

## Review

**Mechanistic implications for the use and monitoring of recombinant activated factor VII in trauma**

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*Critical Care* 2005, **9(Suppl 5)**:S15-S24 (DOI 10.1186/cc3781)**Abstract**

As interest in the use of activated recombinant factor VII (rFVIIa) in trauma grows, questions arise regarding how best to monitor rFVIIa therapy and when rFVIIa may be expected to improve hemostasis. Knowledge of the mechanisms of action may be combined with available data on laboratory monitoring and efficacy in various coagulopathic states in coming to clinically relevant conclusions. This review addresses the physiology of hemostasis, placing emphasis on how rFVIIa influences the process by both tissue factor dependent and tissue factor independent mechanisms. This is extended to a mechanistic consideration of how rFVIIa may function under acidotic, hypothermic, and hemodilutional and/or consumptive conditions of trauma related coagulopathy. When these considerations are viewed alongside the available clinical data, it becomes apparent that rFVIIa has potential to improve hemostasis during trauma coagulopathy, within limitations. Common laboratory procedures are discussed with reference to mechanisms of action of rFVIIa and the available clinical data. Although there is no single assay that can predict rFVIIa efficacy in trauma, the prothrombin time (PT) is recommended as a minimum. Although a shortened PT does not predict success, correction of PT into the normal range may be a better indicator. A nonresponding PT appears to indicate that rFVIIa alone will not lead to hemostasis, and that additional blood products and other measures must be applied. Once the patient is more stable, PT and thromboelastography are recommended.

**Introduction**

In 1983, Hedner and Kisiel [1] reported the successful use of plasma derived activated factor VII (FVIIa) for the control of bleeding in two patients with factor VIII (FVIII) antibodies. This was followed by successful treatment in other patients [2]. However, the procedures required for purification of FVIIa from plasma precluded extensive use. The shortage was addressed when NovoNordisk Pharmaceuticals developed a method to produce recombinant human FVIIa (rFVIIa) using a transfected baby hamster kidney cell line [3]. The drug NovoSeven® (Novo Nordisk A/S, Bagsvaerd, Denmark) is

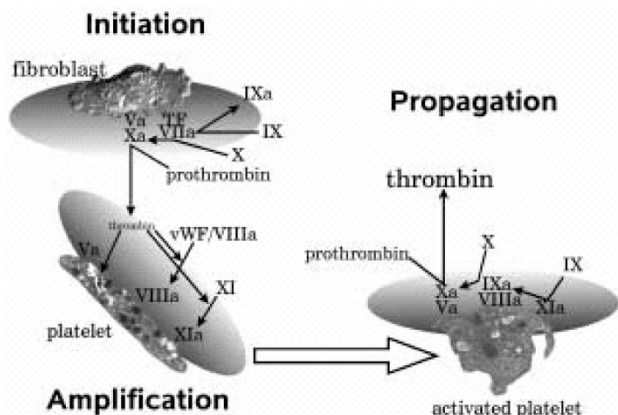
now registered in many countries worldwide for the treatment of spontaneous and surgical bleeding in patients with inhibitors against FVIII or factor IX (FIX) [3]. Over 700,000 standard doses (90 µg/kg) have been administered for acquired and congenital hemophilia [4]. Recently, there has been growing interest in the use of rFVIIa to control hemorrhage in patients without hemophilia. There are numerous reports of rFVIIa use in patients under a variety of circumstances, including trauma [5], as reviewed by Grounds and Bolan in this supplement. As interest in the use of rFVIIa in trauma grows, questions arise pertaining to how best to monitor rFVIIa therapy and when rFVIIa may be expected to work. Regarding the use of rFVIIa in trauma, knowledge of the mechanism of action may be combined with available data on laboratory monitoring and efficacy in various coagulopathic states in coming to clinically relevant conclusions.

**Cell based model of hemostasis**

Over 40 years ago the cascade model of blood clotting was introduced [6], a model that was later expanded to include both the intrinsic and extrinsic (tissue factor [TF]) pathways. Over the years, observations such as the fact that factor XII deficiency does not result in a hemorrhagic disease and the identification of important links between the two pathways [7,8] pointed to the importance of the TF pathway. By the 1990s it became clear that the TF pathway (extrinsic) of coagulation was of primary importance in normal hemostasis, and it became questionable whether the intrinsic pathway was relevant to *in vivo* hemostasis [9]. Lawson and coworkers [10] reported a model of TF initiated coagulation that described an initiation or lag phase, during which initiating enzymatic events result in the assembly of highly efficient enzyme complexes on phospholipid surfaces, followed by a propagation phase, during which an exponential burst of thrombin generation occurs. More

ACT = activated clotting time; aPTT = activated partial thromboplastin time; FIX = factor IX; FV = factor V; FVII:C = factor VII clotting activity; FVIII = factor VIII; FX = factor X; FXI = factor XI; PFA = platelet function analyzer; PT = prothrombin time; rFVIIa = activated recombinant factor VII; TEG = thromboelastography; TF = tissue factor.

Figure 1



A cell-based model of coagulation. The three phases of coagulation occur on different cell surfaces: initiation on the tissue factor bearing cell; amplification on the platelet as it becomes activated; and propagation on the activated platelet surface. vWF, von Willebrand factor. Reproduced with permission from Hoffman and Monroe [11].

recently, a cell based model has been proposed that differs from previous models primarily by emphasizing the cellular control of coagulation *in vivo* (Fig. 1) [11]. An understanding of this model is important in understanding the mechanism of action of rFVIIa. The cell based model includes three overlapping phases that describe events that take place on different cell surfaces: initiation, amplification, and propagation.

**Initiation**

TF is the primary physiologic initiator of coagulation [9] and is present in subendothelium and other tissues that are not normally exposed to blood [12,13]. TF is also expressed on monocytes following trauma [14] and on endothelium in response to activation by inflammatory mediators [15]. It may also be present in plasma [16] and circulating microparticles under some circumstances [17].

The process begins when vascular damage exposes blood to subendothelium, which is rich in TF and collagen. Both FVIIa (which circulates as 1% of total FVII) and FVII bind to TF on subendothelial cells. The FVIIa/TF complex activates both factor X (FX) and FIX [10]. TF bound FVII is activated by this small amount of FXa, producing more TF/FVIIa in a feed forward manner. FXa also activates plasma factor V (FV) [18], allowing the formation of the prothrombinase complex (FXa/FVa/calcium) on the TF bearing cell surface and leading to the production a small amount of thrombin [10,19].

**Amplification**

At the site of vascular injury, platelets adhere to the subendothelium and become partially activated. The small amount of thrombin produced during initiation fully activates

platelets [20]. This enhances adhesion [21], causes release of FV from platelet  $\alpha$ -granules, and induces a procoagulant configuration of cell membrane phospholipids and receptors [19]. On the activated platelet surface, thrombin produced during the initiation phase activates FV, FVIII, and factor XI (FXI) [20], which subsequently activates FIX to FIXa. The result is that the coagulation process has moved to the activated platelet surface, where the required components of the tenase complex (FIXa/FVIIIa/calcium) are assembled and ready to produce FXa, which will allow formation of the prothrombinase complex on the same surface in preparation for large scale production of thrombin [19].

**Propagation**

Small amounts of FIXa produced by the TF/FVIIa on the TF bearing cell diffuse to the surface of the activated platelet. This FIXa, along with FIXa produced by FXIa on the platelet, binds to the platelet surface and combines with FVIIIa, forming the tenase complex. The tenase complex converts FX to FXa, which then forms the prothrombinase complex in combination with FVa. The formation of these complexes makes possible the highly efficient generation of large amounts of thrombin from prothrombin [19]. The 'burst' of thrombin results in recruitment of more platelets as well as the production of fibrin polymers, activation of factor XIII, and other related events.

**Localization of the procoagulant process**

The coagulation process is localized to the site of vascular injury by several mechanisms [19]. The first is localized exposure of subendothelial elements. FVII and FVIIa have negligible enzymatic activity in the absence of TF. The binding of FVIIa to TF provides the enhanced FVIIa activity specifically where required. Binding and activation of platelets at the wound site, in close proximity to cells bearing the TF/FVIIa complex, provides the required procoagulant surface. Other mechanisms involve three key inhibitors of procoagulant enzymes and cofactors, antithrombin III, protein C, and tissue factor pathway inhibitor. FXa and thrombin that diffuse away from the protected surface of the cell are rapidly inactivated by antithrombin III [22]. Thrombin binds to thrombomodulin on adjacent undamaged endothelial surfaces. The thrombin/thrombomodulin complex activates protein C, which binds to a specific endothelial receptor and, with its cofactor protein S, inactivates FVa and FVIIIa, further controlling the spread of the coagulation process [23]. TF pathway inhibitor directly inhibits TF/FVIIa [24]. Other interactions among these enzymes and inhibitors are also involved. Additionally, endothelial cells possess a cell surface ADPase, which inhibits recruitment of platelets to bind to intact endothelium [25].

**Mechanisms of action of activated recombinant factor VII**

The normal ratio of FVII to FVIIa in the circulation is 100:1 (10 nmol/l and 0.10 nmol/l, respectively). Following rFVIIa administration the circulating total FVIIa (rFVIIa and native

FVIIa) concentration is increased approximately 100-fold (to 3–20 nmol/l) such that the FVII:FVIIa ratio is approximately 1:1. It is generally agreed that rFVIIa acts by enhancing thrombin generation at the site of injury, but there is controversy over the exact mechanism(s) of action by which this is accomplished. Current knowledge of the action of rFVIIa comes primarily from work using systems of simulated or actual hemophilia conducted to study the ability of rFVIIa to enhance thrombin production by 'bypassing' FVIIIa or FIXa and the need for the tenase complex. It appears that there are two principal mechanisms by which rFVIIa acts [26]: a TF dependent and a TF independent mechanism.

In both mechanisms, rFVIIa augmented coagulation is initiated at the site of vascular injury by the interaction of rFVIIa with TF. This results in initial FX activation followed by thrombin generation. Along with the exposed sub-endothelium, this initial thrombin acts to recruit and activate platelets at the site of injury. At this point the mechanisms diverge.

Under the TF independent mechanism, rFVIIa binds with low affinity to the surface of activated platelets. This platelet bound rFVIIa then activates FX to FXa. Although the enzymatic efficiency of this reaction is low relative to that of the TF/rFVIIa complex, the pharmacologic concentration of rFVIIa offsets this inefficiency. Therefore, FXa production becomes independent of TF with the accumulation of platelets at the site of injury [19,27]. Under the TF dependent mechanism, pharmacologic concentrations of rFVIIa result in an increased proportion of TF occupancy by (total) FVIIa (either FVIIa or rFVIIa), resulting in a higher concentration of TF/(r)FVIIa complex at the site of vascular damage [28]. The rFVIIa must bind to TF to enhance FXa production and ultimately thrombin generation [29,30]. Under either mechanism, the remaining steps involve combination of FXa with FVa on the surface of activated platelets to form the prothrombinase complex, which converts prothrombin to thrombin. The mechanisms are not mutually exclusive, and it is possible that both play a role [26]. Data suggest that the response in nonhemophilic blood may be slightly different, involving the additional activity of rFVIIa activating FIX [31].

The localized generation of large amounts of thrombin as a result of rFVIIa may have the additional beneficial effects of enhancing platelet adhesion and aggregation [32] and making formed clots more resistant to fibrinolysis by increasing activation of thrombin activatable fibrinolysis inhibitor [33] and/or producing thinner and more tightly packed fibrin fibers [34].

### Activated recombinant factor VII in trauma coagulopathy

With the rapidly expanding interest in the use rFVIIa in patients without hemophilia has come debate over whether rFVIIa may be considered a universal hemostatic agent

[35,36]. Both pro and con arguments in this debate acknowledge that hemorrhagic situations are not all the same. Even within the trauma setting a variety of situations exist, and the risks as well as potential efficacy may vary with the situation. With respect to the multifactorial coagulopathy associated with trauma, acidosis, hypothermia, hemodilution, and/or consumption of factors and platelets are important factors to consider [37].

### Activated recombinant factor VII and acidosis

The coagulopathy associated with acidosis appears to involve changes in both enzymatic rates [38] and platelet function [39,40]. *In vitro*, a reduction in pH from 7.4 to 7.0 reduces TF independent activity of rFVIIa by over 90% and TF dependent activity by over 60% [38]. Therefore, it is clear that reduced pH will result in some reduction in efficacy of rFVIIa. However, considering the fact that rFVIIa administration increases total circulating FVIIa levels by 100-fold, it is not clear at what level of acidosis the beneficial effect of rFVIIa would be nullified. The decrement in the TF independent mechanism is greater than the effect on the TF dependent mechanism. To the extent that one mechanism may be more important than the other in a given situation, the effects of pH may be variable. In addition to decrements in enzymatic efficiency, impaired platelet function during acidosis would also be expected to affect rFVIIa efficacy.

In one report [41] the clinical response to rFVIIa in trauma patients tended to decline at pH below 7.2 but the effect was only significant at or below 7.0. Dutton and coworkers [42] found that mean blood pH was 7.29 in the group of trauma patients who responded to rFVIIa whereas the pH in the nonresponders was 7.02 ( $P < 0.05$ ). However, six out of 20 nonresponders had a pH greater than 7.10, while five patients with pH at or below 7.10 responded to rFVIIa therapy. In a case report of a trauma patient who had an arterial pH of 7.09 at the time of rFVIIa administration [43], the drug was deemed efficacious. In an early report of coagulopathic trauma patients [44], rFVIIa was also effective during acidosis.

Based on currently available information, pH cannot be used as an absolute guide as to whether rFVIIa therapy will be effective, but it can be expected that efficacy will be reduced as pH declines. It is clear that blood pH should be taken into account when considering use of rFVIIa for management of trauma coagulopathy, and the recommendation to correct pH to at least 7.2 before rFVIIa administration is a reasonable guideline [41].

### Activated recombinant factor VII and hypothermia

It is well known that hypothermia decreases the activity of coagulation enzymes and impairs platelet function such that blood clotting is slowed. TF dependent activity of rFVIIa declines by 20% with a drop in temperature from 37°C to 33°C [38]. Interestingly, TF independent activity increases

under the same experimental conditions [38]. With the assumption that both the TF dependent and TF independent activities of rFVIIa play a role, it seems possible that the TF independent mechanism may make rFVIIa somewhat resistant to decreases in temperature in terms of hemostatic effect. On the other hand, rFVIIa cannot be expected to be effective for all degrees of hypothermia. For the initiation of blood clotting (initiation phase) at the site of injury, formation of the TF/FVIIa complex and generation of a small amount of FXa by the TF dependent mechanism is required. Initiation by this mechanism would diminish with decreasing temperature. The other theoretical option is for platelets to adhere to the subendothelium and become sufficiently activated for rFVIIa to bind and activate FX by a TF independent mechanism. The limiting factor in this case could become the lack of platelet activation under hypothermic conditions [45]. Therefore, it appears that there will be a level of hypothermia at which rFVIIa is no longer effective.

rFVIIa appears to be effective at temperatures normally encountered in trauma, although this has not been fully studied. Martinowitz and Michaelson [41] reported efficacy in trauma patients with an average core temperature of 34.1°C. Similar findings were reported by others [46,47]. In one study a hemostatic effect at a body temperature as low as 30°C was reported [44]. Two studies in swine [48,49] also suggested efficacy in hypothermia, although a third swine study [50] failed to confirm those findings.

Based on currently available information, rFVIIa can be expected to be useful for the control of nonsurgical bleeding under hypothermic conditions encountered in trauma (e.g. 33°C), but loss of TF dependent activity and decreased platelet function will likely lead to reduced rFVIIa efficacy as temperature approaches 30°C.

#### **Activated recombinant factor VII and hemodilution/consumption**

Examination of the model of blood coagulation and an inventory of the normal concentrations of coagulation factors makes it clear that the process is exquisitely balanced [22]. Changes in the concentrations of various components of the system can alter greatly thrombin generation and the clotting process as a whole [51]. Therefore, it is expected that sufficient dilution or consumption of various components of the coagulation system will result in coagulopathy. The potential for efficacy of rFVIIa will be eliminated when components other than FVIIa become rate limiting. For example, in the absence of FX or FV, rFVIIa does not shorten *in vitro* plasma clotting times [52]. There could also be a point when fibrinogen [37], prothrombin, or platelets become rate limiting. This notion is supported by data from 13 patients who were treated with rFVIIa for life threatening coagulopathy [53]. All patients received blood products before rFVIIa administration. However, patients who responded to treatment had a better coagulation status (as

measured by fibrinogen and platelet concentrations, PT, and activated partial thromboplastin time [aPTT]) at the time of treatment than did nonresponders.

Nonetheless, rFVIIa does appear to improve hemostasis in the presence of hemodilution/consumption, within limits. The trauma cases reported to date have included patients who received crystalloid and colloidal fluids as well as blood products in various combinations. It is expected that all of these patients would have some degree of consumption and/or hemodilution. There have been a number of reports of successful use of rFVIIa for patients in consumptive states (disseminated intravascular coagulation or similar states) resulting from trauma or other causes [54-60]. In several cases in which hemodilution was clearly identified, rFVIIa was effective in controlling bleeding [43,46,60-62]. Two studies in swine [48,49] also suggest efficacy in hemodilution, although a third study [50] failed to confirm those findings.

Further studies are needed to elucidate more fully the combinations of coagulopathic conditions under which rFVIIa will and will not be effective with regard to trauma. At the present time, to optimize the opportunity for rFVIIa to enhance hemostasis, it is recommended that factors and platelets be replaced to the extent possible before rFVIIa is used [37,41,57,63]. As a guideline, platelets should be maintained at a minimum of 50,000/ $\mu$ l and fibrinogen at a minimum of 50 mg/dl. Although the lower limits of pH and temperature under which rFVIIa may be effective are not clearly defined, efforts should be made to warm the patient and correct acidosis before administering rFVIIa treatment [41].

#### **Laboratory monitoring of activated recombinant factor VII**

There is currently no single laboratory test that is satisfactory for monitoring the clinical efficacy of rFVIIa [41,64,65]. By far the most commonly used tests have been the PT and the aPTT, but it has been suggested that the shortening of these two tests does not necessarily reflect clinical effectiveness [65]. A variety of other laboratory tests have been used to monitor or study the effects of rFVIIa under various conditions. When one is assessing the utility of a laboratory test in monitoring rFVIIa therapy, it is important to consider factors such as availability, turnaround time, and relevance of assay end-points to clinical outcomes, among others [66]. The following is a brief discussion of the potential applicability of selected laboratory tests in the trauma setting.

##### **Plasma coagulation assays**

###### *Prothrombin time*

The PT assay measures time to clot formation by the TF pathway. The assay is performed on a citrated plasma sample and takes only a few minutes to perform, although logistical considerations drive local turnaround times. The clotting reaction is initiated by adding TF and phospholipids (usually in the form of thromboplastin), and calcium. By the most

common method, clot formation is detected by the change in turbidity as the fibrin mesh forms. Because PT specifically examines the TF pathway, it is sensitive to rFVIIa. Many studies have demonstrated that rFVIIa shortens PT when coagulation is normal and in coagulopathy due to trauma and many other nonhemophilic conditions. The maximal degree of PT shortening is limited not only by *in vivo* parameters but also by the concentration of TF and phospholipids present in the assay reagents, and by the settings of the instrument.

PT is sensitive to both temperature and pH. Standard PT assays are run at 37°C and the pH dictated by the reagents used. Therefore, the correction in PT may not reflect the actual status of the patient under coagulopathic conditions. Some automated instruments now perform PT at the patient's temperature, which assists in interpretation (perhaps future advances in instrumentation will account for patient pH).

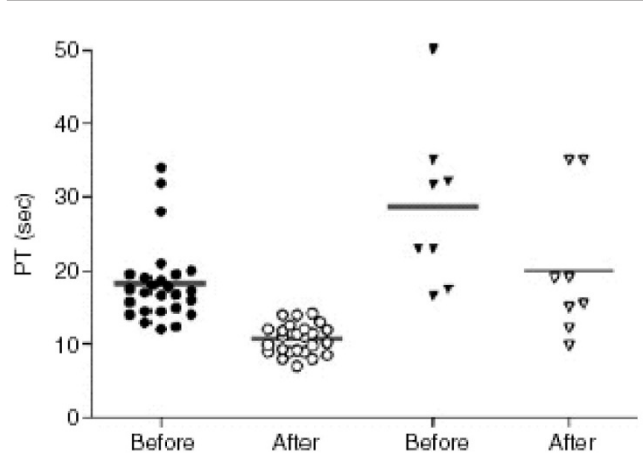
Simple improvement (shortening) of the PT cannot be used as a reliable indicator of rFVIIa hemostatic efficacy in trauma. Both Dutton and coworkers [42] and Eikelboom and colleagues [67] noted that all patients treated with rFVIIa had improved PT, even those who did not respond in terms of hemostasis. There is some indication that the degree of shortening of PT may correlate with hemostatic efficacy. Although the PT shortened in all patients following rFVIIa, Dutton and coworkers [42] found that the post-rFVIIa PT was shorter in patients who responded hemostatically than in those who did not. Martinowitz and Michaelson [41] reported similar findings (Fig. 2). Although currently available data are limited, it appears that correction of the PT into or below the normal range may be a better indicator of expected rFVIIa hemostatic efficacy than a simple numeric reduction in PT.

Although there have been many reports of rFVIIa shortening PT, we know of only one report in which hemostasis was attained without a shortening in PT following rFVIIa administration [68]. Therefore, the PT may be useful in identifying patients in whom rFVIIa will not be expected to work. If PT is not shortened after rFVIIa administration, then replacement of one or more factors in addition to FVIIa may be indicated.

The PT assay is useful in monitoring rFVIIa treated patients after a damage control or definitive surgical procedure, or after re-dosing based on the clinical scenario. The time course of PT correction follows that of increased plasma FVII activity after rFVIIa treatment and is dose dependent [48,49,69-79]. As an alternative to the FVII assay (e.g. if this is not available), PT may be used to monitor duration of expected rFVIIa correction of coagulopathy.

In summary, a simple reduction in PT is not useful in identifying responders to rFVIIa but the lack of a reduction may identify nonresponders. A reduction in PT into the normal range may prove to be a better indicator of potential hemo-

**Figure 2**



Shortening of prothrombin time (PT) values before and after administration of activated recombinant factor VII (rFVIIa) was significant in both 'responders' (circles;  $P = 0.0001$ ) and 'nonresponders' (triangles;  $P = 0.016$ ). However, in the latter group the mean PT values before and after treatment were significantly higher. Reproduced with permission from Martinowitz and Michaelson [41].

static effect. The PT can be useful in monitoring the duration of expected rFVIIa efficacy. Considering these factors, as well as the universality of the PT test, it is recommended that PT be used as a standard laboratory test in trauma patients receiving rFVIIa.

#### *Activated partial thromboplastin time*

This assay measures the time to clot formation by the intrinsic pathway and is performed using methods similar to the PT. The difference is that the clotting reaction is initiated by a source of celite or kaolin, a source of phospholipids (historically referred to as partial thromboplastin), and calcium. No TF is added. Often, rFVIIa treatment shortens aPTT. This phenomenon is due to TF independent activity of rFVIIa such that rFVIIa generates FXa independently of TF under the conditions of the assay [52,80]. The aPTT is useful for monitoring rFVIIa efficacy in clinical hemophilia [81], but this is not necessarily so in other patients. In some cases aPTT has been shortened following rFVIIa administration in trauma patients, whereas in others it has not. Therefore, we do not consider the aPTT to be a useful assay for making clinical decisions regarding rFVIIa use in trauma.

#### *Other standard plasma coagulation assays*

It is not normally expected that rFVIIa treatment will affect thrombin time, fibrinogen concentration, or levels of coagulation factors, with the exception of FVII. However, it has been observed that administration of rFVIIa may interfere with results of some factor assays, producing artificially elevated levels [82]. With the exception of the FVII assay described below, the standard laboratory coagulation tests that are based on plasma clot formation (thrombin time,

fibrinogen, clotting factors) are not useful in monitoring specifically the effectiveness of rFVIIa in trauma. Although rFVIIa treatment may interfere with the results of some of these assays, this phenomenon should not preclude monitoring of these parameters as necessary to assess overall patient status, for example to determine the need for blood products.

The most relevant of the standard factor assays for monitoring rFVIIa therapy is the FVII assay (factor VII clotting activity [FVII:C]). This assay does not measure specifically FVIIa or rFVIIa, but instead it measures total plasma FVII activity [83]. In a dose dependent manner, rFVIIa increases FVII activity in a sample far above normal levels [44,48,49,69, 71-73,75-79,82,84-86]. Therefore, the sample must be diluted by the laboratory in order to calculate an accurate value. The laboratory should be notified that this is expected. Although levels are related to the duration of PT correction, no study has demonstrated that FVII:C levels are predictive of hemostatic response to rFVIIa in trauma. The value of this assay in the management of rFVIIa in trauma is limited, but it can be used to confirm that the dose entered a patient's blood stream or to monitor the time course of FVII:C elevation.

#### *Thrombin generation test*

Thrombin generation tests have been used in research but clinical use has been very limited. In one version, the assay measures the amount of thrombin generated over time in platelet poor or platelet rich plasma by monitoring the change in fluorescence as a fluorogenic substrate molecule is cleaved by thrombin. rFVIIa increases thrombin generation as detected using this method [87]. Issues related to availability and complexity currently limit the use of this assay. However, as automated versions become available, it may become a useful assay for monitoring rFVIIa therapy in trauma.

### **Whole blood clotting assays**

#### *Thromboelastography*

Thromboelastography (TEG) is performed most commonly in North America using the Thromboelastograph (Haemoscope, Skokie, IL, USA). In other regions, a slight variation of this assay is performed using the roTEG Coagulation Analyzer (PentaPharm, Marburg, Germany). The same general principals hold for both instruments, although different nomenclature is used for the various clotting parameters. The nomenclature for the thromboelastograph is used for the purpose of this discussion.

TEG monitors changes in the viscoelastic properties of a forming clot. Either unaltered or citrated blood is delivered into a sample cup and clotting initiated spontaneously or using various agonists. A pin is suspended in the blood by a torsion wire and monitored for motion as the pin is repeatedly rotated through the blood in a 10 s cycle. Increased blood viscosity increases resistance to pin rotation and deflects the

pin. The deflection is translated into output measured in millimeters of amplitude, which increases as blood viscosity increases. Various standard parameters are calculated, including reaction time (R), coagulation time (K),  $\alpha$  angle, maximum amplitude (MA), time to reach MA (tMA), and per cent lysis at 30 or 60 min after MA. R reflects the time to initial clot formation. K reflects the time from R until a standardized level of clot firmness is reached (amplitude 20 mm). The  $\alpha$  angle is a measure of the kinetics of clot development. MA reflects the maximum firmness of the clot. Per cent lysis measurements assess the rate of fibrinolysis. Recently, two additional TEG parameters – maximum velocity of clot formation (MaxVel) and time to reach MaxVel (tMaxVel) – were reported for both versions of the TEG instrument [77,88]. These parameters appear more sensitive to rFVIIa than the standard kinetic parameters [88]. It is important to use dilute TF as the agonist to initiate clotting for best sensitivity [89,90]. TEG has more traditionally been performed using agonists that initiate clotting via the intrinsic pathway, but these agonists yield variable results with rFVIIa and are not optimal for monitoring this drug.

The most consistent effect of rFVIIa on TF initiated TEG is a shortening of the R [42,50,77,91-93], but this is not always observed [49]. Increased MaxVel,  $\alpha$ -angle and MA, and decreased K have also been observed [42,77,90-92]. Hendriks *et al.* [92] reported no change in rate of fibrinolysis after rFVIIa treatment. The principal advantage of the TEG is that it can assess the rate of whole blood clot initiation, rate of clot development, maximum clot strength, and fibrinolysis in a single assay. Because it is a whole blood assay it accounts for the number and function of platelets, as well as other cellular and plasma phase components. TEG is not a standard instrument in many hospitals, and the assay normally takes about 1 hour to perform, even when the instrument is located in the operating room. However, it can be performed much more rapidly if one is interested only in R. These drawbacks notwithstanding, TEG using dilute TF to initiate clotting can be a useful and sensitive method to monitor rFVIIa therapy.

#### *Other whole blood clotting assays*

The activated clotting time (ACT) assay measures the time required for clot formation using whole blood, usually with clotting initiated via the intrinsic pathway. Although the ACT is commonly available, reports of use of the ACT after rFVIIa treatment are limited. One study reported a faster clot time in blood from pigs treated with rFVIIa [77]. Recently available modified ACT assays that use a TF reagent to initiate clotting may prove useful in monitoring rFVIIa treated patients. The platelet function analyzer (PFA) assesses primarily the ability of platelets to form a hemostatic plug and has been related to bleeding time [94]. In one study [95] PFA values before and after rFVIIa treatment was not related to efficacy in patients with platelet defects. Current data do not support the use of PFA as a standard parameter in monitoring rFVIIa therapy in

trauma. The Hemodyne hemostasis analyzer (Hemodyne Inc., Richmond, VA, USA) monitors platelet contractile force over time and clot elasticity. Thrombin generation time can also be determined [96]. Although this assay offers some advantages over other whole blood assays and appears useful for rFVIIa monitoring, it is not widely available.

### Recommendations for monitoring and interpretation of laboratory values

Recently, several articles have examined the issue of rFVIIa monitoring [66,81,89,96]. Although numerous methods are available, the issue remains that there is no currently available single laboratory monitor that predicts rFVIIa efficacy [41,64,65]. There are factors related to trauma that make monitoring more problematic than for other patients. The most important of these is the issue of turnaround time. The rapidly changing status of the patient during the use of multiple blood products, colloids, and crystalloids during active resuscitation and ongoing hemorrhage is another factor, as is the uncertain half-life of a given dose of rFVIIa under these conditions. When a trauma patient has uncontrollable coagulopathic bleeding, it is usually not possible to wait for laboratory results before taking further action. Instead, empiric treatment with rFVIIa and direct observation must be used. Nonetheless, comparison of laboratory values after treatment with those before treatment can provide valuable information. Especially in cases in which continued bleeding may be obscured by packing, when intracranial hemorrhage is the target for hemostasis, or when rFVIIa treatment is administered under relatively more controlled circumstances, laboratory monitoring can be very useful.

The use of the PT is recommended. Although a simple shortening of PT does not predict hemostatic efficacy, correction of PT into the normal range may be a better indicator. It appears that a nonresponding PT indicates that rFVIIa alone will not lead to hemostasis, and that additional blood products and other measures must be applied. Once the patient is more stable, PT and TEG are recommended. FVII:C may also be useful in some situations. Other laboratory measurements, although useful for answering specific questions and monitoring overall patient status, are not necessary for basic rFVIIa monitoring in trauma.

### Conclusion

Whether by a TF dependent or TF independent mechanism, or by a combination, it is agreed that the hemostatic efficacy of rFVIIa is due to its ability to increase thrombin generation in a localized manner at the site of vascular injury. Thrombin generation is dependent on the assembly and activity of key enzyme complexes on the membranes of activated platelets. Mechanistic considerations and available clinical reports are consistent with the concept that rFVIIa administration can improve hemostasis under conditions of acidosis, hypothermia, and hemodilution/consumption, within limits. Because of the effects of acidosis and hypothermia on

enzymatic rates and platelet function, it is expected that extremes of acidosis and hypothermia will be reached at which rFVIIa can no longer be expected to function. Evidence for efficacy at least to a pH in the region of 7.2 and to a core temperature of 34°C is growing, although it is likely that the limits of efficacy are somewhat lower. When hemodilution/consumption reaches a point at which a component other than FVIIa becomes rate limiting, rFVIIa efficacy will be impossible without replacement of other components, such as FV, FX, prothrombin, fibrinogen, and platelets. Current recommendations to give blood products and to attempt to correct acidosis and hypothermia before rFVIIa administration are consistent with the current understanding of rFVIIa mechanisms of action [37,41,63] but further research is needed to define more clearly the limitations of rFVIIa efficacy under the multifactorial coagulopathic conditions associated with trauma.

During active resuscitation and damage control procedures, laboratory monitoring may be too slow to permit its meaningful use in clinical decision making. There is currently no replacement for direct observation of hemostatic effect. Comparison of laboratory values after treatment with those before treatment can provide valuable information. Especially when continued bleeding may be obscured by packing, when intracranial hemorrhage is the target for hemostasis, or when rFVIIa treatment is administered under relatively more controlled circumstances, laboratory monitoring is essential. Although no laboratory test can predict the hemostatic efficacy of rFVIIa, the use of the PT is recommended. A shortened PT does not predict success, but correction of PT into the normal range may be a better indicator. A nonresponding PT appears to indicate that rFVIIa alone will not lead to hemostasis, and that additional blood products and other measures must be applied. Once the patient is more stable, PT and TEG are recommended. FVII:C may also be useful in some situations.

### Competing interests

The author(s) declare that they have no competing interests.

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