

# Acquired isolated factor VII deficiency associated with severe bleeding and successful treatment with recombinant FVIIa (NovoSeven)

Charles G. Mullighan<sup>a</sup>, Amanda Rischbieth<sup>b</sup>, Elizabeth M. Duncan<sup>a</sup> and John V. Lloyd<sup>a</sup>

Acquired isolated FVII deficiency not due to vitamin K deficiency or liver disease is rare and often associated with severe bleeding. We present a case of transient acquired factor VII deficiency associated with major bleeding, successfully treated with twice daily intermittent intravenous recombinant activated factor VII (rFVIIa) (NovoSeven; Novo Nordisk). The severe transient reduction in factor VII coagulant activity (FVII:C) levels, unresponsive to fresh frozen plasma and vitamin K administration, raise the possibility of an acquired inhibitor to factor VII. However, no inhibitor to factor VII could be demonstrated using protein G sepharose adsorption, or a Bethesda assay using IgG purified from patient plasma. There are few reports of the use of rFVIIa in this setting and this case suggests that rFVIIa is effective therapy, and

should be considered early when acquired factor VII deficiency is associated with severe bleeding. *Blood Coagulation and Fibrinolysis* 15:347–351 © 2004 Lippincott Williams & Wilkins.

*Blood Coagulation and Fibrinolysis* 2004, 15:347–351

**Keywords:** factor VII, deficiency, inhibitor, recombinant, NovoSeven

<sup>a</sup>Haematology, Institute of Medical and Veterinary Science, Adelaide, Australia, and <sup>b</sup>Intensive Care Unit, Wakefield Hospital, Adelaide, Australia.

Correspondence and requests for reprints to Dr Charles G. Mullighan, Haematology, Institute of Medical and Veterinary Science, PO Box 14, Rundle Mall, Adelaide, SA, 5000, Australia.

Tel: +61 8 8222 4000; fax: +61 8 8222 3162; e-mail: cmull@senet.com.au

Received 8 October 2003 Revised 16 February 2004

Accepted 17 February 2004

## Introduction

Acquired isolated factor VII (FVII) deficiency not due to liver disease or vitamin K antagonists is rare, is often associated with severe bleeding, and has been described in patients with malignancy, sepsis, post-operatively and in patients undergoing bone marrow transplantation [1–15]. Proposed mechanisms include inhibitors of FVII or activated FVII function, inhibitors that accelerate FVII clearance, or granulocyte-associated proteases that cleave FVII. Treatment with conventional factor replacement or immunosuppression is often unsuccessful. We describe a case of severe isolated FVII deficiency associated with severe bleeding occurring in the context of post-operative sepsis. Bleeding refractory to conventional therapy was successfully treated and prevented with recombinant activated factor VII (rFVIIa) (NovoSeven; Novo Nordisk, Bagsvaerd, Denmark). We describe attempts to demonstrate the presence of an inhibitor *in vitro*, review the existing literature and discuss the role of rFVIIa in this setting.

## Materials and methods

The International Normalized Ratio (INR) was measured using Dade-Behring Innovin on an MLA Electra-1000 (New York, USA). The activated partial thromboplastin (aPTT) was measured using Dade-Behring Actin FSL on the MLA. One-stage factor assays were performed using

Dade-Behring factor-deficient plasma and the standard protocol for the MLA.

## Assays for FVII-containing immune complexes

Immune complex formation was investigated by assaying factor VII coagulant activity (FVII:C) activity of patient plasma before and after removal of free and complexed immunoglobulin using the method of Weisdorf *et al.* [6], although protein G sepharose was used instead of protein A sepharose. Protein G Sepharose (GammaBind G Sepharose; Amersham Biosciences, Uppsala, Sweden) was washed three times in 150 mmol/l phosphate-buffered saline (PBS) (pH 7.5) before use, and then reconstituted to a suspension of 75% gel:25% PBS. Aliquots of sepharose (300 µl) were then allowed to settle, the suspension buffer removed, and plasma (300 µl) was added and mixed with it for 45 min at room temperature. After centrifugation at 11 500 × *g* for 10 min, the supernatant was assayed for FVII activity. Untreated plasma was also re-assayed to allow direct comparison of FVII levels.

## Assays for inhibitor activity of purified patient immunoglobulin

To determine whether plasma and purified IgG from the patient at presentation demonstrated FVII inhibitor activity, a FVII inhibitor assay was used (Bethesda Assay, Nijmegen modification) [16,17]. IgG

was purified from 2.5 ml plasma (collected on presentation) using a protein G Sepharose column (Immuno-pure (G) IgG Purification kit; Pierce, Rockford, Illinois, USA), following the manufacturer's instructions. The peak IgG fraction (1.25 ml), as determined by absorbance at 280 nm, was desalted using an excellulose column, also supplied in the kit. The final pool of four fractions was estimated to contain 4.8 mg/ml IgG in 2 ml PBS. Purified IgG from a FVIII inhibitor patient was also assayed as a negative control, and showed no inhibitory activity.

### Presentation of case

The patient was a 53-year-old male who underwent coronary artery bypass surgery for unstable angina. Past medical history included hypertension and hypercholesterolaemia. There was no personal or family history of a bleeding diathesis. Preoperative coagulation studies were normal, and the patient's initial perioperative course was unremarkable, with no excessive haemorrhage, and no use of homologous blood products. A methicillin-sensitive *Staphylococcus aureus* sternal wound infection developed on the third postoperative day (D3), which progressed to empyema and *Staphylococcal* bacteraemia. Intravenous antibiotic therapy was commenced. The prothrombin time became progressively prolonged (INR = 2.2 on D7), with normal aPTT time. Results of investigations performed on day 10 showed further prolongation of the INR (3.5) with a normal aPTT (33 s,  $R = 22-34$ ) and isolated profound FVII deficiency (0.02 IU/ml,  $R = 0.4-1.45$ ). Other specific factor assays were normal (FII:C, FV:C, FVIII:C, FIX:C, FX:C, FXI:C, FXII:C, antithrombin, protein C and protein S). There was no evidence of disseminated intravascular coagulation (normal fibrinogen and D-dimer levels), and a lupus anticoagulant screen was negative. There was mild elevation of liver function tests (alanine aminotransferase, lactate dehydrogenase and bilirubin less than twice normal, and aspartate aminotransferase 129 U/l,  $R = 0-45$ ). A mixing study showed near complete correction of the prothrombin time with the addition of 20% control plasma, suggestive of coagulation factor deficiency.

The patient was treated with vitamin K (10 mg twice daily), fresh frozen plasma (FFP) (3 U every 8 h) and a continuous infusion of aminocaproic acid (1 g/h intravenously after a loading dose of 5 g), with incomplete and transient improvement in the INR (1.8) and FVII:C level (0.23 IU/ml). Further major surgery, including sternectomy and placement of an omental flap, was performed without excessive bleeding. Subsequently the INR rose to 3.1 and FVII:C fell to 0.15 IU/ml, and attempted reconstructive surgery was complicated by severe haemorrhage despite FFP, vitamin K and antifibrinolytic therapy. rFVIIa (90 µg/kg daily for 2 days) was commenced on D28, and surgery performed

on D30 under rFVIIa cover (90 µg/kg rFVIIa at 0900 and 1100 h on the day of surgery, thereafter 45 µg/kg at 0900 and 1100 h), with excellent haemostasis. Close peak and trough monitoring of the INR and FVII:C (using a PT-based one-stage FVII:C assay) was performed, showing brisk transient rises in FVII:C and falls in the INR after each dose of rFVIIa (Fig. 1).

Bleeding with soft tissue and intrathoracic haematoma formation recurred soon after cessation of rFVIIa. rFVIIa was recommenced, and further surgery attempted but abandoned soon after induction of anaesthesia due to fulminant multiorgan failure and polymicrobial systemic sepsis (*S. aureus* and *Escherichia coli*). rFVIIa was continued at the 45 µg/kg dose at 0900 and 1100 h with no further bleeding and slow resolution of the haematomata. Further wound revision surgery was successfully performed under rFVIIa cover, and the patient made a full recovery and was discharged on D67. He was readmitted 3 months later for minor plastic surgical revision of his sternal scar. Pre-operative coagulation studies (INR, aPTT and FVII:C) were normal, and the operation was uncomplicated.

## Results

### Anti-FVII immunoglobulin studies

The severe acquired FVII deficiency in this case raised the possibility of an acquired immunoglobulin inhibitor to FVII. To investigate this, FVII:C activity of patient plasma was assayed before and after incubation with protein G sepharose (PGS) to deplete reactive immunoglobulin or immunoglobulin complexes containing FVII. The assays were performed three times (except in the post-FFP sample due to insufficient plasma) and results are presented in Table 1. The ratio of FVII:C activity post to pre-PGS treatment was similar for nadir, post-FFP, recovery and control samples (61–81%). All samples showed minor reductions in FVII:C activity after PGS treatment but this was comparable between patient and control samples, and may be due to non-specific binding or entrapment of FVII by sepharose. These results do not suggest the presence of FVII complexed with anti-FVII IgG.

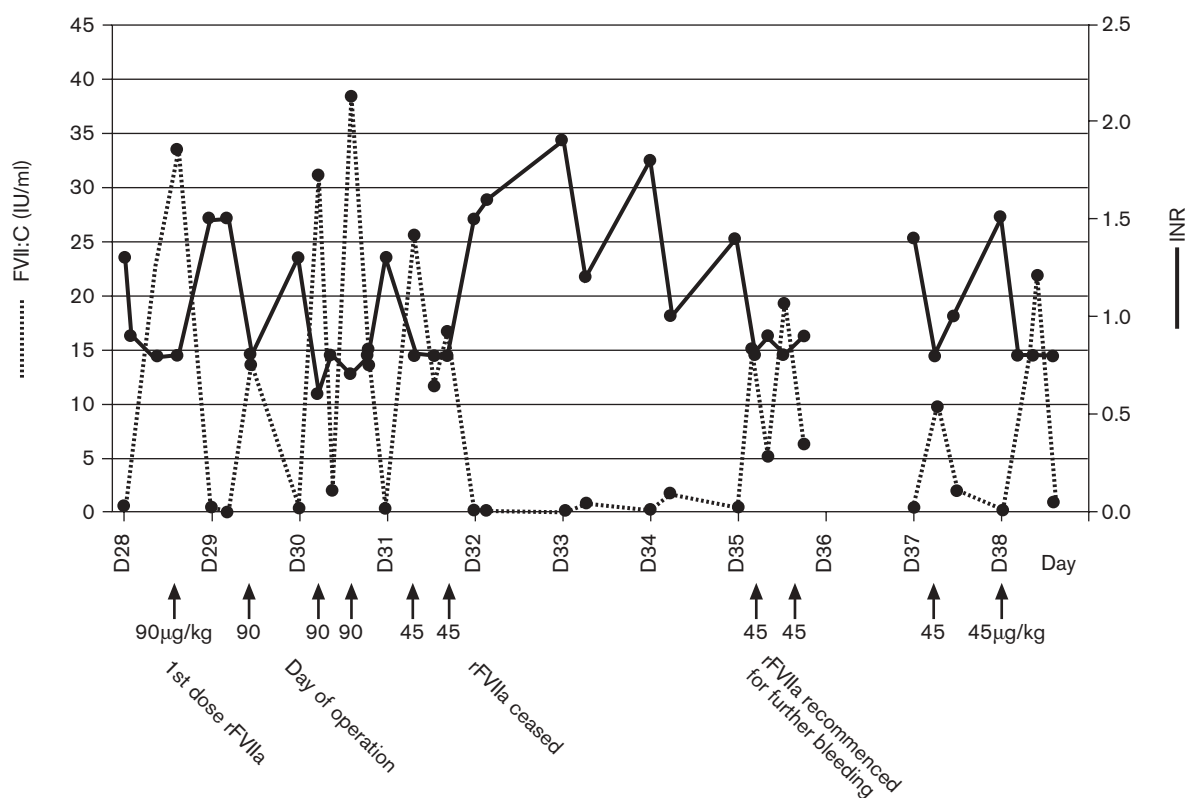
### Assays for inhibitor activity of plasma and purified immunoglobulin

Both purified IgG and plasma were tested for FVII antibody activity by a standard Bethesda assay for coagulation inhibitors, modified to detect FVII inhibitors. No inhibitory activity could be detected.

## Discussion

This case illustrates several points regarding acquired isolated FVII deficiency, including the apparent rarity of this condition, the mechanism of deficiency, recovery of administered rFVIIa and response to therapy. There are relatively few published reports of this condition

Fig. 1



Combined plot of one-stage factor VII coagulant activity (FVII:C) levels and International Normalized Ratio (INR) pre and post recombinant activated factor VII (rFVIIa) doses. Note the brisk rise (to over 30 IU/ml) of FVII:C levels followed by a swift decline after each dose of rFVIIa (each dose is indicated by an arrow, with the dose indicated in  $\mu\text{g}/\text{kg}$  beneath). Days refer to days post the first operation (coronary artery bypass grafting). The transient falls in the INR without a corresponding rise in FVII:C levels on postoperative day 33 and postoperative day 34 represent the incomplete response to continued fresh frozen plasma administration. Due to errors in sample collection, no levels are available for postoperative day 36.

**Table 1** Factor VII coagulant activity (FVII:C) results before and after removal of free and bound IgG

Sample	Postoperative day	Comment	Original FVII:C assay	FVII:C pre PGS [mean (SD)]	FVII:C post PGS [mean (SD)]	Ratio post/pre (SD)
Patient	10	Baseline	0.07	0.077 (0.010)	0.060 (0.018)	81% (36.3)
Patient*	11	Post FFP	0.23	0.26*	0.175*	67%*
Patient	113	Recovery	0.52	0.48 (0.043)	0.31 (0.012)	65% (3.5)
Normal	110	Donor	Not done	1.10 (0.15)	0.67 (0.10)	61% (4.6)

FVII:C levels are shown for patient plasma samples at baseline, post fresh frozen plasma (FFP) and at recovery. Results are shown as mean and standard deviation (SD) for three experiments. PGS, protein G sepharose.

\* Only sufficient sample for one experiment.

[1–15], although the description of multiple cases in two published reports [9,14] suggests the condition may be under-recognized. Previous case reports are presented in Table 2. The clinical contexts vary, including individuals who are critically ill for other reasons (sepsis, malignancy, transplantation) and individuals presenting *de novo* with bleeding, and no other apparent comorbidity.

The mechanism of FVII deficiency in these cases is frequently unclear, but two main explanations have been advanced, primarily based on mixing study results. One is that a transient inhibitor is formed that results in either inhibition of FVII function or accelerated FVII clearance. In those cases where mixing studies were performed, several show an inhibitor pattern but others show a deficiency pattern, as in our

Table 2 Prior reports of isolated acquired factor VII deficiency

Author, year	Age (years), sex	Comorbidity	FVII:C (%)	Mixing study pattern	Bleeding	Therapy, comments
Sleese and Shumacher, 1977 [1]	NS	Hodgkin lymphoma	NS	NS	Yes	Chemotherapy, with resolution
Campbell <i>et al.</i> , 1980 [2]	66, male	Lung carcinoma	30	Inhibitor	Nil	No treatment
Delmer <i>et al.</i> , 1989 [3,5]	62, male	Nil known	11	Inhibitor	Gut, urinary, biliary, intracranial	IVIg, cyclophosphamide, corticosteroids, apheresis, azathioprin
Ndimbie <i>et al.</i> , 1989 [4]	37, male	AIDS	14	Inhibitor	Nil	No treatment
Weisdorf <i>et al.</i> , 1989 [6]	18, female	Aplastic anaemia	16	Deficiency	Intracranial	BMT; corticosteroids for GVHD resulted in correction of FVII level
Mehta <i>et al.</i> , 1992 [7]	NS	Penicillin	NS	NS	Fatal bleeding	NS
de Raucort <i>et al.</i> , 1994 [8]	30, male	Pleural liposarcoma	15	Inhibitor	Nil	IVIg, FFP; response with chemotherapy, then relapse with tumour recurrence
Biron <i>et al.</i> , 1997 [9]	Multiple	Sepsis	~30	Deficiency	Yes	11 cases with mild 'heterozygous-like' FVII deficiency associated with sepsis
Brunod <i>et al.</i> , 1998 [10]	60, male	Orchitis	12	Inhibitor	Gastric	FFP, FVII concentrate, corticosteroids
Muleo <i>et al.</i> , 1998 [11]	36, male	Hepatic lymphoma with abnormal LFTs	10	Deficiency	None	10 mg/kg rFVIIa pre liver biopsy, and at 6 and 12 h post biopsy
White <i>et al.</i> , 1999 [13]	Male	Acute leukaemia, <i>Aspergillus</i> infection	35	Deficiency	Haemoptysis	rFVIIa
Okajima and colleagues, 2000 [12,18]	66, male	Nil	< 1	Inhibitor	Gut, urinary	Corticosteroids
Toor <i>et al.</i> , 2002 [14]	7-43, 4 female, 4 male	BMT (6 allogeneic, 2 autologous)	8-35 (mean 22)	Deficiency	Frequent major bleeding	Lupus anticoagulants present in four patients; poor outcome no response to FFP and fatal bleeding in two patients
Aguilar <i>et al.</i> , 2003 [15]	80, male	Chronic lung disease, TCC bladder	38	Inhibitor	Cutaneous, soft tissue, gut	Tranexamic acid, corticosteroids

AIDS, Acquired immune deficiency syndrome; BMT, bone marrow transplantation; FVII:C, factor VII coagulant activity; GVHD, graft versus host disease; IVIG, intravenous immunoglobulin; LFTs, liver function tests; NS, not stated; TCC, transitional cell carcinoma.

case [6,9,11,13]. Few reports have attempted to formally demonstrate the presence of an inhibitor [5,6,18].

Weisdorf *et al.* [6] reported the case of an 18-year-old female with aplastic anaemia and profound FVII deficiency, eventually corrected with bone marrow transplantation. As in our case, there was no evidence of an inhibitor on mixing studies. The authors found that significantly more FVII:C activity was removed after incubation with protein A sepharose in comparison with a normal control, suggesting the presence of an IgG inhibitor accelerating FVII clearance, but not inhibiting FVII:C activity [6]. We repeated this experiment, but found much higher post:pre PGS treatment FVII:C ratios than Weisdorf *et al.*, with only a mild reduction in activity that was comparable between patient and control. The experiment was performed three times and, although the standard deviation was high, this was comparable with that of Weisdorf, indicating that this technique gives variable results. It is thus likely that the plasma FVII activity measured in the untreated samples reflects FVII circulating in unbound form. No evidence could be found for FVII retaining coagulant activity but bound in a complex with anti-FVII IgG (as hypothesized by Weisdorf *et al.*). Likewise, no evidence of inhibitory activity could be detected in a modified Bethesda assay using patient plasma and purified IgG.

If there is no evidence of an inhibitor in these experi-

ments, what is the pathogenesis of the severe FVII deficiency in this case? It remains possible that an inhibitor was responsible, but not present in excess and rapidly cleared after binding FVII, as previously suggested [6]. This would explain the deficiency pattern in mixing studies, the lack of inhibitory activity in the Bethesda assay, and the lack of differential FVII:C activity after PGS incubation. In other reports of acquired FVII deficiency in the context of sepsis, FVII-cleaving proteases liberated by pathogens or granulocytes have been postulated [9,13]. Of note, FVII:C levels were substantially higher in cases thought to be protease-related (rather than inhibitor related) than in this case. Furthermore, this hypothesis is speculative, and the liberation of proteases has not been formally demonstrated in previous reports.

The recovery of FVIIa (as measured by FVII:C assay) also provides insights into the mechanism of FVII deficiency. The recovery of rFVIIa was comparable with previous reports [19,20] and with recovery in patients with inherited FVII deficiency that we have treated with rFVIIa (unpublished data). *In vivo* recovery (IVR) was also calculated by the methods of Nilsson and Hedner [21] (mean IVR, 14.6%; standard deviation, 4.5) and Prowse [22] (mean IVR, 0.38 U/dl per U/kg; standard deviation, 0.11). These results are comparable with published rFVIIa IVR data in inherited FVII deficiency [23]; however, the half-life in our

case is shorter, less than 30 min in our patient compared with 2.82–3.1 h in patients with inherited FVII deficiency (as measured by the FVII:C assay) [23]. This may be compatible with an inhibitor either accelerating FVII clearance or not present in excess, although an inhibitor has not been formally demonstrated.

In many previous reports of inhibitor-related FVII deficiency, a variety of immunomodulatory therapies have been used with variable success [3,5,6,8,10,12,15,18]. Very few patients with isolated acquired FVII deficiency have been treated with rFVIIa [11,13]. As in these prior reports, haemostasis was excellent in this patient while the drug was administered. Early reports of rFVIIa use frequently described 2-hourly dosing until haemostasis was achieved. Such indefinite 2-hourly dosing was not feasible in this case due to the high cost and limited availability of the drug at the time. We had previously found a regimen of two 90 µg/kg doses per day, 2 h apart to provide satisfactory haemostasis in other clinical contexts (e.g. inherited factor VII deficiency). It is possible that this brief peak of rFVIIa results in intense thrombin generation at the site of tissue injury and cessation of bleeding. This regimen achieved satisfactory haemostasis quickly in this case, although repeat treatment was required due to recurrence of bleeding. Our experience suggests that rFVIIa is a highly useful therapy to control major bleeding in the context of acquired severe FVII deficiency, and that therapy should be continued until the risk of haemorrhage has passed, in order to minimize the risk of rebleeding.

## References

- Sleese RB, Schumacher HR. Deficiency of coagulation factors VII and XII in a patient with Hodgkin's disease. *Arch Intern Med* 1977; **137**: 1633–1635.
- Campbell E, Sanal S, Mattson J, Walker L, Estray S, Mueller L, *et al.* Factor VII inhibitor. *Am J Med* 1980; **68**:962–964.
- Delmer A, Andreu G, Horellou MH, Lecompte T, Samama M, Zittoun R. Acquired factor VII inhibitor: treatment using high-dose immunoglobulins, corticotherapy and plasma exchange. *Ann Med Interne (Paris)* 1988; **139**(suppl 1):48–50.
- Ndimbie OK, Raman BK, Saeed SM. Lupus anticoagulant associated with specific inhibition of factor VII in a patient with AIDS. *Am J Clin Pathol* 1989; **91**:491–493.
- Delmer A, Horellou MH, Andreu G, Lecompte T, Rossi F, Kazatchkine MD, *et al.* Life-threatening intracranial bleeding associated with the presence of an antifactor VII autoantibody. *Blood* 1989; **74**:229–232.
- Weisdorf D, Hasegawa D, Fair DS. Acquired factor VII deficiency associated with aplastic anaemia: correction with bone marrow transplantation. *Br J Haematol* 1989; **71**:409–413.
- Mehta J, Singhal S, Mehta BC. Factor VII inhibitor. *J Assoc Physicians India* 1992; **40**:44.
- de Raucourt E, Dumont MD, Tourani JM, Hubsch JP, Riquet M, Fischer AM. Acquired factor VII deficiency associated with pleural liposarcoma. *Blood Coagul Fibrinolysis* 1994; **5**:833–836.
- Biron C, Bengler C, Gris JC, Schved JF. Acquired isolated factor VII deficiency during sepsis. *Haemostasis* 1997; **27**:51–56.
- Brunod M, Chatot-Henry C, Mehdaoui H, Richer C, Fonteau C. Acquired anti-factor VII (proconvertin) inhibitor: hemorrhage and thrombosis. *Thromb Haemost* 1998; **79**:1065–1066.
- Muleo G, Santoro R, Iannaccaro PG, Papaleo P, Leo F. The use of recombinant activated factor VII in congenital and acquired factor VII deficiencies. *Blood Coagul Fibrinolysis* 1998; **9**:389–390.
- Okajima K, Ishii M. Life-threatening bleeding in a case of autoantibody-induced factor VII deficiency. *Int J Hematol* 1999; **69**:129–132.
- White B, Martin M, Kelleher S, Browne P, McCann SR, Smith OP. Successful use of recombinant FVIIa (Novoseven) in the management of pulmonary haemorrhage secondary to Aspergillus infection in a patient with leukaemia and acquired FVII deficiency. *Br J Haematol* 1999; **106**:254–255.
- Toor AA, Slungaard A, Hedner U, Weisdorf DJ, Key NS. Acquired factor VII deficiency in hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2002; **29**:403–408.
- Aguilar C, Lucia JF, Hernandez P. A case of an inhibitor autoantibody to coagulation factor VII. *Haemophilia* 2003; **9**:119–120.
- Kasper CK, Aledort L, Aronson D, Counts R, Edson JR, van Eys J, *et al.* Proceedings: a more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh* 1975; **34**:612.
- Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; **73**:247–251.
- Kamikubo Y, Miyamoto S, Iwasa A, Ishii M, Okajima K. Purification and characterization of factor VII inhibitor found in a patient with life threatening bleeding. *Thromb Haemost* 2000; **83**:60–64.
- Erhardttsen E. Pharmacokinetics of recombinant activated factor VII (rFVIIa). *Semin Thromb Hemost* 2000; **26**:385–391.
- Ingerslev J. Efficacy and safety of recombinant factor VIIa in the prophylaxis of bleeding in various surgical procedures in hemophilic patients with factor VIII and factor IX inhibitors. *Semin Thromb Hemost* 2000; **26**:425–432.
- Nilsson IM, Hedner U. Characteristics of various factor VIII concentrates used in treatment of haemophilia A. *Br J Haematol* 1977; **37**:543–557.
- Prowse CV. In vivo recovery of factor VIII following transfusion: a survey of recent data and publications to assess the influence of standards used for potency assignment. On behalf of the Subcommittee on Factor VIII and IX of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1995; **74**:1191–1196.
- Berrettini M, Mariani G, Schiavoni M, Rocino A, Di Paolantonio T, Longo G, *et al.* Pharmacokinetic evaluation of recombinant, activated factor VII in patients with inherited factor VII deficiency. *Haematologica* 2001; **86**:640–645.