Case Report Form - hemorheological studies

HERMES

Date and time (MM:HH DD:MM:YYYY): ……..

Hematocrit (%): .....

Red blood cell aggregation (Myrenne aggregometer)[1]:

	First sample			Sacond sample			Mean
M index							
M1 index							

Red blood cell aggregation (LORCA)[2]:

AI (%): ..... t ½ (s): ..... ɣ: (1/s) .....

Red blood cell deformability (LORCA)[3]:

EI_{30}	$EI_{16.87}$	$EI_{9.49}$	$EI_{5.33}$	EI_{3}	$EI_{1.69}$	$EI_{0.95}$	$EI_{0.53}$	$EI_{0.3}$

Viscosity of whole blood (Brookfield viscometer, mPa·s)[4]: ……

Viscosity of plasma (Brookfield viscometer, mPa·s)[4]: ……

[1]The Myrenne aggregometer (model MA-1, Myrenne GmbH, Roetgen, Germany) consists of a laser diode, a transparent upper plate, and a transparent lower rotating cone (i.e., shearing unit). A sample (30 µl red blood cell suspension) is placed between the gap of the upper plate and the rotating cone. Then, the rotating cone shears the red blood cells at 600 s^{-1} to disaggregate pre-existing aggregates. The cone then suddenly stops moving (**M index or aggregation at stasis**) or continues to rotate (**M1 index or aggregation at low shear**) and shear at 3 s^{-1} while measuring the intensity of transmitted infrared light for 10 s. If the red blood cells tend to aggregate, the light transmittance of the sample increases (as well as M and M1 indices). Measurements are carried out at room temperature three times from two samples.

[2]The Laser-assisted Optical Rotational Cell Analyzer (LORCA, R&R Mechatronics, Hoorn, The Netherlands) consists of a glass cup and a perfectly fitting bob with a 0.3 mm gap in between. The red blood cell suspension is placed in this gap. The disaggregation of the sample is carried out at 500 s^{-1} shear. Following the release, a red laser beam is directed to the sample and the reflection is measured by photodiodes for 120 s. **Aggregation index (AI)** is calculated by the integral of the change in in reflection (intensity of light) during the first 10 s corrected to the possible maximal change. **T½** defines the time required for achieving the half of the maximal aggregation. The threshold shear rate is the lowest shear rate that can maintain complete disaggregation (**$\dot{\gamma}$**). Measurements are carried out at room temperature.

[3]Red blood cells are exposed to shear stress on ektacytometry (with LORCA, R&R Mechatronics, Hoorn, The Netherlands), then the change in shape is quantified. LORCA consists of a rotating glass cup with a fitting bob, which provides a nearly homogenous shearing field. Low hematocrit (0.2%) is required for the measurement; therefore, we suspend them in a medium with known viscosity (25 µl blood/5 ml volume). The exposure of red blood cells to shear stress results in the transition of the normally biconcave shape to an ellipsoid one. The deformed cells are examined with a laser beam causing diffraction. The **elongation index (EI)** quantifies the deformation of the red blood cells (EI=(A-B)/(A+B), where A and B represent the major and the minor axes of the ellipsoids). EI can be determined at various shear stresses, the set of shear stress-elongation index is the so-called ektacytogram. Shear stresses range from 0.3 to 30.0 kPa (e.g., **$EI_{0.3}$, EI_{3}**). Measurements are carried out at room temperature.

[4]Viscosity of the whole blood and plasma is determined by Brookfield DV-III Ultra LV Programmable rotational viscometer (Brookfield Engineering Labs; Middleboro, Mass., USA). A spindle is immersed in the sample that the torque required to rotate it is measured. The **torque** is proportional to the viscosity measured at mid-shear (90 s^{-1} or 12 rpm) in our study. Measurements are carried out at 37°C .