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D-Dimers Predict Stroke Subtype when Assessed Early

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Key Words
Acute stroke · Stroke subtype · Biomarkers · Coagulation · D-dimers

Abstract
Background: Early classification of ischemic stroke subtype is important for secondary stroke prevention and may guide further investigations. ***Methods:*** Levels of coagulation activation [fibrinopeptide A (FPA), prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT)] and fibrinolysis activation [plasmin-α_2-antiplasmin complex (PAP), D-dimers] markers were measured in 98 consecutive patients with a first-ever acute ischemic stroke admitted within 12 h after symptom onset. ***Results:*** Median age was 67 years and 44% were women. Median time from symptom onset to blood sampling was 4 h. Stroke subtype was classified as 'cardioembolic' (54%), 'large-artery atherosclerosis' (11%), 'small-vessel disease' (5%), 'other determined' (9%) or 'undetermined etiology' (20%). Patients with cardioembolic stroke suffered more often from coronary artery disease than patients with other stroke etiologies (40 vs. 22%, p = 0.019). There were no differences in age, sex, stroke severity, time to blood sampling, frequency of hypertension, diabetes mellitus or current smoking. D-dimers (medians) were higher in patients with cardioembolic strokes than in those with other etiologies (615 vs. 322 μg/l, $p < 0.001$). No differences in F1+2, FPA, TAT or PAP levels were found. After multivariate analysis, higher D-dimer levels remained independently associated with cardioembolic stroke (p = 0.022). When measured within 6 h, D-dimers below 300 μg/l excluded cardioembolic stroke with a sensitivity of 100% and a specificity of 52%. ***Conclusions:*** Low D-dimer levels in the first few hours make a cardioembolic stroke unlikely, and may be useful to guide further investigations. Other coagulation markers were not useful in differentiating between different stroke etiologies.

Introduction

Several studies have demonstrated differences in coagulation and fibrinolysis markers between various ischemic stroke subtypes [1–10]. One difference between atherothrombotic and cardioembolic stroke is the duration from thrombus formation to symptom onset, which is longer in the latter. Therefore, we hypothesized that differences in the levels of fibrin formation and fibrinolytic markers might be detectable in acute stroke.

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Limited data are available on coagulation markers very early after symptom onset, as the majority of previous studies were performed >12 h after symptom onset [3, 5–8, 10–14]. D-dimer levels have been shown to be associated with cardioembolic strokes [4, 6–10, 12, 14], yet their significance in acute stroke remains to be determined.

The aim of the present study was to analyze different coagulation parameters [thrombin-antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2), fibrinopeptide A (FPA), D-dimers and plasmin-α_2-antiplasmin complex (PAP)] very early after stroke onset in relation to stroke etiology.

Materials and Methods

Patient Selection

One hundred and sixty-three consecutive patients with a first-ever acute ischemic stroke with a symptom duration of <12 h were screened. Exclusion criteria were: transient ischemic attack, intracranial hemorrhage, seizure, intracranial neoplasm, migraine, intoxication, metabolic or psychiatric disorders, and conditions affecting coagulation (anticoagulants, sepsis, malignancy, deep venous thrombosis, major surgery or trauma within 30 days, and myocardial infarction within 10 days). Informed consent was obtained, and the study was approved by the local ethics committee.

Patient Workup

Stroke severity was graded using the National Institutes of Health Stroke Scale [15]. In addition, cardiovascular risk factors and history of coronary artery disease were assessed.

Blood Sampling

Blood was drawn (S-Monovette; Sarstedt, Nuembrecht, Germany) immediately after admission. A 10-ml Monovette containing 1 ml of 0.106 M trisodium citrate and a 5-ml tube containing 0.5 ml CTAD-PPACK were collected. Blood samples were centrifuged twice at 3,000 rpm. The plasma was snap-frozen and stored at –70°C.

Coagulation and Fibrinolysis Markers

Measurements were performed using the PAP Complex ELISA Kit (Technoclone, Dorking, UK) for PAP, the FPA ELISA Kit (Vitrochemie, Nijmegen, The Netherlands) for FPA, Enzygnost Micro (Dade Behring, Eschborn, Germany) for TAT and F1+2, and Asserachrom D-dimer (Diagnostica Stago, Asnières sur Seine, France) for D-dimers. All assays were performed twice.

Stroke Subtype Classification

A neurologist, blinded to the laboratory results, classified stroke subtype according to the Trial of ORG 10172 in Acute Stroke Therapy [16] criteria into: (1) cardioembolic; (2) large artery atherosclerosis; (3) small vessel occlusion; (4) other determined etiology; and (5) undetermined etiology. The following examinations were used: trans-esophageal (n = 58) and/or transthoracic echocardiography (n = 74), MRI/MRA (n = 55) and/or cranial CT/CTA (n = 94), 24-hour electrocardiography (n = 50), neurovascular ultrasound (n = 61) and/or digital subtraction arteriography (n = 54).

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 (SPSS, Chicago, Ill., USA). Patients were grouped according to stroke etiology into cardioembolic and non-cardioembolic causes. Incompletely evaluated patients (n = 7) were not included. Differences between groups were examined by the χ^2 test, Mann-Whitney rank-sum test and Student's t test. To identify variables independently associated with cardioembolic stroke etiology, a multivariate analysis (backward stepwise method) was performed. Only variables with a value of $p < 0.15$ in univariate analysis were evaluated. Since D-dimers and PAP are co-linear, these variables were entered separately into the multivariate analysis. To assess the value of D-dimers, receiver operating characteristic (ROC) curves, sensitivity, and specificity were calculated. Prespecified subgroup analyses were performed for D-dimers analyzed within 3 and 6 h of symptom onset.

Results

Ninety-eight patients were enrolled in the study. Patient characteristics and stroke subtypes are presented in table 1. Patients with cardioembolic stroke tended to be older than those with other stroke subtypes ($p = 0.053$), and they more often had a history of coronary artery disease ($p = 0.042$). The latter remained statistically significant after multivariate analysis ($p = 0.019$), whereas no differences were found with regard to other variables. Blood samplings for coagulation analysis were performed a median of 4 h (range 1–12) from onset of symptoms.

Differences in coagulation activation and fibrinolytic markers are summarized in tables 2 and 3.

Patients with cardioembolic strokes had significantly higher D-dimer levels than those with other etiologies ($p < 0.001$). There was a trend towards higher PAP levels in the cardioembolic group ($p = 0.106$). No differences in FPA, F1+2 or TAT levels were found. After multivariate analysis, higher D-dimers remained independently associated with cardioembolic stroke ($p = 0.022$), whereas PAP did not. No patient with D-dimer levels below 300 μg/l measured within 6 h had a cardioembolic stroke. D-dimers below 300 μg/l excluded a cardioembolic stroke with a sensitivity of 100% and a specificity of 52%. When analyzed within 3 h, sensitivity and specificity were 100 and 57%, respectively. ROC curves for D-dimers within 3 and 6 h are shown in figures 1 and 2. Area under the ROC curve for measurement within 6 h was 0.83 (95% CI 0.73–0.93), and for measurement within 3 h it was 0.87 (95% CI 0.77–0.97).

Table 1. Patient characteristics according to stroke subtype

	All patients	Cardio-embolic	Large artery athero-sclerosis	Other determined etiology	Small vessel disease	Undeter-mined etiology (evaluation negative)	Undeter-mined etiology (>1 possible cause)	Incomplete evaluation	p value (uni-variate)	p value (multi-variate)
Number of patients	98 (100)	53 (54)	11 (11)	9 (9)	5 (5)	12 (12)	1 (1)	7 (7)		
Median age, years (range/IQR)	67 (16–79/19)	70 (16–79/17)	66.5 (43–78/20)	49 (38–68/12)	63 (62–71/7)	62 (40–79/20)	63	70 (41–73/14)	0.053	0.364
Male gender	55 (56)	29 (55)	8 (73)	7 (78)	2 (40)	5 (42)	0	4 (57)	0.761	0.988
Hypertension	59 (60)	32 (60)	6 (55)	3 (33)	3 (60)	8 (67)	1	6 (86)	0.794	0.536
Diabetes mellitus	14 (14)	9 (17)	1 (9)	0	0	2 (17)	0	2 (29)	0.408	0.559
Current smoker	23 (23)	12 (23)	3 (27)	1 (11)	0	4 (33)	1	2 (29)	0.96	0.44
Coronary artery disease	31 (32)	21 (40)	1 (9)	0	0	4 (33)	1	4 (57)	0.042	0.019
Median time to blood sampling, h (IQR)	4 (2)	3 (2)	5.5 (5.4)	3 (1.8)	3 (2.1)	3 (3)	2.5	2 (3)	0.138	0.053
Median NIHSS score on admission (IQR)	9 (12)	10 (12)	10.5 (9)	9 (13)	2 (3)	7 (8)	5	19 (10)	0.154	0.085

Figures in parentheses are percentages, unless otherwise indicated; p values refer to cardioembolic vs. all non-cardioembolic strokes.
IQR = Interquartile range.

Table 2. Levels of different hemostatic markers according to stroke subtype

	All patients	Cardioembolic	Large artery atherosclerosis	Other determined etiology	Small vessel disease	Undetermined etiology (evaluation negative)	Undetermined etiology >1 possible cause)	Incomplete evaluation
n	98	53	11	9	5	12	1	7
FPA, μg/l	14.3 (21.0)	14.3 (21.3)	15.1 (25.8)	3.8 (10.2)	22.0 (21.9)	9.2 (15.4)	14.9	39.8 (37.8)
F1+2, nmol/l	1.3 (0.5)	1.3 (0.4)	1.3 (0.9)	1.1 (0.6)	1.0 (1.0)	1.3 (0.5)	1.18	1.5 (0.5)
TAT, μg/l	5.8 (11)	6.1 (12)	6.0 (27)	3.1 (17)	3.7 (17)	4.6 (5)	5.3	8.4 (34)
D-dimers, μg/l	545 (452)	615 (405)	465 (835)	202 (417)	290 (217)	320 (373)	437	709 (1,153)
PAP, μg/l	269.8 (172.6)	286 (188.3)	235.6 (179.5)	222.5 (231.7)	228.6 (131.5)	219.6 (332.0)	84.9	262.0 (134.3)

Data are presented as medians with IQR in parentheses.

Discussion

Compared to other etiologies, in 1,153 cardioembolic strokes thrombus generation and consecutive fibrinolysis occur well before symptomatic thrombus embolization. However, with time, D-dimer levels are likely to rise in all patients as a result of fibrinolysis of the clot which caused the stroke symptoms, regardless of etiology. Therefore, our hypothesis was that cardioembolic strokes can be distinguished from other stroke etiologies by measuring D-dimer levels very early. The results of our study confirm our hypothesis: D-dimers were significantly higher in patients with cardioembolic strokes. The most striking finding was that no patient with D-dimers below 300 μg/l measured within 6 h of symptom onset had a cardioembolic stroke (sensitivity 100%, specificity 52%).

Table 3. Comparison of different hemostatic markers

	Cardioembolic (n = 53)	Non-cardio-embolic (n = 38)	p value (uni-variate)	p value (multi-variate)
FPA, μg/l	14.3 (21.3)	11.2 (17.3)	0.292	
F1+2, nmol/l	1.3 (0.4)	1.2 (0.6)	0.344	
TAT, μg/l	6.1 (12)	4.5 (8)	0.187	
D-dimers, μg/l	615 (405)	322 (363)	<0.001	0.022
PAP, μg/l	286.0 (188.3)	223.7 (250.5)	0.106	0.568

Data are presented as medians with IQR in parentheses.

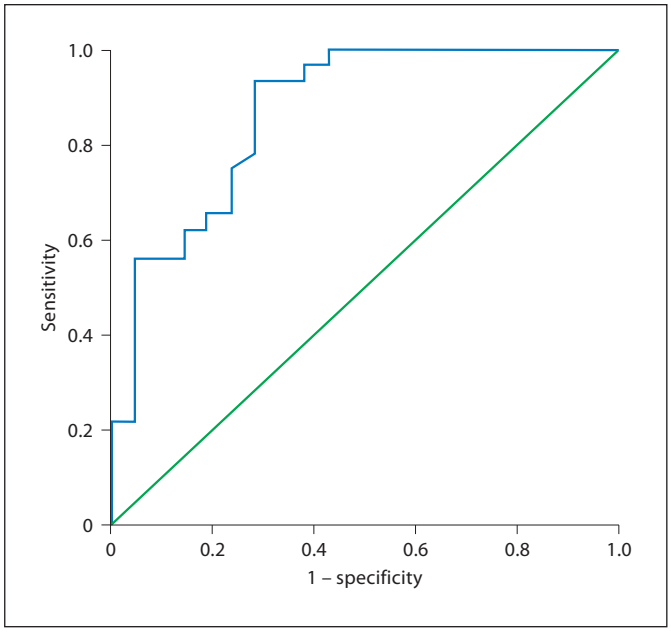

Fig. 1. ROC curve for D-dimer levels analyzed within 3 h of symptom onset (n = 57).

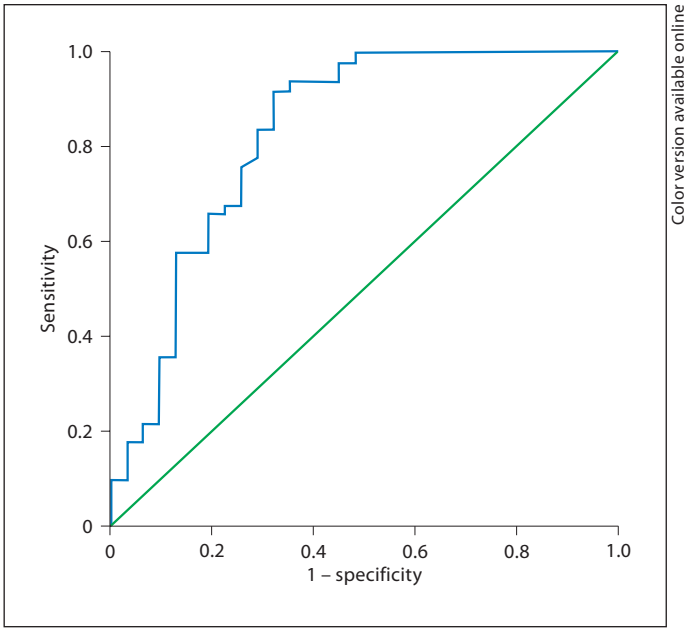

Fig. 2. ROC curve for D-dimer levels analyzed within 6 h of symptom onset (n = 87).

This association was even stronger when D-dimers were measured within 3 h (sensitivity 100%, specificity 57%). The other markers were less accurate for distinguishing between cardioembolic and non-cardioembolic strokes. There was a trend towards higher PAP levels in the cardioembolic group, whereas F1+2, TAT and FPA levels were not different between the groups. This is most likely due to the small sample size.

Differences in coagulation/fibrinolysis activation in cardioembolic stroke have been demonstrated before, yet time to coagulation analyses varied considerably [4, 7, 10, 12–14, 17]. The strength of the present study and the major difference to earlier studies is the very short interval from symptom onset to blood sampling (median 4 h). The fact that there was a relatively high number of cardioembolic strokes in our study might be due to a selection bias, i.e., as a tertiary care referral center for stroke and interventional stroke therapy, our patients are younger and suffer severer strokes than average stroke patients. Younger patients are more likely to suffer a stroke of cardioembolic origin. In addition, cardioembolic strokes might be severer than other stroke etiologies (e.g. small vessel disease) due to larger clot volumes with obstruction of larger vessels. Due to this potential limitation and the small sample size, further and larger studies are needed to confirm our results.

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References

1 Altes A, Abellan MT, Mateo J, Avila A, Marti-Vilalta JL, Fontcuberta J: Hemostatic disturbances in acute ischemic stroke: a study of 86 patients. Acta Haematol 1995;94:10–15.
2 Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ: Hemostatic function and progressing ischemic stroke: D-dimer predicts early clinical progression. Stroke 2004;35:1421–1425.
3 Berge E, Friis P, Sandset PM: Hemostatic activation in acute ischemic stroke. Thromb Res 2001;101:13–21.
4 Dahl T, Kontny F, Slagsvold CE, et al: Lipoprotein(a), other lipoproteins and hemostatic profiles in patients with ischemic stroke: the relation to cardiogenic embolism. Cerebrovasc Dis 2000;10:110–117.
5 Ince B, Bayram C, Harmanci H, Ulutin T: Hemostatic markers in ischemic stroke of undetermined etiology. Thromb Res 1999;96:169–174.
6 Kataoka S, Hirose G, Hori A, Shirakawa T, Saigan T: Activation of thrombosis and fibrinolysis following brain infarction. J Neurol Sci 2000;181:82–88.

7 Takano K, Yamaguchi T, Uchida K: Markers of a hypercoagulable state following acute ischemic stroke. Stroke 1992;23:194–198.
8 Tombul T, Atbas C, Anlar O: Hemostatic markers and platelet aggregation factors as predictive markers for type of stroke and neurological disability following cerebral infarction. J Clin Neurosci 2005;12:429–434.
9 Uchiyama S, Yamazaki M, Hara Y, Iwata M: Alterations of platelet, coagulation, and fibrinolysis markers in patients with acute ischemic stroke. Semin Thromb Hemost 1997;23:535–541.
10 Yamazaki M, Uchiyama S, Maruyama S: Alterations of haemostatic markers in various subtypes and phases of stroke. Blood Coagul Fibrinolysis 1993;4:707–712.
11 Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ: D-dimer predicts early clinical progression in ischemic stroke: confirmation using routine clinical assays. Stroke 2006;37:1113–1115.
12 Ageno W, Finazzi S, Steidl L, et al: Plasma measurement of D-dimer levels for the early diagnosis of ischemic stroke subtypes. Arch Intern Med 2002;162:2589–2593.
13 Takano K, Yamaguchi T, Kato H, Omae T: Activation of coagulation in acute cardioembolic stroke. Stroke 1991;22:12–16.
14 Montaner J, Perea-Gainza M, Delgado P, et al: Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 2008;39:2280–2287.
15 Brott T, Adams HP Jr, Olinger CP, et al: Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–870.
16 Adams HP Jr, Bendixen BH, Kappelle LJ, et al: Classification of subtype of acute ischemic stroke: definitions for use in a multicenter clinical trial. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35–41.
17 Anzola GP, Magoni M, Ascari E, Maffi V: Early prognostic factors in ischemic stroke: the role of protein C and protein S. Stroke 1993;24:1496–1500.