Biomarkers to predict clinical progression in small vessel disease strokes: Prognostic role of albuminuria and oxidized LDL cholesterol

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A B S T R A C T

Objective: Clinical progression in lacunar strokes (LS) is an unpredictable and fearful complication. Endothelial dysfunction (ED) is believed to be the first step in the pathophysiology of LS therefore we aimed to analyze the association of three markers of ED: albuminuria, von Willebrand factor (vWF), and oxidized LDL cholesterol (ox-LDL) with LS progression.

Methods: From December 2007 to December 2010, 127 LS patients admitted within 6 h of symptom onset were prospectively assessed. Progression was defined as initial NIHSS score worsening ≥4 points within the first 72 h. Analysis of vWF and ox-LDL was done at admission. Albuminuria was measured in the first morning spot urine. Association between 3 biomarkers and progression was tested using logistic regression analysis. Other clinical variables of interest were also studied. Discriminative power was analyzed with a receiver operator curve.

Results: Twenty-two patients (17.3%) progressed. Progression was associated with worse outcome at 90 days. Albuminuria and ox-LDL were associated in univariate analysis; vWF was not. Adjusted OR were: ox-LDL [OR: 1.03; 95% CI: 1.01–1.07, p = 0.019], albuminuria [OR: 2.07; 95% CI: 1.04–4.13, p = 0.039]. Association was linear without a cut-off point. Clinical variables were not associated with progression. The model including albuminuria and ox-LDL had a good predictive value [AUC: 0.80 [0.70–0.89]]

Conclusions: Albuminuria and ox-LDL levels are independently associated with higher risk of progression in LS. The lack of reliable clinical predictors makes biomarker research a priority to improve progression detection in this subtype of ischemic strokes.

1. Introduction

Lacunar strokes (LS), or small-vessel disease strokes, result from occlusion of a single penetrating artery and account for one quarter of cerebral infarctions [1]. Despite current therapies, LS are the major cause of progressive motor deficits, which are closely associated with a worse clinical outcome [2]. Although inflammation and excitotoxicity related molecules have been associated with LS progression [3–5], no biomarker is available for routine use in clinical practice to identify patients at higher risk of progression.

Endothelial dysfunction (ED) plays a key role in the development of systemic vascular disease [6]. Recently ED has been suggested as the primary step in the pathogenesis and severity of small vessel disease, although it is unclear which mechanisms promote ED in LS [7,8]. ED means the loss of the vascular homeostasis, which leads to an array of endothelial responses such as vasoconstriction, increased inflammation and cell permeability, enhanced oxidative stress, impaired coagulation, and vascular cell proliferation [6]. ED might be assessed through functional methods that can be invasive, such as coronary vasodilatation in response to acetylcholine, or non-invasive, such as ultrasound flow-mediated dilation (FMD) of the brachial artery and non-functional methods, including identification of certain endothelium-released molecules involved in coagulation and inflammation, endothelial circulating cells and markers of vascular damage.

In previous studies, markers of vascular tone, thrombogenesis, and inflammation have been found elevated in LS patients compared with control subjects [8]. However, the association between these markers and lacunar progression has not been assessed.

We hypothesized that markers of systemic ED could help to predict progression in LS. Therefore the aim of the study was to analyze the predictive value of 3 well-studied biomarkers: albuminuria [9], von Willebrand Factor (vWF) [10], and oxidized low-density lipoprotein (ox-LDL) [11] to detect LS patients with high risk of progression.
2. Materials and methods

2.1. Patients and methods

All patients admitted in the Stroke Unit from December 2007 to December 2010 within the first 6 h of symptoms onset were included in a prospective study designed to evaluate clinical progression in acute LS patients. Patients were evaluated at hospital admission and at least twice a day during the first 3 days by a trained neurologist who established initial severity using the NIH stroke scale (NIHSS). All patients had an initial brain CT. Lacunar stroke was defined using the SSS-toast criteria [12] as a single clinically relevant acute infarction of less than 20 mm in greatest diameter, within the territory of basal or brainstem penetrating arteries, in the absence of any other pathology and confirmed by DWI-MRI study, which in our case was performed between 72 h and 6 days of admission.

B-mode color Doppler carotid ultrasound and transcranial Doppler were performed in all patients to rule out stenosis. Angio MRA or Angio CT was performed in selected cases during hospitalization to confirm the results. Patients with significant intra or extracranial stenosis (≥50%) in the symptomatic artery were not included in the study. Moreover all patients had a cardiology study to rule out embolic etiologies.

Cardiovascular risk factors and previous treatments were registered in a structured questionnaire (Basicmar) according to previously reported methodology [13].

Treatment was decided in each case following international consensus in neurovascular diseases. Initially, antiplatelet therapy was started with aspirin (300 mg/day). Patients with aspirin intolerance received other antiplatelet drugs, such as clopidogrel (75 mg/day) or triflusal (600 mg/day). In patients already receiving aspirin treatment, 75 mg clopidogrel was initiated. Glycemia and temperature were monitored every 6 h. Insulin and antipyretic treatment were given according to international guidelines. Blood pressure (BP) was determined each hour during at least the first 48 h. Antihypertensive agents were administered in patients with heart failure and if the diastolic BP was >120 mm Hg or systolic BP was >220 mm Hg.

Clinical progression was defined as a worsening by at least 4 points in the initial NIHSS score within the first 72 h. This methodology had been previously validated [13,14].

Outcome was assessed at 90 days by a neurologist examination. Poor outcome was defined as a modified Rankin scale score (mRs) of 3–6 at 90 days.

We analyzed the associations between albuminuria, plasma vWF, and ox-LDL levels for evidence of progression. We also studied potential associations with other factors previously associated with progression, such as age, history of hypertension and diabetes, stroke severity and initial blood pressure (BP), calculated as the mean arterial pressure (MAP) with the formula \((2/3 \times \text{diastolic BP} + 1/3 \times \text{systolic BP})\). Other factors analyzed were: plasma glucose levels, leucocyte count, sex, vascular risk factors and previous medications including statins, antiplatelet medication, angiotensin converting enzyme inhibitors (ACEI) and/or angiotensin II receptor antagonists (ARA-II).

Data collection for the study followed local ethics committee guidelines. Patient data was analyzed with complete anonymity. All patients signed an informed consent.

2.2. Laboratory measurements

Blood samples were obtained in all patients at hospital admission, within the first 6 h of symptoms onset. For the measurements of ox-LDL and vWF concentrations, blood was drawn into tubes containing K₂EDTA (1.8 mg/mL) and plasma was separated by centrifugation at room temperature (15 min at 1500 \(\times\) g). Plasmas were kept frozen at −80 °C until their use. The concentration of ox-LDL in plasma was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) procedure (ox-LDL; Mercodia AB, Uppsala, Sweden). Intra- and interassay coefficients of variation (CVs) were 6.02 and 10.42%, respectively.

VWF was measured using a commercial ELISA (DG-EIA vWF; Diagnostic Grifols, #218008). Intra- and interassay CVs were 0.40 and 6.6%. Results are given as a percentage from reference plasma (%vWF).

Albuminuria was measured in the first 24 h morning spot urine sample, which was obtained either from spontaneous urination or by urethral catheterization, using a commercial immunoturbidimetric assay (Hitachi P-800, Roche). Intra- and interassay coefficients of variation (CV) were 1.3% and 4.3%, respectively.

2.3. Statistical analysis

Data were presented as means ± SD for continuous variables and as frequencies and percentages for categorical variables. \(T\) test and \(\chi^2\) test were used to evaluate the differences in means for continuous variables and in percentages for categorical variables, respectively. Albuminuria, vWF, and ox-LDL were analyzed as continuous variables. Logarithmic transformation of albuminuria was performed to achieve a normal distribution. Pearson coefficient correlation (\(r\)) was used to assess collinearity between the 3 biomarkers. Univariate analyses were performed between the biomarkers and the study variables and between the study variables and clinical progression. Multivariate OR with 95% CI was estimated by a logistic regression model. We analyzed 2 adjusted models. The first included only variables that obtained a \(P\) value < 0.05 in the univariate analysis. The second model also included interest variables according to previous studies, such as age, initial NIHSS, and history of hypertension and diabetes. Linear effect of the 3 molecules was tested under a Generalized Additive Model (GAM). The discriminative power of the model was analyzed using a receiver operator curve (ROC) between predicted risk and observed status. Two-sided \(P\) values < 0.05 were considered statistically significant. All statistical analyses were performed by a biostatistician (I. Subirana) with SPSS (version 15.0) and R software (version 2.11.1).

3. Results

From December 2007 to December 2010, 625 ischemic strokes were attended in the acute phase and 135 (21.6%) fulfilled LS criteria. Four patients were excluded by previous conditions that could influence the concentration of the 3 biomarkers of interest, such as active malignancies at the time of stroke (\(n = 2\)) and plasma creatinine levels ≥1.5 mg/dL (\(n = 2\)). Four patients were excluded due to missing data or plasma samples. Baseline characteristics of these patients were similar except for older age.

The final cohort was 127 patients. Demographic data, cardiovascular risk factors, and pretreatments are summarized in Table 1.

During the first 72 h, 22 patients (17.3%) progressed, most of them within the first 24 h (\(n = 16, 72.7\%). No differences in the distribution of the main cardiovascular risk factors or pretreatments were found between patients with and without progression (Table 1).

Poor outcome was found in 28 (22%) patients. Although initial severity was not statistically different, poor outcome was higher in patients that had clinical progression (40.9% vs. 18.1%, \(p = 0.019\)). Similarly, in univariate analysis initial MAP [123.65 (17.92) vs. 110.05 (17.53) mm Hg, \(p = 0.002\)], albuminuria [1.65 (0.73) vs. 1.07 (0.62) mm Hg, \(p < 0.001\)], and ox-LDL levels [76.39 (17.94) vs. 62.42 (14.67) U/L, \(p < 0.001\)] were associated with progression. However,
Table 1
Univariate comparison according to progression.

<table>
<thead>
<tr>
<th></th>
<th>Total n = 127</th>
<th>With progression n = 22</th>
<th>Without progression n = 105</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>89 (70.1)</td>
<td>13 (59.1)</td>
<td>76 (72.4)</td>
<td>0.216</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>70.05 (11.82)</td>
<td>72.77 (10.85)</td>
<td>69.49 (11.98)</td>
<td>0.237</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>92 (72.4)</td>
<td>16 (72.7)</td>
<td>76 (72.4)</td>
<td>0.974</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>38 (29.8)</td>
<td>6 (27.3)</td>
<td>32 (30.5)</td>
<td>0.765</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>32 (25.2)</td>
<td>7 (31.8)</td>
<td>25 (23.8)</td>
<td>0.431</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>8 (63)</td>
<td>1 (4.5)</td>
<td>7 (6.7)</td>
<td>0.710</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>9 (7.1)</td>
<td>3 (13.6)</td>
<td>6 (5.7)</td>
<td>0.188</td>
</tr>
<tr>
<td>Previous LS, n (%)</td>
<td>21 (16.5)</td>
<td>3 (13.6)</td>
<td>18 (17.1)</td>
<td>0.687</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>23 (18.1)</td>
<td>3 (13.6)</td>
<td>20 (19.0)</td>
<td>0.549</td>
</tr>
<tr>
<td>Antiplatelet use, n (%)</td>
<td>31 (24.4)</td>
<td>4 (18.2)</td>
<td>27 (25.7)</td>
<td>0.455</td>
</tr>
<tr>
<td>ACE/ARAII use, n (%)</td>
<td>28 (22)</td>
<td>5 (22.7)</td>
<td>23 (21.9)</td>
<td>0.933</td>
</tr>
<tr>
<td>Initial severity median (q 1–3)</td>
<td>3 (2–5)</td>
<td>4 (2–6)</td>
<td>3 (2–5)</td>
<td>0.318</td>
</tr>
<tr>
<td>MAP mm Hg, mean (SD)</td>
<td>112.30 (18.24)</td>
<td>122.60 (18.16)</td>
<td>110.14 (17.59)</td>
<td>0.003</td>
</tr>
<tr>
<td>Glycemia, mg/dL, mean (SD)</td>
<td>136.98 (66.03)</td>
<td>148.00 (79.59)</td>
<td>134.67 (63.02)</td>
<td>0.397</td>
</tr>
<tr>
<td>Leucocyte count</td>
<td>7802.16 (2248.3)</td>
<td>8250.0 (3004.83)</td>
<td>7802.16 (2248.3)</td>
<td>0.426</td>
</tr>
<tr>
<td>vWF %, mean (SD)</td>
<td>136.09 (48.2)</td>
<td>132.12 (45.28)</td>
<td>136.92 (48.96)</td>
<td>0.673</td>
</tr>
<tr>
<td>ox-LDL, mean (SD)</td>
<td>64.73 (16.05)</td>
<td>75.74 (17.78)</td>
<td>62.43 (14.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albuminuria, mean (SD)</td>
<td>1.17 (0.72)</td>
<td>1.65 (0.73)</td>
<td>1.07 (0.68)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

vWF levels were not different in patients with and without progression [132.55 (46.35) vs. 136.79 (48.74), p = 0.714].

There was a positive correlation between albuminuria and ox-LDL levels (r = 0.255, p = 0.004) but not between vWF and the other biomarkers. Albuminuria and ox-LDL were also correlated with MAP (r = 0.23, p = 0.009 and r = 0.20, p = 0.019, respectively). Regarding vascular risk factors, vWF was associated with age (r = 0.27, p = 0.002) and ox-LDL was associated with female sex [70.30 (16.73) vs. 62.36 (15.23), p = 0.010] and dyslipidemia [70.01 (18.12) vs. 62.21 (14.40), p = 0.019]. No association was found between the biomarkers and previous treatments.

The logistic regression model that included only the variables associated in the univariate analysis showed an independent association between clinical progression and ox-LDL levels [OR: 1.03; 95% CI: 1.01–1.07, p = 0.019] and albuminuria [OR: 2.07; 95% CI: 1.04–4.13, p = 0.039], while the association with MAP [OR: 1.03; 95% CI: 0.99–1.06, p = 0.060] did not reach significance (Table 2, model 1). The linearity of adjusted effect of albuminuria and ox-LDL levels on progression was confirmed in a GAM model regression (p = 0.411 and p = 0.343, respectively), therefore we did not look for a cut-off point. However, the risk of progression for patients in the highest quartile of ox-LDL (>75.24 U/L) and albuminuria (>45.30 mg/L) was notably higher than for patients in the lower quartiles [34.4% vs. 11.6%, unadjusted OR: 4.0; 95% CI: 1.52–10.47, p = 0.003]. In addition, for every 10 U/L of ox-LDL adjusted risk increased 46% [OR: 1.46; 95% CI: 1.06–2.03, p = 0.019].

We performed a second analysis including variables of interest in the multivariate models (Table 2, model 2). These adjustments did not change the estimated effects of ox-LDL or albuminuria substantially. Discrimination power in predicting clinical progression by the model including albuminuria, ox-LDL and MAP (Table 2, model 1) was accurate [AUC: 0.80 (0.70–0.89)] (Fig. 1). Adding other clinical variables (Table 2, model 2) did not improve risk prediction.

4. Discussion

The present study shows an association between the risk of progression and 2 biomarkers related to ED, albuminuria and ox-LDL, in a prospective cohort of LS monitored acutely and intensively. On the other hand the most specific marker of systemic ED, vWF, was not associated with progression.

Clinical progression was found in 17.5% of the patients, a slightly lower percentage than in other studies [3,15–17], probably due to our strict definition of progression in an effort to avoid fluctuation.

Table 2
Multivariate analysis and progression.

<table>
<thead>
<tr>
<th></th>
<th>Progression</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ox-LDL</td>
<td>1.03 (1.01–1.07)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>Albuminuria</td>
<td>2.07 (1.04–4.13)</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>1.03 (0.99–1.06)</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ox-LDL</td>
<td>1.05 (1.02–1.10)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Albuminuria</td>
<td>2.53 (1.22–5.24)</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Initial NIHSS</td>
<td>1.08 (0.93–1.26)</td>
<td>0.246</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (0.99–1.10)</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.41 (0.44–4.49)</td>
<td>0.560</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.24 (0.38–4.05)</td>
<td>0.719</td>
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</tr>
</tbody>
</table>
ing symptoms and interobserver variability. Prognosis at 90 days was similar to previous reports [3,17], with higher incidence of disability in patients that suffered progression [15].

Several clinical variables such as age, history of diabetes or hypertension, and stroke severity have been associated with progression [3,4,15,18]. Our study found no significant association between progression and vascular risk factors or previous medications; however, the limited size of the study might have influenced these results.

According to our results high BP might have a key role in the development of progression. However, previous studies have shown conflicting results probably due to the inclusion of different stroke subtypes. Whereas BP drops seem to be deleterious in strokes with large hyperperfused areas, the effect of BP changes in lacunar strokes is unknown [19].

In the last decade various studies have searched for biomarkers of LS progression. Although more research is needed to fully establish the mechanisms involved, enlargement of the infarcted area due to inflammation or excitotoxicity has been suggested as a marker. In a prospective cohort of 113 LS patients, plasma concentrations of glutamate >200 μmol/L and GABA <240 nmol/L had a positive predictive value for LS progression of 67% and 84%, respectively [3]. Inflammatory markers such as tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and intercellular adhesion molecule 1 (ICAM-1) were also independently associated with progression and poor outcome [4]. Other studies report increases in systemic inflammatory parameters such as leucocyte count, fibrinogen and matrix metalloproteinase 9 (MMP-9) in patients with progressive LS [5,20]. In our cohort the leucocyte count was higher in the progressive group, although the difference was not statistically significant.

Endothelial dysfunction has been implicated in the pathophysiology of small-vessel disease [8,21], although the possible impact on progression has not been addressed. The present study was designed to test the predictive role of 3 biomarkers related to systemic ED through different molecular mechanisms in the development of progression.

Albuminuria results from higher transvascular leakiness due to a malfunction of the glomerular endothelium that has been associated with systemic vascular disease through oxidative stress, higher permeability, and arterial stiffness [9,22,23]. ox-LDL is considered a marker of oxidative stress that has also been associated with arterial stiffness and atherogenesis [24]. Finally, vWF plays a crucial role in platelet adhesion and aggregation and is considered a robust marker of systemic ED [10].

Whereas albuminuria and ox-LDL concentration were independently associated with progression, vWF levels did not have predictive value. The adjusted model that included albuminuria, ox-LDL and initial MAP showed an accurate discriminative power to predict clinical progression; inclusion of other clinical variables did not significantly improve the predictive power. Moreover, the predictive effect of albuminuria and ox-LDL was linear without a cut-off point, although progression risk was notably higher for the upper two quartiles.

To our knowledge the role of albuminuria and ox-LDL in LS progression had not been previously studied and vWF had only been analyzed in a study of 46 LS patients that found lower vWF levels in patients that suffered progression (147.3 ± 59 vs. 193.5 ± 60.5, p = 0.041). However, plasma was taken one day after admission and results were not adjusted [5].

Our study design does not permit any pathophysiological explanation because we are studying a cerebral process using systemic biomarkers. Correlation of these biomarkers with the status of the cerebral microvasculature is unknown. Nevertheless, our results would suggest that systemic ED is not implicated in the progression of LS and that other molecular mechanisms related to oxidative stress and arterial stiffness could be more useful for progression prediction.

The study has several limitations. The sample size might have influenced the magnitude of association between study variables. Although the number of patients included is low, all were admitted during the first 6 h after onset of symptoms, which is of crucial importance because progression occurs more frequently during the first 24 h. BP was measured only upon the patient's arrival. Measurement of BP changes between the first clinical evaluation and when progression was diagnosed could have been more informative to establish the role of BP in acute LS. Albuminuria was measured the morning after admission; by then, 8 patients (6.3%) already showed clinical progression. Measurement at patient admission is feasible, correlates well with the 24-h absolute urinary albumin concentration [25], and would be more useful in clinical practice. Since the ability to predict progression on the basis of a clinical profile is low [26], the use of biomarkers to identify patients at higher risk would be very helpful. Moreover, measuring different types of biomarkers is interesting because the mechanisms involved in progression are not clearly established. In previous studies the markers of excitotoxic damage were determined by high-performance liquid chromatography of plasma samples, a technique that is not useful in the clinical practice. Albuminuria and ox-LDL are biomarkers that are easy to measure with the techniques used in our study. Results might be ready in just a few hours after the patient's arrival. Our results should be confirmed in other cohorts, testing all biomarkers on admission in order to establish cut-off points that would be more helpful in routine clinical practice. Inflammatory markers such as TNF-α, ICAM-1, IL-6 and MMP-9 should also be included to determine what markers are necessary to measure in order to predict progression.

5. Conclusions

Clinical progression of LS is a frequent complication with an important impact on functional outcome. Albuminuria and ox-LDL levels have an independent and linear association with higher risk of clinical progression in LS patients and might be used in clinical practice in the future. The lack of predictive clinical models gives a priori importance to continued research on biomarkers to develop a panel that will help to detect patients at high risk.

Conflict of interest

All the authors report no conflicts of interest.

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