RESEARCH LETTER

Plasma levels of the A subunit of factor XIII in patients undergoing off-pump coronary artery bypass surgery

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Introduction Coagulation factor XIII (FXIII) is the zymogen precursor of an active transglutaminase (FXIIIa); it circulates in blood as a heterotetramer consisting of 2 catalytic A subunits (FXIII-A) and 2 noncatalytic B subunits (FXIII-B). In the presence of thrombin and calcium, the A subunits are released and activated. The enzyme plays a crucial role in clot stabilization by the formation of covalent crosslinks between fibrin monomers in the final stage of the blood coagulation pathway.[1]

Previous research has demonstrated a significant association between FXIII-A and inflammation.[2,3] Studies performed in animal models have shown that the inflammatory process is enhanced via fibrin stabilization by FXIII.[3] Recent data have suggested that, in addition to its importance in coagulation, FXIII may be involved in the heart healing process.[4-6] However, these studies investigated FXIII levels in patients with myocardial infarction (MI). In contrast, remarkably little is known about the effect of cardiac surgery on FXIII. For instance, FXIII dynamics among patients scheduled for off-pump coronary artery bypass (OPCAB) surgery has not yet been examined. Therefore, the aim of this preliminary study was to measure the levels of FXIII-A in relation to fibrinogen and high-sensitivity C-reactive protein (hs-CRP) levels before and 1 week after OPCAB surgery. Additionally, we aimed to investigate whether FXIII-A levels are affected by previous MI and intraoperative transfusions.

Patients and methods The analysis included 29 men who underwent elective first-time isolated OPCAB surgery. The inclusion criteria were as follows: coronary artery disease with indication for OPCAB surgery, male sex, and age between 45 and 80 years. The exclusion criteria were as follows: emergency, redo, or concomitant valvular surgery; recent MI (≤1 month); a history of systemic malignancy, known bleeding disorders, renal insufficiency (serum creatinine upper normal limit [≥1.3 mg/dl; ≥115 μmol/l] or estimated glomerular filtration rate of less than 60 ml/min/1.73 m^2), liver insufficiency, active hepatitis, preoperative anemia (hemoglobin <130 g/l), severe obesity (body mass index [BMI] >40 kg/m^2); and anticoagulant therapy, except for low-molecular-weight heparin bridging started 5 to 7 days prior to the index procedure. Patients with known mental disorders and unable to provide written consent were also excluded. The final exclusion criterion was the macroscopic evidence of hemolysis or lipemia in obtained plasma samples. All patients were treated before and after OPCAB surgery according to the 2014 European Society of Cardiology / European Association for Cardio-Thoracic Surgery Guidelines on myocardial revascularization.[7] The study was approved by the Ethics Committee of the Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland.

Fasting blood was drawn twice: before the surgery (day 1) and 1 week after the surgery (day 7) from a 21-G needle into 3.2% sodium citrate vacuum tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, New Jersey, United States) and centrifuged at 2500 × *g* for 20 minutes. The resulting plasma was stored at –80 °C until analysis. The FXIII-A antigen and hs-CRP levels were assessed using enzyme-linked immunosorbent assay kits (Zymutest Factor XIII-A, Hyphen BioMed SAS, France and Human hsCRP ELISA, BioVendor Laboratory Medicine Inc., Czech Republic, respectively). The measurements were performed according to the manufacturer's instructions, and researchers running the assays were blinded to patients'

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data. The levels of FXIII-A were expressed as percentage. The 100% FXIII-A corresponds to the levels in a normal human citrated plasma pool, obtained by pooling plasma samples from healthy men or women aged from 18 to 55 years and free from any disease or not using any medication. The hs-CRP levels were expressed in mg/l. The lower detection limits were 10% or lower for FXIII-A and 0.02 mg/l for hs-CRP. The intraassay and interassay coefficients of variation for tests were below 10%. Fibrinogen levels were determined according to the Clauss method on a coagulometer K-3002 Optic (Kselmed s.c., Poland). The results were expressed as the average of the 2 measurements in g/l.

Statistical analysis Statistical analyses were carried out using STATISTICA® 12.0 (StatSoft, Tulsa, Oklahoma, United States). The Wilcoxon test was used to compare the plasma levels of the parameters between days 1 and 7. The groups were compared using the Mann–Whitney test for numerical data and the Fisher exact test for qualitative data. All correlations were analyzed with the Spearman nonparametric correlation coefficient. A P value of less than 0.05 was considered significant.

Results The clinical characteristics of the patients are described in Supplementary material online, *Table S1*. The definitions of comorbidities and surgical procedures were described in detail elsewhere.[8] The most prevalent cardiovascular risk factors were hypertension (n = 27) and dyslipidemia (n = 18). Of all patients, 12 had undergone aortic no-touch OPCAB surgery. Pre- and postsurgical pharmacological treatment and data concerning surgical procedure are shown in Supplementary material online, *Table S1*. No patients received oral anticoagulant therapy during the study.

First, we used the Wilcoxon test to compare presurgical (day 1) and postsurgical (day 7) FXIII-A levels in relation to fibrinogen and hs-CRP levels. The data are summarized in **FIGURE 1**. At day 7, median FXIII-A levels were lower (101.41% vs 81.03%, $P = 0.03$), while median hs-CRP and fibrinogen levels were higher (2.40 mg/l vs 13.11 mg/l, $P < 0.001$ and 2.99 g/l vs 4.89 g/l, $P < 0.001$, respectively) compared with day 1.

Although it is not clear what mechanism controls FXIII-A levels following OPCAB surgery, it has been proposed that they might be affected by previous MI.[9] To address this issue, we divided patients into 2 groups: with and without previous MI (16 and 13 patients, respectively). There were no differences between the groups in terms of age, BMI, left ventricular ejection fraction, blood pressure, renal function, complete blood count, comorbidities, intraoperative transfusion data, pre- and postoperative drug use, and surgical procedure parameters (Supplementary material online, *Table S2*). The non-MI patients had lower median FXIII-A levels before surgery compared with patients with previous MI (84.53% vs 105.33%, $P = 0.04$; Supplementary material online, *Table S3*). Additionally, our results showed that only patients with previous MI had lower median levels of FXIII-A after surgery compared with day 1 (105.33% vs 81.58%, $P = 0.02$; Supplementary material online, *Table S3*). In both groups, hs-CRP and fibrinogen levels were found to be higher after surgery ($P < 0.05$ for all; Supplementary material online, *Table S3*).

Subsequently, we wanted to verify whether transfusion during surgery might affect the levels of the measured plasma parameters. Ten patients received at least 1 unit of packed red blood cells or fresh frozen plasma during surgery (Supplementary material online, *Table S1*). In 19 patients, surgery could be completed without transfusion of packed red blood cells or fresh frozen plasma. The analysis showed that transfusion had no significant effect on FXIII-A, hs-CRP, or fibrinogen levels. In addition, the within-group analysis showed that FXIII-A levels were similar between days 1 and 7. However, hs-CRP and fibrinogen levels were significantly higher 1 week after the surgery in both groups (Supplementary material online, *Table S4*).

To determine whether FXIII-A levels correlated with fibrinogen and hs-CRP levels, we performed a Spearman rank correlation analysis. However, we found no significant correlations between the measured parameters on days 1 and 7 or between the 2 measurement timepoints (Supplementary material online, *Tables S5–S7*). Furthermore, we correlated the clinical parameters such as age, BMI, left ventricular ejection fraction, and systolic and diastolic blood pressure with the levels of FXIII-A on day 1. In addition, data concerning the surgical procedure (number of grafts, procedure duration, postprocedural hospital days, and total volume of blood products) and the postprocedural number of white blood cells were correlated with the reduced levels of FXIII-A at day 7. However, there were no significant correlations between any of these parameters (Supplementary material online, *Tables S8–S9*).

Discussion To the best of our knowledge, this study is the first to show that OPCAB surgery may result in reduced FXIII-A antigen levels. Previous publications have shown that both the levels and activity of FXIII were decreased after traditional coronary artery bypass grafting. These changes were associated with hemodilution and FXIII consumption during cardiopulmonary bypass.[10,11] Nonetheless, our patients underwent the OPCAB surgery without conventional cardiopulmonary bypass, and the above results[10,11] were obtained shortly after the surgery (up to 24 hours after surgery). Importantly, the lower FXIII-A levels after the surgery may be a response to surgical trauma; however, a detailed study is needed to verify this hypothesis.

The precise mechanisms behind a decrease in FXIII-A antigen levels after OPCAB surgery have not been defined. However, we speculate that it

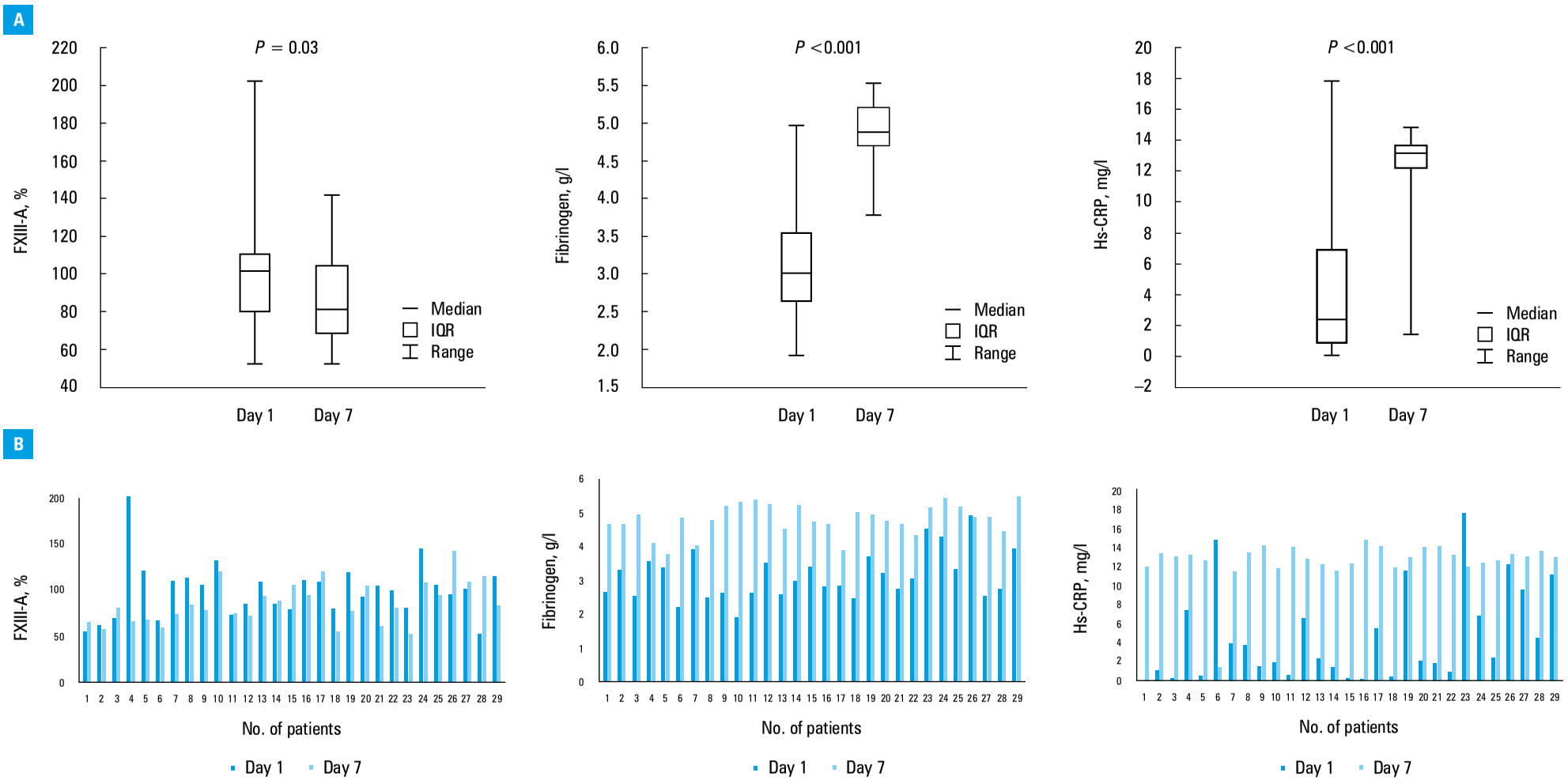

FIGURE 1 **A** – box plots representing the plasma levels of coagulation factor XIII A-subunit (FXIII-A), fibrinogen, and high-sensitivity C-reactive protein (hs-CRP) before (day 1) and 1 week after (day 7) off-pump coronary artery bypass surgery (OPCAB); **B** – individual values for plasma levels of FXIII-A, fibrinogen, and hs-CRP before (day 1) and 1 week after (day 7) OPCAB surgery.

may be explained by the consumption of FXIII during myocardial wound healing after the surgery. Nahrendorf et al[4-6] provided experimental and clinical evidence for a central role of FXIII in infarct healing processes. Further investigation by Gemmati et al[9] revealed a remarkable drop in FXIII-A antigen levels at day 5 after MI. The authors suggested that this phenomenon was compatible with the processes of cardiac healing and scar formation. Nevertheless, the mechanisms by which MI leads to alteration of FXIII-A levels are not completely understood. Our data indicating that patients with previous MI have higher levels of FXIII-A before the surgery compared with non-MI individuals suggest that MI could be an important factor influencing plasma FXIII-A levels. However, these results should be interpreted with caution as the time between MI and surgery varies between patients from month to several years. Undoubtfully, this issue should be the focus of future studies.

Moreover, in our patients, a decline in FXIII-A levels 1 week after OPCAB surgery were accompanied by increased levels of inflammatory markers, namely, fibrinogen and hs-CRP. Importantly, the negative effect of inflammation on FXIII levels has been reported previously.[12,13] Consequently, in patients with inflammation of various etiologies, a lower plasma level or activity of FXIII was observed.[12,13] Miscellaneous proinflammatory mediators that work antagonistically to the FXIII synthesis have been implicated in this process.[13] Nonetheless, despite higher fibrinogen and hs-CRP levels observed 1 week after the surgery, we did not show significant correlations between these inflammatory markers and the plasma levels of FXIII-A. A possible explanation for this finding may be that we measured only the levels of FXIII-A antigen. Thus, future studies should investigate FXIII-A activity in patients undergoing OPCAB surgery. Another important challenge is to understand the relationship between other subunits of FXIII and cardiac surgery. Nonetheless, it was reasonable to measure the levels of FXIII-A as they have catalytic activity for fibrin crosslinking.[1]

This study has some limitations. It was a preliminary investigation in a small number of subjects, which may have affected the results. However, it should be noted that the number of patients was similar to that in studies involving patients after coronary artery bypass grafting,[14,15] and the current analysis may be useful for other researchers who are planning a study in individuals undergoing OPCAB surgery.

Together, our data suggest that OPCAB surgery has a significant impact on the circulating A subunits of FXIII antigen levels. Nonetheless, further research is required to confirm our observation.

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