitment of stem cells and myocardial regeneration after myocardial infarction [1,2]. CXCL12 mediates homing of progenitor cells to areas of ischemic tissues [3]. It is expressed on the surface of platelets and endothelial cells and is secreted in plasma after activation, facilitating mobilization, migration, and domiciliation of progenitor cells in ischemic tissues [4,5]. On the other hand, CXCL12 by activating several signaling pathways has been shown to induce an inflammatory response by activation of chemotaxis, cell migration, and secretion of several inflammatory biomarkers [6]. Limited numbers of clinical studies have reported differences in CXCL12 levels in patients with a variety of clinical manifestations of coronary artery disease (CAD) and with varied exposure to traditional cardiovascular risk factors [7,8]. However, the data on prognostic role of CXCL12 level, a key

* Corresponding author. Emory Clinical Cardiovascular Research Institute, 1462 Clifton Road NE, Suite 507, Atlanta GA 30322, USA.
E-mail address: aquyyum@emory.edu (A.A. Quyyumi).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.10.094
0021-9150/

modulator of circulating progenitor cells, in patients with CAD is limited [9]. The goal of the present study was to investigate the prognostic role of plasma CXCL12 levels on long-term cardiovascular outcomes in patients with suspected or confirmed CAD, with the hypothesis that higher CXCL12 would be associated with higher incidence of adverse cardiovascular events.

2. Methods

2.1. Study population

785 patients, aged 63 ± 12 years, undergoing cardiac catheterization were enrolled independently into discovery ($N = 186$) and replication ($N = 599$) cohorts. The discovery cohort was established at the Atlanta Veterans Affairs and Emory University hospitals between years 2004–2006 and includes patients with stable CAD undergoing percutaneous coronary intervention and stenting. The replication cohort was a nested study within the Emory Cardiovascular Biobank with subjects enrolled between years 2008–2011. Demographics, medical, and behavioral characteristics as well as risk factor prevalence were documented as previously described [10]. Subjects were classified as current or non-smokers. Acute MI at enrollment was defined using universal criteria for diagnosis [11]. Subjects were noted to have hypertension or dyslipidemia if they had a documented history or they were on treatment. Subjects were excluded if they had a history of heart transplantation, immunosuppressant use, malignancy, or significant infections. The Institutional Review Board at Emory University approved both cohorts and all subjects provided written informed consent.

2.2. Follow-up data collection

Outcomes data were collected by independent personnel who were blinded to the study data. Record of death was obtained from the Social Security Death Index and/or via direct contact with subjects' family members. Cause of death was adjudicated from medical records or direct contact. Follow-up was conducted at 1 and 5 years from the date of enrollment to identify cases of myocardial infarction. MI occurring within a month of enrollment was not included in the final analysis.

2.3. Identification of CAD and severity scoring

Coronary angiograms were scored for luminal narrowing based on the modified AHA/ACC classification of the coronary tree [12]. Patients were classified as having non-obstructive (visible plaque resulting in <50% luminal stenosis) or obstructive CAD plaque resulting in ≥50% stenosis.

2.4. Sample collection

Fasting arterial blood samples for plasma were drawn at cardiac catheterization and stored at −80 °C before analysis. CXCL12 levels were measured using the Human CXCL12 Quantikine ELISA kit (R&D systems). Discovery cohort samples were processed at the time of enrollment and replication cohort samples were processed after an average 3.2 years. The inter-assay precision for the CXCL12 assay varies between 8.2% and 13.4% for the different concentrations. In a batch of 20 samples from the discovery cohort that were re-analyzed with the assay kits used for the replication cohort, an insignificant 3.48% ($p = 0.56$) difference in means of CXCL12 level was found. Mean CXCL12 was 1623 ± 606 pg/ml in the discovery cohort compared to 2534 ± 1011 pg/ml in the replication cohort.

2.5. Statistical analysis

Continuous variables are presented as means ± SD and categorical variables as proportions (%). The student *t*-test and Chi-square analyses were performed when appropriate. Univariate predictors of the primary endpoint were identified using univariate Cox proportional hazard models. Multivariate Cox regression models were created adjusting for traditional cardiovascular risk factors as well as medication use, serum creatinine, LVEF, history of CABG, and presence of ≥50% stenosis in any major epicardial vessel. The best discriminatory cutoff for CXCL12 in association with outcomes was determined using the Youden's index (Sensitivity − (1-Specificity)) from the Receiver Operating Characteristic (ROC) analysis to identify “high” and “low” CXCL12 levels in both cohorts separately. Both cohorts were then pooled to determine cases with “high” or “low” CXCL12 based on the same method of categorization. Discrimination analysis for prediction of the primary endpoint was calculated as the difference in C-statistic comparing baseline model with a model containing CXCL12 (high/low) variable in addition to the baseline variables. Using multivariate Cox models with the clinical covariates noted above, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) metrics were calculated [13,14]. Annual event rates for each outcome measure were calculated by dividing the observed number of events by the observed event-specific number of person-years of follow-up. *P* values <0.05 from two-sided tests were considered statistically significant. Analyses were performed with SAS (Version 9.3; SAS Institute, NC, USA).

3. Results

Baseline characteristics of the 186 subjects in the discovery and 599 subjects in the replication cohorts are presented in Table 1. In the combined cohort, the mean age was 63 ± 12 years, 76% were

Table 1
Baseline characteristics.

	Discovery ($N = 186$)	Replication ($N = 599$)	Pooled ($N = 785$)
Age, years	61 ± 10	63 ± 12	63 ± 12
Male gender (%)	84	74	76
African American race (%)	19	22	22
Serum creatinine (mg/dL)	1.08 ± 0.31	1.43 ± 175	1.35 ± 1.55
Diabetes mellitus (%)	44	39	40
Hypertension (%)	83	89	88
Dyslipidemia (%)	89	78	81
LVEF (%)	53 ± 9.2	53 ± 13	53 ± 12
History of CABG (%)	23	23	23
Active smoking (%)	27	19	21
Aspirin use (%)	97	60	69
ACE-inhibitor/ARB use (%)	71	52	57
Beta blocker use (%)	75	66	68
Statin use (%)	78	71	72
Total cholesterol (mg/dL)	172 ± 41	161 ± 50	164 ± 48
LDL (mg/dL)	94 ± 33	92 ± 43	92 ± 41
HDL (mg/dL)	43 ± 13	40 ± 15	40 ± 14
Triglyceride (mg/dL)	187 ± 168	170 ± 109	175 ± 127
Presence of CAD ≥ 50% (%)	100	69	76
Acute MI (%)	0.0	9.0	7.0
Management strategy			
Medical management only (%)	0	61	47
PCI (%)	100	33	49
CABG (%)	0	6	4
High CXCL12 (%)	35	34	34

Data presented as Mean ± SD. CAD: coronary artery disease, LVEF: left ventricular ejection fraction, CABG: Coronary artery bypass surgery, PCI: Percutaneous Coronary Intervention, LDL: low density lipoprotein, HDL: high density lipoprotein, MI: myocardial infarction, High CXCL12 based on based discriminatory cutoff for the composite cardiovascular death/MI outcome.