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Circulating Secretory Phospholipase A2 Activity Predicts Recurrent Events in Patients With Severe Acute Coronary Syndromes

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OBJECTIVES The purpose of this study was to determine the prognostic value of circulating secretory phospholipase A2 (sPLA2) activity in patients with acute coronary syndromes (ACS).

BACKGROUND The plasma level of type IIA sPLA2 is a risk factor for coronary artery disease (CAD) and is associated with adverse outcomes in patients with stable CAD. The prognostic impact of sPLA2 in patients with ACS is unknown.

METHODS Secretory phospholipase A2 antigen levels and activity were measured in plasma samples of 446 patients with ACS, obtained at the time of enrollment.

RESULTS Baseline sPLA2 activity was associated with the risk of death and myocardial infarction (MI). The unadjusted rate of death and MI increased in a stepwise fashion with increasing tertiles of sPLA2 activity ($p < 0.0001$). The association remained significant in the subgroup of patients who had MI with ST-segment elevation ($p = 0.014$) and the subgroup of patients who had unstable angina or non–ST-segment elevation MI ($p < 0.002$). After adjustment for clinical and biological variables, the hazard ratios for the combined end point of death or MI in the third tertile of sPLA2 compared with the first and second tertiles was 3.08 (95% confidence interval, 1.37 to 6.91, $p = 0.006$).

CONCLUSIONS A single measurement of plasma sPLA2 activity at the time of enrollment provides strong independent information to predict recurrent events in patients with ACS. (J Am Coll Cardiol 2005;46:1249–57) © 2005 by the American College of Cardiology Foundation

Phospholipase A2 (PLA2) enzymes hydrolyze phospholipids at the *sn*-2 position to generate lysophospholipids and fatty acids (1). One of the most extensively studied PLA2 is a low-molecular-weight (14 kDa) group IIa secretory PLA2 (sPLA2), expressed in normal human arteries and atherosclerotic plaques (2), and associated with enhanced susceptibility to atherogenesis in animals (3). The plasma level of sPLA2 is elevated in patients with coronary artery disease (CAD) and predicts coronary events in stable patients (4). Increased plasma level of sPLA2 in stable patients undergoing percutaneous coronary angioplasty also provides independent prognostic information over other classic biological and clinical variables (5). Studies evaluating the prognostic value of sPLA2 in patients with acute coronary syndromes (ACS) are limited to a small study of patients with unstable angina (UA), where increased plasma levels of sPLA2 predicted recurrent coronary events, mainly revascularization procedures, independently of other risk factors (6). Noteworthy, sPLA2 activity has not been directly determined in the previously published studies, and no information is available on the prognostic implications of sPLA2 activity in patients with ACS. These include patients with ST-segment elevation myocardial infarction (STEMI), patients with myocardial infarction (MI) but no ST-segment elevation (NSTEMI), and patients with UA. We sought to evaluate the prognostic implications of plasma sPLA2 activity across the entire spectrum of ACS by recruiting patients from the Global Registry of Acute Coronary Events (GRACE). In parallel, we measured plasma levels of two other systemic inflammatory markers, the classic high-sensitivity C-reactive protein (CRP), and interleukin (IL)-18, a potent pro-atherogenic cytokine recently shown to provide strong independent prognostic

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Abbreviations and Acronyms

ACS = acute coronary syndromes
CAD = coronary artery disease
CRP = C-reactive protein
GRACE = Global Registry of Acute Coronary Events
hsCRP = high-sensitivity CRP
IL = interleukin
MI = myocardial infarction
NSTEMI = non–ST-segment elevation MI
STEMI = ST-segment elevation MI
sPLA2 = secretory type II phospholipase A2
UA = unstable angina

information in patients with coronary heart disease but whose prognostic value in patients with ACS has not been evaluated.

METHODS

Study sample. Full details of the GRACE methodology have been published (7–9). Patients included in this substudy (from September 28, 2000, to October 24, 2002) were recruited in three centers in France and one center in Scotland. The study was approved by institutional review committees and the subjects gave informed consent. Inclusion criteria for the present study were age ≥18 years, a presumptive diagnosis of ACS, and at least one of the following criteria: dynamic electrocardiographic (ECG) changes consistent with ACS, serial increases in serum biochemical markers of cardiac necrosis, and/or documentation of CAD. Exclusion criteria were recent MI with persistent elevation of serum biochemical markers of cardiac necrosis, Killip class IV, trauma, surgery, significant comorbidity, life expectancy of less than six months, a diagnosis of cancer, human immunodeficiency virus positivity, nocturnal paroxysmal hemoglobinuria, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, autoimmune thrombocytopenia, heparin-induced thrombocytopenia, anti-phospholipid syndrome, systemic lupus erythematosus, and Crohn's disease. Patients were followed up by telephone, clinic visits, or calls to the primary care physician. Standardized definitions of all patient-related variables and clinical diagnoses were used as well as hospital complications and outcomes. All cases were assigned to one of the following categories: STEMI, NSTEMI, or UA. These definitions take into account clinical presentation, ECG findings, and the results of serum biochemical markers of necrosis. Unstable angina was defined as ACS with normal biochemical markers of necrosis (particularly troponin I).

Blood sampling and biochemical analyses. Blood samples were collected in heparin tubes at the time of enrollment (the first working morning following admission) and immediately centrifuged at 3,000 rpm at 4°C for 10 min. The resulting plasma was aliquoted, frozen, and shipped on dry ice to Hôpital Bichat-Claude Bernard (Paris, France), where they were maintained at −70°C until analyzed. C-reactive protein was measured by a high-sensitivity test, performed on a Behring BN II analyzer (Behring Diagnostics, Deerfield, Illinois), and cardiac troponin I was quantified with an automated system (RXL HM analyzer, Dade Behring). High-sensitivity IL-18 was measured with a human ELISA kit (MBL Co., Nagoya, Japan) with a detection limit of 12.5 pg/ml. Levels of immunoreactive sPLA2 in plasma were measured by an immunometric assay on the basis of a double-antibody "sandwich" technique, with a monoclonal antibody specific for sPLA2 (Cayman Chemical Company, Ann Arbor, Michigan). This antibody has no cross-reactivity with type I, IV, or type V sPLA2 (Cayman Chemical Company). Cross-reactivity with type X sPLA2 has not been tested. The minimum detectable concentration was 15.6 pg/ml. and the intra and interassay coefficient of variation (CV) was <10%. Plasma sPLA2 activity was measured by a selective fluorimetric assay of Radvanyi et al. (10), as modified by Pernas et al. (11). The sPLA2 activity was measured with fluorescent substrate 1-hexadecanoyl-2-(1-pyredecanoyl)-sn-glycero-3 phosphomethanol, sodium salt (Interchim, Montluçon, France). Of the fluorescent substrate, 100% hydrolysis was measured with 0.1 U PLA2 from bee venom (Sigma Chemical Co., Lyon, France). The hydrolysis of substrate in the absence of plasma was used as negative control and deduced from PLA2 activity. All samples were tested in duplicate and plasma activity was expressed as nmol/min/ml. The minimum detectable activ-

Table 1. Sample Characteristics

French recruitment center (%)	63
Male (%)	76.6
Age, yrs (mean ± SD)	61.1 ± 13
Medical history (%)	
Infarction	22.9
Congestive heart failure	5.6
Coronary artery disease	28.5
Cerebrovascular disease	5.6
Diabetes	16.6
Hypertension	40.2
Hyperlipidemia	49.6
PCI	16.4
CABG	6.9
Current or former smoker (%)	72.9
Index diagnosis (%)	
STEMI	38.5
NSTEMI	52.5
Unstable angina	9.0
Killip class >1 (%)	20.5
Troponin I, ng/ml (median–interquartile range)	2.55–17.3
CRP, mg/l (median–interquartile range)	8.45–15.7
IL18, pg/ml (median–interquartile range)	195–120
Cholesterol, mg/dl (median–interquartile range)	197.4–70.4
Creatinine, mg/dl (median–interquartile range)	1.1–0.26
sPLA2 activity, nmol/min/ml (median–interquartile range)	2.3–1.78
sPLA2 antigen, ng/dl (median–interquartile range)	279–459

CABG = coronary artery bypass graft surgery; CRP = C-reative protein; IL = interleukin; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; sPLA2 = secretory type II phospholipase A2; STEMI = ST-segment elevation myocardial infarction.

Table 2. Association Between Plasma sPLA2 Activity and Baseline Clinical Variables

	Mean (SD) of sPLA2 Activity	Univariate p Value	Multivariable p Value
Recruitment center			
Scotland	3.07 (1.9)	<0.0001	0.0006
France	2.36 (1.5)		
Gender			
M	2.59 (1.6)	0.4	
F	2.77 (1.9)		
Age	*	<0.0001	0.002
Medical history			
Infarction			
Yes	2.48 (1.4)	0.3	
No	2.67 (1.8)		
Coronary artery disease			
Yes	2.47 (1.4)	0.15	
No	2.7 (1.8)		
Cerebrovascular disease			
Yes	2.79 (1.3)	0.6	
No	2.63 (1.7)		
Diabetes			
Yes	3.03 (2.2)	0.08	
No	2.55 (1.6)		
Hypertension			
Yes	2.81 (1.8)	0.07	
No	2.51 (1.6)		
Hyperlipidemia			
Yes	2.36 (1.3)	0.001	0.04
No	2.88 (1.9)		
PCI			
Yes	2.33 (1.4)	0.06	
No	2.69 (1.7)		
CABG			
Yes	2.33 (1.3)	0.3	
No	2.65 (1.7)		
Smoking status			
Current or former smoker	2.58 (1.7)	0.3	
Never smoked	2.77 (1.8)		
Index diagnosis			
Myocardial infarction with ST-segment elevation	2.99 (2.2)		
Myocardial infarction without ST-segment elevation	2.42 (1.3)	0.002	0.01
Unstable angina	2.31 (1.4)		
Killip class			
1	2.43 (1.5)		
2	3.46 (2.2)	<0.0001	0.04
3	3.24 (1.8)		
Treatment within first 24 hr hospital or chronic use			
Aspirin			
Yes	2.65 (1.7)	0.2	
No	2.29 (1.2)		
Ticlopidine/clopidogrel			
Yes	2.54 (1.7)	0.3	
No	2.70 (1.7)		
LMW heparin			
Yes	2.36 (1.4)	0.001	
No	2.90 (2.0)		
Unfractionated heparin			
Yes	2.93 (2.0)	0.0008	0.03
No	2.35 (1.2)		
GP IIb/IIIa			
Yes	2.71 (1.9)	0.6	
No	2.60 (1.6)		
Statin			
Yes	2.53 (1.6)	0.09	
No	2.84 (1.9)		
ACE inhibitor			
Yes	2.79 (1.9)	0.3	
No	2.59 (1.6)		
Beta blocker			
Yes	2.62 (1.8)	0.5	
No	2.77 (1.5)		

Variables with a $p < 0.05$ in univariate analysis were studied in multivariable analysis by performing an analysis of covariance (see Methods). *Correlation coefficient with sPLA2: 0.2.

ACE = angiotensin-converting enzyme; GP = glycoprotein; LMW = low molecular weight; other abbreviations as in Table 1.

ity was 0.10 nmol/min/ml and the intra- and interassay CV was <10%.

Statistical analysis. Power calculations were conducted to detect an adjusted hazard ratio of 3. This estimation was on the basis of the results of the study by Kugiyama et al. (4). The number of events planned to provide the study with 80% power with a risk alpha equal to 5% was calculated to be 42. Assuming a six-month cumulative incidence of 10%, with unbalanced group sizes (one-third, two-third), 435 patients should be included.

We first studied relationships between plasma sPLA2 activity and clinical parameters: for binary variables, the means of sPLA2 levels were compared with the Student *t* test. For index diagnosis, means of sPLA2 activity were compared with an analysis of variance. Relationships between sPLA2 activity and continuous parameters were tested with Spearman's rank correlation. To identify clinical independent predictors of sPLA2 levels, variables with a p value <0.05 in univariate analysis were studied in multivariable analysis by performing an analysis of covariance. We used a forward selection with a significance level of 0.05 for entering an explanatory variable into the multivariable model.

The predictive factors of death or MI were determined with survival analysis. Survival curves were derived from Kaplan–Meier estimates. In univariate analysis, comparisons of the survival distributions were made by log-rank test for clinical parameters and by univariate Cox regression for biological markers (CRP, Troponin I, IL-18, sPLA2 activity, and sPLA2 antigen). These markers were a priori divided into tertiles and coded to two dummy variables. For each factor, hazards ratios and their confidence limits were estimated with a univariate Cox model. Univariate predictors with a p value <0.05, were included in a stepwise

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Table 3. Univariate Survival Analysis

	Cumulative Incidence of Death/MI (SEM)*	Hazard Ratio (95% Confidence Interval)	p Value
Recruitment center			
Scotland	10.5% (0.02)	1.81 (0.93–3.53)	0.08
France	6.0% (0.01)		
Gender			
F	7.9% (0.03)	1.13 (0.53–2.41)	0.7
M	7.7% (0.01)		
Age			
≥60 yrs	13.2% (0.02)	5.4 (2.26–13)	<0.0001
<60 yrs	2.3% (0.01)		
Medical history			
Infarction			
Yes	14.9% (0.04)	3.2 (1.65–6.13)	0.0002
No	5.6% (0.01)		
Congestive heart failure			
Yes	27.9% (0.09)	4.41 (1.93–10.1)	0.0001
No	6.5% (0.01)		
Coronary artery disease			
Yes	12.1% (0.03)	2.27 (1.18–4.36)	0.01
No	6.1% (0.01)		
Cerebrovascular disease			
Yes	16.0% (0.07)	2.06 (0.73–5.83)	0.16
No	7.2% (0.01)		
Diabetes			
Yes	12.5% (0.04)	1.95 (0.94–4.05)	0.07
No	6.8% (0.01)		
Hypertension			
Yes	10.2% (0.02)	1.90 (1.01–3.55)	0.05
No	6.1% (0.01)		
Hyperlipidemia			
Yes	8.8% (0.02)	1.44 (0.74–2.8)	0.28
No	6.8% (0.02)		
PCI			
Yes	4.4% (0.02)	1.6 (0.57–4.52)	0.37
No	8.4% (0.01)		
CABG			
Yes	18.2% (0.07)	2.67 (1.11–6.41)	0.02
No	6.9% (0.01)		
Smoking status			
Never smoked	10.6% (0.03)	1.67 (0.86–3.27)	0.13
Current or former smoker	6.6% (0.01)		
Index diagnosis			
MI with ST-segment elevation	9.9% (0.02)	1.93 (0.96–3.86)	0.06
Other	5.7% (0.02)		
Killip class			
>1	21.4% (0.04)	5 (2.62–9.7)	<0.0001
1	4.3% (0.01)		
Creatinine			
>1.1	14% (0.02)	6.2 (2.39–15.95)	<0.0001
≤1.1	2% (0.01)		
Total cholesterol			
≤197	8.4% (0.02)	1.34 (0.59–3.06)	0.5
>197	5.2% (0.02)		
Treatment			
LMW heparin			
No	8.7% (0.02)	1.12 (0.57–2.2)	
Yes	6.8% (0.02)		0.7
Unfractionated heparin			
No	8.4% (0.02)	1.27 (0.64–2.52)	
Yes	6.8% (0.02)		0.5
Revascularization (in hospital)			
No	10.5% (0.02)	2.42 (1.16–5.05)	0.01
Yes	4.3% (0.01)		

Continued on next page

Table 3. Continued

	Cumulative Incidence of Death/MI (SEM)*	Hazard Ratio (95% Confidence Interval)	p Value
Troponin I			
Tertile 1	6.2% (0.02)	$HR_{2,3\ vs.\ 1}$:1.39 (0.59–3.30)	0.5
Tertile 2	7.8% (0.02)	$HR_{3\ vs.\ 1,2}$:1.22 (0.57–2.61)	0.6
Tertile 3	9.4% (0.02)		
CRP			
Tertile 1	7.7% (0.02)	$HR_{2,3\ vs.\ 1}$:0.99 (0.43–2.3)	0.9
Tertile 2	7% (0.02)	$HR_{3\ vs.\ 1,2}$:1.28 (0.58–2.81)	0.5
Tertile 3	8.8% (0.02)		
IL-18			
Tertile 1	7.3% (0.02)	$HR_{2,3\ vs.\ 1}$:0.99 (0.43–2.28)	0.9
Tertile 2	7% (0.02)	$HR_{3\ vs.\ 1,2}$:1.31 (0.6–2.89)	0.5
Tertile 3	9.8% (0.02)		
PLA2 antigen			
Tertile 1	7.8% (0.02)	$HR_{2,3\ vs.\ 1}$:1 (0.51–2)	0.9
Tertile 2	5% (0.02)	$HR_{3\ vs.\ 1,2}$:1.9 (0.96–3.6)	0.06
Tertile 3	11.3% (0.03)		
sPLA2 activity			
Tertile 1	3% (0.01)	$HR_{2,3\ vs.\ 1}$:1.8 (0.5–6.2)	0.3
Tertile 2	5.1% (0.02)	$HR_{3\ vs.\ 1,2}$:4.3 (2.1–8.7)	<0.0001
Tertile 3	14.4% (0.03)		

*Six months cumulative incidence of death/MI (SEM).
MI = myocardial infarction; HR = hazard ratio; other abbreviations as in Tables 1 and 2.

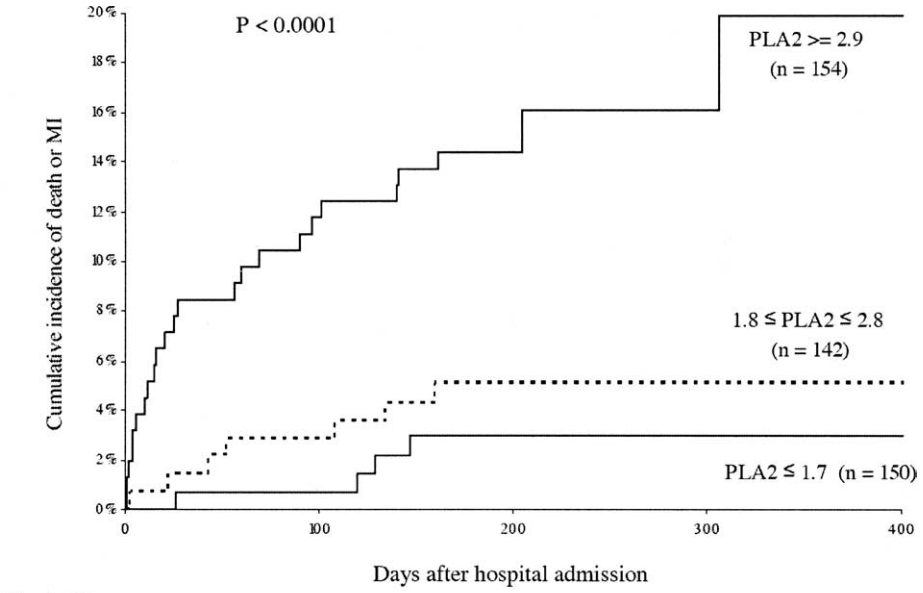
multivariable Cox regression model. The selection method was a forward selection with a significance level of 0.05 for entering an explanatory variable into the model. The final model only includes significant variables with a p value <0.05 with the Wald test. Analyses were performed with the SAS software package V8.2 (SAS Institute, Cary, North Carolina).

RESULTS

The study sample consisted of 446 patients with ACS, 38.5% with STEMI, 52.5% with NSTEMI, and 9% with UA. The median follow-up after admission was 6.5 months (25th percentile: 6 months; 75th percentile: 7.5 months). The rate of in-hospital revascularization (percutaneous coronary intervention or coronary artery bypass graft) was high: 48.3%. One year cumulative incidence of death or MI was 9.63% (SEM: 0.02). Plasma sPLA2 activity ranged from ≤0.1 to 16.9 nmol/min/ml with a mean of 2.63 ± 1.69 nmol/min/ml. The time interval from onset of clinical symptoms to blood sampling was not associated with plasma levels of sPLA2 activity ($p = 0.45$).

Association of plasma sPLA2 activity with baseline clinical and biological variables. Sample characteristics are shown in Table 1 and clinical variables associated with sPLA2 activity are shown in Table 2. In multivariable analyses, significant predictors of high sPLA2 activity were recruitment center, age, diagnosis of STEMI at admission, presentation with Killip class >1, and treatment by unfractionated heparin (Table 2). A history of hyperlipidemia was significantly associated with lower sPLA2 activity (Table 2). Plasma sPLA2 activity showed moderate association with CRP ($r = 0.35$, $p < 0.0001$). Although statistically significant, the associations between sPLA2 activity and troponin I ($r = 0.23$, $p < 0.0001$), total cholesterol ($r = -0.24$, $p < 0.0001$), and low-density lipoprotein (LDL) cholesterol ($r = -0.24$, $p = 0.0003$) were weak. There was no association between sPLA2 activity and IL-18 levels ($r = 0.03$, $p = 0.53$). Secretory PLA2 activity showed moderate association with sPLA2 antigen levels ($r = 0.37$, $p < 0.0001$). This was not unexpected, because sPLA2 activity is that of the various sPLA2 subtypes, whereas sPLA2 antigen levels only reflect the levels of the type IIA sPLA2.

Association of baseline plasma sPLA2 activity with clinical outcomes. There were 35 major adverse events (20 deaths and 15 MI). No significant association was seen between clinical outcomes and either CRP or IL-18 levels (Table 3). The rate of death and new or recurrent MI increased according to increasing tertiles of sPLA2 activity. Patients in the highest tertile of sPLA2 activity had a hazard ratio of 4.30 (95% confidence interval [CI], 2.1 to 8.7) for death or MI ($p < 0.0001$) (compared with the other two tertiles) (Fig. 1, Table 3). Adjustment for potential confounders in a stepwise Cox regression model, including age, a history of hypertension, MI, heart failure, CAD, coronary artery bypass surgery, Killip class>1, and creatinine level >1.1 did not alter the strong association between high baseline levels of sPLA2 activity and increased risk of major coronary events at follow-up (Table 3). The adjusted hazard ratio for the combined end point of death or MI in the third tertile of sPLA2 activity, compared with the first and second tertiles, was 3.08 (95% CI, 1.37 to 6.91) ($p = 0.006$) (Table 4). No significant association was found between sPLA2 antigen level and the composite end point of death or MI (adjusted hazard ratio, 1.44, $p = 0.3$). Finally, we verified that the GRACE score, previously shown to be highly predictive of event recurrence (12), significantly predicted



No. at risk					
0 - 1.7	150	134	94	25	21
1.8 - 2.8	142	133	63	19	19
≥ 2.9	154	135	60	23	17
Total	446	402	217	67	57

Figure 1. Kaplan-Meier curves showing the cumulative incidence of death or myocardial infarction (MI) according to the tertile of secretory type II phospholipase A2 (PLA2) activity.

adverse outcomes (MI/death) in the present study (hazard ratio for each 1-point increase in score, 1.03; 95% CI, 1.02 to 1.04, $p < 0.0001$). Interestingly, sPLA2 activity remained an independent predictor for recurrent events after adjusting for the GRACE score for recurrent events in a Cox regression model. The adjusted hazard ratio for the combined end point of death or MI in the third tertile of sPLA2 activity was 2.78 (95% CI, 1.30 to 5.93) ($p = 0.006$).

DISCUSSION

Inflammatory and thrombotic processes are major determinants of atherosclerotic plaque complications leading to ACS and sudden death. The last decade has witnessed an increasing interest in the study of the role of systemic inflammatory markers and their relation to the severe clinical complications of atherosclerosis (13). Several circulating inflammatory markers, including CRP, IL-6, vascular cell adhesion molecule (VCAM)-1, and, more recently, myeloperoxidase (14), have been shown to be elevated in patients with ACS and to be associated with adverse clinical outcomes (MI/death) at follow-up. In this study, we show that plasma sPLA2 activity, in contrast to CRP or IL-18, is a major independent predictor of death and new or recurrent MI in patients with ACS. In addition, whole sPLA2 activity has a better prognostic value than sPLA2 type IIA antigen level. Since the landmark study by Liuzzo et al. (15), numerous studies have addressed the prognostic value of CRP in patients with ACS. Higher CRP levels were associated with increased risk at follow-up of patients with UA or NSTEMI in several randomized trials, including Thrombolysis In Myocardial Infarction Phase IIA (TIMI IIA) (16), c7E3 Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) (17), Fragmin during Instability in Coronary Artery Disease (FRISC) (18), and Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO-IV) (19). C-reactive protein levels in ACS patients assigned to early invasive revascularization procedures also predicted adverse outcomes (20); however, the independent predictive value of CRP for major adverse cardiac events in patients with STEMI or patients with ACS, recruited outside randomized clinical trials or treated with early revascularization, remains uncertain (21–23). In the present observational substudy from GRACE, CRP measured after admission for ACS (mostly STEMI and NSTEMI) was not associated with adverse outcomes (MI/death). It should be noted, however, that the present study

Table 4. Multivariable Analysis of the Predictors of Death or MI

Death or MI	HR	95% CI	p
Killip class >1	3.09	1.50–6.30	0.002
Age ≥60 yrs	2.96	1.12–7.83	0.03
Medical history of CABG	3.46	1.34–8.93	0.01
Creatinine >1.1	3.23	1.22–8.57	0.02
sPLA2 activity, third tertile	3.08	1.37–6.91	0.006

Univariate predictors with a $p < 0.05$ (Table 3) were included in a stepwise multivariable Cox regression model. The selection method was a forward selection with a significance level of 0.05 for entering an explanatory variable into the model. The final model only includes significant variables with a $p < 0.05$ with the Wald test.
CI = confidence interval; other abbreviations as in Tables 1 and 3.

does not allow the detection of moderate effect sizes like the 1.28 hazard ratio associated with high CRP levels.

On the basis of experimental data by our group and others showing a potent proatherogenic role for endogenous IL-18 (24–26), plasma IL-18 level has been measured in patients with a history of CAD and in healthy middle-aged men and was found to be of predictive value for the occurrence of coronary events (27,28). In the present study, we found no association between IL-18 level and adverse outcomes in patients with ACS. These results, taken together, suggest that the prognostic value of IL-18 varies according to disease activity at the time of sampling. This could be related to multiple factors, including untested confounding variables, a differential role of IL-18 in different clinical settings, or to differences in the production of IL-18 binding protein, the endogenous inhibitor of IL-18. These considerations should be taken into account in future studies when evaluating the prognostic implications of IL-18 in patients with ACS.

The most important and novel finding in the present study is the demonstration that a single determination of sPLA2 activity, obtained during the two days after the onset of ischemic symptoms, provides powerful prognostic information in patients with ACS. The association between sPLA2 activity and the risk of subsequent death or MI was independent of the other known predictors of major adverse outcomes in patients with ACS, including the presence or absence of a history of MI and the presence or absence of clinical signs of heart failure at admission (9). Interestingly, this finding of a strong predictive value of sPLA2 was obtained despite the heterogeneity of the sample under study regarding clinical presentation, pathophysiology, the risk associated with each type of ACS, and the high rate of in-hospital revascularization, consistent with the current everyday practice. This suggests that activation of sPLA2 might be a critical and common mechanism among patients at risk for death or MI after an acute coronary syndrome. Secretory PLA2 activity was higher in patients recruited in Scotland than in patients recruited in France (Table 2). This might be related, at least in part, to the higher age of patients recruited in Scotland ($p < 0.0001$ in comparison with France) and the higher percentage of Scottish patients with a Killip class >1 at admission (34.52% vs. only 12.02% in the French sample of patients) ($p < 0.0001$).

A previous small study has demonstrated that in patients with UA, a higher plasma level of sPLA2 was associated with a higher probability of developing clinical coronary events, mainly coronary revascularizations and readmissions, during a follow-up period of two years (6). This previous study focused on a limited number of homogeneous patients and included only 52 patients with UA who had no increase in biochemical markers of necrosis. In addition, no direct measurement of sPLA2 activity was performed. Our study extends these previous findings to the whole spectrum of ACS and shows that sPLA2 activity is a strong and independent predictor of death or MI.

Unlike traditional cardiac biomarkers used to predict adverse outcome in patients with ACS, sPLA2 activity has been shown to act at multiple critical pathways involved in atherogenesis (3), from lipid oxidation (29) to modulation of vascular and inflammatory cell activation and apoptosis (30–32). Interestingly, sPLA2 is already expressed in the normal arterial wall (2,33), and its expression is readily up-regulated by inflammatory stimuli (30,31,34–36), suggesting a potential role for sPLA2 beginning in the early phases of the vessel response to injury. Secretory PLA2 stimulates the oxidation of native LDL (29) and is involved in the release of polyunsaturated fatty acids, leading to the production of biologically active phospholipids (37). These oxidation products play important roles in platelet, monocyte, and endothelial activation, processes known to be critical steps in atherogenesis (38). Lysophospholipids, including lysophosphatidylcholine and lysophosphatidic acid, released from the cell membrane of platelets and other cells in the form of microvesicles following sPLA2 activation (39), can influence cellular functions (40,41) and lead to the generation of platelet-activating factor, a potent cell activator and a potentially proatherogenic factor. Of major importance, sPLA2 is virtually inactive on phospholipids from intact cells, and several studies suggest that only those membranes where the transverse distribution of phospholipids has been disturbed offer a convenient surface able to interact with the enzyme (42). We have previously shown increased levels of intraplaque and circulating shed-membrane microparticles presenting an altered phospholipid distribution, with phosphatidylserine exposure at the outer leaflet of the membrane, in patients with ACS (43,44). We believe that these microparticles might constitute an important substrate for sPLA2, both at the site of plaque complication and in the circulating blood. Taken together, these data suggest that sPLA2 activity, in the arterial wall and the circulating blood, might play a critical role in inflammatory pathways leading to ACS, and might explain, at least in part, the more than three-fold increase in the risk of death or new or recurrent MI in patients with ACS.

There are a number of study limitations that should be acknowledged. The relationship between sPLA2 activity and inflammatory mediators has not been assessed in detail. Particularly, measurement of serum amyloid A levels, an acute phase protein whose transport is partially regulated by LDL cholesterol and whose serum levels are known to be enhanced in ACS (45), could have provided additional insights into the interaction between lipids, inflammatory mediators, and sPLA2. Another limitation is the relatively small number of patients in our study. Indeed, the study was planned to detect an adjusted relative risk of three. Moderate effect sizes could not reach statistical significance.

In conclusion, the level of sPLA2 activity, measured at the time of enrolment in patients with ACS, is a strong predictor of death and MI. This study should be confirmed in other populations of patients with ACS before level of

sPLA2 activity can be considered as valuable additional information for use in risk stratification.

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