Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke

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ABSTRACT

Background Classification defined in the Trial of Org10172 in Acute Ischaemic Stroke (TOAST) is widely used in trials and practice. Previous studies on pathophysiology suggest a role for endothelial inflammation in atherothrombotic strokes and intracardiac thrombosis in cardioembolic strokes. Data on lacunar and undetermined strokes are limited. The aim of the study was to assess non-specific inflammatory and thrombogenic parameters in patients with ischaemic stroke.

Methods This was a prospective controlled clinical study involving 200 patients with ischaemic stroke and 50 controls. Patients were classified following the TOAST criteria. Plasma levels of fibrinogen, D-dimer, C reactive protein and values for D-dimer/fibrinogen ratio and erythrocyte sedimentation rate were assessed over 48 h after admission. Clinical severity was measured using the National Institutes of Health Stroke Scale and the Oxfordshire Community Stroke Project classification. Patients with severe systemic disorders were excluded.

Results The assessed parameters were significantly higher in patients versus controls. Cardioembolic stroke patients showed increased D-dimer, fibrinogen and D-dimer/fibrinogen ratio. Patients with atherothrombotic stroke showed raised fibrinogen and erythrocyte sedimentation rate. Patients with lacunar and undetermined stroke showed intermediate values of markers. Total anterior cerebral infarction syndrome was related to D-dimer.

Discussion Patients showed analytical modifications during the acute phase of stroke, both related to acute response and mechanism. The results suggest that the biochemical profile may be prothrombotic in patients with cardioembolism and inflammatory in those with atherothrombotic stroke. Patients with lacunar and undetermined stroke showed intermediate profiles. Assessment of the studied parameters is not expensive, widely available and may proportionate information about pathophysiology in stroke patients without severe systemic conditions.

BACKGROUND

Large vessel atherothrombosis or thromboembolism from arterial or cardiac sources are the main mechanisms in approximately 80% of acute ischaemic stroke cases.[1] The development of atherosclerotic lesions is regarded as a systemic inflammatory disease. The first stages are characterised by endothelial dysfunction, and progression of the disease is linked to inflammatory processes into the plaque mediated by cytokines and leucocyte recruitment.[2 3] Several studies have described that leucocyte count and levels of fibrinogen and C reactive protein (CRP) may serve as markers of inflammatory activity and a risk of developing a vascular event or worse prognosis after stroke.[4–11] Lacunar infarcts are related to occlusion of penetrating arteries when lipohyalinosis or microatheromatosis affects the smaller arteries, less than 200 μm in diameter. Inflammatory cytokines such as interleukin 6, intercellular adhesion molecule-1 and tumour necrosis factor α have been implicated in the pathophysiology of early neurological deterioration in lacunar stroke patients, suggesting a role for inflammation in this subtype of infarct.[12 13] Hence it is probable that low grade systemic inflammation is relevant for progression of microatheromatosis and macroatheromatosis in lacunar and atherothrombotic strokes, respectively. The association between atherothrombotic stroke in the carotid territory and chronic periodontal disease supports the role of inflammation.[3]

In contrast, atrial fibrillation and other cardiac disorders have been related to intracardiac activation of coagulation.[14] Different authors reported raised levels of fibrinogen, CRP, D-dimer, thrombin–antithrombin III complex and β-thromboglobulin, and reduced levels of antithrombin III, C and S proteins, and plasminogen activator inhibitor-1.[15–17]

In clinical practice, the best treatment for stroke requires the correct classification of aetiology and mechanism. The categories of stroke defined in the Trial of Org 10172 in Acute Ischaemic Stroke (TOAST) are widely used in clinical trials and routine practice.[18] The application of TOAST criteria requires an exhaustive cardiac and vascular paraclinical evaluation, but biomarkers are not used. Some studies demonstrated that early raised levels of D-dimer and brain derived natriuretic peptide may be useful to diagnose a cardioembolic stroke[19 20] but there are not biomarkers to support the diagnosis of atherothrombotic and lacunar stroke in routine clinical practice.[19 21 22]

The aim of the present study was to assess easily available plasma markers of thrombogenesis (D-dimer and fibrinogen) and inflammation (CRP levels and erythrocyte sedimentation rate (ESR)) during the acute phase of ischaemic stroke classified using the TOAST criteria.

PATIENTS AND METHODS

This was a prospective, controlled, clinical study which included 200 patients admitted to the Stroke Unit of a teaching hospital with a diagnosis of

ischaemic stroke or transient ischaemic attack, and 50 controls without evidence of cerebrovascular disease from a neurology outpatient consultation.

The main aim of the study was to determine plasma levels of D-dimer, fibrinogen, D-dimer/fibrinogen ratio, CRP and ESR during the acute phase of ischaemic stroke to assess the differences between stroke patients and controls. D-dimer, fibrinogen and D-dimer/fibrinogen ratio were used to assess thrombotic activity whereas CRP, fibrinogen levels and ESR value were used as unspecific markers of systemic inflammation.[13 15] The ratio between D-dimer and fibrinogen was previously defined as a marker of thrombotic activity in patients with deep venous thrombosis.[23] This ratio was calculated to determine its applicability to stroke. A total of 200 patients with a diagnosis of ischaemic stroke (50 cardioembolic, 50 atherothrombotic, 50 lacunar and 50 undetermined classified following the TOAST criteria) were consecutively included.[18]

Patients were excluded when one of the following criteria was present: more than 24 h since the stroke when the patient arrived at the emergency department; diagnosis of haemorrhagic stroke; presence of fever and/or signs of active infection on admission; presence of anaemia on the first analysis performed in the emergency department, following the WHO definition of anaemia (haemoglobin <13 g/dl in men and <12 g/dl in women); previous diagnosis of active chronic liver disease; active malignant neoplasm or monoclonal gammopathy diagnosed before admission or during inhospital evaluation; evidence of venous thromboembolic disease on admission; fibrinolysis; and diagnosis of 'other aetiology' of stroke.

Evaluation of patients

Demographic variables, pathological antecedents and cardiovascular risk factors were recorded on admission. Analytic parameters were determined in two separate venous samples. The first sample was obtained in the emergency department to determine blood count, glycaemia, CRP and fibrinogen. The second sample was obtained during the 48 h after admission to determine the lipid profile, ESR and D-dimer.

Following the Stroke Unit protocol, arterial blood pressure, heart rate, oximetry, tympanic temperature and ECG were semi-intensively monitored during the first 48 h after admission. The clinical severity of stroke on admission was assessed using the National Institutes of Health Stroke Scale (NIHSS). All patients were clinically classified using the Oxfordshire Community Stroke Project (OCSP) as total anterior carotid infarction (TACI), partial anterior carotid infarction, lacunar infarction and posterior infarction.[24] As TACI syndromes represent greater infarcts, the OCSP classification was used to indirectly correlate biochemical variables with presumed size of lesion. The protocol of paraclinical examinations included transthoracic echocardiogram and carotid eco-Doppler and transcranial Doppler studies. Additional examinations, such as transoesophageal echocardiogram, Holter monitoring of blood pressure or heart rate, hypercoagulability and autoimmunity analysis, and vascular imaging, were performed when indicated. The mechanism of stroke was classified using the TOAST criteria. The modified Rankin Scale was calculated on discharge.

A generic Stroke Unit informed consent form was obtained from patients or their relatives. The present study was approved by the ethics commission of the hospital.

Laboratory methods

Fibrinogen concentration was quantitatively determined in plasma by the Clauss method using the Hemos IL Fibrinogen-C Kit (Beckman Coulter, Brea, CA, USA), which uses an excess of thrombin to transform the fibrinogen in fibrin in diluted plasma. The normal range used in the laboratory is 203–472 mg/dl. D-dimer concentration was quantitatively measured in plasma using an automated latex turbidimetric immunoassay kit (D-dimer Kit, Beckman Coulter). This assay uses a suspension of latex particles with a monoclonal antibody against the D-dimer dominion of soluble fibrin derivates. The normal range used in the laboratory and indicated by the manufacturer is 0–255 ng/ml. Plasma concentrations of CRP were quantitatively determined using a latex enhanced turbidimetric immunoassay technique (Cobas Integra C Reactive Protein, Roche Diagnostics GMBH, Mannheim, Germany). The normal range is 0–0.75 mg/dl. ESR was measured following the method described by Westergren in 1924 and recommended by the WHO. This method evaluates aggregation of red cells in the pipette after 1 h and is less sensitive to factors that potentially may affect the ESR value. The normal range used in the laboratory is 0–20 mm/h.

Statistical analysis

Licensed statistical software SPSS (V.15.0) was used to elaborate the database and to analyse the data. The assumption of normal distribution of continuous variables was assessed by the Kolgomorov–Smirnov test. Continuous quantitative variables were compared using the Student's t test for those normally distributed and the Mann–Whitney U test for non-parametric data. Comparisons among subtypes of ischaemic stroke and clinical syndromes of OCSP classification were performed by one way ANOVA for normally distributed variables and by the Kruskal–Wallis test for non-normally distributed variables. Following ANOVA and Kruskal–Wallis tests, post hoc analyses were applied to significantly different variables using Tukey and Mann–Whitney U tests, respectively. Qualitative variables were compared using the χ^2 test. The relationships between subtypes of stroke and covariates of interest were assessed using multinomial logistic regression in order to adjust for other factors in the model.

In all analyses a bilateral α level ≤ 0.05 was chosen to define statistical significance.

RESULTS

Characteristics of included and excluded patients

Patients were included between November 2007 and September 2009. During this period, 469 patients were admitted to the Stroke Unit with a diagnosis of stroke. The main causes for exclusion were haemorrhagic stroke (n=72), evolution of stroke more than 24 h or unknown (n=49), fibrinolysis (n=35), lack of second sample for the analytic study (n=31), signs of infection on admission (n=21), diagnosis of neoplasm (n=20), anticoagulant therapy (n=16), presence of anaemia (n=12), diagnosis of monoclonal gammopathy (n=10), recent surgery (n=2) and diagnosis of stroke of 'other aetiology' (n=1).

In the group of patients with a final diagnosis of stroke of undetermined mechanism, 45 had no aetiology after examination and five had atrial fibrillation and coexistent significant carotid stenosis homolateral to stroke.

Controls were recruited between January 2008 and March 2009. The diagnoses of controls were: mild cognitive impairment/Alzheimer's disease (n=18), idiopathic or non-vascular secondary epilepsy (n=8), essential tremor (n=7), primary headache tension or migraine type (n=6), Parkinson's disease and other parkinsonian syndromes (n=6), mononeuropathy, idiopathic dysgeusia, idiopathic syncope, peripheral vertigo and generalised anxiety disorder (n=1).

As NIHSS score and levels of triglycerides, fibrinogen, D-dimer, ESR and CRP followed a non-parametric distribution, Kruskal–Wallis and Mann–Whitney U tests were applied. Other quantitative variables (age and levels of total and low density lipoprotein (LDL) cholesterol) followed a normal distribution and comparisons were done using ANOVA and the Student's t test. Figure 1 shows the box plots of primary laboratory variables in controls and in the subtypes of ischaemic stroke.

Controls versus patients

Both groups showed a similar distribution for sex and age. As expected, the prevalence of cardiovascular risk factors was significantly higher in stroke patients.

The control group showed values for all analytical parameters within the normal range. Table 1 summarises the demographical characteristics, cardiovascular risk factors and laboratory variables in the controls and patients.

Patients by stroke subtype

Patients with cardioembolic stroke were significantly older than patients with atherothrombotic and lacunar infarcts ($p<0.0001$ and $p=0.023$, respectively). Male gender was significantly more frequent in the atherothrombotic subtype compared with the other subtypes ($p=0.003$ vs cardioembolic, $p=0.041$ vs lacunar and $p=0.025$ vs undetermined). Clinical severity on admission was significantly higher in patients with cardioembolic stroke compared with patients with atherothrombotic ($p=0.05$) and lacunar ($p=0.013$) strokes. Fifty-five patients had a TACI syndrome, 55 had a partial anterior carotid infarction, 71 had a lacunar infarction and 18 had a posterior infarction. Older age ($p=0.040$, ANOVA), atrial fibrillation ($p=0.002$, χ^2 test) and higher D-dimer level ($p=0.041$, Kruskal–Wallis test) were related to TACI syndrome.

The prevalence of diabetes mellitus was higher in patients with lacunar and undetermined strokes. Differences were significant compared with patients with cardioembolic stroke ($p=0.021$ and $p=0.004$ vs lacunar and undetermined strokes, respectively). Atrial fibrillation was the most frequent cardiac condition in cardioembolic stroke patients ($p<0.0001$ vs others subtypes), followed by ischaemic heart disease associated with left ventricular hypo/akinesia. The prevalence of smoking and alcohol abuse were higher in patients with stroke related to large vessel disease compared with patients with cardioembolic ($p<0.0001$ for both comparisons), lacunar ($p=0.001$ and $p=0.086$ for smoking and alcohol consumption, respectively) and undetermined strokes ($p<0.0001$ and $p=0.003$ for smoking and alcohol consumption, respectively). The prevalence of alcohol consumption was also higher in patients with lacunar versus cardioembolic strokes ($p=0.003$).

Patients with atherothrombotic and lacunar strokes showed the highest levels of triglycerides and total and LDL cholesterol. Considering patients with atherothrombotic stroke, differences were significant compared with patients with cardioembolic ($p=0.001$, $p=0.002$ and $p<0.0001$ for total cholesterol, LDL cholesterol and triglycerides, respectively) and undetermined strokes ($p=0.007$, $p=0.022$ and $p=0.010$ for total cholesterol, LDL cholesterol and triglycerides, respectively). Considering

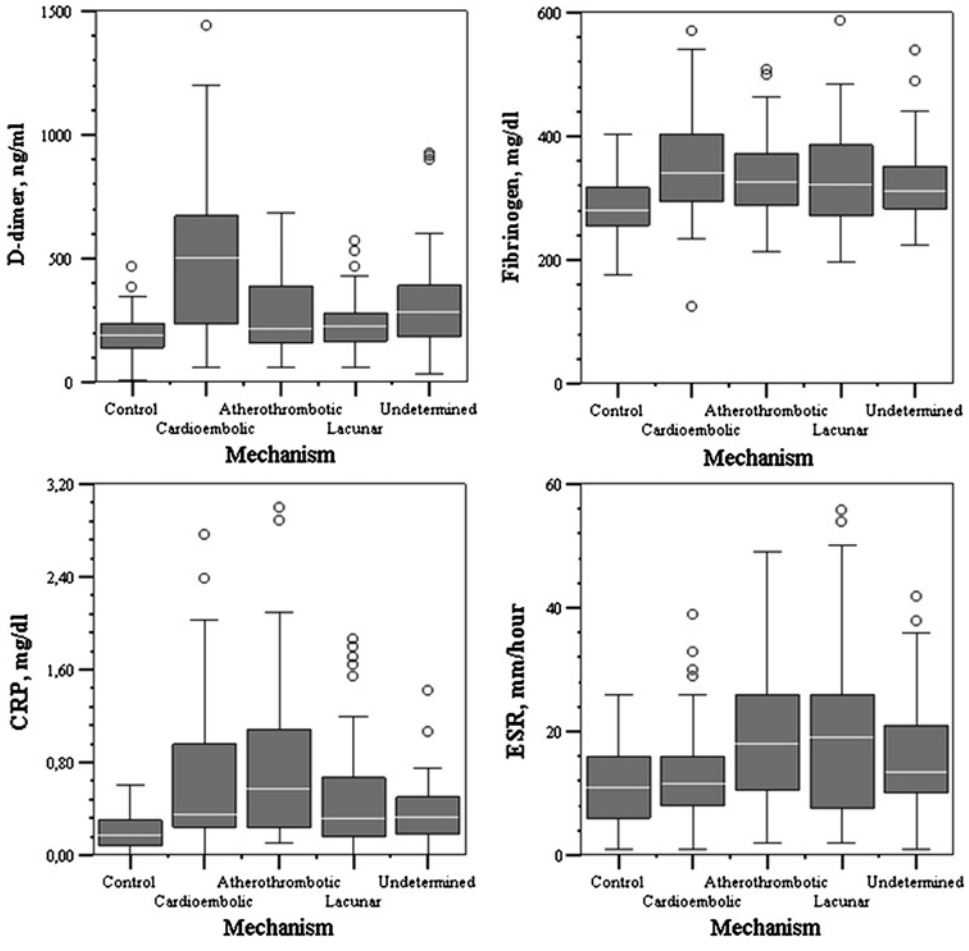

Figure 1 Box plots of primary laboratory variables in controls and subtypes of ischaemic stroke. CRP, C reactive protein; ESR, erythrocyte sedimentation rate.

Table 1 Demographical characteristics, cardiovascular risk factors and biochemical parameters in stroke patients and controls

	Cases (n=200)	Controls (n=50)	p Value
Age (years)	72±11.5	69.2±9.9	0.107†
Male (n (%))	104 (52)	21 (42)	0.206
NIHSS on admission	4 (2–9)‡		
Hypertension (n (%))	153 (85.5)	26 (52)	*<0.0001*
Atrial fibrillation (n (%))	43 (21.7)	0	*<0.0001**
Diabetes mellitus (n (%))	48 (24.2)	8 (16)	0.213
Coronary heart disease (n (%))	22 (11.2)	6 (12)	0.868
HF with EF <50% (n (%))	5 (2.5)	0	0.586*
Previous stroke (n (%))	42 (21.2)	0	*0.001**
Smoking (n (%))	54 (30.7)	11 (22.9)	0.293
Alcohol consumption (n (%))	58 (33)	14 (29.2)	0.618
Total cholesterol (mg/dl)	188.3±47.3	201.5±41.4	0.086†
LDL cholesterol (mg/dl)	112.5±41.7	121.4±32.6	0.183†
Triglycerides (mg/dl)	132 (99–182)	114 (85–153)	*0.032*‡
Fibrinogen (mg/dl)	323 (286–381.5)	281 (255–319.5)	*<0.0001*‡
D-dimer (ng/ml)	272.5 (181.2–471.5)	190.5 (140–240.5)	*<0.0001*‡
CRP (mg/dl)	0.36 (0.19–0.77)	0.18 (0.08–0.30)	*<0.0001*‡
D-dimer/fibrinogen ratio	0.78 (0.56–1.32)	0.71 (0.49–0.90)	*0.030*‡
ESR (mm/h)	14 (9–23)	11 (6–16)	*0.002*‡

*Dichotomous variables are expressed as n (%) and comparisons between cases and controls were done with the χ^2 test or Fisher's exact test.
†Normal continuous variables are expressed as mean±SD and comparisons were done using the Student's t test.
‡Non-parametric continuous variables are expressed as median (IQR) and comparisons were done with the Mann–Whitney U test.
CRP, C reactive protein; EF, ejection fraction; ESR, erythrocyte sedimentation rate; HF, heart failure; LDL, low density lipoprotein; NIHSS, National Institutes of Health Stroke Scale.

patients with lacunar stroke, total cholesterol level was significantly higher compared with patients with cardioembolic stroke (p=0.016) and triglycerides level was greater compared with patients with cardioembolic (p<0.0001) and undetermined events (p=0.001). No other differences were found for demographic characteristics of patients, stroke severity or risk factors when all subtypes were compared.

The highest median level of fibrinogen was found in patients with cardioembolic stroke followed by patients with atherothrombotic stroke, but differences among subtypes were not significant.

Median values for D-dimer and D-dimer/fibrinogen ratio were higher in patients with cardioembolic stroke (p<0.0001 for both parameters vs controls and patients with atherothrombotic and lacunar stroke; p=0.002 for D-dimer and p=0.004 for D-dimer/fibrinogen ratio vs patients with undetermined stroke). Compared with controls, the median level of D-dimer was significantly higher in patients with atherothrombotic (p=0.026), lacunar (p=0.042) and undetermined (p=0.001) strokes.

The median level of CRP was higher in patients with atherothrombotic stroke, followed by cardioembolic stroke. Differences were significant when patients with atherothrombotic stroke were compared with those with lacunar (p=0.010) and undetermined (p=0.003) strokes.

The ESR value was highest in patients with lacunar stroke, followed by patients with stroke related to large vessel disease. Differences were significant when controls were compared with patients with atherothrombotic (p<0.0001), lacunar (p<0.0001) and undetermined (p=0.040) strokes but not with cardioembolic stroke (p=0.253). Differences among subtypes of stroke were not significant, and only the comparison between patients with cardioembolic and lacunar strokes was close to significance (p=0.064).

No other differences were found in analytical parameters among the subtypes of stroke. Table 2 summarises the demographic characteristics, NIHSS, cardiovascular risk factors and biochemical parameters in the different subtypes of stroke patients.

Multinomial logistic regression

The model included variables significantly different among subtypes of stroke. Because NIHSS was not available in the controls, cardioembolic stroke was used as the reference category. The median level of D-dimer was consistently and independently associated with presumed mechanism of stroke; less constant was the relationships between subtype and median value of ESR, mean level of cholesterol and smoking. Compared with patients with cardioembolic stroke, as the level of D-dimer decreased, there was an increased probability of diagnosing a different mechanism of stroke. OR values and complete model details are given in table 3.

DISCUSSION

The results of the present study showed specific analytical modifications during the acute phase of ischaemic stroke and a different biochemical profile depending on the mechanism of the disorder. As expected, patients showed a significantly higher prevalence of previous stroke, hypertension and atrial fibrillation. No other differences were observed in age, gender or cardiovascular risk factors between controls and patients. Mean levels of variables defined as inflammatory (ESR, CRP) and prothrombotic markers (D-dimer, fibrinogen, D-dimer/fibrinogen rate) were significantly higher in patients compared with controls.

As patients had no systemic disorders when they were included in the study, analytical differences were probably associated with the mechanism of stroke and with specific biochemical modifications developed during the first 48 h after the ischaemic episode. The lack of association between positive acute phase parameters and TACI syndromes supports the fact that these modifications may be partially related to the mechanisms of stroke. Although volumes of infarct were not assessed, there is a reasonable correlation between TACI syndromes and large middle cerebral artery lesions.[25] It suggests that TACI could be associated with higher levels of the analysed positive acute phase parameters (fibrinogen, D-dimer, CRP) but only D-dimer was in the present study. This was probably related to the higher prevalence of atrial fibrillation in patients with this syndrome.

Comparisons among subtypes of stroke suggested three biochemical profiles which may be related to the mechanism of ischaemia: thrombogenic, inflammatory and non-specific. The thrombogenic profile was characterised by raised fibrinogen, D-dimer and D-dimer/fibrinogen ratio, and was found in patients with cardioembolic stroke. The inflammatory profile was observed in patients with atherothrombotic stroke and was defined by raised mean levels of fibrinogen, CRP and ESR. The non-specific profile was found in lacunar and undetermined stroke patients. Patients with lacunar stroke showed a higher value for ESR and lower D-dimer and D-dimer/fibrinogen ratio, suggesting a predominance of inflammatory mechanisms. In common with patients with cardioembolic stroke, patients with undetermined stroke showed higher levels of D-dimer and D-dimer/fibrinogen ratio, and lower CRP and ESR values, suggesting a predominance of thrombogenic activity. Multinomial logistic regression analysis confirmed that associations

Table 2 Demographic characteristics, cardiovascular risk factors and biochemical parameters in stroke patients classified by stroke subtype

	Cardioembolic (n=50)	Atherothrombotic (n=50)	Lacunar (n=50)	Undetermined (n=50)	p Value
Age (years)	76.9±7.6	66.7±12.5	70.6±11.9	73.9±10.9	*<0.0001**
Male (n (%))	20 (40)	35 (70)	25 (50)	24 (48)	*0.021*
NIHSS on admission	6 (2–14)	4 (2–8)	4 (2–6)	6 (2–10)	0.067†
Hypertension (n (%))	41 (83.7)	38 (77.6)	36 (73.5)	38 (76)	0.660
Atrial fibrillation (n (%))	36 (72)	0	0	5 (10)	*<0.0001*
Diabetes mellitus (n (%))	5 (10)	12 (24)	14 (28.6)	17 (34)	*0.039*
Coronary heart disease (n (%))	6 (12.5)	4 (8)	5 (10)	7 (14)	0.791
HF with EF <50% (n (%))	5 (10)	0	0	0	*0.001*
Peripheral arteriopathy (n (%))	2 (4.1)	4 (8.2)	1 (2)	3 (6)	0.553
Previous stroke (n (%))	12 (24)	9 (18)	7 (14)	14 (28.6)	0.333
Smoking (n (%))	7 (14)	26 (53)	12 (24)	9 (18)	*<0.0001*
Alcohol consumption (n (%))	5 (10)	24 (47)	17 (34)	12 (24)	*<0.0001*
Total cholesterol (mg/dl)	170.8±40.9	207.2±49.9	198.2±49	177.3±40.4	*<0.0001**
LDL cholesterol (mg/dl)	98.9±37	128±45	119.1±42	104.3±36.7	*0.001**
Triglycerides (mg/dl)	103.5 (82.5–134)	164 (108–214.5)	155.5 (119–228)	117.5 (89.3–144.5)	*<0.0001*†
Fibrinogen (mg/dl)	340 (293–408.5)	325 (287–374)	322.5 (270–390)	312 (280.5–352)	0.397†
D-dimer (ng/ml)	500 (235.5–675)	218 (156.8–406)	224.5 (162–281)	283 (182.3–394)	*<0.0001*†
CRP (mg/dl)	0.36 (0.24–0.97)	0.58 (0.24–1.09)	0.32 (0.16–0.68)	0.33 (0.18–0.51)	*0.013*†
D-dimer/fibrinogen	1.26 (0.73–2.01)	0.66 (0.41–1.25)	0.65 (0.50–1.02)	0.81 (0.58–1.24)	*<0.0001*†
ESR (mm/h)	11.5 (8–16.3)	18 (10.3–26)	19 (7.3–27)	13.5 (10–21.5)	*0.002*†

*Dichotomous variables are expressed as n (%) and comparisons were done with the χ^2 test. Normal continuous variables are expressed as mean±SD and comparisons were done using ANOVA.
†Non-parametric continuous variables are expressed as median (IQR) and comparisons were done with the Kruskal-Wallis test.
CRP, C reactive protein; EF, ejection fraction; ESR, erythrocyte sedimentation rate; HF, heart failure; LDL, low density lipoprotein; NIHSS, National Institutes of Health Stroke Scale.

between D-dimer and cardioembolism and between ESR and lacunar and atherothrombotic stroke were independent and reasonably excluded the influence of other relevant variables such as age and gender.

Raised D-dimer is a constant finding in thrombogenic disorders, especially when they affect the venous system. However, published data in patients with ischaemic stroke are limited and the assessment of D-dimer is not routine in these patients. Previous studies reported increased levels of D-dimer during the acute phase of ischaemic stroke and related this to worse vital and functional prognosis and to a higher risk of recurrence during follow-up.[26] In addition, the highest level was associated with a cardioembolic mechanism. A controlled study reported that patients with cardioembolic stroke had raised mean levels of D-dimer, and the greatest level of the parameter defined patients with a higher risk of new embolisms during follow-up. The authors concluded that there was continuous activation of coagulation and fibrinolysis characterised by raised D-dimer.[15] Compared with the present study, they used a different method to assess D-dimer, included fewer patients and did not exclude patients with severe systemic disorders. Other studies were methodologically closer to the present, because authors used an immunoturbidimetric method, included a relatively high number of patients and excluded patients with systemic diseases. Independent of methods, they agree that a higher level of D-dimer is related to stroke of cardioembolic aetiology.[16 21 22]

This was the first study evaluating the D-dimer/fibrinogen ratio in patients with stroke. A Swiss study showed that patients with pulmonary thromboembolic disease diagnosed by thoracic angio-CT had higher values for this ratio (median value 1.22 in patients and 0.25 in controls). The authors suggested that raised values of this quotient may define prothrombotic activity in conditions with excessive fibrinogen consumption and D-dimer formation, independent of absolute values of both.[23] In the present study, the D-dimer/fibrinogen ratio was higher in patients with cardioembolic stroke and lower in patients with atherothrombotic or lacunar stroke. Because fibrinogen increases during both inflammatory and thrombotic processes, this ratio suggests that a raised level of fibrinogen is related to thrombogenic activity in cardioembolic strokes and to inflammation in the other two subtypes.

Fibrinogen participates in thrombogenesis, platelet aggregation, blood viscosity, atherogenesis and inflammation. Raised plasma levels of fibrinogen have been firmly related to a higher

Table 3 Multinomial regression logistic results for mechanism of stroke using cardioembolic category as the reference

	Atherothrombotic versus cardioembolic			Lacunar versus cardioembolic			Undetermined versus cardioembolic		
	B	OR (95% CI)	p Value	B	OR (95% CI)	p Value	B	OR (95% CI)	p Value
Age (years)	−0.035	0.965 (0.909 to 1.025)	0.245	−0.021	0.979 (0.925 to 1.036)	0.468	0.011	1.011 (0.958 to 1.066)	0.695
Male gender	0.255	1.291 (0.274 to 6.734)	0.762	0.260	1.296 (0.311 to 5.399)	0.721	0.222	1.249 (0.353 to 4.415)	0.730
NIHSS on admission	−0.072	0.931 (0.832 to 1.041)	0.211	−0.126	0.882 (0.783 to 0.994)	*0.039*	−0.041	0.960 (0.877 to 1.050)	0.368
Diabetes mellitus	0.359	1.432 (0.335 to 6.127)	0.628	0.624	1.867 (0.481 to 7.249)	0.367	1.027	2.791 (0.822 to 9.484)	0.100
Smoking	2.671	14.455 (2.299 to 90.865)	*0.004*	1.279	3.593 (0.655 to 19.698)	0.141	0.333	1.396 (0.289 to 6.748)	0.678
Total cholesterol (mg/dl)	0.024	1.024 (1.008 to 1.040)	*0.004*	0.014	1.015 (0.999 to 1.030)	0.069	0.008	1.008 (0.994 to 1.022)	0.272
D-dimer (ng/ml)	−0.003	0.997 (0.994 to 0.999)	*0.025*	−0.005	0.995 (0.992 to 0.999)	*0.004*	−0.003	0.997 (0.995 to 0.999)	*0.015*
CRP (mg/dl)	0.086	1.090 (0.737 to 1.610)	0.666	−0.084	0.920 (0.480 to 1.762)	0.801	0.040	1.040 (0.835 to 1.296)	0.725
ESR (mm/h)	0.066	1.068 (1.020 to 1.118)	*0.005*	0.052	1.053 (1.006 to 1.102)	*0.026*	0.001	1.001 (0.959 to 1.046)	0.951
Intercept	−2.805		0.366	−0.281		0.926	−1.114		0.689

Goodness-of-fit: χ^2 (Pearson): 480.032 (p=0.158). Pseudo R^2 (Cox and Snell): 0.467. N=200. B, β coefficient.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; NIHSS, National Institutes of Health Stroke Scale.

risk of developing coronary heart disease and ischaemic stroke.[10 11] Previous studies found increased levels of fibrinogen during the acute phase of stroke. The authors related it to the pathophysiological substrate of stroke and/or the acute response to tissue damage, but they did not assess correlations with the mechanism.[16 27] Later, weak associations between fibrinogen (or some polymorphisms) and atherothrombotic and lacunar strokes and development of carotid stenosis ≥50% were reported.[28 29] In contrast, raised fibrinogen may represent, similar to D-dimer and von Willebrand factor, intracardiac thrombogenesis in patients with chronic atrial fibrillation.[15]

Consistent with previous data, the present study found higher fibrinogen in patients with cardioembolic and atherothrombotic strokes. The role of fibrinogen is probably different in the pathophysiology of cardioembolic and atherothrombotic stroke. In the first subtype, the increased level of D-dimer and D-dimer/fibrinogen ratio suggest an association with intracardiac thrombogenesis. However, in atherothrombotic stroke, the low D-dimer/fibrinogen ratio and the raised levels of CRP and ESR may relate to systemic inflammation. Obviously there is a close relationship between thrombosis and inflammation, and both processes are present in all strokes; the isolated assessment of fibrinogen is insufficient to characterise this.

The raised level of CRP was related to inflammation and progression of endothelial damage in atherosclerotic disease.[30] This parameter may be raised in patients at risk of suffering an acute coronary syndrome, worse outcome after myocardial ischaemia, ischaemic stroke and worse functional outcome and higher mortality after a cerebrovascular event.[31–34]

Several studies found an increase in CRP during the acute phase of stroke and some suggested that its level may be higher in patients with TACI syndrome and patients with cardioembolic stroke.[7 22 35] These studies related the early increase in CRP to acute phase response after ischaemia and/or pre-existing inflammatory conditions but patients with systemic complications were not excluded. This is especially relevant because these complications are more frequent in patients with cardioembolism or TACI syndrome.[36] The present study found an association between raised CRP and atherothrombotic and cardioembolic strokes, which may characterise both the acute phase response and the endothelial inflammatory processes underlying atherosclerosis and some cardiopathies.

ESR represents the velocity of red cell precipitation. Fibrinogen is one of its determinants but both are used separately to assess cardiovascular risk. Epidemiological studies related raised ESR to a higher risk of suffering a fatal or non-fatal coronary event.[4] In patients with acute ischaemic stroke, higher values of ESR during the first 72 h were associated with the risk of early neurological deterioration and worse functional state on discharge.[5] Raised values for leucocyte count and ESR showed a positive correlation with modified Rankin scale scores at 30 days, but not with the size of infarct or acute phase reaction.[6] The results excluded a correlation between ESR and TACI syndromes and showed an association between higher values for ESR and atherothrombotic and lacunar strokes, suggesting that ESR is independent of fibrinogen, and it may be a surrogate marker of inflammatory processes underlying micro and macroangiopathy.

The present study had several limitations. Firstly, the lack of follow-up may imply that patients with recurrent stroke and additional clinical information remained with a diagnosis of undetermined stroke. Secondly, the analysed markers were unspecific and evaluated superficially the role of inflammation and thrombogenesis. Thirdly, serial determinations of parameters were not performed. These evaluations may be useful to characterise the acute phase response after stroke although previous studies reported that the analysed parameters remain relatively stable during the first week after the episode.[37] Finally, there were no data about the final volume of infarct to correlate tissue damage with the assessed parameters and the acute phase response.

In summary, the present study identified analytical changes characteristic of the acute phase of ischaemic stroke. As patients suffering severe systemic conditions were excluded, these findings must be related to stroke. Because there was no correlation between TACI syndromes and the markers, it is reasonable to hypothesise that the analysed parameters represent both the mechanism of the stroke and the acute response to ischaemia. Three analytical profiles were suggested: thrombogenic, inflammatory and non-specific.

Determination of the assessed parameters is not expensive and is available in most centres. They may proportionate physiopathological information useful for diagnosis when assessed during the first 72 h of ischaemic stroke in patients without severe systemic complications.

Competing interests None.

Ethics approval This study was conducted with the approval of the Hospital of Covilhã.

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Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke

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