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Role of lipoprotein-associated phospholipase A_2 activity for the prediction of silent brain infarcts in women

Iolanda Riba-Llena [a], Anna Penalba [a], Dolors Pelegrí [b], Andrea Vilar [a], Carme I. Jarca [c], Josefina Filomena [d], Joan Montaner [a, e], Pilar Delgado [a, *], on behalf of the ISSYS Study Group

[a] Neurovascular Research Lab, Institut de Recerca Vall d'Hebron (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
[b] Clinical Biochemistry Unit, Hospital Universitari Vall d'Hebron, Barcelona, Spain
[c] Emergency Department, CUAP Horta, Barcelona, Spain
[d] Clot Primary Care Center, Institut Català de Salut, Barcelona, Spain
[e] Stroke Unit and Neurology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

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ABSTRACT

Objectives: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), predicts risk of coronary events and stroke and might be associated with cerebral small vessel disease. We aimed to determine whether silent brain infarcts relate to Lp-PLA_2 activity and also, whether the addition of Lp-PLA_2 activity to prognostic clinical models improves silent brain infarcts' discrimination. **Methods**: Cross-sectional study in 921 stroke-free individuals. On baseline, demographic and vascular risk factors were collected and a brain magnetic resonance was performed to assess for the presence of silent brain infarcts. Serum Lp-PLA_2 activity was tested by an enzymatic assay (PLAC Test for activity) for all study participants and 49 healthy individuals free of vascular risk factors. Multivariate analysis and Integrated Discrimination Improvement were performed to assess whether Lp-PLA_2 activity was independently associated with silent brain infarcts and improved their discrimination added to clinical variables. **Results**: Lp-PLA_2 activity was independently associated with silent brain infarcts in women (OR per one standard deviation increase: 2.14, from 1.31 to 3.50) but not in men (OR = 1.09, from 0.81 to 1.48) after adjustment by age, diastolic blood pressure, total cholesterol, statin treatment and other potential confounders. Adding Lp-PLA_2 to clinical information for SBIs diagnosis resulted in a non-significant and mild improvement in discrimination (IDI = 3.1%) in women. **Conclusions**: Although Lp-PLA_2 is independently associated with silent brain infarcts in women, its addition to clinical variables does not lead to any improvement in their prediction.

1. Background

Silent brain infarcts (SBIs) are well known markers of stroke and dementia [1]. They constitute a significant burden of brain vascular disease and given their association with increasing age, their prevalence and consequences are expected to grow in the near future.

The recognition of the association with increased risk of strokes and dementia in those with SBIs represents an opportunity for intervention and prevention of future poor outcomes. However, future trials are needed to demonstrate the therapeutic gain of this approach by reducing the occurrence of strokes [2]. In this setting, the use of blood biomarkers may facilitate early detection of SBIs and the implementation of preventive treatments in populations at risk.

Some cardiovascular biomarkers, such as troponin T and N-terminal brain natriuretic peptide have been associated not only with the presence but also with the appearance of new SBIs [3]. Other circulating proteins, related to systemic inflammation, have been extensively studied in relation to silent brain infarcts and to other markers of cerebral small vessel disease, such as white matter lesions or measures of brain atrophy. The best known inflammatory biomarkers are C-reactive protein (CRP) and interleukin-6 (IL-6), which have been associated with cerebral small vessel disease in most (though not all) of the studies performed to date [4–13].

* Corresponding author. Neurovascular Research Lab, Vall d'Hebron Research Institute (VHIR), Passeig Vall d'Hebron, no. 119-129, CP 08035 Barcelona, Spain.
E-mail address: pilar.delgado@vhir.org (P. Delgado).

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Other less explored biomarkers in this field are myeloperoxidase, and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) mass which were associated with white matter lesions volumes [14].

Regarding Lp-PLA_2, this is a serine lipase which circulates mainly linked to low-density lipoproteins and, in a small fraction, to high-density lipoproteins. Lp-PLA_2 is known to be a good predictor of both first-ever coronary events and ischemic stroke in population-based studies [15] and it is approved by the Food and Drug Administration as a predictor of vascular risk. Also, it has been associated with the risk of recurrent strokes [16–18]. Lp-PLA_2 is suggested to be involved in the development of atherosclerotic stroke [19], but there are also some evidences linking Lp-PLA_2 with cerebral small vessel disease [14,20].

In this study, we hypothesized that Lp-PLA_2 activity might be as well associated with the presence of silent brain infarcts, as markers of cerebral small vessel disease and further we explored whether the addition of Lp-PLA2 activity to prognostic models containing clinical information improves SBIs discrimination.

2. Materials and methods

Subjects for this study are included within the ISSYS (Investigating Silent Strokes in hYpertensives: a magnetic resonance imaging Study), a cohort study on a randomly selected sample of hypertensive individuals, aged 50 to 70 and without prior stroke or dementia. Details on subject selection and baseline procedures have been published elsewhere [21,22].

Briefly, subjects in this study were randomly recruited from primary care centers in the city of Barcelona, between November 2010 and May 2012. Inclusion criteria which were verified first by the responsible primary care physicians and further by trained investigators after reviewing clinical records and interviewing the patient during the baseline visit, consisted on: 1) Patients with essential hypertension diagnosed at least one year earlier; 2) Age comprised between 50 and 70 years; and 3) Patients who gave their informed consent to participate in the study. Patients were excluded when: 1) they had history of previous stroke or dementia; 2) brain MRI was contraindicated; 3) there was a suspicion of white coat hypertension syndrome or 4) patients were affected by a terminal illness preventing any future follow-up examination, based on the investigator criteria. In case of suspicion of secondary hypertension, the patients were referred to other medical specialists (i.e. endocrinologist, nephrologist, etc.) for further evaluation and were not included in this study.

After inclusion, participants were asked about demographical data and personal medical history, including questions about the presence of other vascular risk factors such as diabetes mellitus, dyslipidemia and smoking habit (current, former, never), among others. Diabetes mellitus was defined as fasting glucose levels over 7 mmol/L or the use of oral antidiabetic drugs or insulin. Dyslipidemia was defined as total cholesterol over 5.2 mmol/L, triglycerides over 2.3 mmol/L and/or the use of lipid lowering treatments. Also, a directed questioning and review of medical records was performed to assess for the existence of a previous cardiovascular or kidney disease. For those participants without previous vascular disease, global vascular risk was estimated by applying the Framingham-calibrated REGICOR function [23] and participants were divided into the following categories depending on their 10-year risk of having a coronary event: Low risk (<5%), moderate risk (5–9.9%), high risk (10–14.9%) and very high risk (≥15%).

Hypertension stage and blood pressure control as defined in the 2007 guidelines of the European Hypertension/Cardiology Societies [24] were also recorded, together with the duration of hypertension for each individual.

A brain MRI was performed to evaluate the presence of SBIs which were defined as lesions of ≥3 mm in their widest diameter, with cerebrospinal fluid-like signal characteristics in all pulse sequences, and a hyperintense rim surrounding the lesion in FLAIR images [25]. All examinations were performed with the same 1.5 T MR (Signa HDx 1.5, General Electrics, Waukeska, WI), including axial T1, T2 and FLAIR (fluid-attenuated inversion recovery) weighted images. SBIs were differentiated from enlarged perivascular spaces based on their size, shape and location. All images were primarily assessed by two neurorradiologists and in a second term by the same readers plus an experienced stroke neurologist, all blinded to clinical characteristics. Intra-rater agreement (which was calculated for each reader in a training set before undertaking the actual reading) and inter-rater agreements ranged from 0.6 to 0.75. Disagreements in assessments were solved by consensus [21].

Blood samples were drawn from each participant at baseline visit and serum was obtained after 15 min centrifugation and frozen for further analysis. Total cholesterol (Olympus, Lismeehan, Ireland) and Lp-PLA_2 activity (PLAC test for activity, diaDexus Inc.) were determined using enzyme assays on a single automated clinical chemistry analyzer (Olympus AU 2700) and in the same laboratory. Lp-PLA2 measurements were obtained once in each patient and were conducted between July and August 2012. For Lp-PLA_2, intra-assay and inter-assay variation were less than 2.5% at the lowest and highest concentrations, as others reported before [26]. In 51 patients (5.2% from the total sample), Lp-PLA_2 activity could not be determined, because of excessive turbidity of the sample or hemolysis. In these conditions, according to the manufacturer's instructions, results might be erroneous and must be discarded.

In addition to ISSYS participants, 49 healthy subjects free of vascular risk factors and vascular diseases, were tested and their values were used as reference group. They were matched by gender with the study participants but significantly younger than them (median age = 51 versus 64 years old, $p = 0.001$).

All statistical analyses were conducted using SPSS version 17.0. For descriptive purposes, normality for continuous variables was assessed by Kolmogorov–Smirnov test. Since Lp-PLA_2 activity was not normally distributed, values are given in median and interquartile range. In univariate analysis, continuous variables were compared using *t*-test and ANOVA or Mann–Whitney *U* test and Kruskal–Wallis test when appropriate. Categorical variables were compared using Pearson χ^2 and continuous data were analyzed using Pearson or Spearman correlation depending on their normality. We set statistical significance at p value <0.05. SBIs were dichotomized into 2 categories (present/absent). We performed univariate and multivariate analysis to relate Lp-PLA_2 activity to the presence of SBIs. Multivariate models were adjusted by age, gender, dyslipidemia, diastolic blood pressure (DBP), diabetes and current smoking (since all have been associated with SBIs in our sample or in other previous studies), and also by total cholesterol, statin treatment and by the presence of an established cardiovascular disease. Moreover, since differences are described for Lp-PLA_2 regarding gender, we tested for interaction with gender in the association between Lp-PLA_2 activity and the presence of SBIs.

Further, to look for the presence of a threshold effect, Lp-PLA_2 was divided into quartiles in women and, in order to compare global diagnostic accuracy of a model with only clinical variables and a model including such clinical variables plus Lp-PLA_2 activity, receiver operator curves (ROC) were configured and the area under the curves compared by means of Delong's method [27] using MedCalc 12.4 software. Improvements on the performance of clinical variables (clinical model) by adding the information on Lp-PLA_2 activity were measured by the Integrated Discrimination