Serum secretory phospholipase A2-IIa (sPLA2-IIA) levels in patients surviving acute myocardial infarction

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Abstract. – BACKGROUND: The increase of secretory phospholipase A2-IIa (sPLA2-IIa) in culprit coronary lesions is associated with myocardial infarction, and the increase of sPLA2-IIa in peripheral plasma levels has a significant risk and prognostic value in patients with coronary artery disease. Little is known about the prognostic significance of elevated serum sPLA2-IIa in post-acute myocardial infarction (post-AMI) patients.

OBJECTIVES: The present study is designed to investigate the potential association between serum sPLA2-IIa and prognosis in post-acute myocardial infarction (post-AMI) patients.

PATIENTS AND METHODS: From Oct 1998 to Sep 2008, a total of 964 post-AMI patients with serum samples collected in the convalescent stage were studied. Serum levels of sPLA2-IIa were measured by ELISA. According to the optimal cut-off value for sPLA2-IIa concentration, patients were then divided into 2 groups. Categorical variables were compared between the 2 groups using the χ^2 test. All continuous variables are described as mean ± SD and were compared using Student's t-test. Statistical associations between clinicopathological observations and sPLA2-IIa levels were determined using the Mann-Whitney U test. The clinical value of sPLA2-IIa level as a prognostic parameter was evaluated using the Cox's proportional hazards model.

RESULTS: During a median follow-up period of 1,462 days, 123 patients (12.7%) had adverse events (all-cause mortality, n=52; non-fatal MI, n=31; readmission for heart failure [HF], n=40). Patients were divided into 2 groups according to a serum sPLA2-IIa level of 360 ng/dl, which was determined to be the optimal cut-off for discriminating all-cause mortality based on the maximum value of the area under the receiver operating characteristic curve. Patients with elevated sPLA2-IIa (> 360 ng/dl, n=164) had a significantly higher prevalence of death (18.3% [30/164] vs. 2.75% [22/800] $p < 0.001$) and readmission for HF (14% [23/164/ vs. 2.1% [17/800], $p < 0.0001$), but not of non-fatal MI (4.88% [8/164]vs. 2.87% [23/800], $p = 0.096$), compared to those with sPLA2-IIa < 360 ng/dl. Multivariate Cox regression analysis indicated that elevated serum sPLA2-IIa was associated with an increased risk of mortality and readmission for HF.

CONCLUSIONS: Elevated serum sPLA2-IIa during the convalescent stage of AMI predicted long-term mortality and readmission for HF after survival discharge in the post-AMI patients.

Key Words:
   Acute myocardial infarction, Secretory phospholipase A2-Iia.

Introduction

Phospholipases A2 (PLA2) is an enzyme that catalyzes the hydrolysis of the fatty acyl ester bond at the sn-2 position of phospholipids to produce free fatty acids and lysophospholipids. In human tissues, PLA2 is present in several cell types and different forms of the enzyme are divided into groups according to the structure of the enzyme molecule[1]. Several studies showed that secretory nonpancreatic type II phospholipase A2 (sPLA2-IIA) might importantly contribute to the pathogenesis of various inflammatory diseases[2-3]. sPLA2-IIA has also been reported to be expressed in human colorectal carcinoma[4], gastric cancer[5], pancreatic cancer[6], prostate cancer[7], and lung cancer[8]. Moreover, abnormal expression of sPLA2-IIA in cancers may be a predictor of poor overall survival.

Previous studies showed that the increase in peripheral plasma levels of sPLA2-IIA is a significant risk factor for the presence of coronary spasm and it may possibly reflect inflammatory activity in spasm coronary arteries[9]. Otherwise, the increase in circulating levels of sPLA2 is a significant risk factor for the presence of coronary artery disease (CAD) and predicts clinical coronary events independent of other risk factors in patients with CAD[10]. Moreover, elevated levels

of sPLA2-IIA were associated with an increased risk of future coronary artery disease (CAD) in apparently healthy individuals[11]. Serum concentrations of sPLA2-IIA could be an important prognosic marker in CAD patients. Nijmeijer et al[12] has recently found sPLA2-IIA is more abundantly present in atherosclerotic culprit lesions that have led to myocardial infarction. This suggests a role for extracellular sPLA2-IIA in the development of complications of atherosclerotic lesions in coronary arteries. Several reports have shown that elevated sPLA2-IIA *in vivo* is associated with in acute myocardial infarction (AMI) and inhibition of type 2A secretory phospholipase A2 reduces death of cardiomyocytes in AMI[13], but few studies have investigated the clinical significance of serum sPLA2-IIA level in the convalescent stage of AMI.

It has found that the circulating levels of sPLA2-IIA was increased in patients with CAD. In the present study we investigated whether elevated serum sPLA2-IIA was a predictor of adverse events in patients surviving AMI.

Patients and Methods

Study Population

From Oct 1998 to Sep the Affiliated Hospital of Medical College Qingdao University. Among the 1793 patients, 964 post-AMI patients fulfilling the following criteria were selected to study in the study: (1) discharge alive over 5 years; and p. 2007, a total of 1793 post-AMI patients in the convalescent stage were collected. The 1793 patients with AMI who were registered in (2) provision of a blood sample before or within 14 days of discharge. Medical Ethical Committee (Qingdao University) approved the protocol. Written informed consent was obtained from each patient.

Data Collection

We recorded data concerning sociodemographic variables, medical history, therapeutic procedures, and clinical events during the hospital stay. Clinical data after discharge were obtained at 3 and 12 months after the onset of AMI, and annually thereafter up to 5 years. The clinical endpoint of this study was primarily all-cause mortality. To explore the underlying mechanism, readmission for heart failure (HF) and non-fatal MI were also included as study endpoints. Non-fatal MI was defined as the occurrence of symptoms used for the diagnosis of AMI[14]. Readmission for HF was defined using the Framingham Heart Study criteria[15].

Serum Collection and Measurement of sPLA2-IIa

Peripheral venous blood after an overnight fast was collected by an antecubital vein with the patient in the supine position. The blood was put into serum separator tubes for assays of sPLA2-IIa levels. After separation, blood samples were separated by refrigerated centrifuge 3000 rpm for 10 min at 4°C. Then the plasma was aliquoted and stored at –80°C until analyzed.

Enzyme-linked Immunosorbent Assay (ELISA)

sPLA2-IIa levels in plasma samples were determined by ELISA kit (Catalog No. 585000, Cayman Chemical Company, Ann Arbour, MI, USA) according to the supplier's instructions. The concentration of sPLA2-IIa in plasma was tested in duplicate and determined against a standard curve for each ELISA assay.

Statistical Analysis

Categorical variables were compared using the χ^2 test. All continuous variables are described as mean ± SD and were compared using Student's *t*-test. For continuous variables not normally distributed, the variables are given as median (25th-75th percentile) and compared using Mann-Whitney U-test. Rates of cardiac events, including all-cause mortality, non-fatal MI, and readmission for HF, following discharge are described using the Kaplan-Meier method and compared using the logrank test. Multivariate logistic regression analysis was used to identify factors associated with elevated sPLA2-IIa. Cox regression models were used to examine whether elevated sPLA2-IIa was associated with an increased risk of all-cause mortality, non-fatal MI, and readmission for HF. All analyses were performed using version 11 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$ or 95% confidence intervals (95% CI) that did not include 1.0.

Results

sPLA2-IIa Levels in the Peripheral Circulation of the post-AMI Patients

A total of 964 post-AMI patients were included in the present study; 64% (617/964) were

male and the mean age was 59±12 years. The median serum sPLA2-IIa concentration collected at a median of 20 days after AMI onset was 270 (92-360) ng/dl (Figure 1). Because the mean serum sPLA2-IIa was significantly higher in patients who died from any cause than surviving patients [410 ng/dl; (318-513) ng/dl vs. 215 ng/dl; (226-317) ng/dl, $p < 0.001$], we determined the optimal cut-off value of serum sPLA2-IIa for discriminating between the risk of all-cause mortality and survival. The optimal cut-off was estimated to be 360 ng/dl (75th percentile of the sPLA2-IIa distribution in patients who died from any cause).

Correlation of Baseline Patient Characteristics and Serum sPLA2-IIa Level

Patients with elevated serum sPLA2-IIa > 360 ng/dl (n=164) were more likely to have diabetes mellitus, hypertension, HF defined as Killip class ≥ 2 on admission, and multivessel disease compared to those with serum sPLA2-IIa ≤ 360 ng/dl (n=800; Table I). In addition, patients with elevated serum sPLA2-IIa had significantly lower HDL-cholesterol and higher LDL-cholesterol levels, compared to sPLA2-IIa ≤ 360 ng/dl subjects (n=800; Table I).

Multivariate Logistic Regression and Kaplan-Meier Curves Analysis

In multiple logistic regression analysis with forward stepwise selection, the higher levels of sPLA2-IIa (> 360 ng/dL), diabetes mellitus, and hypertension were the variables differing significantly and independently between the patients who died from any cause than surviving patients, as shown in Table II. During a median follow-up period of 1,462 days, 52 patients died, 31 had non-fatal reinfarction, and 40 were rehospitalized for HF (Table II). Kaplan-Meier curves showed that patients with elevated sPLA2-IIa had a significantly higher incidence of death (18.3% [30/164] vs. 2.75% [22/800] $p < 0.001$; Figure 2A) and readmission for HF (14% [23/164 vs. 2.1% [17/800], $p < 0.0001$; Figure 2B) than those without, although no significant differences in the rate of nonfatal MI was detected between the 2 groups (4.88% [8/164] vs. 2.87% [23/800], $p = 0.096$; Figure 2C). Multivariate Cox regression analysis also showed that elevated serum sPLA2-IIa was associated with an increased risk of all-cause mortality and readmission for HF (Table III); no association was observed between elevated serum sPLA2-IIa and the risk of non-fatal MI (Table III).

Discussion

In the present study, we found that elevated sPLA2-IIa is associated with an increased risk of all-cause mortality, and readmission for HF following AMI, but not of non-fatal MI. From the present analysis of 964 AMI patients, the opti-

Table I. Correlation of baseline patient characteristics and serum sPLA2-IIa level.

	Serum sPLA2-IIa levels		
	≤ 360 (n=800)	> 360 (n=164)	*p*-value
Age (years)	60 ± 11	59 ± 12	0.543
Male sex	64.6%	60.9%	0.128
Smoking	63.1%	68.3%	0.105
BMI (kg/m^2)	23.6 ± 3.3	24.4 ± 3.4	0.563
Diabetes mellitus	23.6%	34.7%	0.013
Hypertension	14.3%	26.8%	0.001
Dyslipidemia	61.8%	59.7%	0.263
Previous MI	58.4%	62.5%	0.462
Onset to admission time < 24 h	85.4%	87.3%	0.768
Peak CK ≥ 3,000 IU/L	36.4%	32.8%	0.452
Killip class ≥ II on admission	12.6%	21.5%	0.014
Reperfusion therapy	90.7%	78.4%	0.03
β-blocker	41.5%	40.16%	0.373
Antiplatelet therapy	93.4%	92.2%	0.843
HDL-cholesterol, mg/dl	51 ± 13	40 ± 12	0.02
LDL-cholesterol, mg/dl	125 ± 38	141 ± 40	0.004
Triglycerides, mg/dl	116 (74, 178)	119 (89, 182)	0.354

Table II. Multiple logistic regression analysis: final significant variables differing between patients who died from any cause with surviving patients.

Variables	β-coefficient	SE	p-value	OR	95% CI
sPLA2-Iia > 360 ng/dL	1.83	0.29	< 0.0001	6.4	2.1-21.5
Diabetes mellitus	1.92	0.41	0.002	7.5	1.8-35.9
Hypertension	1.24	0,37	0.06	4.6	1.2-7.8

Forward, stepwise, multiple logistic regression analysis was performed using the following factors as categorical covariates: sPLA2-Iia > 360 ng/dL, Diabetes mellitus, Hypertension, BMI, Killip class ≥ II on admission, Smoking, Dyslipidemia, Previous MI, Male, Peak CK ≥ 3,000 IU/L, Reperfusion therapy and age.

Table III. Outcome following discharge for AMI.

|	sPLA2-Iia (ng/dL)		
	≤ 360 (n=800)	> 360 (n=164)	p-value
Outcome			
All-cause mortality, n (%)	22 (2.75)	30 (18.3)	< 0.001
Non-fatal MI, n (%)	17 (2.1)	23 (14)	< 0.001
Readmission for HF, n (%)	8 (4.88)	23 (2.87)	> 0.05

mal cut-off value for serum sPLA2-IIa was 360 ng/dl. The factors associated with elevated sPLA2-IIa were diabetes mellitus, hypertension, HDL-cholesterol, LDL-cholesterol, and Killip class ≥ II on admission. To our knowledge, this is the first study to investigate the clinical impact of serum sPLA2-IIa concentration at the convalescent stage in post-AMI patients. sPLA2-IIa level in the present patients was skewed towards lower concentrations, given that the median and mean were 92 ng/dl and 360 ng/dl,respectively (Figure 1). Considering that the cut-off of sPLA2-IIa to diagnose AMI is >

360 ng/dl in the clinical setting, as measured by commercially available kits, 18.3% of the present patients had elevated sPLA2-IIa in the 20 days after AMI onset. Although it is presently unclear why sPLA2-IIa remains high in the convalescent stage of AMI, it is possible that myocardial damage is ongoing during this stage[16-17].

The present findings are of marked clinical significance in the setting of secondary prevention after AMI, because the prognostic impact of elevated sPLA2-IIa on subsequent adverse events in post-AMI patients was examined during a relatively long follow-up period of ap-
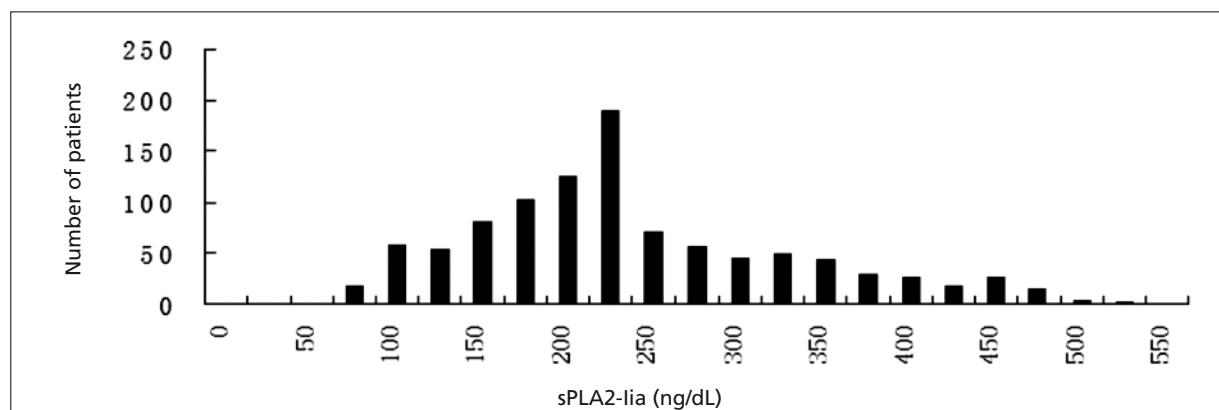
Figure 1. Serum sPLA2-IIa distribution in post-acute myocardial infarction patients.
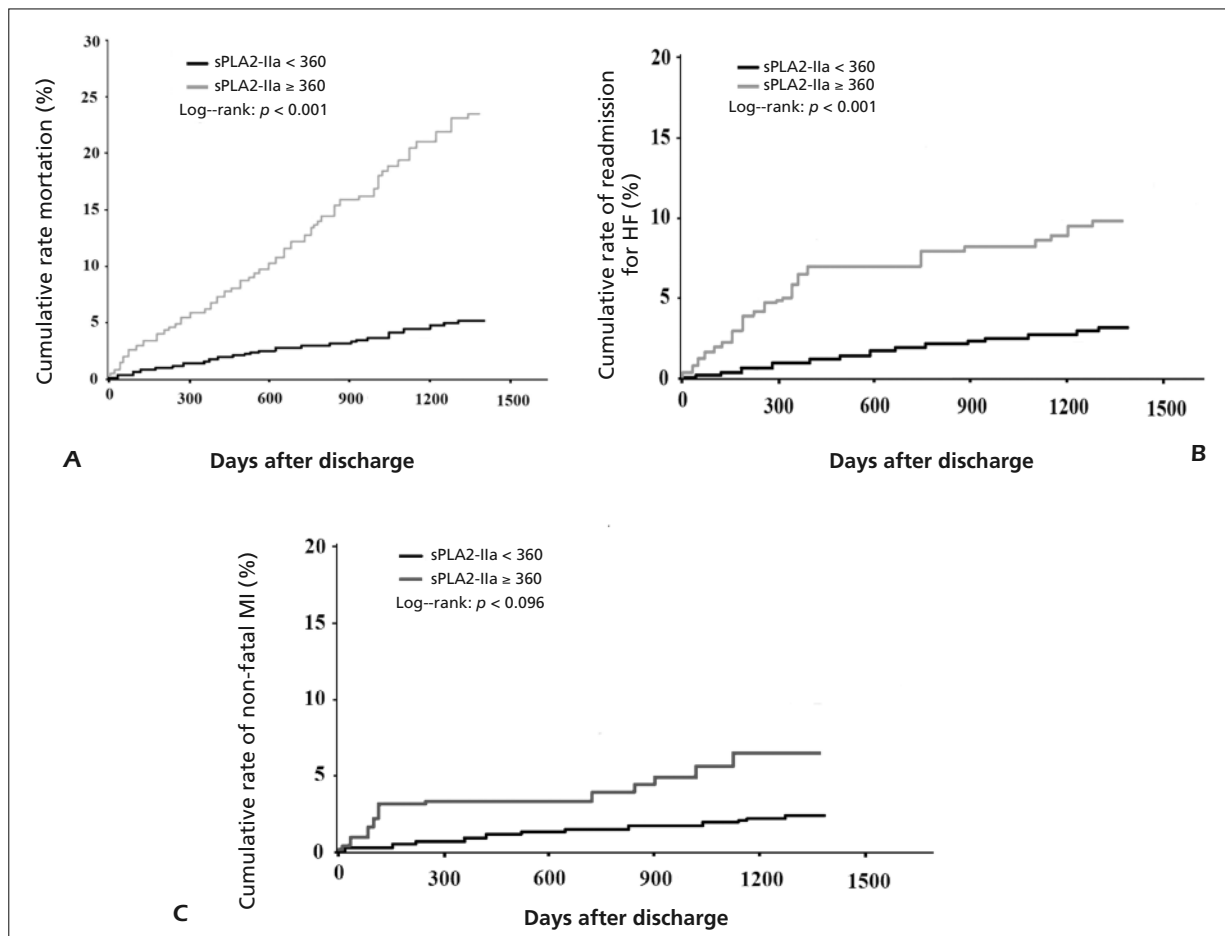
Figure 2. Kaplan-Meier curves showing event rates for patients (*gray*) with and (*black*) without elevated sPLA2-IIa: *(A)* all-cause mortality; *(B)* readmission for heart failure (HF); and *(C)* non-fatal myocardial infarction (MI).

proximately 5 years. Notably, we found that elevated serum sPLA2-IIa is associated with an increased risk of all-cause mortality and readmission for HF. We, therefore, suggested that sPLA2-IIa is good marker to predict subsequent adverse events in post-AMI patients. The present findings suggest that the measurement of serum sPLA2-IIa in a single blood sample collected during the convalescent stage may predict long-term outcome, such as all-cause mortality and rehospitalization for HF.

This study showed that diabetes mellitus, hypertension, and high sPLA2-IIa levels were significant and independent variables differing between patients who died from any cause with surviving patients. However, in the present prospective study, only high sPLA2-IIa levels (but not other traditional coronary risk factors including diabetes mellitus and hypertension) had predictive values for post-AMI events. The lack

of the predictive significance of diabetes and hypertension in the present prospective study may be partly explained by the modification of these traditional risk factors during the follow-up by medications and improvement of lifestyle. Other associated risk factors may additionally contribute to the significant probability because the extensive coronary diseases did not remain significant in the multivariate analysis.

Conclusions

Elevated serum sPLA2-IIa served as an accurate predictor of long-term outcome, including all-cause mortality and readmission for HF, in the 964 present post-AMI patients. Patients with sPLA2-IIa > 360 ng/dl during the convalescent stage of AMI may be treated as at high risk for subsequent adverse events.

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