

Procoagulants and anticoagulants in fetal blood. A literature survey

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Abstract: In intrauterine life, hemostasis is maintained by the same components as in extrauterine life (blood platelets, coagulation and fibrinolysis systems, involvement of the vascular wall); in the fetus, however, these components show significant differences of a quantitative/qualitative nature. In the present study, we surveyed the literature on the coagulation system in the fetus. We focused on the velocity of development of the coagulation system, being reflected in the increased concentration of all procoagulants and anticoagulants (a rise from approximately 20% in the middle of pregnancy to about 60% or more in the period of labor; exceptions: factors V, VIII and XIII which in the labor period reach the adult level) and screening test results (prothrombin time, aPTT - activated prothrombin time, and thrombin time). Reference values were given for the 19-38 weeks of pregnancy and the labor term. Biochemical features of fetal fibrinogen and PIVKA factors were also discussed. The role of activated protein C (APC) in the maintenance of balance between procoagulants and anticoagulants was postulated as well as the role of APC in the formation of thrombin activatable fibrinolysis inhibitor (TAFI).

Key words: procoagulants, anticoagulants, fetus, cord blood

Introduction

According to many authors, the hemostatic system in the fetus has unique features. The distinctions do not only concern differences in the concentrations of hemostatic components, but also in the ability to generate and inhibit thrombin, as compared to adults. Two diagnostic methods that have been developed in the last two decades allow further growth of knowledge on fetal hemostasis, particularly on the coagulation and fibrinolytic systems. Thus, sampling the cord blood during pregnancy (cordocentesis) enables investigation of the fetal coagulation system throughout intrauterine life and due to the micromethods, even tiny samples can be used for testing. In Poland, T. Wyrzykiewicz and M. Kurzawa developed their own micromethods for neonatological purposes as early as in 1981 [1]).

The new methodological possibilities coincided with a negative assessment of then-current analyses of

hemostasis in the fetus and infant. In 1991, the Subcommittee on Neonatal Hemostasis of the Scientific and Standardization Committee (abbreviation of the earlier name: ICTH) informed that the analyses carried out until then had had numerous methodological errors (heterogeneity of the material and methods, other inadvertences) and therefore had to be considered not fully reliable [2].

The well known components of hemostasis include (i) the coagulation and fibrinolysis systems, (ii) blood platelets, and (iii) vascular endothelium. The formation of a hemostatic plug occurs due to interactions between these components. Procoagulants and anticoagulants contribute to the generation of thrombin, the key coagulation enzyme.

The aim of the following article is to provide a survey of the literature concerning procoagulants and anticoagulants in the blood of the fetus, particularly changes in their concentration during pregnancy, to present selected referential values that have been established in the last decade and to discuss some mechanisms of interactions between these components.

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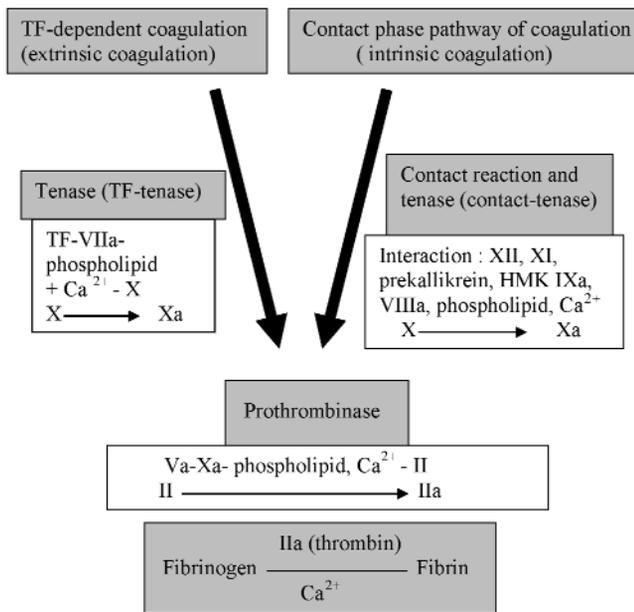


Fig. 1. Basic mechanism of coagulation cascade: action of the tenase and prothrombinase complexes and thrombin formation. Abbreviations: TF – tissue factor, HMK – high molecular weight kininogen.

Definition and a list of procoagulants and anticoagulants

The term procoagulants pertains to all coagulation factors (procoagulants *sensu stricto*) marked with Roman numerals (I-III, V, VII-XIII) and two coagulation factors without such numerals (prekallikrein and high molecular weight kininogen - HMWK). It is also used with reference to phospholipids as an indispensable component of the coagulation process (involved in the formation of two types of the tenase complex - VIIIa-IXa-X+phospholipid; TF-VIIa-X+phospholipid, and the prothrombinase complex: Va-Xa-II+phospholipid).

The coagulation factors include a group of the contact phase factors (factors XI,XII, kallikrein and HMWK), a group of vitamin K-dependent procoagulants (factors II, VII, IX and X) and a group consisting of fibrinogen, factor V, factor VIII, and factor XIII; tissue factor (TF) (factor III, earlier called tissue thromboplastine) is not considered as belonging to any of these groups. TF and contact phase factors are responsible for the initiation of coagulation (or more precisely for the initiation of thrombin generation), called TF-dependent or TF-independent coagulation (contact phase coagulation), respectively.

For decades, the interaction of the coagulation factors was presented as the cascade system. The classic cascade schema was later supplemented with information about the role of these factors in complexes (two tenase complexes and a prothrombin complex (Fig.1). The above mentioned interaction results in the genera-

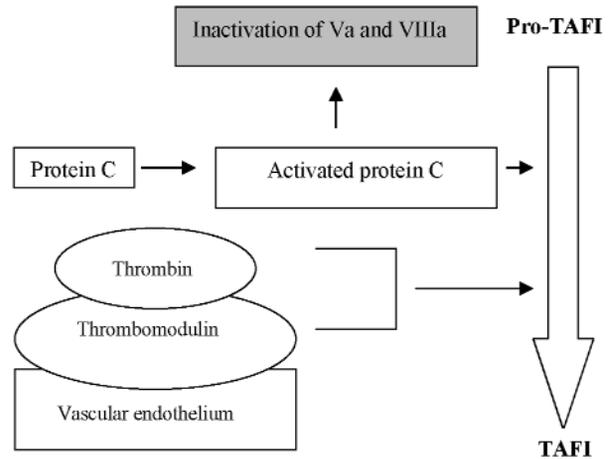


Fig. 2. Protein C pathway: generation of activated protein C (APC) under the effect of the thrombin/thrombomodulin complex; activity of APC: (a) inactivation of Va and VIIIa factors, (b) generation of TAFI (thrombin activatable fibrinolysis inhibitor).

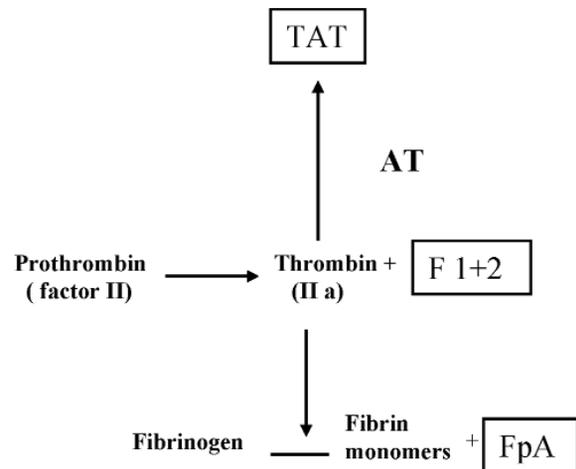


Fig. 3. A fragment of the coagulation cascade illustrating the formation of three markers of thrombin generation: thrombin-antithrombin complexes (TAT), prothrombin fragments 1+2 (F1+2) and fibrinopeptides A (FpA).

tion of thrombin, the coagulation enzyme that can transform fibrinogen into fibrin, activate blood platelets, participate in the formation of antithrombotic APC (Activated Protein C) and Thrombin Activatable Fibrinolysis Inhibitor (TAFI) (Fig. 2).

The term anticoagulants is assigned to proteins inhibiting thrombin generation or inactivating the already generated thrombin (Fig. 3). The mechanism of action is a criterion for the distinction between indirect and direct anticoagulants:

(a). Indirect anticoagulants include tissue factor pathway inhibitor, TFPI, and activated protein C (APC), the last being transformed by thrombin/ thrombomodulin complex from inactive protein C to serine enzyme APC with participation of protein S, PS, as a

Table 1. The levels of coagulation factors in cord blood (normal values). Ranges are given only for values that are most important for clinicians; more detailed data can be found in the publications cited [3-6]. The percentage values have been taken from literature or calculated based on this literature [3-6].

Coagulation factor	Gestation period		
	Gestational age: weeks 19–38	At delivery	Adults
Fibrinogen – antigen (f. I)	1.08 (range: 1.30–2.40) g/l	2.65 (range: 1.68–3.60) g/l	Adults: 3.5 (range: 2.50 – 5.20) g/l
Vitamin K-dependent factors:			Adults: 98.7 % (range: 70 – 125%)
– f. II	19.9% → 27.9%	43.5 %	
– f. VII	27.4% → 45.9%	52.5 %	
– f. IX	10.1% → 12.3%	31.8 %	
– f. X	20.5% → 28.0%	39.6 %	
F. V	32.1% → 48.9%	50-140%	f. V, VIII and XIII can reach the adult level at delivery
F. VIII	34.5% → 50.1%	38-150%	
F. XIII	Lack of information	c. 1 U/ml	
Factors of contact phase			
– f. XI	13.2% → 14.8%	37.2 %	
– f. XII	14.9% → 25.8%	69.8 %	
– prekallikrein	12.8% → 18.1%	35.4 %	
– high molecular kininogen (HMK)	15.4% → 23.6%	38.9 %	
Tissue factor (TF)	Lack of information	Lack of information	3.91 ng/mg protein [20]

cofactor. Both anticoagulants suppress thrombin generation - TFPI by forming a quaternary TF/VIIa/TFPI/Xa complex, whereas APC by inactivating Va and VIIIa factors;

(b). Direct anticoagulants include antithrombin III (AT III) and heparin cofactor II (HC II); they inactivate the existing thrombin to form stable complexes. The thrombin-antithrombin III complexes (TAT) are useful for clinical purposes as markers of thrombin.

Concentrations of procoagulants and anticoagulants in fetal blood

Basic information on hemostasis in the fetus and infant is included in publications by Andrew M. *et al.* [3,4], Reverdiau-Moalic P. *et al.* [5] and Kuhle S. *et al.* [6].

The hemostatic proteins in fetal blood are synthesized by the fetus itself as they do not cross the placental barrier from the mother.

The synthesis of fibrinogen - factor I - begins during the 6th week of intrauterine life, i.e. the embryo period (the time from the zygote stage to the 8th week of pregnancy). The fetal blood starts to coagulate around the 11th week of intrauterine life (after Kuhle *et al.* [6]), which means that at that time all the factors indispensable for thrombin generation as well as the coagulation protein fibrinogen are already present in the blood. It is difficult, however, to precisely deter-

mine the time at which particular coagulation factors or fibrinolysis components appear for the first time in the blood. All authors agree that around the 34th week (30th to 38th week) of pregnancy the concentration of the coagulation factors rapidly increases. Delivery is probably an additional accelerating impulse, which, however, has not been satisfactorily documented yet.

The most important data from the clinical point of view are presented in Table 1 (procoagulants), Table 2 (anticoagulants) and Table 3 (selected tests). The cited authors have formulated the following conclusions:

- The earlier is the pregnancy age of the fetus, the lower the concentration of procoagulants and anticoagulants; thus, in fetuses at around 20 weeks of age, the concentrations of the respective procoagulants and anticoagulants are most often below 20% of the values for adults.
- A significant increase in the concentration of the procoagulants occurs in the period of the 30th - 38th week of pregnancy, although only three factors - V, VII and XIII - reach the level of adults at delivery time.
- An increase is also noted in the level of anticoagulants, but only antithrombin (earlier called antithrombin III) and heparin cofactor II reach about 60% of the adult value at the time of delivery, while the concentration of other anticoagulants is around 50%. The levels corresponding to adult values are reached about the 6th month of life [3], though that of protein C - as late as in teenagers

Table 2. The levels of anticoagulants in cord blood. Ranges are not given for all the values (the Reader is referred to the original manuscript [5]). The percentage values have been taken from literature or calculated based on the literature data [3-6]; *[21].

Anticoagulant	Gestation period		
	Gestational age: weeks 19 – 38	At delivery	Adults
Tissue factor pathway inhibitor (TFPI)	~20%	38.1%	73.0 (50.9 – 90.1)%
Antithrombin (AT)	20.2% → 37.1%	59.4%	99.8 (65 – 130)%
Heparin cofactor II (HC II)	10.3% → 21.1%	52.1%	101.4 (70 – 128)%
Protein C (antigen)	9.5% → 15.9%	32.5%	100.8 (68 – 125)%
Protein S (total activity)	15.1% → 21.0%	38.5%	99.6 (72 – 118)%
Thrombomodulin (TM)	Lack of data	11.35±3.7 ng/ml*	4.51±0.71 ng/ml*
Endothelial protein C receptor (EPCR)	Lack of data		

Table 3. Cord blood - normal screening test results [5].

Test	Gestation period		
	Gestational age weeks 19 – 38	At delivery	Adults
Prothrombin time	32.5 s. → 22.6 s.	16.7 s.	13.5 s. (range: 11.4 – 14.0 s.)
Activated prothrombin time (aPTT)	168.8 s. → 104.8 s.	44.3 s.	33.0 s. (range: 25 – 39 s.)
Thrombin time	Lack of data		

- Delivery accelerates an increase in the level of procoagulants and anticoagulants, which can be observed after delivery [7].
- vWF has not been analyzed with the fetus yet, though in infants the concentration is low in comparison with adults [8].

Are there fetal forms of the coagulation factors?

Most probably all fetal procoagulants and anticoagulants show certain structural and functional differences as compared to adults. It can be expected that the fetal features are most pronounced in the early period of development. The most frequently mentioned is fetal fibrinogen, whereas PIVKA-type factors (Protein Induced in Vitamin K-Absence) and other coagulation proteins seem to be seldom reported. It is known that structural defects result in divergence between measurements of antigen and activity (higher immunological than functional value). This proves poorer hemostatic utility of the fetal factors.

Fetal fibrinogen increases the content of sialic acid and reduces susceptibility to thrombin as compared to adults. PIVKAs, i.e. vitamin K-dependent coagulation factors, are deprived of COOH groups in the so called gamma position, thus being unable to form complexes with calcium and phospholipids during thrombin generation. The effect of PIVKA on fetal hemostasis has not

been investigated by the authors cited above [3,4,5,6]. However, fetal hemostasis in the newborn has been the subject of congress discussion (Haemostasis 1986;16: suppl 5, p. 85-86). Most likely, PIVKA factors have not been searched for in the fetal blood yet.

In adult humans, PIVKA factors are known to originate as a result of high deficiency of vitamin K, e.g. when cumarine or phenylindandione derivatives are administered. PIVKA factors are detected in the blood of healthy newborns, mainly as PIVKA-II (nearly in 50% of the examined). It is not clear whether PIVKA production is caused by vitamin K deficiency only or by liver immaturity? Nevertheless, administration of vitamin K is recommended in the prophylactic treatment of perinatal bleeding in women undergoing antiepileptic therapy [9].

Thrombin generation in fetal blood

Thrombin generation in the fetus has been studied using standard screening tests (activated partial thromboplastin time - aPTT, and prothrombin time) [4] and by measurements of prothrombin fragments F 1+2 [10]. The ability of the fetus to form thrombin is found to be from 1/3 to 1/2 of that of adult. The immediate cause of the lowered thrombin generation in the fetus is claimed to a low concentration of contact factors and vitamin K-dependent factors. The most important cause is said to be a low prothrombin level (prothrom-

bin deficit). When the deficiency was supplemented to the adult level, the activity of the generated thrombin was similar to that of adults [4]. Thrombin generation in fetal blood occurs with low concentrations of anticoagulants, which according to some authors allows sufficient production of thrombin [10].

Inactivation of thrombin in fetus blood

Many authors have investigated anticoagulant synergism in the newborn and infant but not in the fetus. It has been demonstrated, among others, that tissue factor pathway inhibitor (TFPI) and antithrombin (AT) amplify the anticoagulant action of activated protein C (APC) [11]. According to clinicians, the anticoagulant effect obtained by means of recombinant human APC is greater in adults than in children, which can be explained by the fact that the levels of TFPI and AT are higher in adults than in children [12].

Each of the anticoagulants shows a specific effect: (i) TFPI regulates the coagulation by forming a quaternary TF/VIIa/TFPI/Xa complex, inhibiting prothrombinase formation; (ii) APC inactivates Va and VIIIa factors; (iii) AT inactivates the already generated thrombin to form inactive complexes (thrombin/antithrombin complexes, TAT).

There are two forms of PS in the plasma: (i) free and functionally active form which can participate in the protein C pathway, and (ii) PS complexed with C4b-binding protein being functionally inactive. The anticoagulant activity of PS becomes higher when the free fraction of PS increases [12,13,14].

Low stability of hemostasis in fetuses and infants

Despite low ability of thrombin generation in healthy fetuses and infants, their hemostasis is efficient, which surprises researchers and clinicians. On the other hand, however, surprising is the passage from the norm (homeostasis) to complications (undercoagulability and bleedings or disseminated intravascular coagulation, DIC). This is discussed in terms of shallow hemostatic equilibrium in fetuses and infants, and explained enigmatically as being a feature of immature hemostatic systems in fetuses and infants (a), a result of low levels of procoagulants in the blood (b) or due to other causes (c) [3,5,6,8,15].

Activated protein C resistance (APCr) in cord blood

Resistance to active protein C is a known risk factor of thromboembolic complications in newborns, infants and adults [16]. APCr is in 90% caused by V Leiden factor - a genetic defect affecting approximately 5% of

the general population [17]. There is also APCr associated with the use of contraceptives and pregnancy (elevated levels of factor VIII, fibrinogen and von Willebrand factor in a pregnant woman being non-genetic causes of APCr) [18].

It can be assumed that the Leiden-type factor is present in fetuses as frequently as in adults, although no research has been conducted to confirm the assumption. Some authors hold that nonspecific APCr can occur also in fetuses and infants, but others have not supported this claim [literature survey according to [19]].

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