Fibrin monomer and factor XIII
A new concept for unexplained intraoperative coagulopathy

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Keywords
Thrombin, fibrin monomer, FXIII, clot firmness, intraoperative blood loss

Summary
Unexplained intraoperative coagulopathies continue to be a diagnostic and therapeutic dilemma. The pathophysiology behind unexplained intraoperative coagulopathies is of great variety and complexity (preexisting coagulopathies, dilutional coagulopathy, interactions of medications etc.). We have shown in prospective studies that patients undergoing elective surgery who develop “unexplained” intraoperative coagulopathies have significantly less FXIII per unit thrombin available at any point in time (i.e. already preoperatively) than patients without such coagulopathies. The consequence is a significant loss of clot firmness associated with an increase in intraoperative blood loss. Thus, these patients have less cross-linking capacity to begin with, which explains their preoperatively increased fibrin monomer concentration. The association of increased preoperative fibrin monomer and increased intraoperative blood loss was prospectively evaluated and confirmed in a separate clinical study. It is important to note that the acquired or (compared to the amount of thrombin generated) relative F. XIII deficiency in situations with surgical stress shows early clinical relevance (even if only mild to moderate changes are present); this differs from the experiences with patients with inborn FXIII deficiency, where a pronounced deficiency must be present to have clinically significant spontaneous bleeding.

Patients undergoing elective surgery, without clinically obvious coagulopathy but increased preoperative fibrin monomer concentration (as a marker of decreased cross-linking capacity) are at risk for increased intraoperative blood loss. This new concept helps to explain the pathophysiology behind unexplained intraoperative coagulopathies and thus allows for corresponding treatment strategies. Further clinical studies for early detection and interventions in patients with such coagulopathies are necessary.

FXIII structure and function
FXIII circulating in the plasma consists of two identical pro-enzyme (A₂) and carrier protein (B₂) subunits. The A-subunit contains the active centre, the activation peptide, a calcium binding site and free sulphydryl groups.

The B-subunit (6, 7) acts as carrier protein to stabilise subunit A, binds the substrate (fibrinogen) and assists in regulating FXIII activity.

While B-subunit is found in plasma in its free form as well as incorporated in the A₂B₂ tetramer, A-subunit is completely complexed with the B-subunits (i.e. in its non-active state, A is found only in the tetramer); FXIII often circulates in association with fibrinogen (8).

The gene for subunit A is located on chromosome 6 (p24–25), consists of 160 kilobases and has 15 exons. The gene for subunit B, consisting of 28 kilobases and having 12 exons, is located on chromosome 1 (q31–32.1) (9–11).

The Val34Leu polymorphism plays no part in severe (congenital) FXIII deficiencies but contributes to the wide range of normal distribution of FXIII activity (12).

FXIII is present in platelets as A₂ homodimers, representing about 50% of the total FXIII activity present in the body. During steady state, FXIII has a long half-life of 9–14 days. Biosynthesis of FXIII subunit A takes place in bone marrow cells, monocytes and macrophages, as well as hepatocytes; subunit B is synthesised in the liver.

An enzyme known as tissue transglutaminase – in fact being the intracellular form of FXIII – is also found as A₂ homodimer in platelets, megakaryocytes, monocytes, macrophages, the liver, placenta and the uterus (13–15).

Thrombin generation is followed by the release of fibrinopeptide A from the α-chain and of fibrinopeptide B from the fibrinogen β-chain. Following release of the fibrinopeptides, the fibrinogen molecule is referred to as fibrin monomer. Fibrin monomers spontaneously associate longitudinally before being cross-linked by FXIIIa. FXIIIa is generated after thrombin-mediated cleavage of an activation peptide. Subsequent calcium ion-dependent dissociation of the B subunit allows the presentation of the active centre through conformational changes (16, 17).

Apart from its clot-stabilising properties, FXIII also anchors α₂-antiplasmin to the fibrin clot (18). In this way, FXIII ensures that the clot has a certain resistance to the fibrinolytic activity generated in parallel.

Hämostaseologie 3a/2006

Hämostaseologie 2006; 26 (Suppl 1): S30 – S 34
FXIII concentration and FXIII activity

FXIII deficiency is not detected through classical global coagulation tests, e.g. PT/INR and aPTT. Normal INR or aPTT values therefore do not allow the conclusion that there is no factor XIII deficiency. A specific chromogenic method which can easily be automated is used to measure FXIII activity in most laboratories at the present time (19). In the low range, however, this assay seems to be less accurate than an incorporation assay based on the natural function of FXIII (20) which is a modified version of earlier assays (21, 22). As for antigen measurements, these seem not relevant outside scientific research.

**FXIII deficiency**

**Inherited FXIII deficiency**

Congenital FXIII deficiency is rare. The prevalence of a severe FXIII deficiency within the population is approx. 1–2/10⁶ and shows an autosomal recessive inheritance. The clinical picture can be variable and depends on the severity of the deficiency (23). It includes

- spontaneous abortions that occur in early pregnancy (24),
- umbilical cord bleeding (up to three weeks after birth),
- an increased tendency to bleed during childhood, especially in response to trauma; bleeding may be immediate, delayed or repeated,
- intracerebral haemorrhage (which may be the first sign of severe FXIII deficien-
cy); in children, this is often preceded by trauma; intracerebral bleeding may recur in one-third of cases, and has a correspondingly high mortality risk,
● menorrhagia (25),
● wound healing disorders (5).

Long-term replacement therapy has to be considered in (severe) cases of congenital FXIII deficiency because of the high risk of CNS haemorrhage. Due to the long half-life of FXIII, replacement is usually only needed every 4 weeks.

Acquired (relative) FXIII deficiency

Surgery causes marked activation of the coagulation system. This trauma-induced activation aims at keeping blood loss at a minimum by immediately closing off small injured vessels. It is well known that tissue factor (TF) / factor VII (FVII) are important stimuli in the initial activation of the clotting system. Interactions between TF and FVII ultimately result in thrombin generation via FX and FIX. Thrombin – the concentration of which is greatly increased by positive feedback loops involving platelet surfaces (26, 27) – acts to release the fibrinopeptides from fibrinogen. The potential of these positive feedback loops to generate a “thrombin burst” is exploited when using recombinant factor VIIa (28).

At this point, release of fibrinopeptides A and B allows the still monomeric fibrin molecules to associate laterally in preparation for cross linking. Just as thrombin generation is important for the conversion of fibrinogen to fibrin, FXIII is important to transform soluble fibrin (fibrin monomers) into cross-linked fibrin and thus form a stable clot. FXIII reinforces clot stability through the formation of covalent bonds between (at that point still) soluble fibrin molecules. It is important to note that FXIII is not just activated by thrombin in an all-or-nothing reaction; FXIII activation seems regulated via thrombin and soluble fibrin (7, 16, 29–33).

As previously mentioned, FXIII also anchors α2-antiplasmin and other proteins (e.g. fibronectin and collagen) to the clot. This increases the resistance of the clot to the physiologically (co-)generated fibrinolytic response; it also facilitates wound healing by modulating already formed “grids” (34–36). These properties of FXIII are therefore of particular relevance in the perioperative and postoperative periods. In this context, the fact that FXIII (A) is present in platelets has an even wider meaning. Platelet numbers are often reduced perioperatively through consumption so that less platelet FXIII is available during these situations.

Given the considerations and explanations mentioned above, a situation with reduced FXIII activity has to be accompanied by a reduction in clot firmness. In this case, the risk of bleeding increases with decreasing FXIII availability. Is there any clinical evidence for this scenario? Although observational trials indicate that FXIII does not play an important role in cardiac surgery (37), therapeutic studies show another picture (38, 39). There is also evidence from neurosurgical procedures that available FXIII activity correlates with the risk of bleeding (41, 42). Given these partially contradictory results, the pathophysiology behind the observations remained unclear.

We therefore evaluated patients who had an apparent tendency to increased unexplained bleeding during surgery. In a sufficiently large prospective controlled trial, we established that these patients do not show a reduction in thrombin formation (as it might be expected at a first glance) but in fact show an increase in thrombin generation (42). As this observation is not easily understood and even seems to be a paradox, we have tried to clarify the background to this finding. We provide evidence that these patients do not only show an increase in throm-
bin generation but also have increased fibrin formation at any point in time (including preoperatively). This is evidenced by a more pronounced formation of fibrin monomers as can be seen in Figure 1a (43).

The reduction in fibrinogen concentration (median 1.4 g/l) did not explain sufficiently the observed haemorrhagic tendency. The question resulting from this is how to explain that patients with increased thrombin generation and more soluble fibrin formation bleed more heavily? One possible explanation is that the transformation of soluble fibrin into cross-linked fibrin, which is mediated by factor XIII, is compromised. In fact, we were able to demonstrate a reduction in FXIII activity in those patients that bled more heavily (Fig. 1b). This finding does not yet explain the reason for the development of this haemorrhagic tendency since the reduction in FXIII was only obvious with the bleeding already being active. However, as FXIII needs first to be activated by thrombin (cleavage of the activation peptide from the A subunit, release of the A from the B subunit), we also have to consider an imbalance in the ratio of available FXIII activity and prior thrombin generation as the reason for impaired cross-linkage. We were able to show that there was a significant difference in the availability of FXIII activity in comparison to the amount of thrombin generated (as quantified by measurement of prothrombin activation by F1 + 2) between patients with and without an unexplained intraoperative bleeding tendency.

This phenomenon, like the increased fibrin monomer formation, was also seen preoperatively (Fig. 1c), now explaining the development of the increased intraoperative bleeding tendency. Finally, it remained to verify that these observed changes indeed have an effect on the quality of the clot. The corresponding reduction of clot firmness was demonstrated using thromboelastography (Fig. 1d) (43). Thereafter, two main points still had to be considered: we still had to confirm that the association of increased preoperative fibrin monomer concentration and increased intraoperative bleeding tendency was not just coincidence; and the question whether an increase in FXIII availability would improve clot firmness (and hence reduce the tendency to bleed) had to be answered.

To address the question on the association of fibrin monomer concentration and intraoperative bleeding tendency, we carried out a further prospective study. In a population of patients undergoing elective visceral surgery, we showed that the fibrin monomer concentration measured preoperatively did, in fact, correlate positively with the intraoperative blood loss (Fig. 2b). Inclusion volume, taken as a biological surrogate marker for intraoperative blood loss, also correlated with the preoperative fibrin monomer concentration (44).

The question whether FXIII replacement in high-risk patients (high preoperative fibrin monomer concentration) improves or maintains clot firmness is subject of a prospective randomised double blind trial that has been performed and is being evaluated at the present time.

FXIII deficiency is also not uncommon seen postoperatively, which can easily be explained by intraoperative consumption (43). There is clear clinical evidence that absolute or relative FXIII deficiency is associated with an increased risk of bleeding and that the administration of FXIII can improve the clinical situation (39, 41–49).

It is therefore worth to measure FXIII activity in cases of unexplained postoperative bleeding (e.g. when standard laboratory tests (INR, aPTT), fibrinogen concentration and platelet count are in the normal range and there is no indication of platelet dysfunction). If FXIII activity is low, replacement (at a dose of 20 U/kg body weight) should be considered. If rapid FXIII measurement is not possible, a trial dose of FXIII is justified if a bleeding tendency persists despite foregoing adequate active haemostatic therapy: an acquired FXIII deficiency might then be assumed in this case.

From these observations, we can conclude that FXIII should by no means be “forgotten” in optimising intraoperative haemostasis. This has to be emphasized given the frequently encountered meaning that minimal FXIII activity is sufficient for adequate haemostasis.

This idea comes from experiences in patients with congenital FXIII deficiency. However, acquired FXIII deficiency in surgical patients seems a different issue. The clinical experience here is very different since even mild acquired FXIII deficiencies respond to replacement (39, 43, 50, 51), whereas patients with mild to moderate congenital FXIII deficiency virtually never show clinically relevant bleeding diatheses (whereas those with a definite bleeding tendency and congenital deficiency are usually severely deficient (52)).

Conclusions

FXIII is a parameter that should not be ignored in intra- and perioperative haemostasis. We have evidence that the use of FXIII in cardiac surgery leads to a reduction in transfusion requirements. In non-cardiac surgical patients with intraoperative coagulopathy a significantly lower FXIII availability could be demonstrated, which led to a defect in cross-linkage, loss of clot firmness, and finally more pronounced blood loss. These patients already show an increased fibrin monomer concentration preoperatively. Postoperative FXIII deficiency due to previous consumption seems common, and the resulting bleeding often responds well to FXIII replacement.

Therefore, the differential diagnosis and treatment of perioperative bleeding should also take FXIII deficiency into consideration. It must be remembered that FXIII has to be measured separately, as its deficiency does not influence the aPTT and PT/INR.

References