	HDL-C	75歳未満	75歳以上
		人数 (%)	人数 (%)
全体 P < 0.01	達成	3462 (77.2)	1770 (70.3)
	未達成	428 (9.5)	303 (12.0)
	未測定	595 (13.3)	444 (17.6)
	計	4485 (100.0)	2517 (100.0)
カテゴリーI	達成	108 (85.0)	0
	未達成	0 (0.0)	0
	未測定	19 (15.0)	0
	計	127 (100.0)	0
カテゴリーII P < 0.01	達成	1653 (84.0)	778 (75.7)
	未達成	62 (3.1)	29 (2.8)
	未測定	254 (12.9)	221 (21.5)
	計	1969 (100.0)	1028 (100.0)
カテゴリーIII P <0.05	達成	1342 (71.2)	650 (65.9)
	未達成	289 (15.3)	187 (18.9)
	未測定	254 (13.5)	150 (15.2)
	計	1885 (100.0)	987 (100.0)
二次予防 NS	達成	313 (62.1)	342 (68.1)
	未達成	164 (32.5)	128 (25.5)
	未測定	27 (5.4)	32 (6.4)
	計	504 (100.0)	502 (100.0)

表13 HDL-C のカテゴリー別管理目標値達成率の年齢による差異

	TG	75歳未満	75歳以上
		人数 (%)	人数 (%)
全体 P < 0.01	達成	2634 (58.7)	1652 (65.6)
	未達成	1553 (34.6)	607 (24.1)
	未測定	298 (6.6)	258 (10.3)
	計	4485 (100.0)	2517 (100.0)
カテゴリーI	達成	78 (61.4)	0
	未達成	36 (28.3)	0
	未測定	13 (10.2)	0
	計	127 (100.0)	0
カテゴリーII P < 0.01	達成	1234 (62.7)	693 (67.4)
	未達成	614 (31.2)	207 (20.1)
	未測定	121 (6.1)	128 (12.5)
	計	1969 (100.0)	1028 (100.0)
カテゴリーIII P <0.01	達成	1009 (53.5)	617 (62.5)
	未達成	739 (39.2)	272 (27.6)
	未測定	137 (7.3)	98 (9.9)
	計	1885 (100.0)	987 (100.0)
二次予防 P <0.05	達成	313 (62.1)	342 (68.1)
	未達成	164 (32.5)	128 (25.5)
	未測定	27 (5.4)	32 (6.4)
	計	504 (100.0)	502 (100.0)

表14 TGのカテゴリー別管理目標値達成率の年齢による差異

副作用	全体		男性		女性	
	件数	(%)	件数	(%)	件数	(%)
なし	6410	(91.4)	2590	(91.1)	3818	(91.6)
筋肉痛	29	(0.4)	12	(0.4)	17	(0.4)
CK 上昇	38	(0.5)	13	(0.5)	25	(0.6)
肝機能異常	56	(0.8)	30	(1.1)	26	(0.6)
その他	19	(0.3)	10	(0.4)	9	(0.2)
不明	465	(6.6)	190	(6.7)	275	(6.6)

表15 治療に伴う副作用

介護保険サービス	全体		男性		女性	
	人数	(%)	人数	(%)	人数	(%)
受けていない	5815	(82.9)	2470	(86.8)	3343	(80.2)
受けている	752	(10.7)	179	(6.3)	573	(13.8)
不明	446	(6.4)	195	(6.9)	251	(6.0)

表16 介護保険によるサービス

	病院	診療所	介護老人保健施設・特別養護老人ホーム	訪問看護ステーション	不明	合計（人）
入院患者数	3602	48	98	0	5	3753
外来患者数	20259	72514	0	0	355	93128
入所・入居患者数	3936	3996	907	0	0	8839
訪問看護数	0	49	0	52	0	101

表17 月間の入院（入所）・外来患者数

厚生労働科学研究費補助金　難治性疾患克服事業
分担研究報告書
原発性高脂血症に関する調査研究
研究分担者　石垣　泰（東北大学大学院医学系研究科・准教授）
研究要旨　家族性高コレステロール血症における頚動脈硬化の特徴

Ａ．研究目的

原発性高脂血症の代表的疾患である家族性高コレステロール血症（ＦＨ）は、早発性の粥状動脈硬化をきたすことが大きな問題である。代表的な動脈硬化診断法であるＢモード超音波で頸動脈の状態を評価すると、ＦＨではプラークの多発が特徴的である。一方、代表的な動脈硬化危険因子である２型糖尿病（ＤＭ）では、びまん性の頸動脈内中膜肥厚（IMT）増大が主たる所見であり、疾患によって動脈硬化病変の性質が異なっていると考えられる。

本学では、工学部と共同で新しい超音波測定法・位相差トラッキング法の臨床応用を進めている。本測定法は、血管壁における数千に及ぶ複数の計測点の、心拍毎の動きを数十μｍ単位で追跡することで、組織性状を非侵襲的に評価可能なことから、動脈硬化診断への有用性が期待されている。

本研究の目的は、新規測定法によってＦＨの動脈硬化病変の性質を検討し、質的診断の体系を確立することで、ＦＨのイベント発症予測・予後向上に繋げることである。

Ｂ．研究方法

ＦＨ４０名と年齢をマッチさせたＤＭ４６名、検診受診健常者３５名を対象に位相差トラッキング法で測定した頸動脈性状、また他の動脈硬化指標を比較検討した。尚、位相差トラッキング法は従来の医療用超音波機器を用いることから、被験者への有害事象の危険性は極めて低いと考えられる。また測定に関しては本学医学部倫理委員会の審査・承認を得ている(2011-335)。

Ｃ．研究結果

両群の検査データを比較すると、平均IMTは健常者:0.54mm, ＤM0.70mm, ＦＨ0.79mm と肥厚度に差を認めた。また頸動脈プラークスコア はDM1.4, ＦＨ3.5とＦＨで頸動脈プラークの多発を認めた。また脈波伝播速度(PWV)は、DM1603 cm/s, ＦＨ1496 cm/s とＤＭで有意に高値を呈した。一方で、位相差トラッキング法で評価した頸動脈測定値はＤM54.6 kPa、ＦＨ39.9kPa とＦＨで有意に低い値であった。ちなみに健常者では、測定値は28.0kPa とさらに低値を示した。次に位相差トラッキング法は血管の組織性状を評価できると考えられることから、測定値150kPa 以上のいわゆる「硬い」と評価される部分の血管壁に存在する割合を定量した。その結果ＤＭの頸動脈では総計測点の 1.20%が 150kPa を上回っていたのに対して、ＦＨではその割合は 0.54%と低値であった。過去に摘出組織標本と位相差トラッキング法測定値を適合させた

報告を鑑みて、DMの頸動脈内中膜は膠原繊維が豊かで、一方FHの頸動脈には脂質成分が多く沈着しているものと推察された。さらに各測定点での計測値のばらつきを評価したところ、DMで変動係数(coefficience of variance)が高く、FHに比較してDMでは頸動脈血管壁を構成する成分が不均一であると考えられた。

D．考察

これまでの検討では、IMT肥厚やプラークの形成はFHで顕著に進行している一方で、PWVはDMで高値を示しており、動脈硬化の質に違いがあることが示唆された。新規測定法である位相差トラッキング法の測定値はDMで高く、しかも各測定点でのばらつきが大きいことから、DMの血管壁には「硬い」と評価される成分が多く存在し、しかも構成が不均一であると考えられた。それに対してFHでは、血管壁に脂質が沈着するといったこれまでの病理像を裏付ける測定値、すなわち「柔らかい」成分が比較的均一に存在していることが示唆された。このように位相差トラッキング法では非侵襲的に血管性状を評価できる可能性があるが、今後は新規測定法で得られた結果が、冠動脈疾患や脳血管障害といった臨床像とどのように関連するか検討を進めていきたい。

E．結論

位相差トラッキング法を用いて頸動脈の血管性状を評価することで、FHの動脈硬化形成の特徴が評価可能と期待され、FHの冠動脈疾患の予防に寄与する方向で研究を発展させていきたい。

F．健康危険情報

なし

G．研究発表

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Ｈ．知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案登録　なし
3. その他　なし

研究成果の刊行に関する一覧表

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Increased circulating plasma lysophosphatidic acid in patients with acute coronary syndrome

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ABSTRACT

Background: The platelet activator lysophosphatidic acid (LPA) has recently been identified as an ingredient in oxidized LDL and it has been isolated from atherosclerotic plaques. The lysophospholipase D activity of autotaxin produces LPA extracellularly from lysophosphatidylcholine (LPC). The present study determines whether circulating LPA is associated with acute coronary syndrome (ACS).
Methods: We enrolled 141 consecutive patients (age, 62.6 ± 3.8 y; male, 69.2%) with ACS (n = 38), stable angina pectoris (SAP; n = 72) or angiographically normal coronary arteries (NCA; n = 31). The relationships between LPA and other established biomarkers were examined. Concentrations of plasma LPA were determined using an enzymatic assay.
Results: Concentrations of LPA significantly correlated with LPC (r = 0.549), autotaxin (r = 0.370) and LDL-C (r = 0.307) (all $p<0.01$). Lysophosphatidic acid concentrations were significantly higher in patients with ACS than with SAP and NCA ($p<0.01$), but did not significantly differ between patients with SAP and NCA. Multivariate logistic regression analyses revealed that the highest LPA tertile was independently associated with ACS (odds ratio 1.99, 95% CI: 1.18–3.39, p = 0.02).
Conclusions: The present study demonstrated that increased circulating plasma LPA concentrations are significantly associated with ACS.

1. Introduction

Lysophosphatidic acid (LPA), although originally viewed as a key intermediate in de novo lipid synthesis, has emerged as an important lipid mediator with various biological activities, which is especially important in the area of vascular biology [1–3]. Atherogenic oxidized low-density lipoprotein (LDL) contains lysophosphatidylcholine (LPC) that serves as a substrate for the production of LPA by autotaxin (lysophospholipase D, LysoPLD) [4]. In addition, LPA, which is a platelet activator and has highly thrombogenic lipid constituent of plaque, accumulates in the lipid core of human atherosclerotic lesions [5]. The platelet-activating effect of the lipid-rich core of atherosclerotic plaques and LPA involvement in this effect has been characterized [6]. Moreover, individual platelet responses to LPA might be influenced by factors that affect the degree of systemic platelet activation, such as vascular disease and blood coagulation [7–9]. Hence, an LPA receptor blockade should be a promising new approach to reducing the risk of thrombosis associated with plaque rupture [10]. We therefore considered that these findings reflect a relationship between plasma LPA concentrations and acute coronary syndrome (ACS) because of the pathophysiology associated with plaque instability and platelet aggregation. However, a relationship between LPA and other established biomarkers in patients with ACS has not been examined.

2. Methods

2.1. Study design and patient population

The present study is a prospective cross-sectional study of consecutive patients who underwent coronary angiography at Juntendo University Hospital (J-Bacchus trial) between July and December 2009. The entry criteria were as follows: no previous examination by coronary angiography, no history of coronary intervention or coronary artery bypass grafting, and having precisely evaluable coronary trees. Patients without significant stenosis according to coronary angiography were placed in a group with normal coronary arteries (NCA), whereas those with significant stenosis were defined as having coronary artery disease and placed in groups with ACS or stable angina pectoris (SAP). Patients

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with acute myocardial infarction (AMI) and unstable angina (UAP) were included in the ACS group. The diagnostic criteria for AMI and UAP were taken from the respective guidelines published by the American College of Cardiology/American Heart Association in 2007 [11,12]. These definitions of ACS depended on the specific characteristics of each element of the clinical presentation, electrocardiographic changes and a marker with high specificity for cardiac injury. Stable angina pectoris was defined as effort angina with a stable profile of symptoms for at least 3 months before admission. Demographic characteristics, medical history and current medications were determined for all participants at the time of enrollment.

We initially screened 158 patients for this study. The exclusion criteria were as follows: 1) maintenance dialysis (n=5), 2) diabetes treated with insulin (n=4), and 3) acute or chronic infectious (n=5) or 4) neoplastic (n=3) diseases. The Ethics Review Committee at our institution approved the study, all participants signed informed consent forms and the study was registered in the UMIN protocol registration system (#UMIN000002103).

2.2. Evaluation of coronary artery disease and renal function

We evaluated the severity of CAD by standard coronary angiography. All angiograms were prospectively evaluated at our angiographic core laboratory. Two expert interventional cardiologists reviewed the angiograms with no knowledge of the biomarker concentrations and patient characteristics. Disagreement over lesion characteristics was resolved by a third expert. Angiographically significant lesions were defined as >50% stenosis in vessels with a diameter ≥2.0 mm. Extensions of coronary artery disease were classified in the standard manner as 1-, 2- or 3-vessel disease.

We evaluated renal function using the estimated glomerular filtration rate (eGFR) based on the new equation published in the Japanese National Kidney Foundation guidelines [13]. The formula is as follows: $eGFR = 194 \times SCr^{-1.094} \times age^{-0.287}$, where age is in years, serum creatinine (SCr) is in mg/dl, and GFR is in ml/min per 1.73 m^2 body surface area. The product of this equation was multiplied by a correction factor of 0.739 in women.

2.3. Blood sampling and laboratory measurements

Arterial blood samples were obtained using a syringe and 18-gauge needles from the arterial sheaths of all patients before they were examined by coronary angiography in the operating room. Blood samples were directly collected into glass vacutainer tubes with or without EDTA to obtain plasma and serum, respectively. The samples were immediately placed on ice. The anticoagulated samples were centrifuged at 1000×*g* for 10 min and then the supernatant comprising plasma was carefully collected to avoid contamination of cell components. Whole blood samples collected without EDTA-2Na were left to clot and then serum was separated by centrifugation at 1000×*g* for 10 min.

Concentrations of plasma LPA and LPC were determined using an enzymatic assay as described [14–16]. In brief, LPA was hydrolyzed with lysophospholipase to glycerol 3-phosphate, followed by enzymatic cycling using glycerol 3-phosphate oxidase and glycerol 3-phosphate dehydrogenase. The amplified concentrations of hydrogen peroxide, a product of enzymatic cycling, were then colorimetrically measured (JCA-BM8040, JEOL, Tokyo, Japan). Lysophosphatidylcholine concentrations in human plasma were measured using our validated enzymatic assay [16] in which LPC is converted by lysophospholipase into glycerophosphophorylcholine, from which glycerophosphorylcholine phosphodiesterase generates choline. The hydrogen peroxide produced from choline by choline oxidase was determined in the presence of peroxidase using an oxidative chromogenic reagent and 4-aminoantipyrine by measuring changes in absorbance. Serum highly sensitive C-reactive protein (hs-CRP) was measured using a validated, highly sensitive immunoassay. The activity of lipoprotein associated phospholipase A2 (Lp-PLA2) in serum was spectrophotometrically assayed as described [17]. Concentrations of serum cardiac troponin T were measured using a chemiluminescent enzyme immunoassay kit (Determiner CL TnT, Kyowa Medex, Tokyo, Japan). Serum autotaxin was quantified using a 2-site immunoenzymetric assay as described [18]. Other markers were determined by routine laboratory methods.

2.4. Statistical analysis

All data were statistically analyzed using SPSS ver. 18.0 (Chicago, IL) and JMP ver. 7.0 (SAS Institute Inc., Cary, NC). The distribution of continuous variables was assessed by visual inspection of frequency histograms and using the Shapiro–Wilk test. Results are presented as medians and inter-quartile ranges (IQR), median ± standard deviation or as ratios (%) and numbers for categorical data. Values obtained from three groups were compared by the one-way analysis of variance (ANOVA), the Kruskal–Wallis test and the χ^2 analysis. The post-hoc Scheffé test compared parameters within groups. Because of the known association between LPA and the other markers, natural log transformation of the LPA data achieved a normal distribution, and thus log-transformed LPA values were used in this study. Correlations were searched using Spearman's rank correlation. The independent effect of the biomarkers on the risk of ACS adjusting for potential confounders was determined using multiple logistic regression analysis. We evaluated the effect of the biomarkers Lp-PLA2, hs-CRP, and LPA in this model according to tertile increments in the concentrations of each. The following variables were initially incorporated into the univariate model: age, sex, diabetes, dyslipidemia, current smoking, angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB), statins, HbA1c, eGFR and tertiles of Lp-PLA2, hs-CRP, and LPA. Variables with p values of <0.20 were then entered into the multivariable model. A $p<0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of study participants

We enrolled 141 patients (age 65.8 ± 11.5 y, male sex 78.0%), all of whom had angiographically documented coronary trees with confirmed clinical features, and a diagnosis of NCA (n=32, 22.7%), SAP (n=71, 50.4%) and ACS (n=38, 27.0%; UAP=17, AMI=21). Table 1 shows the baseline characteristics of the three groups. Briefly, the three groups were similar with respect to age and body mass index but differed in terms of cardiovascular risk factors, which were more frequent in patients with SAP and ACS than with NCA. The patients with ACS tended to have lower eGFR concentrations than the NCA and SAP groups. Moreover, patients with SAP more frequently received cardiovascular therapy than those with NCA and ACS.

3.2. Plasma LPA concentrations

Circulating plasma LPA concentrations did not significantly differ between males and females (median: 0.375 vs. 0.47 μmol/l, p=0.102), or between those with or without conventional risk factors such as diabetes (median: 0.36 vs. 0.41 μmol/l, p=0.408), current smoking (median: 0.365 vs. 0.42 μmol/l, p=0.156), dyslipidemia (median: 0.37 vs. 0.435 μmol/l, p=0.125) or hypertension (median: 0.38 vs. 0.435 μmol/l, p=0.174). The concentration of autotaxin, which produces LPA from LPC through its lysoPLD activity, was higher in females than in males (median: 0.90 vs. 0.65 mg/l, p<0.001) [18].

Table 1
Baseline demographic and clinical characteristics of all patients.

	NCA (n=32)	SAP (n=71)	ACS (n=38)	p value
Age, y	64.8±11.1	67.4±10.3	63.8±13.6	NS
Sex, male (%)	20 (62.5)	61 (85.9)	29 (76.3)	0.032
Body mass index, kg/m^2	23.5±3.4	24.3±3.4	24.2±5.0	NS
Waist circumference, cm	87.1±7.4	89.0±7.96	88.0±9.7	NS
Systolic blood pressure, mm Hg	132.3±17.8	138.4±22.8	137.6±24.8	NS
Diastolic blood pressure, mm Hg	73.7±14.4	74.0±13.3	78.5±17.6	NS
Hypertension, n (%)	22 (68.8)	55 (77.5)	24 (63.2)	NS
Diabetes, n (%)	5 (15.6)	26 (36.6)	8 (21.1)	0.045
Dyslipidemia, n (%)	17 (53.1)	52 (73.2)	22 (57.9)	NS
Current smoking, n (%)	8 (25.0)	29 (40.9)	11 (29.0)	NS
Family history, n (%)	9 (28.1)	29 (40.8)	15 (39.5)	NS
eGFR, ml/min/1.73 m^2	74.6±18.7	73.7±19.7	66.3±16.4	NS
ACE-I or ARB, n (%)	11 (34.4)	37 (52.1)	11 (29.0)	0.039
Statins, n (%)	7 (21.9)	30 (42.3)	9 (23.7)	0.046
Beta-blockers, n (%)	7 (21.9)	33 (46.5)	6 (15.8)	0.004
Aspirin, n (%)	9 (28.1)	57 (80.3)	10 (26.3)	<0.001
Angiographic degree of CAD				<0.001
1-vessel disease	0 (0)	31 (43.6)	26 (68.4)	
2-vessel disease	0 (0)	26 (36.6)	9 (23.7)	
3-vessel disease	0 (0)	14 (19.7)	3 (7.9)	

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate.

3.3. Comparison of LPA, Lp-PLA2, hs-CRP concentrations and other markers

The plasma concentrations of LPA significantly increased in the group with ACS (median 0.54 μmol/l; IQR, 0.32–0.87) compared with the SAP (0.36 μmol/l; IQR, 0.3–0.47) and NCA (0.41 μmol/l; IQR 0.28–0.54) groups ($p=0.006$ and $p=0.008$, respectively). However, LPA concentrations did not significantly differ between the SAP and NCA groups ($p=0.919$). On the other hand, serum concentrations of Lp-PLA2 were significantly higher in the group with documented coronary atherosclerosis, namely the ACS (255.5 IU/l; IQR, 159.5–317.5) and SAP (252 IU/l; IQR, 179–367) groups, than in the NCA (151.5 IU/l; IQR, 108–260.5; $p=0.026$ and $p=0.004$, respectively) group, whereas the ACS and SAP groups did not significantly differ ($p=0.944$). Serum concentrations of hs-CRP were also significantly higher in the ACS (0.08 mg/dl; IQR, 0.03–0.15) than in the SAP (0.06 mg/dl; IQR, 0.02–0.16) and NCA (0.03 mg/dl; IQR, 0.01–0.16; $p=0.031$ and $p=0.042$, respectively) groups, with no difference between the SAP and NCA groups ($p=0.623$). Plasma concentrations of LPC significantly increased in the group with ACS (190.5 μmol/l; IQR, 159.8–238.8) compared with the SAP (166 μmol/l; IQR, 151–190) and NCA (177 μmol/l; IQR, 142.5–194.8] groups ($p=0.024$ and $p=0.025$, respectively; Fig. 1). Serum concentrations of autotaxin did not differ among the groups with ACS (0.76 mg/l; IQR, 0.56–0.91), SAP (0.67 mg/l IQR, 0.56–0.81) and NCA (0.72 mg/l IQR, 0.63–0.90; $p=0.218$). The lipid profile revealed significantly more LDL-C in the ACS (120 mg/dl IQR, 105–136.5) than in the SAP (106 mg/dl IQR, 88–118) and NCA (102 mg/dl IQR, 78.3–117.3; $p=0.007$ and $p=0.001$, respectively) groups. Cardiac troponin T concentrations were significantly higher in the ACS (105.3 pg/ml IQR, 14.6–373.6) than in the SAP (4.4 pg/ml IQR, 2.9–9.7) and NCA (4.0 pg/ml IQR, 2.6–6.4; $p<0.001$ and $p<0.001$, respectively) groups, whereas those in the SAP and NCA groups did not significantly differ ($p=0.928$; Table 2).

3.4. Relationship between LPA values and other markers

We analyzed the correlation between plasma log-LPA concentrations and the other important markers in all patients. The results showed that log-LPA was positively and significantly associated

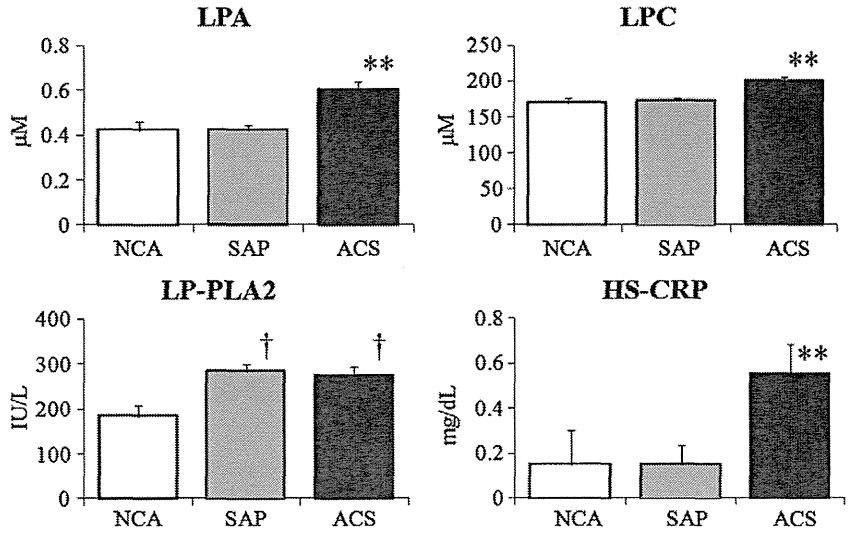

Fig. 1. Levels of LPA, LPC, Lp-PLA2 and hs-CRP in patients with NCA, SAP and ACS. Data are mean±SE; **$p<0.05$, compared with NCA and SAP; †$p<0.05$, compared with NCA.

Table 2
Laboratory characteristics of patient population.

	NCA (n=32)	SAP (n=71)	ACS (n=38)	P value
Fasting blood glucose, mg/dl	94 (88.3–117)	94 (87–117)	102.5 (87.5–137.3)	NS
HbA1C (%)	5.3 (5.2–5.6)	5.7 (5.3–6.4)	5.4 (5.2–5.9)	0.031
Total cholesterol, mg/dl	180 (161–191)	176 (163.5–198)	193 (169–214)	NS
Triglyceride, mg/dl	93.5 (81.5–153.8)	118 (89–167)	112 (78–150.5)	NS
LDL-C, mg/dl	102 (78.3–117.3)	106 (88–118)	120 (105–136.5)	0.001
HDL-C, mg/dl	47.5 (39–58.3)	45 (39–52)	47 (35.5–56.5)	NS
hs-CRP, mg/dl	0.03 (0.01–0.16)	0.06 (0.02–0.16)	0.08 (0.03–0.15)	0.039
Lp-PLA2, IU/l	151.5 (108–260.5)	252 (179–367)	255.5 (159.5–317.5)	0.004
Autotaxin, mg/l	0.72 (0.63–0.90)	0.67 (0.56–0.81)	0.76 (0.56–0.91)	NS
LPC, μM	177 (142.5–194.8)	166 (151–190)	190.5 (159.8–238.8)	0.002
LPA, μmol/l	0.41 (0.28–0.54)	0.36 (0.3–0.47)	0.54 (0.32–0.87)	<0.001
Troponin T, pg/ml	4.0 (2.6–6.4)	4.4 (2.9–9.7)	105.3 (14.6–373.6)	<0.001

hs-CRP, high sensitivity C-reactive protein; LPA, lysophosphatidic acid; Lp-PLA2, lipoprotein associated phospholipase A2; LPC, lysophosphatidylcholine.

with plasma concentrations of LPC (r=0.549, p<0.001). Moreover, log-LPA significantly correlated with serum concentrations of autotaxin (r=0.370, p<0.001). The correlation analysis with cholesterol showed that log-LPA significantly but weakly correlated with LDL-C (r=0.307, p<0.001) and total cholesterol (r=0.348, p<0.001), but not with HDL-C (r=0.094, p=0.271). Log-LPA also did not significantly correlate with either Lp-PLA2 (r=−0.061, p=0.473), hs-CRP (r=−0.180, p=0.067) or troponin T (r=0.157, p=0.084) (Fig. 2).

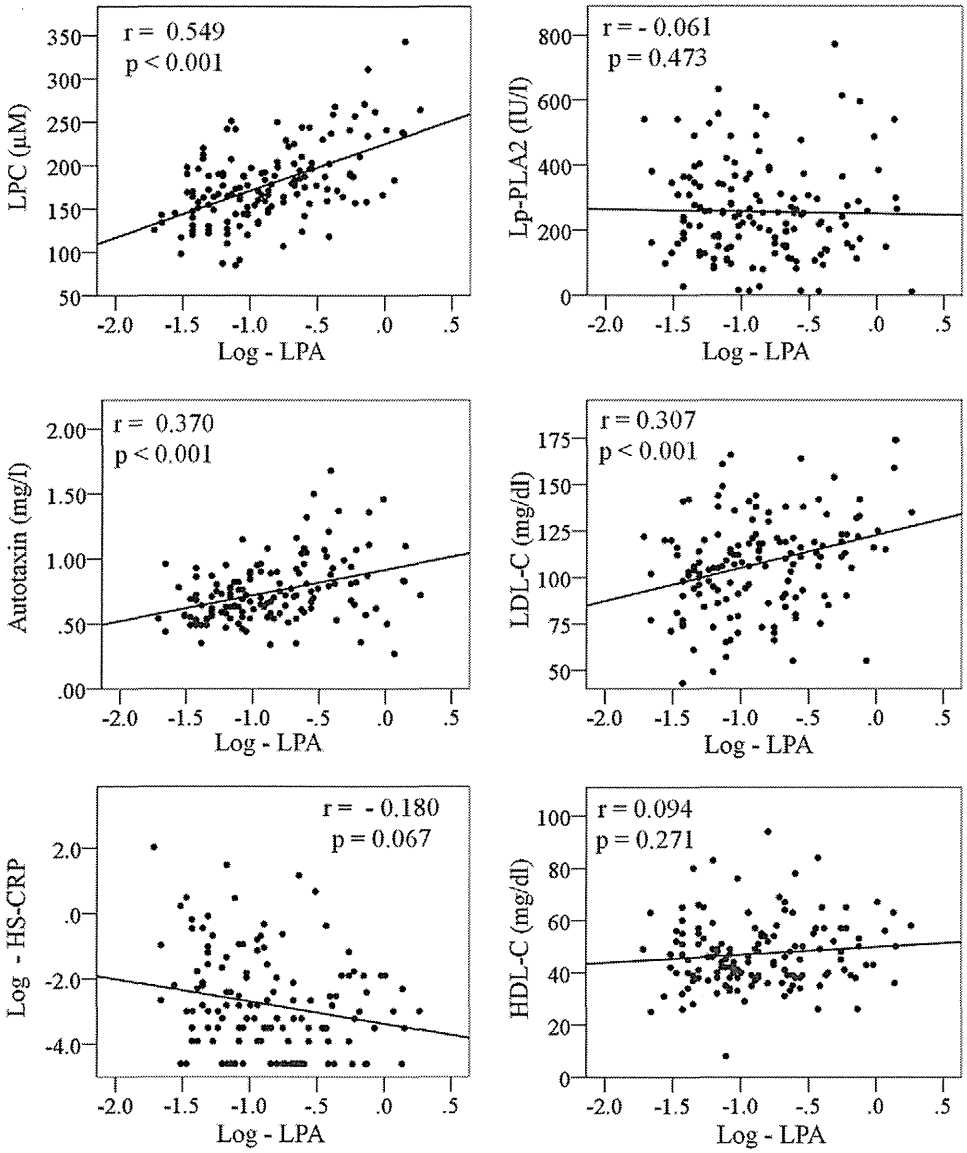

Fig. 2. Correlation between log-LPA and LPC, autotaxin, log-hs-CRP, Lp-PLA2, LDL-C and HDL-C in all patients.

Table 3
Univariate and multivariate logistic regression model for prediction of acute coronary syndrome.

	Univariate analysis OR (95 % CI)	P	Multivariate analysis OR (95 % CI)	P
Age, years	0.980 (0.948–1.012)	0.159	0.959 (0.922–0.997)	0.035
Sex, male	0.767 (0.362–2.118)	0.767	Not selected	
Diabetes, yes	1.615 (0.666–3.917)	0.152	1.650 (0.632–4.308)	0.307
Dyslipidemia, yes	1.476 (0.688–3.168)	0.318	Not selected	
Current smoking, yes	0.727 (0.324–1.631)	0.439	Not selected	
ACE-I or ARB, yes	0.467 (0.210–1.040)	0.062	0.499 (0.193–1.289)	0.409
Statin use, yes	0.554 (0.237–1.294)	0.236	Not selected	
HbA1C (%), /%	0.802 (0.506–1.272)	0.240	Not selected	
eGFR, 1 ml/min/1.73 m^2 increase	0.978 (0.958–0.999)	0.063	0.970 (0.945–0.996)	0.035
High-LPA, tertile increment	1.808 (1.111–2.940)	0.009	1.999 (1.180–3.387)	0.018
High-LP-PLA2, tertile increment	1.117 (0.704–1.772)	0.759	Not selected	
High-hs-CRP, tertile increment	1.325 (0.824–2.113)	0.163	1.408 (0.865–2.292)	0.169

ACE-I, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; hs-CRP, high sensitivity C-reactive protein; LPA, lysophosphatidic acid; Lp-PLA2, lipoprotein associated phospholipase A2.

3.5. Predictive value of increased LPA concentrations for ACS

We constructed a logistic regression analysis model to evaluate predictors of ACS for the entire study population. Age, diabetes, ACE-I or ARB use, eGFR, LPA and hs-CRP that were predictive in the univariate analysis were introduced into the multivariate model. After adjustment, only LPA (OR 1.999, 95%CI 1.180–3.387, $p=0.018$), age (OR 0.959, 95%CI 0.922–0.997, $p=0.035$) and eGFR (OR 0.970, 95%CI 0.945–0.996, $p=0.035$) emerged as being significantly predictive for ACS and remained when LPA was entered into the multivariate analysis as a continuous variable (log-LPA: OR 4.746, 95%CI 1.834–12.282, $p=0.001$; Table 3).

4. Discussion

The present study demonstrated that concentrations of circulating plasma LPA are significantly higher in patients with ACS than with SAP or NCA. We also showed that plasma LPA concentrations closely correlated with LPC, autotaxin and LDL-C, findings that are consistent with those of recent studies of LPA production mechanisms [2,3].

Accumulating evidence indicates that LPA can promote cardiovascular diseases by virtue of its atherogenic as well as thrombogenic activity [1–3,19]. Bot et al. recently found that LPA metabolism is associated with changes in plaque formation. The increased deposition of potent platelet-activating and proinflammatory LPC species in advanced atherosclerotic lesions indicates that thin cap fibroatheromas can be characterized not only by cellular and morphological features but also by their prothrombotic lipid profiles [20]. Lysophosphatidic acid is also abundant in the lipid-rich core of human atherosclerotic plaque. After plaque rupture or erosion, exposure to LPA in the lipid-rich core might play a key role in triggering or potentiating platelet responses during acute thrombosis [21,22]. In addition, Chen et al. found a significant increase in serum LPA concentrations in patients with acute myocardial infarction [23]. We therefore believe that circulating LPA has potential as a biomarker of atherothrombotic vascular diseases such as ACS that is pathophysiologically related to plaque rupture and platelet activation.

Although one report has indicated that the concentration of LPA is more closely related to that of the enzyme autotoxin than to the substrate LPC [24], the present study of ACS identified closer correlation between LPA and LPC. We consider that LPA can be produced by several intracellular as well as extracellular platelet-dependent and -independent pathways. In the platelet-dependent pathway, activated platelets release large amounts of phospholipids that are then converted by phospholipase A1 (PLA1) and phospholipase A2 (PLA2) to lysophospholipids (LPLs) such as LPC, lysophosphatidylethanolamine (LPE), and lysophosphatidylserine (LPS). Subsequently, LPA is generated from LPLs by autotaxin. About half of all circulating LPA is produced by a platelet-independent pathway from LPC. Plasma LPC is synthesized mainly by lecithin-cholesterol acyltransferase (LCAT), which catalyzes the transesterification of phosphatidylcholine and free cholesterol [25]. From the viewpoint of the mechanistic pathway, we consider that LPA production associated with autotaxin is a common pathway among healthy individuals [15]. We also believe that the atherogenic effects of LPC might be partly ascribed to its conversion to LPA, the verification of which will be an important task. In fact, this question of enzyme activity will be the theme of our next population study.

The present study found that an increased LPA concentration was a more powerful predictor of ACS than high concentrations of hs-CRP or Lp-PLA2. Many of the factors involved in ACS can be systemically and sensitively assayed, and increased circulating concentrations are associated with plaque destabilization and eventual plaque rupture. C-reactive peptide (CRP) belongs to the pentraxin family and is the most extensively studied proinflammatory factor. C-reactive peptide has a potential pathogenic role in atheromatous plaque vulnerability, since higher CRP concentrations closely correlate with increased numbers of thin cap fibroatheromas [26]. Although concentrations of LPA and hs-CRP did not significantly correlate in the present study, concentrations of both were significantly higher in patients with ACS. On the other hand, Lp-PLA2 is a novel biomarker and participant in vascular inflammation that is found in human atherosclerotic plaques; it hydrolyzes the sn-2 fatty acids of oxidized phospholipids to yield oxidized fatty acid and LPC [27,28]. The latter plays an important part in the effect of Lp-PLA2 on endothelial function [29]. Lysophosphatidylcholine also stimulates both the proliferation and apoptosis of endothelial and smooth muscle cells at low and high concentrations, respectively [30,31]. Furthermore, high concentrations of Lp-PLA2 and LPC are associated with coronary atherosclerosis and endothelial dysfunction in humans [32]. Therefore, we considered that higher concentrations of LPC as well as of LPA might reflect systemic atherosclerotic instability through the mechanism of systemic endothelial dysfunction. Concentrations of Lp-PLA2 were significantly higher in patients with, than without documented coronary atherosclerosis. However, Lp-PLA2 concentrations did not differ between patients complicated with plaque instability and patients with stable disease. Another study found that Lp-PLA2 concentrations are not increased in patients with ACS compared with those without ACS, unlike acute-phase reactants such as CRP [33]. Consequently, we considered that Lp-PLA2 might be an important marker of, or play an active role in, the atherosclerotic process, whereas it does not play a causative role in creating susceptibility to plaque rupture. That is, not only higher concentrations of Lp-PLA2 but also of LPA might imply plaque instability in patients with coronary atherosclerosis. Based on these findings, we believe that circulating concentrations of the lysophospholipids LPA and LPC can identify patients who are complicated with vulnerable plaque and have unstable cardiovascular disease. Furthermore, a recent study found that LPA is involved

in atherogenic monocyte recruitment mediated by hyperlipidemia and modified LDL [34]. We consider that the present results support these findings and further indicate the importance of LPA signaling as a target for treating coronary artery disease.

The present study discovered an association between increased systemic circulating LPA concentrations and ACS. Concentrations of LPA significantly and positively correlated with autotaxin and even more closely with those of LPC. The results of our multivariate analysis indicated that higher LPA concentrations could be a powerful predictor of ACS. Given that circulating LPA concentrations are increased during the clinical course of plaque instability, the present findings suggest a novel biological mechanism that might contribute to the accelerated development of plaque in coronary artery disease. Thus, LPA might play an important role in patients with ACS. We also believe that LPA could serve as a new systemic biomarker of ACS and that various biomarkers reflect different phases of atherosclerotic plaque progression.

Abbreviations

LPA	lysophosphatidic acid
LPC	lysophosphatidylcholine
LPS	lysophosphatidylserine
Lp-PLA2	lipoprotein associated phospholipase A2
NCA	normal coronary arteries
SAP	stable angina pectoris

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Probucol therapy improves long-term (>10-year) survival after complete revascularization: A propensity analysis

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ABSTRACT

Objective: Probucol has anti-atherosclerotic properties and has been shown to reduce post-angioplasty coronary restenosis. However, the effect of probucol therapy on long-term (>10 years) outcome following coronary revascularization is less well established. Accordingly, we sought to determine if probucol therapy at the time of complete coronary revascularization reduces mortality in patients with coronary artery disease (CAD).

Methods: We collected data from 1694 consecutive patients who underwent complete revascularization (PCI and/or bypass surgery). Mortality data were compared between patients administered probucol and those not administered probucol at the time of revascularization. A propensity score (PS) was calculated to evaluate the effects of variables related to decisions regarding probucol administration. The association of probucol use and mortality was assessed using 3 Cox regression models, namely, conventional adjustment, covariate adjustment using PS, and matching patients in the probucol and no-probucol groups using PS.

Results: In the pre-match patients, 231 patients were administered probucol (13.6%). During follow-up [10.2 (SD, 3.2) years], 352 patients died (including 113 patients who died of cardiac-related issues). Probucol use was associated with significant decrease in all-cause death (hazard ratio [HR], 0.65; $P=0.036$ [conventional adjustment model] and HR, 0.57; $P=0.008$ [PS adjusted model]). In post-match patients ($N=450$, 225 matched pair), the risk of all-cause mortality was significantly lower in the probucol group than in the no-probucol group (HR, 0.45; $P=0.002$).

Conclusion: In CAD patients who had undergone complete revascularization, probucol therapy was associated with a significantly reduced risk of all-cause mortality.

1. Introduction

Probucol, a cholesterol lowering drug, has anti-oxidant [1–3] and anti-inflammatory [4,5] properties and has been shown to have clinical benefits such as regression of atherosclerosis in carotid arteries [6] and reduction of post-angioplasty restenosis in coronary arteries [7–11]. However, probucol use has recently declined with the introduction of statins and due to several concerns regarding its potential role in reducing serum high-density lipoprotein (HDL) cholesterol levels as well as QT interval prolongation [12]. As a result, probucol is now unavailable in many Western countries.

The specific mechanisms of the low-density lipoprotein (LDL) cholesterol lowering effect of probucol are uncertain. However, previous studies showed that the anti-atherosclerotic effects of probucol are independent from the cholesterol lowering effect [1], suggesting that inhibition of LDL cholesterol oxidative modification may play an important role [13]. On the other hand, recent studies have suggested that enhanced reverse cholesterol transport (RCT) caused by activation of cholesteryl ester transfer protein (CETP) and scavenger reverse cholesterol class B type I (SR-BI) is the major mechanism responsible for both the anti-atherosclerotic effect and paradoxical lowering of HDL cholesterol levels by probucol [14–19]. The apparent HDL level lowering induced by probucol may be associated with the remodeled function of HDL, including an increase in preβ1-HDL (i.e., lipid-poor apoA-1), which participates in the cholesterol efflux [20]. Therefore, HDL lowering induced by probucol may not be an adverse effect (i.e., not harmful), but may instead reflect its primary effect (i.e., increase in cholesterol efflux). Recent clinical data from Japan, where probucol is available for clinical use and currently administered to patients [12], suggest that long-term probucol treatment may be beneficial for preventing secondary

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cardiovascular events in patients with heterozygous familial hypercholesterolemia [21]. However, the effect of probucol therapy in patients after coronary revascularization over a long-term period is less well established. Thus, we sought to determine if probucol therapy at the time of complete revascularization is associated with reduced mortality.

2. Methods

2.1. Subjects

Data from consecutive patients who had undergone surgical and/or percutaneous coronary revascularization at Juntendo University Hospital between February 1985 and December 1992 were analyzed. Patients who had achieved complete revascularization, defined as those who had no un-bypassed major vessels with ≥50% stenosis [22,23] were enrolled. Patients with untreated neoplasm at baseline and those with associated complex cardiac procedures, such as valve replacement or aneurysm repair at the time of surgical revascularization, were excluded.

This study was performed according to the principles expressed in the Declaration of Helsinki and the ethics policies of the institution and was approved by an internal review board.

2.2. Baseline data collection

Demographic data, including age, gender, and body mass index (BMI), coronary risk factors, including blood pressure, lipid profile, fasting plasma glucose, smoking status, and family history of coronary artery disease (CAD), medication use, revascularization procedure-related factors, and comorbidities (prior myocardial infarction [MI] or stroke, dialysis history, and atrial fibrillation [AF]), were collected in the database at our institution.

For all analyses, patients were divided into two groups according to probucol use or non-use at the time of complete revascularization. Each patient was further categorized based on the presence of coronary risk factors using the following criteria during the study period: hypertension was defined as systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg or treatment with antihypertensive medications. A current smoker was defined as a patient who smoked at the time of complete revascularization or who had quit smoking within 1 year before complete revascularization. Diabetes mellitus (DM) was defined as a fasting plasma glucose level of ≥126 mg/dl or treatment with oral hypoglycemic drugs or insulin injections. AF was defined as persistent or permanent AF at the time of the procedure. Patients with isolated percutaneous coronary intervention (PCI) were those in whom complete revascularization was achieved by PCI without bypass grafting.

2.3. Outcomes

Survival data and date of death of subjects who died were collected by serial contact with patients or their families until September 30, 2000 and from medical records of patients who had died and of those who continued to be followed up at our hospital. Information regarding the continuation of probucol use and the circumstances and date of death was obtained from families of patients who had died at home, and details of cardiac events or cause of death were supplied by other hospitals or clinics where patients had been admitted. Mortality data were categorized according to the cause of death, such as death from all-causes or cardiac death due to CAD, cardiogenic shock, and sudden death.

2.4. Statistical analysis

Continuous variables are expressed as mean (SD) and compared using the Student's *t*-test or Mann–Whitney *U*-test as appropriate. Categorical data are displayed as frequencies and percentages and were compared using the Chi-square test or Fisher's exact test.

Because patients were not randomly assigned to the probucol or no-probucol groups, there were significant differences in baseline covariates between the two groups. A propensity score (PS) was used to account for this selection or predisposition bias. Details regarding estimation of propensity score are described in the online Supplementary data.

We assessed the relationship between probucol therapy and study outcome variables by using 3 separate analytical techniques: (1) conventional adjustment, (2) covariate adjustment using PS, and (3) matching patients in the probucol group and those in the no-probucol group using PS. Kaplan–Meier survival analysis with a log-rank test was used to compare study outcome variables between the probucol and no-probucol groups in both entire (pre-match) and post-match datasets.

For all patients, in addition to the crude model, two multivariate Cox proportional hazards models (conventional adjustment and adjustment using PS) were used to determine the benefits of probucol therapy against study outcome variables. For conventional adjustment of baseline covariates used in the logistic regression model for the PS, variables regarded as significant ($P<0.10$) in univariable analyses were included in multivariate analysis in addition to the use of probucol. For the adjustment, adjustment using PS and use of probucol were included.

Details regarding propensity score matching are described in the online Supplementary data. To determine whether PS matching produced balanced distributions of baseline characteristics across the probucol and no-probucol groups, we compared the balance of baseline covariates between the two groups before and after matching by using absolute standardized differences that describe the observational selection bias in the means or proportions of covariates across two groups and expressed these values as percentages of the pooled SD. Absolute standardized differences of <10% suggest substantial balance across groups. Cox proportional hazards regression stratified on the matched pairs were used to estimate the association of probucol therapy with study outcome variables in matched patients, accounting for matched-pair natures of the sample.

To assess potential heterogeneity of treatment effect on all-cause mortality, we conducted subgroup analyses using matched patients. We formally tested for first order interactions using multivariable Cox proportional hazards models by entering interaction terms between probucol use and the subgroup variables. We also showed the effect of probucol therapy on all-cause mortality in each subgroup. Other details regarding subgroup analyses are described in the online Supplementary data.

P-values <0.05 were considered significant. All data were analyzed using Dr. SPSSII for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Baseline characteristics in entire patients

Overall, complete revascularization was achieved in 1694 patients during the study period. Baseline characteristics and clinical events during follow-up [mean, 10.2 (3.2) years] were collected for all patients. Among these patients, 231 (13.6%) received probucol at the time when complete revascularization was achieved. All patients underwent PCI with balloon angioplasty. No patients received stent implantation, since stents were not available at

Table 1
Baseline characteristics of the entire subjects (pre-match) and matched-pair (post-match).

	Pre-match			Post-match	
	No-probucol $N=1463$	Probucol $N=231$	P	No-probucol $N=225$	Probucol $N=225$
Age (years)	59.5 (9.0)	58.1 (8.5)	0.024	58.9 (9.4)	58.1 (8.5)
Male (n, %)	1229 (84.0%)	198 (85.7%)	0.572	197 (87.6%)	193 (85.8%)
BMI (kg/m^2)	23.5 (2.6)	24.1 (2.7)	0.002	24.0 (2.9)	24.0 (2.7)
Hypertension (n, %)	981 (67.1%)	153 (66.2%)	0.864	146 (64.9%)	148 (65.8%)
Diabetes mellitus (n, %)	576 (39.4%)	88 (38.1%)	0.767	82 (36.4%)	83 (36.9%)
Total cholesterol (mg/dl)	218.9 (47.5)	221.0 (59.5)	0.790	222.9 (54.3)	220.6 (58.3)
HDL cholesterol (mg/dl)	43.2 (12.2)	38.4 (14.3)	<0.001	38.6 (11.0)	38.7 (14.3)
Triglyceride (mg/dl)	166.2 (93.1)	163.5 (144.8)	0.058	167.8 (83.3)	163.4 (146.1)
Current smoker (n, %)	1087 (74.3%)	177 (76.6%)	0.501	167 (74.2%)	172 (76.4%)
Family history of CAD (n, %)	462 (31.6%)	85 (36.8%)	0.134	77 (34.2%)	80 (35.6%)
Prior MI (n, %)	732 (50.0%)	73 (31.6%)	<0.001	70 (31.1%)	72 (32.0%)
Prior stroke (n, %)	70 (4.8%)	6 (2.6%)	0.186	9 (4.0%)	6 (2.7%)
AF (n, %)	193 (13.2%)	17 (7.4%)	0.017	21 (9.3%)	17 (7.6%)
On dialysis (n, %)	21 (1.4%)	5 (2.2%)	0.583	7 (3.1%)	5 (2.2%)
LVEF (%)	64.2 (13.0)	66.0 (12.0)	0.054	65.3 (12.9)	65.8 (11.9)
LMT lesion (n, %)	128 (8.7%)	12 (5.2%)	0.090	13 (5.8%)	12 (5.3%)
Number of diseased vessels	2.31 (0.80)	1.87 (0.88)	<0.001	1.84 (0.95)	1.89 (0.88)
Multivessel disease (n, %)	1149 (78.5%)	126 (54.5%)	<0.001	124 (55.1%)	125 (55.6%)
Arterial bypass graft to LAD (n, %)	569 (38.9%)	36 (15.6%)	<0.001	39 (17.3%)	36 (16.0%)
Isolated PCI (n, %)	378 (25.8%)	139 (60.2%)	<0.001	127 (56.4%)	134 (59.6%)
Procedure date ≥ median (n, %)	748 (51.1%)	99 (42.9%)	0.023	94 (41.8%)	96 (42.7%)
Use of medications					
Aspirin (n, %)	1068 (73.0%)	179 (77.5%)	0.174	174 (32.9%)	174 (32.9%)
Nitrates (n, %)	1310 (89.5%)	209 (90.5%)	0.751	200 (88.9%)	204 (90.7%)
Nicorandil (n, %)	146 (10.0%)	44 (19.0%)	<0.001	38 (16.9%)	39 (17.3%)
ACE inhibitors (n, %)	60 (4.1%)	8 (3.5%)	0.780	8 (3.6%)	8 (3.6%)
Calcium channel blockers (n, %)	299 (20.4%)	57 (24.7%)	0.167	62 (27.6%)	55 (24.4%)
Beta blockers (n, %)	393 (26.9%)	62(26.8%)	0.999	51 (22.7%)	58 (25.8%)
Statins (n, %)	300 (20.5%)	28 (12.1%)	0.004	34 (15.1%)	28 (12.4%)

BMI, body mass index; HDL, high-density lipoprotein; CAD, coronary artery disease; MI, myocardial infarction; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LMT, left main trunk; LAD, left anterior descending; PCI, percutaneous coronary intervention; ACE, angiotensin converting enzyme.
Values are represented as mean (SD) or number (%).

the time of complete revascularization. All coronary artery bypass graft procedures were performed under on-pomp conventional cardiopulmonary bypass. Probucol (500–1000 mg/day) was administered. During the total follow-up period, 139 patients (60.2%) in the probucol group stopped probucol therapy, and 171 patients (11.7%) in the no-probucol group started probucol therapy. Baseline characteristics of pre-match patients with and without probucol use are shown in Table 1. Patients using probucol were older, had higher BMIs, and lower HDL cholesterol levels than patients not using probucol. More cases with a history of MI, AF, multivessel disease, and arterial bypass graft to left anterior descending artery were included in the no-probucol group. Furthermore, more cases in no-probucol group underwent complete revascularization by isolated PCI, and these cases occurred more recently. More patients were administered nicorandil in the probucol group; however, fewer patients were administered statins. There were no significant differences between the two groups in any other variables.

3.2. Propensity score and matching

The discriminatory power of the logistic regression model used to derive the PS was confirmed on the basis of the area under the receiver operating characteristics curve (0.75). The 4 thresholds used to determine the quintiles of the PS were 0.072, 0.125, 0.182, and 0.276. Probucol administration rates within each PS quintile were 4.1, 7.4, 7.7, 15.0, and 34.0% from the lowest to highest quintiles, respectively.

PS matching resulted in the creation of 225 matched pairs of patients in the probucol and no-probucol groups. Thus, for 6 patients in the probucol group, no suitable control was identified. This resulted in elimination of 6 patients in the probucol group and 1238 patients in the no-probucol group from the matched analysis. Other details regarding PS matching are described in the online Supplementary data. Baseline characteristics of matched patients are shown in Table 1. PS matching reduced the standardized difference for all variables to an absolute value below 10% (Fig. 1).

3.3. Survival analyses

Among all patients, 352 patients died during follow-up (326 in the no-probucol group and 26 in the probucol group). Of these, 113 were cardiac deaths (105 in the no-probucol group and 8 in the probucol group) and 239 were non-cardiac deaths (221 in the no-probucol group and 18 in the probucol group). For non-cardiac deaths, deaths associated with cancer were the most frequent (70 in the no-probucol group and 11 in the probucol group). The Kaplan–Meier curves are shown in the online Supplementary data. In pre-match patients, in addition to the crude model, both conventional adjustment and PS adjusted models showed that probucol use at the time of complete revascularization was a significant predictor of long-term survival with respect to all-cause death (hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.43–0.97; $P=0.036$ in the conventional adjustment model and HR, 0.57; 95% CI, 0.38–0.87; $P=0.008$ in the PS adjusted model) (Table 2). In post-match patients, risk of all-cause mortality was also significantly lower in the probucol group than in the no-probucol group (HR, 0.45; 95% CI, 0.27–0.75; $P=0.002$) (Table 2). However, probucol use was not an independent predictor of long-term survival with respect to cardiac death in all models for pre-match analyses and in post-match analysis, although the risk of cardiac death tended to be lower in the probucol group than in the no-probucol group (Table 2). Risk of non-cardiac death was significantly lower in the probucol group than in the no-probucol group in PS adjusted and

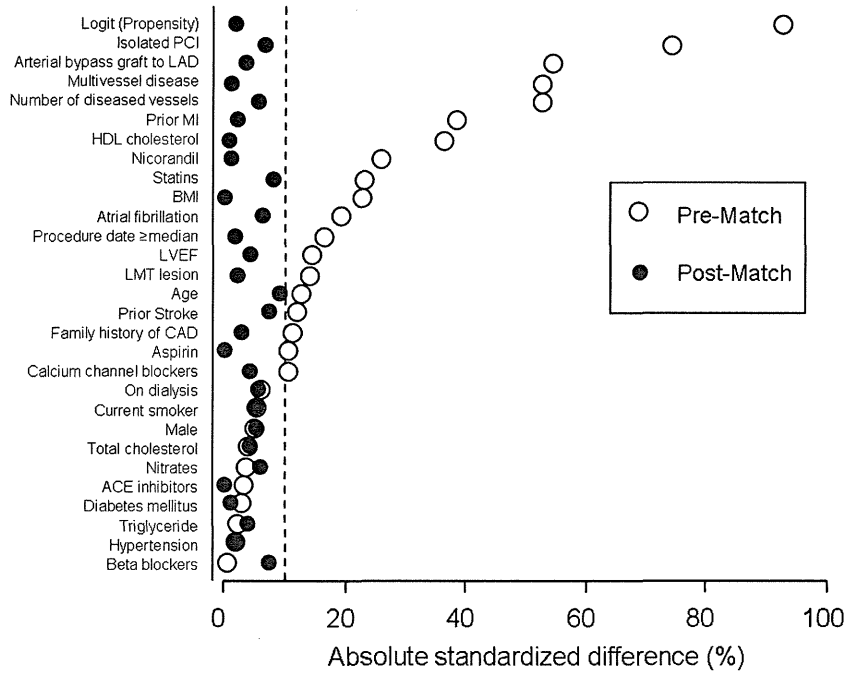

Fig. 1. Absolute standardized differences before and after propensity score matching comparing covariate values for patients receiving and not receiving probucol.

post-match analyses (Table 2). However, there were no significant differences in the risk of cancer-related deaths between the two groups in all analyses (Table 2).

3.4. Subgroup analyses

There were no significant interactions between probucol use and any of subgroups, suggesting that there are no differences in treatment effect on all-cause mortality across subgroups (Fig. 2). However, patients using probucol did not show a lower all-cause mortality risk than those not using probucol in women, patients with atrial fibrillation, and patients using statins in which only a small number of patients was included (Fig. 2).

4. Discussion

This study showed that probucol treatment at the time of revascularization is associated with reduced long-term (>10 years) all-cause mortality risk in patients who have undergone complete revascularization, using 3 separate analytical techniques: conventional adjustment, covariate adjustment using PS, and matching based on the PS. Consistent results in all 3 analyses would support a causal effect of probucol therapy and reduce the possibility of the observed effects of probucol resulting from confounding by indication (i.e., selection bias). Furthermore, results of tests for subgroup-treatment effect interaction suggested that the treatment effect on all-cause mortality was not different across subgroups.

Previous clinical data have suggested that probucol is beneficial in regression of atherosclerosis [6], although another study revealed no obvious effect on femoral atherosclerotic lesions [24]. In addition, several studies, including those by our group, have shown reduction of post-angioplasty coronary restenosis [7–11]. However, no large-scale randomized clinical studies assessing the benefits of probucol use on morbidity and mortality risk have been conducted. The following two clinical studies have assessed different aspects of probucol use. The Aggressive Reduction of Inflammation Stop Events (ARISE) trial [25] is a

Table 2
Hazard ratio of probucol use mortality.

	Pre-match						Post-match	
	Crude		Conventional adjustment[a]		Adjustment using PS[b]			
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
All-cause	0.52 (0.35–0.77)	0.001	0.65 (0.43–0.97)	0.036	0.57 (0.38–0.87)	0.008	0.45 (0.27–0.75)	0.002
Cardiac	0.51 (0.25–1.04)	0.064	0.61 (0.29–1.29)	0.198	0.52 (0.25–1.10)	0.085	0.54 (0.22–1.35)	0.187
Non-cardiac	0.52 (0.32–0.84)	0.008	0.66 (0.41-1.08)	0.102	0.60 (0.36–0.99)	0.044	0.41 (0.22–0.77)	0.005
Cancer	1.00 (0.53–1.89)	0.999	1.22 (0.64–2.34)	0.551	1.10 (0.56–2.17)	0.776	2.00 (0.68–5.85)	0.206

HR, hazard ratio; CI, confidence interval; PS, propensity score.

[a] For conventional adjustment of baseline covariates used in the logistic regression model for the PS, variables regarded as significant ($P < 0.10$) in the univariable analyses were included in the multivariate analysis in addition to the use of probucol.

[b] For the adjustment using PS, along with use of probucol, PS was included.