CXCL16 is an independent predictor of cardiovascular death and morbidity in acute coronary syndromes

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ABSTRACT

Objective: The chemokine C-X-C motif ligand 16 (CXCL16) is a scavenger receptor for oxidized low-density lipoproteins and involved in inflammation at sites of atherosclerosis. This study aimed to investigate the association of CXCL16 with clinical outcome in patients with acute coronary syndrome (ACS).

Approach and Results: Serial measurements of CXCL16 were performed in a subgroup of 5142 patients randomized in the PLATelet inhibition and patient Outcome (PLATO) trial. Associations between CXCL16 and a composite of cardiovascular (CV) death, spontaneous myocardial infarction (sMI) or stroke, and the individual components were assessed by multivariable Cox regression analyses. The hazard ratio (HR) per 50% increase in admission levels of CXCL16 analyzed as continuous variable was 1.64 (95% confidence interval [95%CI]: 1.44-1.88), $p<0.0001$. This association remained statistically significant after adjustment for randomized treatment, clinical variables, C-reactive protein, leukocytes, cystatin C, NT-proBNP, troponin T, Growth Differentiation Factor 15 and other biomarkers; HR 1.23 [1.05-1.45], $p=0.0126$. The admission level of CXCL16 was independently associated with CV death (1.50 [1.17-1.92], $p=0.0014$), but not with ischemic events alone, in fully adjusted analyses. No statistically independent association was found between CXCL16 measured at 1-month, or change in CXCL16 from admission to 1-month, and clinical outcomes.

Conclusions: In patients with ACS, admission level of CXCL16 is independently related to adverse clinical outcomes, mainly driven by an association to CV death. Thus, CXCL16 measurement may enhance risk stratification in patients with this condition.

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ABBREVIATIONS

ACS – Acute coronary syndrome
Apo-A1 – Apolipoprotein A1
Apo-B – Apolipoprotein B
CABG - Coronary artery bypass grafting
CAD – Coronary artery disease
CHF - Congestive heart failure
CKD - Chronic kidney disease
CRP - C-reactive protein
CV - Cardiovascular
CXCL16 – C-X-C ligand 16
CXCR6 - C-X-C receptor 6
DM - Diabetes mellitus
GDF-15 - Growth Differentiation Factor 15
HR - Hazard ratios
hs-CRP - High-sensitivity CRP
hs-TnT - High-sensitivity troponin T
HT – Hypertension
IL-6 – Interleukin 6
IL-18 – Interleukin 18
ln – Natural logarithm transformed
LR-test – Likelihood Ratio Test
NT-proBNP - N-terminal pro-brain natriuretic peptide
NSTE-ACS - Non-ST-elevation acute coronary syndrome
oxLDL - Oxidized low-density lipoproteins
PAD - Peripheral arterial disease
PCI - Percutaneous coronary intervention
PLATO - PLATelet inhibition and patient Outcome trial
Q1-4 – Quartile 1-4
sMI - Spontaneous myocardial infarction
STE-ACS - ST-elevation acute coronary syndrome
WBC - Leukocytes

INTRODUCTION

Ischemic heart disease remains the leading cause of death world-wide despite substantial recent improvements in therapeutic options and risk stratification, including the use of biomarkers.[1] Several inflammatory biomarkers, of which C-reactive protein (CRP) is the most extensively investigated, have been shown to be independent predictors of adverse outcome in patients with coronary artery disease (CAD). [2-6] This includes those with acute coronary syndrome (ACS), and several studies have identified CRP as an independent risk factor for the development of CAD.[2-6]

Chemokines, including the chemokine C-X-C motif ligand 16 (CXCL16), play an important role in atherosclerosis and plaque destabilization. Physiological research indicates that CXCL16, and its receptor C-X-C receptor 6 (CXCR6), are involved in several aspects of atherogenesis. CXCL16 is involved in formation of foam cells through the ability of membrane bound CXCL16 to function as a scavenger receptor for internalizing oxidized lipids.[7] It also promotes leukocyte

infiltration into the atherosclerotic lesion, directly linking lipids to inflammation.[7] It seems that the soluble and membrane form of CXCL16 in part possess different biological functions. Thus, whereas enhanced expression of membrane-bound CXCL16 on endothelial cells, smooth muscle cells, and macrophages within the vessel wall has been shown to promote binding and adhesion of lymphocytes and platelets, soluble CXCL16 acts as a classical chemoattractant for various leukocyte subsets.[8] Soluble CXCL16 may also promote smooth muscle cell proliferation and enhance extracellular matrix remodelling.[9, 10] CXCL16 is released from activated platelets and may also amplify platelet activation, potentially representing a vicious circle that may be operating during plaque destabilization and ACS.[11-14] Taken together CXCL16 seems to be involved in or reflect changes in several important pathways in atherothrombotic disease.

CXCL16 gene polymorphisms have been implicated in increased risk of both CAD, stroke and carotid artery plaque formation, but the results of different studies are partly conflicting, and serum levels of CXCL16 has been suggested as a better marker.[15-20] Several previous studies have found higher serum levels of CXCL16 in ACS versus stable CAD and controls.[17, 21-23] There are, however, only a few prospective studies investigating the use of CXCL16 as a risk marker in CAD[6, 24-27], and only one of these included patients from an ACS population[6, 24].

The aims of the present work were to investigate, in a contemporary cohort of ACS patients, the levels of CXCL16 in the acute and chronic phases of ACS and to assess the associations between CXCL16 concentrations and fatal and non-fatal cardiovascular (CV) outcomes, adjusted for other biomarkers reflecting inflammation, kidney dysfunction, myocardial necrosis or dysfunction.

MATERIAL AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Design and study population

The PLATelet inhibition and patient Outcome trial (PLATO) (NCT00391872) is an international phase III multicenter, double-blind trial of 18624 patients with ACS (i.e. non-ST-elevation ACS (NSTE-ACS), or ST-elevation ACS (STE-ACS) scheduled for primary percutaneous coronary intervention (PCI)) randomized to either ticagrelor or clopidogrel for 6-12 months on top of optimal medical therapy and invasive strategy as appropriate[28, 29]. The present work was a prespecified substudy in the biomarker project included in the PLATO program.[28] All included patients at selected sites were invited on a consecutive basis to participate in this project which aimed to obtain serial venous blood samples from at least 4000 patients at randomization, discharge and 1 month and at least 2000 at 6 months. The trial adhered to the Helsinki Declaration and informed written consent was obtained from all patients. The study protocol was approved by the local ethics committees.

Study endpoints

The primary efficacy endpoint of the present study was a composite of CV mortality, spontaneous myocardial infarction (sMI) or stroke within 12 months of follow-up and have been defined in an earlier publication.[28, 29] Secondary endpoints

were the individual components of the primary outcome measure. All endpoints were assessed by a central, independent, blinded clinical event adjudication committee.[28]

Blood sampling and laboratory analysis

Baseline venous blood samples were drawn within 24 hours of symptom onset of the index event and before study medication was administered. Serial blood samples were obtained at discharge, 1-month and 6-months follow-up. The samples were centrifuged, and serum and plasma aliquots frozen locally before transport to a central biobank. Measurement of soluble serum CXCL16 was performed by enzyme immunoassay using commercially available matched antibodies (R&D Systems Minneapolis, Minnesota, USA) in a 384-format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, Vermont, USA) dispenser/washer (EL406). Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Synergy H1 Hybrid, BioTek, Winooski, Vermont, USA). Intra- and interassay coefficient of variation were <10%, and this assay has been validated previously.[24] We have shown that serum CXCL16 measurement to only a minor degree is influenced by 3 freeze and thaw cycles, fasting or non-fasting condition, the timing of blood collection during daytime, and by storage time of frozen samples.[24] Plasma high-sensitivity troponin T (hs-TnT), N-terminal pro-brain natriuretic peptide (NT-proBNP) and cystatin C were analyzed by immunoassays on Cobas® Analytics e601 Immunoanalyzer (Roche Diagnostics, Mannheim, Germany). High-sensitivity CRP (hs-CRP) and leukocytes (WBC) were measured with a spectrophotometric analyzer (Architect, Abbott, Chicago, Illinois, USA). Analysis of Growth Differentiation Factor 15 (GDF-15) was performed with a pre-commercial assay (Roche Diagnostics) using monoclonal murine antibodies. Interleukin 6 (IL-6) was measured using high-sensitivity enzyme-linked immunosorbent assays (Quantikine high sensitivity IL-6 kit, R&D Systems, Minneapolis, Minnesota, USA). Interleukin 18 (IL-18) was measured in EDTA plasma using human IL-18 ELISA (Medical and Biological Laboratories Co. Ltd, Naka-ku, Nagoya, Japan. Oxidized Low-Density Lipoproteins (OxLDL) was measured in EDTA plasma using an enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden). D-dimer was measured in plasma using a commercial kit (Asserachrom, Stago, Asnieres, France). Apolipoprotein A1 (ApoA1) and Apolipoprotein B (ApoB) was analyzed using an immunoturbidimetric analysis (Architect, Abbott, Chicago, Illinois, USA) using Apolipoprotein A1 and Apolipoprotein B kit from Abbott.

Statistical analyses

Baseline characteristics were presented by quartile groups of CXCL16. Categorical baseline variables were shown as frequencies and percentages and compared by quartile groups of CXCL16 using Chi-square test. Continuous baseline variables were presented as medians and 25^{th} to 75^{th} percentiles (Q1-Q3) and compared by quartile groups of CXCL16 using Kruskal-Wallis tests. Biomarkers were logarithmic transformed to approximate normal distribution. The relationship between natural log -transformed (ln) CXCL16, baseline characteristics and other biomarkers were analyzed by multivariable linear models. Geometric means were calculated by the antilogarithms of the model-adjusted means and compared between groups using ratios. Correlations between analyzed biomarkers and CXCL16 were assessed by Spearman rank correlation coefficient. Changes in CXCL16 levels over time were analyzed by Wilcoxon signed rank test.

Crude event rates at 12 months as well as Kaplan-Meier event rates were estimated by quartiles of CXCL16 at baseline. Multivariable Cox proportional hazards models were used to assess association between ln(CXCL16) and endpoints, with hazard ratios (HR) based on 50% increase in CXCL16 level. An 8-step incremental model of covariates was used. In **model 0** only randomized treatment was included. **Model 1** added clinical variables; age, sex, body mass index, diabetes mellitus (DM), dyslipidemia, hypertension (HT), chronic kidney disease (CKD), congestive heart failure (CHF), ST-elevation ACS (STE-ACS)/Non-ST-Elevation ACS (NSTE-ACS) at randomization, smoking, aspirin at entry, previous atherosclerotic disease (MI, peripheral arterial disease (PAD), coronary artery bypass grafting (CABG), Percutaneous Coronary Intervention (PCI) or non-hemorrhagic stroke). **Model 2** added ln(hs-CRP) and ln(WBC). **Model 3** added ln(cystatin C). **Model 4** included ln(NT-proBNP) and ln(hs-TnT). **Model 5** also included ln(GDF-15). **Model 6** included ln-(Apo-A1) and ln(IL-6). The fully adjusted **Model 7** also included ln(D-dimer), ln(IL-18), ln(oxLDL) and ln(Apo-B). The same models were used in assessment of CXCL16 at 1-month. The models used in analyzing change in CXCL16 levels from admission to 1-month were adjusted for both admission levels of CXCL16, and for change in CXCL16 levels from admission to 1-month follow-up, in addition to the models 0-7 described above.

The effects of CXCL16 levels on outcomes in relation to randomized treatment were evaluated using Cox proportional hazards models including CXCL16, randomized treatment and the treatment by CXCL16 interaction term. CXCL16 was included as a continuous, log transformed variable and fitted using restricted cubic splines with 4 knots located at the 5^{th}, 35^{th}, 65^{th} and 95^{th} percentiles.

To assess the discriminatory ability of the models, Harrell's *C*-index was estimated. Models with CXCL16 level added were compared with models without CXCL16 in terms of global model fit using likelihood ratio (LR) tests.

The proportional hazards assumption was assessed by visual inspection of Schoenfeld residual plots. A two-sided p-value of <0.05 was considered statistically significant and there were no adjustments for multiple comparisons, due to the exploratory nature of the study.

All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, North Carolina, USA)

RESULTS

Admission levels of CXCL16 in relation to baseline characteristics and other biomarkers

A total of 5142 patients were included in the study with a median admission level (Q1-3) of CXCL16 of 5.24 ng/mL (4.51-6.17). Several of the baseline characteristic variables differed significantly across the quartiles of CXCL16 (tab. 1). However, in multivariable regression analyzis only female gender, CKD and smoking remained associated with higher CXCL16 values, while increasing age and STE-ACS were associated with lower values (tab. 2A). CXCL16 levels by statin use at inclusion are shown in Suppl.fig I and shows slightly lower levels in the statin group. Spearman correlation analyzes revealed a significant positive correlation of CXCL16 with hs-

TnT, NT-proBNP, GDF-15, cystatin C, D-dimer and hs-CRP, but not with WBC, with the strongest correlation with cystatin C (R=0.3047, p<0.0001) (Suppl.tab. I). Multivariate regression analyzes revealed a strong association of CXCL16 with GDF-15, cystatin C, D-dimer, ApoA1 and ApoB (p<0.0001 for all), whereas no association was seen with NT-proBNP and hs-TnT (tab.2B).

Association of admission levels of CXCL16 with clinical outcome

Of 5142 patients, 434 (8,4%) suffered a primary event of CV death, sMI or stroke (191 CV deaths [3,7%], 243 sMI [4,7%] and 62 strokes [1,2%]). The crude event rate of the primary composite endpoint per quartile of CXCL16 was 5.6%, 7.3%, 8.7% and 12.1%, respectively. Kaplan-Meier curves, presenting the unadjusted association between quartile groups of CXCL16 and primary endpoint, revealed an increasing risk with higher quartile groups (Fig.1).

The admission level of CXCL16 (with HR per 50% increase in concentration analyzed as continuous variable) was significantly associated with the primary composite endpoint, HR: 1.64 (95%CI: 1.44-1.88), p<0.0001. This association remained significant in fully-adjusted analyses that, in addition to clinical characteristics, also included hs-CRP, hs-TnT, NT-proBNP, cystatin C, GDF-15, Apo-A1, IL-6, D-dimer, IL-18, oxLDL and Apo-B; HR 1.23 [1.05-1.45], p=0.0126. This association was mainly driven by the independent relation to CV-death, HR 1.50 [1.17-1.92], p=0.0014. There was, however, no significant association with sMI or stroke alone. (tab.3) The full stepwise analyses are shown in Suppl.tab II. The restricted cubic spline analysis (Fig. 2) showed that the associations of CXCL16 with the outcomes were non-linear, with relative higher risk of adverse events at high levels of CXCL16. There was no significant interaction between the CXCL16 levels on admission, randomized treatment and the primary composite endpoint (p=0.7578).

The CXCL16 level contributed independently to discrimination of the risk of the primary composite endpoint as the C-index increased from 0.703 [0.675-0.731] to 0.706 [0.679-0.734], p=0.0115. This improvement in discrimination was driven by CV-death, where C-index improved from 0.798 [0.760-0.836] to 0.808 [0.771-0.845], p=0.0013. C-indices and LR-tests for all endpoints are shown in Suppl.tab III

CXCL16 in the chronic phase

CXCL16 levels at follow-up were available at discharge in 4576 patients, at 1 month in 4256 patients and at 6 months in 3100 patients. There was an initial increase in the CXCL16 concentrations from admission to a median level (Q1-3) of 5.85 (5.05-6.79) ng/ml at discharge, and 5.31 (4.61-6.19) ng/ml at 1-month (p<0.0001 for both time points vs. baseline) and thereafter a decrease to 5.10 (4.45-5.90) ng/ml at 6 months (p<0.01 vs. baseline) (fig. 3). The median level of CXCL16 at discharge was significantly lower in the ticagrelor group as compared with the clopidogrel group, but this difference was not sustained at 1- and 6-months follow-up (Suppl.tab. IV).

Of the 4234 patients with 1-month measurements and no new CV events since baseline, 240 patients (5.7%) suffered a primary endpoint, of which 85 were CV deaths, 154 sMI and 36 strokes. Crude event rates of the primary composite endpoint per quartile of CXCL16 were 4.2%, 5.8%, 4.6% and 8.1%, respectively. The HR (per 50% increase in CXCL16 concentration) at 1-month for the primary endpoint (in model 0) was 1.53 [1.25-1.86], p<0.0001. This association remained statistically

significant after adjustment for clinical variables, hs-CRP and WBC (HR 1.30 [1.05-1.61], p=0.0144), but not in the fully-adjusted model. The fully-adjusted analysis did not reveal any significant association between CXCL16 levels at 1 month and secondary endpoints (Suppl.tab. V). There was no interaction between CXCL16 values at 1 month, randomized treatment and the primary endpoint (p=0.0936).

Change in CXCL16 over time and outcome

The change in CXCL16 from admission to 1-month and the association to outcomes were assessed in 4191 patients. The HR (per 50% increase of CXC16 at 1-month for a given value of CXCL16 on admission) for the primary endpoint (in model 0) was 1.27 [0.97-1.67], p=0.0843. There was no significant association between change in CXCL16 levels from admission to 1-month and outcomes in further adjusted models, neither for the primary endpoint, nor for any secondary endpoints. The full stepwise analyses are included in the suppl.tab VI.

DISCUSSION

In the present study, serum CXCL16 measured in patients early after admission for ACS was independently associated with the composite of CV death, sMI or stroke. This association remained statistically significant in the fully adjusted analyses, i.e. including both the important prognostic biomarkers NT-proBNP, hs-TnT, cystatin C and GDF-15, and several inflammatory markers (e.g. hsCRP, IL-6, IL-18 and D-dimer) known to be elevated and involved in the pathogenesis of CAD. The relation to outcome was mainly driven by an association with CV-death, while no independent association was seen between CXCL16 levels and other outcome measures. The level of CXCL16 at admission independently contributed to the discrimination between patients concerning the risk of the composite endpoint, again driven by an improved discrimination in the risk of CV death. Our results are supported by the only previous study investigating CXCL16 and risk after ACS, as they also found that CXCL16 predicted long-term mortality in ACS.[24]

CXCL16 measured at 1 month was predictive of subsequent adverse outcome, but this association did not remain significant in the fully-adjusted model. In an earlier study, CXCL16, in combination with osteoprotegerin, was found to predict CV death, MI or heart failure at 3 months.[6] Both this double-biomarker approach, as well as partially different outcome measures and differences in adjustment variables in the statistical analyses, may well explain these results as opposed to our findings.

The predictive value of CXCL16 has also been studied in stable CAD, showing an association with a composite of all-cause death, non-fatal MI, revascularization and hospitalization for angina pectoris during 24 months of follow-up.[25] CXCL16 has furthermore been found to predict the risk of MI in healthy volunteers.[26] Accordingly, CXCL16 may be a new predictor of adverse outcome across the whole spectrum of CAD.

Serial measurements of CXCL16 in the present study showed a rise and fall, with higher values at discharge and a gradual decline over 6 months thereafter. Although the absolute changes are small, the temporal pattern we found is similar to that found in an earlier study.[24] This is in contrast to the pattern found in a small study in STE-ACS patients which found steadily decreasing values after primary PCI.[30] As the fluctuations in CXCL16 levels in our study was generally modest, the

data should be interpreted with caution. The rise from baseline to discharge could potentially indicate a prolonged release of CXCL16 during PCI as a response to ischemia/reperfusion injury and may also reflect the dynamics of CXCL16 with a more gradual increase and decline during acute events, potentially mirroring activation of several up-stream inflammatory pathways.[24, 31] Indeed, while CRP mainly reflects interleukin-6 activation, higher levels of interleukin-1, tumor necrosis factor and interferon-ɣ has been shown to contribute to the release of soluble CXCL16 in endothelial cells and leukocytes.[9, 32]

Change in CXCL16 levels over time has previously been shown to correlate with adverse outcome in stroke patients, but to our knowledge this has never been evaluated in ACS-patients.[31] In stroke patients, the change from day 1 to day 4 was a significant predictor of both all-cause and CV-mortality in adjusted analyses. Our data included only CXCL16 levels on admission, discharge, 1-month and 6-month. Since discharge levels of CXCL16 was significantly associated with randomized treatment, and follow-up was very short with few new adverse events after 6-months, we chose to include only baseline and 1-month levels in our analysis of changes of CXCL16 levels. However, we were not able to confirm any association between change in CXCL16 levels over time and adverse outcome in patients with ACS.

Medical therapy of CAD patients has changed substantially over time. This might have influenced the results of the available studies on CXCL16. Statins (simvastatin and atorvastatin)[9], angiotensin II receptor blockers (irbesartan)[33] and peroxisome proliferator-activated receptor gamma (PPAR-gamma) agonists (rosiglitazone and pioglitazone)[21] have all been shown to decrease the levels of CXCL16 and, as such, may have influenced the results of earlier studies. Still, other studies have reported no difference in levels of CXCL16 between statin-users and others.[21] In our study, patients admitted with prior statin use had lower median levels of CXCL16, although the absolute difference was small and unlikely to be relevant for the associations to clinical outcomes. Furthermore, there were lower levels of CXCL16 at discharge in the ticagrelor group as compared with the clopidogrel group, but this difference was not sustained at 1 and 6 months follow up. However, there were no statistical interaction between CXCL16 levels at any time-point, randomized treatment and outcome. Even though our findings might be without influence of confounding medication, the question of medication use as a bias must be taken into consideration in further studies of CXCL16.

STRENGHTS AND LIMITATIONS

Our study has the strength of being a large international multicenter trial that includes a broad ACS-population treated according to contemporary guidelines. The long-term storage of the serum samples in the PLATO biobank before CXCL16 measurement may have affected our results. It is difficult to assess the impact of up to 9 years of storage, but the storage time is similar for all samples and hence a potential decay should be similar in all. Moreover, in a previous study, we have to some extent investigated this issue by comparing samples from the initial half of the inclusion period (1995-2001) with the samples from the second half, and found no significant difference.[24] Moreover, our CXCL16 assay have been shown to be robust and only in a minor degree influenced by freeze and thaw cycles, fasting or no fasting condition, and the timing of blood sampling during the day.[24]

CXCL16 levels may be affected by drugs used for ACS or co-morbidities in this population. In our study we have adjusted for randomized treatment and co-morbidities, and have also examined the influence of statins, but still there might be remaining confounders represented by other medication.

CONCLUSION

In patients with ACS, the admission level of CXCL16 is an independent predictor of the composite of CV death, sMI, or stroke, driven mainly by an association with CV death. The strong relation to fatal outcome remains statistically significant in multivariable analyzes adjusted for other potent predictors such as hs-CRP, NT-proBNP, hs-TnT and GDF-15. Thus, CXCL16 may contribute to improved understanding of the pathophysiology of ACS, as well as to enhanced risk stratification beyond that of established biomarkers in patients with this condition.

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HIGHLIGHTS:

- CXCL16 is an inflammatory marker produced locally at sites of inflammation.
- CXCL16 levels are independently associated with the primary composite endpoint of cardiovascular death, spontaneous myocardial infarction or stroke within 12 months after acute coronary syndrome, mainly driven by an association with cardiovascular death.
- Associations between CXCL16 and outcome are adjusted for clinical variables and other biomarkers, including hs-CRP, hs-TnT, NT-proBNP, cystatin C and GDF-15.
- This is the largest study to examine CXCL16 as a risk marker in a broad acute coronary syndrome cohort

FIGURE LEGENDS:

Figure 1: Kaplan-Meier estimated event rates of the primary outcome measures per quartile group of the admission levels of CXCL16

Figure 2: Restricted cubic splines of the association between the admission levels of CXCL16 and 12-months estimated event rates of the individual outcome measures in the whole study population

Figure 3: Dynamic changes in CXCL16 levels from admission through 6 month's follow-up

APPENDICES:

Supplementary figure and tables

Table 1: Baseline characteristics of the study population and biomarkers levels by quartile groups of admission levels of CXCL16

Characteristic		CXCL-16 (ng/mL) Q1 <4.5 n=1286	Q2 4.5-5.2 n=1285	Q3 5.2-6.2 n=1286	Q4 >6.2 n=1285	P-value*
Demographics	Age years, median (Q1-Q3)	61 (54-69)	62 (54-70)	62 (53-71)	64 (55-72)	<.0001
	Female	340 (26.4%)	354 (27.5%)	385 (29.9%)	459 (35.7%)	<.0001
	Weight kg, median (Q1-Q3)	80 (71-90)	80 (71-90)	80 (70-90)	80 (70-90)	0.5224
	BMI kg/m^2, median (Q1-Q3)	27.5 (24.9-30.4)	27.7 (25.2-30.4)	27.5 (24.8-30.7)	27.8 (25.1-31.1)	0.0849
Risk factor	Habitual smoker	429 (33.4%)	459 (35.7%)	523 (40.7%)	484 (37.7%)	0.0012
	Hypertension	811 (63.1%)	839 (65.3%)	837 (65.1%)	904 (70.4%)	0.0009
	Dyslipidemia	538 (41.8%)	537 (41.8%)	533 (41.4%)	563 (43.8%)	0.6088
	Diabetes mellitus	247 (19.2%)	262 (20.4%)	293 (22.8%)	341 (26.5%)	<.0001
Medical history	Angina pectoris	581 (45.2%)	580 (45.1%)	593 (46.1%)	640 (49.8%)	0.0556
	Myocardial infarction	213 (16.6%)	248 (19.3%)	260 (20.2%)	287 (22.3%)	0.0029
	Congestive heart failure	43 (3.3%)	54 (4.2%)	75 (5.8%)	124 (9.6%)	<.0001
	PCI	148 (11.5%)	137 (10.7%)	151 (11.7%)	199 (15.5%)	0.0010
	CABG	63 (4.9%)	53 (4.1%)	54 (4.2%)	86 (6.7%)	0.0088
	TIA	24 (1.9%)	22 (1.7%)	31 (2.4%)	36 (2.8%)	0.2106
	Non-hemorrhagic stroke	36 (2.8%)	42 (3.3%)	54 (4.2%)	43 (3.3%)	0.2628
	Peripheral arterial disease	62 (4.8%)	71 (5.5%)	93 (7.2%)	123 (9.6%)	<.0001
	Chronic renal disease	15 (1.2%)	22 (1.7%)	51 (4.0%)	92 (7.2%)	<.0001
Type of ACS	ST-elevation MI	687 (53.4%)	614 (47.8%)	554 (43.1%)	498 (38.8%)	<.0001
Risk scores	TIMI risk score median (Q1-Q3)	3.0 (2.0-4.0)	4.0 (2.0-5.0)	4.0 (2.0-5.0)	4.0 (3.0-5.0)	<.0001
	GRACE risk score median (Q1-Q3)	132 (116-147)	133 (116-151)	133 (117-151)	139 (120-156)	<.0001
Anti-thrombotic treatment in hospital	Aspirin	1266 (98.4%)	1266 (98.5%)	1273 (99.0%)	1254 (97.6%)	0.0406
	Unfractioned heparin	725 (56.4%)	697 (54.2%)	702 (54.6%)	682 (53.1%)	0.4059
	LMW heparin	684 (53.2%)	701 (54.6%)	708 (55.1%)	684 (53.2%)	0.7098
	Fondaparinux	18 (1.4%)	23 (1.8%)	16 (1.2%)	16 (1.2%)	0.6096
	Bivalirudin	16 (1.2%)	19 (1.5%)	18 (1.4%)	23 (1.8%)	0.7066
	Gp 2b/3a inhibitor	401 (31.2%)	350 (27.2%)	312 (24.3%)	303 (23.6%)	<.0001
Other medication in hospital	Beta-blockade	1144 (89.0%)	1128 (87.8%)	1104 (85.8%)	1098 (85.4%)	0.0255
	ACE-inhibition and/or ARB	1127 (87.6%)	1119 (87.1%)	1106 (86.0%)	1113 (86.6%)	0.6541
	Cholesterol lowering (Statin)	1238 (96.3%)	1216 (94.6%)	1196 (93.0%)	1166 (90.7%)	<.0001
	Calcium inhibitor	263 (20.5%)	263 (20.5%)	257 (20.0%)	292 (22.7%)	0.3157
	Diuretic	406 (31.6%)	467 (36.3%)	490 (38.1%)	591 (46.0%)	<.0001
	Proton pump inhibitor	575 (44.7%)	553 (43.0%)	556 (43.2%)	577 (44.9%)	0.6870
Biomarkers	Troponin T ng/L median (Q1-Q3)	119.0 (29.3-400.0)	138.0 (37.7-508.0)	198.0 (42.7-651.0)	203.0 (55.8-691.0)	<.0001
	NT-proBNP pmol/L median (Q1-Q3)	262.0 (96.0-734.0)	387.0 (126.0-1020)	453.0 (152.0-1163)	641.0 (225.0-1925)	<.0001

Characteristic		CXCL-16 (ng/mL) Q1 <4.5 n=1286	Q2 4.5-5.2 n=1285	Q3 5.2-6.2 n=1286	Q4 >6.2 n=1285	P-value*
	Cystatin C mg/L median (Q1-Q3)	0.74 (0.61-0.88)	0.80 (0.65-0.95)	0.83 (0.67-1.02)	0.94 (0.76-1.18)	<.0001
	GDF-15 ng/ml median (Q1-Q3)	1336 (1031-1772)	1455 (1096-1987)	1562 (1195-2176)	1897 (1344-2797)	<.0001
	CRP mg/L median (Q1-Q3)	2.4 (1.2-5.6)	3.1 (1.5-7.0)	3.8 (1.7-9.2)	6.0 (2.5-16.0)	<.0001
	IL-6 pg/ml median (Q1-Q3)	2.6 (1.6-4.9)	3.2 (1.9-6.8)	3.5 (1.9-7.5)	4.6 (2.4-10.0)	<.0001

* P values from the Chi-square test (categorical variables) or Kruskal-Wallis test (continuous variables)
Abbreviations: BMI: Body Mass Index, PCI: Percutaneous Coronary Intervention, CABG: Coronary Artery Bypass Grafting, TIA: Transient Ischemic Attack, MI: Myocardial Infarction, TIMI: Thrombolysis In Myocardial Infarction, GRACE: Global Registry of Acute Coronary events, LMW: Low Molecular Weight, ACE: Angiotensin Converting Enzyme, ARB: Angiotensin Receptor Blocker, NT-ProBNP: N-terminal ProBrain Natriuretic Protein, GDF-15: Growth Differentiation Factor 15, CRP: C-Reactive Protein, IL-6: Interleukin 6

Table 2: Multivariable linear regression analyses of the association of admission levels of CXCL16 with both clinical and laboratory variables

A: Clinical variables

Background characteristic	Relative increase*	95% C.I.	P-value
Age, 10-year increase	0.9771	(0.9675 - 0.9867)	<.0001
Female vs male	1.0460	(1.0262 - 1.0662)	<.0001
Diabetes	0.9811	(0.9612 - 1.0014)	0.0680
Congestive heart failure	1.0339	(0.9970 - 1.0721)	0.0720
ST-elevation myocardial infarction	0.9722	(0.9543 - 0.9905)	0.0030
In-hospital invasive	0.9675	(0.9485 - 0.9868)	0.0010
Percutaneous coronary intervention	1.0150	(0.9861 - 1.0447)	0.3127
Chronic renal disease	1.0645	(1.0173 - 1.1139)	0.0069
Hypertension	0.9887	(0.9708 - 1.0069)	0.2223
Coronary artery bypass grafting	1.0120	(0.9733 - 1.0522)	0.5483
Myocardial infarction	0.9952	(0.9716 - 1.0192)	0.6905
Non-hemorrhagic stroke	0.9678	(0.9264 - 1.0110)	0.1415
Peripheral arterial disease	1.0318	(0.9987 - 1.0659)	0.0595
Habitual smoker	1.0353	(1.0157 - 1.0553)	0.0004
Dyslipidemia	1.0036	(0.9865 - 1.0211)	0.6814
Body mass index, >= 30 kg/m2	1.0121	(0.9938 - 1.0308)	0.1969
Aspirin at entry	0.9678	(0.9241 - 1.0136)	0.1653

B: Laboratory variables

Background Characteristic	Relative increase*	95% C.I.	P-value
NT-proBNP	0.9999	(0.9991 - 1.0006)	0.7770
Troponin T	1.0003	(0.9998 - 1.0009)	0.2495
GDF-15	1.0056	(1.0037 - 1.0075)	<.0001
Cystatin C	1.0178	(1.0144 - 1.0211)	<.0001
hsCRP	1.0012	(1.0004 - 1.0019)	0.0017
WBC	0.9997	(0.9970 - 1.0024)	0.8241
D-dimer	1.0026	(1.0014 - 1.0037)	<.0001
IL-6	1.0019	(1.0007 - 1.0031)	0.0020
IL-18	1.0008	(0.9989 - 1.0027)	0.4196
Ox-LDL	0.9963	(0.9938 - 0.9988)	0.0038
Apo-A1	0.9851	(0.9817 - 0.9884)	<.0001
Apo-B	1.0077	(1.0044 - 1.0109)	<.0001

*The relative increase is the adjusted geometric mean ratio for 10% increase in biomarker levels.
Abbreviations: NT-ProBNP: N-Terminal ProBrain Natriuretic Peptide, GDF-15: Growth Differentiation Factor 15, hsCRP: High Sensitivity C-Reactive Protein, WBC: White Blood Cell Count; IL-6: Interleukin 6, IL-18: Interleukin 18, Ox-LDL: Oxidized Low-Density Lipoprotein, Apo-A1: Apolipoprotein A1, Apo-B: Apolipoprotein B

Table 3: Association of admission levels of CXCL16 with the primary composite endpoint and its individual components. Fully-adjusted multivariable Cox regression analyses with HR per 50% increase of CXCL16 as continuous variable.

Clinical endpoint	HR (95%CI)	P-value
CV death / sMI / stroke	1.23 (1.05 - 1.45)	0.0126
CV death	1.50 (1.17 – 1.92)	0.0014
CV death / sMI	1.20 (1.01 - 1.43)	0.0360
CV death / all MI / stroke	1.25 (1.07 - 1.45)	0.0043
sMI	1.01 (0.82 - 1.25)	0.8980
Stroke	1.18 (0.77 - 1.80)	0.4530

Abbreviations: CV: Cardiovascular; sMI: Spontaneous myocardial infarction