Lorrca®
Mastering RBC analysis

RR Mechatronics
Masters of Measurement

Lorrca® MaxSis Osmoscan

Laser-assisted Optical Rotational Cell Analyzer

The Lorrca® Maxsis osmoscan (Lorrca®) is a unique instrument which combines Red Blood Cell (RBC) deformability by ektacytometry, osmoscan and aggregometry; all temperature controlled. It is capable of fully automated measurement and calculation of various phenomena of RBC's by analysis of their rheological behavior. The technique accurately detects deformability as a function of shear stress, pre-hemolytic stability and aggregation of the RBC's, which should be standard in the hematology laboratory. Therefore, the Lorrca® is not only vital in research, but also in the clinical diagnostic fields.

Fig. 1: Lorrca®: The basic instrument (right) and the computer (left). The Lorrca® is a versatile small footprint instrument that fits on every desk.

Scope

The Lorrca® has been developed in close collaboration with our science[1] and technology partners, e.g. Academic Medical Center (AMC) in Amsterdam. Scientists in over 20 countries use the Lorrca® to contribute to research, often in research environments[1]. The scope of the Lorrca®'s capabilities is still growing. Besides the use in research, the clinical diagnostic fields become of great importance. Prominent reference laboratories and other experts already make intensive use of the Lorrca®[2]:

- Hereditary RBC disorders[3]
  - Spherocytosis, Elliptocytosis, Stomatocytosis, Pyknocytosis, Ovalocytosis, South-east Asian Ovalocytosis
- Sickle cell disease and Thalassemia
- Pre-hemolytic quality control test (blood banks)[4]
- Malaria tropica[5,6]
- Diabetes mellitus
- Obesity
- Sepsis
- Microcirculation
- Influence of oxidative stress on RBC's
- Influence of drugs (in development) on the RBC's
  - Aggregation
  - Deformability
  - Condition at the different osmotic values

Selected Examples

Typical osmoscan curves

The different hemolytic anemias can unambiguously be determined, using the basis of typical deformability (osmoscan) curves of RBC's[6] *(Fig. 2)*.
Other specific diagnostic tests are not capable of doing this, they are insensitive or do not discriminate for the various membrane disorders.

Storage lesions

RBC deformability assays and osmoscans have been shown to be of additional value in studies on the storage lesions of stored RBC's.

- It has been demonstrated that the deformability of the RBC's already decreases during the first three days of processing from blood to blood bag in the blood bank. During the five weeks of storage itself, there was no significant decrease observed in the deformability of the RBC's[5].

Typical osmoscan curves

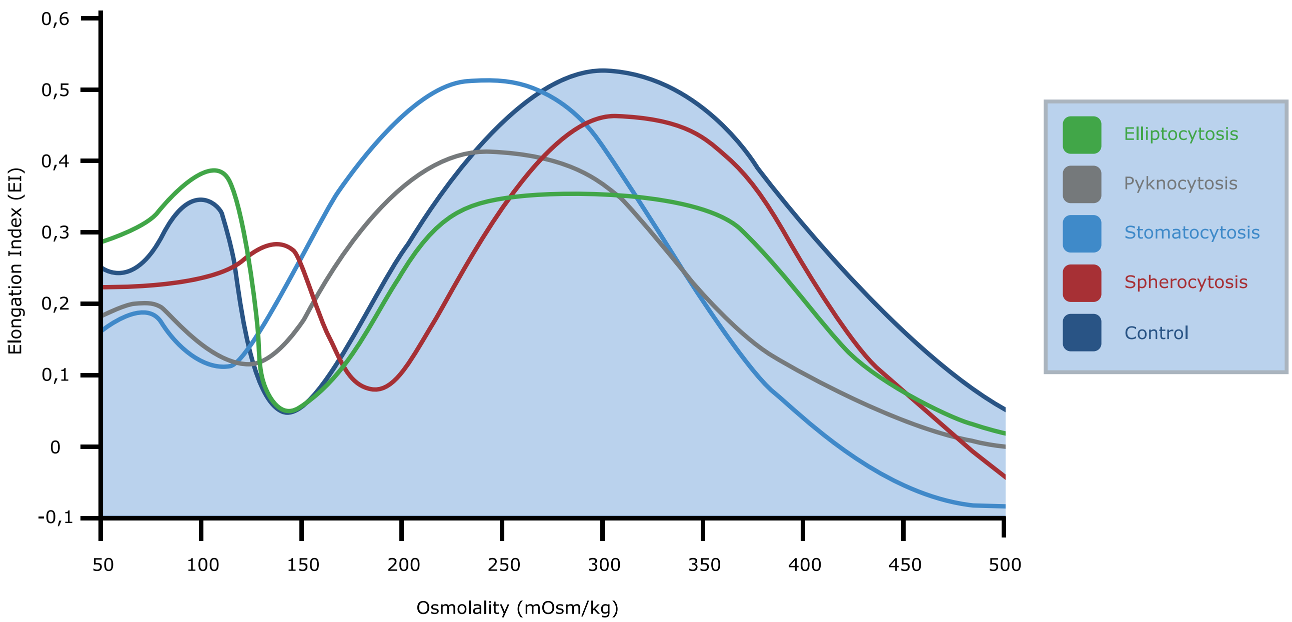

Fig. 2: The typical osmoscan curves of the different hemolytic anemias. The technique and measurement of these curves will be explained on pages 4 and 5.

Measurement potential of the instrument

Evaluation of the rheology of blood in different parts of the body is a very specific task, therefore it is important to have a technique available that can accurately measure the RBC properties. The Lorrca® incorporates a variety of techniques to perform these specific measurements:

- Deformability: Laser diffraction ektacytometry[1], Parameterization of deformation curve[7], Cell- and cell membrane stability[8,9]
- Osmoscan[10]: Deformability under an osmotic gradient, Osmotic Resistance Test
- Aggregation[11]: Syllectometry – Extent of aggregation, aggregation kinetics and tendency

Deformability

The capacity of RBC's to deform is a powerful indicator of their viability as it is essential for the optimal circulation of these cells both in micro- and macrocirculation. This deformability depends on three important cell properties[12,13].

- Surface area to volume ratio
- Viscoelastic properties of the membrane
- The viscosity of the intracellular fluid

Measurement

Red blood cell deformability is expressed as the Elongation Index (EI) measured in polyvinylpyrrolidone (PVP) medium of known viscosity and presented in a EI versus shear stress deformability curve *(Fig. 3)*. A thin layer of RBC's is sheared between two concentric cylinders and a certain, but exactly known, shear stress is applied to the cells (by rotation of the outer cylinder; the cup).

The RBC's will change, in an iso-osmotic environment, from oval biconcave disks to its elongated shape. Because of the osmotic variety of the medium (50-500 mOsm/kg) during the measurement, continuous deformation occurs which is measured by a laser beam diffraction pattern; captured with a video camera and analyzed by a computer[1]. For this test only 25 μl of blood is needed.

RBC deformability curve

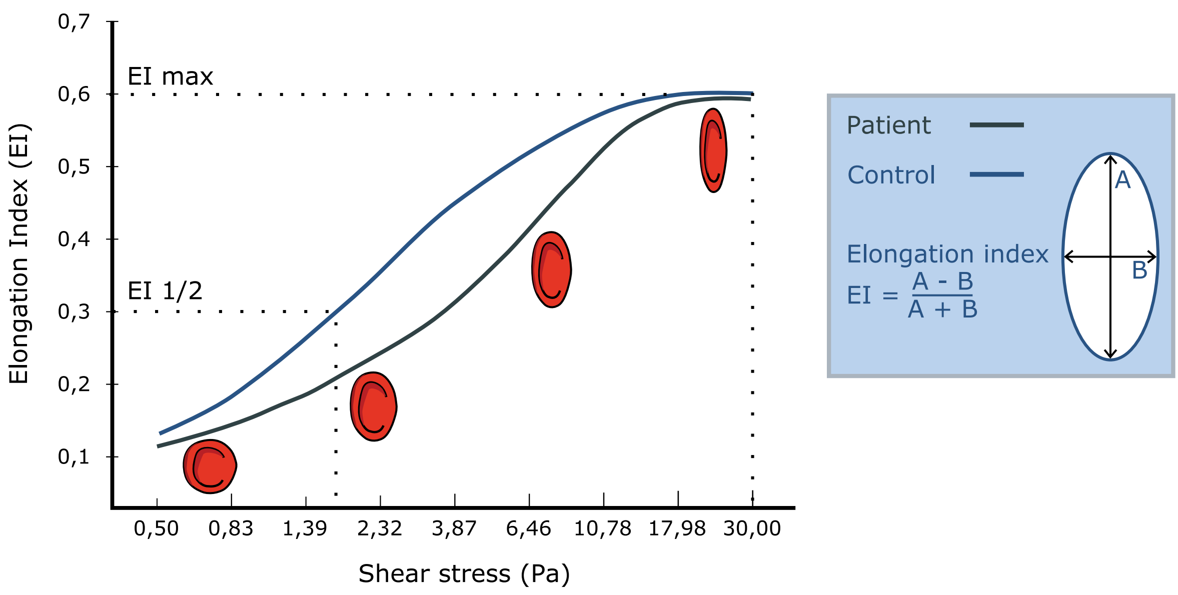

Fig. 3: Change in Elongation Index (EI) with applied shear stress; Parameterization of deformability curve.

Osmoscan

The osmoscan measures the deformability under an osmotic gradient *(Fig. 4)*.

Osmotic conditions

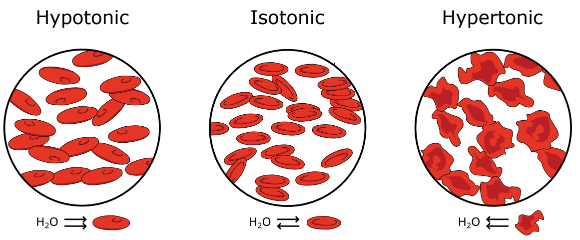

Fig. 4: Cell condition under different osmotic values.

Measurement

The Lorrca® has the ability to automatically measure RBC deformability over a gradient of osmotic values. This results in a continuous curve; *Osmoscan* *(Fig. 5)* which shows the cell's condition at the different osmotic values. The osmoscan provides information about the cell's deformability and membrane rigidity, depending on both the shape and the position along the osmolality axis[7]. For this test 200 µl of whole blood is needed.

Osmoscan

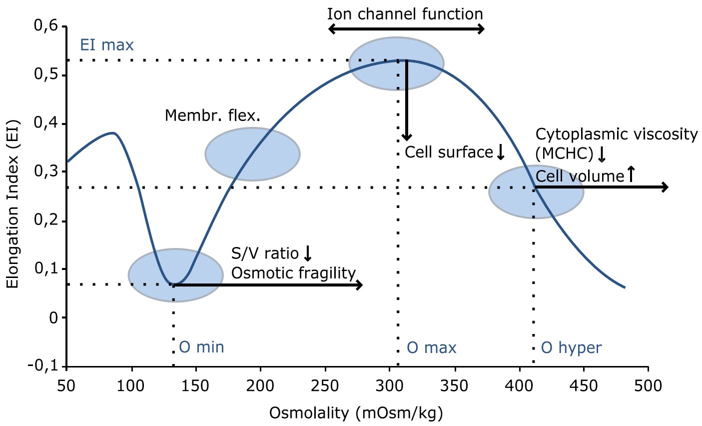

Fig. 5: With the Osmoscan 4 parameters are defined:
Omin (osmolality at EImin, hypotonic region),
EImax (maximal EI of the total curve), Omax (osmolality at EImax) and Ohyper (osmolality corresponding to 50% of the EImax; hypertonic region).

Aggregation

RBC aggregation is an important, shear dependent, determinant of blood viscosity. Hyperaggregability can be detected in various diseases (e.g. inflammatory states, vascular pathologies and tissue perfusion problems)[14].

Rouleaux formation

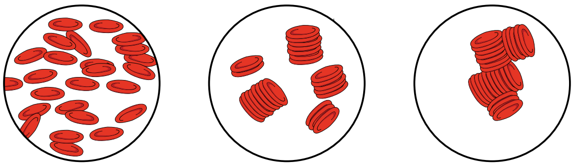

Fig. 6: At low shear stress, RBC's form larger stacks, called rouleaux, followed by side-to-end connection creating 3D-aggregates (Rouleaux formation).

Measurement

Complete disaggregation of the RBC's is induced by applying a high shear rate; followed by an abrupt stop, which causes the elongated and orientated RBC to retake their normal, biconcave, shape and orientate randomly. Then the aggregation process starts, at which rouleaux formation occurs *(Fig. 6)*. A syllectogram is created by plotting the laser backscattered intensity (Isc) versus time *(Fig. 7)*. By means of a photodiode (which measures the change in light backscatter) the total extent and kinetics of aggregation are displayed, as well as the minimal shear rate (threshold) needed to prevent RBC aggregation, i.e. the tendency for aggregation[11]. For this test ~ 1 ml of whole blood is needed.

RBC Syllectogram

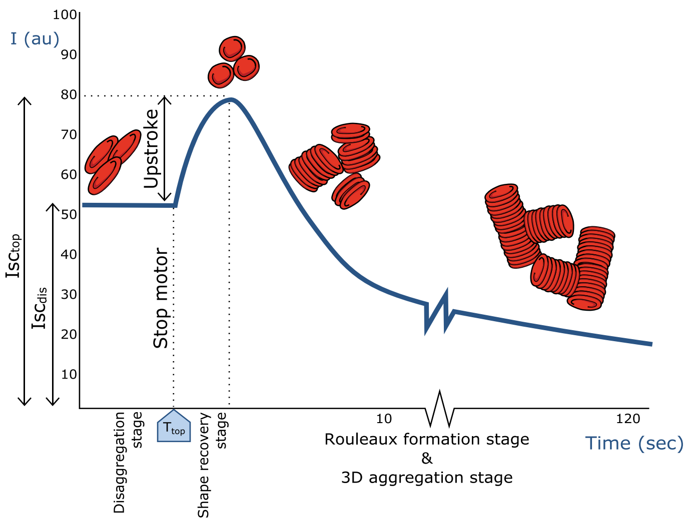

Fig. 7: The Lorrca® measures the total extent of aggregation of the RBC's[11].

Further applications and potential future applications

Measuring under various conditions

In addition to various osmotic gradients, the Lorrca® also allows investigators to vary other modalities such as pO2, viscosity, acidity or pharmaceutical concentrations[7].

- By means of adding a device which creates the possibility of measuring under various pO2 conditions, sickle cell disease can be further investigated:
  - Using the gradient forming unit, the Lorrca® can perform a PO2-scan with the optional pO2-probe and measure the sickling tendency of RBC's[3,15]. These results are especially useful for the development of sickle cell medication and patient monitoring.

Deformability of different sub-populations of the RBC's

When measuring the deformability, the obtained deformability curve shows the mean deformability of all RBC's present in the sample. By adjusting the gain of the CCD camera (the electronic diaphragm), it is possible to detect the deformability of sub-populations of the RBC's. This can be refined to the percentage of RBC's with this decreased deformability, e.g. in sickle cell disease: the percentage of irreversibly sickled cells[16].

Microparticles

During the process of reduction of the RBC deformability, scientists observed the possible loss of microparticles (MPs) on the way. MPs are small plasma membrane-derived vesicles that can expose molecules originating from their parental cells[17]. They play an active role in homeostasis and pathogenesis, the latter including atherosclerosis, various malignancies, autoimmune disorders and infection[18,19]. Besides that, during storage in the blood bank, RBC's and platelets continuously shed MPs, which might be responsible for some of the side effects observed after transfusion[20]. By applying stress to the RBC's, using the Lorrca®, these MPs can be created and further examined[21].

Enzyme deficiencies

Different pathways in which the RBC's receive their metabolic needs are identified. The hereditary enzyme deficiencies of these pathways can cause non-spherocytic anemia, of which glucose-6-phosphate dehydrogenase (G6PD) is the most common deficiency[22].

- In G6PD deficient individuals RBC deformability was found to be significantly impaired[23]. Carriers have two RBC populations present in their blood: one with and one without G6PD activity, there is evidence that these RBC's differ in their deformability, this can be further investigated[24].

Malaria tropica

Regarding the instrument's value in clinical pathology, it was demonstrated that in malaria tropica the mean RBC deformability (mainly of non-parasitized RBC) is the most important predictor of, and most probably a contributor to, mortality[13]. The Lorrca® could determine the threshold at which complete blood transfusion is the only lifesaving treatment possible[5,6]. Above this threshold, oral medication is still an adequate treatment. Below this threshold, without intervention, the mortality-rate is up to a 100%[5].

Immunosuppressive therapy

Cyclosporin is an increasingly popular drug in immunosuppressive treatment, especially after organ transplantation. Unfortunately, serious adverse reactions, like nephrotoxity, can occur which are possibly dose related[25]. It is suggested that this slow, but continuously increasing, RBC rigidification plays a role in the early pathogenesis of the adverse nephrotoxic complications frequently associated with this immunosuppressive regimen[13]. Therefore, regular screening on a loss of RBC deformability and eventually subsequent intervention by lowering the dose or by changing to another drug, could prevent irreversible nephrotic damage.

Different other possible applications are still explored. Further large scale clinical investigations may clarify the hemorheological involvement in the pathogenesis of impaired microcirculatory flow[13].

New insights and references to related scientific publications are available on our website: portal.rrmechatronics.com

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About RR Mechatronics

Since being founded in The Netherlands in1986, we have adhered to the belief in the exponential strength of technological advancement. RR Mechatronics has a track record of thinking in terms of solutions, not problems. We do not have all the answers, but persistently strive to find them in close collaboration with research scientists and our other partners. These partnerships significantly contribute to our high quality work.

Our people are passionate about the invention and development of highly precise analytic instruments. RR Mechatronics focuses on medical analytics laboratory instruments. We serve laboratory customers and other partners all over the world.

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