Collection biomarkers
- Pre-operative = time-point 0
- Directly post-operative = time-point 1
- Day 5 post-operative = time-point 5

1. Blood withdrawal
Via venous canule (venflon):
- Apply tourniquet
- Introduce the venous canule (at least, 20G, 32 mm, pink. Preferably larger)
- Loosen the tourniquet, but do not remove completely (gentle pressure)
- Withdraw blood directly out of the venflon, without the aid of a long plastic extension piece
- Withdraw 1 vacuum “Citrate tube” (blue, plastic, 2.7 ml, BD 0.109 M citrate);
- Withdraw 1 vacuum “EDTA tube” (purple, plastic, 4 ml)
- Withdraw 1 vacuum “Heparine tube” (green, plastic, 4.5 ml)
- N.B. Take one vacuum precursor tube in case the venflon was installed more than 5 minutes ago or when the venflon was washed with saline (0.9% NaCl).
- Mix gently without shaking.
- Bring to the laboratory within 1 hour
- Date of birth and the date and time of blood withdrawal, as well as any peculiarities during blood withdrawal (e.g. difficult to obtain blood etc)

Via vein puncture (in case a venous canule will not be installed):
- Apply tourniquet
- Introduce needle (21 G green)
- Loosen the tourniquet, but keep gentle pressure
- Withdraw 1 vacuum “citrate tube” (blue, plastic, 2.7 ml, BD 0.109 M citrate);
- Withdraw 1 vacuum “EDTA tube” (purple, plastic, 4 ml)
- Withdraw 1 vacuum “Heparine tube” (green, plastic, 4.5 ml)
- Mix gently without shaking
- Bring to the laboratory within 1 hour
- Date of birth and the date and time of blood withdrawal, as well as any peculiarities during blood withdrawal (e.g. difficult to obtain blood etc)
2. **Laboratory procedures**

A. **Preparation of platelet poor plasma (PPP)**
   - Bring freshly collected, citrate-anticoagulated (0,109 M BD) whole blood as soon as possible (without shaking, etc) to the lab within 1 hour after blood withdrawal.
   - Centrifuge the blood for 15 minutes at 1500 - 2000 x g without brake in a swing-out rotor, at room temperature.
   - Register the time at which the centrifugation of the blood is started.
   - When (part of) the samples are haemolytic, please note this on the sample log.
   - Carefully collect the upper 2/3 of the (platelet-poor) plasma.

B. **Preparation of EDTA and Heparine**
   - Bring freshly collected, EDTA and Heparine whole blood as soon as possible (without shaking, etc) to the lab within 1 hour after blood withdrawal.
   - Centrifuge each tube of blood for 15 minutes at 1500 - 2000 x g without brake in a swing-out rotor, at room temperature.
   - Register the time at which the centrifugation of the blood is started.
   - When (part of) the samples are haemolytic, please note this on the sample log.
   - Carefully collect the upper 2/3 of the plasma of both tubes.

C. **Storage of material**
   - Aliquot the plasma aliquots in conical centrifuge micro tubes of 2.0 ml.
     - Citrate ≥ 0.5 ml of PPP in each tube – divided in 2 tubes.
     - EDTA ≥ 1.5 ml in each tube (total 2 tubes).
     - Heparine ≥ 1.5 ml in each tube (total 2 tubes).
   - Store the microtubes at -80 °C.
   - Mark the tubes with a sticker, which includes information on the **Institute, Patient Identification Number** = PIN (ask Local Investigator), and **time-point of blood collection**, to be one of the following:
     - time-point 0 (= pre-operative)
     - time-point 1 (= direct post-operative)
     - time-point 5 (= day 5 post-operative)