

# A comparative study of the protein C system in mother's blood, cord blood and amniotic fluid

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**Abstract:** Activated protein C (APC) is an important anticoagulant which plays a role in pathophysiology of pregnancy, *e.g.* in maintenance of the uteroplacental circulation and development of the fetus as well as in pathogenesis of preeclampsia. The study objective was to compare the levels of the respective components of the protein C system (protein C, PC; protein S, PS; thrombomodulin, TM) as well as thrombin activatable fibrinolysis inhibitor – TAFI in mother's blood, cord blood and amniotic fluid. The study group consisted of 136 healthy parturients at term, divided into subgroups of 30-35. The immunoenzymatic method (ELISA) was used to measure the antigens of the components studied. The concentrations of PC and PS antigens were the highest in the mother's blood plasma ( $135.11 \pm 1.05\%$  and  $92.0 \pm 13.24\%$ , respectively), lower in cord blood plasma ( $57.60 \pm 10.32\%$  and  $33.19 \pm 4.96\%$ , respectively) and the lowest in amniotic fluid ( $6.75 \pm 3.50\%$  and  $2.40 \pm 1.64\%$ , respectively); the differences between the levels of that of mother, fetus and amniotic fluid were statistically significant ( $p \leq 0.0001$ ). The TM and TAFI antigen concentrations were the highest in cord blood plasma ( $11.35 \pm 3.71$  ng/ml and  $91.50$  (median; range:  $71.76-160.77$ ) ng/ml, respectively) and lower in maternal plasma ( $4.51 \pm 0.71$  ng/ml and  $55.46$  – median; range:  $39.77-68.54$  ng/ml, respectively); the differences between the levels of that of cord blood plasma and maternal plasma were statistically significant ( $p \leq 0.0001$ ). Of the three protein C system components, PC and PS occur in relatively high concentrations in maternal blood, being lower in fetal blood and the lowest in amniotic fluid. On the other hand, as an exception, the concentrations of TM and TAFI are the highest in fetus blood.

**Key words:**

## Introduction

Activated protein C (APC) is an anticoagulant, resembling serine enzyme, which is generated from protein C (PC) under the effect of the thrombin/thrombomodulin (TM) complex, with the involvement of protein S (PS) as a co-factor. Researchers frequently refer to the protein C system or protein C anticoagulant pathway. A further component of the protein C anticoagulant pathway is the endothelial protein C receptor (EPCR)

that augments protein C activation and potentiates the activity of APC. Moreover, the protein C system contains protein C inhibitor (PCI).

Proteins C and S occur in the plasma. These vitamin K-dependent proteins are synthesized in the liver. TM is synthesized within endothelial cells and expressed on endothelial surface (cellular TM) or found outside the cells as soluble TM (sTM). TM is a thrombin receptor and forms TM/thrombin complexes, which convert non-active protein C (zymogen) into its highly active form (APC).

It is believed that APC is the most important vaso-protective protein due to its anti-thrombotic and anti-inflammatory as well as anti-apoptotic properties. In the course of spectacular interactions taking place in

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the protein C system, thrombin changes its procoagulant activity into anticoagulant activity, and activation of the proteinase-activated receptor 1 (PAR-1) occurs, being a link between coagulation and inflammation responses. APC degrades clotting factors Va and VIIIa in tenase and prothrombinase complexes, in this way attenuating thrombin generation, and in addition it transforms pro-TAFI into TAFI (Thrombin Activatable Fibrinolysis Inhibitor) [1,2] (Fig. 1).

Due to high TM level in the placenta and myometrium [3] as well as to the placental concentration of EPCR receptors, APC is probably the major anticoagulant of the uteroplacental circulation potentiating the antithrombotic effect of APC [4,5]. Also in general circulation, APC plays a role of an important anticoagulant – the second after antithrombin (AT) or even the first one, making up the antithrombotic activity of the vascular wall. In a pregnant woman, a decrease in vascular antithrombotic activity (a drop in AT and APC) may lead to preeclampsia (assumption of a preeclampsia hypothesis [6]). According to diabetologists, the pathogenesis of diabetic nephropathy indicates a pathogenetic relationship with reduced APC activity in the kidney (a direct effect of TM deficiency in glomerular endothelium) [7].

Although relatively much has been known about APC function in the blood of pregnant women, the knowledge of APC in fetal blood and amniotic fluid is still preliminary, perhaps due to the fact that the data concerning the concentrations of this system components are still incomplete.

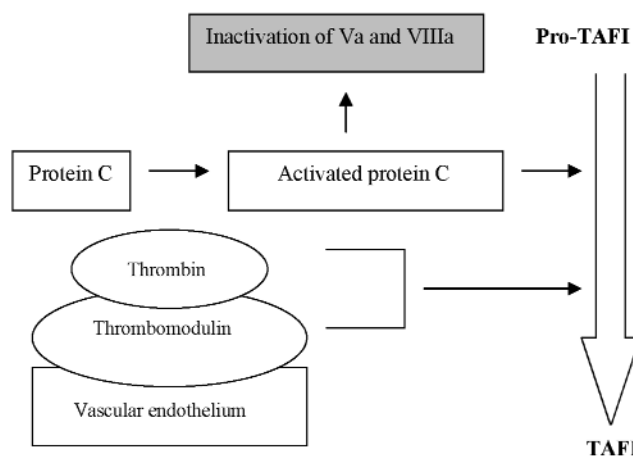
The objective of the current study was to compare the levels of proteins C and S and thrombomodulin (TM) in three fluid compartments of the mother and the fetus, i.e. in maternal blood, in fetal blood (cord blood) and amniotic fluid, as well as TAFI concentrations in these compartments.

## Material and methods

**Patients.** The material sample included 136 parturients at term, 22.1±3.2 years of age, 88 primiparas and 48 multiparas, with a singleton and uneventful pregnancy (complicated cases were excluded from the analysis). To study the components of the protein C system: protein C, protein S, thrombomodulin and TAFI the women were divided into groups of 30-35. Each group had a control counterpart (15-20 non-pregnant healthy women, on day 18-26 of menstrual cycle, age-matched with the study group).

**Sampling of mother's blood, cord blood and amniotic fluid.** The materials were collected immediately after delivery of the fetus, before placenta expulsion and before clumping of the umbilical cord.

Maternal venous blood was obtained by puncturing the antecubital vein without occlusion. Sodium citrate (3.2%) was used as anticoagulant (one part of anticoagulant to nine parts of blood). Control blood was obtained in a similar way from the control group. Cord blood was obtained by puncturing the umbilical vein.



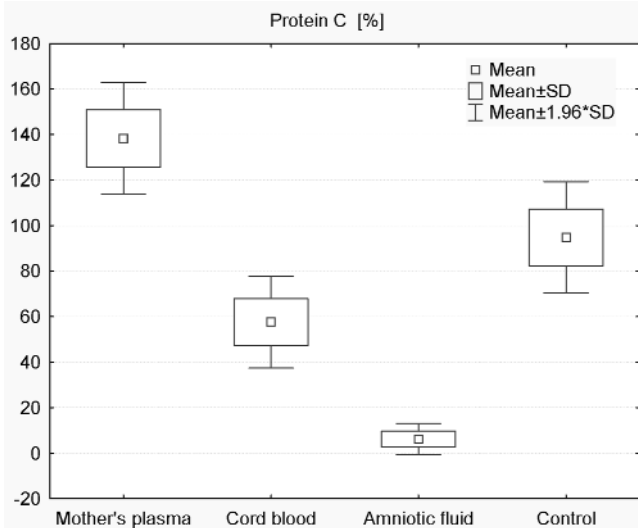
**Fig. 1.** Protein C pathway: 1) generation of activated protein C (APC) under the effect of the thrombin/thrombomodulin complex, 2) activity of APC: (a) inactivation of Va and VIII a factors, (b) generation of TAFI (thrombin activatable fibrinolysis inhibitor).

Sodium citrate in the same concentration and the anticoagulant/blood ratio as in the mother were used. Amniotic fluid was collected from the waters that were flowing out from the uterus after fetus delivery (fluid contaminated with blood was excluded from analysis). The syringe used to collect the fluid contained the same amount of sodium citrate as for blood collection. All the samples (mother's blood, cord blood, amniotic fluid, control blood) were placed into ice water, transported to the laboratory and then centrifuged (2500 × g for 20 min at +4°C); 200 µl portions of plasma and the supernatant of amniotic fluid were transferred into plastic test-tubes, which were tightly closed and stored for 2-3 weeks at -70°C.

**Laboratory measurements.** The concentrations of protein C (PC), protein S (PS) and soluble thrombomodulin (sTM) were determined by the immunoenzymatic method (ELISA), using respectively ASSERACHROME Protein C, ASSERACHROME Protein S from Diagnostica Stago Roche, and IMUBIND Thrombomodulin ELISA Kit of American Diagnostica. The reference values for adults (plasma) were: protein C – 70-140%, protein S – 70-140%, thrombomodulin in the plasma of non-pregnant women from 2.73 ng/ml (21-30 years) to 4.79 ng/ml (61-70 years). Thrombin activatable fibrinolysis inhibitor (TAFI) antigen was measured also by ELISA, using IMUBIND TAFI a/ai Antigen ELISA kit from American Diagnostica Inc., which measures TAFIa and TAFIai (inactive TAFIa). The lower limit of TAFI antigen detection recommended by the manufacturer is 10 ng/ml. In our laboratory, the inter-assay and intra-assay coefficients of variability were lower than 10%. The level of protein (total protein) was measured by BCA method.

**Ethical issues.** All patients were informed about the research and they gave informed consent for sampling of the material.

**Statistical analysis.** The results are presented as mean values with standard deviation for normally distributed variables (PC, PS and TM), and as median and range for those different from normally distributed variables (TAFI). Statistical analysis was performed using two tests: (i) the ANOVA Friedman's test for the comparison of paired data (mother's plasma vs. cord blood, plasma or amniotic fluid; (ii) non-parametric Mann-Whitney and Wilcoxon tests for evaluation of unpaired data (pregnant vs. non-pregnant). The significance limit was chosen at p value ≤0.05.



**Fig. 2.** The concentration of protein C antigen in maternal blood plasma, cord blood plasma and in amniotic fluid.

## Results

### Protein C

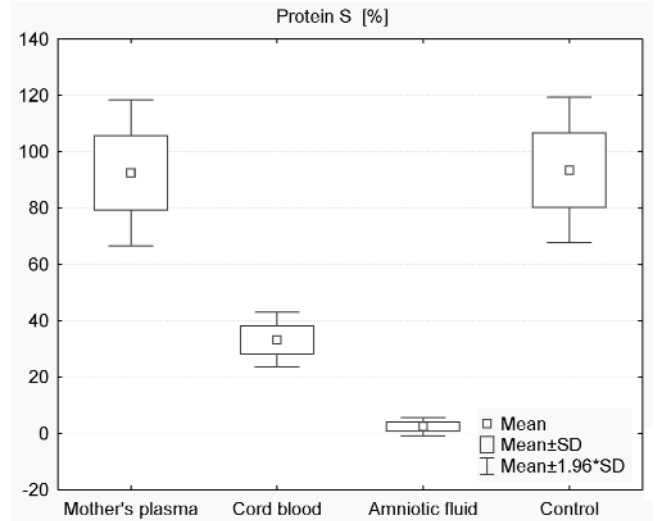
The concentration of protein C antigen in mother's plasma was  $135.11 \pm 10.32\%$ , whereas in cord plasma  $57.60 \pm 10.32\%$ , thus it was lower by over a half (42.67%) in the fetus as compared to the mother ( $p \leq 0.0001$ ). In the amniotic fluid, the level was  $6.75 \pm 3.50\%$ , i.e. only a fraction of the values found in the mother and fetus (4.99% and 11.72%, respectively) ( $p \leq 0.0001$ ). In control (non-pregnant women), the level was  $94.78 \pm 12.46\%$  (Fig. 2).

### Protein S

The level of total protein S in mother's plasma was  $92.49 \pm 13.24\%$ , whereas in cord plasma  $33.19 \pm 4.96\%$ , thus being almost threefold lower (35.88%) in the fetus than in the mother ( $p \leq 0.0001$ ). In amniotic fluid, it was  $2.40 \pm 1.64\%$ , i.e. only a fraction of the concentrations detected in the mother and fetus (2.59% and 7.23%, respectively). In control (non-pregnant women), the level was  $93.47 \pm 8.36\%$  (Fig. 3).

### Thrombomodulin

The level of TM antigen in mother's plasma was  $4.51 \pm 0.71$  ng/ml and in cord plasma  $11.35 \pm 3.71$  ng/ml, i.e. it was almost three times higher in the fetus than in the mother (251.66% of the value found in the mother) ( $p \leq 0.0001$ ). In amniotic fluid, the level of TM was  $2.71 \pm 1.21$  ng/ml, which accounted for 60.08% of the maternal concentration value ( $p \leq 0.018$ ). In control (non-pregnant women), it was  $3.86 \pm 0.92$  ng/ml (Fig. 4).



**Fig. 3.** The level of total protein S in maternal plasma, cord blood plasma and in amniotic fluid.

### TAFI

The level of TAFI antigen was  $55.46$  (39.77-68.54) ng/ml in the mother's plasma and  $91.50$  (71.76-160.77) ng/ml in cord plasma, thus being nearly twice as high in the fetus than in the mother (164.98% of the mother's level) ( $p \leq 0.0001$ ). However, in amniotic fluid it was similar to that in the mother's plasma ( $p \geq 0.3389$ ). In control (non-pregnant women), the concentration was  $72.55$  (67.50-76.69) ng/ml (Fig. 5).

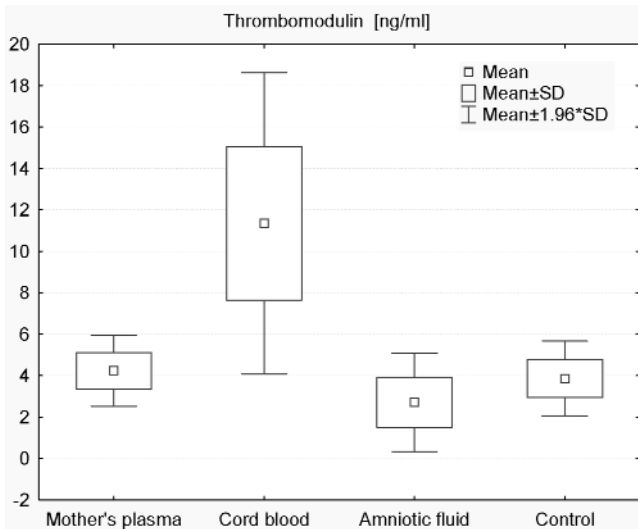
### Total protein

Total protein in mother's plasma was  $83.27 \pm 11.54$  mg/ml, in cord blood plasma was  $85.82 \pm 21.87$  mg/ml, and  $2.96 \pm 1.05$  mg/ml in amniotic fluid.

## Discussion

APC, an anticoagulant with the activity of the serine enzyme is generated in the dynamic system called the protein C system or protein C anticoagulant pathway. The major components of this system are PC, PS and TM, in which PC undergoes conversion into APC under the influence of the thrombin/thrombomodulin complex.

We knew about the presence of PC, PS and TM as well as TAFI – one of the APC activity products – in fluid compartments of the mother and fetus from earlier investigations performed by our team [9,10], or from studies carried out by other researchers [11-14]. In the current study, we examined the levels of the four above mentioned antigens simultaneously in all the three compartments, which allowed making comparisons. Our results are either similar [11] or compatible with the studies mentioned above. However, altogether the studies should be treated as preliminary.

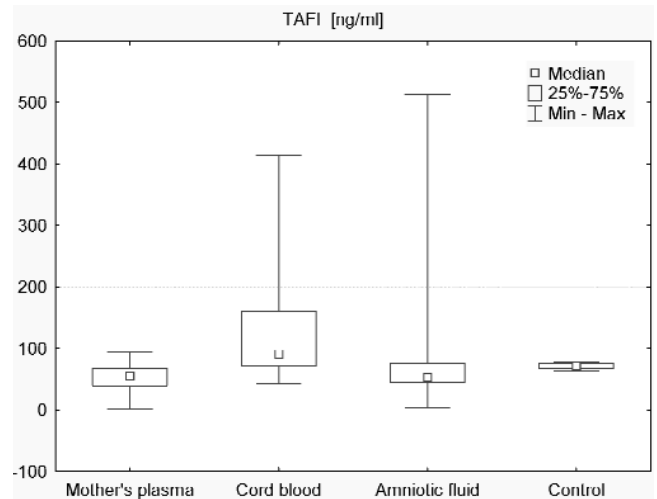


**Fig. 4.** The level of thrombomodulin (TM) antigen in maternal plasma, cord blood plasma and in amniotic fluid.

It is known that the fluid compartments of the mother and the fetus are functionally different and at the same time related, which shows the anatomical and functional interrelationship of the fetus and the mother, referred to as the fetal-maternal unity.

The concentration of proteins is one of the distinctive features of these compartments. The so called placental barrier hinders protein permeation through the placenta and fetal membranes both from the mother to the fetus (permeation of maternal IgM antibodies to the fetus is a known exception) and in the opposite direction. Because of this, the levels of the respective proteins in maternal and fetal blood provide evidence of their synthesis in these compartments. Our findings do not deny the previously accepted rules that: (i) the levels of total protein and coagulation proteins are higher in maternal blood than in fetal blood (rule 1); (ii) the levels of total protein and coagulation proteins in amniotic fluid are lower than in maternal blood and in fetal blood (rule 2). However, we have found a few exceptions to these, noting that the level of TM is more than twofold higher in fetal than in maternal blood and that the level of TAFI is nearly twice as high. These two observations require a comment.

Two other teams examined the level of TM in fetal blood [13,14]. According to Menashi *et al.* [13], between week 23 and week 26 of gestation the level of TM reached a peak of approximately 165 ng/ml, being 108 ng/ml of plasma at term delivery; according to Orbe *et al.* [14], TM concentration at term delivery was  $53.6 \pm 16.4$  ng/ml. We found this concentration to be  $11.35 \pm 3.71$  ng/ml at term delivery, and twofold higher than in mother's blood. The results appear to vary, which is undoubtedly due to the application of different reagents and methods of measurement. Nev-



**Fig. 5.** The level of thrombin activatable fibrinolysis inhibitor (TAFI) in maternal plasma, cord blood plasma and in amniotic fluid

ertheless, these investigations provide evidence of a relatively high TM level in the fetus. Worthy of note is also the fact that the cited authors made no comparison with adult plasma, which has been a research requirement since 1991 [8].

The role of TM in the fetus can be diverse: (i) TM can up- or down-regulate APC production depending on the levels of TAFI and active fraction of PS, as reported in literature [15-18]; (ii) the premises of the studies conducted on the animal model (mice) [19,20] suggest that TM exerts not only a hemostatic effect but also has an essential impact on fetal development (ontogenetic effect); (iii) it can be assumed that TAFI is produced more intensively in fetal than in maternal blood and is associated with APC activity or the factors/products of the protein C system. Undoubtedly, further studies are necessary to elucidate the problem.

## Conclusions

Protein C (PC), protein S (PS) and thrombomodulin (TM) – the proteins of the protein C anticoagulant pathway – as well as thrombin activatable fibrinolysis inhibitor (TAFI) – the product of activated protein C (APC) – are present in mother's blood, cord blood and in amniotic fluid. There are exceptions to the rule according to which the level of procoagulants and anticoagulants in fetal blood is lower than in mother's blood: it has turned out that the levels of TM and TAFI are higher in fetal than in maternal blood. According to literature survey, certain components of the protein C system (TM) may have an effect on the development of the fetus (ontogenetic effect).

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Submitted: 25 September, 2009

Accepted after reviews: 15 January, 2010