Global fibrinolytic capacity in healthy newborn infants

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The fibrinolytic system removes the fibrin clot once it has been activated. Fibrinolysis uses elements from plasma, platelets, tissue, and other blood cells to regulate the degradation of fibrin. This is brought about by the conversion of plasminogen to plasmin. The process of plasminogen activation can occur through two distinct pathways including the extrinsic activator system [tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA)] that exists in tissues throughout the body and the intrinsic (plasma) activator system (kallikrein, factor IXa and factor XIIa). The pathway employed in vivo appears to be the extrinsic pathway, however, both the intrinsic as well as exogenous-associated systems could play an important role in the activation of plasminogen. A wide variety of natural inhibitors of fibrinolysis exist in plasma, blood cells, tissues, and other extracellular matrices. These natural inhibitors can act to either inhibit plasmin directly (α_2-antiplasmin, α_2-macroglobulin, antithrombin-III and C1 esterase inhibitor), or block the conversion of plasminogen to plasmin (plasminogen activator inhibitors). At least four plasminogen activator inhibitors (PAI) have been identified. These include PAI-1, PAI-2, PAI-3, and protease nexin-1. The PAI-1 is the main plasminogen activator inhibitor that exists in plasma and serves an important role in the regulation of fibrinolysis[1].

Evaluation of the body's fibrinolytic potential is difficult in contrast to blood clotting in vitro because fibrinolysis is only activated after coagulation. Fibrinolysis is generally initiated by the local release of t-PA in the vascular micro-environment. In this micro-environment, the concentrations of t-PA and of PAI-1 are considerably higher than in the blood circulation. When a fibrin clot is present, t-PA binds to it and becomes protected from inhibition by PAI-1 and other inhibitors. By contrast, free t-PA in plasma is rapidly inactivated by PAI-1. In addition, t-PA and PAI-1 have short half-lives in plasma. For this reason, determinations of plasma levels of plasminogen, its activators and inhibitors do not reflect fibrinolytic activity properly.

This fundamental principle has limited the ability of investigators to define the nature of plasminogen activators and the regulation of fibrinolysis for years. To resolve this problem Amiral et al.[2] developed a standardized assay to measure global fibrinolytic capacity (GFC) in plasma. This assay is a sensitive and reliable parameter to evaluate the fibrinolytic potency of plasma in vitro. GFC assay also allows us to evaluate the sum of the known and unknown factors that regulate plasminogen activators and inhibitors.

The fibrinolytic mechanism of the normal newborn infant is poorly understood. Numerous investigators have reported conflicting results such as reduced plasminogen activity and antigen, normal t-PA antigen, normal to increased t-PA activity, normal to increased PAI-1 antigen, normal to decreased PAI-1 activity, and normal to reduced α_2-antiplasmin[3-5]. All these indirect findings of the fibrinolytic system suggest that there is reduced fibrinolysis in the first days of life. However, abnormally short whole blood clotting times, short euglobulin lysis times, and increased plasma concentrations of the Bβ 15-42 fibrin-related peptides all suggest that the fibrinolytic system is activated at birth[6]. At the same time, the capacity of the fetal fibrinolytic system to generate plasmin in response to stimulation with a thrombolytic agent is decreased compared with adults[7]. We therefore studied GFC in the first six hours of life in newborn infants. As far as we know, this is the first report on GFC of newborn infants in the medical literature.

Twelve preterm and 13 full-term healthy infants, who were consecutively admitted to the Neonatal Care Unit of Hacettepe University Children's Hospital, Ankara, Turkey, were studied. The mothers of the infants showed no amnionitis, prolonged rupture of membranes (24 h before delivery), toxicosis, the syndrome of hemolysis, elevated liver enzymes, low platelet count (HELLP) nor any other diseases that could influence the activation of clotting and fibrinolysis in their infants. Dexamethasone,

which may inhibit the fibrinolytic system[8], was given to the mothers of four infants in the preterm group more than two days before delivery. No other specific drug that could influence the activation of clotting and fibrinolysis was given to the mothers. For ethical reasons, all neonates received vitamin K1 2 mg intramuscularly upon delivery.

In all infants, blood samples for GFC testing were taken within six hours after birth before any medical teratment. In preterm infants, blood samples were obtained from an indwelling umbilical arterial catheter. Peripheral arterial blood samples were used in full-term infants, for ethical reasons. None of the infants had severe acidosis (umbilical arterial blood pH < 7.10) at the time of blood sampling, and no clinical abnormalities were observed during the first month of life. All samples were collected in soft plastic tubes containing an appropriate anticoagulant to prevent activation of blood clotting before analysis. At each sampling 1 ml of blood was anticoagulated with citrate (0.3%), immediately centrifuged (1500 x g, 20 min) and the supernatant stored at -20 °C until determination of GFC.

Global fibrinolytic capacity was determined by the method of Amiral et al.[2] Briefly, a freeze-dried fibrin clot (obtained with fibrinogen, depleted from all the lysine binding proteins, then clotted with thrombin in the presence of silica, calcium and factor XIII-A), is used for preparing standardized fibrin tablets. These latter are introduced in 200 µl test plasma, supplemented with a constant and limited amount of tPA, and the mixture is incubated for 1 hour at 37 °C. Fibrinolysis is then stopped with 50 µl of an aprotinin solution. Generated D-dimer is measured and its concentration is directly related to GFC.

Data are presented as mean ± SD. Gestational age, birth weight and D-dimer concentrations were compared using Mann-Whitney U tests. Correlations were determined by calculating Spearman's rank correlation coefficient. Statistical significance was assumed when the p value was less than 0.05.

Global fibrinolytic capacity, which is expressed as generated D-dimer concentrations, was significantly higher in preterm infants compared to full-term infants ($q < 0.001$, Table I). The D-dimer concentration was negatively correlated with the gestational age ($r = -0.38$; $p < 0.05$), but was not correlated with the birth weight ($r = -0.22$; $p > 0.05$).

Table I. Global Fibrinolytic Capacity in the First Six Hours of Life in Healthy Full-term and Preterm Infants

	Full-term infants (n = 13)	Preterm infants (n = 12)
Gestational age (weeks)	39.2 ± 1.1	32.5 ± 2.5
Birth weight (g)	3332 ± 440	1683 ± 713
Generated D-dimer (µg/ml)	3.8 ± 2.6*	9.9 ± 8.1*

* $p < 0.001$ by Mann-Whitney U test.

Our findings suggest that GFC is increased in preterm infants when compared to full-term infants. Normally plasma t-PA plasminogen concentrations of preterm infants are lower than in term infants[6]. Increased fibrinolytic activity in preterm infants in our study seems to contrast with low plasminogen levels in these infants. However, GFC assay allows evaluation of not only plasminogen levels, but also the sum of the known and unknown factors that may regulate plasminogen activators and inhibitors. Although indirect parameters of fibrinolysis (e.g. plasma plasminogen, t-PA, PAI-1, etc) could not be determined in this study, simultaneous determination of these parameters may not clarify the cause(s) of increased GFC. In addition, increased GFC can be responsible for the low levels of plasma plasminogen due to consumption in preterm infants. On the other hand, increased thrombin-antithrombin III complex and plasmin-antiplasmin complex have been reported in healthy preterm infants without clinical evidence of thrombosis[9]. We may hypothesize that the increased thrombotic activity may be balanced by increased GFC activity. The cause(s) of the increased GFC in preterm infants remains to be determined. Further studies are needed to assess the increased GFC in preterm infants before making a reliable conclusion.

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