Complete remission* achieved by steroid pulse therapy following rituximab treatment in a case with autoimmune haemorrhaphilia due to anti-factor XIII antibodies

Yoshiyuki Ogawa1; Masahiro Mihaara1; Masayoshi Souri2,3; Kunio Yanagisawa1; Toshimasa Hayashi1; Nobuhiko Kobayashi1; Hiroki Shimizu1; Hirono Iriuchijima1; Takuma Ishizaki1; Hiroshi Handa1; Tukasa Osaki1,2; Yoshihisa Nojima1,1; Akitada Ichinos2,3,†

1Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Japan;
2Department of Molecular Patho-Biochemistry and Patho-Biology, Yamagata University School of Medicine, Yamagata, Japan;
3Japanese Collaborative Research Group on Autoimmune Hemorrhaphilia XIII/13

Dear Sirs,

Coagulation factor XIII (FXIII or F13) is a plasma fibrin-stabilising factor which crosslinks fibrin monomers, and fibrin and $\alpha_1$-plasmin inhibitor, and thus plays an essential role in haemostasis. Therefore, its congenital deficiency results in a life-long haemorrhagic tendency (1, 2). In contrast, acquired coagulation FXIII deficiency is a common abnormal condition secondary to its hyper-consumption or hypo-synthesis (2, 3), while autoimmune haemorrhaphilia due to anti-FXIII antibodies (AHXIII) is a rare life-threatening bleeding disorder mostly in the elderly (3, 4). In the 21st century, the number of AHXIII cases has been on the increase at least in Japan (unpublished data), because Japan has become a super-aging society first in the world. Therefore, it is most important to save patients’ lives by its prompt examinations, correct diagnosis, and proper treatment. Here, we report such an excellent example.

A 70-year-old Japanese male had no personal and family history of bleeding tendency. He had general fatigue and arthralgia of the right wrist from the second half of May 2013, and then diffuse swelling developed in his right back in the middle of June. Because the swelling and pain of his right back increased, he visited a local hospital. Since a hematoma was found by CT imaging and severe anaemia (Haemoglobin 6.7 g/dl) was detected by haematological analyses, he was referred to our hospital. Hand X-ray showed non-destructive synovitis and laboratory examinations revealed severe anaemia (haemoglobin 6.7 g/dl) and haemolysis (LDH 1,469 IU/l; total bilirubin 1.9 mg/dl; haptoglobin, <6.6 mg/dl) as well as positive Coombs’ tests (direct 3+, indirect 1+), antinuclear antibody (ANA, 1:2,560 dilution) and anti-DNA antibody (34 IU/ml). Accordingly, he was diagnosed as having systemic lupus erythematosus and autoimmune haemolytic anaemia.

Furthermore, a physical examination on admission confirmed extensive swelling from the right scapular to lumbor portions and CT imaging revealed a huge intramuscular haematoma in his right back (data not shown). Although PT, APTT and platelet count were normal, his FXIII activity and antigen turned out to be as low as 4% and 5% of normal, respectively. The reduced activity was not corrected (6% residual FXIII activity) by a normal plasma (97% FXIII activity) in a 1:1 cross-mixing test, and a five-step dilution cross-mixing test of the patient’s plasma showed a concaved “inhibitor” pattern (Suppl. Figure 1A, available online at www.thrombosis-online.com), clearly indicating the presence of anti-FXIII inhibitor. Finally, all three immunological tests, ELISA and dot blot assay (Figure 1 and Suppl. Figure 1B, available online at www.thrombosis-online.com), and immuno-chromatography (data not shown), detected anti-FXIII A subunit (FXIII-A) autoantibodies. Thus, he was definitely diagnosed as AHXIII. Detailed experimental studies also demonstrated the absence of both $\gamma$-dimerisation and $\alpha$-polymerisation of fibrin (Suppl. Figure 1C, available online at www.thrombosis-online.com), indicating that fibrin-stabilisation was highly impaired.

Whereas he was initially treated with prednisone (PSL; 0.8 mg/kg/day) from hospital day 2, there was no evidence of its efficacy on either his FXIII activity or anti-FXIII inhibitor potency (residual FXIII activity in the 1:1 cross-mixing test), for 28 days (Figure 1). Because he had repeated bleeding episodes, he was infused with plasma-derived FXIII concentrates (27–45 U/kg). Pharmacokinetic studies revealed extremely low recovery rates and shortened half-lives of exogenous FXIII (Suppl. Figure 2, available online at www.thrombosis-online.com), suggesting the presence of a large amount of anti-FXIII inhibitor (i.e. “free” neutralising anti-FXIII-A autoantibodies). Accordingly, he was treated with rituximab (375 mg/m²/week) for four consecutive weeks. Since his plasma FXIII activity and residual FXIII activity in the cross-mixing test only slightly increased after the rituximab therapy, he was given methyl-PSL (1,000 mg/day) for three days (steroid pulse therapy) from day 57. Thereafter, residual FXIII activity in the cross-mixing test started to increase rapidly, indicating a rapid decrease in the neutralising anti-FXIII-A autoantibodies. The patient’s FXIII activity also gradually increased and reached to normal levels about 50 days after the steroid pulse therapy (Figure 1). All immunological tests confirmed the disappearance of the anti-FXIII-A autoanti-

Correspondence to:
Akitada Ichinos, MD, PhD
Department of Molecular Patho-Biochemistry and Patho-Biology
Yamagata University School of Medicine
Yamagata, 990–2331, Japan
Tel: +81 23 628 5276, Fax: +81 23 628 5280
E-mail: aichinos@med.id.yamagata-u.ac.jp

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† Both YN and AI contributed to this research project as senior authors.

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bodies (▶ Figure 1 and Suppl. Figure 1D, available online at www.thrombosis-online.com), and thus he was discharged on day 116 (hospital week 17, ▶ Figure 1). Until the time of submission of this revised manuscript, he is still in complete remission (CR) for more than six months.

Haemostatic therapy is essential in the management of AHXIII, because it is a life-threatening disease. In fact, three out of 12 Japanese AHXIII cases had deceased before the arrival of test plasma samples to the last author’s laboratory („dead-on-arrival” of test samples) in 2013 (unpublished data). Fortunately, the present case was promptly diagnosed and properly treated under continuing close monitoring of FXIII activity, antigen, cross-mixing tests, anti-FXIII-A antibodies, etc. Especially, pharmacokinetic analyses were carried out as many as four times to estimate recovery rates, peak times, as well as half-lives of administered FXIII. These results clearly indicated the rapid neutralisation and enhanced clearance of the infused exogenous FXIII in the present patient in vivo (Suppl. Figure 2, available online at www.thrombosis-online.com). All these detailed examinations made it possible to rapidly achieve CR of AHXIII without any further bleeding episodes or complications after the steroid pulse therapy.

Figure 1: Clinical course of the present AHXIII case. A) The patient underwent immune-suppressive therapies firstly with regular-dose steroid (PSL), secondly rituximab, and thirdly steroid pulse (methyl-PSL; mPSL). Plasma-derived FXIII concentrates were given together with an anti-fibrinolytic agent (tranexamic acid). B) A Japanese version of ISTH/SSC Bleeding Assessment Tool (JBAT; shadowed column) was applied to objectively evaluate the patient’s bleeding symptoms. Bleeding score was 6 at admission to our hospital and gradually deceased to 0 following medication, in the opposite direction to haemoglobin levels (closed circle). LDH (closed square) rapidly decreased following the immune-suppressive therapies. C) Both FXIII activity (closed circle) and antigen (shadowed column) only slightly increased following rituximab treatment, and rapidly rose after steroid pulse therapy. Residual FXIII activity in a 1:1 cross-mixing test (closed triangle) also increased after steroid pulse therapy. *; This relatively high FXIII antigen level was obtained 24 hours after the infusion of FXIII concentrates (2,400 U), and the resulting low FXIII specific activity (i.e. FXIII activity/antigen ratio) indicated the formation of FXIII antigen-antibody complexes between “free” anti-FXIII-A autoantibodies and exogenous FXIII concentrates, as shown in Suppl. Figure 2D (available online at www.thrombosis-online.com). D) Anti-FXIII-A IgG (open triangle) measured by ELISA decreased in parallel with anti-DNA antibody (closed diamond). The amount of anti-FXIII-A IgG on June 28 (hospital day 1), 2013 is defined as 100%.
Steroid pulse was given (in combination with calcineurin inhibitors) to acquired haemophilia A (AHA) cases, and sustained response was achieved in 10 of 11 patients in a median time of three weeks (5). Several AHA cases were also successfully treated with steroid pulses alone (6) or followed by regular-dose steroid therapy (7–9). In contrast, two AHXIII cases were treated with steroid pulse, but there was no evidence of response (10, 11). Since FXIII activity had not improved following rituximab treatment in the present case, steroid pulse was added (Figure 1). However, we cannot attribute the patient's CR to steroid pulse alone, because rituximab may require more time than other regimens to achieve remission, as reported in 51 AHA cases (12). Complete (13) and partial responses (14, 15) were obtained by rituximab treatment in one and two AHXIII cases, respectively, while no response was achieved in two cases (11, 16). Accordingly, it would be proper to conclude that a combination of steroid pulse and rituximab treatment achieved CR in this AHXIII case.

It is also important to note that both ANA and anti-DNA antibodies disappeared in concert with anti-FXIII autoantibodies (Figure 1), indicating the efficacy of the combination therapy on all the aforementioned autoimmune comorbidities.

Finally, it is essential to raise the awareness of AHXIII to save its patients' lives by prompt diagnosis and treatment. We would also like to emphasise that detailed characterisation of AHXIII, especially by both the mixing study for the detection of neutralising antibodies against FXIII-A (e.g. functional 1:1 and five-step dilution tests) and the binding assays for the detection of non-neutralising antibodies against FXIII-A and FXIII-B (e.g. immunological tests), “must” be carried out, as per recommendation of the FXIII/Fibrinogen subcommittee of ISTH/SSC (1), in order not to overlook any AHXIII patients.

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Conflicts of interest
None declared.

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