Soluble CXCL16 Predicts Long-Term Mortality in Acute Coronary Syndromes

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Background—CXCL16/SR-PSOX is an interferon-γ-regulated chemokine and scavenger receptor for oxidized low-density lipoprotein that is expressed in atherosclerotic lesions. Proteolytic cleavage of membrane-bound CXCL16 releases soluble CXCL16, which may promote migration of effector T cells and augment a proatherogenic inflammatory response. We hypothesized that soluble CXCL16 concentrations are associated with long-term outcome in patients with acute coronary syndromes.

Methods and Results—We assessed the association between circulating CXCL16 levels obtained within 24 hours after admission and time to death in 1351 patients (median age 67 years, 30% female) with a diagnosis of unstable angina, non-ST-segment–elevation myocardial infarction, or ST-segment–elevation myocardial infarction. During a median follow-up time of 81 months, 377 patients died. Increased levels of CXCL16 were prognostically unfavorable; the fourth versus first quartile was associated with higher risk of death (hazard ratio 2.1; 95% CI 1.8 to 2.8; P<0.0001), triple risk of developing heart failure (hazard ratio 3.0; 95% CI 1.8 to 5.1; P<0.0001), and a doubling of the risk of rehospitalization for myocardial infarction (hazard ratio 2.1; 95% CI 1.3 to 3.3; P=0.002). After adjustment for conventional risk markers, logarithmically transformed CXCL16 level remained a strong independent indicator of long-term mortality (hazard ratio 1.21; 95% CI 1.09 to 1.36 per 1 SD increase in CXCL16; P=0.0006) and congestive heart failure development (hazard ratio 1.25; 95% CI 1.05 to 1.48; P=0.01). In a subsample of 714 patients, after further adjustment for troponin T, high-sensitive C-reactive protein, pro–B-type natriuretic peptide, and left ventricular ejection fraction, CXCL16 still provided significant additional prognostic information on mortality (hazard ratio 1.21; 95% CI 1.02 to 1.42 per 1 SD increase in CXCL16; P=0.02).

Conclusions—In patients with an acute coronary syndrome, CXCL16 levels obtained within 24 hours of admission are associated with long-term mortality after adjustment for other risk factors. (Circulation. 2009;119:3181-3188.)

Key Words: angina pectoris ■ peptides, natriuretic B-type ■ proteins, C-reactive ■ echocardiography ■ myocardial infarction ■ prognosis

The prognosis of patients with acute coronary syndrome (ACS) varies widely, and a number of clinical, electrocardiographic, and biochemical markers have been shown to predict adverse short-term and long-term outcome. Markers of myocyte necrosis such as cardiac troponin are invaluable diagnostic tools and are routinely used for risk stratification. However, many patients with troponin-negative ACS who have vulnerable coronary plaques remain at high risk for future ischemic events, and additional tests to improve risk stratification could prove valuable in clinical practice. More recently, B-type natriuretic peptide (BNP) has emerged as a prognostic marker, not only in patients with congestive heart failure, but also in those with ACS. Given the important role of inflammation in atherogenesis, much attention has lately been directed against the potential role of plasma markers of inflammation, and in particular C-reactive protein (CRP), as cardiovascular risk predictors. However, the inflammatory processes that underlie atherosclerosis are mediated by a multitude of cytokines and are unlikely to be reflected by CRP levels alone.1

Clinical Perspective on p 3188

Chemokines, such as monocyte chemoattractant protein-1, interleukin 8, and fractalkine (CX3CL1), are thought to play an important pathogenic role in atherogenesis and plaque destabilization by activating and directing leukocytes into the

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atherosclerotic lesion.\textsuperscript{2,3} However, the role of chemokines as prognostic markers has been questioned.\textsuperscript{4} Recently, CXCL16, a newly discovered chemokine of the CXC family, has been proposed as a pathogenic mediator in atherosclerosis.\textsuperscript{5–7} CXCL16, which is expressed in soluble and transmembrane forms, has been found to guide migration of activated T cells into inflamed tissue through interaction with its receptor, CXCR6. CXCL16 is identical to scavenger receptor SR-PSOX, which mediates uptake of oxidized low-density lipoprotein (oxLDL), suggesting the involvement of CXCL16 in both inflammation and lipid metabolism,\textsuperscript{8} and more recently, this chemokine has been shown to promote matrix degradation.\textsuperscript{6} These properties may indicate a role for CXCL16 in atherogenesis, and it is found to be upregulated in atherosclerotic plaques by the proatherogenic cytokine interferon-\(\gamma\),\textsuperscript{7} potentially acting in a positive feedback loop to increase inflammation in the atherosclerotic lesion. Enhanced expression of both CXCL16 and CXCR6 has been found in atherosclerotic lesions from humans\textsuperscript{5,7} as well as from apolipoprotein-E-deficient mice,\textsuperscript{7} but its role in atherogenesis is far from clear.

Thus a recent murine study suggests that targeted disruption of CXCL16 accelerates atherosclerosis.\textsuperscript{8} Also, both decreased\textsuperscript{9} and increased\textsuperscript{8,10} plasma concentrations have been reported in patients with coronary artery disease (CAD), and, at present, prospective studies on the association of CXCL16 with risk of developing CAD or clinical events in those with overt CAD are lacking.

The ability to reflect upstream inflammatory activity is an important criterion for an ideal biomarker in cardiovascular disease. Because of the link between several inflammatory mediators and the cleavage of the membrane-bound to the soluble form of CXCL16,\textsuperscript{11} it has been suggested that soluble CXCL16 could serve as a reliable marker of inflammation. Given its capability to reflect upstream inflammation, as well as its ability to promote downstream inflammatory and matrix-degrading responses, and thereby not only reflect, but also promote, atherogenesis and plaque destabilization, we hypothesized that a single measurement of circulating CXCL16 is predictive of long-term survival and nonfatal cardiovascular events in patients with ACS. To test this hypothesis, we measured CXCL16 in serum samples obtained from a large series of patients with ACS admitted to a Scandinavian teaching hospital.

### Methods

#### Study Design

As previously described, patients with ACS, defined as a diagnosis of unstable angina pectoris, non–ST-segment–elevation myocardial infarction (MI), or ST-segment–elevation MI, admitted to the coronary care unit of the Sahlgrenska University Hospital, Gothenburg, Sweden, during the period of September 1995 to March 2001 were eligible for participation in an ongoing prospective risk stratification program, PRACSIS (Prognosis and Risk in Acute Coronary Syndrome In Sweden).\textsuperscript{12} The main exclusion criteria were age <18 or ≥80 years, non-CAD associated with a life expectancy <1 year, residence outside the hospital’s catchment area, unwillingness, and prior admission resulting in inclusion in the study. The patients included in the current study were those with available blood samples from day 1 after admission. The primary outcome measure was all-cause mortality, with a median follow-up time of 81 months. Survival confirmation and date of death were obtained from the Swedish National Population Registry. Eleven patients, who emigrated from Sweden, were lost to follow-up and censored alive at the day of emigration. Secondary outcome measures were the incidence of acute MI (International Statistical Classification of Disease, Ninth Revision [ICD-9 code 410 or ICD-10 code I21 or I22]), congestive heart failure (ICD-9 code 428 or ICD-10 code I50), and stroke (ICD-9 codes 431, 432, 433, or 436 or ICD-10 codes I61, I62, I63, or I64) during a median follow-up time of 50 months, as obtained from the Swedish Hospital Discharge Registry. Morbidity data were checked against information in the patients’ medical records for quality control purposes. Patients were prospectively classified according to maximum Killip class on admission and during the index hospitalization. Electrocardiographic findings on admission were classified according to the presence or absence of ST-segment elevation and ST-segment depression. On the basis of hospital records and personal interview, patients were classified as having or not having a history of MI, angina pectoris, congestive heart failure, diabetes mellitus, hypercholesterolemia, or arterial hypertension. Glomerular filtration rate was estimated by the Cockcroft-Gault formula. The Regional Ethics Committee approved the study protocol, and informed consent was obtained from all individuals.

#### Echocardiography

An experienced investigator performed echocardiographic investigation within 5 days of hospital admission. Biplane left ventricular ejection fraction (LVEF) was calculated by the disc sum method and tracings were checked in motion mode for accuracy, as described previously.\textsuperscript{13}

#### Blood Sampling Procedures

Peripheral venous blood was obtained within 24 hours of admission by direct venipuncture of an antecubital vein after the patients had been supine for >30 minutes. Blood samples for CXCL16 determination were drawn into serum tubes and centrifuged within 1 hour. Blood samples for high-sensitivity determination of CRP and pro-BNP were drawn into pyrogen-free tubes with EDTA as anticoagulant, immediately immersed in ice water, and centrifuged within 1 hour. All plasma and serum samples were stored at −70°C and thawed <3 times before analysis.

#### Biochemical Analysis

Serum CXCL16 was quantified by an enzyme immunoassay using commercially available matched antibodies (R&D Systems, Minneapolis, Minn) after dilution (factor 10). No patient had a CXCL16 value less than the detection limit (11 pg/mL). The intraobserver coefficient of variation was 3.3±2.2% (mean±SD). Further, the coefficients of variation (n=8) were 3.9±3.7% for 3 freeze and thaw cycles, 8.8±3.0% for variation during daytime (nonfasting at 8 AM, noon, and 3 PM), and 9.7±3.9% for influence of food intake (fasting and nonfasting in the morning). In order to check the stability of the stored samples, we also calculated the CXCL16 level during the initial half of the study compared with the second half of inclusion time as mean±SD (1561±750 versus 1493±436 pg/mL) and median [25th to 75th percentile] values (1456 [1262 to 1720] versus 1444 [1209 to 1699] pg/mL), with neither showing a difference over time (\(P=0.11\)).

Troponin T (TnT) and creatinine kinase MB (CK-MB) fractions in serum were measured on a Modular platform (Roche Diagnostics, Mannheim, Germany). CRP and pro-BNP were measured using immunofluorescent assays calibrated with spiked plasma (Biosite Inc, San Diego, Calif).\textsuperscript{14} Samples for CRP analyses were diluted (factor 1600) to get the concentration into the measurable range. The minimal detectable concentration to upper range was 400 to 30,000 pg/mL for pro-BNP and 0.3 to 100 mg/L for CRP. All samples were run in duplicates in a blinded fashion. Creatinine and total cholesterol concentrations in serum were determined by routine laboratory methods. Glomerular filtration rate (GFR) in milliliters per minute was estimated using the Cockcroft-Gault formula \([140−\text{age}×\text{weight (kg)/serum creatinine (\(\mu\text{mol}/\text{L})\)}]\) multiplied by a constant of 1.23 in men and 1.04 in women.
Statistical variables

categorical variables are reported as proportions and continuous
variables as median values or mean±SD. The association between
CXCL16 and baseline demographic variables and cardiovascular risk
factors was tested using the Mann–Whitney U test and Spearman
rank correlation statistics for categorical and continuous variables,
respectively. The relationship between CXCL16 quartiles and long-
term prognosis was visualized by Kaplan–Meier plots.

For calculation of crude and adjusted risk estimates associated
with a 1-SD increase in logarithmically transformed CXCL16 levels
for the end points mortality from all causes and the first hospital-
ization due to MI, stroke, or congestive heart failure, respectively,
Cox proportional hazards regression analysis was used. In the
multivariable analyses, we adjusted for potential confounders
deemed to be clinically relevant. These clinical variables were age,
gender, index diagnosis, smoking status, prior MI, diabetes, hyper-
tension, angina, congestive heart failure, Killip class, heart rate,
estimated GFR, and peak CK-MB. In addition, for the subgroup for
which measurements of TnT, pro-BNP, CRP, and LVEF were
available (n=714), we performed a corresponding analysis of all-
cause mortality also adjusting for these variables. In the analyses,
TnT values <0.05 μg/L were set to 0.0 in 250 patients, pro-BNP was
set to the detection limit of 400 pg/L in 203 patients, and CRP was
set to 0.3 mg/L in 5 patients. The assumption of proportional hazard
regarding the natural logarithm of CXCL16 was assessed by study-
ing whether the addition of the interaction term [logarithm of
time×ln(CXCL16)] significantly improved the −2 log likelihood of
the model.

Similarly, the assumption of linearity was checked by entering the
squared transformation of ln(CXCL16) into the model and consid-
ering a significant change in the −2 log likelihood of this expanded
model as a sign of nonlinearity. The interactions between CXCL16
and CRP, pro-BNP, and low-density lipoprotein (LDL) cholesterol,
respectively, were tested by calculating the difference in the −2 log
likelihood of the model with, and the model without, the interaction
term in question. Probability values <0.05 were considered statisti-
cally significant.

Results

Baseline Characteristics

A total of 1351 patients (30% female, median age 67 years,
interquartile range 58 to 73 years) had CXCL16 values
obtained from blood sampled within 24 hours of admission.
The natural logarithm of our primary objective variable
CXCL16 did not show any sign of nonproportionality
(P=0.23) or nonlinearity (P=0.74) over time. The mean±SD
(range) of CXCL16 in all patients was 1529±622 (20 to
14 497) pg/L, whereas the corresponding data for
Ln(CXCL16) were 7.28±0.32 (3.00 to 9.58). The CXCL16
level was significantly increased within 24 hours of admi-
sion compared with 3 months later, as determined from 155
patients with serum samples on 4 occasions, and illustrated
separately for patients with ST-segment–elevation MI and
non–ST-segment–elevation MI/unstable angina in Figure 1.

The characteristics of patients at baseline according to
CXCL16 quartile are presented in Table 1. Patients with
higher levels of CXCL16 were more likely to be older, to be
female, to have a history of hypertension, and to be current
smokers. They were also more likely to have a diagnosis of
ST-segment–elevation MI, whereas lower levels of CXCL16
were associated with an index diagnosis of unstable angina.
Higher CXCL16 levels were associated with the presence of
ST-segment elevation and Q wave on the admission ECG, as
well as with higher peak levels of markers of myocardial
injury (ie, TnT and CK-MB) and treatment with

thrombolysis. No significant association with primary percu-
taneous coronary intervention (PCI) was seen. Lower
CXCL16 levels were associated with PCI at a later stage
during hospitalization. Higher CXCL16 levels were also
associated with elevated CRP and pro-BNP levels, a higher Killip
class at admission, and a higher maximum Killip class during
hospitalization, as well as impaired LVEF as assessed by
echocardiography. There was an association between higher
levels of CXCL16 and decreased renal function (GFR), but
not with previous hypercholesterolemia, preadmission lipid-
lowering treatment, or the actual level of total and LDL
cholesterol. In a subsample of patients with data on coronary
anatomy (n=294), there was a trend for association between
CXCL16 levels and extent of CAD in terms of 3-vessel
disease (P=0.07).

CXCL16 and Long-Term Mortality

During a median follow-up time of 81 months (interquartile
range 61 to 99 months), 377 patients died. Using the first
quartile as the reference, a CXCL16 level in the fourth
quartile (hazard ratio 2.1; 95% CI 1.6 to 2.8; P<0.0001), and
even in the third quartile (hazard ratio 1.4; 95% CI 1.0 to 1.9;
P=0.04), was associated with an increased risk of death.
Long-term mortality for the 4 quartiles of CXCL16 levels,
sampled within 24 hours of admission for ACS, is graphically
illustrated in Figure 2.

We further evaluated the relationship between mortality
and increasing level of CXCL16 in the whole group and in
subgroups. An increase in CXCL16 by 1 SD in the natural
logarithm of CXCL16 corresponded to an increased hazard
ratio (1.33; 95% CI 1.21 to 1.46; P=0.004), and even in the third quartile (hazard ratio 1.4; 95% CI 1.0 to 1.9;
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Table 1. Characteristics at Baseline

<table>
<thead>
<tr>
<th>CXCL16 Quartile</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum level of CXCL16 (pg/mL) ( \leq 1236 )</td>
<td>1237–1450</td>
<td>1451–1706</td>
<td>&gt;1706</td>
<td>( &lt;0.0001 )</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>338</td>
<td>338</td>
<td>338</td>
<td>337</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean±SD)</td>
<td>63±10</td>
<td>65±10</td>
<td>66±9</td>
<td>66±10</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Female, %</td>
<td>23</td>
<td>27</td>
<td>33</td>
<td>36</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Previous MI, %</td>
<td>23</td>
<td>25</td>
<td>23</td>
<td>17</td>
<td>0.08</td>
</tr>
<tr>
<td>Previous angina, %</td>
<td>42</td>
<td>47</td>
<td>46</td>
<td>47</td>
<td>0.24</td>
</tr>
<tr>
<td>Previous congestive heart failure, %</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>0.31</td>
</tr>
<tr>
<td>Previous diabetes, %</td>
<td>18</td>
<td>14</td>
<td>16</td>
<td>19</td>
<td>0.59</td>
</tr>
<tr>
<td>Previous hypertension, %</td>
<td>37</td>
<td>40</td>
<td>36</td>
<td>48</td>
<td>0.02</td>
</tr>
<tr>
<td>Previous hypercholesterolemia, %</td>
<td>28</td>
<td>28</td>
<td>26</td>
<td>30</td>
<td>0.83</td>
</tr>
<tr>
<td>Current smoker, %†</td>
<td>22</td>
<td>32</td>
<td>35</td>
<td>36</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Lipid lowering at admission, %</td>
<td>13</td>
<td>14</td>
<td>11</td>
<td>16</td>
<td>0.65</td>
</tr>
<tr>
<td>ST-segment-elevation MI, %</td>
<td>35</td>
<td>36</td>
<td>43</td>
<td>51</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Non-ST-segment-elevation MI, %</td>
<td>38</td>
<td>35</td>
<td>37</td>
<td>30</td>
<td>0.19</td>
</tr>
<tr>
<td>Unstable angina, %</td>
<td>27</td>
<td>29</td>
<td>20</td>
<td>19</td>
<td>0.0004</td>
</tr>
<tr>
<td>ST-segment elevation at admission, %</td>
<td>33</td>
<td>34</td>
<td>40</td>
<td>45</td>
<td>0.001</td>
</tr>
<tr>
<td>ST-segment depression at admission, %</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>0.17</td>
</tr>
<tr>
<td>Q wave at admission, %</td>
<td>6</td>
<td>10</td>
<td>13</td>
<td>19</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Systolic BP &lt;100 mm Hg, %</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>0.09</td>
</tr>
<tr>
<td>Heart rate at admission, bpm, median</td>
<td>70</td>
<td>72</td>
<td>73</td>
<td>74</td>
<td>0.0007</td>
</tr>
<tr>
<td>CK-MB maximum, ( \mu )g/L, median</td>
<td>46</td>
<td>50</td>
<td>65</td>
<td>57</td>
<td>0.002</td>
</tr>
<tr>
<td>TnT maximum, ( \mu )g/L, median‡</td>
<td>0.7</td>
<td>0.8</td>
<td>1.0</td>
<td>1.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Estimated GFR, ( \mu )mol/L, median†</td>
<td>70</td>
<td>67</td>
<td>64</td>
<td>59</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Pro-BNP, pg/mL, median‡</td>
<td>1408</td>
<td>1557</td>
<td>2014</td>
<td>2107</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>CRP, mg/L, median‡</td>
<td>10.6</td>
<td>11.2</td>
<td>15.2</td>
<td>18.0</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L, median‡</td>
<td>5.2</td>
<td>5.3</td>
<td>5.6</td>
<td>5.4</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L, median‡</td>
<td>3.4</td>
<td>3.4</td>
<td>3.6</td>
<td>3.4</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI, kg/m², median†</td>
<td>25.7</td>
<td>25.7</td>
<td>25.6</td>
<td>26.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Killip class II–IV at admission, %</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Maximum Killip class II–IV, %</td>
<td>12</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>Thrombolysis, %</td>
<td>14</td>
<td>17</td>
<td>17</td>
<td>23</td>
<td>0.001</td>
</tr>
<tr>
<td>Primary PCI, %</td>
<td>15</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>0.54</td>
</tr>
<tr>
<td>Other PCI during hospitalization, %</td>
<td>20</td>
<td>22</td>
<td>18</td>
<td>14</td>
<td>0.02</td>
</tr>
<tr>
<td>CABG during hospitalization, %</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>0.11</td>
</tr>
<tr>
<td>No thrombolysis or revascularization during hospitalization, %</td>
<td>44</td>
<td>48</td>
<td>47</td>
<td>42</td>
<td>0.65</td>
</tr>
<tr>
<td>LVEF, median‡</td>
<td>55</td>
<td>55</td>
<td>54</td>
<td>51</td>
<td>( &lt;0.0001 )</td>
</tr>
<tr>
<td>3-vessel disease, %§</td>
<td>35</td>
<td>33</td>
<td>38</td>
<td>49</td>
<td>0.07</td>
</tr>
<tr>
<td>Proximal LAD stenosis, %§</td>
<td>25</td>
<td>38</td>
<td>32</td>
<td>35</td>
<td>0.31</td>
</tr>
<tr>
<td>Left main coronary artery stenosis, %§</td>
<td>12</td>
<td>6</td>
<td>12</td>
<td>7</td>
<td>0.68</td>
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</tbody>
</table>

BMI indicates body mass index; BNP, brain natriuretic peptide; BP, blood pressure; CABG, coronary artery bypass grafting; CK-MB, creatine kinase MB fraction; CRP, C-reactive protein; GFR, glomerular filtration rate; LAD, left anterior descending; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; and TnT, troponin T.

*Actual CXCL16 value used in the statistical calculations.
†1% to 5% missing.
‡5% to 25% missing.
§>25% missing.
quartile: 1.7; 95% CI 1.2 to 2.3; \( P = 0.004 \)). In the group of patients for whom all of TnT, CRP, pro-BNP, and LVEF were available (\( n = 714 \)) and also adjusted for, the logarithmically transformed CXCL16 levels still predicted mortality (hazard ratio per 1 SD increase in \( \ln \) CXCL16: 1.21; 95% CI 1.02 to 1.42; \( P = 0.02 \)). Among these patients, quartile analyses showed borderline significance (third versus first quartile hazard ratio 1.7; 95% CI 1.0 to 2.7; \( P = 0.04 \)); and fourth versus first quartile hazard ratio 1.5; 95% CI 0.9 to 2.5; \( P = 0.13 \)).

In subgroups, as shown in Table 2, we found a significant association between CXCL16 at baseline and prognosis, not only in the complete ST-segment–elevation MI group and the non–ST-segment–elevation ACS group, but also in the subgroups of patients with ST-segment–elevation MI undergoing primary PCI or thrombolysis and the subgroup of non–ST-segment–elevation ACS not undergoing revascularization. After adjustment for clinical variables, the predictive value of CXCL16 remained largely unaltered in patients undergoing primary PCI in ST-segment–elevation MI and in patients with non–ST-segment–elevation ACS not undergoing revascularization. In the latter group, CXCL16 retained its predictive value after adjustment for LVEF, TnT, pro-BNP, and CRP.

**CXCL16 and CRP Combinations and Long-Term Mortality**

We found no significant interaction between CXCL16 and pro-BNP or LDL cholesterol regarding long-term mortality. However, there was a significant interaction with CRP (\( P = 0.002 \)). Therefore, the joint effects of CRP and CXCL16 are described in Figure 3. Patients in the fourth quartile of CXCL16, but not of CRP, had a hazard ratio in the same order (1.4; 95% CI 1.0 to 1.9; \( P = 0.06 \)) as those with CRP but not CXCL16 in the fourth quartile (1.6; 95% CI 1.1 to 2.2; \( P = 0.005 \)) when compared with patients with neither CXCL16 nor CRP in the fourth quartile. However, a considerably higher hazard ratio was noted for patients with both CXCL16 and CRP in the fourth quartile (3.3; 95% CI 2.3 to 4.6; \( P < 0.0001 \)). This relationship of mortality to the biochemical markers persisted after adjustment for clinical variables (hazard ratio 2.3; 95% CI 1.6 to 3.4; \( P < 0.0001 \)) and also when including TnT, pro-BNP, and LVEF (hazard ratio 1.8; 95% CI 1.1 to 2.9; \( P = 0.02 \)).

**CXCL16 and Nonfatal Cardiovascular Events**

Patients with CXCL16 in the fourth quartile were 3 times as likely as those with CXCL16 in the first quartile (hazard ratio 3.0; 95% CI 1.8 to 5.1; \( P < 0.0001 \)) to become hospitalized as a result of congestive heart failure and twice as likely to be hospitalized as a result of MI (hazard ratio 2.1; 95% CI 1.3 to 3.3; \( P = 0.002 \)) or stroke (hazard ratio 2.0; 95% CI 1.0 to 3.9; \( P = 0.06 \)). In a Cox proportional hazards model, there was a significant univariate association between the logarithmically transformed CXCL16 level and new admission for acute MI as well as for congestive heart failure, as shown in Table 2. After adjustment for clinical variables, the associations remained significant for both. Likewise, patients in the fourth quartile had a higher adjusted risk compared with those in the first quartile (hazard ratio for MI 2.0; 95% CI 1.2 to 3.3; \( P = 0.01 \)); and for congestive heart failure, hazard ratio 2.7; 95% CI 1.5 to 4.9; \( P = 0.0007 \)). However, in the group in which TnT, CRP, pro-BNP, and LVEF were also all available (\( n = 714 \)) and adjusted for, the initial CXCL16 value was not significantly associated with readmissions for MI nor congestive heart failure.

**Discussion**

In the present study, examining a large population of ACS patients, we report an association between high serum levels of CXCL16 and disease severity as assessed by ECG abnormalities (ST-elevation and Q-wave at admission), maximum level of markers of myocardial injury (CK-MB and TnT) as well as impaired left ventricular systolic function in terms of reduced LVEF. Moreover, we demonstrate, for the first time, that high CXCL16 levels during the initial 24 hours after admission for an ACS significantly predict long-term mortality. The results remained significant after adjustment for conventional risk factors as well as for contemporary laboratory tests used for risk stratification. These findings suggest that CXCL16 should be added to the list of risk markers that could give prognostic information in patients with ACS beyond that of traditional risk indicators.

Given the link between inflammatory mediators and the cleavage of the membrane-bound to the soluble form of CXCL16, it has been suggested that soluble CXCL16 could serve as a reliable, stable, and robust marker of inflammation, being detectable in plasma/serum in concentrations regularly greater than 1 ng/mL. Our findings in the current study showing high stability of the actual protein (eg, little circadian variation, minor influence of food intake, and little variation after freeze and thaw cycle) further support a potential role of CXCL16 as a biomarker in clinical samples. Increased joint fluid levels of soluble CXCL16 without increased serum levels have been reported in rheumatoid arthritis,\(^{15}\) and an increased serum level in systemic lupus erythematosus was associated with nephritis.\(^{16}\) However, conflicting data exist on plasma levels of CXCL16 in CAD. Thus although Sheikine et al\(^{9}\) found decreased CXCL16 levels in both stable and unstable angina, others have reported increased CXCL16 levels in patients with CAD, and in the study by Lehrke et al,\(^{10}\) particularly...
Recently, Aslanian and Charo\(^8\) reported that CXCL16-deficient LDL receptor\(^{-/-}\) mice were burdened with accelerated atherosclerosis. This study, suggesting antiatherogenic effects of CXCL16, may seem in conflict with the present study. However, as the membrane-bound and soluble forms of CXCL16 seem to have different biological functions, enhanced atherogenesis in CXCL16 knockout mice may not necessarily argue against a proatherogenic effect of soluble CXCL16. We have recently shown that soluble CXCL16 induces inflammatory responses in vascular smooth muscle cells and peripheral-blood mononuclear cells, with particularly prominent effects in patients with CAD, and with relevance to plaque destabilization, soluble CXCL16 has recently been shown to enhance matrix metalloproteinase activity.\(^6,17\) Also, substantial evidence indicates that CXCL16 may have constitutive functions, such as promotion of cell survival and normal leukocyte recruitment. Thus although too much CXCL16 may be harmful, too little may not necessarily be beneficial. Moreover, a recent study by Galkina et al\(^18\) reported that CXCR6-deficient (ie, lacking the CXCL16 receptor) apoE\(^{-/-}\) mice showed attenuated atherosclerosis, accompanied by a decreased percentage of CXCR6\(^+\) T cells within the aortas, indicating a proatherogenic role of CXCL16/CXCR6 interaction. Taken together, although further studies are needed, our data in the present study lend further support for a role of soluble CXCL16 as a marker as well as a mediator of CAD.

It may be argued that the association between CXCL16 and outcome is due to confounding factors such as oxLDL. However, despite the fact that the membrane-bound form of CXCL16 is a scavenger receptor for oxLDL,\(^9\) no correlation between circulating CXCL16 and oxLDL was observed in patients with rheumatoid arthritis.\(^15\) This also concords with

### Table 2. Associations Between CXCL16 Concentrations and Events During Follow-Up in Acute Coronary Syndromes

<table>
<thead>
<tr>
<th>Event</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
<th>Adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete cohort</td>
<td>1.33 (1.21–1.46)</td>
<td>&lt;0.0001</td>
<td>1.21 (1.09–1.36)</td>
</tr>
<tr>
<td>(n=1351, 377 end points)</td>
<td></td>
<td></td>
<td>1.21 (1.02–1.42)</td>
</tr>
<tr>
<td>ST-segment–elevation MI</td>
<td>1.34 (1.15–1.57)</td>
<td>0.0001</td>
<td>1.17 (0.98–1.41)</td>
</tr>
<tr>
<td>(n=561, 152 end points)</td>
<td></td>
<td></td>
<td>1.27 (0.91–1.78)</td>
</tr>
<tr>
<td>Primary PCI</td>
<td>1.66 (1.11–2.47)</td>
<td>0.01</td>
<td>1.96 (1.15–3.35)</td>
</tr>
<tr>
<td>(n=192, 36 end points)</td>
<td></td>
<td></td>
<td>1.70 (0.76–3.84)</td>
</tr>
<tr>
<td>Thrombolyis</td>
<td>2.16 (1.51–3.07)</td>
<td>&lt;0.0001</td>
<td>1.44 (0.96–2.18)</td>
</tr>
<tr>
<td>(n=223, 55 end points)</td>
<td></td>
<td></td>
<td>1.39 (0.62–3.10)</td>
</tr>
<tr>
<td>None of above</td>
<td>1.01 (0.81–1.25)</td>
<td>0.94</td>
<td>0.98 (0.78–1.22)</td>
</tr>
<tr>
<td>(n=146, 61 end points)</td>
<td></td>
<td></td>
<td>1.45 (0.83–2.53)</td>
</tr>
<tr>
<td>Non–ST-segment–elevation MI</td>
<td>1.32 (1.18–1.48)</td>
<td>&lt;0.0001</td>
<td>1.25 (1.09–1.44)</td>
</tr>
<tr>
<td>(n=790, 225 end points)</td>
<td></td>
<td></td>
<td>1.24 (1.01–1.52)</td>
</tr>
<tr>
<td>MI/unstable angina</td>
<td>1.17 (0.83–1.66)</td>
<td>0.38</td>
<td>1.00 (0.74–1.34)</td>
</tr>
<tr>
<td>(n=286, 54 end points)</td>
<td></td>
<td></td>
<td>1.09 (0.62–1.91)</td>
</tr>
<tr>
<td>No PCI/CABG</td>
<td>1.31 (1.17–1.47)</td>
<td>&lt;0.0001</td>
<td>1.31 (1.13–1.52)</td>
</tr>
<tr>
<td>(n=504, 171 end points)</td>
<td></td>
<td></td>
<td>1.33 (1.05–1.68)</td>
</tr>
<tr>
<td>New admissions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute MI</td>
<td>1.25 (1.08–1.44)</td>
<td>0.002</td>
<td>1.18 (1.01–1.38)</td>
</tr>
<tr>
<td>(n=1351, 175 end points)</td>
<td></td>
<td></td>
<td>1.05 (0.83–1.34)</td>
</tr>
<tr>
<td>CHF</td>
<td>1.31 (1.12–1.52)</td>
<td>0.0005</td>
<td>1.25 (1.05–1.48)</td>
</tr>
<tr>
<td>(n=1351, 142 end points)</td>
<td></td>
<td></td>
<td>1.02 (0.76–1.36)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.09 (0.86–1.39)</td>
<td>0.48</td>
<td>0.98 (0.77–1.25)</td>
</tr>
<tr>
<td>(n=1351, 76 end points)</td>
<td></td>
<td></td>
<td>1.21 (0.84–1.74)</td>
</tr>
</tbody>
</table>

CABG indicates coronary artery bypass grafting; CHF, congestive heart failure; HR, hazard ratio per 1 SD increase in the natural logarithm of CXCL16; LVEF, left ventricular ejection fraction; MI, myocardial infarction; and PCI, percutaneous coronary intervention.

*Adjusted for age (in quartiles), gender, previous MI, previous angina, previous congestive heart failure, previous diabetes, previous hypertension, current smoking, index diagnosis, heart rate (in quartiles), peak creatine kinase-MB (in quartiles), estimated glomerular filtration rate (in quartiles), and Killip class (>1).

†Adjusted also for troponin T (in quartiles), pro–B-type natriuretic peptide (logarithmically transformed), C-reactive protein (logarithmically transformed), and LVEF (%).
the lack of correlation between CXCL16 and LDL cholesterol in our patients with ACS. Furthermore, in vitro experiments suggest that no stable binding occurs between soluble CXCL16 and oxLDL. Although we found a strong correlation between serum levels of CRP and CXCL16, both being markers of upstream inflammation, they may reflect different aspects of the atherosclerosis-related inflammatory responses, and indeed, CXCL16 was able to predict total mortality in patients with ACS also after adjustment for CRP. The strong predictive effect of combining CRP and CXCL16 as biomarkers further underscores that these markers, at least partly, reflect different upstream inflammatory pathways.

**Possible Future Clinical Role for CXCL16 in ACS**

It has become evident that plaque rupture in nonstenotic lesions with subsequent thrombotic occlusion is a common cause of ischemic events. A rapid progression of insignificant plaques to those requiring PCI has been observed. Consequently, it may be difficult to predict the risk of a new event despite a multitude of imaging techniques that could be applied in such attempts. Not only for economical reasons, biochemical risk markers therefore seem warranted. BNP and pro-BNP are also strong prognostic markers across the spectrum of ACS after adjustment for left ventricular systolic function. Different models relying on combinations of CRP, BNP/pro-BNP, creatinine/GFR, and TnT/I have been shown to predict death and recurrent MI better than just one of the markers by itself. A prognostic yield may also be obtained in the elderly, even without overt cardiovascular disease. Previous conflicting data have been reported regarding circulating CXCL16 levels in human atherosclerosis, but these studies have been hampered by small sample size and lack of follow-up data. In the present study, we show the capability of a single measurement of CXCL16 at admission for ACS to predict long-term mortality, and we suggest that this is based on the ability of CXCL16 to reflect a broad range of inflammatory and matrix-degrading activity, being part of the inflammatory loop that contributes to atherogenesis and plaque destabilization. We hypothesize that increased CXCL16 levels during ACS are related to an inflammatory phenotype that predisposes to enhanced atherogenesis, plaque destabilization, and increased mortality.

Possibly, CXCL16 could help enhance risk stratification further, because in this study, it contributed prognostic information even when accounting for pro-BNP, CRP, TnT, GFR, and a number of clinical variables, including LVEF. The ability of CXCL16 to predict total mortality in patients with troponin-negative ACS (ie, unstable angina) may be of particular importance. However, although we found a strong association between CXCL16 levels and total mortality after adjustment for other risk factors, the clinical implications of our findings remain at present somewhat unclear, and recommendations for implementing CXCL16 measurements in ACS must await further studies in larger patient populations testing the prognostic value of a model consisting of conventional risk markers and of conventional risk markers plus CXCL16.

**Strengths and Limitations**

Important strengths of this single-center prospective study include a large sample size, long duration of follow-up, and a large number of end points. However, for some subgroup analyses, fewer end points may explain lack of significance, and these data should be interpreted cautiously. Echocardiographic information is a strength, although it was not available in all patients. Objective measures concerning left ventricular systolic function are not commonly obtained or adjusted for in biomarker substudies of major pharmaceutical multicenter trials in patients with ACS. The lack of complete laboratory data, including lipid data during hospitalization, in all subjects is clearly a limitation. Although our data may suggest a pathogenic role of CXCL16 in CAD, correlation does not necessarily mean causal relationship, and the study design does not answer the question of whether circulating CXCL16 plays a pathophysiological role or is merely a marker of severity of atherosclerosis. Furthermore, the predictive value of CXCL16 needs to be confirmed in other ACS patient groups.

**Conclusions**

In the present study, we demonstrate that CXCL16 levels are associated with long-term mortality across the spectrum of ACS. Notably, an association was still found after adjustment for prognostic markers used in clinical cardiology today, such as index diagnosis, conventional risk factors, CRP, TnT, and proBNP. These findings suggest that CXCL16 should be further investigated as a potential risk marker in even larger populations of patients with CAD as well as in those at risk for development of such disorders.

**Acknowledgments**

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Disclosures
None.

References

CLINICAL PERSPECTIVE
CXCL16/SR-PSOX is an interferon-γ-regulated chemokine involved in inflammation, lipid metabolism, and matrix degradation. Enhanced expression of CXCL16 has been found in atherosclerotic lesions. On the basis of its potential proatherogenic properties as well as the ability of soluble CXCL16 to reflect upstream inflammation, we hypothesized that soluble CXCL16 concentrations are associated with long-term outcome in patients with acute coronary syndromes. In a large group of patients with acute coronary syndromes, we found that the CXCL16 level during the initial 24 hours of admission predicted long-term mortality. This was the case even when accounting for a number of clinical variables, in addition to troponin T, C-reactive protein, pro-B-type natriuretic peptide, and ejection fraction. Rehospitalization for an acute myocardial infarction or congestive heart failure was also predicted by CXCL16 level, even when adjusting for clinical variables. Our findings suggest that CXCL16 should be further investigated as a potential risk marker in even larger populations of patients with coronary artery disease, as well as in those at risk for development of such disorders. Although we found a strong association between CXCL16 and total mortality, recommendations for implementing CXCL16 measurements in acute coronary syndromes must await further studies in larger patient populations, testing the prognostic value of a model consisting of conventional risk markers and CXCL16.
Soluble CXCL16 Predicts Long-Term Mortality in Acute Coronary Syndromes
Anna M. Jansson, Pål Aukrust, Thor Ueland, Camilla Smith, Torbjørn Omland, Marianne Hartford and Kenneth Caidahl

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