INTRODUCTION

An intact microcirculation is mandatory for effective oxygen delivery. The widespread formation of microthrombi in small capillaries in critically ill patients may occur in any scenario of endothelial injury with subsequent exposure of blood cells and plasma proteins to procoagulant surfaces ***(Levi, 2007)***. In combination with consecutive consumption of coagulation factors and platelets, this series of events is called disseminated intravascular coagulation (DIC). In some cases, acute hemorrhage also ensues ***(Alex et al., 2010)***.

Newborns, especially those born preterm, are probably one of the groups with the highest risk among intensive care patients to develop DIC, as the following factors combine to create a dangerous situation: asphyxia, hypoxia, labile blood pressure and cardiac output, intravascular volume contraction after birth, immature mucosal and skin barriers, as well as multiple invasive procedures and devices ***(Williams et al., 2002)***. In addition, reserves of pro- and anticoagulant coagulation factors are very limited; thus the overall results are vulnerable endothelium, early microvascular thrombus formation and hemorrhage ***(Alex et al., 2010)***.

The true incidence of DIC in the newborn is not easily defined by the literature, because DIC may remain undetected until the neonate presents with severe hemorrhage or the disease is determined at autopsy. Despite evidence that

newborns are in a hypercoagulable state, there does not seem to be clear or universally accepted criteria to diagnose DIC in the neonatal ICU. In 1998, The International Society on Thrombosis and Haemostasis (ISTH) developed a DIC scoring system for low birth weight preterms, but it has not been proved to be reliable or valid**.** Hence, the score currently is not in widespread clinical use ***(Daijiro et al., 2013)***.

Hypercoagulation and secondary fibrinolysis are the major pathologic mechanisms in DIC. Laboratory data based on the clinical condition might be critical for the diagnosis of DIC ***(Remkova, 2006)***. Among the coagulation markers (prothrombin time, platelets, fibrinogen, fibrin- related markers (FRMs) in the DIC scoring system, FRMs such as fibrin monomer (FM) and D-dimer best reflect the underlying pathology of DIC. D-dimer was reported to be very sensitive but not specific. As for FM, little is known about its diagnostic performance in patients with DIC ***(Kyoung et al., 2011)***.

FM appears in the bloodstream during the extremely early stage of blood coagulation. FM is formed of fibrinopeptides (desA, desAA, and desAABB fibrin monomers) that are cleaved from fibrinogen under the effect of thrombin. FM rapidly polymerizes to form a clot. Small amounts can circulate in plasma as "soluble fibrin", which may have a complex composition, and are named as soluble fibrin monomer complexes (SFMC). SFMC include fibrinogen and a variable amount of cross-linking and has been found in high

concentrations in hypercoagulable state blood. Therefore, FM and SFMC are well known to be very sensitive for the diagnosis of conditions of hypercoagulability such as DIC ***(Horan and Francis, 2001)***.

AIM OF THE WORK

1. To identify the presence of a hypercoagulable state in sick newborns by studying the behavior of SFMC with special reference to those of other coagulation tests.
2. To evaluate the role of SFMC in early diagnosis of DIC in sick newborns.

HYPERCOAGULABLE STATES

The function of hemostatic system depends on complex reactions among its components: the platelets, vessel wall, coagulation factors with their inhibitors, and the fibrinolytic proteins with their inhibitors. Disturbance in the balance between coagulation and fibinolysis makes the blood prone to thrombosis (Figure1). Hypercoagulable state is a disorder of the blood coagulation with a shift of the hemostatic balance towards the enhancement of procoagulant forces ***(Mannucci, 2005)***.

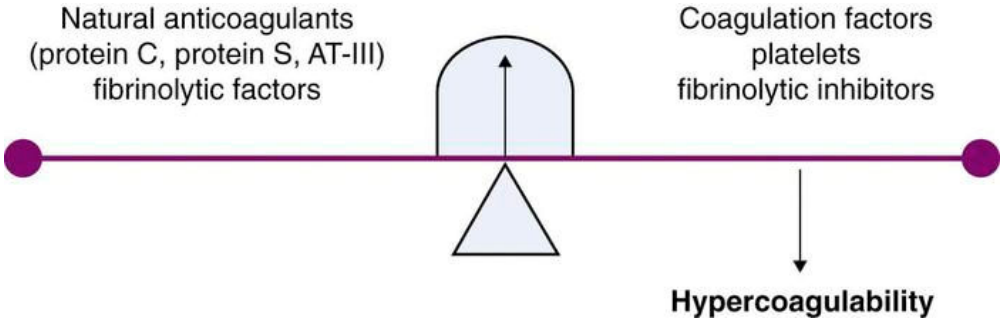

Figure (1): Hemostatic balance. AT-III: antithrombin III ***(Deitcher, 2010).***

Etiology:

Multiple genetic and environmental factors contribute to the development of thrombosis. According to this concept, thrombosis is either a congenital disorder or an acquired disorder through triggering the mechanism of coagulation and/or reduces the natural anticoagulant activity. These factors can be classified as follows ***(Favaloro, 2005)***:

1. ***Inherited (Primary) hypercoagulable states:***

   a. Antithrombin III deficiency

   b. Protein C deficiency

   c. Protein S deficiency

   d. Protein C resistance due to factor Vgene mutation (factor V Leiden)

   e. Prothrombin gene mutation

   f. Dysfibrinogenemia (rare)

2. ***Acquired (Secondary) hypercoagulable states:***

   a. Antiphospholipid syndrome

   b. In association with other physiologic or thrombogenic stimuli (e.g., injury, postoperative state, etc.)

   c. In association with some clinical disorders (e.g., malignancy, congestive heart failure, nephrotic syndrome, etc.)

3. ***Mixed hypercoagulation states:***

Hyper homocysteinemia

Worth mentioning, critical illness is a well-recognized risk factor for thrombophilia in all age groups. Immobilization,

rapid changes in intravascular volume and extensive intravascular instrumentation contribute to the enhanced risk of venous and arterial thrombosis in patients in intensive care units ***(Johnson et al., 2005)***. Additional risk factors include, but are not limited to, asphyxia, maternal diabetes, poor cardiac output, septicaemia and dehydration. Neonates are born with a high hematocrit and tend to contract their intravascular volume within the first days of life, making them even more prone to thromboembolic events ***(Angus et al., 2001)***.

Pathophysiology:

Reviewing the physiology of how the human body maintains normal hemostasis is important to understand the pathogenesis of cases of coagulopathy.

- ***Mechanisms of normal hemostasis:***

Upon vessel damage, platelets adhere to the damaged site and aggregate through interactions of platelet receptors with extracellular ligands and soluble proteins. Vascular damage-induced exposure of subendothelial tissue factor (TF) generates trace amounts of thrombin with multiple effects on other coagulation factors and platelets. Via multiple enforcement loops in the coagulation system and in platelet activation, large amounts of fibrin are formed stabilizing earlier formed platelet thrombi ***(Versteeg et al., 2013)***. Inhibitors of the coagulation cascade limit and confine the coagulation response, and

activation of the fibrinolysis pathway results in the dissolution of fibrin clots to maintain and/or restore blood vessel patency ***(Shoshana, 2012)***.

- ***The cascade model of coagulation:***

In the 1960s, two independent groups constructed a model for coagulation that resembled a waterfall or cascade. Therefore, this model was named the coagulation cascade model (Figure 2). Herein, each clotting factor consists of a proenzyme that is converted to an active enzyme by the upstream activated clotting factor ***(Davie and Ratnoff, 1964)***.

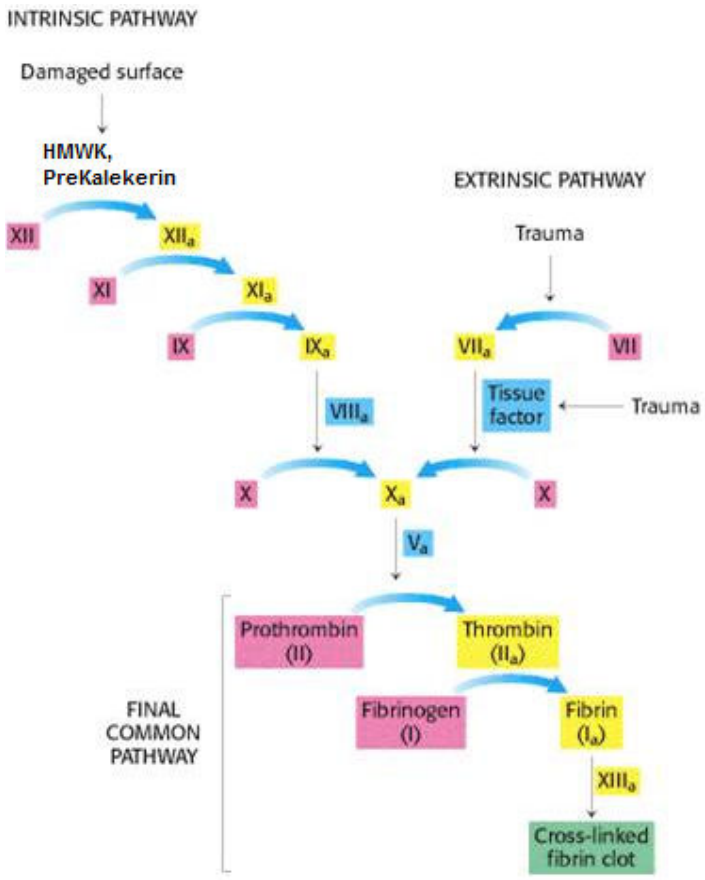

Figure (2): The coagulation cascade. The traditional concept of blood coagulation with separate intrinsic system (left) and extrinsic/cellular injury system (right), and the final common pathway (bottom) ***(http://cancergrace.org/cancer-101/2009/12/22/cancer-and-clotting-protecht) (accessed 10/6/2014)***

Defects in the cascade model:

Although this model explains the laboratory coagulation tests, the separation into intrinsic and extrinsic pathways, as well as the absence of platelets and other cellular elements from

the overall framework, limited the model's application to in vivo hemostasis and thrombosis. For that reason, in the last decade Hoffman and Monroe have proposed a new model of homeostasis (A Cell-based Model of Hemostasis) that establishes physiological, integrated, and functional view of complex biochemical events occuring on cellular surfaces rather than independent cascades ***(Hoffman and Monroe, 2001)***. It also provides a scientific foundation for understanding the importance of specific platelet binding sites for coagulation proteases, the non hemostatic role of coagulation factors, the dynamic nature of cellular interactions and the inter-individual variability of platelet procoagulant activity ***(Becker, 2005)***.

- ***The cell-based model of hemostasis:***

According to the cell-based model of hemostasis, coagulation can be divided into three overlapping phases: 1) an initiation phase, in which low amounts of active coagulation factors are generated; 2) an amplification phase, in which the level of active coagulation factors is boosted; and 3) a propagation phase, in which coagulation factors bind to highly procoagulant membranes of activated platelets and fibrin clots are formed ***(Mackman, 2009)*** (Figure 3).

1. Initiation phase

The initiation phase, classically referred to as the extrinsic pathway of coagulation, starts when the vasculature is disrupted,

and sub-endothelial cells like smooth muscle cells and fibroblasts become exposed to the bloodstream ***(Monroe and Hoffman, 2006)***. These cells expose a key initiator of the coagulation cascade, TF, which binds coagulation FVII. By acting as a cofactor for FVII, TF promotes proteolysis and activation to FVIIa. The TF/FVIIa complex proteolytically cleaves traces of FIX and FX into FIXa and FXa, respectively. This allows FXa to associate with cofactor FVa to form a prothrombinase complex on TF-expressing cells, which serves to convert prothrombin (FII) into thrombin ***(Versteeg et al., 2013)***.

2. Amplification phase

The slowly accumulating amounts of thrombin will further activate platelets that have adhered to a site of injury. In parallel, thrombin will convert platelet-derived FV into FVa, thus amplifying prothrombinase activity, and convert FVIII into FVIIIa, which acts as a cofactor to FIXa on the surface of activated platelets to support FXa generation. In addition, thrombin converts FXI into FXIa ***(Versteeg et al., 2013)***.

3. Propagation phase

This phase occurs on surfaces containing procoagulant phospholipids such as activated platelets. Activated FXI converts FIX into FIXa, which then associates with thrombin-cleaved FVIII. The tenase complex of FIXa/ FVIIIa catalyzes the conversion of FX to FXa, after which the FXa/FVa complex

produces sufficient amounts of thrombin to massively form fibrin fibers. As a final step, the thrombin-activated plasma transglutaminase FXIIIa catalyzes the formation of covalent crosslinks between adjacent fibrin chains to yield an elastic, polymerized fibrin clot **(Ariens et al., 2002)**.

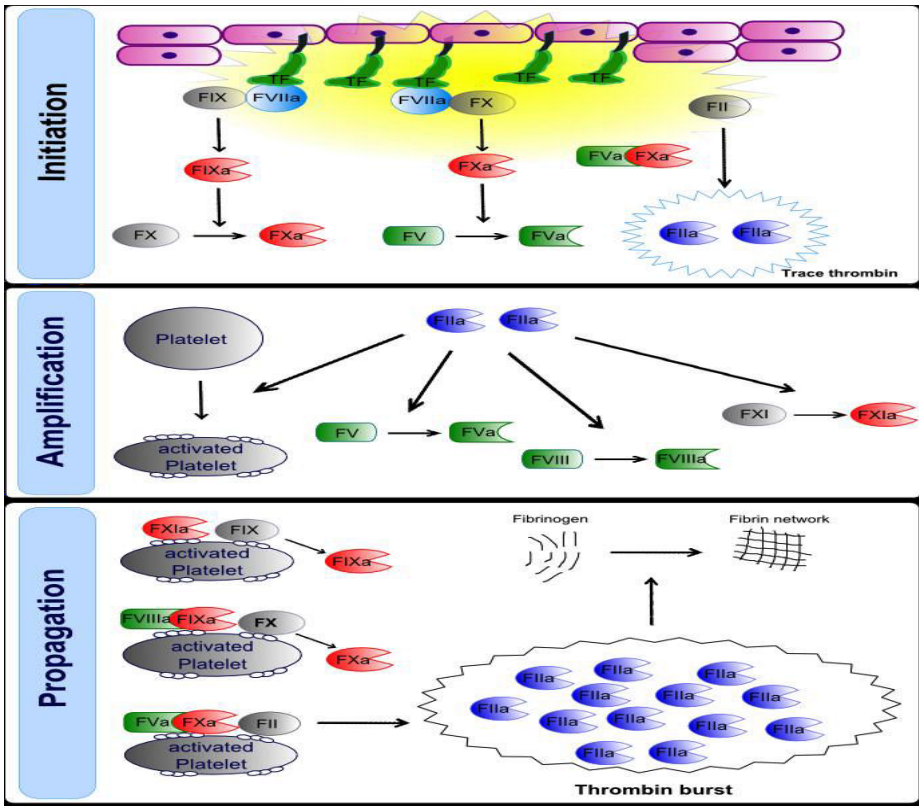

Figure (3): Cell based model of coagulation consists of 3 phases: initiation, amplification and propagation ***(Palta et al., 2014).***

▪ ***Inhibition of the Coagulation Process***

Considerable efforts have been made in recent years to figure out the suppressor mechanism of the coagulation process, which is essential to prevent uncontrolled, widespread clot formation. First, circulating protease inhibitors eliminate activated coagulation factors by attacking their active sites. The second anticoagulant modality is provided by the enzyme-based protein C/protein S pathway ***(Versteeg et al., 2013)***.

1. The protein C/protein S pathway

Since the 1980s, it is known that activated protein C (APC) in complex with protein S establishes proteolytic inactivation of FVIIIa and FVa, thus suppressing tenase and prothrombinase actions. Protein C is an anticoagulant factor with high homology to the vitamin K-dependent procoagulant factors. For full anticoagulant control, it needs to be cleaved into APC and bind to its cofactor, protein S, also a vitamin K-dependent protein ***(Dahlback and Villoutreix, 2005)***.

As the concentrations of thrombin gradually rise during coagulation, it binds to thrombomodulin, a transmembrane protein that is expressed on endothelial cells. Once bound to thrombomodulin, thrombin proteolytically cleaves and activates protein C that is bound to nearby endothelial protein C receptor (EPCR) ***(Koutsi et al., 2008)***.

2. Coagulation protease inhibitors

Out of the many plasma proteins that exert negative regulatory control on the coagulation process, tissue factor pathway inhibitor (TFPI) and antithrombin (AT) are the most studied and best understood inhibitors ***(Versteeg et al., 2013)***.

TFPI is present in platelets and on the microvascular endothelium, where it remains associated with the cell surface in a yet poorly characterized manner ***(Broze and Girard, 2012)***.

AT is considered one of the most important inhibitors of thrombin generation and function. This is exemplified by its high affinity towards three key coagulation proteases: FIXa, FXa, and thrombin. Clinically, heterozygous deficiency in AT confers a 10-fold higher risk of venous thrombosis, while true homozygous deficiency has never been observed in humans, probably because it is lethal ***(Ishiguro et al., 2002).*** The natural inactivation of coagulation proteases by AT is strongly enhanced by heparin. Long-chain heparins function to bind AT as well as protease FIXa, FXa, or thrombin, thereby bringing these components together in close proximity ***(Olson et al., 2004)***.

▪ ***Fibrynolysis:***

Vascular endothelial disruption triggers not only coagulation reactions but also the fibrinolytic pathways (Figure 4). Physiologic fibrinolysis is initiated by endothelial cell-derived tissue plasminogen activator (tPA)-mediated

conversion of plasminogen to plasmin. Plasmin can degrade fibrinogen and fibrin, thus limiting the size of a thrombus and helping to clear a thrombus once the vascular injury has been repaired. The fibrinolytic pathways are regulated by the inhibitory proteins α_2-antiplasmin and plasminogen activator inhibitor-1(PAI-1) ***(Kriz et al., 2009)***.

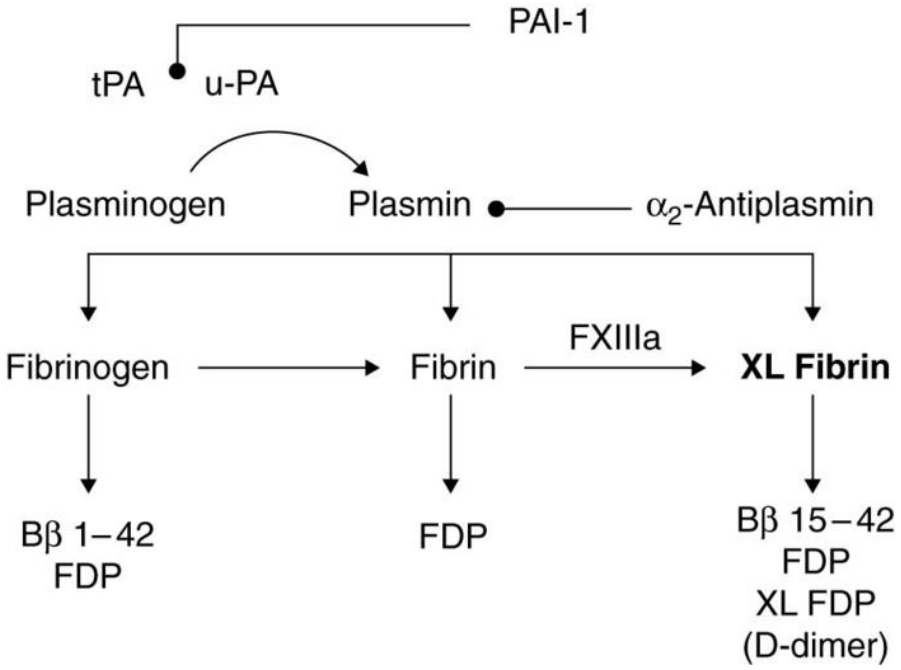

Figure (4): The fibrinolytic pathways**.** PAI-1: plasminogen activator inhibitor-1; tPA: tissue plasminogen activator; u-PA: urokinase plasminogen activator; FDP: fibrin(ogen) degradation product; XL: cross linked; Bβ 1-42/ 15-42: fibrinopeptide β 1-42/15-42 ***(Palta et al., 2014)***.

- ***Hypercoagulability in neonates:***

Notably, labor and parturition are already associated with a higher risk for thromboembolic complications in both