Protein Z and its Role in Venous and Arterial Thrombosis

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Protein Z is a vitamin K-dependent glycoprotein synthesized in the liver. The structure of protein Z is similar to other vitamin K-dependent coagulation factors, with highest homology to factors VII, IX and X. However, it lacks the active center in its amino acid sequence and therefore does not function as a serine protease [1]. Protein Z circulates in plasma in association with the recently characterized protein Z-dependent protease inhibitor [2]. In the presence of Ca^{+2} and phospholipids, protein Z forms a complex with activated coagulation factor X and serves as a co-factor for the rapid inhibition of factor Xa by PZI, enhancing PZI activity more than 1000-fold. While PZI was also shown to inactivate other coagulation factors, i.e., IXa [3] and XIa [4] even in the absence of protein Z, it is believed that normal protein Z levels are necessary for proper factor Xa inhibition [2].

Although protein Z was characterized already in the 1980s, its role in normal and pathologic coagulation is still somewhat controversial. In recent years studies have suggested an association between protein Z deficiency and thrombosis, and these are reviewed here.

Plasma protein Z levels

Protein Z levels are antigenically determined by enzyme-linked immunosorbent assay. There is currently no established assay for the measurement of protein Z activity. Miletich and Broze [5] established a normal range for protein Z antigen in healthy blood donors. Levels were found to have a wide variation, 2.9 ± 1.0 μg/ml, with a 95% confidence interval of 32–168%. There was no difference in protein Z levels with regard to age or gender.

Protein Z gene is localized to chromosome 13q34 [6]. Several polymorphisms were found in a healthy population and are correlated with protein Z levels, suggesting a partial explanation for the wide variation in its normal range [7]. Inflammation may decrease protein Z levels; i.e., increased interleukin-6 levels were correlated with low protein Z levels in patients with hematologic malignancies [8]. The magnitude of this effect, however, may be quite small [9]. Hemodialysis and peritoneal dialysis patients have high protein Z levels [10], while in patients with nephrotic syndrome low protein Z levels were observed [11]. Like all other vitamin K-dependent coagulation factors, protein Z levels are significantly decreased in patients treated with coumarin derivatives [5].

Protein Z in ischemic stroke

The results of studies on protein Z in patients with ischemic stroke are summarized in Table 1. Vasse et al. [12] and Heeb et al. [13] reported that low protein Z levels were associated with an increased risk for ischemic stroke. In contrast, Kobelt and collaborators [14] found that high rather than low protein Z levels were associated with an increased risk for ischemic stroke. Lopaciuk [15] and McQuillan [16] and their teams found no correlation between protein Z levels and history of ischemic stroke.

Lichy et al. [17] investigated polymorphisms in the protein Z gene in patients with a history of ischemic stroke. They found a significantly lower frequency of the A allele of intron F polymorphism in the group of patients compared to controls. This polymorphism was associated with decreased protein Z levels in the healthy controls. The results suggest that the presence of this polymorphism may have a protective effect against stroke and that high protein Z levels may represent a prothrombotic condition.

Table 1. Studies of protein Z in ischemic stroke

Study	Vasse [12]	Heeb [13]*	Lopaciuk [15]	McQuillan [16]*	Kobelt [14]
Population	Caucasians	Hispanic (54%)	Caucasians	Caucasians	Caucasians
N (Patients/controls)	169/88	85/86	99/100	79/186	125/192
Age (yrs)	33	58	38	66	40
Protein Z (μg/ml)					
Patients		1.98 ± 0.92	1.56 ± 0.62	1.14± 0.18	
Controls		2.40 ± 0.97**	1.64 ±0.68	1.16± 0.09	
Protein Z (%)‡					
Patients	20%				100†
Controls	5% §				94†

* Excluding patients in the acute episode
** $P = 0.0003$. Results not significant in diabetics and females.
† Levels presented as percent of normal human plasma
‡ Percent of patients with levels < 5th percentile of control levels
§ $P < 0.01$

Xa = activated coagulation factor X
PZI = protein Z-dependent protease inhibitor

The different results of these studies are intriguing. A partial explanation could be the different populations that were studied. The patients in the series of Vasse and co-workers [12] were younger than in the other studies, and patients with hyperlipidemia and hypertension were not included. In contrast, 15–65% of patients in the other studies had at least one of these risk factors. Moreover, Kobelt et al. [14] suggested that protein Z levels are higher in hypertensive patients. Therefore, it is plausible that by excluding patients with hypertension the magnitude of protein Z effect will be higher.

Protein Z and cardiac disease

Fedi and colleagues [18] found significantly lower protein Z levels in patients with acute coronary events. In a multivariate analysis, low protein Z levels remained an independent risk factor for an acute coronary event. In contrast, an analysis of protein Z levels in the epidemiologic PRIME cohort did not show an association between protein Z levels and future development of coronary disease [19]. There are major differences between the two studies. In particular, Fedi et al. studied protein Z levels in the acute episode, therefore it may have been influenced by factors such as inflammation; while in the PRIME study the levels were obtained several years prior to the ischemic episode.

Marco et al. [20] studied protein Z levels in patients with atrial fibrillation who were not taking oral anticoagulants. They found similar protein Z levels in the study group compared to controls, and concluded that protein Z was not involved in the prothrombotic risk associated with atrial fibrillation.

Protein Z and pregnancy-related complications

Gris et al. [21] found an association between low protein Z levels and first unexplained early fetal loss. Paidas and researchers [22] found significantly lower first-trimester protein Z levels in a group of patients with complicated pregnancy outcomes such as bleeding, preeclampsia, preterm delivery, premature rupture of membranes and intrauterine growth restriction, as compared to women with normal pregnancy outcomes.

Gris et al. [21] and Grandone et al. [23] found no association between protein Z deficiency, recurrent abortions and late fetal loss. Interestingly, in another report, Gris et al. [24] analyzed anti-protein Z antibodies in women with pathologic pregnancies and reported that high levels of immunoglobulin G or M anti-protein Z antibodies were associated with recurrent embryo loss or fetal death [24]. However, in women with recurrent embryo loss there was no correlation between anti-protein Z titer and protein Z levels.

Protein Z in venous thromboembolism

Protein Z levels determined in unselected patients with venous thromboembolism did not significantly differ from levels in healthy controls [12]. The combination of protein Z deficiency with other thrombophilia may, however, influence the thrombotic risk.

Protein Z deficiency in mice heterozygous for the factor V Leiden mutation significantly increased their prothrombotic phenotype [25]. In 46 consecutive VTE patients with factor V Leiden mutation 26% had significantly low protein Z levels vs. 4.3% of control subjects [26]. Patients with protein Z deficiency experienced their first VTE episode at an earlier age and tended to have more proximal deep vein thrombosis than patients with higher protein Z levels. Moreover, in a recent study, two patients with factor V Leiden mutation, early-onset VTE and low protein Z levels were found to have a *R225H* substitution in the protein Z gene. This mutation was further found in 12 of 132 additional patients and was associated with an increase in frequency of thromboembolic events [27].

Protein Z and antiphospholipid antibodies

McColl and co-workers [28] found significantly lower protein Z levels in women with increased levels of antiphospholipid antibodies compared to controls, irrespective of clinical manifestations. Similar results were reported by Steffano et al. [29]. Forastiero and team [30] studied the degree of factor Xa inhibition by the protein Z/ZPI complex in the presence of APA.

Protein Z has a role in inactivation of the activated coagulation factor X. Protein Z deficiency may enhance the risk for thrombosis in certain situations.

Protein Z/ZPI activity was significantly reduced in the presence of IgG APA and beta-2 glycoprotein 1, while it remained almost unchanged in the presence of normal IgG or IgG APA and modestly reduced with β2GPI alone. In that study, significantly lower protein Z levels were found in patients with increased levels of APA and APA syndrome compared to controls, but not in patients with increased levels of APA and no symptoms. Protein Z deficiency was significantly correlated with an increased risk for arterial thrombosis.

The mechanism by which low protein Z levels develop in patients with increased levels of APA is not known. The abnormal levels may be independent events. However, it seems that one complicates the other and that low protein Z levels may contribute to the manifestations of APA syndrome in patients with APA.

Protein Z and other thromboses

Ozturk et al. [32] found significantly low protein Z levels in patients with Bechet syndrome, a disease complicated by venous and arterial thromboses, compared to controls. Koutroubakis et al. [33] reported that protein Z levels were significantly lower in

VTE = venous thromboembolism
APA = antiphospholipid antibodies
Ig = immunoglobulin
β2GPI = beta-2 glycoprotein 1

patients with ischemic colitis compared to healthy controls and to patients with diverticulosis

We investigated protein Z levels in patients with central retinal vein or central retinal artery occlusion [31]. Protein Z levels did not differ significantly in the whole patient group compared with controls. However, in a subgroup of patients with no traditional risk factors for the development of retinal vessel occlusion, protein Z levels were significantly lower than in the group of patients with risk factors and in the control group.

Conclusions

There is evidence that decreased protein Z levels are associated with an increased risk for thrombosis in certain situations and in specific subgroups of patients. These include: young patients with ischemic stroke and no risk factors for atherosclerosis, patients with VTE and factor V Leiden mutation, and patients with APA syndrome, in particular those with arterial thrombosis. In these situations determination of protein Z levels may be justified. However, there is still insufficient evidence with regard to protein Z levels and other thrombotic events discussed in the present review. Further investigation with a larger cohort of patients is needed.

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