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# Life-threatening bleeding under vitamin K antagonists in spite of an INR in the therapeutic range

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The major risk associated with the use of vitamin K antagonists (VKA) is haemorrhage, which might be severe or even life-threatening. In clinical studies where anticoagulation intensity was carefully monitored, treatment with VKA increases the risk of major bleeding by 0.3–0.5% per year when compared to controls [1]. In randomized trials including patients with mechanical heart valves, VKA treatment was associated with a risk of major bleeding ranging between 1 and 8.3% [1]. However, in clinical practice, the rates are less consistent [1]. The major determinants of VKA-induced bleeding are the intensity of the anticoagulant effect, the patient characteristics, the concomitant use of drugs that interfere with hemostasis, and the length of therapy [1].

Here, we report a case of a rare hypersensitivity to VKA due to a mutation of factor IX (FIX) propeptide in the context of a prosthetic mechanical heart valve.

In November, 2008, a 62-year-old man presented with an obstructive laryngeal hematoma (Fig. 1a, b) after tongue biting requiring intratracheal intubation. His medical history revealed prosthetic mechanical aortic valve replacement in July 2008 (Bentall procedure, Cardiomedic prosthesis). Oral anticoagulation with VKA (acenocoumarol) was initiated post-operatively. When the laryngeal hematoma appeared, the patient's International Normalized Ratio (INR) was 3.5. In December, 2008, the patient was admitted to the intensive care unit with a massive hemothorax (Fig. 1c) occurring 10 days after a reintervention to cure a paraprosthetic leak, and requiring surgical drainage. INR was 1.3. The patient's and his relatives reported no history of bleeding. Aspirin, the only patient's medication favoring bleeding, had been stopped 4 days before the first bleeding episode. Before the introduction of acenocoumarol, blood count, prothrombin time (PT) and activated partial thromboplastin time (APTT) were within the normal range.

Coagulation tests are listed in Fig. 1d. Both major bleeding episodes were accompanied by INR values within or even below the therapeutic range (therapeutic range for a mechanical valve carrier: 2.5–3.5) [2]. However, APTT was abnormally prolonged because FIX was exaggeratedly lower than the other vitamin K dependant factors (II, VII, X). Factor V, VIII, XI and XII were within the normal range during both bleeding episodes. APTT and FIX both normalized after vitamin K supplementation and bleeding did not recur.

Genomic DNA was purified from the patient whole blood, and FIX exon 2 was amplified using the forward primer 5′-catgccctaaagagaaattggct-3′ and the reverse primer 5′-tgcatctgaagggtattatgtgg-3′. The thermocycling conditions of the PCR were 30 s 94°C, 1 min 53°C, and 1 min 72°C, for 40 cycles. Direct sequencing of the PCR product showed a single guanosine-to-adenosine transition at nucleotide 109 (numbered from genBank accession gi180552) causing the substitution of alanine at locus-10 (Ala-10) by threonine in the FIX propeptide (Fig. 2a).

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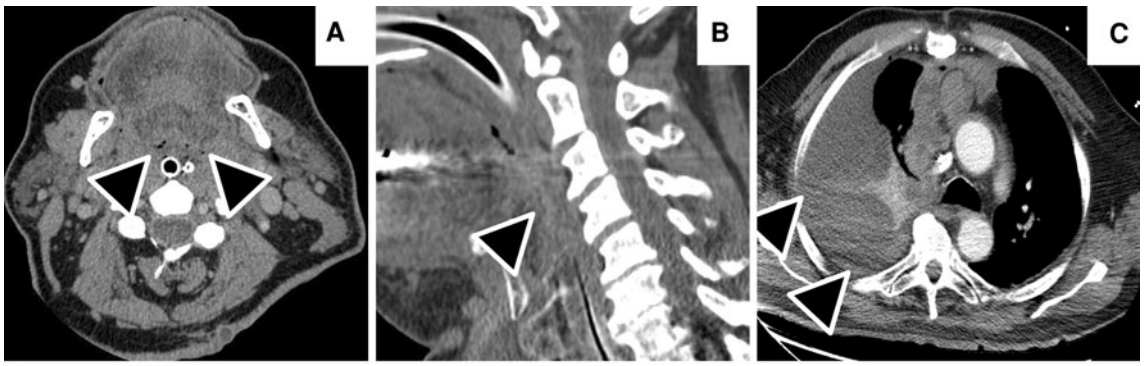


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| Coagulation test (unit) | Normal range | July 2008 pre-operative values | November 2008 1st bleeding episode | After vitamin K supplementation | December 2008 2nd bleeding episode | December 2008 Immediately before discharge |
|---|---|---|---|---|---|---|
| INR | 1 | 1.0 | 3.5 | 1.1 | 1.3 | 1.0 |
| APTT (sec.) | 20-30 sec | 29 | 142 | 38 | 49 | 22 |
| Fibrinogen (g/l) | 2.0-4.0 g/l | | 4.4 | | 7.5 | |
| Factor VII (%) | 70-120 % | | 11 | | 65 | |
| Factor II (%) | 70-120 % | | 20 | | 75 | |
| Factor X (%) | 70-120 % | | 11 | | 39 | |
| Factor IX (%) | 70-120 % | | 1 | 80 | 10 | |
| Factor V (%) | 70-120 % | | 145 | | 175 | |
| Factor VIII (%) | 70-120 % | | 185 | | 245 | |
| Factor XI (%) | 70-120 % | | 110 | | 95 | |
| Factor XII (%) | 70-120 % | | 110 | | 67 | |

INR, international normalized ratio; APTT, activated partial thromboplastin time.

**Fig. 1** **a** (axial) and **b** (sagittal) CT of the neck, showing an obstructive laryngeal hematoma (*arrow heads*). **c** CT of the chest showing a right hemothorax (*arrow heads*). **d** Table including coagulation tests

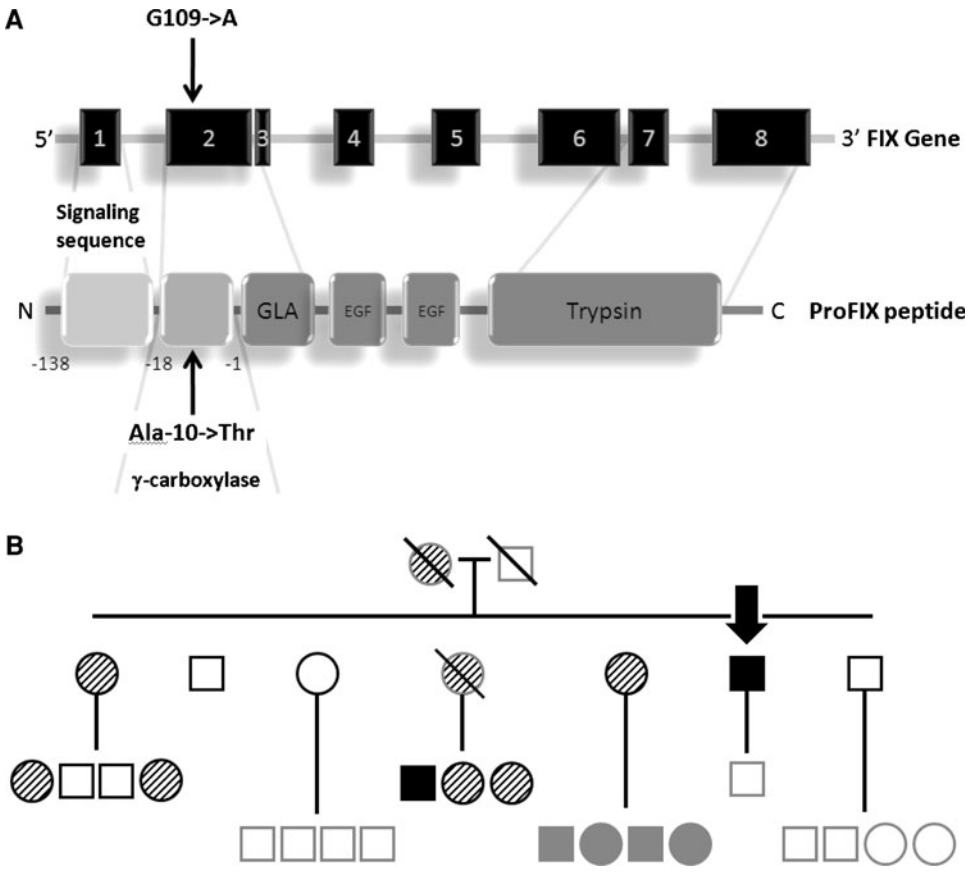


**Fig. 2** **a** Schematic representations of, *upper line*, FIX gene (accession number M11309), and, *lower line*, FIX propeptide. The mutation is indicated by an *arrow line* [6]. Exons are numbered. *G* guanosine, *A* adenosine, *GLA* Vitamin K-dependent carboxylation/gamma-carboxyglutamic domain, *EGF* Epidermal growth factor-like domain, *B* family pedigree. Affected males in *black*, female carriers in *stripes*, wild type phenotype in *white*, unknown phenotype in *grey*, deducted phenotypes circled with *grey*. *Arrow head* indicates the index patient

**Table 1** Summary of all similar cases published

| # | Reference | Date | Origin | Age at Dn | Anticoagulation indication | Bleeding sites | Basal FIX level (%) | FIX Level under anticoagulation (%) | Other coagulation tests under anticoagulation | Mutation type | Alternative therapy proposed/used |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 1996 | USA | 49 | Mechanical prosthetic aortic valve replacement | Hemothorax, hematuria, and ecchymoses over the arms, legs, and trunk | 120–148 | <1 | PT 23.7%, APTT > 150 s, FVII 42%, FVIII > 200% | Ala-10 → Thr | Low dose warfarin, with target: no PT prolongation (FIX 16%, FVII 80%, FX 43%). C: free of thrombotic and hemorrhagic complications. |
| 2 | 4, 13 | 1997 | Germany | 66 | Mechanical prosthetic aortic valve replacement | Severe muscle bleeding | 55 | <1 | PT 45%, APTT 70.9 s, FII 50%, FVII 31%, FX 22% | Ala-10 → Val | Long term heparin. |
| 3 | 4 | 1997 | Germany | 38 | Mechanical prosthetic aortic valve replacement | Bleeding in scrotum and muscle | 55 | <1 | PT 50%, APTT 59.1 s, FII 66%, FVII 44%, FX 46% | Ala-10 → Thr | Long term heparin. |
| 4 | 4, 13 | 1997 | Germany | 30 | Deep vein thrombosis | Severe muscle bleeding | 125 | 1 | PT 29%, APTT 106 s, FII 35%, FVII 19%, FX 13% | Ala-10 → Val | Long term heparin. |
| 5 | 14, 13 | 1997 | Germany | 66 | Mechanic prosthetic aortic valve replacement | Major epistaxis, severe muscle bleeding | 80 | 3 | INR 1.6, APTT 70.9 s, FV 92%, FVII 31%, FVIII 260%, FX 22%, FXI 79%, FXII 122% | n.a. | Long term heparin. |
| 6 | 14, 13 | 1997 | Germany | 38 | Mechanic prosthetic aortic valve replacement | Muscle and scrotal bleeding | 48 | 2 | INR 1.4, APTT 59.1 s, FV 69%, FVII 44%, FVIII 112%, FX 46%, FXI 75%, FXII 66% | n.a. | Long term heparin. C: no complication. |
| 7 | 8 | 199S | Germany | 29 | Deep vein thrombosis, as part of an antipholphol ipid-antibody syndrom | Severe muscle bleeding | 120 | 1 | PT 29%, APTT 106 s, FV 94%, FVII 19%, FVIII 69%, FX 13%, FXI 138%, FXII87% | Ala-10 → Val | Aspirin 100 mg/d plus low molecular weight heparin 5000 lU/d. |
| 8 | 7, 13 | 2000 | UK | 56 | Deep vein thrombosis secondary to carcinoma of the stomach | Severe muscle bleeding | 65 | <1 | INR 2.2, APTT 104–170 s, FVII 20% | Ala-10 → Thr | Low molecular weight heparin. |
| 9 | 12 | 2001 | Germany | 66 | Mechanical prosthetic aortic valve replacement | Severe muscle bleeding | 88 | 2 | APTT > 140 s, other vitamin K dependant factors 20–50% | Ala-10 → Val | Replacement of the prosthetic mechanical valve with a biological device. |
| 10 | 13 | 2001 | Germany | 45 | Pulmonary embolism | Epistaxis, hematuria, muscle bleeding and atraumatic haemarthrosis | Normal | <5 | APTT > 150 s, FII 18%, FVII 11%, FX 6% | Ala-10 → Val | Alternative anticoagulation therapy. |

**Table 1** continued

| # | Reference | Date | Origin | Age at Dn | Anticoagulation indication | Bleeding sites | Basal FIX level (%) | FIX Level under anticoagulation (%) | Other coagulation tests under anticoagulation | Mutation type | Alternative therapy proposed/used |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 13 | 2001 | Germany | 66 | Mechanical prosthetic aortic valve replacement | Muscle and soft tissue bleeding | Normal | <l | APTT>l20sec,FII 34%, FVII 64% | Ala-10 → Val | Alternative anticoagulation therapy. |
| 12 | 13 | 2001 | Switzerland | 77 | Mechanical prosthetic aortic valve replacement | Muscle and retroperitoneal bleeding | Normal | 3 | INR 1.9, APTT 80 s | Ala-10 → Thr | Alternative anticoagulation therapy. |
| 13 | 13 | 2001 | USA | l3 | Mechanical prosthetic aortic valve replacement | n.a. | Normal | 6 | "disproportional" APTT | Ala-10 → Thr | Alternative anticoagulation therapy. |
| 14 | 10 | 2002 | Denmark | 62 | Mechanical prosthetic aortic valve replacement | Severe muscle bleeding | n.a. | 4 | INR 1.9, APTT 128 s | Ala-10 → Thr | (1) Aspirin 100 mg/d. C: cerebral thrombosis, acute myocardial infarction, and a transient ischemic attack. (2) Aspirin 100 mg/2d + low dose warfarin with FIX monitoring (target 10–20%) C: 4 episodes of gastrointestinal bleedings. (3) Low dose warfarin with FIX monitoring (target 8–16%). C: neither bleedings nor thromboses. |
| 15 | 6, 11, 13 | 2001 | Switzerland | 58 | Mechanical prosthetic aortic valve replacement | Muscle bleeding, heamarthrosis, intestinal wall bleeding, laryngeal hematoma | 95 | 3 | INR 1.5, APTT 77.3 s, FII 56%, FV 90%, FVII 78%, FVIII 187%, FX 27%, FXI 102% | Ala-10 → Val | (1) Full dose low molecular weight heparin. C: thrombus on the artificial heart valve with recurrent cerebral ischemia episodes. (2) Aspirin 100 mg/d added. C: idem. (3) Aortic valve replacement with biological prosthesis. C: no further complication. |
| 16 | 6, 13 | 2001 | Switzerland | 63 | Thrombosis prophylaxis after hip replacement surgery (severe heparin allergy) | Major subcutaneous hematoma | n.a. | 2 | INR 2.8, APTT 164 s, FVIII 270% | Ala-l0 → Thr | Stop anticoagulation (prophylaxis). C: no further bleeding complication. |

**Table 1** continued

| # | Reference | Date | Origin | Age at Dn | Anticoagulation indication | Bleeding sites | Basal FIX level (%) | FIX Level under anticoagulation (%) | Other coagulation tests under anticoagulation | Mutation type | Alternative therapy proposed/used |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | 15 | 2008 | Switzerland | 70 | Biological prosthetic aortic valve replacement | Soft tissue, muscle and joint bleeding | n.a. | 2 | INR 2.1, APTT 78 s, FII 46%, FVII 29% | Ala-10 → Thr | Stop anticoagulation. |
| 18 | 9 | 2010 | Switzerland | 53 | Mechanical prosthetic mitral valve replacement | Subcutaneous bleeding, hemarthrosis and haematoma of the epiglottis | n.a. | <1 | INR 2.9, APTT 76 s, FII 30%, FV 112%, FVII 16%, FVIII 263%, FX 8%, FXI 135% | Ala-10 → Thr | Low dose phenprocoumon with monitoring of FIX level (target 5–15%). |
| 19 | Present case | 2011 | Switzerland | 62 | Mechanical prosthetic aortic valve replacement | Laryngeal hematoma and postoperative hemothorax | 80 | 1 | INR 3.5, APTT 142 s, FII 20%, FV 145%, FVII 11%, FX 11%, FVIII 185%, FXI 110%, FXII 110% | Ala-10 → Thr | Full dose low molecular weight heparin. |

When publication mentions a whole family, only the index case is listed. *Dn* diagnosis, *C* comment on the outcome, *INR* international normalized ratio, *PT* prothrombin time, *APTT* activated partial thromboplastin time, *FII* factor II, *FV* factor V, *FVII*, factor VII, *FVIII* factor VIII, *FIX* factor IX, *FX* factor X, *FXI* factor XI, *FXII* factor XII. Ala-10 → Thr alanine residue at position-10 to threonine substitution. Ala-10 → Val alanine residue at position-10 to valine substitution. *n.a.* Information not available

Screening of patient's relatives allowed to identify seven further asymptomatic carriers (two males, five females) (Fig. 2b).

Because of the severe bleeding complications presented by our patient, the oral anticoagulation was replaced by low molecular weight heparin at therapeutic dose monitored by anti-Xa activity in order to be sure that anti-Xa activity levels were maintained within the therapeutic range. 15 months later no recurrence of bleeding nor thrombotic complication were observed.

In the literature, two point mutations causing Ala-10 substitution by either a valine (Ala-10Val) or a threonine (Ala-10Thr) have been described [3, 4]. Ala-10 is located at the essential recognition site for the $\gamma$-carboxylase [5] and is highly conserved in all vitamin K-dependent coagulation factors [6]. Ala-10Val and Ala-10Thr FIX variants display a more than 30-fold lower affinity for the $\gamma$-carboxylase than the wild type propeptide [3]. The affinity of the mutated enzymatic peptide complex for reduced vitamin K is decreased when compared to the enzymatic complex comprising the wild type peptide [3]. Consistantly, the $\gamma$-carboxylation of these FIX variants is less efficient and becomes dramatically impaired in patients with VKA medication [3].

Male patients have a normal or slightly lowered basal FIX level. However, under VKA therapy, they display a dramatic decrease of FIX activity that is out of proportion with the other vitamin K dependant coagulation factors (II, VII, X). APTT is thus abnormally prolonged, while PT and INR remain within, or even below, the therapeutic range; and patients exibit severe and sometimes life-threatening soft tissue and muscle bleeding [3, 4, 7–16]. From time to time misdiagnosed for mild hemophilia B, patients carrying a FIX propeptide mutation can be distinguished by an absence of self or family bleeding history as well as normalization of APTT and FIX level after vitamin K administration. Because FIX gene is located on chromosome X, males are more affected than females. One female patient carrier of the Ala-10Val mutation was treated by VKA [17]. When INR was 2.3, FIX level was 16%. This patient did not develop bleeding complications [17].

So far 18 similar cases have been reported in the literature worldwide [3, 4, 7–16] (Table 1). Systematic screening in 4039 individuals yielded no further case [4, 18–20]. Therefore, prevalence of Ala-10Thr or Ala-10Val variants in the population is estimated to range from 1/1,000 to 1/10,000 and systematic screening prior to initiating oral anticoagulation is not indicated. However it is worth noticing that about one-third of these patients (6/19) originate from Switzerland including our current reported case. Although selection bias is possible, one study using haplotype analysis revealed a founder effect in the five German

and Swiss patients carrying a FIX variant (1 Ala-10Val and and 4 Ala-10Thr) derived from a common founder [14]. We postulate that the prevalence of this genetic variant may be higher in the Swiss population. APTT screening is certainly of interest in any bleeding patient with INR values within the therapeutic range, especially in male patients. If abnormally prolonged, quantitative determination of FIX level can lead to the diagnosis. Further use of VKA is not recommended. For the present being, low molecular weight heparins represent an inconvenient but safe alternative. An alternative could be to monitor FIX activity targeting a level of 5–15%, as previously described [3, 10, 11]. We did not choose this therapeutic option for our patient as he presented a severe bleeding episode with a FIX level at 10% (Fig. 1d). The replacement of the prosthetic mechanical valve by a biological graft in order to avoid anticoagulation was discussed but the risk of morbidity and mortality associated with cardiac surgery was considered to be too high. In the future, patients with FIX propeptide mutation may benefit from the novel oral anti-thrombin and anti-Xa inhibitors, yet further validation studies are needed.

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