Appendix, Supplemental Digital Content 1

Analysis of tranexamic acid in serum

Reference standard of TXA and the internal standard TXA-$^{13}C_2$,^{15}N were purchased from Toronto Research Chemicals (Toronto, Canada). For the preparation of spiked standards and quality controls, blank human serum was collected from healthy medication-free blood donors.

After thawing, automatic sample preparation was performed on Hamilton Microlab STAR, pipetting robot (Hamilton, Bonaduz, Switzerland). Aliquots of 50 µl standard, quality control, or patient sample in addition to internal standard (50 µl), were pipetted onto an Ostro^{TM} 96-well plate (Ostro Protein Precipitation & Phospholipid Removal Plate, 25 mg, Waters, Milford, MA, USA). Ice-cold acetonitrile with formic acid (1% v/v, 800 µl) was added and mixed with the sample for protein precipitation. A positive pressure unit (Positive pressure processor-96, Waters, Taunton, MA, USA) was used to facilitate the filtration of the samples in order to reduce the content of phospholipids in the eluates. The eluates were collected in 2 ml sample collecting well plate (96-well Square collection plate, Waters) and sealed with cap-mat square plugs (silicone/PTFE-treated preslit, Waters).

TXA concentrations in serum were determined by an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method. A Waters Acquity UPLC I-class FTN system (Waters, Milford, MA, USA) equipped with Acquity UPLC HSS T3 (2.1 mm × 100 mm, 1.8 µm) column was used for chromatographic separation. Mobile phase consisted of 0.1 % formic acid in water (A) and acetonitrile (B) with a flow rate of 0.6 ml/min. The gradient run started from 1% to 20% B during the first 1.2 min and thereafter from 20%

to 90% B from 1.2 min to 1.5 min, until it was kept constant at 90% B from 1.5 to 1.9 min. From 1.91 min the composition was reset to the initial condition. Injection volume was set to 0.2 µl, and total run time was 2.5 min. Column temperature was kept at 50 °C, and autosampler temperature was set to 10 °C. A Xevo TQ-S tandem–quadrupole tandem mass spectrometry (Waters, Manchester, UK) equipped with a Z-spray electrospray interface. Positive electrospray ionization was performed in the multiple reaction monitoring (MRM) mode. The capillary voltage was set to 1.0 kV, the source block temperature was 120 °C and desolvation gas (nitrogen) was heated to 650 °C and delivered with a flow rate of 1000 L/h. Mass transitions were *m/z* 158.2 > 123.0 (cone voltage: 40 V, collision energy: 8 eV) for TXA and *m/z* 161.2 > 125.0 for the internal standard TXA-$^{13}C_2$,^{15}N (cone voltage: 40 V, collision energy: 8 eV).

The method was validated according to the US Food and Drug Administration guidelines[72]. The limit of quantification was 0.10 µg/ml and the method was linear at least up to 200 µg/ml. Recoveries were 90-97% at concentrations of 1.0 and 150 µg/ml, and between-day coefficients of variations were < 1.6% at concentrations of 0.25, 1.0 and 150 µg/ml.