Supplemental Materials to Ogawa et al. “Complete remission* achieved by steroid pulse therapy following rituximab treatment in a case with autoimmune haemorrhaphilia due to anti-factor XIII antibodies” (Thromb Haemost 2014; 112.4)

Suppl. Figure 1: Analyses of the patient’s plasma and detection of anti-FXIII antibodies. A) A 5-step dilution cross-mixing test by an amine incorporation assay was performed using the patient’s plasma at the ratios of 0:1, 1:3, 1:1, 3:1, and 1:0 with a normal plasma. The mixed samples clearly showed an “inhibitor” pattern, because there was a large downward deviation. A broken line depicts a theoretical “deficient” pattern. B) A dot blot assay was performed using recombinant FXIII-A (rFXIII-A), recombinant FXIII-B subunit (rFXIII-B), and their complexes (A2B2) at the indicated amounts shown as antigen (ng). The results showed the presence of anti-FXIII-A antibodies. The positive controls stand for AHXIII patients’ plasmas identified previously. C) A fibrin cross-linking study was performed by the addition of 1 unit/mL thrombin and 5 mM CaCl2 into the patient’s plasma and a normal control plasma. The clots were recovered
at the indicated time intervals and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. The results demonstrated the lack of both γ-dimerization and α-polymerization reactions. D) The dot blot assay was repeated to compare the reactivity of the patient’s plasma to rFXIII-A before and after the antibody eradication therapy (day 1 vs. day 74). His anti-FXIII-A autoantibodies had completely disappeared by day 74, which is consistent with the anti-FXIII IgG data obtained by ELISA (Figure 1).
Suppl. Figure 2: Pharmacokinetic studies by infusion of exogenous FXIII. Plasma-derived FXIII concentrates were infused (open arrows in Figure 1) at a dose of 1,440 U (27 U/kg, equivalent to about 55% increase) on day 4 (A) and day 19 (B), and at 2,400 U (45 U/kg, equivalent to about 90% increase) on day 25 (C) and day 39 (D). Both FXIII activity and antigen levels were measured before and after 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h of the FXIII infusion. Pharmacokinetic study was not carried out thereafter, because of the absence of bleeding episode as well as the limitations of public medical insurance. Broken lines depict expected FXIII activities after the infusion of exogenous FXIII concentrates. Discrepancies between FXIII activity and antigen levels indicate the formation and existence of FXIII antigen-antibody complexes between “free” anti-FXIII-A autoantibodies and exogenous FXIII concentrates.