The protein Z/protein Z-dependent protease inhibitor complex
Systemic or local control of coagulation?

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Keywords
Protein Z, protein Z-dependent protease inhibitor, thrombosis, cancer

Summary
Protein Z (PZ) is a vitamin K-dependent factor identified in human plasma in 1984 but it has no enzymatic activity. It is a cofactor of a serpin, the protein Z-dependent protease inhibitor (ZPI), and the complex PZ/ZPI inhibits activated factor X on phospholipid surfaces. In mice, the disruption of PZ or ZPI gene is asymptomatic, but enhances the thrombotic phenotype and mortality of other thrombotic risk factors. Most of the clinical studies focused on PZ. Despite conflicting results, a recent meta-analysis indicated that PZ deficiency could be a risk for venous and arterial thrombosis and early fetal loss. However, these conclusions are drawn from case-control studies of small size, constituting an important limitation. Recently, it was shown that PZ and/or ZPI are synthesized by normal kidney and different cancer cells, suggesting that the complex PZ/ZPI could play a role in inhibiting the tissue deposition of fibrin. The physiopathological consequences of these observations remain to be established. At this time, the measurement of plasma PZ and ZPI or analysis of their gene polymorphisms should not be performed routinely for the exploration of thrombophilia.

Keywords
Protein Z, Protein-Z-abhängiger Protease-Inhibitor, Thrombose, Krebs

Zusammenfassung

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Biochemistry
PZ synthesis and secretion

In contrast to other vitamin-K dependent factors, plasma PZ levels are characterized...
by a wide concentration range in normal subjects as well as a dramatic decrease induced by oral anticoagulants (10). The importance of the Gla-domain for the secretion of PZ was suspected by the analysis of two mutations within this region of PZ. An E30Q mutation was reported for a patient who had a low level of plasma PZ (11). Expression studies of this mutated PZ in baby hamster kidney cells showed that this mutation prevented the secretion of this PZ variant, but could also inhibit the secretion of normal PZ. Similarly, the substitution of Gla-30 by a Lys residue (E30K) was also associated with a defective secretion of PZ (12). In an elegant study using a secretory form of luciferase and various chimeric proteins, Souri et al. demonstrated the importance of vitamin K for PZ secretion and the specific and unique role of the Gla-domain for the secretion of PZ (13). PZ secretion by HEK293 transfected cells was much less efficient than factor X (FX) and was totally dependent upon vitamin K, whereas FX secretion was not. In addition, almost all secreted PZ was γ-carboxylated, while the majority of secreted FX was non-carboxylated. Of course, warfarin blocked the secretion of PZ while FX secretion was hardly modified. The secretion of a chimeric PZ with the Gla-domain of FX was increased as compared to wild-type PZ and was less sensitive to warfarin, while the secretion of a chimeric FX containing the Gla-domain of PZ was lower as compared to wild-type FX and became vitamin K-dependent. As the Gla-domain of PZ is a major determinant for the secretory mechanism. As vitamin K is influenced by dietary intake and plasma levels greatly vary among subjects (14), this could contribute in part to explain the wide distribution of plasma PZ in the healthy population.

Among other environmental factors which could also influence PZ and ZPI levels, a near-consensus estrogen response element was identified for PZ in mouse and human genome, and 17α-ethinylestradiol was shown to decrease hepatic mRNA of PZ and ZPI in a murine model (15). In contrast, in humans, the use of oral contraceptives is associated with higher plasma levels of both PZ and ZPI (16). Concerning physiological levels of estrogens, lower concentrations of PZ were reported in women than in men in some studies (17, 18), but most of the clinical studies did not identify significant variations. Heeb et al. reported significantly lower levels of PZ in post-menopausal women than in younger females (= 57 years), whereas no significant variations were detected between younger and older men (19), suggesting an influence of estrogen on plasma PZ levels. However, the use of oral contraceptives or hormonal therapy was not reported in their study and this conclusion should be considered with caution.

Plasma contains molar excess of ZPI relative to PZ and all the PZ was shown to circulate in a complex with ZPI. A chronic warfarin treatment, which dramatically reduces PZ plasma levels, is associated with a decrease of ZPI by 45% (3), and it was suggested that the PZ concentration may affect the ZPI level by acting either on ZPI secretion or clearance. In agreement with this hypothesis, different clinical studies showed a significant correlation between PZ and ZPI levels (16, 20). In PZ -/- mice, the decrease in plasma ZPI was less pronounced than in warfarin-treated patients (24% reduction), whereas in ZPI -/- mice, the plasma ZPI deficiency was more pronounced (43% reduction) (5) indicating that ZPI could be a limiting factor for the formation and/or the stabilization of the plasma PZ/ZPI complex. This is consistent with the observation that in patients with anti-ZPI antibodies and low plasma PZ levels, plasma ZPI remained unaffected (20).

Genetic control of PZ and ZPI

The gene for human protein Z (PZ) is localized to chromosome 13q34, where the genes for factors VII and X exist side by side, and it spans approximately 14 kb, consisting of 9 exons, including an alternative exon (21). Due to the weak regulation of hepatic PZ synthesis, we suggested that a genetic control of PZ was a major determinant of plasma PZ level (22). The estimation of heritability of coagulation factor levels in a large kindred of French-Canadian origin demonstrated a relevant variability in PZ and ZPI levels that showed a high heritability for both proteins ($h^2 = 0.66$ and 0.42 for PZ and ZPI, respectively) (23). Regarding single nucleotide polymorphisms (SNP) as established by the National Institute of Health 110 SNP in the human PZ gene were indicated, and 14 additional ones in close proximity to the gene.

Based on the identification of SNP in the PZ gene (24) two of them, A-13G and G79A, which have a high degree of linkage disequilibrium, were shown to influence plasma levels of PZ: The lowest levels of plasma PZ were associated with the GG and AA genotypes for the A-13G and the G79A polymorphisms, respectively (17, 25). A polymorphism in the intron C (G-42A), not in linkage disequilibrium, could be also associated with differences in plasma levels of PZ, with the lowest PZ level for the genotype AA (26).

Recently, it was shown that three polymorphisms in the PZ gene (A-13G, G-103A and G79A) had both an additional and combined influence on plasma PZ levels (27): In a Caucasian population of 306 healthy donors, 6.9% had rare genotypes, and a PZ plasma level of 1.53 μg/ml versus 2.32 μg/ml for the more frequent (59.1%) genotypes (AA/GG/ GG). Nowak-Göttl et al. identified three haplotype-tagging SNP (rs3024718, rs3024731 and rs3024772), which were in tight linkage disequilibrium and captured 97% of the genetic variation in PZ gene of a Caucasian cohort (28). Rs3024718 and rs3024772 were in accordance with polymorphisms previously reported (29, 30). In subjects with the PZ ATG haplotype, PZ levels were significantly higher compared with those without this haplotype (1.64 μg/ml versus 1.27 μg/ml; p < 0.001). Six genetic modifications affecting the ZPI gene were related to five haplotypes (31), but the relationship between ZPI haplotypes and ZPI plasma levels have so far not been published.

Measurement of PZ and ZPI

Recently, a functional test for plasma ZPI was described (32). It is based on the capacity of ZPI to inhibit FXIa, an inhibition which is independent of the presence of PZ (6). A highly significant correlation (p < 0.0001) was found between ZPI antigen and ZPI activity, but the cor-
relation coefficient was only 0.68. Further studies are needed to understand if these discrepancies are due to ZPI polymorphisms which are recognized differentially in the monoclonal antibody sandwich system, related to the interference of cleaved forms of ZPI which are quantified by the ELISA assay but lack functional activity (6), or due to ZPI polymorphisms with a gain or loss of function. However, as FXa is certainly the main target for ZPI, it would be useful to have a functional assay which quantifies the inhibition of FXa by the complex PZ/ZPI. To our knowledge, no functional assay is available to quantify the cofactor activity of PZ. Difficulties in obtaining a specific and reliable functional assay arise from the fact that PZ has no direct enzymatic activity, that PZ and ZPI form a complex in plasma and that the PZ/ZPI complex acts only in the early phases of coagulation, prior to thrombin generation (6). Lastly, we hypothesised that, as observed for TAFI (thrombin activatable fibrinolysis inhibitor), some polymorphisms in the PZ gene could interfere with the reactivity towards the antibodies in immunological assays (7).

PZ and ZPI molecular interactions with factor Xa

PZ serves as a critical protein cofactor for the inhibition of factor Xa by ZPI, enhancing the rate of factor Xa inhibition more than 1000-fold in the presence of calcium and phospholipids (2). Different studies for the last three years focused on the interaction between PZ and ZPI for the inhibition of FXa. PZ is composed of a Gla-domain, followed by an α-helical region containing a cluster of aromatic residues, two epidermal growth factor (EGF)-like modules, and a serine protease-like module (33) without catalytic function, because the critical histidine and serine residues of the catalytic triad are missing (34). Chimeric mutants of PZ (one lacking the Gla-domain and another in which the Gla-domain and the first EGF domain of PZ were substituted with identical domains of FXa) allowed a better understanding of the interaction between PZ and ZPI. Both mutants did interact with ZPI with a similar dissociation constant, indicating that the C-terminal part of PZ is involved in its interaction with ZPI. It was also shown that a specific interaction between both the Gla-domains of PZ and FXa may accelerate the inhibition of FXa by ZPI on phospholipid membranes by facilitating the formation of a ternary complex between PZ, ZPI and FXa (35). In addition, the authors hypothesised that PZ may induce a structural change in ZPI, which could optimize the interaction between ZPI and FXa (Fig. 1).

Recent analysis of the crystal structure of the PZ-ZPI complex confirmed the importance of the interaction of the Gla-domains of PZ and FXa, bringing bound ZPI into close proximity to the Gla-anchored FXa (36) as well as approximating the C-terminal part of PZ with ZPI. Ten residues (6 on PZ and 4 on ZPI) were identified to form three clusters of salt bridges, and the interface PZ-ZPI was shown to be stabilized by nine additional hydrogen bonds. In addition, the residues Asp 213 (instead of an Asn in other serpins) of ZPI was shown to play a critical role for FXa inhibition.

Using a similar crystallographic approach, the importance of four charged residues (Asp 74, Asp 238, Lys 239 and Asp 293) at the PZ-ZPI interface was evidenced for the activation of ZPI by PZ (37), these charged residues being conserved in the ZPI sequence of five species other than humans. In contrast to antithrombin, a serpin which is a potent inhibitor of FXa, the reactive centre of ZPI is a Tyr and not an Arg residue (38). An Arg variant of ZPI reacted more rapidly with FXa, even in the absence of cofactors (PZ, phospholipids and cal-

Fig. 1 Factor Xa inhibition by protein Z inhibitor (ZPI): Protein Z circulates in plasma associated with ZPI (A). In the presence of procoagulant phospholipids (PL), the Gla-domain of Protein Z (in grey) interacts with the Gla-domain of factor Xa (B), which optimizes the inhibition of membrane-associated factor Xa by ZPI (35, 36). ZPI can be activated by glycosaminoglycans present on the endothelial cell surface (C) and inhibits factor Xa that escapes from procoagulant PL. Calcium ions are required to maintain an extended conformation of free factor Xa (40). Protein Z is represented in dashed line, since it is not known if its presence is necessary for this alternative pathway.
hemostasis but became a substrate for FXa and was rapidly cleaved. Therefore, it was concluded that the “unfavourable” Tyr in the reactive centre of the ZPI impairs its spontaneous interaction with FXa, protects it from proteolysis by FXa, and is responsible for the specificity of this coagulation regulator system on cellular membranes.

A detailed analysis of the interaction kinetic between ZPI and FXa indicated that, despite its unusual active centre, ZPI functions like other serpins to regulate the activity of FXa, with the formation of an acyl-intermediate and the subsequent cleavage of ZPI. The high affinity interaction between PZ and ZPI is disrupted following reaction of ZPI with FXa, either in the ZPI-FXa acyl-intermediate complex or with the cleaved ZPI (39), reminiscent of the dissociation of heparin from the antithrombin-protease complex. More recently, it was also shown that heparin serves as an activator of ZPI (40). This was not really surprising, since heparin was used as an affinity ligand to purify ZPI from blood plasma (2). Interaction of ZPI with heparin requires the presence of calcium, is more pronounced with unfracti- onated heparin (~100 fold acceleration of the ZPI-FXa reaction) than with low molecular weight heparin (~8 fold acceleration of the ZPI-FXa reaction) and is not dependent on the pentasaccharide presence. Heparin favours the formation of a ternary complex together ZPI and FXa whereas inhibition of FXa by ZPI was only slightly increased by heparin. The binding site for heparin on ZPI has not been identified, but is distinct from the binding site for PZ on ZPI. Consequently, these recent data suggest that, in addition to the inhibition of membrane-associated FXa, the PZ-ZPI complex could also inhibit FXa that escapes from a membrane site (Fig. 1).

Although the mechanism of inhibition of FXa by the PZ/ZPI complex is being clarified, several aspects remain obscure and the physiological importance of this complex remains unclear. The complex between ZPI and FXa is less stable than other serpin-protease complexes and is unable to fully inactivate the catalytic activity of FXa. This could be due to the inability of ZPI to induce a sufficient FXa distortion which is needed for a complete inhibition of a protease by a serpin. However, the PZ/ZPI complex promotes the assembly of a high affinity Michaelis complex with FXa on the membrane. In addition to crucial residues such as Arg-143 on FXa (41), the existence of other exosite determinants on PZ or ZPI involved in the formation of this high affinity ternary complex are likely to exist but remain to be identified.

**PZ/ZPI and Disease**

**Thrombosis**

Since our first report showing an increased frequency of PZ deficiency in young patients with a previous history of an ischaemic stroke but not in patients with venous thrombosis (42), several studies investigated the role of PZ thrombotic disease, reaching conflicting results (7). The confusing results reported are likely due at least in part to the limited number of individuals included and the choice of the control groups (healthy individuals or sex- and age-crossed controls). In order to overcome the limitation of the weak number of cases of the hitherto published studies, Sofi et al. (9) performed a meta-analysis including 2054 patients with arterial vascular events (compared to 3033 controls) and 1297 patients with venous thromboembolic diseases (compared to 1399 controls). They concluded that there is a significant association between low PZ levels and arterial vascular diseases (OR 2.67, 95% CI 1.6–4.48, p = 0.0002) and venous thrombotic disease (OR 2.18, 95% CI 1.19–4, p = 0.01). However, an important limitation of this meta-analysis is that it is based only on case-control studies, and therefore the conclusions have to be interpreted carefully.

In addition, it is clear that the relationship between venous thrombosis (VT) and PZ deficiency is weaker than for arterial disease. It is of note that this meta-analysis for VT included studies with selected population of patients [patients with Factor V Leiden mutation (43) or patients with an unusual frequency of constitutive thrombotic risk factors (44)]. Lastly, no cut-off value for PZ deficiency could be concluded from this study.

As suggested by Martinelli et al. (45), if only a very low level of PZ could serve as an isolated risk factor of VT, moderate PZ deficiency certainly would increase the venous thrombo-embolic risk of other well identified prothrombotic risk factors.

As some polymorphisms are important regulators of plasma PZ levels, but as PZ levels could be influenced by dyslipidaemia (46) or inflammation (47, 48), different studies evaluated different polymorphisms of PZ genes and their association with arterial thromboembolic events. One study found that the frequency of the A allele of the polymorphism G79A was significantly lower than in controls, suggesting that low levels of plasma PZ could have a protective role against ischemic stroke (25), whereas other studies observed a similar distribution between cases and controls for the different alleles analysed. Only a few studies evaluated actually both PZ polymorphisms and plasma PZ levels (26, 49, 50): They confirmed the influence of the A-13G and G79A polymorphisms on plasma PZ levels, and one study (49) reported lower levels of plasma PZ in patients with arterial thrombotic events, despite a similar frequency of polymorphisms between the patients and the controls, suggesting that plasma PZ deficiencies observed were acquired, and could constitute a marker of vascular disease.

In contrast, Nowak-Göttler et al. (28) observed that the haplotype ATG, associated with higher levels of plasma PZ, was more frequent in children with a previous history of stroke. A meta-analysis on 131 adults did not identify the G79A polymorphism as a risk factor for cerebral venous thrombosis (51), but this study did not include a series of 54 patients where the presence of at least one A allele led to an odds ratio of 2.57 (95% IC:1.23–5.34) (52). However, in this last study, plasma levels of PZ were not reported.

Only a few studies evaluated plasma ZPI levels either in arterial and or venous thrombotic diseases (16, 20, 32, 53). As for PZ, discrepant results were obtained and additional studies are certainly needed in order to ascertain a possible role of ZPI in thrombotic events. Within the coding region of ZPI 16 mutations/polymorphisms were identified by van de Water et al. (54) and two mutations generating stop codons at R67 and W303 were identified, possibly
associated with VT. The role of these nonsense polymorphisms in VT was further investigated with conflicting results (31, 55), but a recent meta-analysis on more than 2000 cases and 3000 controls failed to demonstrate a role of these mutations in VT (56). Surprisingly, no studies analysed both plasma ZPI levels and these mutations, and consequently, it is not known if these mutations are actually associated with low plasma levels of ZPI. In addition, studies on ZPI polymorphisms and ischemic arterial events are still missing.

**PZ deficiency and obstetrical pathologies**

Gris et al. reported a high frequency of protein Z deficiency in women with a first primary episode of early fetal death from the 10th to the end of the 15th week of gestation (57). It was hypothesised that the PZ deficiency could impair the invasion of the spinal uterine arteries by the cytotrophoblast. Interestingly, PZ was detected at high expression in villous trophoblasts (58, 59). The results of this first study were not confirmed by an Italian study on a lower number of patients (60) where the cut-off of plasma PZ was particularly low. The meta-analysis of Sofi et al. (9) confirmed the strong relationship between low PZ levels and pregnancy complications (OR 4.17, 95% CI 2.31–7.52, p < 0.00001). The report by Gris et al. showing an enhanced frequency and high levels of anti-PZ antibodies (both IgG and IgM) in women with pathological pregnancies (61) was confirmed by Sater et al. (62). The absence of statistical significance of anti-PZ antibodies in the study of Sailer et al. is certainly due to the rather weak number of cases included, but the authors reported a trend to significance (63).

A last study including 51 women did not evidence increased levels of anti-PZ antibodies in cases of fetal death, but a significant increase of IgG anti-PZ antibodies in case of fetal growth retardation (64). All these studies detected anti-PZ antibodies in non-pregnant patients and were suggested to be natural antibodies (64). This affirmation should be tempered by the fact that all these studies were performed with the same kit to detect anti-PZ antibodies, and therefore a bias in the methodology can not be excluded. If it is confirmed, the role of these natural antibodies remains to be identified. It is important to note that no correlation was found between plasma PZ and the titer of anti-PZ antibodies (61, 62), but the combination of both high titer of PZ antibody and PZ deficiency was shown to be associated with a poor efficiency to anticoagulant treatment and an increased risk for recurrent fetal death (65). Lastly, only high titers of anti-PZ antibodies constitute a risk factor for pregnancy complication (61–63).

More conflicting results are obtained when PZ polymorphisms were considered. In a small series of 48 Austrian and 40 Egyptian women, the frequency of the 79A allele, associated with low levels of PZ, was lower in cases than in controls (66, 67). Plasma PZ levels were not determined in these studies, whereas both G79A allele and plasma PZ levels were determined in the study by Topaliou et al. (68): Plasma PZ levels were significantly lower in their series of 51 Greek women as compared to controls, but the frequency of the G79A polymorphism was not different between both groups. In addition, the polymorphism G-42A of PZ gene was shown to be significantly associated with fetal losses (69) and could be also a risk factor for pulmonary embolism (PE) associated with a deep VT during pregnancy (70). These studies are certainly too small to draw firm conclusions but it is possible that the low levels of plasma PZ generally observed in the different studies so far performed in women with obstetrical complications are related to acquired PZ deficiency, by a mechanism which needs to be identified.

**PZ and inflammation**

The role of inflammation on PZ or ZPI plasma levels is debatable: some studies reported lower levels of PZ in patients with high plasma IL-6 or fibrinogen levels (47, 71), whereas a significant increase (from 1.54 ng/ml to 1.91 ng/ml, p < 0.04) of plasma PZ was described at 72 hours after a percutaneous coronary intervention (72), strengthening the possible role of inflammation on PZ levels during an acute vascu-
lar event (48). In vitro studies did not evidence a role of inflammatory cytokines on PZ biosynthesis by cultured hepatocytes (22), whereas Oncostatin M, a cytokine of the IL-6 family, increased PZ biosynthesis by microvascular endothelial cells (73), suggesting a possible but weak induction of PZ levels by inflammatory mediators. The divergent results from the clinical studies could be related to the comorbidities of the patients tested. The negative correlation between IL-6 and plasma PZ described in patients with acute leukaemia or lymphoma (47) could be due to a discrete consumption coagulopathy (common in malignant haemopathies) inducing a decrease of PZ levels (74), independently of the elevation of IL-6 plasma level, which is a marker of aggressivity in lymphomas (75). In a similar way, hypertriglyceridaemia was shown to be associated with elevated levels of PZ (46). In the study by McQuillan et al., 26% of the patients were diabetic at the time of diagnosis (48), a pathological condition associated with hypertriglyceridaemia. However, neither the percentage of these patients was reported, nor if there was an improvement of the diabetes equilibrium during the follow-up. Consequently, the conclusion that PZ is increased during the acute phase of an ischemic stroke is perhaps mainly related to a higher prevalence of hypertriglyceridaemia at diagnosis rather than to inflammation, followed by an improvement of this parameter during the follow-up of the stroke.

**PZ/ZPI and cancer**

Thrombosis is the second leading cause of death in cancer, and the identification of patients at high risk for venous thromboembolism is a true challenge (76). No significant differences between cases and controls of plasma PZ levels were detected in a small series of patients with malignant haemopathies (47), while another study observed a significant decrease of PZ in patients with cancer, with the lowest levels observed in locally advanced tumours (stages III and IV), suggesting that a decrease of PZ during the follow-up of a cancer could be a criteria of a poor evolution (77). The frequency of the −13A/G and G79A polymorphisms of PZ gene were identical in patients with cancers of different origin (78, 79) and was not estimated as a risk factor for breast cancer, but the identification of patients at high risk for venous thromboembolism is a true challenge (76). No significant differences between cases and controls of plasma PZ levels were detected in a small series of patients with malignant haemopathies (47), while another study observed a significant decrease of PZ in patients with cancer, with the lowest levels observed in locally advanced tumours (stages III and IV), suggesting that a decrease of PZ during the follow-up of a cancer could be a criteria of a poor evolution (77). The frequency of the −13A/G and G79A polymorphisms of PZ gene were identical in patients with cancers of different origin (78, 79) and was not estimated as a risk factor for breast cancer, whereas they were absent from normal breast tissue (82, 83).

Synthesis of PZ was also detected in tumour-associated macrophages as well as in neovessels of the tumours (83). All tumours types are not always positive for the expression of PZ and/or ZPI: During analysis of lymphoblasts from 12 children with acute lymphoblastic leukaemia we failed to detect PZ mRNA, while some low levels of ZPI mRNA was observed in just 2 cases (unpublished observation).

**PZ/ZPI and kidney**

Tissue northern blot analysis detected PZ mRNA in the kidney, and the presence of immunoreactive PZ was observed in distal and collective tubules (84). ZPI protein but no ZPI mRNA was detected in these structures, suggesting that ZPI was produced

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ref.: reference; N.I: not indicated; N.D: not determined; p75/90/99: 75th/90th/99th percentile; ns: not significant; * p < 0.05; ** p < 0.01; *** p < 0.001

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elsewhere but could have interacted with PZ synthesised by renal cells. PZ was shown to be secreted into the urine, and small amounts of PZ were detected in the urine of healthy volunteers (2.4% of the plasma level), whereas ZPI was absent. Consistent with a physiological urinary elimination of PZ, PZ was found in kidney stones (85). Interestingly, plasma ZPI was shown to increase in patients with early acute renal allograft rejection (86), but the origin of ZPI remains to be established. Does it come from injured kidney or from the immune cells?

Conclusions

The analysis of the phenotype of PZ(-/-) and ZPI(-/-) mice gave interesting clues as to the physiological role of PZ and ZPI. Whereas mice deficient in both physiological coagulation inhibitors (antithrombin, protein C and protein S) die in utero or at birth (87 – 89), a complete deficiency in PZ or ZPI in mice is viable, but increases the severity of other thrombotic risk factors. Clinical studies are in agreement with these data: PZ deficiency is common in the general healthy population and increases the thrombotic risk associated with the FV Leiden, the FII mutations or hyperhomocysteinemia (43, 45). Consequently, the determination of PZ and/or ZPI in patients with a previous history of VT should only be performed if a first thrombotic risk factor has been evidenced, in order to analyse if an additional deficiency of the PZ/ZPI system worsen the thrombotic risk or could be a factor of recurrence. A more significant association between PZ deficiency and arterial thrombosis was shown by the meta-analysis of Sofi et al. (9). It is unclear why PZ deficiency could preferentially be a risk factor for arterial than VT, but arterial clots are mainly composed of platelets recruiting large amounts of phospholipids, which are necessary for the maximal anticoagulant activity of the PZ/ZPI complex (36, 37). The existence of a PZ receptor with a selective distribution on arterial endothelium may also be hypothesised. Lastly, there is a clear link between inflammation and ischemic arterial disease (90). Therefore it can be supposed that the PZ/ZPI complex may provide an anti-inflammation role independently of its anticoagulant activity, also observed for other physiological anticoagulants (91). However, we were unable to detect any inhibitory activity of PZ on the synthesis of cytokines of the IL-6 family by monocytes (unpublished observation).

The analysis of both plasma PZ levels and PZ polymorphisms in patients with arterial disease evidenced that, for each genotype, plasma levels were significantly lower than in controls (49). It was also observed that PZ and ZPI levels decreased with the clinical severity of arterial disease (32). Taken together, these data suggest that plasma PZ deficiencies could be acquired, and could constitute a marker of vascular disease. However, a recent study failed to detect any correlation between plasma PZ levels and the intima-media thickness of the common carotid arteries (92).

In contrast to the conflicting data observed for arterial or VT, different studies confirmed that PZ deficiency could be a risk factor for early fetal loss, and that only a high titer of anti-PZ antibodies could be involved in miscarriages. To our knowledge, no studies evaluated the association between ZPI deficiency and early fetal loss, while analysis of ZPI-deficient mice revealed that some ZPI (-/-) mice are lost during gestation or the perinatal period (5), in contrast to PZ (-/-) mice. As PZ and ZPI circulate in complex in plasma and their plasma levels are correlated in humans, we can hypothesise that the PZ deficiency associated with fetal loss in humans appears to be the consequence of a ZPI deficiency. This could explain why no differences in PZ polymorphism frequency were observed in some studies analysing PZ and fetal loss.

The most exciting data are certainly that the synthesis of PZ and/or ZPI are detectable in different pathological tissues, suggesting that PZ and/or ZPI behave as responsive proteins. Immunoreactive PZ was first described in atherosclerotic vascular lesions of diabetic and non-diabetic patients, but not in the subendothelial space and microvascular endothelial cells of healthy controls, suggesting that this protein could be involved in arterial disease (93). More recently, the synthesis of PZ or ZPI was described in different cancer tissues, as well as in the normal kidney or in trophoblasts. All these data are reminiscent of the distribution and behaviour of urokinase, which is present in normal tissues (94), but overexpressed in atherosclerotic lesions (95) and in cancer tissues (96). Therefore, the major role of the PZ/ZPI system could be to avoid the local tissue deposition of fibrin. As fibrin plays an important role in tumour angiogenesis (97), it would be interesting to compare neovessel density in tumours with weak or high expression of PZ and/or ZPI, and to study if the presence of a high expression of these proteins may influence the prognosis of cancer development. Lastly, it would also be interesting to know if the local production of PZ or ZPI by tumour cells may have any influence on their plasma levels, and consequently if the follow-up of these proteins could constitute a marker of tumour burden.

In conclusion, the physiological role of the PZ/ZPI complex remains to be established. Does it play a systemic or a local role to dampen coagulation processes? In addition to their genetic control, what are the other parameters that can influence the plasma levels of PZ and ZPI? The importance of increased triglyceride levels were well identified, but the role of other circumstances such as inflammation, cancer, immune reactions, remain to be established. The major limitation of studies analysing the role of PZ and ZPI in humans is related to the low number of patients included, the choice of the control population, and usually the absence of clinical data which could have an impact on plasma PZ and ZPI levels. Thus, to assess the role of PZ and ZPI in pathology, both PZ levels and polymorphism analysis must be performed only in adequately powered prospective trials with homogeneous groups of patients and controls, and taking into account other comorbidities.

References


