Persistent hyperfibrinogenemia in acute ischemic stroke / transient ischemic attack (TIA)

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Summary

Increased fibrinogen concentration is a well known phenomenon following acute ischemic stroke. However, the natural course of this hyperfibrinogenemia is uncertain. We aimed to clarify whether it is of a transient or more persistent nature in patients who harbor an underlying morbid biology of atherothrombo-inflammation. Venous blood for fibrinogen measurements was obtained from the control group participants and from stroke patients within 24 hours of admission, as well as 12 months following the acute event. In order to perform a time course analysis, we divided our cohort into tiles of time from symptoms' onset and compared the fibrinogen concentrations using ANOVA. Elevated fibrinogen concentrations were found in stroke patients on admission compared with matched controls ($p<0.001$). Analysis of variance in the different tertiles of time from symptoms' onset identified that fibrinogen concentrations were already relatively high during the initial phase of the event and did not differ significantly between the tiles ($p=0.268$). Moreover, when we calculated the absolute differences between the patients' fibrinogen concentrations and that of the matched controls there was clearly a minor increment during the time course from symptoms' onset in the stroke patients group. In conclusion, persistent hyperfibrinogenemia is present in patients with acute ischemic cerebral events and it might be present during the earlier stages of the disease as presently shown. Prompt and long-term, rather than short term, interventions to reduce the concentrations of this protein might therefore be of relevance.

Keywords

Stroke, fibrinogen, inflammation

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Introduction

There is a wealth of evidence to support the role for hyperfibrinogenemia in the pathogenesis of acute ischemic vascular diseases (1–4), including acute ischemic stroke (5–7).

As fibrinogen is an acute phase protein, the hyperfibrinogenemia associated with stroke could at least in part be a response to an underlying vessel wall disease, which has many of the characteristics of an inflammatory process. Alternatively, this raised plasma fibrinogen may be the epiphenomenon of the severity of the vascular injury, the response to vascular injury and the plaque rupture which is taking place(8). Whether it is a background process or a result of the acute event, elevated fibrinogen levels shortly after acute ischemic stroke are associated with a worse prognosis (9), recurrent stroke (10) and an increased risk of death within one year following the event(11).

Together, this suggests that reduced fibrinogen concentrations in stroke patients may improve their condition. However, studies which employed defibrinogenating enzymes (12, 13) or thrombolysis in the presence of increased fibrinogen concentrations (14, 15) did not show clear evidence of an improved prognosis.

In this study we addressed the natural course of the increased fibrinogen concentration that has been detected in acute ischemic stroke/transient ischemic attack (TIA). Our aim was to check whether it has a transient or a more persistent nature in this particular group of patients who harbor an underlying morbid biology of atherothrombo-inflammation.

The results of the present study might be relevant for potential therapeutic approaches due to the fact that the observed hyperfibrinogenemia might represent a profound and persistent alteration of the haemostatic balance and might not represent a transient phenomenon. If further confirmed by other studies, we predict that this persistent hyperfibrinogenemia could potentially be the reason why a single intervention to reduce fibrinogen concentrations is not a sufficient strategy.

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Materials and methods

Patients

We conducted a hospital-based case-control study. One hundred ninety-six patients with a documented event of acute ischemic stroke were enrolled in the study. The patients were all admitted to our medical center between January 2002 and January 2006. Inclusion criteria were patients with acute ischemic stroke or TIA, aged >18 years and within 24 hours from hospital admission. Exclusion criteria included stroke resulting from trauma or an invasive procedure, cerebral hemorrhage, history of malignant tumor, chronic inflammatory diseases, autoimmune diseases, coagulation disorders, signs and symptoms of acute or chronic infections or treatment with anti-inflammatory agents, excluding acetylsalicylic acid (up to 325 mg/day). Written informed consent was obtained from all participants. When aphasia was present, a signature was obtained from an immediate family member in compliance with the instructions of the local Institutional Ethics Committee. The neurological state of the patients was assessed using the National Institutes of Health Stroke Scale (NIHSS) (16) and the modified Rankin Scale (mRS) (17) by a certified senior vascular neurologist.

Controls

The methods of recruitment and enrollment of the control subjects have been described elsewhere (18). We included neurologically asymptomatic subjects who were in routine follow-up in the various outpatient clinics of the medical center between February 2001 and September 2004. These include diabetes, hypertension, metabolic disorders and dyslipidemia clinics. Excluded were individuals with known inflammatory diseases (arthritis, inflammatory bowel disease, etc), history of cerebral or cardiac ischemic event during the previous 12 months, history of acute febrile disease or infection during the previous three months, known malignancy, pregnancy, steroidal or nonsteroidal treatment (except for aspirin at a dose of ≤ 325 mg/day) and invasive procedures (surgery, catheterization, etc) during the prior six months. Written informed consent was obtained from all participants.

Matching procedure

From the entire control group, subjects were matched to the case subjects by gender, age, body mass index (BMI) and vascular risk factors.

Table 1: Population characteristics.

	Stroke/ TIA patients	Controls	p-value
n	196	196	
Gender, males (%)	61	61	1
Age, years (SD)	68.31 (12.4)	68.34 (11.2)	0.921
BMI, kg/m^2	26.83 (4.6)	26.88 (4.1)	0.705
Current smokers (%)	10.3	7.2	0.281
Diabetes Mellitus (%)	31.1	25.8	0.246
Hyperlipidemia (%)	45.4	49.7	0.387
Hypertension (%)	62.1	53.8	0.101

TIA indicates transient ischemic attack; BMI, body mass index.

Definitions of risk factors

Diabetes mellitus was defined as a fasting blood glucose of ≥ 126 mg/dl or the use of insulin or oral hypoglycemic agents; hypertension as intermittent blood pressure measurements of ≥ 140/90 mmHg or the use of anti-hypertensive medications; For individuals without a fasting lipid profile, hyperlipidemia was recorded if the diagnosis of hyperlipidemia was included in their medical records or if they were receiving lipid-lowering medication (HMG-CoA reductase inhibitors or fibrates). For individuals with valid lipid profiles, it was defined by the low-density lipoprotein (LDL) or non-high-density lipoprotein (non-HDL) cholesterol concentrations (in individuals displaying elevated triglyceride concentrations of ≥ 2·26 mM) above the recommended levels according to the risk profile defined by the updated ATP III recommendations (19), or the intake of lipid-lowering medication; and current smokers as those smoking at least five cigarettes per day.

Laboratory methods

Venous blood was obtained from the control group participants and from all stroke patients within 24 hours of admission (mean time ± S.D.: 25.5±13.1 h from onset of stroke symptoms, 'Time 1') as well as at 12 months following the acute event ('Time 2').

The intensity of the acute phase response was determined by assessing the levels of inflammatory biomarkers at Time 1 and at Time 2. The biomarkers tested included the quantitative fibrinogen by employing Clauss's (20) method on a Sysmex 6000 analyzer (Sysmex Corporation, Hyaga, Japan), erythrocyte sedimentation rate (ESR) by Westergen's (21) method, concentrations of high sensitive C-reactive protein (hsCRP) by the Behring BN II Nephelometer (Dade Behring, Marburg, Germany) (22), and white blood cell count (WBCC) determined by the Coulter STKS electronic analyzer (Beckman Coulter, Nyon, Switzerland).

Statistical analysis

All continuous data was summarized and displayed as mean ± SD. Results of fibrinogen, ESR, hs-CRP and WBCC were compared using the paired *t* test. In addition, we further divided our group of patients into three groups (tiles) according to the time from the onset of stroke symptoms. Analysis of variance (ANOVA) conducted by the Dunnett's T3 post hoc comparison tests were used to determine the statistical differences between the tiles. The χ^2 test was used to assess associations among categorical variables, Correlation between the absolute difference between fibrinogen concentration of stroke patients and that of their matched control with time from symptoms onset was determined using the two tailed-Spearman rank correlation. $p<0.05$ was considered statistically significant. SPSS/WIN (version 13.0, SPSS INC, Chicago, IL, USA) software was used to carry out all statistical analyses.

Results

For the 196 acute patients enrolled in this study the mean NIHSS was 4.185 ± 4.1 and the mean mRS was 1.95 ± 1.6.

We analyzed the concentrations of clottable fibrinogen in this group of patients and in the same number of age, gender, BMI and

Table 2: Fibrinogen concentrations of stroke patients on admission, 12 months thereafter and in their matched controls, mg/dl.

Group	Patients on admission (1)	Patients at 12 months (2)	p Value (paired t test) (1–2)	Matched controls (3)	p Value (paired t test)	
The entire cohort n=196	336.0 (73.2)	328.6 (65.9)	0.974	308.0 (61.2)	1–3 2-3	**<0.001** **0.008**
Stroke patients only n=157	343.6 (75.7)	329.3 (66.8)	0.402	306.0 (61.8)	1–3 2-3	**<0.001** **0.032**
TIA patients only n=39	311.3 (60.1)	322.6 (62.3)	0.232	312.7 (56.4)	1–3 2-3	0.916 0.263

TIA indicates transient ischemic attack.

Table 3: Additional inflammatory markers of stroke/TIA patients on admission, 12 months thereafter and in their matched controls.

Variable	Patients on admission (1)	Patients at 12 months (2)	p Value (1–2)	Matched controls (3)	p Value (paired t test)	
hs-CRP, mg/l	9.00 (24.9)	3.86 (4.8)	**0.028**	3.65 (4.4)	1–3 2-3	**0.004** 0.482
ESR, mm/h	28.04 (18.7)	22.01 (16.4)	**0.012**	20.69 (1.16)	1–3 2-3	**<0.001** 0.407
WBC, $10^3/\mu l$	8.62 (2.6)	6.89 (1.7)	**<0.001**	6.89 (1.8)	1–3 2-3	**<0.001** 0.760

Hs-CRP indicates high-sensitive C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell.

vascular risk factors matched controls. Details regarding the population characteristics are shown in Table 1. The mean age, male:female ratio, BMI (body mass index), incidence of current smoking and prevalence of hypertension, hyperlipidemia and diabetes mellitus did not significantly differ between the two groups.

The mean ± SD of fibrinogen concentrations in the entire cohort, in patients with stroke and those with TIA are presented in Table 2. Fibrinogen concentrations of stroke patients as well as TIA patients were elevated on admission compared with matched controls ($p<0.001$). Furthermore, we report that the fibrinogen concentrations 12 months following the acute event did not significantly differ from the initial measurements.

To assess the putative association of hyperfibrinogenemia with neurological scores, we divided up our patients according to a fibrinogen concentration below or equal to and above 375 mg/dl. Patients with a fibrinogen level equal to and above 375 mg/dl had significantly poorer neurological scores upon admission to hospital ($p=0.007$ for NIHSS and $p=0.017$ for mRS). The patients with a fibrinogen level equal and above 375 mg/dl also presented a worse neurological prognosis 12 months thereafter

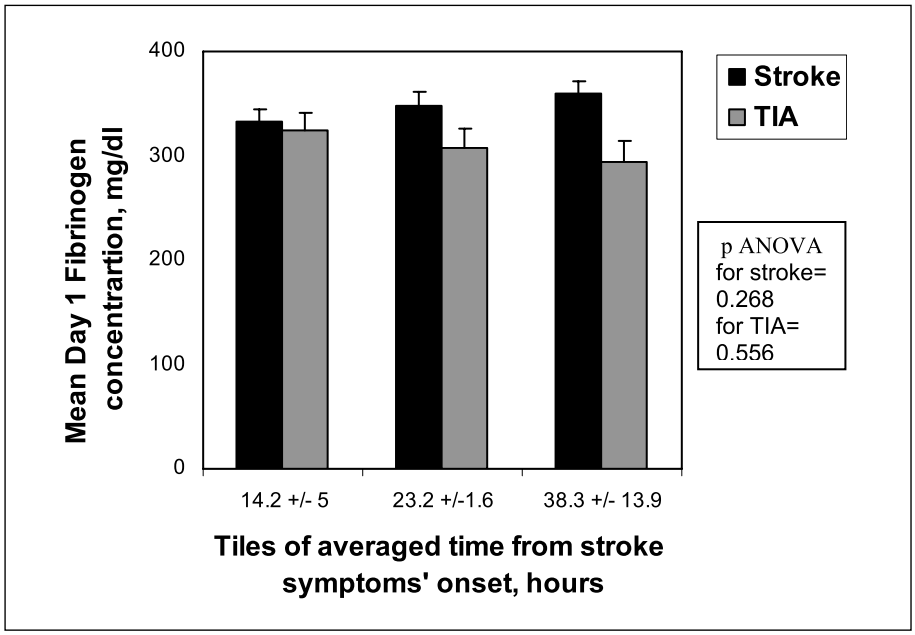

Figure 1: Fibrinogen concentrations of stroke/TIA patients on admission according to tiles of time from symptoms' onset.

compared to patients with a fibrinogen level below 375 mg/dl, but the difference was insignificant (p=0.089 for NIHSS and p=0.069 for mRS), likely due to the small sample size. Moreover, when we compared the percentage of individuals with a fibrinogen concentration ≥ 375 mg/dl we found that 22% of the stroke group had a fibrinogen concentration ≥ 375 mg/dl compared to only 10% in the control group (p=0.08).

In addition to fibrinogen, we evaluated the acute phase response using the hs-CRP, ESR and WBCC levels. These biomarkers were significantly elevated in patients during the acute phase compared to their levels 12 months thereafter as well as to matched controls. However, unlike fibrinogen, these parameters returned to basal levels 12 months following the event (Table 3).

Due to the possibility that fibrinogen is an acute response marker of the ischemic event itself, we divided our cohort into tiles of time from symptoms' onset. The results are presented in Figure 1. Although higher levels were observed in patients with stroke, these were small in absolute values and were not statistically significant.

In order to further elucidate the question of a changing difference in fibrinogen concentration with the passage of time from the event onset we calculated for each patient the absolute difference between their fibrinogen concentration and that of their matched control. There is clearly only a minor increment along the time course from symptoms' onset in the group of stroke patients (r=0.149, p=0.113) and a complete lack of increment in individuals with TIA (r=0.035, p=0.848).

Discussion

We have presently looked at fibrinogen concentrations during the relatively early stages of acute ischemic stroke/TIA and in addition compared them to those obtained one year later as well as to matched controls. The results showed that fibrinogen concentrations were already relatively high during the initial phase of the event and remained elevated 12 months thereafter compared to the matched controls. These findings raise the possibility that individuals with cerebrovascular ischemic events harbor a long and persistent stimulus for fibrinogen synthesis that is not necessarily associated solely with the acute event.

Fibrinogen is a known risk marker for future vascular events (23–25) and the first question that we addressed was whether the hyperfibrinogenemia, known to be present in acute ischemic stroke/TIA patients, might be a factor that contributes to the etiopathogenesis of the event or whether it is a consequence of the acute phase response that results from the ischemia/infarction of the brain tissue.

In order to respond to this question, at least in part, we divided our cohort into tertiles of time from symptoms' onset. It emerged that the concentrations of fibrinogen were already relatively high from the time of onset of disease symptoms (first tile), suggesting the possibility that relatively enhanced fibrinogen concentrations were already present before the appearance of symptomatology. From the time course analysis of the acute phase response it is known that maximal fibrinogen concentrations are not expected earlier than 72 hours from the onset of the inflammatory process (26). Thus, it is highly unlikely that the increased concentrations of fibrinogen observed in the first tile of time (Fig. 1) are mainly the result of the acute event. The possibility exists, therefore, that these relatively high concentrations were already present before the onset of symptomatology. This is especially true in the group of patients with TIA whose concentrations of fibrinogen did not increase along with the time tiles from symptoms' onset.

Support for the concept of enhanced background fibrinogen concentrations in stroke/TIA patients comes from the findings that they did not return to the expected baseline as determined by the use of matched controls. However, it was noted that there was a decline in the concentrations of this protein relative to the acute phase, suggesting that fibrinogen presents in higher concentrations during the period of time surrounding the acute event.

Our potential conclusions from the present investigation would be that acute stroke/TIA patients might harbor a hitherto unknown background inflammatory process and that this is further enhanced during the acute phase of the event. These findings are relevant to the better understanding of the biology of this protein in the context of cerebrovascular events. For example, it might explain, at least in part, the failure of short term defibrinogenating treatment (12) as well as thrombolysis (14, 15).

Multiple mechanisms have been suggested for the harmful effects of hyperfibrinogenemia and these include increased viscosity (27) and platelet aggregability(28), enhanced red blood cell aggregation (29) and clot firmness (30) as well as reduced susceptibility to fibrinolysis (14, 15). Therefore, the finding of hyperfibrinogenemia during the course of symptomatic cerebrovascular disease might rationalize therapeutic interventions. Moreover, the potential importance of this finding is exemplified by a recent study documenting the negative consequences of higher fibrinogen concentrations patients treated with antiplatelet aggregation agents (31). If confirmed in future studies, persistent hyperfibrinogenemia might be a target for long- rather than short-term interventions(32).

Of special interest is the fact that with the use of a fibrinogen concentration of 375 mg/dl as a cut-off, as found in the study of Mahmud et al. (31), a worse neurological outcome was noted in our cohort.

In conclusion, persistent hyperfibrinogenemia is present in patients with acute ischemic stroke/TIA. This hyperfibrinogenemia might be present during the earlier stages of the disease as presently shown. Prompt and long- rather than short-term interventions to reduce the concentrations of this protein might therefore be of relevance in secondary stroke prevention.

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