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sTWEAK is a marker of early haematoma growth and leukoaraiosis in intracerebral haemorrhage

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ABSTRACT

Objective To study the association between early growth of haematoma with biomarkers of endothelial dysfunction such as leukoaraiosis (LA) and the soluble tumour necrosis factor-like weak inducer of apoptosis (sTWEAK) in patients with intracerebral haemorrhage (ICH).

Methods This is a retrospective observational study of patients with nontraumatic ICH. Clinical and biochemical parameters were analysed. sTWEAK levels were measured by ELISA. LA was analysed in the hemisphere without haemorrhage to avoid interference with the acute injury. The main endpoint was the haematoma growth evaluated by the difference in volume between the second and the initial neuroimage. Poor functional outcome, defined as a modified Rankin Scale >2 at 3 months, was considered as secondary endpoint. Receiver operating characteristic curve analysis was performed to stablish the best cut-off for sTWEAK levels associated with haematoma growth.

Results We included 653 patients with ICH in our analysis (71.1±11.9 years, 44% women). Haematoma growth was observed in 188 patients (28.8%). sTWEAK levels ≥5600 pg/mL predicted ICH growth with a sensitivity of 84% and a specificity of 87%. sTWEAK levels ≥5600 pg/mL and the presence of LA were associated with haematoma growth (OR: 42.46; (CI 95% 22.67 to 79.52) and OR: 2.73 (CI 95% 1.39 to 5.34), respectively). Also, the presence of LA (OR: 4.31 (CI 95% 2.89 to 6.42)) and the interaction between ICH growth and sTWEAK (OR: 2.23 (CI 95% 1.40 to 3.55)) were associated with poor functional outcome at 3 months.

Conclusion sTWEAKs, together with the presence and grade of LA, are biomarkers able to predict ICH growth and poor functional outcome in patients with ICH.

INTRODUCTION

Intracerebral haemorrhage (ICH) accounts for 10%–15% of all strokes and is associated with poor prognosis.[1 2] Leukoaraiosis (LA), also referred to as white matter lesions, has been linked to spontaneous ICH[3] and also to the ICH volume in anticoagulated patients and in patients treated with fibrinolytic drugs.[4 5] LA has been associated with the early growth of ICH,[6 7] which is observed in 10%–30% of patients with ICH[1] and is related with both early neurological deterioration and poor outcome at 3 months.[8] Conversely, other studies concluded that LA is not associated with neither the ICH volume[9–11] nor the ICH growth;[12 13] therefore, further studies are still needed to explain the actual implication of LA in the evolution of the ICH lesion.

LA is considered a surrogate marker for small vessel disease,[14] and there are several evidences in the literature that show the association of LA with endothelial dysfunction and blood brain barrier (BBB) damage.[15 16] Based on these evidences, in this work, we have tested an endothelial dysfunction marker, the soluble tumour necrosis factor-like weak inducer of apoptosis (sTWEAK), as a potential biomarker associated with the presence of LA. sTWEAK is constitutively expressed by monocytes and endothelial cells and binds to the Fn14 receptor resulting in the stimulation of signalling pathways and the release of proinflammatory molecules.[17–20] The TWEAK-Fn14 pathway is involved in a considerable number of pathologies, and sTWEAK is released under several inflammatory and degenerative diseases of the central nervous system such as ischaemic stroke,[21] subarachnoid haemorrhage,[22] traumatic brain injury,[23] neuroinflammation[24] and could have important implications in the progression of ICH, as demonstrated in preclinical studies.[25] As such, we have recently shown the independent association between sTWEAK and LA with haemorrhagic transformation and poor outcome in patients with ischaemic stroke undergoing reperfusion therapies.[26]

In this work, we hypothesize that sTWEAK could act as a mediator in the early growth of haematoma in patients with ICH and with LA, and consequently may be a biomarker to predict the risk of haematoma growth in patients with ICH and with LA. In this regard, we evaluated the association of serum sTWEAK levels in patients with ICH and with different degrees of LA and in patients with no LA, in relation to early

growth of haematoma and the poor functional outcome at 3 months.

METHODS

Study design

This is a retrospective observational study of patients with nontraumatic ICH included consecutively and prospectively in a data bank.

The main objective of this study was to analyse the association between sTWEAK and the presence of LA with the early growth of haematoma. The secondary objectives were to analyse the association between sTWEAK and LA with the basal volume of ICH and functional outcome at 3 months.

Standard protocol approvals, registration and patient consents

Patients were treated in the emergency service of a single tertiary university hospital and later admitted to the stroke unit. All patients were treated by neurologists with special training in cerebrovascular diseases and managed according to protocols based on the Spanish and international clinical guidelines.[27 28] This study was carried out in accordance with the Declaration of Helsinki of the World Medical Association (2008).

Inclusion and exclusion criteria

From January 2008 to December 2018, 1100 patients with spontaneous ICH were included in the data bank. The inclusion criteria were: (1) authorization for the anonymous use of the data for research (n=1023), (2) CT study at inclusion and between the second and fourth day (n=895), (3) hemispheric location (n=1019), (4) no ICH surgery (n=1089), (5) follow-up (face-to-face or telephone) within a minimum of 3 months (n=1066), (6) no known comorbidity with a life expectancy of less than half a year (n=996), (7) stored serum sample in the stroke biobank (n=986). The final number of included patients was 653.

Clinical and analytical variables

The severity of the neurological deficit was determined by the National Institute of Health Stroke Scale (NIHSS) on admission. Functional outcome was assessed by the modified Rankin Scale (mRS) at discharge and at 3 months±15 days. Both scales were evaluated by internationally certified neurologists and supervised by the same neurologist. Poor outcome was defined as an mRS >2 at 3 months.

Blood samples, obtained from all patients at admission, were collected in test tubes, centrifuged at 3000 g for 15 min and immediately frozen and stored at −80°C. Serum levels of sTWEAK were measured using commercial ELISA kits (Elabsciences, USA) following the manufacturer instructions. The intra and interassay coefficients of variation were 5.1% and 5.2%, respectively. All determinations were performed in a laboratory blinded to clinical and radiological data. The other laboratory tests were conducted by the Central Laboratory of the University Clinical Hospital of Santiago de Compostela and performed on fresh blood samples at the time of diagnosis or shortly after.

Neuroimaging studies

All neuroimaging studies were supervised by the same neuroradiologist who was blinded to this study. The images of LA were stratified according to the Fazekas scale.[29] LA was analysed in the initial CT scan in the hemisphere without ICH to avoid interference with the acute injury. The volumes were determined using the ABC/2 method[30] until 2016 and through automated planimetric method afterwards. The ICH growth (difference in volume between the second and the initial neuroimage) was categorised in ≤33% versus >33% for its sensitivity and specificity according to the literature.[31 32]

Statistical method

We have described the variables, either categorical (described with frequency and percentage) or continuous (represented as mean±SD or median and IQR (25th and 75th percentiles)). The adjustment to normal distribution was determined with the Kolmogorov-Smirnov test with the Lilliefors correction. χ^2 tests were performed to determine differences in the variables between patients with and without ICH early growth. A receiver operating characteristic (ROC) curve was performed to stablish a cut-off point for sTWEAK levels to predict haematoma growth. Logistic regression analyses were performed to identify those variables independently associated with haematoma growth, initial haematoma volume and poor functional outcome after adjustment by those variables with statistical significance in the bivariate analysis. These results were shown as ORs with 95% CI . A p value <0.05 was considered statistically significant in all analyses. All statistical analyses were conducted in SPSS V.21.0 (IBM, USA).

RESULTS

We analysed a sample of 1100 patients with ICH admitted in a tertiary hospital between January 2008 and December 2018. Among them, 653 were included in our study, with an average age of 71.1±11.9 years and 44% women. We did not find significant differences with the excluded patients in any of the clinical or analytical variables analysed (online supplemental table S1). This analysis demonstrates the lack of bias in the selection of admitted patients, despite the retrospective nature of this study.

Association between haematoma growth with sTWEAK levels and LA

Haematoma growth, defined as an increase of more than 33% in the haematoma volume measured in a second neuroimage, was observed in 188 patients (28.8%). Figure 1 shows a representative example of haematoma growth in three different patients. Taking into consideration that the average latency time (or the time from the

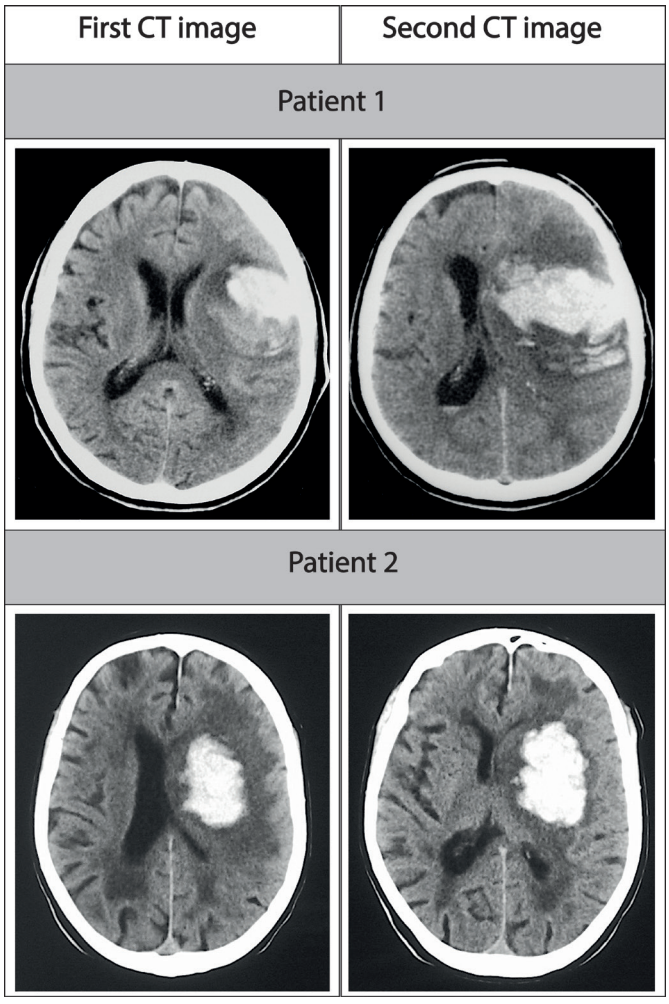

Figure 1 Representative CT images of early haematoma growth for two patients with grade II leukoaraiosis.

ICH symptom onset to the first scan) was 233±208 min, the percentage of patients with ICH growth fits the probability predicted by mathematical models.[1] We compared the clinical and analytical variables between patients with and without ICH growth, and significant differences were found in several parameters (listed in table 1) including, among others, the presence and degree of LA (p<0.0001) and the basal volume of ICH (p<0.0001).

In agreement with our previous study, in this cohort of patients, we found a correlation between sTWEAK levels and the degree of LA (figure 2, Spearman's rho=0.321; p<0.001). sTWEAK levels were analysed in relation to the ICH early growth (figure 3A). It was observed that sTWEAK levels were significantly lower in those patients without ICH growth compared with patients with ICH growth (1916 (1299 to 3892) versus 9037 (6099 to 13240) pg/mL, p<0.0001). Patients with ICH with no LA had significantly lower levels of sTWEAK compared with patients with LA (1894 (1167 to 3715) vs 4838 (1877 to 8207) pg/mL, p<0.001).

Next, a ROC analysis was carried out to define the best cut-off for sTWEAK levels to predict haematoma growth, obtaining an area under the curve of 0.929 (CI 95% 0.911

Table 1 Clinical variables, biochemical parameters and neuroimaging values of patients classified according to early haematoma growth

	Early haematoma growth		
	No (N=465)	Yes (N=188)	P value
Age, years	71.5±11.6	70.2±12.5	0.200
Women, %	48.4	43	0.061
Previous Rankin scale	1(0, 1)	0(0, 1)	0.415
Latency time, min	237.2±207.4	223.5±212.2	0.49
Wake-up stroke, %	5.6	4.8	0.848
Arterial hypertension, %	70.5	70.7	0.919
Diabetes, %	21.9	21.8	0.931
Smoking, %	8.8	11.7	0.305
Enolism, %	13.5	18.6	0.115
Hyperlipidaemia, %	41.1	39.4	0.725
Peripheral arterial disease, %	4.5	6.4	0.328
Atrial fibrillation, %	16.6	36.2	<0.0001
Ischaemic heart disease, %	9.2	12.2	0.254
Heart failure, %	2.4	5.9	0.032
Previous antiaggregants, %	17	12.8	0.194
Previous anticoagulants, %	11.6	38.3	<0.0001
Axillary temperature at admission, °C	36.6±0.8	36.9±0.7	0.003
Glucose, mg/dL	141.3±49.2	136.8±46.7	0.297
Glycosylated haemoglobin, %	5.9±1.0	5.9±0.9	0.85
Leucocytes, $\times 10^3$/mL	9.2±3.2	8.9±3.3	0.272
Haemoglobin, g/dL	13.6±4.7	13.1±3.3	0.319
Platelets, $\times 10^3$/mL	212.1±71.7	209.8±80.6	0.422
Fibrinogen, mg/d	448.3±103.2	432.5±99.9	0.101
LDL cholesterol, mg/dL	108.9±34.6	112.5±36.9	0.354
HDL cholesterol, mg/dL	39.7±21.3	36.6±17.6	0.152
Triglycerides, mg/dL	104.3±51.9	104.6±48.2	0.947
Sedimentation rate, mm	30.5±22.8	24.9±19.7	0.004
Leukoaraiosis, %	57.8	85.1	<0.0001
Degree of leukoaraiosis			<0.0001
Grade I, %	68.6	31.4	
Grade II, %	62	38	
Grade III, %	46.8	53.2	
Basal volume ICH, mL	44.7±38.8	29.6±28.8	<0.0001
Location of the ICH			0.018
Lobar, %	43.9	35.5	
Deep, %	56.1	66.5	
Ventricular/subarachnoid contamination, %	19.4	17.6	0.659
NIHSS at admission	14 [10, 19]	13 [9, 17]	<0.0001
Rankin scale at 3 months	3 [1, 6]	3 [2, 6]	0.007
Poor outcome at 3 months, %	59.8	71.3	0.007
Etiopathogenesis			<0.0001
Hypertensive, %	57.8	36.7	
Amyloid, %	14.4	4.3	
Antiplatelet drugs/ anticoagulants, %	11.4	39.9	
Indeterminate/others, %	16.3	19.1	

HDL, High Density Lipoprotein; ICH, intracerebral haemorrhage; LDL, Low Density Lipoprotein; NIHSS, National Institute of Health Stroke Scale.

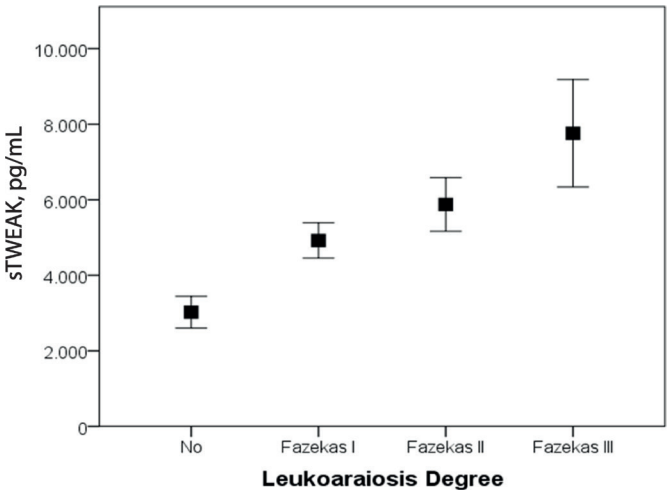

Figure 2 Variation of the serum sTWEAK levels in patients with ICH with different leukoaraiosis degrees. ICH, intracerebralhaemorrhage; sTWEAK, soluble tumour necrosisfactor-like weak inducer of apoptosis.

to 0.948, p<0.0001). The optimal sTWEAK operating point from the ROC curve corresponded to 5600 pg/mL, with a sensitivity of 84% and a specificity of 87% (figure 3B). This value was, therefore, taken as a reference for the subsequent multivariate analysis.

The association of sTWEAK and LA with the haematoma growth was evaluated with a logistic regression analysis (table 2). sTWEAK levels ≥5600 pg/mL were associated with haematoma growth (OR: 42.46 (CI 95% 22.67 to 79.52)) as well as the presence of LA (OR: 2.73 (CI 95% 1.39 to 5.34)) and the ICH basal volume (OR: 0.96 (CI 95% 0.95 to 0.97)). A second logistic regression model was carried out to evaluate the association of haematoma growth with the three degrees of LA severity in relation with the sTWEAK levels. Here, a similar association was observed between haematoma growth and sTWEAK levels (OR: 46.63 (CI 95% 24.18 to 89.9)) and ICH basal volume (OR: 0.96 (CI 95% 0.94 to 0.97)); however, we found differences regarding the three grades of LA, since grade I LA was not associated haematoma growth while grades II and III LA were associated with LA with higher

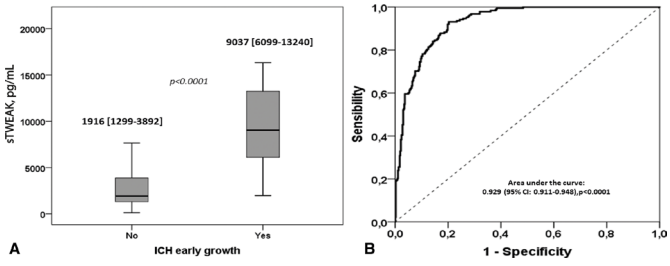

Figure 3 (A) Median values of serum sTWEAK levels measured in patients without and with early growth of ICH. Mann-Whitney test was used to evaluate the p value. (B) ROC curve analysis illustrating the ability of sTWEAK to diagnose early ICH growth. ICH, intracerebral haemorrhage; ROC, receiver operating characteristic; sTWEAK, soluble tumour necrosis factor-like weakinducer of apoptosis.

OR values (OR: 3.42 (CI 95% 1.59 to 7.35), and 7.79 (CI 95% 2.75 to 22.08), respectively).

Association between the basal volume of ICH with sTWEAK levels and LA

The association of sTWEAK levels and the presence of LA were analysed in relation with the ICH basal volumes measured at admission. We did not find a correlation between sTWEAK and ICH volumes (p=0.231). In addition, we did not find significant differences in basal ICH volume neither between patients without and with LA (p=0.163) nor among patients with different degrees of LA (p=0.539).

Association between the functional outcome of patients with ICH with serum sTWEAK levels and LA

Finally, we analysed the association between the functional outcome of patients with ICH and with the sTWEAK levels and the degree of LA (figure 4A). Poor functional outcome was defined as an mRS >2 evaluated at 3 months after hospital discharge. Patients with poor outcome showed higher levels of sTWEAK than patients with good outcome (4150 (1617 to 8109) vs 2035 (1310 to 4558) pg/mL, p<0.0001). In addition, the presence and degree of LA also had a significant influence on the outcome of patients, as illustrated in figure 4B.

Analogous to the analysis performed for haematoma growth, a bivariate analysis was carried out using the functional outcome as the dependent variable (table 3). Next, we carried out a logistic regression analysis using the clinical outcome as the dependent variable. The clinical and analytical variables that showed significant differences between patients with good and poor outcomes were included in the analysis, and the results were adjusted using the presence of LA or the degree of LA (table 4). In this case, the model showed that the presence of LA was associated with poor outcome (OR: 4.31 (CI 95% 2.89 to 6.42)). In this model, we observed that neither sTWEAK levels ≥5600 pg/mL (OR: 1.57 (CI 95% 0.87 to 2.83)) nor the early haematoma growth OR: 1.61 (CI 95% 0.88 to 2.94)) was associated with poor outcome; however, the interaction of both parameters was significantly associated with poor outcome (OR: 2.23 (CI 95% 1.40 to 3.55)). The second logistic regression model including the three degrees of LA showed a similar association between the clinical outcome with the three degrees of LA, being lower for patients with grade I LA (OR: 2.63 (CI 95% 2.66 to 4.14)), compared with grade II (OR: 6.05 (CI 95% 3.67 to 9.98)) and grade III LA (OR: 17.03 (CI 95% 5.72 to 50.68)). The interaction between sTWEAK and early haematoma growth associated with poor outcome was also observed in the second logistic regression model.

DISCUSSION

The goals of this study were to demonstrate the association between the presence of LA and the high levels of sTWEAK in patients with ICH and with the early growth of haematoma and poor functional outcome at

Table 2 Logistic regression model for factors related with early haematoma growth

	Unadjusted			Adjusted*		
	OR	95% CI	P	OR	95% CI	P
sTWEAK ≥5600 pg/mL	29.29	18.54 to 46.30	<0.0001	42.46	22.67 to 79.52	<0.0001
ICH basal volume	0.98	0.98 to 0.99	<0.0001	0.96	0.95 to 0.97	<0.0001
Leukoaraiosis	4.16	2.68 to 6.48	<0.0001	2.73	1.39 to 5.34	0.003
	Unadjusted			**Adjusted†**		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
sTWEAK ≥5600 pg/mL	29.29	18.54 to 46.30	<0.0001	46.63	24.18 to 89.9	<0.0001
ICH basal volume	0.98	0.98 to 0.99	<0.0001	0.96	0.94 to 0.97	<0.0001
Leukoaraiosis						
No LA	Ref.	–	–	Ref	–	–
Grade I	3.2	1.94 to 5.29	<0.0001	1.55	0.7 to 3.43	0.275
Grade II	4.24	2.61 to 7.06	<0.0001	3.42	1.59 to 7.35	0.002
Grade III	7.97	4.21 to 15.06	<0.0001	7.79	2.75 to 22.08	<0.0001

*Adjusted by atrial fibrillation, heart failure, previous anticoagulants, temperature at 183 admissions, sedimentation rate, deep ICH, NIHSS at admission, ICH for anticoagulants, basal ICH volume, presence of LA and sTWEAK ≥5600 pg/mL.
†Adjusted by the same variables; the presence of LA was replaced by the three grades of LA.
ICH, intracerebral haemorrhage; LA, leukoaraiosis; NIHSS, National Institute of Health Stroke Scale; sTWEAK, soluble tumour necrosis factor-like weak inducer of apoptosis.

3 months. In this work, we observed a relation between sTWEAK and LA, in agreement with our previous studies in patients with ischaemic stroke undergoing reperfusion treatments.[26]

Our results demonstrate the association between LA and early haematoma growth in ICH and also suggest that this relation is mediated by sTWEAK, a soluble cytokine that is expressed in many inflammatory and degenerative diseases of the central nervous system.[19 24] The association of sTWEAK or the TWEAK/Fn14 pathway with neuronal cell death and oedema progression has been reported before. In this regard, previous studies have reported the presence of elevated sTWEAK levels in the serum of patients with ischaemic stroke, suggesting its potential application as a blood biomarker able to detect ischaemic stroke and responsiveness to therapeutic intervention.[33 34] Regarding haemorrhagic stroke, although preclinical studies demonstrated that sTWEAK contributes to cerebral endothelial dysfunction and oedema progression in animal models of ICH,[25] the association of

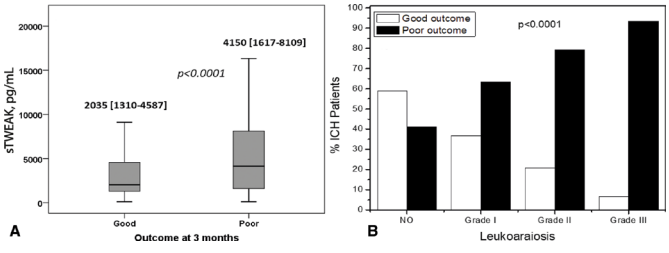

Figure 4 (A) Median values of serum sTWEAK levels measured in patients with good and poor functional outcome at 3 months. Mann-Whitney test was used to evaluate the p value. ((B) Percentage of patients with good and poor outcome at 3 months in relation with the grade of leukoaraiosis. Kruskal-Wallis test was used to evaluate the p value. sTWEAK,soluble tumour necrosis factor-like weakinducer of apoptosis.

sTWEAK with the progression of the lesion has not been demonstrated yet. In our study, we observed a remarkable increase in the risk of developing early ICH growth in patients with high sTWEAK levels. Moreover, sTWEAK serum levels were able to predict the development of early ICH growth with a sensitivity and specificity of 85%, highlighting the value of sTWEAK as a reliable biomarker for ICH growth.

The relationship between LA and ICH growth is still not well understood and the clinical data available so far are still controversial. While some authors have reported a direct association between LA and ICH growth,[6] other studies have showed the opposite, no relation between the haematoma expansion and LA.[12 13] In our series, we found a direct association of early ICH growth with the presence of LA in patients with ICH, being this relationship higher for patients with and advanced LA (grades II and III). An important factor that should be taken into consideration when evaluating ICH growth is the time from ICH symptom onset to baseline scan, since the probabilities to observe ICH growth decrease over time. Our data fit the predicted percentage of patients with ICH growth, according to the mathematical models,[1] and the association observed between LA and ICH growth was not influenced by the time between the symptom's onset and the time to hospital arrival and ICH image. Furthermore, our multivariate regression model showed that the association of the ICH growth and the poor outcome could be mediated by sTWEAK in patients with LA. Here, we have demonstrated the association between serum sTWEAK levels and LA, which could be a consequence of cerebral endothelial dysfunction,[35 36] so it was expected to observe an association between LA and ICH growth independent on sTWEAK levels. The increase in the OR values of sTWEAK after including LA in the model could be derived from the inflammatory response that triggers

Table 3 Clinical variables, biochemical parameters and neuroimaging values of patients classified according to the clinical outcome at 3 months

	Outcome at 3 months		
	Good (242)	Poor (411)	P value
Age, years	67.9±12.5	72.8±10.9	<0.0001
Women, %	45.5	43.1	0.568
Previous Rankin scale	0 (0, 1)	1 (0, 1)	0.015
Latency time, min	251.6±216.3	221.1±203.5	0.113
Wake-up stroke, %	5.4	5.4	1
Arterial hypertension, %	69.4	71.3	0.657
Diabetes, %	19.4	23.4	0.281
Smoking, %	12	8.3	0.132
Enolism, %	14	15.6	0.651
Hyperlipidaemia, %	43	39.2	0.364
Peripheral arterial disease, %	3.7	5.8	0.27
Atrial fibrillation, %	14.9	26.5	0.001
Ischaemic heart disease, %	7	11.9	0.059
Heart failure, %	2.1	4.1	0.183
Previous antiaggregants, %	14.9	16.3	0.658
Previous anticoagulants, %	11.2	24.1	<0.0001
Axillary temperature at admission, °C	36.5±0.7	36.8±0.8	<0.0001
Glucose, mg/dL	128.2±45.7	146.6±48.8	<0.0001
Glycosylated haemoglobin, %	5.8±0.9	5.9±1.0	0.286
Leucocytes, $\times 10^3$/mL	8.6±2.7	9.4±3.4	0.001
Haemoglobin, g/dL	13.2±3.9	12.9±6.5	0.407
Platelets, $\times 10^3$/mL	210.9±75.8	207.5±91.7	0.514
Fibrinogen, mg/dL	436.2±89.6	447.9±109.9	0.203
LDL cholesterol, mg/dL	114.8±33.3	106.9±36.4	0.032
HDL cholesterol, mg/dL	37.4±16.8	39.4±22.1	0.346
Triglycerides, mg/dL	104.3±50.1	104.5±51.3	0.98
Sedimentation rate, mm	25.0±17.6	31.2±24.1	0.001
Leukoaraiosis, %	45.5	77.6	<0.0001
Degree of leukoaraiosis			<0.0001
Grade I, %	28.5	29	
Grade II, %	15.8	34.5	
Grade III, %	1.7	14.1	
Basal volume ICH, mL	25.6±20.8	49.0±41.2	<0.0001
Location of the ICH			0.458
Lobar, %	38.8	42.1	
Deep, %	61.2	57.9	
Ventricular/subarachnoid contamination, %	13.6	21.9	0.01
NIHSS at admission	10 [6, 14]	16 [13, 20]	<0.0001
Etiopathogenesis			<0.0001
Hypertensive, %	56.2	49.1	
Amyloid, %	11.2	11.7	
Antiplatelet drugs/anticoagulants, %	11.6	24.3	
Indeterminate/others, %	21.1	14.8	
sTWEAK (cat) ≥5600 pg/mL, %	18.7	41.7	<0.0001
Early haematoma growth %	21.1	33.3	0.001

HDL, High Density Lipoprotein; ICH, intracerebral haemorrhage; LDL, Low Density Lipoprotein; NIHSS, National Institute of Health Stroke Scale; sTWEAK, soluble tumour necrosis factor-like weak inducer of apoptosis.

Table 4 Logistic regression model for factors related with poor clinical outcome at 3 months

	Unadjusted			Adjusted*		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
sTWEAK ≥5600 pg/mL	3.12	2.13 to 4.55	<0.0001	1.57	0.87 to 2.83	0.129
ICH basal volume	1.03	1.03 to 1.04	<0.0001	1.04	1.03 to 1.05	<0.0001
Leukoaraiosis	4.16	2.92 to 5.86	<0.0001	4.31	2.89 to 6.42	<0.0001
ICH growth	1.87	1.29 to 2.71	0.001	1.61	0.88 to 2.94	0.123
sTWEAK×ICH growth				2.23	1.40 to 3.55	0.001
	Unadjusted			**Adjusted†**		
	OR	95% CI	P value	OR	95% CI	P value
sTWEAK ≥5600 pg/mL	3.12	2.13 to 4.55	<0.0001	1.67	0.93 to 2.99	0.086
ICH basal volume	1.03	1.03 to 1.04	<0.0001	1.03	1.03 to 1.04	<0.0001
Leukoaraiosis						
No LA	Ref.	–	–	Ref	–	–
Grade I	1.43	1.06 to 1.92	0.019	2.63	1.66 to 4.14	<0.0001
Grade II	2.47	1.79 to 3.39	<0.0001	6.05	3.67 to 9.98	<0.0001
Grade III	3.07	1.89 to 4.97	<0.0001	17.03	5.72 to 50.68	<0.0001
ICH growth	1.87	1.29 to 2.71	0.001	1.49	0.82 to 2.73	0.193
sTWEAK×ICH growth				2.01	1.27 to 3.23	0.003

*Adjusted by age, previous mRS, atrial fibrillation, previous anticoagulants, temperature at admission, glucose, leucocytes, C reactive protein, LDL cholesterol, sedimentation rate, basal ICH volume, presence of LA, ICH growth and sTWEAK ≥5600 pg/mL.
†Adjusted by the same variables; the presence of LA was replaced by the three grades of LA.
HDL, High Density Lipoprotein; ICH, intracerebral haemorrhage; LA, leukoaraiosis; LDL, Low Density Lipoprotein; mRS, modified Rankin Scale; sTWEAK, soluble tumour necrosis factor-like weak inducer of apoptosis.

the expression of sTWEAK and the subsequent release of other cytokines such as metalloproteases and interleukins,[17 25 37] with deleterious consequences in the clinical outcome of patients, as discussed next.

The secondary objectives of this study were to analyse the association of LA and sTWEAK with both ICH basal volume and the clinical outcome at 3 months. We did not find an association between LA and ICH basal volume. Our results are in agreement with the previous studies,[9–13] although some authors have found an association between LA and ICH volume in anticoagulated patients and in patients treated with fibrinolytics.[4 5] Regarding the clinical outcome at 3 months, we observed an association between both LA and sTWEAK with a poor prognosis in ICH, which hindered the influence of the basal ICH volume over the outcome at 3 months. sTWEAK is known to activate the Fn14 receptor in endothelial cells, triggering the release of several proinflammatory cytokines, matrix metalloproteases (MMP9) and the leucocyte adhesion.[24] These mechanisms can exacerbate the deleterious effect of the ICH and increase bleeding; indeed, the activation of the TWEAK-Fn14 signalling pathway has a pro-inflammatory effect that could be blocked during the recruitment phase of immune cells across the BBB,[37] which could yield new therapeutic options for patients with ICH.

This work has the common limitations of retrospective studies, and there is a lack of a validation cohort to support the predictive value of sTWEAK in haematoma growth. Besides, there are no previous preclinical data supporting the molecular mechanisms involved in the interactions of sTWEAK with haematoma growth. Experimental studies to confirm the observed relationships are still necessary. Another weakness is the analysis of the soluble levels of sTWEAK and not the TWEAK-Fn14 association, which could have more implications in the development of ICH. Also, we only have evaluated the association of LA and sTWEAK with the evolution of the ICH volume, while other neuroimage markers of haematoma growth could have some implications in the sTWEAK levels. The strength of this work is the unbiased selection of patients, the high number of included patients and the blinded analysis of sTWEAK.

In conclusion, our study shows that sTWEAK and LA are biomarkers with capacity to predict haematoma growth and poor functional outcome in patients with ICH.

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from patients or their relatives at the time of inclusion in the registry, authorising the anonymous use of data for further studies.

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Supplementary Information

sTWEAK is a marker of early hematoma growth and leukoaraiosis in intracerebral hemorrhage.

Table S1. Clinical variables, biochemical parameters, neuroimaging values of included and excluded patients.

	Included patients (653)	Excluded patients (447)	p
Age, years	71.1 ± 11.9	70.9 ± 14.5	0.912
Women, %	44	42.7	0.71
Previous Rankin scale	1 [0, 1]	1 [0, 1]	0.091
Latency time, min	233.2 ± 208.7	228.8 ± 202.5	0.75
Wake-up stroke, %	5.4	4	0.305
Arterial hypertension, %	70.6	68.7	0.463
Diabetes, %	21.9	18.1	0.128
Smoking, %	9.6	12.3	0.166
Enolism, %	15	15.9	0.734
Hyperlipidemia, %	40.6	40.3	0.95
Peripheral arterial disease, %	5.1	4	0.468
Atrial fibrillation, %	22.2	25.7	0.193
Ischemic heart disease, %	10.1	13.6	0.084
Heart failure, %	3.4	3.8	0.741
Previous antiaggregants, %	15.8	17.4	0.508
Previous anticoagulants, %	19.3	18.6	0.815
Axillary temperature at admission, ºC	36.6 ± 0.8	36.5 ± 0.7	0.101
Glucose, mg/dL	139.9 ± 48.4	137.2 ± 47.6	0.405
Glycosylated hemoglobin, %	5.9 ± 1.0	5.9 ± 0.8	0.94
Leukocytes, $x10^3$/mL	9.1 ± 3.2	9.1 ± 3.4	0.913
Hemoglobin, g/dL	13.7 ± 4.3	13.5 ± 2.1	0.265
Platelets, $x10^3$/mL	215.1 ± 63.8	203.4 ± 77.9	0.314
Fibrinogen, mg/dL	443.3 ± 102.3	437.5 ± 100.1	0.432
LDL-cholesterol, mg/dL	108.8 ± 35.5	110.1 ± 35.4	0.64
HDL-cholesterol, mg/dL	38.6 ± 20.1	39.0 ± 15.4	0.771
Triglycerides, mg/dL	104.4 ± 50.7	105.8 ± 50.7	0.721
Sedimentation rate, mm	28.5 ± 22.0	28.8 ± 24.6	0.963
Leukoaraiosis, %	65.7	60.2	0.065
Degree of leukoaraiosis			0.219
Grade I, %	28.8	28.6	
Grade II, %	27.4	23.3	
Grade III, %	9.5	8.3	
Basal volume ICH, mL	40.3 ± 36.8	40.1 ± 64.1	0.942
Location of the ICH			0.069
Lobar, %	40.9	35	
Deep, %	59.1	65	
Ventricular/subarachnoid contamination, %	18.8	23.8	0.129
NIHSS at admission	14 [9, 18]	12 [8, 21]	0.931

Rankin scale at 3 months	3 [2, 6]	3 [1, 6]	0.071
Poor outcome at 3 months, %	64.3	58	0.095
Etiopathogenesis			0.114
Hypertensive, %	51.8	46.7	
Amyloid, %	11.5	10.4	
Antiplatelet drugs/anticoagulants, %	19.6	17	
Indeterminate/others, %	17.2	25.9	