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Letter to the Editors-in-Chief

Severe bleeding due to hypersensitivity to vitamin K antagonist caused by the c.109G > A (p.Ala37Thr) mutation in the *F9* gene in a patient with mechanical heart valve prosthesis

Dear Editors,

Factor IX (FIX) is a vitamin K dependent plasma glycoprotein important for blood coagulation as evidenced by significant bleeding diathesis observed in patients with severe hemophilia B – inborn lack of FIX plasma activity. The gene encoding FIX (*F9*) is located on the X chromosome (q27.1), contains eight exons and spans 34 kb of genomic DNA. Apart from numerous mutations across the *F9* resulting in hemophilia B there are two peculiar genetic alterations localized in the *F9* propeptide that may lead to significant FIX deficiency but only in patients who receive vitamin K antagonists (VKAs). These unique *F9* variants are point mutations (substitutions) at amino acid position 37 in the exon 2 (previous legacy −10), either c.109G > A (p.Ala37Thr) or c.110C > T (p.Ala37Val) [1–3]. Both mutant FIX proteins have reduced binding capacity to the hepatic γ-carboxylase which per se does not affect FIX plasma activity. A severe deficiency of FIX may however occur in patients affected by the above mentioned alterations in the FIX propeptide if they receive VKAs, since VKA further reduce the hepatic γ-carboxylation of vitamin K-dependent proteins [4]. This is known as "warfarin hypersensitivity". It is worth mentioning that bleeding complications have been observed mainly in males affected by the "warfarin hypersensitivity" which implies that wild type *F9* copy on the second X chromosome prevents most females from significant FIX activity decline during VKA therapy [5]. The prevalence of both *F9* variants among healthy blood donors was estimated at < 1 per 1000 [4]. To the best of our knowledge, up to date there have been < 30 cases of c.109G > A (p.Ala37Thr) or c.110C > T (p.Ala37Val)-associated bleeding diathesis reported in the literature [1–4,6–9].

1. Case description

A 74 year-old male was admitted to our Centre for investigation of recently revealed bleeding diathesis. Several months prior to admission the patient had a mechanical heart valve implantation for aortic stenosis and since then had been receiving acenocumarol to prevent thromboembolic complications. Soon after VKA administration he reported easy bruising on arms, legs and trunk which he did not associate with any major injury. Local laboratory blood tests revealed anemia (hemoglobin concentration 8 g/dl; ref. range 14–18 g/dl), prolongation of prothrombin time (PT) with International Normalized Ratio (INR) within the therapeutic range (between 2.0 and 3.0). Interestingly, his activated partial thromboplastin time (APTT) was significantly (> 2-folds) prolonged and FIX activity markedly decreased.

The patient presented massive hematoma of the left thigh and buttock as well as large areas of ecchymosis on extremities and trunk with no mucosal bleeding. He denied bleeding tendency prior to VKA therapy initiation. His family history of bleeding diathesis was negative. His vital signs were normal. Apart from acenocumarol he received doxazosin, furosemide, spironolactone and iron supplementation. Initial blood studies revealed mildly lower glomerular filtration rate (59.35 ml/min/1.73 m^2; ref. range > 60) and higher bilirubin concentration (1.86 mg/dl; ref. range < 1 mg/dl). He required blood transfusion due to severe anemia (hemoglobin 6.9 g/dl). Leukocytes count was slightly elevated but with normal differential white blood cell counts. Platelet count was within normal range.

2. Laboratory tests description

The patient gave his informed consent for molecular studies as well as all other laboratory tests required for final diagnosis. Blood samples were collected via venipuncture: blood was drawn into tubes with trisodium citrate (0.109 M) for coagulation tests and into tubes with EDTA for molecular studies.

Plasma coagulation studies comprised: PT, APTT, thrombin time (TT), fibrinogen concentration, one-stage coagulation activity of factors: II, V, VII, VIII, IX, X, XI, XII and screening for the presence of lupus anticoagulant (LA). All the above were performed on the BCS XP Coagulation Analyzer (Siemens Healthcare GmbH) using Siemens reagents. The PT was 21.65 s (ref. range 9.4–14.1), INR ratio 1.95 – slightly below the recommended therapeutic range. The APTT was significantly prolonged - 61.18 s (ref. range 25–33), while fibrinogen and TT were within normal ranges. Other laboratory findings were unremarkable.

Our attention was drawn by significantly prolonged APTT not explainable by almost adequate VKA therapy and so we assayed activities of clotting factors. Factor VIII (206 IU/dl; ref. range 50–150 IU/dl) and von Willebrand factor (181 IU/dl, ref. range 50–150 IU/dl) activities were slightly elevated, whereas the activity of the vitamin K-dependent clotting factors was reduced: factor II - 51 IU/dl, factor VII - 26 IU/dl, factor X - 26 IU/dl (ref. range for all 70–120 IU/dl) and factor IX - 1.76 IU/dl (ref. range 50–150 IU/dl). Factor V, XI, XII activities and the results of PT and APTT-based plasma mixing studies were normal. The presence of lupus anticoagulant was ruled out as well.

The results clearly showed more pronounced deficiency of FIX than other vitamin K-dependent coagulation factors. Severe deficiency of FIX was the obvious cause of significant APTT prolongation. At this stage it was highly probable that the bleeding tendency observed in our patient might have been caused by hypersensitivity of his FIX to VKA.

In light of the bleeding complications and laboratory findings, VKA therapy was discontinued and treatment with an alternative anticoagulant (low-molecular weight heparin, LMWH) was initiated due to the high risk of thromboembolic complications. Our preliminary diagnosis was confirmed by normalization of the coagulation tests several days after VKA was discontinued (Fig. 1).

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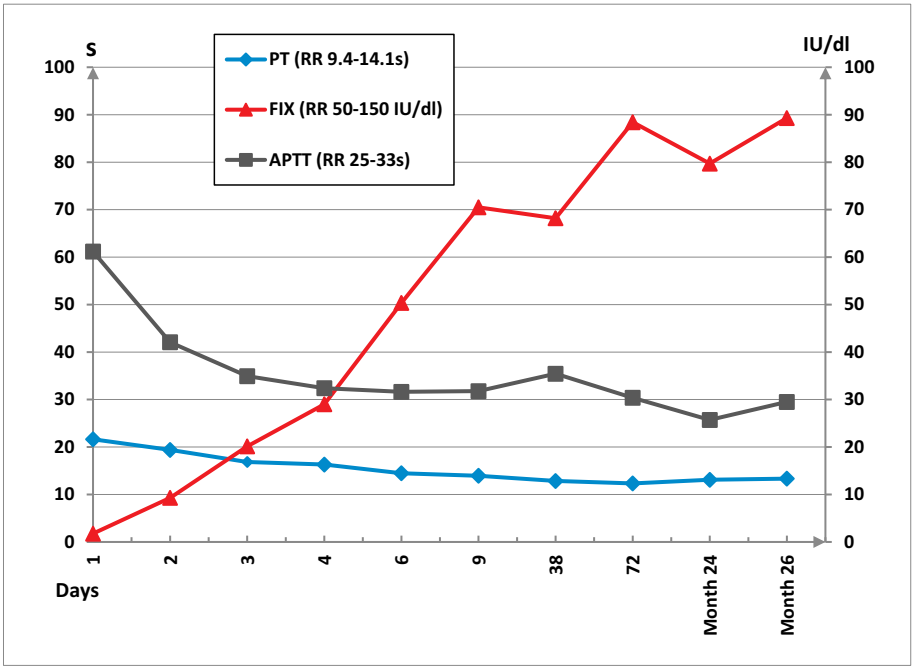

Fig. 1. Graph illustrates laboratory monitoring of PT, APTT and FIX activity during anticoagulation therapy. Day 1 represents day of vitamin K antagonist discontinuation and low molecular weight heparin administration. RR – reference range; FIX – factor IX coagulation activity; PT – prothrombin time; APTT – activated partial thromboplastin time.

3. Molecular tests description

The last step in the diagnostic process was identification of the causative mutation in the *F9* gene to confirm the genetic background of FIX hypersensitivity to VKA. Molecular studies were conducted on genomic DNA isolated from peripheral blood leukocytes by standard salting-out method. Exon 2 of the *F9* gene was amplified by PCR using specific oligonucleotide primers as previously described [4]. Mutation analysis was performed by automated direct sequencing on the ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, USA) using the BigDye Terminator Cycle Sequencing v.1.1. Ready Reaction kit (Applied Biosystems, USA) according to the manufacturer's instructions. Sequencing of the *F9* gene allowed to identify the hemizygous substitution of alanine by threonine at the position 37 (c.109G > A; p.Ala37Thr). This *F9* variant [rs367569299] was already described as the cause of the increased sensitivity to the VKA treatment and registered in The Factor IX Variant Database (http://www.factorix.org/) and The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/).

4. Discussion

Many risk factors of bleeding complications have been reported for patients receiving anticoagulants one of which is anticoagulant overdose [10]. In patients receiving VKA, INR is the primary laboratory test to monitor the level of anticoagulation. In the case of our patient the INR value (1.95) was close to the recommended therapeutic range (between 2.0 and 3.0). During VKA therapy the APTT is usually within normal range or slightly prolonged but the APTT of our patient was almost twofold prolonged. FIX is the only vitamin K dependent clotting factor whose deficiency may be the cause of APTT prolongation with no impact on PT. Very low level of FIX coagulation activity in our patient's plasma strongly suggested a hypersensitivity to VKA as the cause of observed bleeding complications. Normalization of the PT, APTT, and FIX after VKA discontinuation proved that disturbances in the patient's coagulation system were solely caused by the VKA.

Coumarin sensitive FIX-variants are uncommon in the general population [11]. Nevertheless, taking into account how common the VKA therapy is (even in the era of direct oral anticoagulants, DOACs) as well as the high frequency of associated bleeding complications, clinicians should take into account “warfarin hypersensitivity”; timely diagnosis enables VKA discontinuation which may protect the patient from life-threatening hemorrhages [12]. Fortunately, significant prolongation of APTT – a widely available and cheap clotting test – gives the physician a clear hint of the most likely diagnosis [4]. Finally, FIX activity much lower than that of other vitamin K-dependent clotting factors during VKA therapy, confirms the presence of the coumarin sensitive FIX-variant. Definitive diagnosis however requires the detection of the genetic defect in the *F9* gene [3]. Noteworthy is also the fact that practically only male patients are affected by FIX-based hypersensitivity to VKA, while females have a compensating normal allele (in theory, a female carrier of mutated *F9* propeptide may be at risk of bleeding during VKA therapy if the compensating allele is not dominant or does not exist, e.g. skewed inactivation of the X chromosome bearing the wild-type allele or the chromosome X monosomy) [3–5,11].

Nowadays, a wide variety of anticoagulants is available as alternative to VKA for patients with “warfarin hypersensitivity”. These include heparins, fondaparinux and DOACs. Most of them can replace VKA in the management of venous thromboembolism and non-valvular atrial fibrillation. The only problem is patients with mechanical heart valves for whom VKA is still the treatment of choice. Our patient belongs to this very group. In one study it was demonstrated that one of the DOACs (dabigatran) is inferior to warfarin in patients requiring anticoagulation for mechanical heart valves [13]. On the other hand one can find in the literature positive data on the use of LMWH in patients with mechanical heart valves e.g. pregnant women [14]. After discontinuation of VKA we decided to administer LMWH and maintain the patient's blood anti-Xa activity within the range of 0.6–1.2 IU/ml. He was discharged from our Centre in very good shape, with no bleeding complications. The LMWH therapy was continued by his cardiologists. The patient's acceptance for the treatment regimen was very high as assessed in our Centre 3, 24 and 26 months after discharge even though frequent subcutaneous injections are not comfortable for those on life-long therapy. During this time the patient's clinical course was uneventful, no bleeding or thrombotic complications occurred and coagulation parameters including FIX, APTT and PT were normal (Fig. 1).

In conclusion, to the best of our knowledge, our patient is the first case of coumarin-sensitive FIX-variant identified in Poland. Should we expect more “warfarin hypersensitivity” cases in our country? We believe so, but the true prevalence of this phenomenon in the Polish

population is still unknown as is the case in other populations. However, even if it turned out to be a very rare condition, we still believe that genetically determined VKA hypersensitivity warrants more attention of numerous healthcare providers who treat patients with VKAs.

Disclosure of conflict of interests

No conflict of interest to be declared.

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