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Lipoprotein-associated phospholipase A_2 testing usefulness among patients with symptomatic intracranial atherosclerotic disease

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ABSTRACT

Background and purpose: Circulating lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has emerged as a novel biomarker for cardiovascular diseases. Our aim was to determine Lp-PLA_2 mass and activity in a selected cohort of first-ever transient ischemic attack (TIA) or ischemic stroke patients with intracranial atherosclerotic disease (ICAD) and to investigate its relationship with the presence of classical vascular risk factors, response to secondary prevention treatments and risk of recurrent vascular events.
Methods: Lp-PLA_2 mass and activity were measured 3 months after TIA or stroke by means of the PLAC test and CAM-assay (diaDexus, Inc.) respectively in 75 patients. Classic vascular risk factors, preventive treatments and clinical characteristics at the time of the index event were recorded. Follow-up transcranial Doppler ultrasonography (TCD) was performed and the presence of a new vascular event was assessed every 6 months.
Results: Several preventive treatments (statins and clopidogrel) were significantly associated with lower Lp-PLA_2 mass and activity. During follow-up (median time 23 months), eighteen patients (24%) suffered a new vascular event. Baseline factors associated with new vascular events were: history of coronary artery disease, number of intracranial stenoses detected by TCD and also Lp-PLA_2 activity, which was the only independent predictor for new vascular events (hazard ratio 2.89; 95% CI 1.029 to 8.096; $p = 0.044$) after multivariate analysis (Cox regression).
Conclusions: Lp-PLA_2 activity might be a useful tool to identify intracranial large-artery occlusive disease patients at higher risk of suffering new vascular events.

1. Introduction

Intracranial atherosclerotic disease (ICAD) is an important cause of stroke worldwide being responsible of about 8% of strokes in Caucasian population and as far as one third of Chinese population strokes [1,2]. Almost a quarter (22%) of these patients will suffer other ischemic events over the next 2 years despite the best preventive treatments [3], a risk that has been related to the progression of intracranial stenoses [4].

This unacceptable prognosis could be improved through the identification of higher risk patients who could benefit from a more intensive preventive approach. In this regard, some inflammatory biomarkers such as C-reactive protein (CRP) have shown the ability to predict future cardiovascular events, ischemic stroke and progression of symptomatic ICAD [5,6].

Similarly, lipoprotein-associated phospholipase A(2) (Lp-PLA_2) has emerged as a novel cardiovascular risk marker which activity seems to be stable over time [7] and unaffected by systemic inflammatory stimulus [8].

Lp-PLA_2 is a calcium-independent phospholipase derived especially from macrophages, that circulates mainly bound to low density lipoproteins (LDL) in plasma and is responsible of LDL metabolism, producing two pro-inflammatory mediators such as lysophosphatidylcholine (lysoPC) and oxidized nonesterified fatty acids (oxNEFAs), which are able to activate inflammatory path-

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ways and elicit proatherogenic effects in endothelial cells, T cells, neutrophils and smooth muscle cells [9].

High levels of peripheral blood Lp-PLA_2 mass and activity are associated with an increased risk of coronary heart disease (CHD) and stroke [10,11] and their measurement improve stroke and cardiovascular risk stratification [12]. For that reason, US Food and Drug Administration approved Lp-PLA_2 evaluation for stroke and coronary disease risk.

However, as far as we know, the relationship between Lp-PLA_2 and vascular recurrence has not been yet evaluated in the specific high risk subgroup of strokes caused by intracranial atherosclerotic disease.

Our aim was to investigate the association between Lp-PLA_2 mass and activity with the recurrence of ischemic events in symptomatic ICAD patients and its relationship to secondary prevention treatments, classical vascular risk factors and intracranial stenoses progression.

2. Subjects and methods

2.1. Study participants

Our study population were selected among 196 consecutive patients with transient ischemic attack (TIA) or ischemic stroke admitted to our stroke unit with intracranial stenoses recorded by transcranial Doppler (TCD) potentially responsible for the cerebral ischemic event.

Our diagnostic protocol during admission has been reported in detail elsewhere [13].

This study included 75 consecutive patients with angiographic confirmed intracranial stenoses by MR-angiography or CT-angiography. The remaining candidates where excluded because the following list of reasons: (1) presence of other potential causes of cerebral ischemia ($n = 46$), (2) non-atherosclerotic origin of intracranial stenoses ($n = 23$), (3) existence of inflammatory conditions ($n = 20$) and (4) impossibility of performing follow-up due to stroke-related death or severe disability ($n = 10$), lack of an adequate acoustic window ($n = 21$) or denial of informed consent ($n = 1$) (Fig. 1). The inclusion visit took place at least 3 months after the qualifying event and at that moment an informed consent and blood samples, always after overnight fast, were obtained from all 75 patients. Furthermore, acute infections, surgery or trauma during the previous 3 months and incident neoplasm or inflammatory conditions were ruled out by a careful medical history and physical examination prior to sampling.

This study was approved by the local ethics committee.

2.2. Clinical variables and long-term follow-up

2.2.1. Vascular risk factors and clinical variables

Cigarette smoking and medical history of hypertension, hypercholesterolemia, type 2 diabetes mellitus, diagnosed coronary artery disease and intermittent claudication were recorded at the inclusion visit, as previously described [14].

Stroke severity was assessed using the maximum National Institutes of Health Stroke Scale score (NIHSS) during admission and functional status at day 90 was assessed by means of the modified Rankin scale score (mRS). Secondary prevention therapies were established following the recommendations of the American Heart Association guidelines available during the study period and antithrombotic treatment was indicated in an individualized manner following the criteria of the stroke team responsible for each patient. The use of acenocumarol, aspirin, clopidogrel, statins, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers was registered.

After inclusion, clinical visits every 6 months were conducted by a stroke neurologist (J.F.A.) who remained unaware of the biochemical data of the patients throughout the study period. The following major vascular events were considered as predefined clinical end points: acute ischemic stroke; TIA diagnosed by a stroke neurologist; acute myocardial infarction or angina requiring hospitalisation, and vascular death.

2.3. Ultrasound protocol

TCD recordings were performed using a Multi-Dop-X/TCD (DWL Elektronische Systeme GmbH, Germany) device, with a hand-held transducer in a range-gated, pulsed-wave mode at a frequency of 2 MHz. We used a standard method of insonation through the temporal, occipital, and orbital windows without compression testing. According to validated criteria, intracranial stenoses were diagnosed if the mean blood flow velocity at a circumscribed insonation depth was >80 cm/s, with side-to-side differences of >30 cm/s and signs of disturbed flow. TCD examinations were carried out on admission and repeated at the inclusion visit to confirm the persistence of stenoses.

Baseline cervical internal cartid artery (ICA) atherosclerosis was categorized as absent; mild, if one or both ICAs had a mild <50% stenoses; moderate, when any of the ICAs presented a moderate <70% stenoses; and severe, if any ICA had a severe asymptomatic stenoses.

2.4. MRA and CTA

MRA was performed with a 1.5 T whole-body imager system with 24-mT/m gradient strength, 300-ms rise time, and an echoplanar-capable receiver equipped with a gradient overdrive (Magnetom Vision Plus; Siemens Medical Systems, Erlangen, Germany). We used a three-dimensional time-offlight sequence with magnetization transfer suppression and tilted optimized nonsaturating excitation, using 1.5-mm-thick sections, 200-mm field of view, 200×512 matrix, and acquisition time that ranged from 7 to 11 min. Maximal intensity projection (MIP) reconstructions were performed at the time of imaging.

The data were reconstructed around the head-to-foot axis and right-to-left axis. If necessary, target MIP reconstructions were performed. MRAs were analyzed by a neuroradiologist who was blind to sonographic and biochemical data and confirmed the presence of intracranial stenoses.

CTAs were performed on a multislice MX8000 Philips spiral CT scanner (Best, the Netherlands) with four rows of detectors.

Ninety milliliters of iodinated contrast medium (320 mg/ml) was administered IV at a rate of 3 ml/s with a 13-s prescan delay. Scanning began at the cranial base and continued cranially for 80 mm. Total acquisition time average was 22 s. Raw data were transferred to a workstation, and MIP reconstructions were obtained. Images were interpreted by a neuroradiologist who identified the presence of intracranial stenoses.

The number of angiographically confirmed intracranial stenoses in every patient was used to assess the extent of intracranial large-artery occlusive disease on baseline.

2.5. Lp-PLA_2 mass and activity determinations

Peripheral blood samples were collected in EDTA-containing tubes and plasma was extracted after 15 min centrifugation (3500 rpm), blind coded and then frozen at −80 °C until testing was performed.

Lp-PLA_2 mass (PLAC Test, diaDexus Inc.) was assayed using a turbidimetric immunoassay on an automated clinical chemistry analyzer (Olympus AU 2700). Intra-assay coefficient of variation

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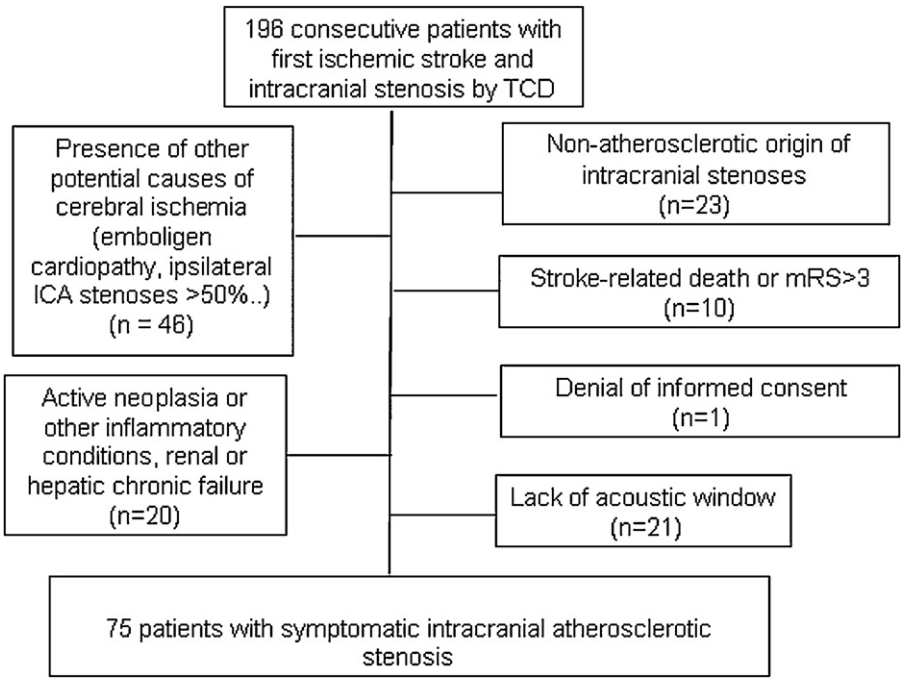

Fig. 1. Description of the study population.

(CV) was determined testing 20 replicates of one human serum sample (CV 1.6%) and 20 replicates of two buffer controls (CV 1.8%) with Lp-PLA_2 at the lowest and highest concentrations. Inter-assay variation was performed during five consecutive days in duplicates for the buffer controls, with values lower than 6.9%. Since our intra and inter-assay variation was low, samples were analyzed by single measurements.

Lp-PLA_2 activity was measured with a colorimetric activity method in a 96-well microplate format (Lp-PLA_2 CAM, diaDexus Inc.), which measures a linear kinetic rate over time. All samples were tested in duplicates, and the coefficient of variation was <20%.

All assays were performed blinded to clinical data.

2.6. Statistical analysis

Analyses were performed with the SPSS statistical package (Chicago, Ill., USA), version 15.0. Statistical significance for inter-group differences was assessed by the χ^2-test or Fisher's exact test for categorical variables and by the Mann–Whitney *U*- and *t*-test for continuous variables, as appropriate.

Cumulative event-free rates for the time to a recurrent vascular event were estimated by the Kaplan–Meier product limit method, and patients with Lp-PLA_2 mass and activity across quartiles were compared by the log-rank test. Time to recurrent vascular event was analyzed with censoring at the time to either non-vascular death or last follow up.

To prevent overmodeling of the data and false-positive results, only clinical recurrence was considered an end point of the study.

Also, a receiver operating characteristic (ROC) curve was performed to identify an optimal cut-off point of Lp-PLA_2 activity that best discriminate between the presence or absence of a new recurrent event.

A Cox proportional hazards multivariate analysis was constructed to identify potential predictors of further vascular events during follow-up, after adjustment for potential confounders such as age, gender, hypertension, diabetes, dyslipidemia and LDL levels. Results were expressed as adjusted hazard ratios and corresponding 95% confidence intervals.

3. Results

3.1. Baseline clinical variables

Baseline characteristics and vascular risk factors of the study population are shown in Table 1. The study sample comprised 55 men (73%) and 20 women (27%). The mean age was 66.2 ± 8.3 years. The qualifying event attributable to a symptomatic intracranial atherostenoses was an ischemic stroke in 54 patients (72%) and a TIA in the remaining 21 (28%). Location of symptomatic intracranial stenosis was recorded; as shown in Table 1, the middle cerebral artery the most affected arterial segment. In 8 patients with multiple stenoses presenting with a TIA, it was not possible to determine which intracranial stenoses had been symptomatic. For stroke patients, the median NIHSS on admission was 2 (interquartile range 0–4). All studied subjects remained free of ischemic events during the period between hospital discharge and the inclusion visit. Regarding secondary prevention therapies, 58 patients (77%) received antiplatelet agents, 17 (23%) received oral anticoagulants and 53 (71%) were treated with statins throughout the follow-up period.

Intracranial stenoses were confirmed by MRA in 66 patients (88%) and by CTA in 9 (12%). Besides the 75 symptomatic stenoses, a total of 165 coexistent asymptomatic stenoses were detected, three being the median number of diagnosed intracranial stenoses per patient (interquartile range 2–4).

3.2. Lp-PLA_2 mass and activity, cardiovascular risk factors and clinical evaluation

Lp-PLA_2 mass was normally distributed (313 ± 100 ng/ml) whereas activity was not [153 (interquartile range: 127–177 nmol/ml/min)], and both were mildly correlated ($r = 0.197$; $p = 0.004$).

Table 2 shows univariate analysis concerning Lp-PLA_2 mass and activity. Several factors were significantly associated with either Lp-PLA_2 mass and/or activity, such as gender, LDL-cholesterol level and baseline vascular status as determined by TCD. Neither Lp-PLA_2

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Table 1
Baseline characteristics of the study sample ($n = 75$).

Characteristic	Value
Demographic characteristics	
Age, years	66.2 ± 8.3
Gender, male	55 (73%)
Risk factors and comorbid vascular diseases	
Current smoking	35 (47%)
Hypertension	60 (80%)
Diabetes mellitus	40 (53%)
Hypercholesterolemia	55 (73%)
Coronary heart disease	13 (17.5%)
Peripheral arterial disease	15 (20%)
>2 vascular risk factors	36 (48%)
Qualifying event	
• Stroke	54 (72%)
• TIA	21 (28%)
Location of symptomatic intracranial stenosis	
• Intracranial ICA	17 (23%)
• MCA	25 (33%)
• ACA	2 (3%)
• PCA	9 (12%)
• VB	14 (19%)
• Undetermined	8 (10%)
Asymptomatic extracranial	
• ICA > 30% stenosis	28 (37%)
Antithrombotic treatment	
• Anticoagulants	17 (23%)
• Aspirin	26 (35%)
• Clopidogrel	43 (57%)
Statins	53 (71%)
ACEI	25 (33%)
ARB	10 (13%)
Lp-PLA_2 mass (ng/ml)	287 (232–396)
Lp-PLA_2 activity (nmol/ml/min)	152 (127–176)

Results are expressed as means ± standard deviation, *n* (percentage) or medians (interquartile range) as appropriate. ACA = anterior cerebral artery; ACEI = angiotensin converting enzyme inhibitors; ARB = angiotensin receptor blocker; ICA = internal carotid artery; MCA = middle cerebral artery; mRS = modified Rankin scale; PCA = posterior cerebral artery; VB = intracranial vertebral and basilar arteries.

mass nor activity were associated with the amount of vascular risk factors.

Noteworthy, ICAD progression assessed by ultrasonographic studies during follow-up visits, was not associated with either Lp-PLA_2 mass or activity, as measured at the time of inclusion into the study.

As expected, significant correlations were found between Lp-PLA_2 mass and activity and total or LDL-cholesterol (all $p < 0.05$). However, after adjustment by sex, age and statins intake, only the relation between Lp-PLA_2 activity and total or LDL-cholesterol remained significant ($r = 0.43$, $p = 0.007$ for LDL-cholesterol; $r = 0.39$, $p = 0.03$ for total cholesterol).

3.3. Lp-PLA_2 mass and activity and preventive treatments during follow-up

At the inclusion visit, 17 (23%) of our patients were under anticoagulants treatment, 26 (35%) took aspirin, 43 (57%) clopidogrel and 53 (71%) statins (Table 1). Some of these preventive treatments were associated with lower Lp-PLA_2 mass and activity levels in the univariate analyses, such as statins [Lp-PLA_2 mass 255 ng/ml in patients on statins vs 327 in patients not on statins ($p = 0.034$); Lp-PLA_2 activity 147 nmol/ml/min in patients on statins vs 160 nmol/ml/min in patients not on statins ($p = 0.049$)] or clopidogrel [Lp-PLA_2 mass 251 ng/ml in patients on clopidogrel vs 336 ng/ml in patients not on clopidogrel ($p = 0.001$)].

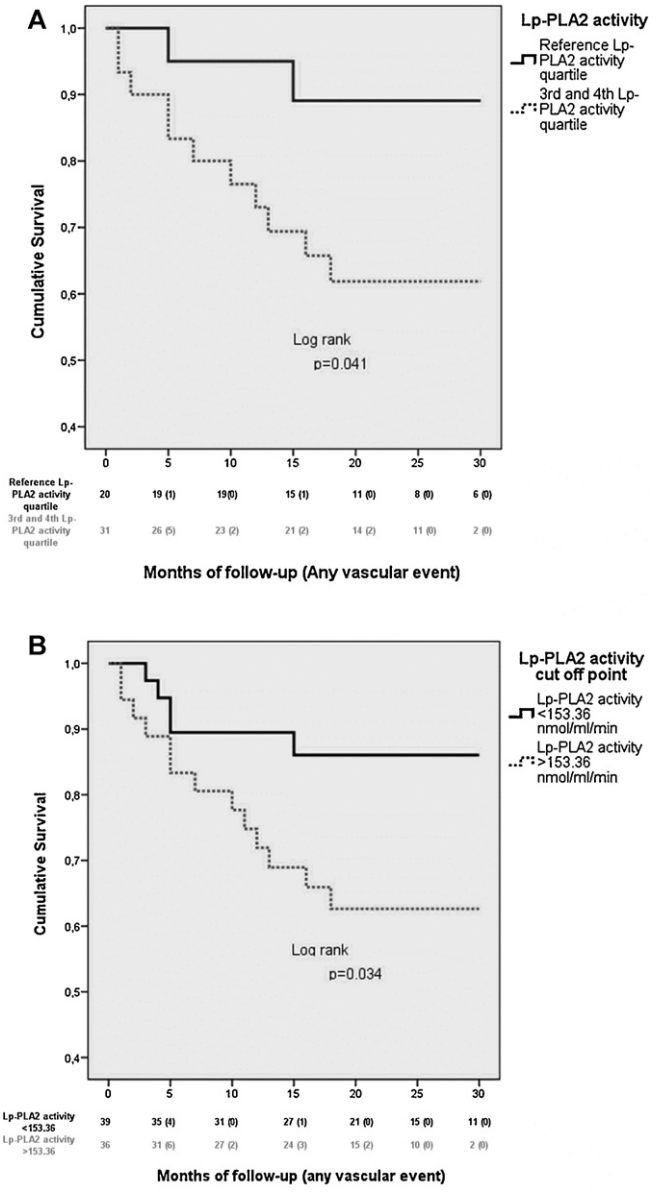

Fig. 2. Panel (A) Kaplan–Meier curves showing the cumulative survival of any vascular event during follow-up between reference quartile (black line) and top 3rd and 4th quartile (grey line). Panel (B) Kaplan–Meier curves showing the cumulative survival of any vascular event during follow-up between groups above (black line) and below (grey line) Lp-PLA_2 activity cut-off point levels.

3.4. Predictors of new major vascular events

During a median follow-up time of 23 months (interquartile range 17–29), 18 patients (24%) suffered a major ischemic event, categorized as follows: 10 ischemic strokes, 3 TIAs and 5 myocardial infarctions (MI). Of these major ischemic events, 9 (50%) occurred within the first 5 months after inclusion and only 4 took place after the first year of follow-up.

Regarding recurrent strokes and TIA, all of them were attributable to intracranial atherostenoses, located in the intracranial internal carotid artery in 5 patients, in the middle cerebral artery in 5 patients, in the basilar artery in 2 and in the posterior cerebral artery in 1.

Survival analyses were performed to identify which, among all baseline factors were associated with the pre-specified combined end-point (stroke/TIA, MI/angina or vascular death). Hypertensive patients and those with more than two vascular risk factors

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Table 2
Demographic characteristics and vascular risk factors according to Lp-PLA_2 levels in patients with symptomatic intracranial stenosis.

Variable	Lp-PLA_2 mass (ng/ml) Median (IQ range)	*p*-Value	Lp-PLA_2 activity (nmol/ml/min) Median (IQ range)	*p*-Value
Gender				
• Men	285 (233-362)	0.951	158 (134–178)	0.02
• Women	297 (219–414)		128 (99–165)	
Age				
• ≤66 years	292 (225–400)	0.953	149 (109–179)	0.167
• >66 years	284 (237–396)		153 (134–177)	
Current smoking				
• No	285 (224–405)	0.562	148 (119–181)	0.246
• Yes	288 (232–393)		157 (134–177)	
Hypertension				
• No	288 (216–425)	0.732	161 (127–183)	0.546
• Yes	286 (235–381)		149 (128–173)	
Diabetes mellitus				
• No	275 (223–384)	0.398	149 (124–177)	0.324
• Yes	303 (240–406)		159 (131–180)	
Hyperlipidemia				
• No	322 (235–412)	0.569	156 (128–187)	0.482
• Yes	286 (232–381)		152 (127–177)	
Basal LDL >130 mg/dl				
• No	281 (220–381)	0.089	141 (119–166)	0.001
• Yes	341 (279–407)		177 (157–189)	
Coronary artery disease				
• No	291 (232–400)	0.498	153 (129–177)	0.743
• Yes	281 (202–382)		163 (131–185)	
Number of cardiovascular risk factors				
• 0–2	302 (230–385)	0.317	158 (131–178)	0.501
• >2	303 (247–400)		158 (133–176)	
Intracranial stenosis by TCD				
• Single	232 (211–425)	0.176	127 (111–156)	0.047
• Multiple.	296 (246–393)		149 (127–178)	
TCD progression				
• No	285 (232–399)	0.841	149 (126–177)	0.598
• Yes	302 (230–385)		158 (131–178)	
Secondary prevention until baseline visit Statins No Yes				
• No	327 (271–396)	0.034	160 (134–179)	0.049
• Yes	255 (216–392)		146 (119–173)	
Aspirin				
• No	280 (232–408)	0.648	151 (126–177)	0.758
• Yes	297 (232–360)		155 (128–178)	
Clopidogrel				
• No	336 (279–422)	0.001	162 (123–180)	0.240
• Yes	251 (217–352)		149 (127–168)	
Baseline episode				
• TIA	304 (225–396)	0.647	152 (120–178)	0.926
• Established infarct	286 (232–399)		152 (129–177)	

Results are expressed as medians (interquartile (IQ) range). LDL = low density lipoprotein; TCD = transcranial doppler; TIA = transient ischemic attack.

showed a trend towards higher recurrence rates ($p = 0.088$ and $p = 0.1$, respectively). Also, the presence of past medical history of coronary artery disease (28% vs 14%, $p = 0.046$) and the increasing number of stenosis detected by TCD ($p = 0.007$) were associated with the presence of the combined end-point. No other baseline factors or preventive treatments were associated with the rate of recurrence.

Regarding Lp-PLA_2 mass, no significant differences in rates of the combined end-point (stroke/TIA, MI/angina or vascular death) were observed across Lp-PLA_2 mass quartiles (combined end-point rates in the first quartile 0%, second quartile 33%, third quartile 28% and fourth quartile 21%, $p = 0.38$).

However, Lp-PLA_2 activity was associated with the presence of new vascular events (combined end-point), which were increased across Lp-PLA_2 activity quartiles [(10% (bottom quartile) vs 21% (second quartile) vs 38% (third quartile) vs 33% (top quartile), $p = 0.04$]. Fig. 2A shows cumulative survival of any vascular event through follow-up between reference quartile and top third and fourth quartiles.

Furthermore, a ROC curve identified 153.36 nmol/ml/min as an optimal cut-off point (sensitivity 0.72 and specificity 0.59, area under the curve = 0.632) to discriminate between the patients who experienced a recurrent vascular event and the patients who did not. Likewise, Kaplan–Meier curve (Fig. 2B) shows a significantly higher rate of vascular recurrence in the group of patients with Lp-PLA_2 activity over 153 nmol/ml/min than in those with levels below that cut-off ($p = 0.034$).

According to the rates of recurrent events individually (stroke/TIA or angina/MI or vascular death alone), no significant association was found with either Lp-PLA_2 mass or activity (data not shown).

Furthermore, in order to find potential predictors for the appearance of the pre-specified end-point, a multivariate proportional hazards regression analysis (Cox Regression) was performed including all associated factors and adjusting for age, gender and vascular risk factors. Among all them, Lp-PLA_2 activity higher than 153 nmol/ml/min was the strongest predictor of vascular recurrence (hazard ratio 2.89; 95% CI 1.029 to 8.096; $p = 0.044$).

Finally, some studies have previously shown how Lp-PLA_2 predictive value might be influenced by LDL levels [15–17] and hormones [18]. Although it would be interesting, our small sample size does not allow to perform stratification analyses exploring these hypotheses.

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4. Discussion

The main finding of the present prospective study is that elevated Lp-PLA_2 activity identifies patients with first-ever stroke or TIA associated with intracranial atherosclerotic disease at higher risk of suffering recurrent vascular events. In our study, patients with Lp-PLA_2 activity over 153 nmol/ml/min had almost three times more risk than patients with activity below that cut-off level, and this risk was independent of other well-known traditional vascular risk factors.

This result is in agreement with most of the previous literature identifying Lp-PLA_2 activity [19,20] as an independent risk biomarker of vascular events and extends previous knowledge in a particularly high-risk group of patients, such as those with intracranial atherosclerotic disease.

Previous stroke studies in healthy population [16,19] or unselected patients during the acute (<72 h), subacute (<6 days) [15] or chronic (6 month) phase [20] after initial stroke, demonstrated that Lp-PLA_2 is a predictor of stroke, coronary heart disease and vascular death [21]. However, to our knowledge, the impact of Lp-PLA_2 levels on the natural history of ICAD had not been addressed so far.

It is well documented that some drugs are Lp-PLA_2 inhibitors: statins, fibrates and nicotinic acid can reduce between 20% and 30% Lp-PLA_2 mass and activity in blood; azetidinones (darapladib, varespladib), combined with statins, achieve reductions in Lp-PLA_2 mass and activity of 66% in blood and 80% in the atherosclerotic plaque; and also angiotensin II type 1 receptor blocker (irbesartan) has shown inhibitory Lp-PLA_2 properties [9,22]. In our study, not only statins but also clopidogrel were associated with lower Lp-PLA_2 levels in univariate analyses. However, the relationship between clopidogrel and Lp-PLA_2 deserves further studies, since it has not been reported previously and might be influenced by potential confounders (other treatments, etc.).

In spite of the predictive value of Lp-PLA_2 activity, Lp-PLA_2 mass levels did not show any association with vascular recurrence in our study population. Therefore, our results could also suggest that activity is a better outcome predictor than mass in certain studies and populations [23,24], and these tests can be complementary in some clinical conditions. However, we have found a weaker significant correlation between Lp-PLA_2 mass and activity ($r = 0.19$, $p = 0.004$) than other previous reports, which have shown correlation between mass and activity ranging from 0.36 to 0.86 [25,26]. This may be the result of a limited sample population of $n = 75$ for the present study.

Unstable atherosclerotic plaques with a rich inflammatory component have been found infrequently in intracranial large vessels, and it has been suggested that the atherosclerotic process in intracranial arteries may have differential characteristics [27].

Furthermore, intracranial stenoses are known to be dynamic lesions whose progression (i.e. worsening degree of stenoses) may determine an increased risk of recurrent ischemic events [4].

In our population Lp-PLA_2 activity was associated with the degree of atherosclerotic intracranial disease at baseline, as the enzyme activity was increased in patients with multiple or bilateral stenoses. However, single measurements at baseline of Lp-PLA_2 mass or activity were not related to disease progression over time as determined by serial TCD examinations, suggesting that mechanisms other than worsening of the stenosis degree might be involved in the risk of recurrent events/are the cause of the recurrent events.

In certain previous reports, Lp-PLA_2 has not been correlated with intima-media thickness (IMT) or atherosclerotic plaque presence [28], but Lp-PLA_2 and its downstream inflammatory mediators LysoPC and oxidized fatty acids (oxFA) have been found in higher amount in symptomatic plaques in association with other instability markers such as macrophages, NAD(P)H oxidase and matrix-metalloproteinase 2 [29]. Therefore, Lp-PLA_2 activity might be associated with vascular recurrence by promoting plaque instability. Similar data had been reported for other inflammatory markers like CRP that determines the risk of ischemic stroke independently of severity of carotid IMT [30].

This study has some limitations. First, the sample size is small and our power is of 75% for the prediction of any ischemic event. Increasing sample size in properly designed multicentre studies would be desirable to improve the power of the study for the prediction of any ischemic event and even more, for the prediction of strokes related to ICAD. Second, since the Lp-PLA_2 mass and activity were measured only once at baseline, intra-individual variation during follow-up could not be assessed. However, very little intra-individual variability in plasma Lp-PLA_2 levels over time [7], as well as between subacute-chronic stages after a vascular event has been described previously [20]. Third, although an extensive work up was done to exclude nonatherosclerotic intracranial stenoses, neither TCD nor MRA nor CTA provides information regarding the histopathological nature of the lesions responsible for vessel narrowing, and we may have included patients with stenoses caused by different underlying vascular pathologies. Finally, we relied on TCD and MRA or CTA for the diagnosis of intracranial stenoses, and not on conventional angiography, considered the gold standard, in order to avoid invasive procedures to our patients.

In conclusion, increased Lp-PLA_2 activity levels strongly predict the risk for new intracranial large-artery occlusive disease-related and other ischemic events in first-ever TIA or stroke patients with intracranial stenoses. Elevated Lp-PLA_2 activity levels in plasma may identify high-risk intracranial large-artery occlusive disease patients, in whom strict vigilance regarding vascular risk factors and therapy combining antithrombotic and anti-inflammatory strategies may be indicated and might be potentially useful in the daily clinics evaluation of vascular recurrence risk, especially in patients without other available instrumental data.

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