Antithrombin plasma levels decrease is associated with preeclampsia worsening

M. MARIETTA*, L. SIMONI†, P. PEDRAZZI*, L. FACCHINI*, R. D'AMICO*, F. FACCHINETTI‡

*Section of Haematology, Department of Oncology and Haematology, University of Modena and Reggio Emilia, Modena, Italy
†Department of Clinical Pathology, Ospedale Policlinico, Modena, Italy
‡Mother-Infant Department, Perinatal Section, University of Modena and Reggio Emilia, Modena, Italy

Correspondence:
Marco Marietta, Section of Haematology, Department of Oncology and Haematology, Ospedale Policlinico, via del Pozzo 71, 41100 Modena, Italy. Tel.: +39 059 4224640; Fax: +39 059 4224429;
E-mail: marietta@unimo.it

doi:10.1111/j.1751-553X.2008.01031.x

Received 7 January 2007; accepted for publication 3 May 2007

Keywords
Antithrombin, D-dimer, HELLP syndrome, platelet count, preeclampsia, pregnancy complications

SUMMARY

Antithrombin plasma levels (AT) have been found decreased in women with preeclampsia (PE), but little is known about the trend of AT during the course of this disease. We prospectively investigated AT in consecutive women admitted to our hospital with a diagnosis of PE, to assess if AT fluctuations could be associated with the evolution of the disease. AT, platelet count and D-dimer levels were determined every other day. In the 73 patients studied, AT, platelet count and fibrinogen progressively reduced during the observational period, reaching a nadir on the day of delivery, whereas D-dimer progressively increased over time. Statistical analysis was restricted to the 39 women that had an AT measurement performed on each of days −1, 0 and +1, with respect to the day of delivery. These subjects showed a significant decrease in AT on the day of delivery compared to the day just before (77.8 ± 15.1% *vs.* 85.4 ± 14.2%, $P = 0.027$), followed by a recovery on the first day after delivery (87.6 ± 21.3% from 77.8 ± 15.1%, $P = 0.005$). Our study demonstrates that a significant drop in AT levels is associated with the clinical worsening of PE, regardless of its severity.

INTRODUCTION

Preeclampsia (PE) is defined as high blood pressure associated with proteinuria (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). It occurs in the second half of pregnancy and complicates between 2% and 8% of pregnancies (World Health Organisation International Collaborative Study of Hypertensive Disorders of Pregnancy, 1988). PE can affect maternal organs, leading to systemic and extremely severe complications such as renal failure, hepatic dysfunction, seizures, disseminated intravascular coagulation and haemolysis-elevated liver enzyme/low platelet

(HELLP) syndrome (Robyn, Taylor & Schellemberg, 1998). However, as the placenta is involved, there is also an increased risk of foetal complications, such as foetal growth retardation (FGR), oligohydramnios, cardiotocographic abnormalities and abruptio placenta (AP; Mushambi, Halligan & Williamson, 1996).

Although PE is considered a multifactorial disease, there is a general consensus that a key role in its pathogenesis is played by endothelial damage (Dekker & van Geijn, 1996), which leads to the activation of platelets and the coagulation system. Therefore a characteristic feature of PE is a marked shift of the haemostatic balance towards hypercoagulability, as reflected by increased levels of platelet activation and thrombin generation markers, such as thrombin–antitrombin complex and fibrinopeptides A and B (Greer, 1994).

Antithrombin (AT) levels have been reported to be decreased in pre-eclamptic women, indicating a more or less compensated coagulopathy (Gilabert *et al.*, 1988; Halligan *et al.*, 1994; Paternoster *et al.*, 1994, 1996; Halim *et al.*, 1995; Savelieva *et al.*, 1995; He, Bremme & Blomback, 1997; Kobayashi *et al.*, 2001, 2002; Osmanagaoglu *et al.*, 2005). Most of these reports, however, referred to case–controls studies regarding small samples of women with PE, in whom the decrease in AT levels was a characteristic feature of overt PE. Indeed, the monitoring of AT activity and platelet count in women with PE who exhibit a gradual decline in these parameters may help to predict the development of severe HELLP (Minakami *et al.*, 1999). No study has examined AT levels longitudinally during the course of the disease, and therefore it could not be shown if the reported decrease was either static or dynamic effect. Such information could be of clinical relevance, as an excess hypercoagulable state, highlighted by a more marked fall in AT levels, could be associated with progression of the disease.

The present study aimed at prospectively investigating AT plasma levels in women with PE, to determine: (i) the behaviour of AT and other associated haemostatic parameters, such as fibrinogen, D-dimer and platelet count, during the course of PE; (ii) if fluctuations in AT levels correlate with the severity of the disease.

MATERIALS AND METHODS

The study population consisted of 96 consecutive patients referred to our Institution between 1999 and 2003 with a diagnosis of mild and severe PE according to the National High Blood Pressure Group Criteria (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). Patients were considered for the study if they met the following criteria: diastolic arterial pressure $\geq$90 mmHg and systolic arterial pressure $\geq$140 mmHg on more than two separate occasions and significant proteinuria (>0.3 g/day). Exclusion criteria were chronic hypertension, kidney or heart disorder, congenital AT deficiency and heparin treatment. The study protocol was approved by the Institutional Review Board. Each participant gave written informed consent.

Upon admission, all subjects underwent 24-h Holter blood pressure monitoring and/or two-hourly blood pressure measurements. In a prospective design, each patient was scheduled for blood sampling every other day. Standard haematological and biochemical blood values included coagulation tests, such as AT plasma levels, platelet count and D-dimer determination. Patients were included in the analysis only if they had at least five blood samples taken on the ten days around the delivery. Both timing and modality of delivery was decided on clinical grounds by the Obstetrician. Clinicians participating to the study were not involved in this decision. The decision to induce delivery was based either on maternal (i.e. lack of control of blood pressure, massive proteinuria, onset of HELLP syndrome) or foetal (i.e. pathological cardiotocography, diagnosis of umbilical and diastolic flow, etc.) clinical worsening.

For AT and D-dimer determinations, 3.5 ml of blood were collected by venepuncture with minimal stasis, using a 19-gauge butterfly needle, into 0.109 mol/l trisodium citrate, nine parts blood to one part anticoagulant. Platelet poor plasma was prepared by double centrifugation of samples at 2700 *g* at room temperature for 20 min and immediately analyzed. Plasma AT activity was measured using a standard chromogenic method (Roche Diagnostics S.p.A., Milan, Italy) considering a range of values between 80% and 120% as normal. Fibrinogen levels were determined by a prothrombin time-derived method (Thromborel S; Dade Behring, Malburg, Germany) using a Behring Coagulation System (BCS) coagulometer (Dade Behring) considering a range of values between 1.4 and 4.0 g/l as normal. The blood level of

D-dimer was assessed by an automated quantitative turbidimetric assay (BC D-dimer PLUS; Dade Behring) using a BCS coagulometer (Dade Behring) considering a value <1000 ng/ml as normal for pregnant women. Platelet count was determined by a routine laboratory procedure (NE-8000 Sysmex; Toa Medical Electronics Co. Ltd, Kobe, Japan). Moreover, every patient included in the study underwent a thorough complete screening for either congenital or acquired thrombophilia at least 60 days after pregnancy, including a search for antiphospholipid antibodies, APC resistance, factor V R506Q and prothrombin G20210A mutations, protein C and S levels and fasting homocysteine level measured by fluorescence polarization immunoassay (FPIA), as previously described (Pabinger *et al.*, 1992; Rosen *et al.*, 1994; Brandt *et al.*, 1995; Kirschbaum & Foster, 1995; Poort *et al.*, 1996; Blanco-Vaca *et al.*, 2000).

Values are reported as mean ± standard deviation (SD); data were compared using the Statistical Package for the Social Sciences (SPSS). Analysis of variance (ANOVA) for within-subjects comparison was applied for continuous variables. Chi-square was used in the case of categorical variables. P-values < 0.05 were considered significant.

RESULTS

Twenty-three of the 96 consecutive women were excluded from the analysis because they did not have the required number of blood samples available. The mean age of the remaining 73 patients considered for the analysis was 31.3 ± 5.4 years (range 19–42); 28 women had mild PE, and the others had severe PE. Among the severe PE patients, 11 had HELLP syndrome, 12 FGR and two abruptio placentae (AP). PE was diagnosed at a mean gestational age of 226 ± 22 days (range 161–252); whereas the mean gestational age at delivery was 232 ± 21 days (range 167–261). Clinical features of study population are reported in Table 1, whereas the indications to delivery are reported in Table 2.

Tables 3 and 4 show the chronological trend of haemostatic variables around the time of delivery. AT (Table 3) progressively reduced during the course of the disease, reaching a nadir on the day of delivery and already recovering the first day postpartum. This pattern was similar among the three subgroups of patients (ANOVA). The platelet count (Table 4) showed a similar trend, although the minimum value was observed in every group of patients on the first day after delivery. This trend was more pronounced, as expected, in HELLP patients. Fibrinogen concentration (Table 4) did not show significant variations, although a small decrease was observed on the day of delivery; on the other hand, D-dimer concentration (Table 4) progressively increased over time, even in the first days after delivery. The temporal trend of the coagulation parameters was unrelated to the severity of PE.

Statistical analysis was restricted to 39 women having had AT measurement performed on each of days −1, 0 and +1 with respect to the day of delivery, to determine a possible trend (Figure 1). Sampling at day 0 was performed at least 3 h before parturition. In this population AT levels significantly decreased on the

Table 1. Clinical features of study population (N = 73)

Age (years)	31.3 ± 5.4 (19–42)
Caucasian (N)	57
Black (N)	16
Inherited thrombophilia	10
Heterozygous Factor V Leiden mutation	6
Heterozygous PT 20210 A mutation	4
Gestational age at delivery (days)	232 ± 21 (167–261)
Birth-weight (kg)	1.732 ± 0.779 (0.240–3.890)
Placental weight (g)	407 ± 168 (110–950)
Perinatal deaths (N)	1
Elective caesarean section (N)	62
Induced labour and delivery	11
Emergency caesarean section	6

Table 2. Indications for delivery

	N (%)
Increased hypertension and/or proteinuria	48 (66)
HELLP Syndrome	11 (15)
Foetal growth restriction (either foetal blood flow worsening and/or nonreassuring foetal heart rate)	12 (16)
Abruptio placentae	2 (3)

Table 3. Antithrombin levels (%) around time of delivery according to preeclampsia severity

PE severity	Days before −1	Day −1	Day of delivery	Day +1	Days after +1
Mild-to-moderate PE	97 ± 15	91 ± 18	85 ± 17	85 ± 12	90 ± 15
Severe PE	95 ± 10	83 ± 14	79 ± 19	92 ± 13	93 ± 11
HELLP syndrome	95 ± 17	83 ± 15	80 ± 18	86 ± 28	89 ± 15

All data are expressed as mean ± SD.

Table 4. Haemostatic parameters around time of delivery according to preeclampsia severity

Haemostatic parameter	PE severity	Days before −1*	Day −1	Day of delivery	Day +1	Days after +1†
Platelets (10^9/l)	Mild-to-moderate PE	162 ± 56	191 ± 63	176 ± 74	181 ± 70	166 ± 71
	Severe PE	175 ± 47	157 ± 33	143 ± 43	142 ± 47	135 ± 58
	HELLP syndrome	154 ± 39	109 ± 34	105 ± 49	76 ± 37	78 ± 48
Fibrinogen (g/l)	Mild-to-moderate PE	4.27 ± 0.74	4.39 ± 1.13	3.82 ± 1.11	3.85 ± 1.01	4.74 ± 1.76
	Severe PE	3.41 ± 0.8	4.06 ± 0.98	3.81 ± 1.15	3.87 ± 0.9	4.72 ± 1.36
	HELLP syndrome	3.19 ± 0.73	3.94 ± 1.25	4.02 ± 1.23	4.1 ± 1.17	4.19 ± 0.9
D-dimer (ng/ml)	Mild-to-moderate PE	1479 ± 903	1408 ± 761	3887 ± 4878	5275 ± 4372	6515 ± 8289
	Severe PE	2205 ± 870	2294 ± 1255	7793 ± 1435	5142 ± 5909	9515 ± 8505
	HELLP syndrome	2173 ± 903	5133 ± 1199	8042 ± 7450	11511 ± 1546	10609 ± 2312

All data are expressed as mean ± SD.
*Days before −1 refer to one sample collected either at day −5 or −4 or −3.
†Days after +1 refer to one sample collected either at day +3 or +4 or +5.

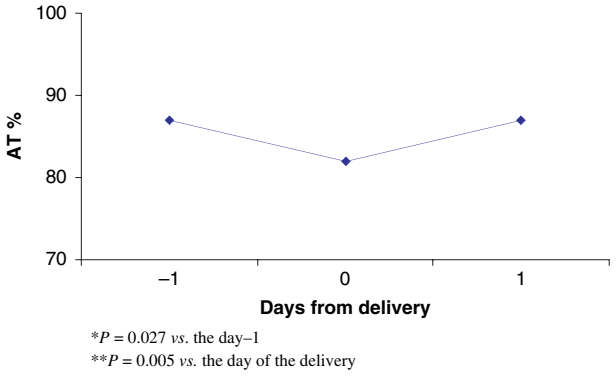

Figure 1. Chronological trend of plasma antithrombin levels in the days around delivery.*$P = 0.027$ *vs.* day −1 **$P = 0.005$ *vs.* the day of delivery.

day of delivery compared with the day just before (77.8 ± 15.1% *vs.* 85.4 ± 14.2, $P = 0.027$). After the delivery, a significant increase in AT levels was observed (87.6 ± 21.3% from 77.8 ± 15.1%, $P = 0.005$).

Among the study population six subjects carried the Factor V Leiden and four were heterozygous for the PT G2010A mutation; no other acquired or inherited thrombophilic conditions were identified. No differences were found in temporal trend of AT plasma levels among thrombophilic and nonthrombophilic subjects; moreover, in the two cases that had AP the peripartal changes in AT levels were similar to those described for the other groups of PE women.

DISCUSSION

A significant reduction in AT plasma levels has already been associated with PE (Halligan *et al.*, 1994; Paternoster *et al.*, 1994, 1996; Halim *et al.*, 1995; Savelieva *et al.*, 1995; He, Bremme & Blomback, 1997; Minakami *et al.*, 1999; Kobayashi *et al.*, 2001, 2002; Osmanagaoglu *et al.*, 2005) and some authors have suggested that the assessment of this parameter could assist in differentiating PE from other forms of gestational hypertension, in which AT

is unchanged (De Boer *et al.*, 1989; Shanklin & Sibai, 1989; Lockwood & Peters, 1990). However, little is known about the trend of AT levels during the course of PE, because most of the available data have arisen from case–controls studies in which AT levels were measured only at hospitalization for PE. The design of the only study in which coagulation parameters were longitudinally evaluated in PE women (Savelieva *et al.*, 1995) was quite different from the present one because it was a case–control, retrospective study including women with overt PE as well as those at risk for developing PE. Moreover, the scheduled timing for blood sampling was every other week, starting from the 10th gestational week, and therefore did not provide information regarding the trend of coagulation parameters in the period close to parturition.

Our study demonstrated that a significant drop in AT levels was associated with the clinical worsening of PE leading to the need for delivery. Of paramount interest, this phenomenon was present regardless of PE severity. AT decrease was also associated with a significant reduction in both platelet count and fibrinogen levels, whereas D-dimer fluctuations were not statistically significant. Interestingly, only AT levels recovered to the predelivery values on the first day after delivery, whereas all the other coagulation parameters recovery occurred only after some days.

This finding has some relevant implications. First, from a pathophysiological point of view, it further supports the hypothesis that the decrease in AT levels in PE women reflects an excess hypercoagulable state that is probably both cause and effect of the disease. However, not every coagulation factor carries the same relevance, and our data suggest that the leading role is played by AT. Indeed its temporal trend exactly paralleled the clinical course of the disease, as it was the only coagulation parameter to recover immediately after parturition. Moreover, as there is increasing evidence that PE is associated with a systemic inflammatory response (Redwan, Sacks & Sargent, 1999), it could be argued that AT be involved in this disease not only because of antithrombotic, but also of anti-inflammatory properties (Uchiba *et al.*, 2004).

These observations strengthen the pathophysiological background for interventional trials that utilized AT concentrates in the treatment of severe PE (Terao *et al.*, 1989; Maki *et al.*, 2000; Paternoster *et al.*, 2004).

Finally, the observation that a drop in AT plasma levels was associated with clinical worsening of PE maintains the clinical relevance of evaluating coagulation and fibrinolytic factors peripartally and suggest that this parameter could become an additional tool for establishing the optimal time of delivery. Such a working hypothesis, although interesting, needs confirmation in further properly designed studies.

REFERENCES

Blanco-Vaca F., Arcelus R., Gonzalez-Sastre F., Ordonez-Llanos J. & Queralto-Compano J.M. (2000) Comparison of the Abbott IMx and a high-performance liquid chromatography for measuring total plasma homocysteine. Clinical Chemistry and Laboratory Medicine 38, 327–329.

Brandt J.T., Triplett D.A., Alving B. & Scharrer I. (1995) Criteria for the diagnosis of lupus anticoagulants: a update. Thrombosis and Hemostasis 74, 1185–1190.

De Boer K., Ten Cate J.W., Sturk A., Borm J.J.J. & Treffers P.E. (1989) Enhanced thrombin generation in normal and hypertensive pregnancy. American Journal of Obstetrics and Gynecology 160, 95–100.

Dekker G.A. & van Geijn H.P. (1996) Endothelial dysfunction in preeclampsia. Part I: primary prevention. Therapeutic perspectives. Journal of Perinatal Medicine 24, 99–117.

Gilabert J., Fernandez J.A., España F., Aznar J. & Estelles A. (1988) Physiological coagulation inhibitors (protein S, protein C and antithrombin III) in severe preeclamptic states and in user of oral contraceptives. Thrombosis Research 49, 319–329.

Greer I.A. (1994) Haemostasis and thrombosis in pregnancy. In: Haemostasis and Thrombosis (eds A. Bloom, C.D. Forbes & E.G.D. Tuddenham), pp. 412. Churchill Livingstone, Edinburgh.

Halim A., Bhuiyan A.B., Azim F.A., Khatun S., Kanayama N., el Maradny E., Maehara K., Hiroshi K. & Terao T. (1995) Blood coagulation and fibrinolysis in eclamptic patients and their correlation with the clinical signs. Gynecologic and Obstetric Investigation 39, 97–102.

Halligan A., Bonnar J., Sheppard B., Darling M. & Walshe J. (1994) Haemostatic, fibrinolytic and endothelial variables in normal pregnancies and pre-eclampsia. British Journal of Obstetrics and Gynaecology 101, 488–492.

He S., Bremme K. & Blomback M. (1997) Acquired deficiency of antithrombin in association with a hypercoagulable state and impaired function of liver and/or kidney in preeclampsia. Blood Coagulation & Fibrinolysis 8, 232–238.

Kirschbaum N.E. & Foster P.A. (1995) The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance. Thrombosis and Hemostasis 74, 874–878.

Kobayashi T., Tokunaga N., Sugimura M., Kanayama N. & Terao T. (2001) Predictive values of coagulation/fibrinolysis parameters for the termination of pregnancy complicated by severe pre-eclampsia. Seminars in Thrombosis and Hemostasis 27, 137–141.

Kobayashi T., Sumimoto K., Tokunaga N., Sugimura M., Nishiguchi T., Kanayama N. & Terao T. (2002) Coagulation index to distinguish severe preeclampsia from normal pregnancy. Seminars in Thrombosis and Hemostasis 28, 495–500.

Lockwood C.J. & Peters J.H. (1990) Increased plasma levels of ED + cellular fibronectin precedes the clinical signs of pre-eclampsia. American Journal of Obstetrics and Gynecology 162, 358–362.

Maki M., Kobayashi T., Terao T., Ikenoue T., Satoh K., Nkabayashi M., Sagara Y., Kajiwara Y. & Urata M. (2000) Antithrombin therapy for severe preeclampsia. Thrombosis and Hemostasis 84, 583–590.

Minakami H., Watanabe T., Izumi A., Matsubara S., Koike T., Sayama M., Moriyama I. & Sato I. (1999) Association of a decrease in antithrombin III activity with a perinatal elevation in aspartate aminotransferase in women with twin pregnancies: relevance to the HELLP syndrome. Journal of Hepatology 30, 603–611.

Mushambi M.C., Halligan A.W. & Williamson K. (1996) Recent developments in the pathophysiology and management of pre-eclampsia. British Journal of Anaesthesia 76, 133–148.

National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (2000) Report of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. American Journal of Obstetrics and Gynecology 183, S1–S22.

Osmanagaoglu M.A., Topcuoglu K., Ozeren M. & Bozkaya H. (2005) Coagulation inhibitors in preeclamptic pregnant women. Archives of Gynecology and Obstetrics 271, 227–230.

Pabinger I., Brüker S., Kyrle P.A., Schneider B., Korninger H.C., Niessner H. & Lechner K. (1992) Hereditary deficiency of venous antithrombin III, protein C and protein S: prevalence in patients with a history of thrombosis and criteria for rational patient screening. Blood Coagulation & Fibrinolysis 3, 547–553.

Paternoster D., Stella A., Simioni P., Trovo S., Plebani P. & Girolami A. (1994) Clotting inhibitors and fibronectin as potential markers in preeclampsia. International Journal of Gynaecology and Obstetrics 47, 215–221.

Paternoster D.M., Stella A., Simioni P., Girolami A. & Plebani M. (1996) Fibronectin and antithrombin as markers of pre-eclampsia in pregnancy. European Journal of Obstetrics, Gynecology, and Reproductive Biology 70, 33–39.

Paternoster D.M., Fantinato S., Manganelli F., Milani M., Nicolini U. & Girolami A. (2004) Efficay of AT in pre-eclampsia: a case–control prospective trial. Thrombosis and Hemostasis 91, 283–289.

Poort S.R., Rosendaal F.R., Reitsma P.H. & Bertina R.M. (1996) A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and increase in venous thrombosis. Blood 10, 3698–3703.

Redwan C.W., Sacks G.P. & Sargent I.L. (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. American Journal of Obstetrics and Gynecology 180, 499–506.

Robyn A.N., Taylor R.S. & Schellemberg J.C. (1998) Evaluation of a definition of preeclampsia. British Journal of Obstetrics and Gynaecology 106, 767–773.

Rosen S., Johansson K., Lindberg K. & Dahlback B. (1994) Multicentre evaluation of a kit for activated protein C resistance on various coagulation instruments using plasmas from healthy individuals. Thrombosis and Hemostasis 72, 255–260.

Savelieva G.M., Efimov V.S., Grishin V.L., Shalina R.I. & Kashezheva A.Z. (1995) Blood coagulation changes in pregnant women at risk of developing preeclampsia. International Journal of Gynaecology and Obstetrics 48, 3–8.

Shanklin D.R. & Sibai B.M. (1989) Ultrastructural aspects of pre-eclampsia: I. placental bed and uterine boundary vessels. American Journal of Obstetrics and Gynecology 161, 735–741.

Terao T., Kobayashi T., Imai N., Oda H. & Karasawa T. (1989) Pathological state of the coagulatory and fibrinolytic system in preeclapsia and the possibility of its treatment with AT concentrate. Asia Oceania International Journal of Obstetrics and Gynaecology 15, 25–32.

Uchiba M., Okajima K., Kaun C., Wojta J. & Binder B.R. (2004) Inhibition of the endotelial cell activation by antithrombin *in vitro*. Thrombosis and Hemostasis 92, 1420–1427.

World Health Organisation International Collaborative Study of Hypertensive Disorders of Pregnancy. (1988) Geographic variation in the incidence of hypertension in pregnancy. American Journal of Obstetrics and Gynecology 158, 80–83.