Protein Z Deficiency in Antiphospholipid-Negative Sneddon’s Syndrome

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Background—Sneddon’s syndrome is characterized by the association of ischemic cerebrovascular events and widespread livedo racemosa. The pathophysiology of Sneddon’s syndrome remains elusive, but various prothrombotic abnormalities have been previously reported in this setting. Low levels of protein Z, a downregulator of coagulation, have been recently linked to an increased risk of arterial thrombosis. The purpose of this study was to investigate the levels of protein Z in a series of Sneddon’s syndrome patients without circulating antiphospholipid antibodies in comparison with an age- and sex-matched control population.

Methods—Twenty-six patients and 78 healthy controls had determination of their protein Z blood levels by an enzyme-linked immunoassay test. Patients’ thrombotic and vascular risk factors, including tobacco smoking, arterial hypertension, oral contraceptive agents, dyslipidemia, factor V Leiden, and factor II mutation were recorded.

Results—Protein Z plasma levels were significantly lower in patients (mean 1.47 mg/L) than in controls (mean 1.93 mg/L) ($P=0.02$). Prevalence of protein Z deficiency (level <1 mg/L) was significantly higher ($P=0.001$) among patients (31%) than among controls (3.8%). Factor V Leiden and heavy smoking were observed in 4 and 7 patients, respectively.

Conclusions—Sneddon’s syndrome could be viewed as the peculiar clinical expression of various and sometimes associated coagulation abnormalities. Low levels of protein Z may account, at least partly, for the thrombotic events observed in Sneddon’s syndrome and shed a new light on its pathophysiology. Clinical implications for protein Z deficiency in this setting deserve further investigations. (Stroke. 2004;35:1329-1332.)

Key Words: protein Z ■ Sneddon’s syndrome ■ livedo racemosa

Sneddon’s syndrome (SNS) is defined by the association of widespread livedo reticularis and ischemic cerebrovascular events.1 It generally affects young adults, with a female preponderance. Two close but relatively distinct subsets of this syndrome may be differentiated according to the presence or absence of circulating antiphospholipid (aPL) antibodies (aPL-positive SNS and aPL-negative SNS, respectively).2 Impairment of coagulation involving factors V, VII, protein S, protein C, or antibodies to prothrombin may be occasionally detected in patients with aPL-negative SNS.3–5 However, many of these young patients have no identified prothrombotic risk factors. Protein Z is a vitamin K-dependant glycoprotein that acts as a downregulator of coagulation. By forming a complex with the plasma protein Z–dependent protease inhibitor, protein Z inhibits activated factor X.6 Evidence in animal models and recent data in humans tend to link protein Z deficiency to an increased risk of arterial thrombophilia.7–9 With respect to arterial occlusive events occurring in SNS, protein Z status of patients with aPL-negative SNS seemed an interesting and yet unexplored perspective. The purpose of this study was to investigate the plasma concentrations of protein Z in patients with aPL-negative SNS in comparison with an age- and sex-matched control population.

Patients and Methods

Twenty-six patients with aPL-negative SNS (20 women, 6 men, mean age 48.9 years, range 36 to 64, mean body mass index 24.8) were enrolled in this study. SNS was diagnosed on a clinical basis (ie, presence of a widespread permanent livedo reticularis involving the trunk or the buttocks, or both, and at least 1 clinical neurologic ischemic event, either completed or transient ischemic attack). Enzyme-linked immunoassay tests (Asserachrom Protein Z, Diagnostica Stago and Serbio) were used to measure protein Z levels in patients and 78 sex- and age (-2 years)-matched healthy controls drawn from a medical labor center (3 matched controls per patient, 60 women, 18 men, mean age 48.1 years, range 34 to 62, mean body mass index 25.1). All included patients were treated with antiplatelet therapy. Patients treated with oral anticoagulants were excluded from the study because of the severe drop in protein Z plasma concentrations induced by these agents.10 Given that nearly all aPL-positive patients with SNS receive long-term oral anticoagulation in our institution,2 the study was restricted to aPL-negative SNS. Protein Z deficiency threshold was defined according to a cutoff value calculated in 200 controls drawn from the same center as controls. Protein Z plasma levels were significantly lower in patients (mean 1.47 mg/L) than in controls (mean 1.93 mg/L) ($P=0.02$). Prevalence of protein Z deficiency (level <1 mg/L) was significantly higher ($P=0.001$) among patients (31%) than among controls (3.8%). Factor V Leiden and heavy smoking were observed in 4 and 7 patients, respectively.
Z deficiency was considered when lower than the mean value $-2$ SDs (1 mg/L).

Patients’ charts were retrospectively reviewed. Clinical features (including age at onset of livedo racemosa and at onset of neurological events) and associated arterial or thrombotic risk factors, both clinical (tobacco smoking, arterial hypertension, and oral contraceptive agents) and biological (thrombocythemia, hyperhomocysteinemia, dyslipidemia, deficiency in antithrombin, protein C, protein S, factor V Leiden mutation, prothrombin gene 20210A mutation, homozygosity for methylenetetrahydrofolate reductase C677T gene mutation [MTHFR], cryoglobulins, and dysfibrinogenemia) were recorded. As indicated above, patients with either lupus anticoagulant (screened by activated partial thromboplastin time and diluted thromboplastin time and confirmed by both mixing studies and demonstration of phospholipid dependance), IgG or IgM anticardiolipin antibodies (measured by ELISA kits, Biomedical Diagnostics SA), anti-β2 glycoprotein1 (detected by an ELISA technique using a purified rabbit β2 glycoprotein1, Behring), or biologic false-positive test for syphilis (defined by a positive venereal disease reaction level, negative pallidum haemagglutination inhibition and fluorescent treponemal antibody absorption tests) were excluded. No paired $t$ test was used to compare quantitative data (patients and controls levels of protein Z). Two-tailed Fisher and Mann–Whitney tests were further performed to compare qualitative data of patients and controls.

### Results

Clinical and biological data of patients are reported in the Table. Patients were 20 women and 6 men. The mean age at livedo onset was available in 25 patients and was estimated at 33.4 years (10 to 53). In the remaining patient, livedo was present since early adulthood. Mean age at first neurological event was estimated at 43.8 years in 24 patients (24 to 57). In the remaining 2 patients, frank cortical and subcortical white matter changes were present on MRI, but exact onset of cognitive decline and transient ischemic attacks could not be specified.

The distribution of protein Z in patients and controls is shown on the Figure. Mean level of protein Z was lower ($P=0.02$) in patients (1.47 mg/L, 0.47 to 3.35) than in controls (1.72 mg/L, 0.5 to 3.2). Prevalence of protein Z deficiency (level <1 mg/L) was significantly higher ($P=0.001$) among patients (8 of 26, 31%) versus controls (3 of 78, 3.8%). Protein Z levels were similar in women and men (1.48 and 1.44 mg/L, respectively). No correlation was observed between protein Z levels and either age at onset of livedo reticularis or age at onset of neurological manifesta-
The eponymous terminology of SNS was coined after Ian Sneddon, who reported 6 patients with widespread livedo reticularis and relapsing cerebrovascular incidents in 1965. The association of widespread livedo reticularis with ischemic cerebrovascular events is currently considered to define this entity. Pathophysiology of SNS remains incompletely understood to date. Based on histologic abnormalities observed on large skin samples taken at the center of livedo racemosa in aPL-negative SNS patients, Zelger et al proposed a sequence of events involving small- to medium-sized arteries. They hypothesized that endothelial dysfunction and cell detachment along with perivascular inflammation represented the *primum movens* of the disease, later followed by the occlusion of the vascular lumen by mononuclear cells, erythrocytes, and fibrin. Subsequent subendothelial proliferation and organization of the plug would ultimately lead to a cellular then fibrotic plug. However, the early stages described by Zegler et al have not been ascertained by others, whereas the late stages may represent nonspecific markers of a thrombotic phenomenon. It is yet to be established whether the indisputable vascular occlusive events occurring in SNS are related to a primary endothelial cell dysfunction, represent an arteriosclerotic-like condition occurring in young adults, result from recurrent thrombotic events in the setting of a hypercoagulable blood state, or are caused by a combination of these conditions. In any case, treatment rationale relies on the observation of microarterial thromboses in the affected tissues and the assumption that SNS is part of the spectrum of vascular occlusive disorders. Corticosteroids and immunosuppressive agents are notably ineffective in SNS, and current therapeutic strategy relies on antiplatelet or anticoagulant therapy to prevent recurrence of vascular occlusive events. Although optimal management of SNS patients is yet to be determined through controlled trials, antiplatelet therapy represents a currently reasonable initial approach in aPL-negative patients, whereas preliminary data seem to indicate that anticoagulation is probably more effective than antiplatelet therapy for secondary prevention in the aPL-positive subset.

Protein Z is a glycoprotein with structural similarities to protein C and coagulation factors VII, IX, and X. A key role for this liver-synthesized protein seems to be the downregulation of coagulation by inhibition of activated coagulation factor X on phospholipid surfaces. Disruption of protein Z gene in mice leads to a prothrombotic phenotype, while in humans, protein Z deficiency has been linked to an increased risk of ischemic stroke, early unexplained fetal loss, and enhanced prothrombotic phenotype in factor V Leiden patients. In addition, evidence of protein Z deposits in arterial lesions suggests that this protein may play a role in atherosclerosis. Our results indicate a high frequency of protein Z deficiency among patients with aPL-negative SNS compared with normal controls. Mean age of patients at onset of ischemic cerebrovascular events in our series tends further support to previous data suggesting that protein Z deficiency plays a role in occlusive vascular events occurring in young adults. We found that, within SNS, low levels of protein Z were not significantly associated with the presence of other risk factors for thrombosis or arterial events. Recent data indicate otherwise that low levels of protein Z are common in the setting of aPL antibodies. If similar results were further confirmed in SNS, protein Z deficiency could thus be regarded as an additional prothrombotic risk factor within SNS and a possibly interesting common denominator for its aPL-negative and aPL-positive subsets.

Our study carries several limitations resulting from: (1) its small sample size and (2) our inability to test the hypothesis that low levels of protein Z could be linked to a more severe prothrombotic phenotype in aPL-negative SNS patients. Indeed, aPL-negative SNS patients who had developed recurrences of neurologic events with antiplatelet therapy had been shifted toward anticoagulants, and their plasma samples were therefore improper for protein Z measurement. A prospective study is currently under way to address this issue. However, our findings shed new light on the pathophysiology of aPL-negative SNS, which should probably be regarded as the consequence of various, and sometimes associated, coagulation abnormalities, including protein Z deficiency.
References

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