Review Article

Principles of hemostasis in children: models and maturation

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Summary
Hemostasis is an active process regulating the formation and dissolution of fibrin clot to preserve vascular integrity. The different phases of hemostasis are coordinated so that effective clotting occurs only at the site of vascular injury while maintaining blood flow in other parts of the circulation. Procoagulant processes culminate in thrombin generation and fibrin clot formation to protect the vasculature against uncontrolled bleeding after injury. Conversely, anticoagulant processes limit clot extension to unaffected portions of the vasculature. Lastly, fibrinolysis is responsible for clot dissolution once tissue repair and regeneration permit the return of normal blood flow. A precise and delicate interplay exists among these processes to ensure normal hemostasis. The hemostatic system is incompletely developed at birth and matures throughout infancy. Both full-term and preterm neonates are born with low levels of most procoagulant proteins including all the contact activation factors and vitamin K-dependent factors. Similarly, levels of the major anticoagulant proteins are low at birth. Although often characterized as ‘immature’, the neonatal hemostatic system is nevertheless functionally balanced with no tendency toward coagulopathy or thrombosis. In this article, we will review the current models of hemostasis and the maturation of the hemostatic system. Our goal is to help clinicians gain a better understanding of the actions of procoagulant agents and of the disruptive effects of serious systemic illnesses on the precarious hemostatic balance of infants.

Keywords: hemostasis; coagulation; children; maturation

Introduction
Hemostasis is a complex physiological process that balances the opposing forces of coagulation and anticoagulation to protect the vasculature from uncontrolled bleeding on the one hand and excessive clotting on the other (1). In the past decade, our understanding of the mechanisms of hemostasis has been refined and new pharmacologic therapies have been developed to help manage coagulation problems. In this review, we will describe the three phases of hemostasis: primary hemostasis or forma-
tion of a platelet plug, secondary hemostasis or coagulation, and tertiary hemostasis or fibrinolysis. We will discuss how, under normal physiologic conditions, these three phases act in a coordinated manner so that effective clotting occurs only at the site of injury while maintaining blood flow in other parts of the vasculature. We will discuss why recombinant activated factor VII (rFVIIa) and other coagulation factor concentrates are increasingly being used to promote coagulation in patients with uncontrolled bleeding from a variety of etiologies. Finally, we will examine the maturation of the hemostatic system. We will discover how the hemostatic system in neonates and young infants differs from the definitive adult system and how this 'immature' system is capable of maintaining normal hemostasis.

Models of hemostasis

Primary hemostasis

Primary hemostasis begins with platelet adhesion (Figure 1). This process is initiated by the interaction of the platelet glycoprotein Ib (GPIb) receptor complex with its primary ligand, von Willebrand Factor (vWF) (2). Although there is debate concerning the precise event triggering this interaction, disruption of the smooth endothelial lining of the blood vessel and the resultant high shear rate of blood flow appear to be required for platelet GPIb-vWF binding (3,4). During platelet adhesion, in addition to binding vWF, platelets bind collagen exposed in the subendothelial matrix by two high-affinity binding receptors. Platelet-collagen binding via these two receptors is essential to platelet adhesion and subsequent activation (2).

Once adherent to the injured vessel wall, platelets become activated by strong agonists present at the site of injury, primarily collagen and thrombin. Upon activation, platelets undergo a change in morphology and expose negatively charged phospholipids, previously unexpressed, on their surface membrane (5). These negatively charged phospholipids play an important role in the adhesion of various coagulation factors to the activated platelet surface. Platelet activation also results in the release of the contents of the platelet α-granules and dense granules into the surrounding environment. These substances promote aggressive platelet aggregation and vasoconstriction of the endothelial smooth muscle cells at the site of injury.

Platelet aggregation, the final step in the formation of a platelet plug, is mediated by the platelet surface receptor, the glycoprotein Ib/IIa (GPIIb/IIIa) receptor, which is switched ‘on’ during platelet activation (6). Fibrinogen is the primary adhesive molecule of the GPIIb/IIIa receptor. It cross-links platelets together by binding receptors on adjacent platelet surfaces. Platelet aggregation occurs in conjunction with the activation of coagulation factors on the platelet surface to support thrombin generation and the formation of a fibrin clot. Formation of a platelet plug is tightly controlled and limited only to areas of vascular injury. Indeed, intact endothelial cells themselves provide the main defense against the propagation of the platelet aggregate beyond the site of injury by producing powerful platelet aggregation inhibitors and vasodilators (3,5).

Secondary hemostasis

In 1964, investigators proposed the cascade model of coagulation, which described the overall structure of coagulation as a series of proteolytic reactions (7). The intrinsic pathway begins with contact activation by negatively charged substances such as kaolin or by the contact activation factors [factor XII (FXII),...
factor XI (FXI), high molecular weight kininogen (HMWK), and prekallikrein (PK) and ends with the activation of factor X (FX). This pathway is assessed by the laboratory test called the activated partial thromboplastin time (aPTT). The extrinsic pathway begins with tissue factor activation of factor VII (FVII) and likewise ends with the activation of FX. It is assessed by the laboratory test called the prothrombin time (PT). Both the intrinsic and extrinsic pathways converge into a final common pathway with the activation of FX. Activated FX (FXa), in complex with its cofactor activated FV (FVa), forms the ‘prothrombinase’ complex which is the main convertor of prothrombin into thrombin (8). At the time, the cascade model was extremely valuable to the understanding of coagulation; however, it was inadequate to explain several important clinical observations. For example, if the activation of FX is indeed part of a final common pathway, then why is the activation of FX through the extrinsic pathway unable to compensate for the lack of factor VIII (FVIII) or factor IX (FIX) in the intrinsic pathway in hemophiliacs (9)?

In 2000, Hoffman and Monroe (7) presented a second model of coagulation termed the cell-based model (Figure 2). This model consists of three overlapping stages: initiation, amplification, and propagation. It differs from the cascade model in that it emphasizes a system regulated by cellular components rather than one regulated by the kinetics of coagulation factors. This model is now recognized as the prevailing model of the coagulation process in vivo.

In the Cell-Based Model, the coagulation process begins with the initiation phase and the primary initiator is tissue factor (TF) (10). When vascular integrity is lost, TF, an extra-vascular protein, is exposed to the circulating blood. Zymogen FVII in the circulation has an extremely high affinity for TF and, once bound to TF, is rapidly converted to its activated form, activated FVII (FVIIa). The resultant TF/FVIIa complex catalyzes two very important reactions (11,12). The first is the activation of FX to FXa. The second is the activation of FIX to activated FIX (FIXa).

In the first reaction, free FXa is rapidly broken down by natural inhibitors which are abundant in the environment of the TF-bearing cell. However, FXa present on the surface of the TF-bearing cell is relatively protected and can catalyze the conversion of a small amount of prothrombin into thrombin (6,13). This small amount of thrombin, although insufficient to sustain the cleavage of fibrinogen into fibrin, is potent enough to fully activate platelets. In the second reaction, FIXa diffuses away from the TF-bearing cell and is attracted to the activated platelet surface. It is the negatively charged phospholipid membrane of the platelet surface, created during platelet activation, which is responsible for assembling coagulation factors on the platelet surface and supporting their reactions. As previously discussed, platelets release various substances from their α-granules during activation. Noteworthy is the release of factor V (FV) onto the platelet surface and its conversion, by thrombin, to activated FV (FVa) (7,8). Thrombin also acts to dissociate vWF from circulating FVIII, thus releasing activated FVIII (FVIIIa) onto the activated platelet surface (7,8). Additionally, thrombin activates factor XIII (FXIIIa), which will eventually be responsible for the cross-linking of soluble fibrin monomers into an insoluble fibrin matrix. Once activated coagulation factors are assembled on the activated platelet surface, the stage is set for a large-scale production of thrombin (7).

Activated platelets express high-affinity binding sites for FIXa and FXa. When FIXa reaches and

Figure 2
A model of tissue factor-initiated, cell-based hemostasis. TF, tissue factor; TFPI, tissue factor pathway inhibitor; vWF, von Willebrand factor. (Reproduced with permission from DM Monroe et al., The Factor VII – Platelet Interplay: Effectiveness of Recombinant Factor VIIa in the Treatment of Bleeding in Severe Thrombocytopenia, Seminars in Thrombosis and Hemostasis, Vol. 26, No. 4, 2000, page 374.)
binds to the activated platelet surface, it joins FVIIIa to form the ‘tenase’ complex (FVIIIa/FIXa). This complex activates large amounts of FXa, which may then join with its co-factor, FVa, to form the ‘prothrombinase’ complex (FXa/FVa). This ‘prothrombinase’ complex is responsible for the large-scale conversion of prothrombin to the thrombin necessary for the formation of a stable fibrin cross-linked clot (7).

Uncontrolled blood coagulation is extremely dangerous thus regulation is exerted at multiple levels of the process. Tissue factor pathway inhibitor (TFPI) regulates the initiation phase of blood coagulation by inhibiting the actions of the TF/FVIIa complex (14). There are no known deficiency states of TFPI in humans perhaps indicating that lack of TFPI is incompatible with life. Indeed, when mice are genetically altered to lack TFPI, they die in utero from uncontrolled coagulation (15). The anticoagulant protein, antithrombin (AT), is a powerful inhibitor of both FXa and thrombin and is designed to limit the coagulation process to the site of vascular injury (1). Protein C (PC), another important anticoagulant protein, is activated by thrombin once it has bound to the membrane protein, thrombomodulin, on the surface of intact endothelial cells. When activated, it inhibits coagulation through the breakdown of FVa and FVIIIa (7).

**Tertiary hemostasis**

The final phase of hemostasis, termed ‘fibrinolysis’, involves the removal of clot once it is no longer needed (Figure 3). Plasmin is the principle enzyme of the fibrinolytic system and is responsible for the cleavage of a mature fibrin clot into its degradation products, the smallest of which are known as D-dimers (9). Plasmin is activated from its zymogen form, plasminogen, by at least three different activating systems (1). The most important is tissue plasminogen activator (t-PA), which binds to fibrin via lysine binding sites and enzymatically converts plasminogen into plasmin. t-PA only activates plasminogen that is bound to fibrin thus limiting fibrinolysis to the site of clot formation.

**Recombinant activated factor VII**

A thorough understanding of the cell-based model of coagulation allows clinicians to appreciate the mechanisms by which high-dose rFVIIa (NovoSeven® RT; Novo Nordisk, Bagsvaerd, Denmark) promotes coagulation and ameliorates bleeding. rFVIIa is a synthetic coagulation factor nearly identical to the human plasma coagulation factor VIIa, but cloned and expressed in hamster kidney cells. Originally, rFVIIa was developed for the treatment of bleeding complications in hemophiliac patients who had developed alloantibodies, also termed inhibitors, against exogenously administered FVIII or FIX (16). Recently, increasingly high-dose rFVIIa has been used to promote coagulation in pediatric patients with nonhemophiliac bleeding from a variety of etiologies (16–18). Although no randomized controlled clinical trial in children has found rFVIIa to be more effective than placebo in reducing surgical bleeding, numerous observational studies have found a strong temporal relationship between rFVIIa therapy and reduced blood loss or blood product transfusion (19–21).

rFVIIa works via two probable mechanisms: one is TF-dependent, the other TF-independent (22). In the former, rFVIIa works synergistically with the patient’s zymogen factor VII to complex all exposed TF. Consequently, more active FVIIa/TF complexes are formed and result in an increase in local thrombin generation. In the TF-independent mechanism, rFVIIa, at sufficiently high doses, binds to negatively charged phospholipids on the surface of activated platelets and activates factor X directly. This provides a platelet surface source of FXa that can catalyze the large burst of thrombin required to support fibrin formation. Recent studies have shown that, while both mechanisms probably work in

![Figure 3](https://example.com/figure3.png)

**Figure 3**

t-PA, tissue plasminogen activator; PAI, plasminogen activator inhibitor; FDP, fibrin degradation products.
concert, TF-dependent thrombin generation activated by rFVIIa is much more efficient than TF-independent thrombin generation (23,24).

Thromboembolic complications are infrequent in hemophiliacs receiving rFVIIa, but occur more frequently in patients receiving rFVIIa for off-label uses (25). In hemophilia, thrombin generation is reduced because of an isolated factor deficiency (either FVIII or FIX). However, these patients have normal levels of anticoagulant factors. Conversely, in patients bleeding after trauma or surgery, the reduction in thrombin generation is accompanied not only by a decrease in procoagulant factor levels but also by a decrease in anticoagulant factor levels. Reduced AT levels with normal or even increased levels of FVIII or FIX may render rFVIIa therapy potentially prothrombotic (26). Thus, monitoring AT levels during rFVIIa therapy may assist in reducing the thrombotic complications occasionally seen in patients receiving rFVIIa for nonhemophiliac bleeding conditions.

Coagulation factor concentrates

Other plasma-derived coagulation factor concentrates are available, among them prothrombin complex concentrates (PCCs), fibrinogen concentrates, and FXIII concentrates. Most commonly, these concentrates are administered for replacement therapy in patients with congenital or acquired coagulation factor deficiencies. PCCs are also indicated to reverse the anticoagulant effects of vitamin K antagonists (27). As with rFVIIa, coagulation factor concentrates are being used more frequently for the treatment of acquired bleeding disorders. In animal models of uncontrolled hemorrhage and dilutional coagulopathy, the administration of PCC and fibrinogen concentrate was able to restore clot formation, reduce blood loss and improve mortality (28,29). Although human data, particularly from pediatric patients, are lacking, PCCs, fibrinogen concentrates, and FXIII concentrates have all been reported to be efficacious in controlling massive intraoperative and postoperative bleeding (30–32).

Coagulation factor concentrates enhance coagulation simply by increasing the in vivo content of coagulation factor(s) present in the concentrate. PCCs contain all the coagulation factors required to promote thrombin generation. The four vitamin K-dependent coagulation factors (FII, FVII, FIX, and FX) as well as trace amounts of FVIII, FVIIa, and FIXa are present in most PCC preparations although the specific composition and amount of each clotting factor in the concentrate varies depending on the manufacturer. Once again, the major adverse effect is thrombosis. Concentrates with high prothrombin (FII) and high FVII activity tend to be associated with a greater thromboembolic risk (27). Some manufacturers have added heparin and AT or PC, or both, in an attempt to reduce the thrombogenicity of the concentrate; however, the effectiveness of this practice has not been clearly demonstrated.

Maturation of hemostasis

Although all the key components of the hemostatic system are present at birth, important quantitative and qualitative differences exist between neonates and adults (Table 1). For procoagulant factors, these differences are primarily quantitative. Levels of the four contact activation factors (FXII, FXI, HMWK, and PK) are low at birth and remain so until approximately 6 months of age (33). This paucity of contact activation factors contributes to the prolonged aPTT seen in neonates and young infants. The vitamin K-dependent factors [prothrombin or factor II (FII), FVII, FIX, and FX] are also low at birth and, similarly, do not reach adult ranges until approximately 6 months of age (33). Levels of FV and FXIII are initially low but increase rapidly to those of an adult by day five of life (33,34). Interestingly, FVIII and vWF are the only two procoagulant proteins that exhibit markedly

Table 1

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<thead>
<tr>
<th>Component</th>
<th>Neonatal vs adult level</th>
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<tr>
<td>Primary hemostasis</td>
<td>platelet count $\downarrow$ vWF</td>
</tr>
<tr>
<td>Coagulation factors</td>
<td>FII, FVII, FIX, FX</td>
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<tr>
<td></td>
<td>FXI, FXII</td>
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<td></td>
<td>$\downarrow$ vWF</td>
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<tr>
<td>Anticoagulant factors</td>
<td>TFPI, AT, PC, PS</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>plasminogen $\uparrow$</td>
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vWF, von Willebrand factor; F, factor; TFPI, tissue factor pathway inhibitor; AT, antithrombin; PC, protein C; PS, protein S; s2M, alpha-2-macroglobulin; PAI, plasminogen activator inhibitor.
elevated levels at birth when compared to adult values (33,34).

Levels of the major anticoagulant factors (TFPI, AT, and PC) are low at birth in both full-term and preterm neonates (33,35). By approximately 3 months of age, mean levels of AT increase to those of adults whereas PC remains low until 6 months of age (33,35). TFPI levels remain low throughout most of childhood (36). Conversely, alpha-2-macroglobulin (α2M), a thrombin inhibitor of secondary importance in adults, is markedly elevated in neonates and often reaches levels twice those measured in adults (37). Some investigators have suggested that the increased levels of α2M in neonatal plasma may compensate to some extent for the decreased levels of other anticoagulants (37,38). Indeed, studies comparing the relative importance of α2M and AT on the inhibition of radio-labeled thrombin confirmed that α2M contributed more to the inhibition of thrombin in neonatal and infant plasma than in adult plasma (39). These findings led investigators to conclude that in neonates α2M is at least as important a thrombin inhibitor as AT. Further studies comparing plasma levels of the thrombin inhibitors between healthy and critically ill neonates found that the latter are often deficient not only in AT but in α2M as well thus significantly impairing normal thrombin inhibition (40,41). The cumulative deficiencies of these thrombin inhibitor proteins may explain the thrombotic occlusion of major vessels often seen in many sick neonates who undergo physiologically challenging surgeries and long postoperative recovery times.

Neonatal platelet counts and mean neonatal platelet volumes do not differ from normal adult ranges (34). However, neonatal platelets do show a notable decrease in function for the first 2–4 weeks after birth. When examined in vitro, platelets from both full-term and preterm newborns show decreased responses to a variety of standard agonists including epinephrine, ADP, collagen, and thrombin (42). This decreased responsiveness is manifest as a decrease in platelet granule secretion, a decrease in the expression of fibrinogen binding sites on the platelet surface, and a decrease in platelet aggregation as reflected in conventional laboratory assays (43). However, most in vivo assays of platelet function do not show platelet dysfunction. Bleeding times, platelet function analyzer closure times (PFA-100®; Dade Behring, Miami, FL, USA) and thrombelastometry coagulation times (ROTEM®; Pentapharm GmbH, Munich, Germany) are all shorter in neonates than adults suggesting that under physiologic conditions neonatal platelets are as efficient as adult platelets in achieving primary hemostasis (44,45). This inconsistency may be explained by the prominent role that vWF plays in neonatal hemostasis (44). As previously discussed, vWF is a large multimeric glycoprotein that assists in the adherence of platelets to areas of vascular injury. Compared with adults, neonates have not only a higher concentration of circulating vWF, but also a greater percentage of the large vWF multimers, the molecules most effective in promoting platelet-vessel wall adhesiveness.

Mean fibrinogen values are comparable between neonates and adults. However, evidence suggests that neonatal fibrinogen is qualitatively dysfunctional and exists in a fetal form until approximately 1 year of age (37). The original idea that a fetal form of fibrinogen might exist was based upon observations that the polymerization of fibrin from cord fibrinogen was slower than that of fibrin from adult fibrinogen (46). Additionally, biochemical studies have proven that neonatal fibrinogen has a different electrical charge and higher phosphorus content than adult fibrinogen (43,47). More recent studies utilizing thrombelastography (TEG®; Hemoscope Corp., Niles, IL, USA) measurements have confirmed the functional differences between fetal and adult fibrinogen (48). In adults, fibrinogen values show excellent correlation with TEG maximum amplitude (MA) after modification with a glycoprotein IIb/IIIa receptor blocker that uncouples platelet-fibrinogen interactions. This correlation is lost in children <1 year of age indicating that a dysfunctional state of fibrinogen exists in these children.

Plasminogen is the only coagulation protein in neonates that exhibits both quantitative and qualitative differences when compared to adult plasminogen. Normal newborn plasma contains approximately 50% of the plasminogen found in adult plasma. Neonatal levels of plasminogen do not reach those of an adult until approximately 6 months of age (37). Neonatal plasminogen itself is qualitatively impaired with slow activation kinetics by its major activator protein, t-PA. One investigation found that five times the amount of t-PA...
was required in the newborn to achieve similar activation of plasminogen to plasmin as seen in adults (49). Conversely, plasminogen activator inhibitor (PAI), a primary inhibitor of fibrinolysis, exhibits normal to elevated values at birth (37). When combined, these differences lead to an overall decrease in plasmin generation and fibrinolytic activity in neonates (50). These findings suggest that fibrinolytic therapy in the neonate may require a much higher concentration of activator to successfully induce a fibrinolytic state.

Conclusion

Blood coagulation is a complex physiologic process in which the proteolytic cleavage of fibrinogen to fibrin by thrombin leads to the formation of a stable fibrin cross-linked clot. Procoagulant proteins support thrombin generation and the formation of clot while anticoagulant proteins promote thrombin inhibition and the breakdown of clot. The specific cellular and protein interactions comprising these processes are interdependent and must operate in a coordinated manner or the entire system will be in jeopardy. Given this delicate balance, more prospective randomized controlled trials are needed to assess the efficacy and safety of rFVIIa and other coagulation factor concentrates when administered to pediatric patients with acquired bleeding conditions.

The hemostatic system of the newborn and young infant is strikingly different from that of older children and adults. However, despite the quantitative and qualitative deficiencies of multiple hemostatic proteins, neonates and young infants have excellent hemostasis. They show no signs of easy bruising nor do they experience increased bleeding during surgery (51). Nevertheless, critical illness often disrupts the hemostatic system altering the balance of procoagulant and anticoagulant factors thus predisposing these patients to hemorrhagic or thrombotic complications. Continuing research into the maturation of the hemostatic system is essential to the development of therapeutic strategies aimed at reducing hemostasis-related complications during episodes of severe illness in children.

Conflict of interest

None.

References


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