Plasminogen activator inhibitor-1: a review

Plasminogen-Aktivator-Inhibitor-1: eine Übersicht

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Abstract

Plasminogen activator inhibitor-1 (PAI-1) is the most potent inhibitor of both tissue type (t-PA) and urokinase type plasminogen activator (u-PA) and thus regulates fibrinolysis as well as proteolysis, cell migration, and tumor cell invasiveness. Stimulated by cytokines, lipopolysaccharide, very low density lipoproteins, and transforming growth factor β -1 (TGF β -1), PAI-1 also influences inflammation, metabolic disorders, and fibrotic diseases. PAI-1 is produced in liver cells, adipocytes, smooth muscle cells, and platelets. In pathological conditions, increased PAI-1 levels mainly result from release by endothelial cells or tumor cells. Elevation of PAI-1 activity is described to be associated with pregnancy complications like recurrent miscarriage, pregnancy-induced hypertension, and preeclampsia. Spontaneous abortion seems to be related to the 4G/4G genotype of the polymorphism in the PAI-1 promoter. Women with polycystic ovarian syndrome, which is associated with anovulatory infertility, also show significantly higher PAI-1 levels than healthy controls. Increased PAI-1 levels are found in a number of malignancies and might give information about prognosis and preferential response to certain therapies especially in patients with primary breast cancer. By influencing extracellular matrix turnover, PAI-1 seems to play a role in fibrotic disorders including nephropathy, chronic lung diseases, cardiac fibrosis, and liver fibrosis. Upregulated by inflammatory mediators, PAI-1 levels are increased in sepsis, trauma, surgery, and

a variety of diseases associated with inflammatory reactions. PAI-1 is suggested to play a functional role in host response to trauma. Inflammatory states are also found in the pathogenesis of atherosclerosis and the metabolic syndrome. Vascular diseases as well as insulin resistance leading to metabolic state are associated with both elevated PAI-1 levels and the 4G/5G polymorphism of the PAI-1 promoter. The 4G/4G and 4G/5G genotypes were observed to be more frequent in patients with obesity, myocardial infarction, and venous thromboembolism. PAI-1 thus represents an important non-invasive diagnostic criterion in a number of diseases and might reveal new therapeutic strategies.

Keywords: cancer; fibrinolysis; fibrosis; inflammation; insulin-resistance; plasminogen activator inhibitor-1; pregnancy.

Zusammenfassung

Plasminogen activator inhibitor-1 (PAI-1) ist der wirkungsvollste Inhibitor des Gewebs-Plasminogen-Aktivators (t-PA) und des Urokinase-Plasminogen-Aktivators (u-PA) und reguliert so neben Fibrinolyse und Proteolyse auch Zellmigration und Tumorzellinvasivität. Stimuliert durch Zytokine, Lipopolysaccharide, very low density-Lipoproteine und Transforming growth factor β -1 (TGF β -1) beeinflusst PAI-1 auch entzündliche Reaktionen, Stoffwechselerkrankungen und fibrotische Erkrankungen. PAI-1 wird in Hepatozyten, Adipozyten, glatten Muskelzellen und Thrombozyten gebildet. In pathologischen Situationen resultieren erhöhte PAI-1-Spiegel aus einer erhöhten Freisetzung aus Endothel- und Tumorzellen. Erhöhte PAI-1-Spiegel wurden im Zusammenhang mit Komplikationen in der Schwangerschaft wie rezidivierende Fehlgeburten, schwangerschaftsinduzierte Hypertonie und Präeklampsie beschrieben. Spontanaborte scheinen mit dem 4G/4G-Genotyp des PAI-1-Promotor-Polymorphismus assoziiert zu sein. Frauen mit polyzystischen Ovarien, welche mit anovulatorischer Infertilität einhergehen, weisen auch signifikant höhere PAI-1-Spiegel auf als gesunde Kontrollpersonen. Erhöhte PAI-1-Spiegel, die bei einer Reihe von malignen Grunderkrankungen vorkommen, können insbesondere bei Mammakarzinom Hinweise auf Prognose und Therapieansprechraten geben. Durch Beeinflussung des Umsatzes extrazellulärer Matrix scheint PAI-1 auch mit fibrotischen Erkrankun-

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gen wie Nephropathie, chronischen Lungenerkrankungen, kardialer Fibrose und Leberfibrose assoziiert zu sein. Die PAI-1-Expression und -Freisetzung wird durch Entzündungsmediatoren hochreguliert und ist somit erhöht bei Sepsis, Trauma, perioperativ und bei einer Reihe von entzündlichen Reaktionen. PAI-1 wird eine funktionelle Rolle in der Reaktion auf traumatische Ereignisse zugeschrieben. Inflammatorische Gewebsreaktionen spielen auch eine Rolle in der Pathogenese der Arteriosklerose und des metabolischen Syndroms. Sowohl Gefäßerkrankungen als auch Insulinresistenz mit Folge einer metabolischen Entgleisung sind mit erhöhten PAI-1-Spiegeln und dem 4G/5G-Polymorphismus des PAI-1-Promotors vergesellschaftet. Die 4G/4G- und 4G/5G-Genotypen wurden häufiger bei Patienten mit Adipositas, Myokardinfarkt und venösen Thromboembolien beobachtet. Der PAI-1 stellt möglicherweise ein wichtiges nicht-invasives Diagnosekriterium bei einer Vielzahl verschiedener Erkrankungen dar und eröffnet neue therapeutische Möglichkeiten.

Schlüsselwörter: Entzündung, Fibrinolyse; Fibrose; Insulin-Resistenz; Malignität; Plasminogen-Aktivator-Inhibitor-1; Schwangerschaft.

Introduction

Plasminogen activators (PA) are serine proteases which are part of an enzyme cascade converting plasminogen into plasmin. The plasminogen activating system consisting of tissue-type (t-PA) and urokinase-type PA (u-PA) plays a role in targeted proteolysis which is necessary for cell invasion and migration as it is found during embryogenesis, angiogenesis, and tumor cell proliferation. On the other hand, this fibrinolytic system initiated by the release of PA is important for the maintenance of vessel patency by removal of fibrin deposits [1]. Altered plasmin formation might thus lead to bleeding tendency and tissue destruction, respectively. PA activity is regulated by hormonal regulation of biosynthesis and release, proteolytic conversion into enzymes with altered activities, interaction with cell surface receptors and proteins, rapid clearance via the liver, and specific PA inhibitors (PAI) [2]. The existence of PAI was first described in 1963 [3]. At least four different types of PAI have been identified: PAI-1, PAI-2, PAI-3, and protease nexin. PAI-1 is present in normal human plasma while PAI-2, which can be found in placenta and leukocytes, is only detected in plasma of pregnant women [4]. PAI-3 was purified from urine and reacts with u-PA and thrombin [5]. Protease nexin found in fibroblasts not only inhibits u-PA and t-PA but also thrombin, trypsin, and plasmin [6]. In normal human plasma, PAI-1 is the primary inhibitor of both t-PA and u-PA and thus not only represents the primary regulator of the fibrinolytic system but has also effects on cell adhesion, detachment, and migration [7]. Elevation of plasma PAI-1 activity levels is associated with vascular diseases like

thrombosis and atherosclerosis as well as with inflammation, malignancy and metastasis, metabolic syndrome and obesity, fibrotic disorders, and with several renal diseases [8]. Methods for the measurement of PAI-1 activity and antigen levels have been standardized and commercialized over the past years. There is controversy about whether these tests are mainly useful in clinical research studies or serve as important diagnostic and therapeutic tools for certain diseases and disorders.

PAI-1-structure and (patho-)physiology

PAI-1 is a member of the serine protease inhibitor (serpin) family including, among others, antithrombin (AT), α 2-antiplasmin and α 1-protease inhibitor. The PAI-1 gene, which is located on the long arm of chromosome 7, is 12.2 kb in length, has nine exons and eight introns [9]. PAI-1 is a single-chain glycoprotein which consists of 379 amino acids carrying arginine at its active site. Like other serpins which are similar in their amino acid sequence, PAI-1 inhibits proteases by imitating the structure of the normal substrate of the target serine protease [10].

Plasminogen activator inhibitors represent nearly 10% of total protein in plasma [11]. In physiological conditions, PAI-1 is released by liver cells, smooth muscle cells, adipocytes, and platelets while in pathological conditions, PAI-1 can also be produced by tumor cells or inflammation-activated cells like endothelial cells [8]. Other cells that were shown to synthesize and release PAI-1 contain hepatoma and melanoma cell lines, cultured endothelial and granulosa cells, and glomerular epithelial cells. Plasma PAI-1 levels in healthy individuals range from 5 to 20 ng/mL. PAI-1 activity is physiologically higher in men than in women and increases with age [12]. A diurnal fluctuation with lowest levels at 3:00 pm was reported [13]. Serum PAI-1 levels were found to be five times higher than plasma levels, which might be due to the large amount of PAI-1 stored in the alpha-granules of platelets [14]. In the clinical setting though, increased plasma PAI-1 activity is due to increased synthesis and release of PAI-1 from endothelial cells rather than from the platelet pool.

PAI-1 is the only serpin able to reversibly switch between active and latent conformational states which have the same molecular weight and are immunologically identical. The active form has a very short half-life of minutes and spontaneously converts into the latent form with a half-life of approximately one hour [8]. The latent form can be converted into the active form by the influence of denaturants, negatively charged phospholipids, or the adhesive glycoprotein vitronectin (VN) to which active PAI-1 binds with high affinity and specificity [7]. In plasma, active PAI-1 circulates as a 1:1 complex with VN resulting in a stabilization and maintenance of PAI-1 activity with a half-life of PAI-1 prolonged more than 10 times. Because of this lability of PAI-1 structure, PAI-1

Figure 1 Plasminogen activator-inhibitor-physiology.

antigen measurements might show large variations and therefore should be interpreted cautiously.

PAI-1 activity is regulated by a number of different mechanisms. During inflammatory reactions, release and gene transcription of PAI-1, respectively, are stimulated either directly by endotoxins like lipopolysaccharide or indirectly by cytokines like tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) released from activated mononuclear cells. At the same time, both TNF- α and IL-1 suppress t-PA production and induce tissue factor (TF) activity in endothelial cells which results in impaired fibrinolytic capacity and increased procoagulant activity (Figure 1). TF, on the other hand leads to increased formation of thrombin which itself also supports PAI-1 activity [8]. Synthesis and expression of PAI-1 on a transcriptional level are, to a large amount, stimulated by transforming growth factor β -1 (TGF β -1) released from the alpha-granules of platelets after vascular injury. TGF- β itself can be activated by the plasminogen activation system [15]. PAI-1 production in hepatocytes and endothelial cells was shown to be upregulated by the influence of very low-density lipoproteins (VLDL) and free fatty acids, possibly due to a VLDL-responsive element in the PAI-1 promoter [16]. By binding p53 at a specific binding site in the region of the PAI-1 promoter, PAI-1 transcription can be stimulated while t-PA and u-PA transcription are repressed. These mechanisms might lead to proteolytic tissue damage and thus influence local tumor cell invasiveness and metastasis [17].

Glucocorticoids and oxidative agents were reported to decrease in PAI-1 synthesis and to inactivate PAI-1, respectively [2]. PAI-1 can be inactivated by thrombin and thrombin-activated protein C (APC) via limited proteolysis. By decreasing PAI-1 activity, APC thus shows profibrinolytic effects [12]. PAI-1 is mainly neutralized by u-PA and t-PA which in their active forms rapidly complex with PAI-1 forming a 10 kD sodium dodecyl sulphate-stable complex [14]. The serpin PAI-1 serves as a pseudo-substrate for the proteinase PA in this process, but a part of the interaction seems to be mediated by the formation of salt bridges between the molecules. PAI-1/PA complexes bound to u-PA receptors can be removed

from the cell surface by members of the low-density lipoprotein (LDLR) family by undergoing lysosomal degradation while the u-PA receptor recirculates and reappears on the cell surface [18].

In normal human plasma, PAI-1 is the primary inhibitor of both t-PA and u-PA and can furthermore inhibit plasmin and trypsin in less efficient reactions. Plasminogen activator inhibitors control a variety of events associated with connective tissue turnover, coagulation, fibrinolysis, complement activation, and inflammatory reactions.

PAI-1 and pregnancy

During normal pregnancy, the hemostatic balance changes in the direction of hypercoagulability and hypofibrinolysis, respectively, in order to minimize bleeding complications during delivery. An impairment of fibrinolytic capacity is the result of PAI-1 release from endothelial cells and PAI-2 release from the placenta [19]. Both PAI-1 and PAI-2 increase with gestational age reaching levels about ten times higher than normal at the end of the third trimester. While PAI-1 decreases rapidly within the first 24 h postpartum, PAI-2 already decreases during the last two weeks of pregnancy, indicating physiological regression of the placenta [20]. Pathological elevation of PAI-1 levels though is reported to be associated with several pregnancy complications.

An association between the 4G/5G polymorphism in the PAI-1 promoter and the risk of recurrent miscarriage was shown. The 4G allele is known to enhance gene expression and thereby increase plasma levels of PAI-1 up to 25% [21]. A number of authors were able to show that women with recurrent pregnancy loss, spontaneous abortion, or infertility present a significantly higher frequency of the 4G/4G mutation of the PAI-1 gene compared to women with uneventful term deliveries [22, 23]. In contrast, neither the PAI-1 4G/5G polymorphism alone nor the combination of different thrombophilic polymorphisms showed a correlation with unexplained late intrauterine fetal death [24]. It is reported that pre-conceptional prophylaxis with acetylsalicylic acid and post-conceptional prophylaxis with heparin or low molecular weight heparin in risk patients might avoid consecutive miscarriage [25]. Anovulatory infertility might as well be associated with PAI-1 activity, as PAI-1 levels in women with polycystic ovarian syndrome were described to be significantly higher than those in healthy controls [26].

There is controversial data about an association between the PAI-1 polymorphism and pregnancy-induced hypertension or preeclampsia, respectively. It was found that the genotype distribution of the PAI-1 4G/5G polymorphism in a group of pre-eclamptic patients was similar to the distribution in the control group of healthy pregnant women and almost identical to that in the general middle-aged population [27]. While another study reported that only the appearance of at least two homo-

zygous genotypes of thrombophilic polymorphisms resulted in a higher risk of preeclampsia [28], there are data indicating that the 4G/4G genotype of the PAI-1 gene is significantly more frequent in pre-eclamptic patients than in normal pregnancies [29]. Circulating levels of PAI-1 increased with increasing severity of pregnancy-induced hypertension [30].

In addition to its inhibitory effects on fibrinolytic capacity, PAI-1 is believed to control proteolysis in the pre-ovulatory follicles leading to rupture of selected follicles during ovulation and in maternal tissue during trophoblast invasion [31]. A lack of PAI-1 might result in uncontrolled placental invasion and thus increase the risk of tubal or molar pregnancies [32].

PAI-1 and cancer

The plasminogen activating system containing serine proteases and their inhibitors as well as a number of metalloproteases is involved in extracellular proteolysis and tissue remodelling. Changes in the expression of components of these systems might reflect uncontrolled tissue remodelling and therefore promote cell invasion, cell migration and proliferation [33]. PAI-1 may contribute to the degradation of extracellular matrix during cancer invasion by decreasing the adhesive strength of cells for their substratum and detaching cells from extracellular matrices by inactivating integrins [34]. A binding site for p53 in the region of the PAI-1 promoter leading to PAI-1 transcription has been identified. Strong expression of PAI-1 in tumor cells like ductal carcinoma in situ cells [35] or tumor tissues of squamous cell cancer [36] has been found while angiogenesis and tumor invasion of specific cancer cell lines are strongly reduced in PAI-1-deficient mice [37]. Myofibroblasts located at the invasive front of colon cancer and malignant melanoma cells have been found to produce significantly higher amounts of PAI-1 than normal surrounding tissue [38, 39]. Inhibition of PAI-1 activity by antibodies or modulators, on the other hand, has been shown to suppress tumor cell invasion and angiogenesis in vitro [40].

Elevated levels of both u-PA and PAI-1 are associated with poor prognosis in a variety of solid tumors. The elevation of u-PA and PAI-1 were shown to have a significant impact on relapse-free and overall survival in patients with primary breast cancer [41, 42]. Besides, high levels of PAI-1 seem to be associated with preferential response to adjuvant chemotherapy, but relative resistance to hormone therapy in these patients [43]. Expression of PAI-1 protein in tumor cells was also described to be a strong independent prognostic factor in cervical cancer [44] and in non-small cell lung carcinoma [45] and is furthermore associated with a higher risk of relapse in squamous cell cancer of the oral cavity.

PAI-1 levels therefore seem to have prognostic value in a number of solid tumors and might help to create individualized therapeutic strategies.

PAI-1 and fibrosis

PA and PAI represent important regulators of the balance between proteolytic and antiproteolytic activities that determine extracellular matrix turnover. Elevated levels of PAI-1 have been demonstrated in keloid fibroblasts which are involved in an excessive accumulation of extracellular matrix [46]. By influencing extracellular matrix turnover as well as activation of proenzymes and growth factors, PAI-1 seems to be involved in a number of pathogenetic processes leading to fibrotic diseases. In this context, PAI-1 expression is not only induced by TGF- β which represents itself a critical mediator of fibrosis, but also by renin, angiotensin II, and aldosterone [47]. Therefore, PAI-1 takes a pivotal role not only in acute renal diseases like thrombotic microangiopathy or glomerulopathy by inhibition of fibrinolysis, but also participates in fibrin accumulation leading to progressive renal diseases like glomerulosclerosis or renal interstitial fibrosis. While undetectable in normal kidneys, PAI-1 was identified in the presence of several renal diseases associated with fibrosis like hypertensive nephropathy, diabetic nephropathy, or lupus nephritis [48]. Normalization of PAI-1 activity correlates with improvement in renal function. Pathogenetic changes in chronic asthma associated with subepithelial fibrosis and extracellular matrix deposition in the airways also seem to be, in part, induced by upregulation of PAI-1. In the airways of patients with severe asthma, PAI-1 expressing mast cells were identified. At the same time, these patients showed a higher frequency of the 4G allele of the PAI gene, which is associated with higher PAI-1 plasma levels [49]. Promoter genotype 4G/4G is also described more often in patients with non-specific interstitial pneumonia than in control populations [50]. PAI-1-deficient mice, on the other hand, presented less extracellular matrix deposition in a murine model of chronic asthma [49] and were shown to develop less severe lung fibrosis after being exposed to bleomycin [51]. In another animal model, excessive increase in cardiac PAI-1 mRNA and plasma PAI-1 antigen levels was found in rats with interstitial and perivascular cardiac fibrosis after myocardial infarction. In this model, PAI-1-deficient animals again developed less severe fibrosis than wild-type animals [52]. Upregulation of PAI-1 expression has also been shown in a variety of other fibrotic diseases like oral submucous fibrosis [53], peritoneal fibrosis in patients with continuous ambulatory peritoneal dialysis [54], and in liver fibrosis [55]. PAI-1 might represent an important therapeutic target in progressive diseases associated with tissue fibrosis.

PAI-1 and inflammation

Inflammatory processes are preceded by activation of the hemostatic system, the vascular endothelium, and the monocyte/macrophage system and are thus characterized by the release of inflammatory mediators like

TNF- α , IL-10, IL-1, IL-6, IL-8, or interferon γ [56]. Some of these acute phase response mediators like IL-1, IL-6 [57], TNF- α and TGF- β [58] as well as lipopolysaccharide have been demonstrated to upregulate PAI-1 expression. The acute phase protein α -1-acid glycoprotein may stabilize the inhibitory activity of PAI-1 via complex binding [59]. Levels of plasma PAI-1 are known to be increased in sepsis, trauma, infection, and surgery and are correlated with the severity of disease [60]. Recently, C-reactive protein (CRP) which indicates inflammatory states was also shown to increase PAI-1 release from endothelial cells and thus to downregulate fibrinolysis, which might explain the negative prognostic value of CRP in critically ill patients [61].

A number of diseases connected with inflammatory reactions are reported to be associated with elevated levels of PAI-1. Overexpression of PAI-1 was observed in patients with rheumatoid arthritis, possibly due to persistent endothelial cell activation [62]. A significant association was found between the PAI-1 promoter 4G/5G polymorphism and IgE-mediated allergic diseases [63]. In inflammatory bowel disease, PAI-1 expression is elevated in active versus inactive disease and is associated with acute vascular lesions [64, 65]. In peritonitis, reduced local fibrinolysis leading to adhesion formation is due to significantly elevated PAI-1 expression [66]. In this context, excessive PAI-1 production was localized in inflamed appendix samples [67]. On the other hand, one group was able to demonstrate that PAI-1-deficient mice show reduced acute phase protein levels and slower recovery after local tissue injury [68]. PAI-1 thus not only seems to be a marker of inflammatory states but is believed to play a functional role in host response to trauma.

Elevation of PAI-1 is found not only in sepsis but also in atherosclerosis which is accelerated by inflammation and impaired fibrinolysis. Overexpression of PAI-1 is furthermore found in the insulin resistance syndrome or diabetes mellitus type 2 and in this context seems to be regulated by proinflammatory cytokines. Inflammatory states leading to upregulation of PAI-1 are thus believed to predict metabolic disorders.

PAI-1 and metabolic disorders

The metabolic syndrome consisting of a combination of obesity, chronic inflammation, and insulin resistance is associated with a hypercoagulable state and an impairment of the fibrinolytic pathway, mainly due to increased PAI-1 activity. This constellation might in part explain the increased risk of venous and arterial thrombotic events in these patients. A subinflammatory state in metabolic disorders may be created by adipose tissue through the release of acute phase proteins like interleukins or PAI-1. IL-8 and PAI-1 release by adipocytes was shown to be increased by insulin and glucose which are found in higher concentrations in plasma of patients with insulin

resistance [69, 70]. An overexpression of PAI-1 in adipocytes was also found to be induced by TNF- α , TGF- β [71], and angiotensin II [72] and was shown to be reduced by the influence of catecholamines and insulin-sensitizing substances like metformin or thiazolidinediones [72].

Diabetes patients were observed to have significantly higher plasma PAI-1 levels than healthy controls [73]. Elevated PAI-1 levels measured during hyperglycemic crises returned to normal levels after insulin treatment [74]. It was described that children with obesity show the 4G/4G genotype of the PAI-1 polymorphism more frequently than control children without obesity [75]. Weight loss and improved metabolic status induced by dietary restriction actually leads to decreased PAI-1 plasma levels in these patients [76].

Increased PAI-1 levels are postulated to be related to insulin resistance and dyslipidemia and thus to contribute to an elevated risk of cardiovascular disease. In an animal model, PAI-1-deficient mice were completely prevented from developing obesity and insulin resistance after high-fat and high-carbohydrate diet. PAI-1 may therefore play a direct causal role in metabolic disorders [77]. The inhibition of PAI-1 might provide new therapeutic strategies both for reducing cardiovascular risk and improving metabolic disorders.

Interestingly, PAI-1 levels are also increased in women with polycystic ovary syndrome (as described above) and are in this context directly correlated with insulin resistance, independently from total or fat-free body mass, which is seen significantly more often in these women than in healthy control persons [26, 78].

PAI-1 and vascular disease

As a fast-acting inhibitor of fibrinolysis by inhibition of t-PA, PAI-1 is supposed to be associated with an increased risk of venous and arterial thrombotic diseases. Altered expression of PAI-1 in vessel walls might affect atherogenesis not only by impairment of fibrinolysis but also by inhibiting vascular smooth muscle cell (VSMC) migration into the neointima and thus leading to exacerbation of vasculopathy. PAI-1 levels which are found to be increased in atherosclerotic lesions are suggested to potentiate VSMC-poor plaques with a higher risk of plaque rupture [79].

Numerous studies were performed to investigate the correlation between PAI-1 levels and/or genetic variations of PAI-1 genotype and the risk of myocardial and cerebral infarction. Sakata et al. described PAI-1 as a useful tool for the detection of early atherosclerosis in men as they found PAI-1 levels independently associated with the degree of intimal medial thickness [80]. In a study of 52 patients who underwent coronary angiography, PAI-1 levels in patients with non-ST-elevation myocardial infarcts were associated with signs of plaque instability and thus with the risk for subsequent adverse

events [81]. Other groups confirmed these data in a study investigating over 150 postmenopausal women with coronary atherosclerosis who showed significantly higher levels of PAI-1 compared to healthy controls [82]. High levels of PAI-1 are also associated with fatal outcome of patients after myocardial infarction. Patients with 30-day mortality and/or reinfarction after myocardial infarction were shown to have significantly higher PAI-1 levels than patients without these complications [83]. Especially the acute release of PAI-1 within the first 24 h after myocardial infarction was described as a strong independent predictor of death at 30 days [84]. Additionally, PAI-1 deficiency seems to protect ApoE-knock-out mice from atherosclerosis progression at sites of turbulent flow [51].

High PAI-1 antigen concentrations are also found in patients with cerebral infarction [85]. In this context, PAI-1 activity was again observed to be associated with high 30-day mortality after stroke [86].

As fibrinolysis is known to be critical to prevent thrombus growth and restore blood flow after thrombotic vascular occlusion, the increase in PAI-1 levels is not only associated with atherosclerosis, but also with deep vein thrombosis. Young patients with recurrent deep vein thrombosis were shown to have a PAI-1 activity more than threefold higher than healthy controls [87]. Increased levels of PAI-1 in venous thromboembolism patients seem to correlate with the development of recurrent venous thromboembolism within the following three to six years [88]. Thrombotic events at unusual sites are also associated with increased PAI-1 activity. PAI-1 levels in patients with central serous chorioretinopathy leading to thrombotic occlusion in choroidal veins were significantly increased compared to those in normal volunteers [89]. On the other hand, PAI-1 was described as an independent laboratory marker in the diagnosis of veno-occlusive disease (VOD) after hematopoietic stem cell transplantation [90]. PAI-1 is suggested to play a major role in the pathogenesis of VOD, which results from endothelial cell damage leading to impairment of fibrinolysis and finally to occlusion of sinusoids of the liver [91]. Recent studies confirmed that PAI-1 mainly serves as an exclusion criterion for the diagnosis of VOD after hematopoietic stem cell transplantation [92].

There are contradictory results though concerning the influence of the PAI-1 genotype on vascular disease. In a large study investigating more than 1,400 men, the distributions of 4G/4G, 4G/5G and 5G/5G genotypes among men who developed myocardial infarction or venous thromboembolism were identical to those of men who remained free of vascular disease [93]. In contrast, the PAI-1 4G/5G gene polymorphism was shown to be associated with a higher risk of myocardial and cerebral infarction in a Chinese population [94] and a higher incidence of VOD after hematopoietic stem cell transplantation [95]. The 4G/4G genotype was also associated with a greater risk of thrombosis both in symptomatic thrombophilic patients and in patients with idiopathic

deep vein thrombosis [96]. A pooled analysis indicated a significant association between the 4G allele and increased risk of myocardial infarction [97]. In groups of high risk, the polymorphism was also a risk factor for severity of disease [98].

Summary

Representing the main inhibitor of t-PA that acts in targeted proteolysis and fibrinolysis, PAI-1 plays a major role in numerous physiological and pathological processes. Increased levels of PAI-1 are associated with arterial and venous vascular disease as well as with malignancy, fibrosis, recurrent miscarriage, and insulin resistance. PAI-1 antigen levels and activity were not only shown to be elevated in a number of diseases, but they were also found to predict the outcome of patients suffering from vascular disease or breast cancer. PAI-1 is thus not only an important non-invasive marker for the diagnosis of thrombotic diseases, malignant diseases, or metabolic states, but it may also predict the risk of subsequent adverse events in these patients and may thus help in making early therapeutic decisions. In mouse models, PAI-1 deficiency was also shown to protect mice from arteriosclerosis or metabolic states suggesting that it plays a causal role in the pathogenesis of several disorders. Development of PAI-1 inhibitors might therefore reveal promising therapeutic tools in a variety of diseases.

Up to now, there have been no general recommendations when to measure PAI-1 levels or PAI-1 genotypes. There might be indications to measure PAI-1 levels in vascular diseases, especially in atherosclerosis or VOD, and in certain malignant diseases, especially in breast cancer. It might also be helpful to determine the PAI-1 genotype in patients with recurrent miscarriage and metabolic syndromes.

References

1. Loskutoff DJ. A slice of PAI. *J Clin Invest* 1993;92:2563.
2. Kruithof EKO. Plasminogen activator inhibitors – a review. *Enzyme* 1988;40:13–121.
3. Brakman P, Astrup T. Selective inhibition in human pregnancy blood of urokinase induced fibrinolysis. *Scand J Clin Lab Invest* 1963;15:603.
4. Lecander I, Åstedt B. Isolation of a new specific plasminogen activator inhibitor from pregnancy plasma. *Br J Haemat* 1986;62:221–8.
5. Stump DC, Thienpont M, Collen D. Purification and characterization of a novel inhibitor of urokinase from human urine. *J Biol Chem* 1986;261:12759–66.
6. Scott RW, Bergman BL, Bajpai A, Hersh RT, Rodriguez H, Jones BN, et al. Protease nexin. Properties and a modified purification procedure. *J Biol Chem* 1985;260:7029–34.
7. Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thromb Haemost* 2005;93:631–40.

8. Binder BR, Christ G, Gruber F, Grubic N, Hufnagl P, Krebs M, et al. Plasmin activator inhibitor 1: physiological and pathophysiological roles. *News Physiol Sci* 2002; 17:56–61.
9. Follo M, Ginsburg D. Structure and expression of the human gene encoding plasminogen activator inhibitor, PAI-1. *Gene* 1989;84:447–53.
10. Lawrence DA, Strandberg L, Ericson J, Ny T. Structure-function studies of the SERPIN plasminogen activator inhibitor type I. *J Biol Chem* 1990;265:20293–301.
11. Travis J, Salvesen GS. Human plasma proteinase inhibitors. *Annu Rev Biochem* 1983;52:655.
12. Krishnamurti C, Alving BM. Plasminogen activator inhibitor type 1: Biochemistry and evidence for modulation of fibrinolysis in vivo. *Sem Thromb Hem* 1992;1:67–80.
13. Kluft C, Verheijen JH, Rijken DC, Chang GT, Jie AFH, One-linx C. Diurnal fluctuations in the activity of the fast-acting t-PA inhibitor. In: Davdison JF, Donati MB, Coccheri S, editors. *Progress in Fibrinolysis Vol VIII*. Edinburgh (Great Britain): Churchill Livingstone 1985:117.
14. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood* 1987;69:381–7.
15. Denner S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad 3 and Smad 4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J* 1998; 17:3091–100.
16. Marx N, Bourcier T, Sukhova GK, Libby P, Plutzky J. PPAR-gamma activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPAR-gamma as a potential mediator in vascular disease. *Arterioscler Thromb Vasc Biol* 1999;19:546–51.
17. Kunz C, Pebler S, Otte J, von der Ahe D. Differential regulation of plasminogen activator and inhibitor gene transcription by the tumor suppressor p53. *Nucleic Acids Res* 1995;23:3710–7.
18. Nykjaer A, Conese M, Christensen EI, Olson D, Cremona O, Gliemann J, et al. Recycling of the urokinase receptor upon internalization of the uPA:serpin complexes. *EMBO J* 1997;16:2610–20.
19. Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost* 2003;29:125–30.
20. Sie P, Caron C, Azam J, Goudemand J, Grandjean H, Boneu B, et al. Reassessment of von Willebrand factor (VWF), VWF propeptide, factor VIII:C and plasminogen activator inhibitors 1 and 2 during normal pregnancy. *Br J Haematol* 2003;121:897–903.
21. Sartori MT, Wiman B, Vettore S, Dazzi F, Girolami A, Patrassi GM. 4G/5G polymorphism of PAI-1 gene promoter and fibrinolytic capacity in patients with deep vein thrombosis. *Thromb Haemost* 1998;80:956–60.
22. Glueck CJ, Wang P, Goldenberg N, Sieve L. Pregnancy loss, polycystic ovary syndrome, thrombophilia, hypofibrinolysis, enoxaparin, metformin. *Clin Appl Thromb Hemost* 2004;10:323–34.
23. Buchholz T, Lohse P, Rogenhofer N, Kosian E, Pihusch R, Thaler CJ. Polymorphisms in the ACE and PAI-1 genes are associated with recurrent spontaneous miscarriages. *Hum Reprod* 2003;18:2473–7.
24. Hefler L, Jirecek S, Heim K, Grimm C, Antensteiner G, Zeilinger R, et al. Genetic polymorphisms associated with thrombophilia and vascular disease in women with unexplained late intrauterine fetal death: a multicenter study. *Soc Gynecol Investig* 2004;11:42–4.
25. Bick RL, Hoppensteadt D. Recurrent miscarriage syndrome and infertility due to blood coagulation protein/platelet defects: a review and update. *Clin Appl Thromb Hemost* 2005;11:1–13.
26. Tarkun I, Canturk Z, Arslan BC, Turemen E, Tarkun P. The plasminogen activator system in young and lean women with polycystic ovary syndrome. *Endocr J* 2004;51: 467–72.
27. Hakli T, Romppanen EL, Hiltunen M, Helisami S, Punnonen K, Heinonen S. Plasminogen activator inhibitor-1 polymorphism in women with pre-eclampsia. *Genet Test* 2003; 7:265–8.
28. Tempfer CB, Jirecek S, Riener EK, Zeisler H, Denschlag D, Hefler L, Husslein PW. Polymorphisms of thrombophilic and vasoactive genes and severe preeclampsia: a pilot study. *Soc Gynecol Investig* 2004;11:227–31.
29. Yamada N, Arinami T, Yamakawa-Kobayashi K, Watanabe H, Sohda S, Hamada H, et al. The 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene is associated with severe preeclampsia. *J Hum Genet* 2000;45:138–41.
30. Ma S, Bai C, Bian X. Investigation of thrombomodulin and plasminogen activator inhibitor type-1 in pregnancy induced hypertension and its clinical significance. *Chin Med Sci J* 2001;16:169–71.
31. Liu YX. Regulation of the plasminogen activator system in the ovary. *Biol Signals Recept* 1999;8:160–77.
32. Floridon C, Nielsen O, Holund B, Sweep F, Sundel L, Thomsen SG, et al. Does plasminogen activator inhibitor-1 (PAI-1) control trophoblast invasion? A study of fetal and maternal tissue in intrauterine, tubal and molar pregnancies. *Placenta* 2000;21:754–62.
33. Dano K, Behrendt N, Hoyer-Hansen G, Johnsen M, Lund LR, Ploug M, et al. Plasminogen activation and cancer. *Thromb Haemost* 2005;93:676–81.
34. Czekay RP, Loskutoff DJ. Unexpected role of plasminogen activator inhibitor 1 in cell adhesion and detachment. *Exp Biol Med (Maywood)* 2004;229:1090–6.
35. Hildenbrand R, Arens N. Protein and mRNA expression of uPAR and PAI-1 in myoepithelial cells of early breast cancer lesions and normal breast tissue. *Br J Cancer* 2004;91: 564–71.
36. Hundsdorfer B, Zeilhofer HF, Block KP, Dettmar P, Schmitt M, Kolk A, et al. Tumour-associated urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 in normal and neoplastic tissues of patients with squamous cell cancer of the oral cavity – clinical relevance and prognostic value. *J Craniomaxillofac Surg* 2005;33:191–6.
37. Maillard C, Jost M, Romer MU, Brunner N, Houard X, Lejeune A, et al. Host plasminogen activator inhibitor-1 promotes human skin carcinoma progression in a stage-dependent manner. *Neoplasia* 2005;7:57–66.
38. Illemann M, Hansen U, Nielsen HJ, Andreasen PA, Hoyer-Hansen G, Lund LR, et al. Leading-edge myofibroblasts in human colon cancer express plasminogen activator inhibitor-1. *Am J Clin Pathol* 2004;122:256–65.
39. Stabuc B, Markovic J, Bartenjev I, Vrhovec I, Medved U, Kocijancic B. Urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 and 2 in stage I malignant melanoma. *Oncol Rep* 2003;10:635–9.
40. Brooks TD, Wang SW, Brunner N, Charton PA. XR5967, a novel modulator of plasminogen activator inhibitor-1 activity, suppresses tumor cell invasion and angiogenesis in vitro. *Anticancer Drugs* 2004;15:37–44.
41. Meo S, Dittadi R, Peloso L, Gion M. The prognostic value of vascular endothelial growth factor, urokinase plasminogen activator and plasminogen activator inhibitor-1 in node-negative breast cancer. *Int J Biol Markers* 2004;19: 282–8.

42. Duffy MJ. Urokinase plasminogen activator and its inhibitor, PAI-1, as prognostic markers in breast cancer: from pilot to level 1 evidence studies. *Clin Chem* 2002;48:1194–7.
43. Duffy MJ, Duggan C. The urokinase plasminogen activator system: a rich source of tumour markers for the individualised management of patients with cancer. *Clin Biochem* 2004;37:541–8.
44. Hazelbag S, Kenter GG, Gorter A, Fleuren GJ. Prognostic relevance of TGF-beta1 and PAI-1 in cervical cancer. *Int J Cancer* 2004;112:1020–8.
45. Werle B, Kotsch M, Lah TT, Kos J, Gabrijelcic-Geiger D, Spiess E, et al. Cathepsin B, plasminogenactivator-inhibitor (PAI-1) and plasminogenactivator-receptor (uPAR) are prognostic factors for patients with non-small cell lung cancer. *Anticancer Res* 2004;24:4147–61.
46. Zhang Q, Wu Y, Ann DK, Messadi DV, Tuan TL, Kelly AP, et al. Mechanisms of hypoxic regulation of plasminogen activator inhibitor-1 gene expression in keloid fibroblasts. *J Invest Dermatol* 2003;121:1005–12.
47. Brown NJ, Vaughan DE, Fogo AB. Aldosterone and PAI-1: implications for renal injury. *J Nephrol* 2002;15:230–5.
48. Eddy A. Plasminogen activator inhibitor-1 and the kidney. *Am J Physiol Renal Physiol* 2002;283:F209–F20.
49. Cho S, Ryu CH, Oh CK. Plasminogen activator inhibitor-1 in the pathogenesis of asthma. *Exp Biol Med* 2004;229:138–46.
50. Kim KK, Flaherty KR, Long Q, Hattori N, Sisson TH, Colby TV, et al. A plasminogen activator inhibitor-1 promoter polymorphism and idiopathic interstitial pneumonia. *Mol Med* 2003;9:52–6.
51. Eitzman DT, Westrick RJ, Xu Z, Tyson J, Ginsburg D. Plasminogen activator inhibitor-1 deficiency protects against atherosclerosis progression in the mouse carotid artery. *Blood* 2000;96:4212–5.
52. Takeshita K, Hayashi M, Iino S, Kondo T, Inden Y, Iwase M, et al. Increased expression of plasminogen activator inhibitor-1 in cardiomyocytes contributes to cardiac fibrosis after myocardial infarction. *Am J Pathol* 2004;164:449–56.
53. Yang SF, Hsieh YS, Tsai CH, Chou MY, Chang YC. The upregulation of type 1 plasminogen activator inhibitor in oral submucous fibrosis. *Oral Oncol* 2003;39:367–72.
54. Higuchi C, Tanihata Y, Nishimura H, Naito T, Sanaka T. Effects of glucose and plasminogen activator inhibitor-1 on collagen metabolism in peritoneum. *Ther Apher Dial* 2005;9:173–8.
55. Zhang LP, Takahara T, Yata Y, Furui K, Jin B, Kawada N, et al. Increased expression of plasminogen activator and plasminogen activator inhibitor during liver fibrogenesis of rats: role of stellate cells. *J Hepatol* 1999;31:703–11.
56. Holler E. Cytokines, viruses, and graft-versus-host disease. *Curr Opin Hematol* 2002;9:479–84.
57. Healy AM, Gelehrter TD. Induction of plasminogen activator inhibitor-1 in HepG2 human hepatoma cells by mediators of the acute phase response. *J Biol Chem* 1994;269:19095–100.
58. Macfelda K, Weiss TW, Kaun C, Breuss JM, Zorn G, Oberndorfer U, et al. Plasminogen activator inhibitor-1 expression is regulated by the inflammatory mediators interleukin-1alpha, tumor necrosis factor-alpha, transforming growth factor-beta and oncostatin M in human cardiac myocytes. *J Mol Cell Cardiol* 2002;34:1681–91.
59. Boncela J, Papiewska I, Fijalkowska I, Walkowiak B, Cierniewski CS. Acute phase protein alpha 1-acid glycoprotein interacts with plasminogen activator inhibitor type 1 and stabilizes its inhibitory activity. *J Biol Chem* 2001;276:35305–11.
60. Leithauser B, Matthias FR, Nicolai U, Voss R. Hemostatic abnormalities and the severity of illness in patients at the onset of clinically defined sepsis. Possible indication of the degree of endothelial cell activation? *Intensive Care Med* 1996;22:631–6.
61. Boudjeltia KZ, Piagnerelli M, Brohée D, Guillaume M, Vincent PJL, Remacle C, et al. Relationship between CRP and hypofibrinolysis: Is this a possible way to explain the association between CRP and outcome in critically ill patients? *Thromb J* 2004;2:2–7.
62. Wallberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapaa-Dahlqvist S. Activation of the immune system and inflammatory activity in relation to markers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. *J Rheumatol* 2002;29:875–82.
63. Buckova D, Izakovicova Holla L, Vacha J. Polymorphism 4G/5G in the plasminogen activator inhibitor-1 (PAI-1) gene is associated with IgE-mediated allergic diseases and asthma in the Czech population. *Allergy* 2002;57:446–8.
64. Vrij AA, Rijken J, van Wersch JW, Stockbrugger RW. Coagulation and fibrinolysis in inflammatory bowel disease and in giant cell arteritis. *Pathophysiol Haemost Thromb* 2003;33:75–83.
65. Desreumaux P, Huet G, Zerimech F, Gambiez L, Balduyck M, Baron P, et al. Acute inflammatory intestinal vascular lesions and in situ abnormalities of the plasminogen activation system in Crohn's disease. *Eur J Gastroenterol Hepatol* 1999;11:1113–9.
66. Ince A, Eroglu A, Tarhan O, Bulbul M. Peritoneal fibrinolytic activity in peritonitis. *Am J Surg* 2002;183:67–9.
67. Whawell SA, Wang Y, Fleming KA, Thompson EM, Thompson JN. Localization of plasminogen activator inhibitor-1 production in inflamed appendix by in situ mRNA hybridization. *J Pathol* 1993;169:67–71.
68. Renckens R, Roelofs JJ, de Waard V, Florquin S, Lijnen HR, Carmeliet P, et al. The role of plasminogen activator inhibitor type 1 in the inflammatory response to local tissue injury. *J Thromb Haemost* 2005;3:1018–25.
69. Fain JN, Madan AK. Insulin enhances vascular endothelial growth factor, interleukin-8, and plasminogen activator inhibitor-1 but not interleukin-6 release by human adipocytes. *Metabolism* 2005;54:220–6.
70. He G, Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Stimulation of PAI-1 and adipokines by glucose in human adipose tissue in vitro. *Biochem Biophys Res Commun* 2003;310:878–83.
71. Pandey M, Loskutoff DJ, Samad F. Molecular mechanisms of tumor necrosis factor-alpha-mediated plasminogen activator inhibitor-1 expression in adipocytes. *FASEB J* 2005 (Epub ahead of print).
72. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Relat Metab Disord* 2004;28:1357–64.
73. Umpaichitra V, Hussain MM, Castells S. Plasminogen activator inhibitor-1 and tissue-plasminogen activator in minority adolescents with type 2 diabetes and obesity. *Pediatr Res* 2005 May (Epub ahead of print).
74. Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress and lipid peroxidation in patients with hyperglycemic crises. *Diabetes* 2004;53:2079–86.
75. Kinik ST, Atac FB, Verdi H, Cetintas S, Sahin FI, Obzбек N. The effect of plasminogen activator inhibitor-1 gene 4G/5G polymorphism on glucose and lipid metabolism in

- Turkish obese children. *Clin Endocrinol (Oxf)* 2005;62: 607–10.
76. Grant PJ. Beneficial effects of metformin on haemostasis and vascular function in man. *Diabetes Metab* 2003;29 (4Pt2):6S44–52.
 77. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, et al. Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 2004; 53:336–46.
 78. Dhindsa G, Bhatia R, Dhindsa M, Bhatia V. Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome. *J Postgrad Med* 2004;50:140–4.
 79. Sobel BE, Taatjes DJ, Schneider DJ. Intramural plasminogen activator inhibitor type-1 and coronary atherosclerosis. *Arteriosclerosis Thromb Vasc Biol* 2003;23:1979–96.
 80. Sakata T, Mannami T, Baba S, Kokubo Y, Kario K, Okamoto A, et al. Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries. *Atherosclerosis* 2004;176:355–60.
 81. Gyongyosi M, Glogar D, Weidinger F, Domanovits H, Lagner A, Wojta J, et al. Association between plasmin activation system and intravascular ultrasound signs of plaque instability in patients with unstable angina and non-ST-segment elevation myocardial infarction. *Am Heart J* 2004; 147:158–64.
 82. Nielsen NE, Logander E, Swahn E. Fibrinolytic variables in postmenopausal women with unstable coronary artery disease. *J Thromb Thrombolysis* 2001;12:217–23.
 83. Sinkovic A, Pogacar V. Risk stratification in patients with unstable angina and/or non-ST-elevation myocardial infarction by Troponin T and plasminogen-activator-inhibitor-1 (PAI-1). *Thromb Res* 2004;114:251–7.
 84. Collet JP, Montalescot G, Vicaute E, Ankri A, Waylo F, Lesty C, et al. Acute release of plasminogen activator inhibitor-1 in ST-segment elevation myocardial infarction predicts mortality. *Circulation*. 2003;108:391–4.
 85. Lindgren A, Lindhoff C, Norrving B, Astedt B, Johansson BB. Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. *Stroke* 1996;27: 1066–71.
 86. Catto AJ, Carter AM, Stickland M, Bamford JM, Davies JA, Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. *Thromb Haemost* 1997;77:730–4.
 87. Swiatkiewicz A, Jurkowski P, Kotschy M, Ciecierski M, Jawien A. Level of antithrombin III, protein C, protein S and other selected parameters of coagulation and fibrinolysis in the blood of the patients with recurrent deep vein thrombosis. *Med Sci Monit* 2002;8:CR263–8.
 88. Schulman S, Wilman B. The significance of hypofibrinolysis for the risk of recurrence of venous thromboembolism. Duration of anticoagulation (DURAC) Trial Study group. *Thromb Haemost* 1996;75:607–11.
 89. Iijima H, Iida T, Murayama K, Imai M, Gohdo T. Plasminogen activator inhibitor 1 in central serous chorioretinopathy. *Am J Ophthalmol* 1999;127:477–8.
 90. Salat C, Holler E, Kolb HJ, Reinhardt B, Pihusch R, Wilmanns W, et al. Plasminogen activator inhibitor-1 confirms the diagnosis of hepatic veno-occlusive disease in patients with hyperbilirubinemia after bone marrow transplantation. *Blood* 1997;89:2184–8.
 91. Pihusch V, Pihusch M, Penovici M, Kolb HJ, Hiller E, Pihusch R. Transforming growth factor beta-1 released from platelets contributes to hypercoagulability in veno-occlusive disease following hematopoietic stem cell transplantation. *Thromb Res* 2005;116:233–40.
 92. Pihusch M, Wegner H, Goehring P, Salat C, Hiller E, Andreesen R, et al. Diagnosis of hepatic veno-occlusive disease by plasminogen activator inhibitor-1 plasma antigen levels: a prospective analysis in 350 allogeneic hematopoietic stem cell recipients. *Transplantation* 2005, in press.
 93. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Miletich JP. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. *Circulation* 1997;95:59–62.
 94. Zhan M, Zhou Y, Han Z. Plasminogen activator inhibitor-1 4G/5G gene polymorphism in patients with myocardial or cerebrovascular infarction in Tianjin, China. *Chin Med J (Engl)* 2003;116:1707–10.
 95. Pihusch M, Lohse P, Reitberger J, Hiller E, Andreesen R, Kolb HJ, et al. Impact of thrombophilic gene mutations and graft-versus-host disease on thromboembolic complications after allogeneic hematopoietic stem-cell transplantation. *Transplantation* 2004;78:911–8.
 96. Sartori MT, Danesin C, Saggiorato G, Tormene D, Simioni P, Spiezia L, et al. The PAI-1 gene 4G/5G polymorphism and deep vein thrombosis in patients with inherited thrombophilia. *Clin Appl Thromb Hemost* 2003;9:299–307.
 97. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Büller HR, Peters RJ. Genetic variations in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction. *Circulation* 2001;104:3063–74.
 98. Lane DA, Grand PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000;95:1517–32.