ORIGINAL ARTICLE

Evaluation of low PAI-1 activity as a risk factor for hemorrhagic diathesis

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Summary. *Background:* Prospective studies of the epidemiology and clinical significance of low plasminogen activator inhibitor type 1 (PAI-1) activity are lacking. *Objective:* To evaluate the prevalence of low PAI-1 activity in patients with a bleeding tendency in comparison with a normal population. *Methods:* In 586 consecutive patients, referred because of bleeding symptoms, we added analyses of PAI-1 activity and tissue plasminogen activator complex with PAI-1 (t-PA–PAI-1) to the routine investigation, consisting of platelet count, bleeding time, prothrombin time, activated partial thromboplastin time, fibrinogen, factor VIII, von Willebrand factor activity, and antigen. Controls were 100 blood donors and 100 age- and sex-matched healthy individuals. The latter were also evaluated regarding the previous bleeding episodes. The bleeding history was classified as clinically significant or not, and the criteria were fulfilled in 75% of the patients and 18% of the healthy controls. *Results:* The routine laboratory investigation of the patients was negative in 57%. Low PAI-1 activity, defined as < 1.0 U mL^{-1}, was found in 23% of the patients and in 13% and 10% of the blood donors and healthy controls, respectively (odds ratio and 95% CI, 2.04; 1.11–3.77 and 2.75; 1.39–5.42, respectively). The difference remained statistically significant after the adjustment for body mass index, use of estrogens, sex and age (odds ratio for patients vs. healthy controls 3.23; 95% CI, 1.22–8.56, $P = 0.019$). The distribution of the 4G/5G genotypes in the patients was not different from that of two control populations. No specific symptom predicted for low PAI-1, which did not aggravate the clinical picture in association with the other hemostatic defects. Low tPA–PAI-1 was not associated with the increased bleeding tendency. *Conclusion:* Low PAI-1 activity is common in patients with a bleeding diathesis, but it is a risk factor of minor clinical importance and not associated with specific bleeding manifestations.

Introduction

Investigations of patients with mild-bleeding disorders yield a diagnosis of a hemostatic defect in approximately half of the cases [1]. Thus, in patients with menorrhagia with normal clinical examination of the pelvis, an inherited bleeding disorder was identified in 10–17% [2,3], and a prolonged-bleeding time was observed in 32% of women with this bleeding manifestation [4]. Investigation for easy bruising demonstrated an abnormality in 54% of the children and in only 6% of the adults [5]. In the remainder, the bleeding manifestations may have been the result of local pathology or acquired hemostatic defects induced by, for example, aspirin or non-steroid anti-inflammatory agents, but there may also be yet unknown or poorly defined reasons for a bleeding tendency.

The vast majority of the diagnoses established by these investigations involve disorders of primary hemostasis [platelet dysfunction and von Willebrand disease (VWD)], followed by the deficiencies of coagulation factors, whereas defects in the fibrinolytic system are rarely identified [2,3,5]. The latter include congenital deficiency of α_2-antiplasmin (α_2-plasmin inhibitor) [6] and of plasminogen activator inhibitor type I (PAI-1), whereas the increased activity of tissue plasminogen activator (t-PA) is almost exclusively iatrogenic. Only a handful of cases with deficiency of PAI-1 in association with a bleeding diathesis have been described in the literature [7–15], but there has not been any systematic study of the prevalence and importance of this condition. In one of the families reported, there was a complete absence of PAI-1 associated with a frame shift mutation in the corresponding gene [16], and in another case with reduced PAI-1 activity a point mutation was identified in the signal peptide [17]. In several of the other

cases, there was a deficient but measurable activity of PAI-1 [9,11,12,15]. It is unclear to what extent a low PAI-1 activity contributes to the bleeding diathesis and, in case this is a risk factor for bleeding, what the prevalence is.

The aim of this study was therefore to prospectively evaluate the prevalence of low PAI-1 activity in a population referred for investigation of bleeding diathesis and to compare with a normal population. Furthermore, we wanted to assess whether any specific symptom, such as delayed or secondary bleeding after surgery or trauma, predicts for this hemostatic defect.

Methods

Subjects

Patients The Coagulation Unit, Karolinska University Hospital has a catchment area of approximately 2 million inhabitants. During the period of October 1998 to October 2002, all patients, 18 years of age or above, referred to this unit because of the increased bleeding tendency, were prospectively included in the study. In addition to the routine laboratory investigation for bleeding, analysis of PAI-1 was added.

Normal controls One hundred blood donors at two donation sites belonging to the Department of Blood Transfusion and Immunology, Karolinska University Hospital provided a blood sample for analysis of PAI-1 at the time of plasma donation. Ten males and 10 females from each of the age groups 21–30, 31–40, 41–50, 51–60, and 61–70 years old were included. The donors were anonymous and the only additional data available for the study were age, height, and body weight. This group of normal controls is hereafter named 'Blood donors'.

In order to obtain a bleeding history from normal controls, an additional cohort of 100 healthy subjects were recruited. These were friends and non-blood relatives of the patients as well as hospital staff without a known hemostatic defect. They were matched to the mean patient population for sex and age. This group of normal controls is hereafter named 'Healthy controls'.

Body mass index (BMI) was calculated from the *body weight (kg) divided by the square of height (m²)*.

The study was approved by the Ethics Committee at Karolinska University Hospital, and all patients and normal controls gave oral informed consent after having read the consent form, which was specifically designed for each population group.

Data collection

In conjunction with the blood sampling, the patients and the Healthy controls filled out a questionnaire, which included data on previous bleeding from nose, gingiva, gastrointestinal canal, urinary tract, vagina, skin, muscles and joints as well as number of surgical procedures, tooth extractions and child deliveries with the number of bleeding complications at each of these. The questionnaire has been used for 6 years at our center, although it has not been specifically validated in a study. Bleeding complication after a tooth extraction was defined as bleeding for more than 5 h or requiring sutures or other interventions. Bleeding complication after surgery was assessed in relation to the severity of the procedure. The investigator reviewed the questionnaire together with the patient to resolve any unclear answers. Patient responses were confirmed by the review of medical records. The bleeding tendency was then classified by two of the investigators (SS and AÅ), who were blinded to the laboratory results but not to the group of subjects (patients vs. Healthy controls), as clinically significant or not. The criteria for 'clinically significant bleeding tendency' included bleeding complications after surgery, tooth extraction or child delivery, bleeding in the central nervous system, gastrointestinal canal, joints or muscles as well as referral for menorrhagia, confirmed as menstrual bleeding requiring change of pads or tampons at least every 2 h (for healthy controls without 'referral for menorrhagia'). Examples of not clinically significant bleeding tendency are subcutaneous hematoma, epistaxis, gingival bleeding and subconjunctival bleeding.

Laboratory analyses

Blood sampling was performed between seven and 10 in the morning on a fasting stomach or after a fat-free breakfast and after at least 15 min of rest. Concomitant use of acetylsalicylic acid or non-steroid anti-inflammatory was not allowed for 10 days prior to the sampling time-point. For patients or healthy controls with infectious disease, as manifested by fever and/or with antibiotic therapy, blood sampling was postponed until they had recovered. Blood donors had to recover for at least 1 week after any symptoms of infection until eligible for donation. Nine volumes of blood were collected in tubes with one volume of 0.129 M trisodium citrate for coagulation analyses. The tubes were centrifuged within 30 min at 2000 *g* for 15 min, after which plasma was separated, immediately frozen and kept at −70 °C until analysis. Activated partial thromboplastin time (APTT), prothrombin time (PT) and platelet count were analyzed on fresh blood samples. Template-bleeding time was performed with the Surgicutt® Adult Device (ITC Europe, Rodano, Italy). In addition to these tests, all patients were routinely investigated with analyses of fibrinogen, factor VIII activity, von Willebrand factor activity (ristocetin co-factor) and von Willebrand factor antigen. In case of normal results at this point, analyses of thrombin time, factor XIII and antiplasmin were added. In case of prolonged APTT or prolonged PT, we analyzed factors XII, XI, IX, X, V and prothrombin, or factors VII, X and prothrombin, respectively. In patients with a bleeding time of more than 900 s and no reduction after a test dose of desmopressin (0.2 μg kg⁻¹ subcutaneously), we proceeded with platelet aggregation and flow cytometry studies. Capillary fragility was assessed by venous occlusion of the forearm for 5 min and counting the petechiae within a 3 cm in diameter circle 3 min after release.

Plasminogen activator inhibitor type I activity was analyzed in all patients and controls using a commercially available kit (Chromolize™ PAI-1 from Biopool, Umeå, Sweden). The manufacturer states that the analysis has a detection range of 2.0–50 U mL^{-1}. Our local laboratory has so far provided a reference range of < 15 U mL^{-1}. In order to improve the precision at low PAI-1 levels, more measuring points were added to the low end of the calibration curve, which was read using a point-to-point approach instead of linear regression. An in-house control with a PAI-1 activity of 0.9 U mL^{-1} was also added. The intra-assay coefficient of variation (C_v) was 11.5% for the low control ($n = 108$) and 5.7% for the high control (PAI-1 19.4 U mL^{-1}; $n = 106$), and the inter-assay C_v was 4.5% and 3.6%, respectively.

tPA–PAI-1 complex was analyzed in all Blood donors and in most of the patients with TintElize® tPA–PAI-1 (Biopool). The reference range of this analyte is 0.6–6.7 ng mL^{-1} according to the manufacturer. During the last part of the study, the manufacturer was not able to supply this reagent anymore and therefore it was not possible to analyze the samples from some of the patients or the Healthy controls. In 52 of the patients with low PAI-1 activity, we also analyzed the PAI-1 antigen in serum with an ELISA method, TintElize® PAI-1, from Biopool to evaluate whether the inhibitor was absent in the platelets.

Genotyping of the PAI-1 4G/5G polymorphism was performed by pyrosequencing using primers and protocols previously described in Ref. [18].

Statistical analyses

Binomial data were analyzed with chi-squared test with Yate's correction. The PAI-1 results were log-transformed to achieve a normal distribution and compared with two-tailed *t*-test for two samples with equal variance. Adjusted odds ratios were calculated by logistic function regression with SAS7STAT 9 software (SAS Institute Inc, Cary, NC, USA).

Results

Study populations

During 4 years, a total of 586 patients were referred and investigated for increased bleeding tendency, and the reasons for referral are shown in Table 1. Demographic data on the patient population and the two control populations are shown in Table 2. Two percent of the patients were of Asian origin and 1% of African origin, whereas of the Healthy controls 10% were of Asian origin and 1% of African origin. Twenty-eight percent of the female patients were receiving estrogen therapy (combined oral contraceptives or replacement therapy) vs. 26% of the Healthy controls at the time of the investigation. Among the patients, 442 (75%) had a history of bleeding symptoms fulfilling our criteria for clinically significant, and the corresponding number among the Healthy controls was 18 (18%). Clinically significant bleeding had occurred among 65% of the

Table 1 Main reason for referral for investigation of bleeding tendency, as stated by the referring physician ($n = 586$)

Reason	n
Perioperative bleeding complication	146
Multiple bleeding symptoms	126
Menorrhagia	117
Subcutaneous hematoma	74
Peripartum bleeding complication	29
Epistaxis	19
Bleeding complication after tooth extraction	15
Intracranial bleeding	8
Abnormal hemostatic screening test	7
Gingival bleeding	6
Gastro-intestinal bleeding	5
Hematuria	4
Hemarthrosis	3
Muscle hematoma	3
Abnormal bleeding during antihemostatic therapy	3
Liver disease	3
Family investigation	3
Other*	15

*Posttraumatic bleeding complication two, bleeding after venipuncture two, petechiae two, hemoptysis two, renal disease two, bleeding into a cyst one, subconjunctival bleeding one, renal hematoma one, hematospermia one, and iron deficiency anemia one.

males and 77.5% of the females in the patient population and among 6% of the males and 20% of the females of the Healthy controls. The proportion of patients with clinically significant bleeding was the same, 75%, among the younger (age 18–41) and older (age 42–81) subset. The Healthy controls with a clinically significant bleeding history were not subjected to further laboratory investigation as part of this study.

The routine investigation of bleeding tendency revealed the following conditions: 143 patients with platelet function defect (133 unspecific – normal von Willebrand factor, flow cytometry, aggregometry; seven drug-induced; and one patient each with Glanzmann thrombasthenia heterozygous, renal disease, and myelodysplastic syndrome), 59 with VWD, 14 with capillary fragility, seven with thrombocytopenia without evidence of a platelet function defect, seven with carriership of hemophilia A, five with factor VII-deficiency, four with factor XI-deficiency, three with hypofibrinogenemia, two with isolated prolonged APTT, and one each with mild hemophilia A, factor V-deficiency, prothrombin deficiency, deficiency of all vitamin K-dependent coagulation factors, Ehlers–Danlos syndrome and Osler–Rendu–Weber syndrome. In 336 cases (57%), the routine investigation was completely negative.

PAI-1 levels

The PAI-1 levels in the different populations are shown in Table 2. After log-transformation the levels attained a normal distribution. In the PAI-1 analysis, the values of absorbance at < 1 U mL^{-1} did not differ significantly from the 0-line, and thus it was not meaningful to set the lower limit of normal below 1.0 U mL^{-1}. The proportion of individuals with PAI-1 levels below 1.0 U mL^{-1} was similar among Blood donors,

Table 2 Demographic characteristics and PAI-1 levels of the populations in the study

Characteristic	Blood donors	Healthy controls	Referred patients
n	100	100	586
Males, *n* (%)	50 (50)[a]	17 (17)	97 (16.6)[a]
Age, median (range)	45 (22–70)	41 (18–78)	42 (18–81)
Body mass index (range)	24.7 (18.3–39.0)	22.6 (18.2–34.6)	24.6 (14.8–59.2)
Clinically significant bleeding tendency, *n* (%)	Unknown	18 (18)[a]	442 (75)[a]
PAI-1 activity (U mL^{-1}), median (range)	6.0 (0–58.5)[d]	3.0 (0.5–43)	3.9 (0–92)[d]
Subjects with PAI-1 activity < 1 U mL^{-1}, *n* (%)	13 (13)[b]	10 (10)[c]	137 (23)[b,c]

[a,b,c]The difference between the indicated data in the same row achieves a *P*-value of [a] < 0.001, [b] 0.03, [c] 0.004 (chi-squared test), and [d] 0.006 (*t*-test after log-transformation). None of the other differences are statistically significant.

13% (95% CI, 6.4–19.6), and Healthy controls, 10% (95% CI, 4.1–15.9). Among the patients, a higher proportion was found, 23% (95% CI, 19.6–26.4). The odds ratio for a PAI-1 level of < 1.0 U mL^{-1} in the patients with a history of bleeding problems compared with Blood donors or to Healthy controls was 2.04 (95% CI, 1.11–3.77) and 2.75 (95% CI, 1.39–5.42), respectively. After adjusting for sex, age, BMI and use of estrogens (not possible for Blood donors as the use of estrogens was unknown), the odds ratio was 3.23 (95% CI, 1.22–8.56, *P* = 0.019) for a low PAI-1 activity among patients compared with Healthy controls. Neither sex nor age had a statistically strong influence on the PAI-1 levels, and when we did not force these variables into the model the adjusted odds ratio was 2.89 (95% CI, 1.31–6.37, *P* = 0.008).

PAI-1 and sex Females had a higher proportion of PAI-1 levels below 1.0 U mL^{-1} than males in all populations. As the ratio of males to females was quite different in the Blood donor population than among the patients, and with the inability to get information on bleeding complications in the past from the blood donors, we subsequently collected samples for PAI-1 analysis from one hundred Healthy controls, who were matched for sex and age to the mean patient population. There was a statistically significant difference in the proportion of females with low PAI-1 between Healthy controls (11%; 95% CI, 4.3–17.7) and patients (25%; 95% CI, 21.2–28.8) (*P* = 0.007), whereas the difference in the proportion of males with low PAI-1 between these groups did not reach statistical significance, but the absolute numbers were also much smaller.

PAI-1 and BMI An association was seen between BMI and PAI-1 levels (Blood donors: r^2 = 0.2, *P* < 0.001), and none of the subjects in the two control populations with a BMI above the normal range (≥25.0) had a PAI-1 level < 1.0 U mL^{-1}. However, 14 patients with overweight (BMI 25–29.9, *n* = 12) or obesity (BMI ≥30, *n* = 2) had PAI-1 levels < 1.0 U mL^{-1}.

PAI-1, age and exogenous estrogens The median PAI-1 level was slightly higher in all populations of at least 43 years of age than among those of < 43 years of age, although with statistically significant difference was only among the patients (*P* = 0.002). This difference seemed to be related to lower PAI-1 levels among females on combined oral contraceptives (median PAI-1 in Healthy controls 1.6 U mL^{-1}, patients 1.25 U mL^{-1}) than among those without exogenous estrogens (Healthy controls 3.35 U mL^{-1}, patients 4 U mL^{-1}), with BMI being similar in the subsets.

PAI-1 and bleeding tendency Of the 586 patients investigated, 137 had a low PAI-1 level (< 1 U mL^{-1}), and out of these, 97 (71%) had a clinically significant bleeding tendency, according to preset criteria. However, of 449 patients with a normal PAI-1 level, 345 (77%) had clinically significant bleeding tendency (not statistically significant). According to the final diagnosis made, clinically significant bleeding tendency had the following prevalence in all major diagnosis groups: 73% among those with PAI-1 < 1.0 U mL^{-1} but otherwise negative results of the investigation and 80% among those with PAI-1 ≥1.0 U mL^{-1} as the only finding; 78% in those with platelet function defects alone, 58% in those with platelet function defect and low PAI-1; 80% in those with VWD alone, and 79% in VWD in combination with low PAI-1 activity (no statistically significant differences). In the Healthy controls population, 18 had a history of clinically significant bleeding, and among these three (17%) had low PAI-1, compared with seven of 72 (8.5%) of those without such a history, a difference that was not statistically significant.

PAI-1 and type of bleeding The prevalence of different bleeding manifestations, according to PAI-1 levels and a concomitant diagnosis of any hemostatic defect, is shown in Table 3. The prevalence of most-bleeding manifestations was slightly lower with a low PAI-1 level alone than when combined with a defined hemostatic defect, and likewise when the investigation was completely negative compared with when a defined hemostatic defect with normal PAI-1 levels was identified. The only exception was peri- or postpartum bleeding, which was associated with a higher risk of bleeding complication per delivery in patients with low PAI-1 than in those with normal PAI-1 and also with a statistically non-significant trend to a higher risk of bleeding at delivery per patient with isolated low PAI-1 than in those with a low PAI-1 combined with another defect or those with a completely negative investigation (*P* = 0.08 for both), but this may be an effect of chance.

tPA–PAI-1 complex

The tPA–PAI-1 antigen levels were similar in Blood donors and patients as well as in the different subsets (data not shown). There was a positive correlation between tPA–PAI-1 levels and PAI-1 activity (r^2 = 0.71, *P* < 0.001), but there was no association between low tPA–PAI-1 levels and increased

Table 3 Prevalence of bleeding manifestations or risk of bleeding complication per event in patients with and without low PAI-1 levels in the absence or presence of a diagnosis of a hemostatic defect

Routine investigation	PAI-1 <1 U mL^{-1}		PAI-1 >1 U mL^{-1}	
	Negative	Another defect*	Negative	Another defect*
Postoperative bleeding[†]	40%	57%	53%	53%
Risk of bleeding per surgical procedure	29%[a]	43%[a]	35%	39%
Bleeding after tooth extraction[†]	29%	40%	24%[b]	44%[b]
Risk of bleeding per tooth extraction episode	29%	39%	20%[b]	38%[b]
Bleeding after child delivery[†]	51%	35%	35%	45%
Risk of bleeding per delivery	32%[a]	28%	21%[a]	33%
Subcutaneous hematoma	63%	75%	55%[b]	75%[b]
Menorrhagia	62%	73%	70%	74%
Gingival bleeding	41%	38%	33%	41%
Epistaxis	20%[c]	42%[c]	26%[b]	46%[b]
Gastro-intestinal bleeding	14%	19%	12%	13%
Hematuria	6%	11%	4%	7%
Abnormal post-traumatic bleeding	3%	9%	7%	7%
Muscle hematoma	4%	2%	3%	2%
Hemarthrosis	3%	3%	2%	1%

*Includes platelet function defect, thrombocytopenia, VWD, coagulation factor deficiency or capillary fragility.
[†]Denotes any occurrence of bleeding complication in a patient, independent of the number of procedures performed.
[a,b,c]The difference between the indicated prevalences or risks in the same row, according to the chi-squared test, achieves a *P*-value of [a]0.02, [b]0.001, [c]0.006. None of the other differences are statistically significant.

bleeding tendency (data not shown). No patient had immeasurable tPA–PAI-1 levels, but in four cases it was very low (<0.1 ng mL^{-1}) and their characteristics are shown in Table 4. None of these patients had other bleeding symptoms than from mucous membranes and none were classified as suffering from a clinically significant bleeding tendency.

The PAI-1 antigen levels in serum were analyzed in 52 patients with PAI-1 activity <1 U mL^{-1}. The concentration was 199 ± 71 ng mL^{-1} with a range of 62–362 ng mL^{-1} except for one patient in whom PAI-1 was not measurable in serum. This patient also had thrombocytopenia (40×10^9 L^{-1}), mild factor V deficiency (0.27 IU mL^{-1}) and suspected Wiscott–Aldrich syndrome.

4G-5G polymorphism

DNA was available from 133 of the patients with low PAI-1 activity and from 48 Healthy Controls for analysis of this polymorphism. We were also able to compare with the results of 1614 controls in a study previously published by us – the SHEEP study [19]. The combinations 4G-4G, 4G-5G, and

5G-5G were seen in 24.8%, 54.1% and 21.1% of the patients; in 27.1%, 45.8% and 27.1% of the Healthy controls; and in 28.6%, 51.0% and 20.4% of the SHEEP-controls, respectively, without any statistically significant differences.

Discussion

In our prospective study of a cohort of 586 patients referred for investigation of various bleeding manifestations, with a predominance of female patients, we found a prevalence of low PAI-1 activity, defined as <1 U mL^{-1}, of 23%. This was significantly higher than in two control populations of 100 Blood donors and 100 Healthy controls, and it can therefore be assumed that low PAI-1 activity might constitute a risk factor for increased bleeding tendency.

In view of the fact that the first case report on bleeding diathesis in association with low PAI-1 activity was published 15 years ago [7], it may seem surprising that no larger studies have been performed until now. One explanation may be that the commercially available reagent kit is intended mainly for evaluation of patients with thrombotic disorders, where

Table 4 Characteristics of patients with low PAI-1 activity and very low tPA–PAI-1 levels

Sex, age	tPA–PAI-1 levels (ng mL^{-1})	PAI-1 activity* (U mL^{-1})	Symptoms	Operations/ bleeding complication	Tooth extractions// bleeding complication	Delivery	Other defect
F, 22	0.02	<1.0; <1.0	Menorrhagia	1/no	1/no	0	None
F, 25	0.05	1.0; <1.0	Menorrhagia	2/no	1/no	0	None
F, 18	0.05	<1.0; <1.0	Menorrhagia	0	1/no	0	Platelet function
F, 18	0.06	0.5; 2.9	Menorrhagia, epistaxis, hemat-uria, bruising	0	0	0	Platelet function

*Repeated determination.

an association with high PAI-1 levels has been described [20,21]. The lower limit of the normal range has been poorly defined, and the manufacturer of the reagent has sometimes not provided it. We have improved the precision of the analysis of low PAI-1 activity by adding more measuring points at the low end of the calibration curve and by using an additional control with a reproducibly low PAI-1 activity (0.9 U mL^{-1}).

Secondly, the PAI-1 activity is influenced by several factors. The diurnal variation with high PAI-1 levels and low t-PA activity in the morning is well known [22], and thus the observation of a low PAI-1 activity in the morning should be a more valuable indication of a deficiency state than when sampled later during the day. Moreover, transforming growth factor β1, tumor necrosis factor α, interleukin 1, 2 and 6 can all induce increased activity of PAI-1 [23–27] and therefore the presence of an infectious or inflammatory disease may mask low levels of PAI-1. A progressive increase of PAI-1 is seen during pregnancy [28] and a much more immediate increase in association with surgery is responsible for the fibrinolytic shutdown [29,30]. Increased levels of PAI-1 have also been associated with the metabolic syndrome [31] and with obesity [32], which can be explained by the fact that significant amounts are secreted by adipocytes [33] and PAI-1 activity is also correlated to serum leptin level [34]. Conversely, a reduction of PAI-1 activity is seen during treatment with estrogens [35]. Finally, the 4G/5G polymorphism in the promoter region of the *PAI-1* gene also has an influence on the activity [36].

In our study, blood sampling was limited to the morning hours and we endeavored to avoid performing the investigation when the patient was suffering from an infection. Patients and Healthy controls were matched for age, gender and had a similar proportion of females on estrogen therapy and a similar BMI. The proportion of individuals with PAI-1 activity < 1 U mL^{-1} in the two control populations (Blood donors 13%; Healthy controls 10%) may seem high and indicating that low PAI-1 activity has no association with a bleeding tendency. However, blood donors are not rejected because of a bleeding propensity as long as their hemoglobin is within acceptable limits. In our study, we had no access to the medical history of the Blood donor population, but among the Healthy controls, we could identify, after asking specific questions, a history of significant bleeding among more of those with low PAI-1 activity (17%) compared with those with higher activity (8.5%), albeit not statistically significant. Thus, we cannot exclude an association between low PAI-1 activity and bleeding tendency in the general population as well as among the patients referred because of bleeding symptoms. Supporting earlier observations, we could confirm the association between PAI-1 levels and BMI [32] as well as the inverse relationship to the use of estrogens [35].

It could be speculated that a deficiency of PAI-1 should promote a specific picture of bleeding diathesis with secondary bleeding complicating surgery, child delivery or trauma. Such events or prolonged bleeding after procedures were described in most of the previous case reports [7–9,11,12], but these patients also suffered from epistaxis or menorrhagia. We could not find any typical features of bleeding among the patients with low PAI-1 levels, except that the risk of bleeding complication after a delivery was higher in patients with low PAI-1 as the only abnormality (32%) vs. patients with a completely negative laboratory investigation (21%), but because of the large number of comparisons made this may be spurious. Moreover, the patients with a low PAI-1 activity in combination with other hemostatic defects did not have a severer bleeding history than those with another hemostatic defect alone.

Normally, most of the tPA is circulating as a tPA–PAI-1 complex. With very low antigen levels of the latter, the free and active proportion of tPA will increase and may cause increased fibrinolysis and accordingly bleeding [8]. In our material, we could not find any association between the tPA–PAI-1 antigen levels in general and the bleeding tendency, and no remarkable bleeding diathesis in four patients with a combination of low PAI-1 activity and very low tPA–PAI-1 antigen levels.

The presence of PAI-1 antigen in platelets, measured as PAI-1 antigen in serum, in all but one of 52 patients with low PAI-1 activity, indicates that the cause of low PAI-1 activity is not to be sought in the *PAI-1* gene. Furthermore, the distribution of the 4G/5G polymorphism did not differ between those with low PAI-1 activity and controls. The inflammatory response includes increased expression of tissue factor, complement activation with increased exposure of negatively charged membrane surfaces, down-regulation of the protein C-pathway, increased platelet count and reactivity as well as elevated PAI-1, all of which promote clot formation [37]. Accordingly, it can be speculated that low PAI-1 activity is an expression of a weak inflammatory potential with the associated increased propensity to bleed.

We conclude that low PAI-1 activity, defined as levels below 1 U mL^{-1}, appears to be a risk factor for bleeding, based on the increased prevalence among the patients referred for investigation of a bleeding diathesis compared with controls. However, the condition is generally mild, not associated with specific bleeding manifestations and does not appear to aggravate the morbidity caused by the common disorders of primary hemostasis.

Addendum

Acknowledgements

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