Supplemental Material to Honickel et al. “Dose requirements for idarucizumab reversal of dabigatran in a lethal porcine trauma model with continuous bleeding” (https://doi.org/10.1160/TH16-11-0824)

**Suppl. Detailed Methods**

**Ethical Considerations**

Pigs were housed in ventilated rooms and allowed to acclimatize to their surroundings for at least 10 days. Examination by a veterinarian ensured that the animals were in good health.

**Preparation and Anesthesia**

Before infusion of idarucizumab or saline, the right carotid artery was cannulated with an 18 G catheter to collect blood samples and measure arterial pressure. Two catheters were placed in each animal's jugular vein for volume substitution and a pulmonary artery catheter was also inserted. A midline laparotomy with cystostomy was performed.

**Liver Injury and Post-Injury Procedures**

To induce the liver injury, a standardized pressure clamp was applied through the parenchyma of the right middle lobe using custom-made forceps. All injuries were generated by the same investigator who was blinded to treatment allocation. Post-injury, after resuscitation with Ringer’s solution, pressure was applied to the wound site using hepatic packing and the abdominal incision was closed. Immediately after death, the abdomen was reopened, the vena cava was clamped cranial to the liver, and intraperitoneal blood was collected to determine total blood loss post-injury.
Laboratory-Based Coagulation Tests and Other Blood Sample Analyses

Blood was collected and arterial blood gas analysis was performed before the first oral dose of dabigatran (baseline), after the last oral dose of dabigatran (-90), after the infusion of dabigatran (0), and 20, 60, 80, 120, 180, 240 and 300 minutes after the first liver injury (Figure 1). For animals dying before 300 minutes, the last assessment was performed immediately after death.

Blood gases were measured on a blood gas analyzer (ABL700, Radiometer, Copenhagen, Denmark) using heparinized blood samples. Hemoglobin and platelets were measured in samples collected in potassium-EDTA-anticoagulant tubes (1.9 mg/mL final concentration, Sarstedt, Nuembrecht, Germany) using a standard hematology analyzer (MEK-6108, Nihon Kohden, Rosbach, Germany). For coagulation assays and measurement of plasma drug concentrations, blood was collected in 3.2% sodium citrate (Sarstedt) and potassium-EDTA-anticoagulant (1.9 mg/mL final concentration, Sarstedt), respectively, centrifuged to obtain platelet poor plasma and stored at -80°C until assayed. Prothrombin time (PT, Innovin® reagent), diluted thrombin time (dTT, Hyphen BioMed, Neuville sur-Oise, France) and D-dimer levels (Innovance® D-dimer assay) were determined by standard laboratory methods using a BCS XP analyzer (Siemens, Erlangen, Germany). Activated partial thromboplastin time (aPTT, CK Prest®, Diagnostica Stago, Asnieres sur Seine, France) and fibrinogen levels (Dade®, Siemens Healthcare, Marburg, Germany) were measured using standard methods with a CL4 coagulation analyzer (Behnk Elektronik GmbH &Co. KG, Norderstedt, Germany). Before determining fibrinogen, all plasma samples were incubated with 3 mg/mL idarucizumab to neutralize dabigatran concentrations in the sample.
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**Plasma Concentrations of Dabigatran and Idarucizumab**

Plasma concentrations of active dabigatran were determined by the calibrated dTT as described above. Plasma concentrations of total dabigatran, which includes active and inactive idarucizumab-bound or protein-bound dabigatran, were analyzed using liquid chromatography-mass spectrometry (LC-MS/MS) methods at Nuvisan GmbH & Co. KG, Neu-Ulm, Germany. Plasma concentrations of idarucizumab were analyzed using a pig enzyme-linked immunosorbent assay (ELISA) at Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA. Briefly, microtiter plates were coated overnight with antihuman IgG antibody (2 µg/mL), then blocked, washed, and incubated with 1:5 diluted samples and appropriate calibration standards and controls. Sample incubation was followed by detection with horseradish peroxidase (HRP)-conjugated sheep antihuman IgG antibody (0.5 µg/mL) and bound HRP-conjugate was detected with tetramethyl benzidine, which was read colorimetrically on a plate reader. Using similar methodology to a previous study performed in humans, the ELISA assay was used to quantify idarucizumab concentrations in potassium-EDTA pig plasma over the validated concentration range (0.1–0.8 µg/mL) with a lower limit of quantification of 0.1 µg/mL.

**Whole-Blood Assays: Thromboelastometry and Activated Clotting Time**

Whole-blood thromboelastometry was performed on a ROTEM analyzer (TEM International GmbH, Munich, Germany) according to the manufacturer’s instructions using blood samples collected in 3.2% sodium citrate. An extrinsically activated assay using recombinant tissue factor (EXTEM) and an intrinsically activated test using ellagic acid and phospholipids (INTEM) were used. The parameters assessed were clotting time (CT, sec), clot formation time (CFT, sec) and maximum clot firmness (MCF, mm).
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Whole-blood samples were collected for measurement of activated clotting time (ACT) using an i-STAT point-of-care device (Abbott, Princeton, NJ) with celite cartridges.

**Thrombin Generation**

Thrombin generation was determined in plasma using calibrated automated thrombinography (Thrombinscope BV, Maastricht, The Netherlands) using 5 pM tissue factor.\(^6,7\) Thrombin generation curves were generated using Thrombinscope software (version 4, Thrombinscope BV) to determine endogenous thrombin potential (ETP), lag time and peak thrombin formation. Prolonged lag time values that could not be detected by the software were defined as having a value of 12 minutes for data analysis.

**Statistical Analysis**

Statistical analysis was performed using SPSS 22 (SPSS, Chicago, IL), and GraphPad Prism (Version 6.0h, GraphPad Software, La Jolla, USA) was used for graphing purposes.

**Suppl. References**

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**Suppl. Figure 1: Haemodynamics.** Cardiac output (A) and lactate concentrations (B). Horizontal thin dashed lines indicate baseline values prior to dabigatran anticoagulation; or, in case of cardiac output, just prior to anesthesia. Data are presented as mean±SD; n=6 per group for each data point except: 180 minutes post-trauma: 60+0 n=5; 240 and 300 minutes post-trauma: 60+0 n=3. *P<0.05 vs. 60+60 and 120+0; § P<0.05 vs. control.

![Suppl. Figure 1: Haemodynamics](image-url)