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Various Local Hemostatic Agents with Different Modes of Action; an in vivo Comparative Randomized Vascular Surgical Experimental Study.

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Published in:
European Journal of Vascular and Endovascular Surgery

DOI:
[10.1016/j.ejvs.2006.10.011](https://doi.org/10.1016/j.ejvs.2006.10.011)

2007

[Link to publication](#)

Citation for published version (APA):
Björkses, K., & Holst, J. (2007). Various Local Hemostatic Agents with Different Modes of Action; an in vivo Comparative Randomized Vascular Surgical Experimental Study. *European Journal of Vascular and Endovascular Surgery*, 33(3), 363-370. <https://doi.org/10.1016/j.ejvs.2006.10.011>

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Citation for the published paper:
Bjorses, K and Holst, J.

"Various Local Hemostatic Agents with Different Modes of Action; an in vivo Comparative Randomized Vascular Surgical Experimental Study."
European Journal of Vascular and Endovascular Surgery, 2007, Vol: 33, Issue: 3, pp. 363-370.

<http://dx.doi.org/10.1016/j.ejvs.2006.10.011>

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Title:

Various local hemostatic agents with different modes of action; an *in vivo* comparative randomized vascular surgical experimental study.

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Abstract

Objectives:

To evaluate the hemostatic effect of principally different local hemostatic agents in a new high flow vascular experimental bleeding model.

Design:

Bovine thrombin combined with collagen matrix (bTcM), microporous polysaccharide hemospheres (MPH), freeze-dried rFVIIa with and without the combination of MPH were compared to a control group (solely compression) in a randomized fashion (20 animals/group). Primary endpoint was hemostasis, and secondary endpoints were time to hemostasis, blood loss, and blood pressure at hemostasis.

Methods:

The common carotid artery of heparinized rats was ligated proximally and transected. Compression was applied for one minute followed by application of the topical hemostatic agent. Compression was maintained for another two minutes followed by re-evaluation of hemostasis: if bleeding continued additional compression was applied and thereafter bleeding was checked every minute until hemostasis.

Results:

All animals in the bTcM group obtained hemostasis compared to 20% in the control group ($p < 0.0001$). The combination of MPH and rFVIIa (70% hemostasis) also showed a significant hemostatic capacity compared to control group ($p < 0.001$). None of the other active treatment groups differed compared to control group. Animals treated with bTcM had a significantly shorter time to hemostasis compared to animals in the other active treatment groups. No significant difference in blood loss and blood pressure at hemostasis was detected.

Conclusions:

The most effective hemostatic agent was bTcM, followed by the combination of rFVIIa and MPH, while neither MPH nor rFVIIa alone displayed any hemostatic capacity compared to compression only.

Key words: local hemostasis, bovine thrombin, microporous polysaccharide hemospheres, recombinant FVIIa

Introduction

Correct surgical technique is a prerequisite for successful vascular surgery. Inadequate hemostasis can jeopardize the outcome of a procedure. In spite of immaculate technique, hemostatic problems may require the use of adjuvant topical hemostatic sealing. In recent years an abundance of topical hemostatic agents have become commercially available.

The sealing products are usually a preparation of a procoagulant substance with a combination of a vehicle such as exogenous thrombin and collagen matrix (i.e. FloSeal[®], Baxter, USA), for example. The thrombin is either derived from human plasma, or of animal origin; currently recombinant coagulation factors are not available for this indication.

Another principle for topical hemostasis is the use of a matrix, such as oxidized cellulose or gelatin sponges, which activates and provides a template for the endogenous coagulation cascade to obtain hemostasis¹. A different matrix has recently been made available: microporous polysaccharide hemospheres (purified starch, Traumadex[®], Medafor, USA). The starch is able to absorb fluid from the blood that causes a concentration of the endogenous coagulation factors and platelets, which in turn facilitate hemostasis. Systemic use of recombinant FVIIa (NovoSeven[®], NovoNordisk, Denmark), originally developed for the treatment of hemophilia patients², has been used successfully off-label to decrease bleeding in trauma patients³.

All hemostatic agents have their clinical shortcomings, however, there is an absence of objective data and comparative studies between modern topical hemostatic agents in a clinical vascular surgical setting. Unquestionably clinical trials are difficult to perform since the cause of bleeding can be diverse, i.e. the origin of the bleeding may be arterial, venous or oozing. Further, the presence or absence of concomitant anticoagulation/antiplatelet therapy may differ, and blood pressure variation, possible acidosis or hypothermia may further cloud the picture. All these factors influence bleeding considerably and make the interpretation of clinical data a considerable challenge.

Therefore we have focused on a new and validated experimental model in the rat to imitate a high flow vascular bleeding to evaluate principally different agents under controlled conditions. The aim of the study was to evaluate the local hemostatic effect of a preparation of bovine thrombin combined with a collagen matrix (bTcM), and to compare it with microporous polysaccharide spheres (MPH) and topical rFVIIa alone and in combination in a randomized experimental setting.

Material and Methods

Animals

Adult acclimatized Sprague-Dawley rats were used. The model development, validation and the comparative study was performed in accordance to the guidelines of good laboratory practice and approved by the Local University Ethics Committee for Animal Experiments.

Model development and validation

There are several bleeding models described in the literature. Parenchymal bleeding models have been used in rat, rabbit and swine⁴⁻⁷. Injuries to the groin by transection of the femoral vessels in swine have been used in models simulating battlefield trauma^{8,9}. Arterial anastomotic leakage has been experimentally simulated in various aortic anastomoses in rabbits^{10,11}.

The aim of our experimental study was to compare different local hemostatic agents in a model imitating a common hazard in vascular surgery: high pressure bleeding from the suture line or stitch holes in anastomoses or patches where additional sutures are difficult to place and compression by itself may be inadequate. Our purpose was to create a high flow arterial bleeding model, which would be repeatable and standardized, with an endpoint variability that was within acceptable limits.

An arterial transection in rat simulates the above-mentioned bleeding well. To avoid a laparotomy, which is likely to cause hypothermia and thus affect the coagulation, we focused on two extra abdominal arteries with suitable dimensions for our purpose: the common femoral and carotid arteries. We evaluated retrograde and antegrade bleeding from these locations in 54 animals with respect to the frequency of obtaining hemostasis, time to hemostasis and blood loss (data not shown). Bleeding from the femoral artery presented larger variation and was less repeatable than bleeding from the common carotid artery. The femoral artery bleeding model was therefore disregarded. The common carotid artery is a larger vessel and easily accessible. The carotid bifurcation is distal of the mandible and the common carotid artery is of a technically acceptable length and facilitates repeatable transections in the middle, between the clavicle and at the bifurcation of the vessel. Of the 54 animals, 46 were evaluated with carotid transections. Retrograde bleeding of the carotid artery was the most suitable model since the bleeding is severe and continuous and without being immediately fatal compared to the antegrade bleeding. We found that the model fulfilled the objective criteria postulated, and it was clearly effective for a high throughput in vivo assay.

Experimental studies of endarterectomies in the common carotid artery have revealed that without heparin the majority of the animals develop thrombi in the damaged vessel^{12,13}. In our validation of transection of the common carotid artery, 80% of the animals obtained hemostasis on solely compression, without heparin administration. Thus we were required to heparinize the animals to create a negative control group and obtain repeatable results. We also wanted to imitate the clinical situation of vascular surgery, where all patients are heparinized. Reviewing the literature of experimental bleeding models, we found the dose of heparin administered in different settings was considerable and variable. Doses between 200 and 2000 IU/kg bw have been used^{4-6,10,11}, but such a high dose in an experimental model must be cautiously extrapolated to the clinical setting. Two different doses of unfractionated heparin, 150 and 300 IU/kg bw, were validated in our model. The two doses did not render any difference in measured bleeding parameters, and gave a consistent and reproducible

prolongation of Activated Clotting Time (ACT). Thus we continued to work with the lower dose (150 IU/kg bw).

No differences in parameters studied, except body weight were noticed between male and female rats during our validation. Due to their more convenient larger size (larger blood volume, larger vessel size), we chose to perform the comparative study with only male rats.

To ensure that bovine thrombin had the intended effect in rat plasma, we performed a dilution series with plasma from five rats and the bovine thrombin used in bTcM. The response in the activated prothrombin time assay was dose dependent and gave a reproducible foreshortening (data not shown).

The comparative study

Study design

Five different treatment groups (20 animal/group) were evaluated. All animals were treated with compression of the bleeding site (the distal part of the transected common carotid artery) for one minute, and then the topical agent was administered in a randomized fashion. Compression was reapplied for two minutes and possible hemostasis was evaluated. Further compression was repeated and controlled every minute until hemostasis was obtained.

- 1) Control group – solely compression: compression was applied with cotton covered application sticks and swabs.
- 2) Combination of bovine thrombin and a collagen matrix (bTcM, Floseal[®], Baxter Inc, USA). bTcM was prepared and used according to the directions provided the by manufacture. One ml of the high viscosity suspension was used for each animal.
- 3) Microporus Polysaccharide Hemospheres (MPH, Traumadex[®], Medafor Inc, USA) MPH was and used according to the directions provided by the manufacture. One ml of the powder was used for each animal.
- 4) Recombinant FVIIa, (rFVIIa, Novoseven[®], NovoNordisk A/S, Denmark) was used in freeze-dried form and it was topically applied at the bleeding site. 0.2 mg of the powder, which is 1/3 ml of the uncompressed freeze-dried powder, was used per animal.
- 5) Combination of MPH and rFVIIa. A mixture of 1/3 ml of the freeze-dried rFVIIa and 2/3 ml of MPH was used per animal.

The dose of the hemostatic agents was chosen by practical reasons; 1 ml of bTcM and MPH was a suitable amount of agent that fits in the operating area, and it also resembles the clinical situation well. Recombinant FVIIa has to our knowledge not been used in freeze-dried form as a topical hemostatic agent. The optimal dose for rFVIIa in this setting is unknown. The dose we evaluated in this study was clearly supraphysiological at the actual bleeding site. The strategy of using freeze-dried rFVIIa topically should be looked upon as a possible proof of concept.

The primary endpoint evaluated was hemostasis, and the secondary endpoints included analysis of time to hemostasis, blood loss, blood pressure at time of hemostasis and rebleeding.

One hundred animals (median weight: 322g, iqr: 145g) were included in the study, and randomized to separate treatment groups. Block randomization was used. One block contained five animals, one animal for each treatment group. In total there were twenty blocks, and twenty animals in each treatment group. The randomization (sealed envelope) was performed after the surgical exposure was performed. One surgeon (KB) performed all the evaluation of bleeding parameters.

Standard operating procedure

The acclimatized animals were administered a subcutaneous injection of neurolept anesthesia, utilizing a mixture of Hypnorm[®] (0.135 mg/ml fentanyl citrate & 10 mg/ml fluanisone, Janssen Pharma Inc, Belgium) and Midazolam Hameln[®] (5 mg/ml, Pharma Hameln, GmbH). The experimental animals never regained consciousness. Rats who survived the bleeding evaluations were euthanized intravenously with an overdose of phenobarbiturate acid and ethanol.

The animals were placed on a heat pad in a supine position with spontaneous respiration. Rectal temperature was measured at the start and end of the operation. One groin was opened and the femoral vessels were dissected, ligated distally and catheterized (PE-50, Becton Dickson Inc, USA). The arterial catheter was used for blood pressure measured by Powerlab[®] (AD-instruments Inc, UK). The venous catheter was used for administration of unfractionated heparin (500 IU/ml, Løvens A/S, Denmark) and saline.

A skin incision was made above the clavicle, and the right common carotid artery was dissected proximal to the carotid bifurcation. Three drops of papaverinsulphate (40 mg/ml, Recip AB, Sweden) was administered topically on the vessel to prevent spasm. Unfractionated heparin 150 IU/kg bw was given iv and allowed to equalize for one minute before start of experiment. The artery was then ligated caudally and transected with scissors, and consequently the artery bled retrograde from the cranial portion.

All animals were treated with compression on the transected artery with gentle pressure using a wooden cotton covered applicator stick (SelefaTrade AB, Sweden) for one minute, and for exact placement of the stick a microscope was used with 10-20 times magnification (Leica, Wild M650, GmbH). The topical agent was then administered in a randomized fashion. Compression was reapplied with a clean non-woven swab (SelefaTrade AB, Sweden) 5x5 cm, folded twice into 2.5x2.5 cm, and kept in place by digital pressure for two minutes. Possible hemostasis was then evaluated and if necessary compression was repeated and controlled every minute until hemostasis was obtained. If the swab was soaked in blood another one was held firmly on top and changed if soaked through. To keep the local hemostatic agent in place at the transection, the innermost compress was not changed during observation. The pressure on the swab was evaluated under the microscope to ensure that it was firm enough to maintain hemostasis without compromising the spontaneous breathing.

Bleeding time was measured from transection of the artery until hemostasis by ocular verification using a microscope (Leica, Wild M650, GmbH). If the animal continued to bleed after twenty minutes of observation primary hemostasis was considered not to be achieved and euthanasia was performed.

If the animal achieved hemostasis within twenty minutes possible rebleeding was checked after another five minutes. Blood loss was measured by the corrected weight of the cotton covered applicator stick and the swabs. The actual bleeding weight was then related to the weight of the animal to correct for different animal weights, which in small animal affect total blood volume and the possible amount of bleeding.

The unfractionated heparin induced anticoagulation level was monitored using Activated Clotting Time (ACT, Hemocrone[®] Jr, Int Technodyne Corp, USA). It was measured as a baseline and at the end of the procedure.

Statistics

The size of the study groups was calculated to confirm a 20% difference of the primary endpoint. Descriptive data are expressed as median and individual observations/interquartile range. Since the distribution of data was skewed non parametric analysis was performed using variance analysis according to Kruskal-Wallis between several groups and, when appropriate, Mann-Whitney U-test for unpaired data between groups and Wilcoxon signed rank-test for paired data within groups. For contingency tables chi-square and Fishers exact test were used. A p-value of <0.05 was considered significant and all test were two-tailed. The software StatView 5.0 (Abacus Concepts, USA) was used.

Results

One animal in the rFVIIa-group died prematurely and was excluded from the analyses. All other animals survived anesthesia and the preparatory surgery. Dissection related blood loss was negligible. The overall operation time was approximately one hour from beginning of anesthesia to the end of the experiment. The body temperature increased (approximately 0.4°C) significantly in all animals during the procedure regardless of whether hemostasis was achieved or not.

The activated clotting time was prolonged at the end of the procedure between 2.5-3 times the baseline values (data not shown).

The overall analysis for the primary endpoint hemostasis (fig. 1) was significant ($p < 0.0001$) and the group treated with bTcM was superior. In the bTcM-group all animals obtained hemostasis, whereas only 1/4 of the animals in the control group (compression) ceased to bleed ($p < 0.0001$). The animals treated with MPH or rFVIIa did not significantly differ from each other or the control group. The hemostatic effect was significantly improved ($p < 0.001$) when MPH and rFVIIa were combined and compared to the control group. When the hemostatic capacity of the combination of MPH and rFVIIa was compared to bTcM a significant difference ($p = 0.02$) was observed in favor of the bTcM treatment.

Time to hemostasis (fig. 2); there was a significant overall treatment effect ($p = 0.0005$). The animals in the bTcM group had the shortest time to hemostasis (median 5 min. 15 sec, iqr 3 min. 40 sec), which was significantly shorter compared to the animals in all the other active treatment groups.

There was no significant difference between the treatment groups with respect to the amount of weight adjusted blood loss during the experiment (fig. 3).

Mean arterial blood pressure before the transection of the carotid artery was 88 mmHg (iqr: 15.5 mmHg) for all animals. In fig. 4 the blood pressure before transection and at the time of hemostasis is shown. There was no significant difference between the different treatment regimes. As anticipated, pair wise comparison between the baseline value and the mean arterial pressure at the time of hemostasis revealed that the APB dropped in all animals irrespective of treatment group.

Rebleeding did not occur in the four animals in the control group that received hemostasis. In the other groups rebleeding did occur (bTcM: 5/20, MPH: 3/8, rFVIIa: 1/4, MPH+rFVIIa 2/14), albeit the frequency of rebleeding was not statistically significant between the active treatment groups. Five of the rebleedings were fatal; three animals in the MPH, one in the bTcM and one in the rFVIIa group respectively.

Discussion

Clinical comparative studies of topical hemostatic agents are as discussed above challenging to interpret. Many of these factors can only be reduced and minimized in an experimental setting. Nevertheless, an *in vivo* experimental setting remains challenging, since the natural variability in all bleeding models is considerable.

During the validation work of the present experimental model both advantages and disadvantages of the model were revealed. Due to the anatomic limitations at the bleeding site, and the profuse bleeding from the carotid artery, the precise administration of the hemostatic agents must be performed with great care. To avoid asphyxia in the animal, compression of the bleeding site must be executed cautiously, especially when the extra volume of the local hemostatic agent is added. Although we used a microscope (6-40 times magnification) it is at times surprisingly difficult to determine the exact time when the hemorrhage gradually diminished and finally ceased. Moreover, the variability becomes greater if more than one person is involved in the assessment of the bleeding. In attempt to reduce this variability only one surgeon did the assessments in the presented study.

A further limitation of the present study is that it could not be performed blinded. Local hemostatic agents have recognizable different physical properties; these differences prevented us from doing a blind study. We chose the block randomization design to minimize potential bias.

The number of animals in each treatment group (20 rats) was adequate to evaluate the primary endpoint with confidence, the interpretations of the secondary endpoints should be done cautiously since a limited number of animals in most treatment regimes got hemostasis and were assessable.

Rebleeding was confirmed by ocular inspection in the microscope, which contrary to the termination of bleeding was easily defined when fresh blood appeared in the previously dry transaction area. An unanticipated 25% of the bTcM group rebled. There is no obvious explanation for this lack of clot stability, and this is interesting observation requires further studies, which are currently underway in our laboratory.

Animals that obtained hemostasis with a blood pressure lower than the prebleeding pressure, received after the five minutes observation time, resuscitation with saline until blood pressure normalized. The aim was to evaluate the stability of the hemostatic clot. Whether the saline itself contributed to any hemostatic deficiency is not possible to evaluate from this study, but it cannot be ruled out. The limited sample size made the resuscitation maneuver difficult to evaluate. It seems, however, that if hemostasis was obtained, the clot was stable to the increased blood pressure.

Various studies of local sealants have been made in recent years^{4,11, 14-17}. Most often fibrin sealants have been compared to cellulose pads and collagen dressings, with and without thrombin of various origins. Fibrin sealants have been superior to all other preparations at all endpoints assessed^{4, 5,10,11,15,17}. Fibrin containing preparations have to be applied on a dry surface, which is not always preferable or even possible to obtain in reality. We wanted to evaluate sealants that interact with the endogenous coagulation, and do not require a dry surface to be effective.

Although several different agents are commercially available, the optimal remains to be found. Such an optimal agent should possess certain qualities. Obviously the hemostatic agent should effectively stop various types of bleedings irrespective of the presence of anticoagulation/antiplatelet therapy. In the present study bTcM and the combination of MPH+rFVIIa were clearly able to stop the experimental vascular surgical bleeding (100 and 70% respectively). bTcM has been evaluated in animal models¹⁰ and in vascular, cardiovascular and spinal surgery studies, and it has proved to be a reliable haemostatic agent^{14, 18,19}, which our study confirms.

Ideally the procoagulant factor should preferably be of a synthetic or recombinant origin. Using a bovine coagulation factor causes an immunologic risk of developing autoantibodies to human coagulation factors, and potentially causing an iatrogenic hemophilia/thrombophilia. Such antibodies are a well-recognized clinical hazard²⁰⁻²². To minimize or avoid such an immunological problem would be to use a human plasma derived procoagulant factor. Interestingly the thrombin used in bTcM for the North American market is plasma derived whereas it is still of bovine origin in Europe. But using human plasma derived coagulation factors have the well-known risk of virus transfection.

For the above reasons rFVIIa seemed especially attractive to evaluate in this setting. The adjunctive intravenous administration of rFVIIa has recently proved capable of significantly reducing blood loss in severe blunt trauma patients in an international multicenter randomized placebo controlled double blind clinical study²³. Recombinant FVIIa has also recently, for the first time in peripheral vascular surgical setting, been reported to significantly decrease transfusion in a retrospective consecutive single center clinical study of intractable bleeding²⁴. In our study topical application of only rFVIIa was not beneficial, whereas the combination of MPH and rFVIIa significantly improved the primary endpoint hemostasis. This may indicate that rFVIIa requires a vector or matrix to be effective as a local hemostatic.

MPH has been studied in traumatic models in swine that involves transection of the femoral vessels. No difference in any endpoint compared to traditional pressure dressing could be registered⁹, which is in accordance with our results. Another study evaluated MPH in partial nephrectomies in swine, and animals treated with MPH had a shorter bleeding time and reduced blood loss compared to cellulose pads⁷. This is, however, contrary to our results, but in the partial nephrectomy study the MPH was applied with a special suction applicator and it is unclear whether the animals were heparinized or not. We observed a clear hemostatic capacity with MPH when it was combined with rFVIIa. Another potential way of increasing the efficiency of the MPH would be to add a fibrinolytic inhibitor such as tranexamic acid or add crystallized calcium ions to the MPH powder since physiologic coagulation requires the presence of calcium.

Several practical features of the topical hemostatic agents also need to be accounted for: the preparation time of the agent, the shelf life and economical aspects. From this point of view MPH has a distinct advantage compared to both bTcM and rFVIIa. The physical properties of the agent are also important; it should be easy to apply correctly. Subjectively we considered the physical properties of bTcM to be more appealing compared to MPH. In our hands in the present model, MPH was less straightforward to apply and tended to dislodge while maintaining pressure on the bleeding site. On the other hand, the time for preparation of bTcM is a shortcoming.

In conclusion: in the present experimental randomized comparative study of different local hemostatic agents in a vascular surgical bleeding model, we were clearly able to show that bTcM (FloSeal[®], Baxter, USA) was capable of obtaining hemostasis whereas MPH (Traumadex[®], Medafor, USA) was no better than solely compression. If MPH was combined with local application of freeze dried recombinant FVIIa (NovoSeven[®], NovoNordisk, Denmark) the hemostatic efficacy was significantly better than solely compression, MPH alone and rFVIIa alone. It remains uncertain to what extent MPH is the ideal vector/matrix for rFVIIa, and further studies are required to find the matrix that is most suitable for rFVIIa.

These data need to be confirmed in other vascular surgical bleeding models where animals not only are heparinized, but also are treated with platelet inhibitors and subjected to hypothermia and/or acidosis before clinical recommendations can be given.

Acknowledgement

The authors wish to greatly acknowledge the important and fruitful collaboration with Dr Bengt Lindblad Dept of Vasc Diseases, Malmö University Hospital, Lund University, Malmö, Sweden. Dr Peter Månsson Dept General Surgery, Halmstad General Hospital, Halmstad, Sweden for generously lending the authors the blood pressure equipment and Cecilie Schwartz at AD Instruments.

The Swedish representatives from V-Tech, Magle Life Sciences, Sweden for supporting us with MPH free of charge and Baxter Medical AB for discounting the purchase of bTcM.

Funding

This work was funded by the foundation of Hulda Almroth, Sweden.

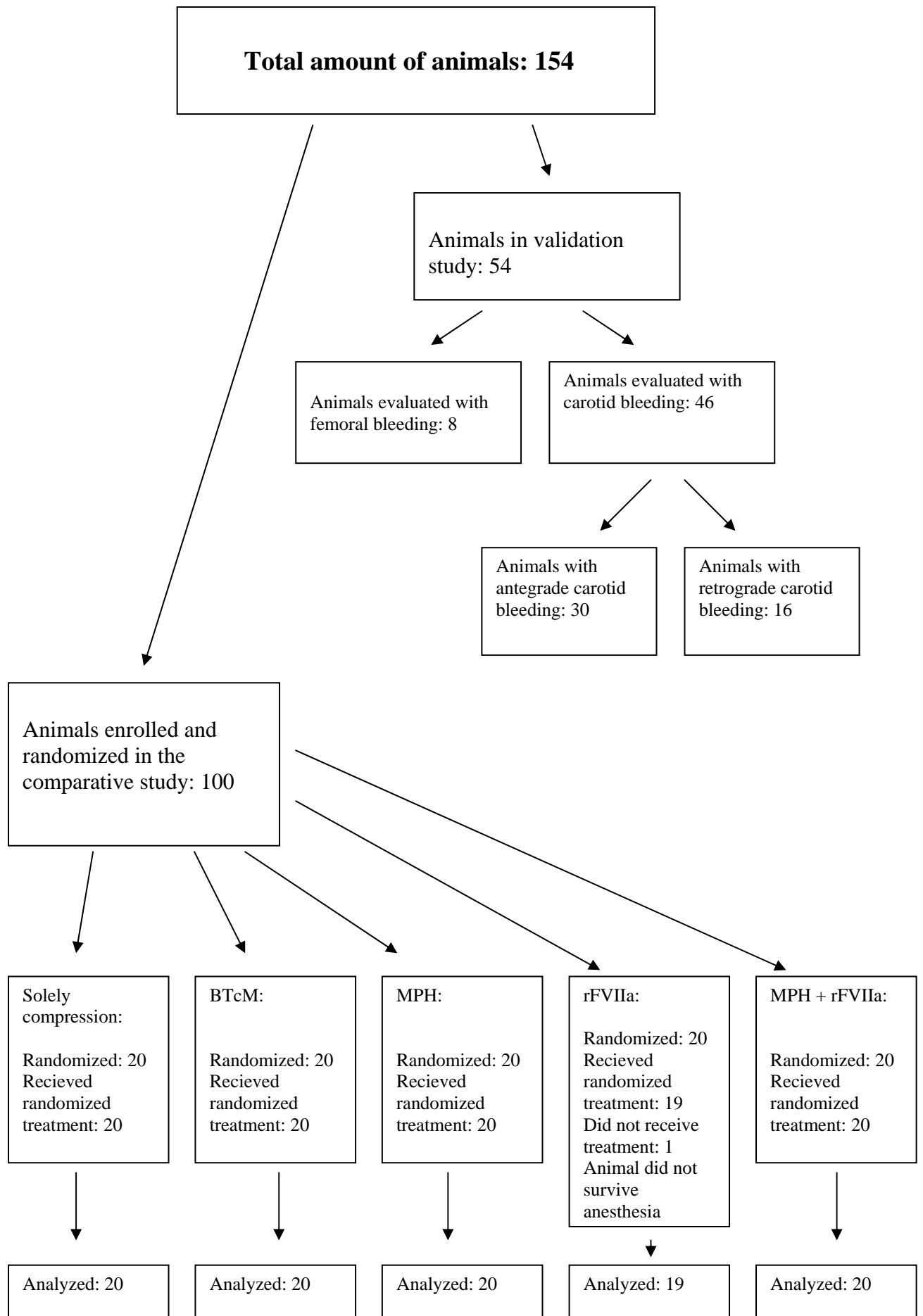
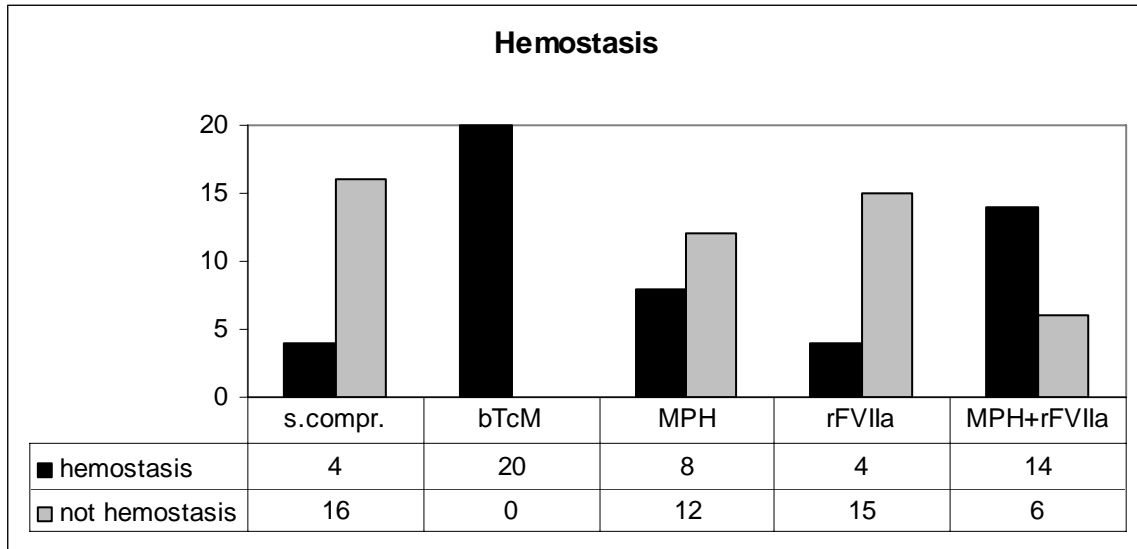
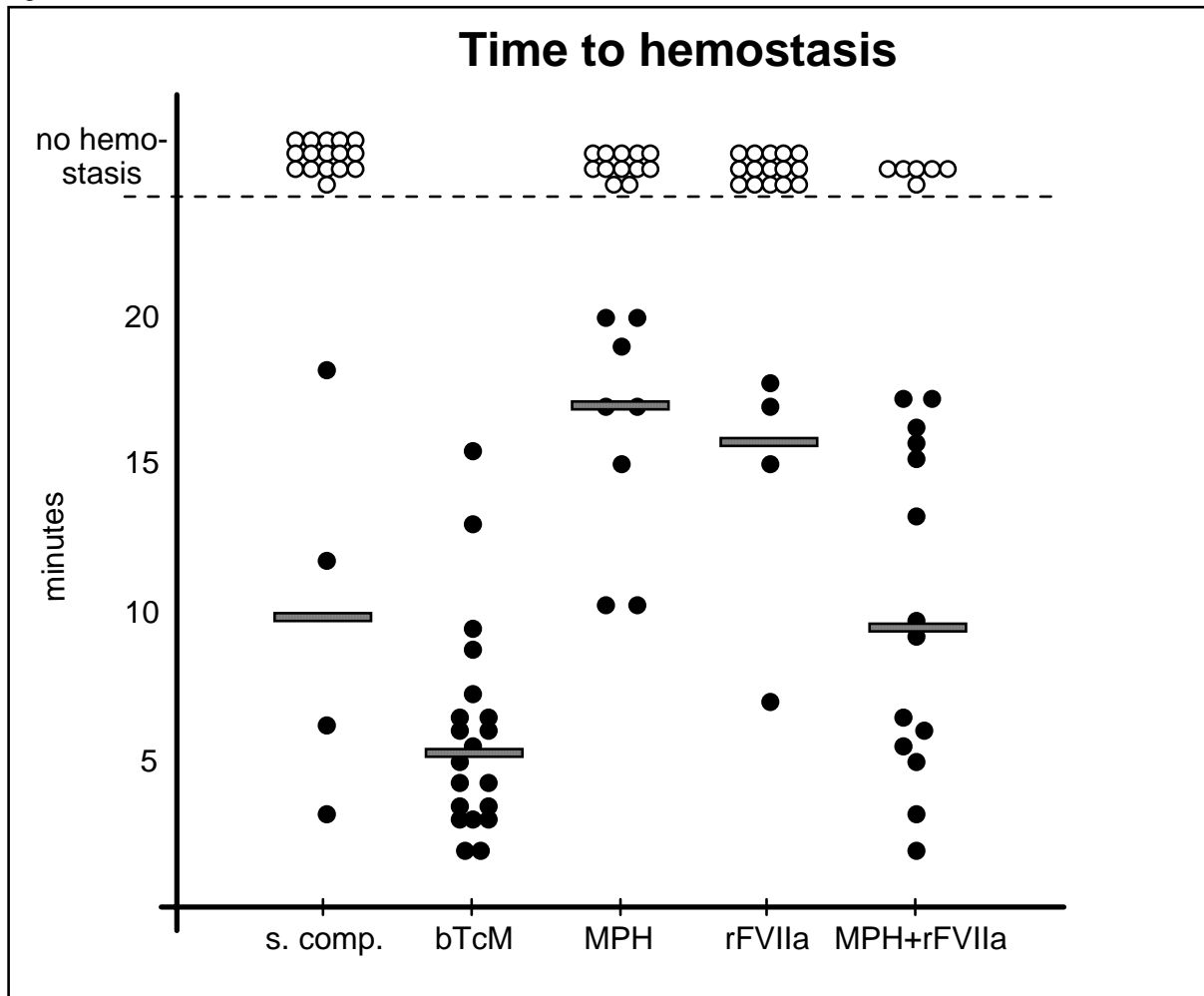


Fig 1



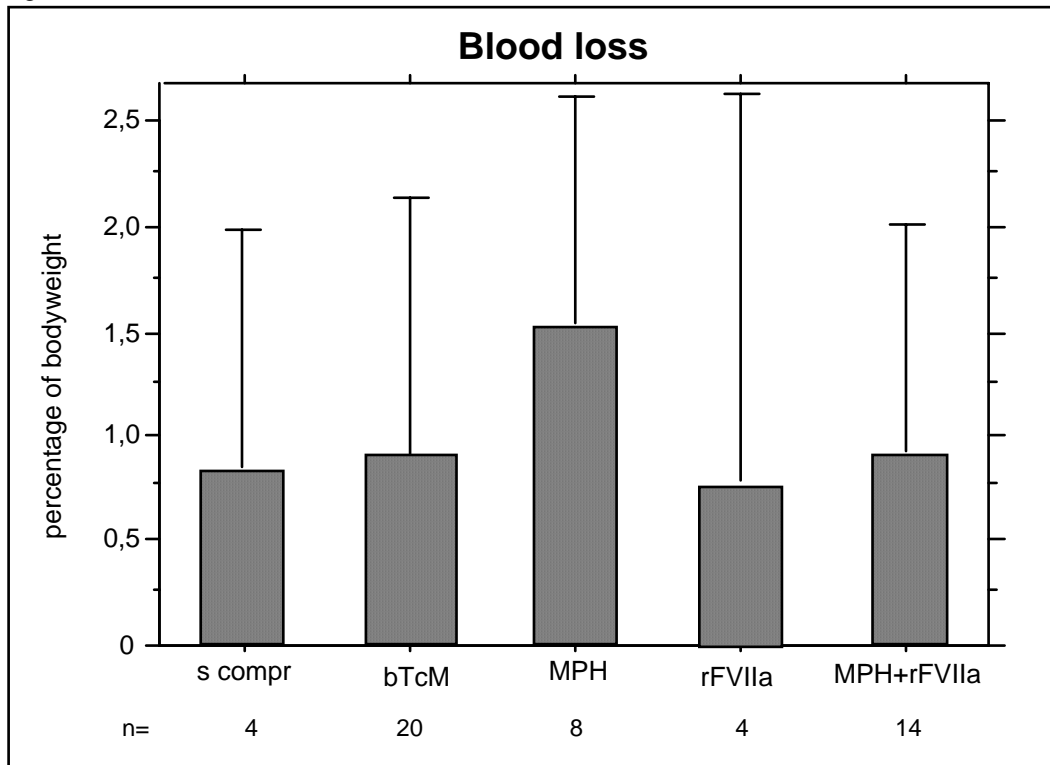
Primary Endpoint: diagram presenting animals obtaining hemostasis or continued to bleed throughout the 20 min observation time. Only animals treated with bTcM or the combination MPH and rFVIIa were significantly superior to surgical compression.

Fig 2



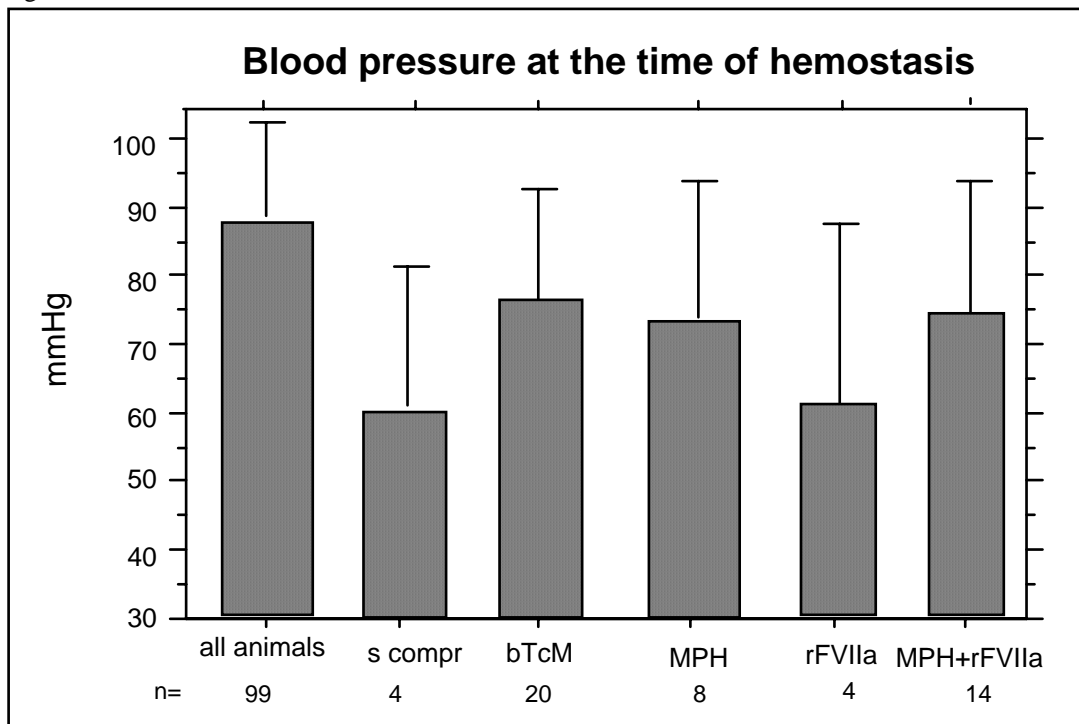
Secondary endpoint: time to hemostasis; individual animals that obtained hemostasis are depicted as filled circles, whereas animals that did not receive hemostasis are depicted with open circles. Numbers of animals are limited (<5) in the two groups receiving surgical compression (control group) and rFVIIa. The bTcM treated animals had a significantly shorter time to hemostasis compared to the other active treated animals.

Fig 3



Secondary endpoint blood loss (median and interquartile range) presented as a ratio of the measured blood loss divided with the bodyweight of the animal. Only animals, which obtained hemostasis, are included in the diagram.

Fig 4



Median arterial blood pressure in all animals prior to transection of the carotid artery compared to the animals obtaining hemostasis. All animals got the anticipated pressure fall after transection but no significant difference was identified between the treatment groups at the time of hemostasis.

References

1. Jackson MR, MacPhee MJ, Drohan WN, Alving BM. Fibrin sealant: current and potential clinical applications. *Blood Coag Fibrinol*. 1996; 7: 737-46.
2. Hedner U, Erhardttsen E. Potential role of rFVIIa in transfusion medicine. *Transfusion* 2002; 42: 114-24.
3. Holcomb JB. Use of recombinant activated FVII to treat the acquired coagulopathy of trauma. *J Trauma* 2005; 58: 1298-303.
4. Tuthill DD, Bayer V, Gallagher AM, Drohan WN, MacPhee MJ. Assessment of topical hemostats in a renal hemorrhage model in heparinized rats. *J Surg Res* 2001; 95: 126-32.
5. Jackson MR, Taher MM, Burge JR, Krishnamurti C, Reid T J, Alving BM. Hemostatic efficacy of a fibrin sealant dressing in an animal model of kidney injury. *J Trauma* 1998; 45: 662-5.
6. Petratos PB, Ito K, Felsen D, Poppas D. Crosslinked matrix tissue sealant protects against mortality and hemorrhage in an acute renal injury model in heparinized rats. *J Urol* 2002; 167: 2222-4.
7. Murat FJL, Ereth MH, Dong Y, Piedra MP, Gettman MT. Evaluation of microporous polysaccharide hemospheres as a novel hemostatic agent in open partial nephrectomy: favorable experimental results in the porcine model. *J Urol* 2004; 172: 1119-22.
8. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, Mosebar RH, Reid TJ, Nelson JH, Goodwin CW, Fitzpatrick GM, McManus AT, Zolock DT, Sondeen JL, Cornum RL, Martinez RS. Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in swine. *J Trauma* 2003; 55: 518-26.
9. Alam HB, Uy GB, Miller D, Koustova E, Hancock T, Inocencio R, Anderson D, Llorente O, Rhee P. Comparative analyses of hemostatic agents in a swine model of lethal groin injury. *J Trauma* 2003; 54: 1077-82.
10. Kheirabadi BS, Field-Ridley A, Pearson R, MacPhee M, Drohan W, Tuthill D. Comparative study of the common topical hemostatic agents with fibrin sealant in a rabbit aortic anastomosis model. *J Surg Res* 2002; 106: 99-107.
11. Ünlü Y, Vural Ü, Kocak H, Ceviz M, Akbulut Ö. Comparison of the topical haemostatic agents for the prevention of suture hole bleeding. An Experimental Study. *Eur J Vasc Endovasc Surg* 2002; 23: 441-4.
12. Malm K, Dahlbäck B, Arnljots B. Low-Molecular-Weight Heparin (Dalteparin) Effectively prevents thrombosis in a rat model of deep arterial injury. *Plast. Reconstr. Surg.* 2003; 111:1659-66
13. Söderström T, Hedner U, Arnljots B. Active site-inactivated factor VIIa prevents thrombosis without increased surgical bleeding: Topical and intravenous administration in a rat model of deep arterial injury. *J Vasc Surg* 2001;33:1072-9
14. Weaver FA, Hood DB, Zalita M, Messina L, Badduke B Gelatin-thrombin based hemostatic sealant for intraoperative bleeding in vascular surgery. *Ann Vasc Surg* 2002; 16: 286-93.
15. Schenk WG, Burks SG, Gagne PJ, Kagan SA, Lawson JH, Spotnitz WD. Fibrin sealant improves hemostasis in peripheral vascular surgery: A randomized prospective trial. *Ann Surg* 2003; 237: 871-6.
16. Glickman M, Gheissari A, Money S, Martin J, Ballard JL. A polymeric sealant inhibits anastomotic suture hole bleeding more rapidly than gelfoam/thrombin. *Arch Surg* 2002; 137: 326-31.
17. Taylor LM, Mueller-Velten G, Koslow A, Hunter G, Naslund T, Kline R, The Beriplast Investigators. Prospective randomized multicenter trial of fibrin sealant versus thrombin soaked gelatin sponge for suture- or needle hole bleeding from polytetrafluoroethylene femoral artery grafts *J Vasc Surg* 2003; 38: 766-71.
18. Oz MC, Cosgrove DM, Badduke BR, Hill DJ, Flannery MR, Palumbo R, Topic N. Controlled clinical trial of a novel hemostatic agent in cardiac surgery. *Ann Thorac Surg* 2000; 69: 1376-82.
19. Renkens KL, Payner TD, Leipzig TJ, Feuer H, Morone MA, Koers JM, Lawson KJ, Lentz R, Shuey H, Conaway GL, Andersson GJ, An H, Hickey M, Rondinone JF, Shargill NS. A multicenter, randomized trial evaluating a new hemostatic agent for spinal surgery. *Spine* 2001; 26: 1645-50.
20. Stricker RB, Lanc PK, Leffert JD, Rodgers GM, Shurnan MA, Corash L. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. *Blood* 1988; 72: 1375-80.
21. Rapaport SI, Zivelin A, Minow RA, Hunter CS, Donnelly K. Clinical significance of autoantibodies to bovine thrombin and factor V after surgical use of bovine thrombin. *Am J Clin Pathol* 1992; 97: 84-91.
22. Streiff MB, Ness PM. Acquired FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. *Transfusion* 2002; 42: 18-26.
23. Boffard KD, Riou B, Warren B, Choong PIT, Rizoli S, Rossaint R, Axelsen M, Kluger Y. Recombinant factor VIIa as adjunctive therapy for bleeding control in severely injured trauma patients: two parallel randomized, placebo-controlled, double-blind clinical trials. *J Trauma* 2005; 59: 8-18.
24. Manning BJ, Hynes N, Courtney DF, Sultan S. Recombinant factor VIIa in the treatment of intractable bleeding in vascular surgery. *Eur J Vasc Endovasc Surg* 2005; 30, 525-7.