Relevant bleeding diathesis due to acquired factor XIII deficiency

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Keywords
Faktor XIII, aortic aneurysm, DIC, fibrinolysis, tranexamic acid

Summary
Background: Acquired factor XIII (FXIII) deficiency is associated with reduced clot firmness and increased bleeding in patients undergoing major surgery. In contrast, only limited information is available on the haemostatic relevance of acquired FXIII deficiency in non-surgical patients. Case report: An 81-year-old patient, who had experienced acute type-A dissection of the aorta eight years earlier, presented with a 3-year history of progressive mucocutaneous and soft-tissue bleeding. Diagnostic work-up was unremarkable for global coagulation tests, but FXIII and alpha2-antiplasmin were decreased to 33% and 27%, respectively, while plasma D-dimer was elevated to >35 mg/l. A FXIII inhibitor was excluded by mixing studies. CT scanning revealed a massively elongated and progressively dilated aorta with a false lumen reaching from the left carotid artery to the iliac bifurcation. Bleeding control was achieved by single doses of FXIII at 20–30 IU/kg body weight and tailored oral tranexamic acid. Conclusion: Acquired FXIII deficiency with activity levels of 30–35% may confer a severe bleeding tendency in non-surgical patients, especially in the context of increased thrombin an fibrin generation.

Schlüsselwörter
Faktor XIII, Aortenaneurysma, DIC, Fibrinolyse, Tranexamsäure

Zusammenfassung

Plasma coagulation factor XIII (FXIII), a tetrameric protransglutaminase, is composed of
- two catalytic A and
- two non-catalytic B subunits (FXIII-A2-B2).

While intracellular FXIII, an A2 homodimer, is involved in cytoskeletal remodeling, plasma FXIII is critical for haemostasis and wound healing (1–3).

Plasma FXIII is activated by thrombin through cleavage of the A subunit (1). Activated FXIII (FXIIIa) stabilizes the forming clot through covalent crosslinking of soluble fibrin mono- and polymers and confers additional clot resistance to fibrinolysis through incorporation of alpha2-antiplasmin (2). FXIIIa also crosslinks several other plasma and matrix proteins, including
- factor V,
- plasminogen activator inhibitor-1,
- fibronectin,
- von Willebrand factor (VWF),
- collagen and
- thrombospordin.

This emphasizes the pleiotropic role of FXIIIa in tissue repair and wound healing (1, 2, 4).

The FXIII A subunit is produced by megakaryocytes and monocytes, whereas the B subunit, which stabilizes the A subunit within the circulation and localizes the FXIII tetramer to the fibrin clot, is synthesized in the liver (1, 5, 6).

In patients with FXIII deficiency, initial clot formation is typically unaltered, but formed clots tend to break down after 24–48 hours, resulting in delayed haemorrhage (1, 2).
There are different forms of FXIII deficiency. 

- Congenital FXIII deficiency is a rare bleeding disorder and mainly due to mutations within the catalytic A subunit. 
  - Patients with homozygous mutations and FXIII plasma activity levels of <1% present with severe bleeding symptoms early after birth (1, 7). AFFECTED women are at an increased risk of spontaneous abortion, particularly during early pregnancy. 
  - Most heterozygous carriers are asymptomatic in everyday life, but may experience pronounced bleeding in certain clinical situations such as surgery or child delivery (2, 3). 

- A variety of disorders can cause acquired FXIII deficiency, either as a result of impaired synthesis (i.e. liver failure) or increased consumption (e.g. haematologic malignancies, ulcerative colitis, major surgery, DIC) (8). FXIII is also susceptible to proteolytic degradation by enzymes such as thrombin, elastases or tumour-derived proteases (9). Very rarely, acquired FXIII deficiency may also be caused by inhibitory autoantibodies, resulting in severely decreased FXIII plasma levels and significant bleeding symptoms (8).

While increasing evidence suggests that acquired FXIII deficiency with residual activity levels of 50–60% may be associated with increased perioperative bleeding in patients undergoing major (i.e. cancer) or intracranial surgery (10, 11), only limited information is available on the haemostatic relevance of acquired FXIII deficiency in non-surgical patients.

**An old man with bleeding**

A man (age: 81 years), who had undergone aortic valve reconstruction and replacement of the ascending aorta eight years earlier due to acute type-A dissection according to the classification of Stanford, presented with a 3-year history of progressive mucocutaneous and soft-tissue bleeding. The patient reported easy bruising and delayed wound healing after minor cuts. In addition, massive bleeding had occurred after a tooth extraction and a facial skin biopsy, and a spontaneous calf-muscle haematoma had developed during regular walking.

The patient's past medical history included uneventful surgery for localized colorectal cancer 20 years earlier with no clinical evidence for relapse ever since. In addition, the patient suffered from chronic renal insufficiency (serum creatinine, 1.5 to 2.0 mg/dl). Arterial hypertension and stable coronary artery disease were also present. A cardiac pacemaker had been implanted nine years earlier for symptomatic second-degree atrioventricular block with no evidence for enhanced bleeding. Besides combination antihypertensive therapy, the patient had been taking 100 mg of acetylsalicylic acid (ASA) per day since the aortic repair, which he had stopped with the worsening of haemorrhagic complications. There was no family history of excessive bleeding. Otherwise, the patient was fit and physically active with an ECOG (Eastern Cooperative Oncology Group) performance status of 1. The patient's day-to-day activity was mainly restricted by his severe bleeding tendency.

At initial presentation to our institution, diagnostic work-up was unremarkable for platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, plasma fibrinogen and VWF as well as for platelet function tests (Tab. 1). In contrast, plasma levels of FXIII and alpha2-antiplasmin were significantly decreased, and plasma D-dimer was excessively elevated. Native whole-blood thromboelastometry documented slightly reduced clot firmness, as indicated by maximal amplitude of 38 mm (normal range, 45–60 mm). The presence of a FXIII inhibitor was excluded by mixing studies (modified Bethesda assay). Further laboratory work-up revealed no evidence for hepatic dysfunction or relevant autoimmune disease. Prostate-specific antigen was normal, and both gastro- and colonoscopy showed no pathologies.

Computed tomography (CT) scanning of the thorax and abdomen was negative for solid-tumour or lymphoproliferative malignancies, but revealed a massively elongated and dilated aorta with a false lumen reaching from the left carotid artery to the iliac bifurcation (Fig. 1). Comparison of the current CT scans with a magnetic resonance imaging (MRI) study from eight years earlier showed that the cranio-caudal extension of the dissection had not changed, but that the aneurysm’s thoracic diameter had increased from 5.6 to 7.7 cm.

Because severe acquired FXIII deficiency has been observed in cancer patients

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<td>platelet aggregation (%)</td>
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<td>60 – 95</td>
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**Tab. 1 Results of coagulation and platelet function tests for diagnostic work-up of the bleeding disorder; abnormal parameters in bold (ADP: adenosine diphosphate; Ag: antigen; EPI: epinephrine; PFA: platelet function analyzer; PTT: partial thromboplastin time; RCo: ristocetin cofactor activity; VWF: von Willebrand factor)**
with chronic DIC due to elevated levels of circulating tissue factor (TF)-bearing microparticles (MPs) (12), we isolated total cellular MPs from the patient’s plasma and measured their procoagulant activity (PCA) in a fluorogenic thrombin generation assay in the presence or absence of inhibitory TF monoclonal antibody (▶Fig. 2). No TF-specific PCA could be detected in association with the patient’s MPs (▶Fig. 2a), and there was no obvious difference with regard to the kinetics or magnitude of thrombin generation when results were compared to those obtained in four healthy individuals (▶Fig. 2b). These findings further implicated the dissected aortic aneurysm, and not circulating TF-positive MPs in the pathogenesis of acquired FXIII deficiency in our patient.

Following a minor cut at the right forearm five days earlier, the patient presented to our institution with persistent bleeding from the cutaneous wound and a significant soft-tissue haematoma. Plasma-derived FXIII (Fibrogammin® P, CSL Behring) was substituted at a single dose of 25 IU/kg of body weight, and oral tranexamic acid (Cyklokapron®, MEDA Pharma) was initiated at a daily dose of 3 x 1 g, resulting in rapid haemostatic control and improved wound healing. Key laboratory parameters obtained immediately before and 1 week into treatment, after which antifibrinolytic therapy was stopped to avoid thrombotic complications, are shown (▶Tab. 2). A marked increase in FXIII activity was associated with an elevation in fibrinogen and platelets and a significant decline in plasma D-dimer.

**Fig. 1** Transaxial and sagittal CT scans demonstrating dissection, elongation and dilatation of the thoracic aorta

**a)** Contrast enhanced CT at the level of the left pulmonary artery (PA) demonstrates an aneurysmatic ascending aorta (AA) with a dissection flap (arrowheads) extending into the descending aorta (DA). **b)** Contrast enhanced sagittal reconstructed CT of the thorax and abdomen shows irregular elongation and dilatation of the thoracic aorta with a maximal diameter of the descending arch of 7.7 cm (double arrow).

**Fig. 2** Analysis of plasma microparticles (MPs) by thrombin generation assay (TGA)

**a)** Total cellular MPs were isolated from the patient’s plasma by high-speed centrifugation (60 min at 16,000 x g) and subsequently resuspended in normal human plasma (NHP) depleted of endogenous phospholipids. Following recalcification, thrombin generation was monitored over a 2-hour period in the presence of 30 µg/ml inhibitory tissue factor monoclonal antibody (anti-TF; American Diagnostica) or isotype-matched control (IgG; Sigma) using the Technothrombin™ TGA (Technoclone).

**b)** For comparison, plasma MPs were isolated from four healthy volunteers and analyzed for procoagulant activity as described before. TGA was carried out in the presence of control IgG only.
Discussion

Acquired FXIII deficiency is increasingly recognized as a risk factor for increased blood loss in surgical patients (10, 11). Particularly, elevated perioperative levels of soluble fibrin monomer and decreased FXIII availability per unit of thrombin generated appear to result in a significant loss of clot firmness (13).

Consequently, prophylactic FXIII substitution has been shown to maintain clot strength and to reduce blood loss in high-risk surgical patients (14, 15).

While in congenital FXIII deficiency residual activity levels of 5–20% may be sufficient to prevent spontaneous bleeding, our case report clearly suggests that acquired FXIII deficiency with activity levels of 30–35% may confer a severe bleeding tendency even in non-surgical patients, especially in the context of increased thrombin an fibrin generation.

A striking feature of the presented case is that FXIII was the only decreased clotting factor during initial evaluation at our institution (Tab. 1). In fact, a similar finding had previously prompted a different institution to initiate molecular testing for hereditary FXIII deficiency in this patient, which had revealed no genetic abnormalities. The excessively elevated D-Dimer and decreased alpha2-antiplasmin levels indicated significant, albeit compensated systemic coagulation activation with hyperfibrinolysis and implied increased consumption and/or proteolytic degradation, rather than impaired synthesis, as the main mechanism of acquired FXIII deficiency in our patient. Although bleeding symptoms (i.e. extensive soft-tissue hematomas with delayed wound healing) were compatible with FXIII deficiency, hyperfibrinolysinaemia with accelerated clot breakdown due to exhaustion of the antifibrinolytic system had most likely contributed to the haemorrhagic phenotype. Consistent with an acquired dynamic coagulation disorder, the patient intermittently developed mild thrombocytopenia and hypofibrinogenaemia (Tab. 2), but still failed to meet the ISTH criteria for overt (i.e. decompensated) DIC. FXIII should thus always be included in the diagnostic work-up of non-surgical patients with an acquired bleeding tendency despite apparently normal global coagulation tests.

Because other potentially underlying diseases such as liver failure, malignancies or autoimmune disorders could be confidently excluded, acquired FXIII deficiency in our patient was likely due to the massive aortic dissection. We hypothesize that the large thrombogenic surface of the perfused false lumen, in concert with turbulent blood flow, was causative of the clotting disorder. The aneurysm's increasing diameter over time may explain why the patient had been asymptomatic during the first five years after surgery and why progressive bleeding symptoms had only recently developed. Our thrombin generation data do not support a relevant contribution of circulating procoagulant MPs to the pathogenesis of acquired FXIII deficiency in this patient.

Aortic (dissecting) aneurysms are a rare, albeit well-recognized cause of decompensated DIC (16). Several reports have emphasized the haemostatic challenges encountered both before and during vascular surgery in such patients (16–19). Administration of clotting factor concentrates and definitive surgical repair were the main treatment approaches. In contrast, the use of low-dose heparin to control the consumptive coagulopathy in a bleeding patient with DIC remains controversial (20).

Tab. 2 Effects of FXIII substitution and oral antifibrinolytic therapy on selected haemostatic parameters: A single dose of plasma-derived FXIII (25 IU/kg of body weight) was administered on day 0, whereas oral tranexamic acid (3 x 1 g per day) was continued for a week (PTT, partial thromboplastin time).

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<tr>
<td>prothrombin time (%)</td>
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<td>activated PTT (s)</td>
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<tr>
<td>thrombin time (s)</td>
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</tr>
<tr>
<td>fibrinogen (g/l)</td>
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</tr>
<tr>
<td>FXIII : C (%)</td>
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</tr>
<tr>
<td>D-dimer (mg/l)</td>
<td>&gt;35</td>
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Acquired FXIII deficiency

What is known about this topic?
- Acquired FXIII deficiency is associated with increased blood loss in surgical patients.
- Elevated perioperative levels of soluble fibrin monomer and decreased FXIII availability per unit of generated thrombin result in a significant loss of clot firmness.
- Prophylactic FXIII substitution has been shown to maintain clot strength and to reduce blood loss in high-risk surgical patients.

What does this paper add?
- Acquired FXIII deficiency with residual activity levels of 30–35% may confer a severe bleeding tendency in non-surgical patients, especially in the context of increased thrombin and plasmin generation.
- Short-term treatment with tranexamic acid and intermittent FXIII substitution were safe and efficacious in an elderly patient with acquired FXIII deficiency and significant cardiovascular comorbidities.
- FXIII should be included in the diagnostic work-up of an acquired bleeding disorder even in the presence of normal global coagulation tests.
Conflict of interest

The authors declare that they have no conflict of interest.

References