SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Species differences and metabolic determinants of osmotic gradient red blood cell deformability

by Ádám Varga

Supervisor: Prof. Norbert Németh, DSc

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2025

Species differences and metabolic determinants of osmotic gradient red blood cell deformability

by Ádám Varga, MSc

Supervisor: Prof. Norbert Németh, DSc

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Defense Committee**:	Prof. Árpád Illés, DSc
Reviewers:	Prof. Lajos Bogár, DSc Harjit Pal Bhattoa, PhD
Members of the Defense Committee:	Prof. László Harsányi, DSc Imre Tóth, PhD

The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen on 2 April 2025 at 13:00.

1. INTRODUCTION

Haemorheology is the study of blood flow characteristics. Parameters of particular interest are blood and plasma viscosity, red blood cell deformability and aggregation. An increase in viscosity impairs blood fluidity, leads to an increase in flow resistance and can increase the workload of the heart, while lower viscosity can also lead to insufficient tissue perfusion. Red blood cell deformability is another crucial factor that describes the ability of cells to passively change shape under shear and compressive forces as they pass through narrow capillaries. Another important micro-rheological factor is red blood cell aggregability, i.e. their reversible binding ability, which can influence the dynamics of blood flow, especially at low-velocity gradients.

In recent decades, haemorheological research has grown considerably with the advent of newer measurement methods. This expanding field of research has provided valuable insights into how pathological processes affect blood flow, with a particular focus on the micro-rheology of red blood cells. Pathological changes in haemorheological parameters have been revealed in several cardiovascular, metabolic and haematological diseases, but inflammatory diseases, sepsis, ischaemia-reperfusion, and tumours are also characterised by significant micro-rheological and microcirculatory changes. Using various measurement techniques, ranging from viscometry to advanced imaging and molecular analysis, not only the macroscopic properties of blood flow but also the microscopic interactions between blood cells and plasma can be studied.

Among micro-rheological parameters, an interesting aspect of red blood cell deformability is the study of the so-called osmotic gradient deformation ("osmoscan"), which is associated with osmotic changes, and for which the newer measurement method of ectacytometry offers the possibility. Despite the wide range of effects of osmolality changes on red blood cells in circulation, this parameter is still poorly studied, as only a few laboratories have the methodological background to study it. It is also of great interest to investigate this parameter between species, since the micro-rheological parameters of red blood cells are influenced by the size, shape, density, viscoelasticity and biomechanical properties of the cells, and these factors are known to show a wide range of heterogeneity between species compared to humans. For animal species involved in biomedical research, comparative haemorheology has important implications for the extrapolation, evaluation and interpretation of results, including their clinical utility. Another aspect is the effect of metabolic variables on micro-rheological parameters. In our research, we aimed to clarify these questions.

2. AIMS

1. As a follow-up to previous comparative haemorheological studies in the Department, we aimed to perform a micro-rheological comparative analysis of human, canine, feline, porcine, ovine, murine, rat and rabbit blood samples, with a special focus on red blood cell osmotic gradient deformability, which has not been studied in such a broad context.

2. Our objective was to investigate the relationship of osmotic gradient deformability with other micro-rheological (red blood cell deformability determined by conventional ectocytometry, static and dynamic aggregation parameters) and with red blood cell quantitative and qualitative haematological parameters.

3. To investigate the effect of blood gas, acid-base and metabolic factors on micro-rheological, including conventional and osmotic gradient deformability in a large animal model.

4. Comparative studies were performed before and after experimental surgical small bowel anastomosis operations to determine the dynamics of changes in samples from four blood sampling sites (femoral artery and vein, portal vein, renal vein).

3. MATERIALS AND METHODS

3.1. INTERSPECIES DIVERSITY OF OSMOTIC GRADIENT DEFORMABILITY OF RED BLOOD CELLS IN HUMAN AND SEVEN VERTEBRATE ANIMAL SPECIES

3.1.1. Volunteer participants

Blood samples were collected from healthy female volunteers in the morning hours after overnight fasting (n = 8, age: 19–40 years; Clinical Ethical Committee Approval No.: DE-RKEB 3625-2012). The samples were taken from the antecubital vein (21 G needle, BD Vacutainer® tubes, 1.5 mg/mL K3-EDTA; Becton, Dickinson and Company, USA).

3.1.2. Laboratory animals

We also examined blood samples taken from male beagle dogs (n = 6, bodyweight: 17.05 ± 1.05 kg), male cats (n = 7, bodyweight: 4.25 ± 0.65 kg), female Hungahib-39 pigs (n = 8, bodyweight: 15.2 ± 1.1 kg), female Merino sheep (n = 8, bodyweight: 71 ± 5.31 kg), female C57BL/b mice (n = 14, bodyweight: 32.1 ± 3.8 g), female Wistar rats (n = 10, bodyweight: 302.8 ± 12 g), and female New Zealand white rabbits (n = 4, bodyweight: 3063.5 ± 230.18 g). The samples were taken from animals without any other interventions (as control groups or control samplings only). None of the animals were sacrificed solely for this comparative study. All animal experiments were carried out according to the Hungarian Animal Protection Act (Law XVIII/1998) and approved by the University of Debrecen Committee of Animal Welfare (Permission Registration No.: 24/2016/UDCAW).

3.1.3. Blood sampling

Blood samples were taken from the dogs and cats via cephalic vein puncture (anesthesia: 10 mg/kg ketamine + 1 mg/kg xylazine, i.m.), from pigs via medial saphenous vein puncture (anesthesia: 15 mg/kg ketamine + 1 mg/kg xylazine, i.m.), and from sheep via external jugular vein puncture. In mice, rats, and rabbits, blood sampling was also carried out under general anesthesia (mice: 60 mg/kg thiopental, i.p., sampling site: inferior caval vein; rats: 60 mg/kg thiopental, i.p., sampling site: caudal caval vein; rabbits: 60 mg/kg thiopental, i.p., sampling site: lateral ear vein). The blood was drawn into standard Vacutainer tubes (anticoagulant: sodium-EDTA, 1.5 mg/mL). We completed all the laboratory measurements within 2 h after the blood sampling.

3.2. LOCAL AND SYSTEMIC MICRO-RHEOLOGICAL CHANGES DURING INTESTINAL ANASTOMOSIS OPERATION: A METABOLIC DEPENDENCE IN AN EXPERIMENTAL MODEL

3.2.1. Experimental animals

Sixteen female Hungahib-39 (Agrargazdasag Ltd., Hungary) hybrid pigs were used in our study (ethical permission registration Nr.: 24/2016/UDCAW, 16/2018/UDCAW). The experiments were performed by the The Hungarian Animal Protection Act (Law XXVIII/1998). The average age of the animals was 7–8 weeks and 18.93±1.88 kg was the average body weight. Before surgery, the animals underwent a standard acclimatization period during which they were deprived of food for 16 hours. The study was performed during the "Advanced Surgical Operative Techniques" compulsory elective course in the 2022 autumn semester.

3.2.2. Surgical protocol

The anesthesia protocol was the following: for pre-medication, i.m. 1–2 mg/kg of azaperone (Stresnil, Elanco GmbH, Cuxhaven, Germany); for induction of anesthesia, i.m. 2 mg/kg of xylazine (CP-xylazine hydrochloride, 2%) and 20 mg/kg of ketamine (CP-ketamine hydrochloride 10%); for maintenance of permanent anesthesia, i.v. 1 mg/kg of xylazine and 10 mg/kg of ketamine, supplemented with i.v. 2 mg/kg of diazepam (Diazepeks 5 mg/ml, AS Grindeks, Riga, Latvia). After anesthesia, a tracheal tube (ID 5.5; Eickemeyer, Tuttlingen, Germany) was inserted for assisted ventilation. Before the surgery the animals were divided into two equal groups randomly: an anastomosis group and a sham-operated control group. Afterward, the left external jugular vein was gently prepared, exposed and cannulated directly for i.v. 10 ml/kg/h fluid re-placement (amount: 452.4±45.9 ml, "Baxter" Sodium Chloride 0.9%, pH= 4.5-7.0, os-molarity: 308 mOsm/l, Baxter Hungary Ltd.). The left femoral artery and the right femoral vein were used as systemic blood sampling sites after cannulation (Certofix Duo S730 B. Braun SE, Hessen, Germany). The paramedian laparotomy started with preoperative preparation. The pig's abdomen was meticulously disinfected and isolated to provide a sterile surgical field followed by an incision through the skin. To facili-tate the exploration, isolation lines were established. Once hemostasis was achieved, we proceeded to make an incision in the peritoneum. In the sham-operated control group, this was the last step of the surgical intervention and the closure of the abdomen.

In the anastomosis group, we performed an end-to-end jejuno-jejunostomy. After intestinal clamps, we resected a bowel segment (the average length of the resected bowel segments was 12±0.65 cm). To perform the anastomosis, we used a one-layer suture line with Mikulicz-stitches (suture material: VITREX Monolac Monofil Violet, USP 4/0). The average

time of the experimental operation was 143±12 minutes (from starting anesthesia until the last blood sampling immediately after completing the anastomosis). The average time of completing the small bowel anastomosis was 38±7 minutes.

3.2.3. Blood sampling

During the experiment 3.5 ml of blood was taken at two time points and from a total of four different locations. Before abdominal surgery, blood was drawn from the femoral artery and the femoral vein using pre-inserted cannulas. The blood was collected into standard Vacutainer tubes (BD Vacutainer® tubes, 1.8 mg/ml K3-EDTA; Becton, Dickinson and Company, USA). The renal vein and portal vein were also sampled but that was done by direct puncture with a 23 G needle and 5 ml syringe after the median laparotomy. Blood samplings were completed after the end-to-end jejuno-jejunostomy starting with the systemic cannulas, then the portal and the renal veins by direct puncture. In the sham-operated control group, we kept the same protocol.

3.3. LABORATORY MEASUREMENTS

3.3.1. Hematological parameters

Sysmex F-800 and Sysmex K4500 microcell counter devices (TOA Medical Electronics Co., Ltd., Kobe, Japan) were used to measure RBC count [10^{12}/μL], white blood cell count (WBC [10^{9}/μL]), hemoglobin concentration (Hgb (g/dL)), and platelet count (Plt [10^{9}/μL]). Calculated values by the automates were hematocrit (Hct [%]), mean corpuscular volume (MCV [fL]), mean corpuscular hemoglobin (MCH [pg]), and mean corpuscular hemoglobin concentration (MCHC [g/L]).

3.3.2. Micro-rheological parameters

3.3.2.1. Red Blood Cell Deformability (Conventional and Osmotic Gradient Ektacytometry)

RBC deformability was determined using a LoRRca Maxsis Osmoscan ektacytometer (RR Mechatronics International B.V., Zwaag, the Netherlands). In this ektacytometry method, RBCs were subjected to shear stress, and their elongation was determined by laser diffraction techniques. The so-called elongation index (EI) was determined by the function of shear stress (SS [Pa]). Shear stress ranged between 0.3 and 30 Pa. For the conventional deformability test, 10 μL of whole blood was gently mixed with 2 mL of polyvinylpyrrolidone (PVP)–PBS solution (PVP: 360 kDa, Sigma-Aldrich Co., USA; PVP-PBS solution viscosity = 28–32 mPas, osmolality = 290–310 mOsmol/kg, pH = 7.3–7.5). All measurements were carried out at 37 °C.

From the EI–SS curves, comparative data were used, such as EI values at 3 Pa, and by parameterization of the entire EI–SS curve, maximal elongation index (EI_{max}) and shear stress at half EI_{max} ($SS_{1/2}$, [Pa]), which were calculated based on the Lineweaver–Burk equation.

Osmotic gradient deformability (osmoscan) measurements were carried out using 5 mL of isotonic PVP-PBS that was mixed with 250 µL of blood. In this module, the determination of EI is performed at constant shear stress (set for 30 Pa), while the osmolality of the suspension changes as the device mixes low-osmolar (0 mOsm/kg) and high-osmolar (500 mOsm/kg) PVP solutions with the whole-blood sample. The blood sample was aspirated to this PVP solution with a gradually increasing osmolality, while the elongation index was continuously registered. The result was a characteristic EI–osmolality (O) curve, with several notable points. EI min represents the minimal elongation index in the low-osmolar environment. The associated osmolality value, O min (osmolality at EI min), roughly corresponds to an osmolality value where 50% of the RBSs hemolyze in the osmotic fragility test. EI max here means the maximal elongation index in the function of osmolality (note: it is not the same as EI_{max} that was calculated by the Lineweaver–Burk equation, see above). Osmolality at EI max (O (EI max) is the value where RBCs deform optimally. EI hyper (half of the maximal elongation index in the high-osmolar environment) and O hyper show the point in the hyperosmolar region where the RBCs are half of their maximal elongation. Another parameter is the Area, which is calculated from the area under the individual EI–O curves.

Besides the standard comparative parameters of the osmoscan curves, further parameters were calculated, such as ΔEI (absolute difference of maximal and minimal EI values), ΔO (absolute difference of osmolality values at maximal and minimal EI), and ratio values: EI max/EI min (rEI), O (EI max)/O min (rO), ΔEI/ΔO, and rEI/rO.

3.3.2.2. Red Blood Cell Aggregation

A Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used to determine the RBC aggregation. The test requires approximately 20 µl of blood. After disaggregation by a controlled shearing system (shear rate: 600 s^{-1}), the light transmission was tested for 5 or 10 seconds at stasis (M values, shear rate: 0 s^{-1}) or at a low shear (M1 values, shear rate: 3 s^{-1}). The higher index values represent enhanced red blood cell aggregation.

3.3.3. Blood gas, acid-base parameters, metabolites and electrolytes

To analyse the blood gas and acid-base parameters, we used the EPOC Blood Analysis System (Siemens Healthineers AG, Germany). The following parameters were measured: pO_2 [mmHg], pCO_2 [mmHg], pH, $cHCO_3^-$ [mmol/L], Na^+ [mmol/L], K^+ [mmol/L], Ca^{2+} [mmol/L], Cl^- [mmol/L], glucose [mmol/L], lactate [mmol/L] and creatinine [μmol/L] concentrations.

3.4. STATISTICAL ANALYSIS

SigmaStat Software (Systat Software Inc, San Jose, CA, USA) was used to carry out the statistical analyses. Data are generally presented as means ± S.D. or median, 25% and 75% percentiles, and maximum and minimum values. To analyze existing differences between the species, the t-test or the Mann–Whitney rank-sum test was used based on the results of the normality test. One-way ANOVA tests (Bonferroni) were also used. After testing the normality of the data distribution by the Kolmogorov–Smirnov test, the correlation of the data was determined using Pearson/Spearman correlation on certain hematological and osmoscan parameters. A value of $p < 0.05$ was considered statistically significant.

4. RESULTS

4.1. INTERSPECIES DIVERSITY OF OSMOTIC GRADIENT DEFORMABILITY OF RED BLOOD CELLS IN HUMAN AND SEVEN VERTEBRATE ANIMAL SPECIES

4.1.1. Hematological parameters

White blood cell counts were significantly lower in rodents compared to humans ($p < 0.001$ vs. mouse, $p < 0.001$ vs. rat, $p = 0.035$ vs. rabbit). Red blood cell count was the highest in cats, followed by sheep, mice, and dogs. In dogs and cats, we found the highest hemoglobin concentration and hematocrit values. These two variables were significantly greater compared to other species, including the human blood samples as well (all: $p < 0.001$). Mean corpuscular volume and mean corpuscular hemoglobin values were significantly higher in humans. Platelet count showed higher values in almost every mammalian blood sample, except cats, which were significantly lower (all: $p < 0.001$ vs. human).

4.1.2. Red blood cell deformability

Comparing the different mammalian species, it is visible that sheep RBCs were deformed the least (all: $p < 0.001$ vs. sheep). In the low-shear section of the curve, human samples were significantly less deformed compared to the other groups, except for sheep samples ($p < 0.05$ vs. dog, cat, pig, mouse, rat, and rabbit). EI at 3 Pa results expressed

significant differences between humans and the other species investigated (human vs. dog: $p < 0.001$, human vs. cat: $p = 0.003$, human vs. pig: $p < 0.001$, human vs. sheep: $p < 0.001$, human vs. mouse: $p < 0.001$, human vs. rat: $p < 0.001$, human vs. rabbit: $p < 0.001$). Rodents had the highest EI at 3 Pa levels (mouse, rat, and rabbit vs. human: $p < 0.001$). By parameterization of the EI–SS curves, we found marked differences in EI_{max} values. The highest EI_{max} values were observed in rats, followed by dog and human samples. It could be seen that the ovine RBCs were deformed the least (sheep vs. all other species: $p < 0.001$). These results are strongly correlated with EI at 3 Pa results as well. The $SS_{1/2}$ [Pa] values significantly differed in the mammalian species. Human $SS_{1/2}$ results were the highest ($p < 0.001$ vs. all), as the slope of the human EI–SS curves notably altered from the other species (Figure 2). These findings were reflected well in $EI_{max}/SS_{1/2}$ values.

4.1.3. Osmotic Gradient Deformability

The shape of the EI–O (osmoscan) curves showed the classic bell shape in all the investigated species. If we compare the rat curve to the human, dog, and rabbit curves, a major difference is visible at the low-osmolar region of the curves. The tendency of the pig and mouse plots was lower, and a characteristic leftward shift of the curves could be observed. The osmoscan parameters showed a great analogy between these two groups, which cannot be stated for the results of the cats and sheep. Osmoscan curves of cats and sheep were shifted to the right on the scale.

The EI min and EI max values showed great interspecies differences between the study groups. Significant differences were found compared to human blood in dogs ($p < 0.001$), pigs ($p < 0.001$), rats ($p = 0.004$), and rabbits ($p < 0.001$). The rabbits have the highest EI min values, being significantly different from the other species, except for rats. The lowest EI min values were detectable in sheep ($p < 0.001$ vs. dog, $p = 0.014$ vs. cat, $p < 0.001$ vs. pig, rat, and rabbit). In the EI max results, these alterations were much more moderate; however, they showed interspecies diversity. In comparison to the human samples, the dog and rodent samples had higher values (dog: $p = 0.003$, mouse: $p = 0.043$), while sheep had the lowest ($p < 0.001$ vs. human, $p = 0.047$ vs. rat, $p = 0.012$ vs. rabbit). EI hyper values were relatively close to each other in the investigated mammalian species, except for sheep ($p < 0.001$ vs. all). Dogs had the highest EI hyper values.

Osmolality parameters (O min, O (EI max), O hyper) also showed colorful diversity. We found the same pattern as in the elongation result. Significantly higher O min values were found in the sheep ($p < 0.001$ vs. all), while the dogs had the lowest values ($p < 0.001$ vs. all). Related

to the O (EI max) parameter that reflects osmolality at the highest elongation index, three groups of the investigated mammalian species can be formed, arbitrarily: human and dog (human: 303.05 ± 10.92 mOsm/kg, dog: 272 ± 6.72 mOsm/kg), rodents with higher values (mouse: 327 ± 14.57 mOsm/kg; rat: 315.3 ± 18.45 mOsm/kg; rabbit: 319.75 ± 10.5 mOsm/kg), and pig, cat, and sheep with the highest values (pig: 360.88 ± 19.28 mOsm/kg, cat: 370.14 ± 19.94 mOsm/kg, sheep: 396.88 ± 15.23 mOsm/kg). The O hyper values of cats, pigs, sheep, and mice were the highest, while human and canine blood samples showed the lowest.

The Area parameter is derived from the area under the EI–O curves. Ascending order of Area parameters was found according to the following: sheep < cat < pig < mouse < human < rat < rabbit < dog.

Further comparative parameters (ΔEI, ΔO, EI max/EI min, O (EI max)/O min, and their ratios) focus on the left region of the EI–O curves, reflecting the magnitude of changes in EI in the function of osmolality at the hypo-osmolar direction. The absolute difference in EI min and EI max values (as ΔEI) was comparable to each other; however, pig, sheep, and mouse values differed significantly from human ($p = 0.027$, $p < 0.001$, and $p = 0.01$, respectively). The difference between O min and O (EI max) (as ΔO) was markedly higher in cats, pigs, and sheep ($p < 0.001$) and moderately higher in mice, rats, and rabbits compared to humans ($p = 0.01$, $p = 0.011$ and $p < 0.001$, respectively). Their ratio (ΔEI/ΔO) was almost identical in all the investigated species. The ratio of EI max and EI min reflects the diversity that was seen in conventional red blood cell deformability measurements (dog: $p < 0.001$, pig: $p < 0.001$, rat: $p = 0.008$, rabbit: $p = 0.004$ vs. human). The ratio of O (EI max) and O min was very close to each other in the species; however, compared to humans, the values of dogs, pigs, rats, and rabbits differed significantly ($p < 0.001$, $p < 0.001$, $p = 0.002$ and $p = 0.004$, respectively).

4.1.4. Correlation of parameters

Since certain osmoscan parameters are related to cell volume (dominantly at hypo-osmolar region), density, and intracellular viscosity (mostly at hyperosmolar region), correlation analysis of mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were also carried out on pooled data. A significant correlation was found between the following variables: MCV–EI min (coefficient: 0.414, $R^2 = 0.1714$, $p = 0.0013$), MCV–O min (coefficient: −0.696, $R^2 = 0.4844$, $p < 0.001$), MCV–ΔO (coefficient: −0.571, $R^2 = 0.326$, $p < 0.001$), MCV–Area (coefficient: 0.562, $R^2 = 0.3158$, $p < 0.001$), MCHC–EI hyper (coefficient: 0.523, $R^2 = 0.2735$, $p < 0.001$), and MCHC–O hyper (coefficient: −0.486, $R^2 = 0.2361$, $p = 0.0014$).

4.2. LOCAL AND SYSTEMIC MICRO-RHEOLOGICAL CHANGES DURING INTESTINAL ANASTOMOSIS OPERATION: A METABOLIC DEPENDENCE IN AN EXPERIMENTAL MODEL

4.2.1. Hematological parameters

White blood cell count significantly decreased in the anastomosis group in every blood sampling site compared to the base measurements (portal and renal venous blood: $p<0.001$, artery: $p=0.023$, vein: $p=0.01$). Red blood cell count increased after the bowel surgery in all samples ($p<0.001$). In the case of hemoglobin concentration, we observed that after the bowel surgery, the systemic arterial, venous, and portal venous samples showed a significant increase compared to the base and control values ($p=0.002$ vs. before operation, $p=0.004$ vs. control femoral artery, $p<0.001$ vs. control femoral vein), while the hematocrit increased mainly in the portal and renal venous blood samples ($p<0.001$ vs. before operation, $p=0.01$ vs. control portal vein, $p<0.001$ vs. renal vein). The mean corpuscular volume increased in every sample after operation in the anastomosis group ($p<0.001$ vs. before operation) and this phenomenon was also present when compared with the control group ($p=0.02$ vs. control femoral artery, $p<0.001$ vs. control femoral vein, $p=0.023$ vs. control portal vein, $p<0.001$ vs. renal vein). There was no significant change in the MCH and MCHC parameters. In contrast, platelet count significantly decreased in both the control and the operated groups, and further changes were observed after the intervention ($p<0.001$ vs. control femoral vein, $p=0.015$ vs. control portal vein, $p<0.001$ vs. renal vein).

4.2.2. Red blood cell deformability

EI at 3 Pa results expressed major significant differences between the control and the anastomosis group after the intervention ($p<0.001$ vs. control femoral artery, $p<0.001$ vs. control femoral vein, $p<0.001$ vs. control portal vein, $p<0.001$ vs. control renal vein) and also all sampling points in the anastomosis group showed a significant decrease compared the before operation values. This difference also persisted, although to a lesser extent for the EI_{max} parameter characterizing the maximum of the EI-SS curve ($p=0.001$ vs. before operation). After the operation only the portal sample showed a significant difference ($p=0.045$ vs. control portal vein). $SS_{1/2}$ parameters deteriorated in the anastomosis group after operation in the case of the systemic and portal sampling point ($p=0.008$ vs. femoral artery, $p<0.001$ vs. femoral vein, $p=0.003$ vs. portal vein) but the difference between groups did not show a significant difference. The ratio slightly increased after the operation, mainly in the portal and renal sample ($p=0.001$ vs. before operation), with a small worsening in the systemic samples.

4.2.3. Osmotic gradient ektacytometry (Osmoscan)

EI min parameter showed a slight decrease in all blood sampling points but the most noticeable difference was seen in the case of the portal vein sample after the operation ($p<0.001$ vs. control portal vein, $p=0.001$ vs. before operation). This downward trend in the EI max continues. The most significant differences were observed in the femoral artery and vein and the portal vein ($p<0.001$ vs. before operation, $p=0.001$ vs. control femoral artery, $p=0.002$ vs. control femoral vein, $p<0.001$ vs. control portal vein). In the hyperosmolar environment, the red blood cell deformability also deteriorated in the anastomosis group but only the sample from the femoral vein showed a significant difference compared to the control group ($p=0.033$ vs. control femoral vein). Osmolality parameters (O min, O (EI max), O hyper) also showed colorful diversity. We found a similar pattern to the elongation results. The most noticeable changes could be observed in the case of the O (EI max) and O hyper results, mainly in the portal vein samples after the operation. The area under the curve is a good description of the size and shape of the osmoscan curves. The smaller this value, the worse the deformability. Significant worsening was observable after surgery at three blood sampling points (femoral artery and vein, portal vein), and this difference is visible when these results are compared to the control group.

4.2.4. Red blood cell aggregation

In the light transmission-based aggregation measurement, significant differences in the aggregation index values were measured in stasis at the 5th second (M 5 sec), 10th second (M 10 sec), and also at low shear rate (M1 5 and 10 sec) after disaggregation were observed between the groups. Interestingly, this increase in red blood cell aggregation levels was only observed in the anastomosed group. In the control group, red blood cell aggregation showed only minimal variation in all the parameters tested, which could be either non-significantly decreasing or increasing. The most marked increases in aggregation were seen for M1 5 sec and M 10 sec parameters in renal venous blood samples (Rel. M1 5 sec: $p<0.001$ vs. before operation and control; Rel M10 sec: $p<0.001$ vs. before operation and control). Notably, the portal and renal blood samples were significantly higher than the baseline and the control group in all aggregation parameters.

4.2.5. Blood gas, acid-base and metabolites

The oxygen partial pressure levels decreased after the bowel surgery in all cases in the anastomosis group, mainly in the femoral artery and portal sampling site (Femoral artery:

$p<0.001$ vs. before operation, $p=0.005$ vs. control; portal vein: $p<0.001$ vs. before operation, $p<0.001$ vs. control). On the other hand, parallel with the oxygen pressure the carbon dioxide levels increased after operation compared to the base values and the control results (Femoral vein: $p<0.001$ vs. before operation, $p=0.005$ vs. control; Portal vein: $p<0.001$ vs. before operation, $p<0.001$ vs. control; Renal vein: $p<0.001$ vs. before operation, $p<0.001$ vs. control). The pH results did not show much variation after blood sampling, only a small change was seen in the femoral vein. Bicarbonate levels were decreased significantly in the portal and renal samples after the operation ($p<0.001$ vs. before operation) but no significant difference was seen compared to the control group.

Sodium and potassium levels also changed during intestinal surgery. In contrast with the bicarbonate level, sodium and potassium levels showed a large difference compared to the control group. Calcium and chloride ion concentrations didn't change significantly compared to the control group or the base measurements. As expected, glucose levels would increase slightly during surgery (both in the control and anastomosed groups), in the portal and renal samples of the anastomosis group, this increase was significant ($p=0.045$ vs. before operation). Examining lactate levels, we found that the effect of surgery showed a significant increase, and this difference was also significantly different compared to the control group (Femoral artery: $p=0.011$ vs. before operation, $p=0.002$ vs. control; Femoral vein: $p<0.001$ vs. before operation, $p<0.001$ vs. control; Portal vein: $p<0.001$ vs. before operation; Renal vein: $p<0.001$ vs. before operation, $p<0.001$ vs. control). A small, non-significant increase in creatinine levels was observed during the study.

We also observed that changes in pH and lactate concentrations, as described, correlated with changes in red blood cell deformability and aggregation. As the pH increased, and reached a physiological limit, the EI_{max} value increased in parallel ($p=0.003$, $R^2=0.151$). As the lactate concentration increased, the deformability of red blood cells deteriorated ($p<0.001$, $R^2=0.773$). Red blood cell aggregation indexes are also correlated with pH and lactate values. In general, red blood cell aggregation values increased with increasing lactate concentration ($p<0.001$, $R^2=0.823$) and decreasing pH ($p=0.002$, $R^2=0.343$).

5. DISCUSSION

5.1. INTERSPECIES DIVERSITY OF OSMOTIC GRADIENT DEFORMABILITY OF RED BLOOD CELLS IN HUMAN AND SEVEN VERTEBRATE ANIMAL SPECIES

The osmoregulatory processes of living organisms are complex, and there has been a long evolutionary journey to the final function of these processes. Osmoregulation is a key process for maintaining electrolyte and water balance (osmotic equilibrium), which enables organisms to maintain internal fluid balance and solute concentration despite external environmental changes. Different organisms use different mechanisms for osmoregulation, which are mainly classified as osmoconformers and osmoregulators. Mammalian homeostasis has evolved not only to regulate the general osmotic state across cell membranes (including the membrane of red blood cells), but also to regulate the specific concentration of important electrolytes in the three main fluid compartments: the blood plasma, the interstitial fluid space and the intracellular space. The water balance of the body can be shifted by a number of pathophysiological processes. Decreases in plasma osmolality are observed in hyponatremia, hyperhydration and SIADH. In contrast, in the presence of chronic renal failure, ketoacidosis, dehydration, hyperatremia and other exogenous substances, osmolality increases. There are also differences in the shape of the osmoscan (elongation-index-osmolality) curve of red blood cells in different diseases, especially in haematological pathologies.

Haemorheological differences between species have long been studied and a wide spectrum of variation has been identified, influenced by several factors (physiological parameters, environmental conditions and specific methods used). These studies have often revealed interesting, colourful differences between species, although typically without precise or consistent explanations, leaving many unanswered questions about the underlying mechanisms. Nonetheless, one key area that has received relatively little attention is the interspecific variation in osmotic gradient deformability. The existing literature contains remarkably little data on this topic, making it one of the less understood aspects of haemorheology. In this descriptive-comparative study, we have sought to build on our previous research by broadening the range of species included in the analysis. In addition, we aim to provide a more comprehensive and detailed examination of osmotic measurements, which we believe could provide valuable insights into nuanced haemorheological characteristics between species.

Similar to the literature and our previous publications, we observed that red blood cells of different mammalian species are deformed differently under shear forces, and the

aggregation and aggregability of erythrocytes also differs between species. The highest EI values were observed in mouse and dog blood, typically above 1 Pa shear stress. In contrast, slightly lower values were observed in rats, this variation was accentuated in pig and cat samples, while the lowest EI values were observed in sheep blood, mostly above 3-5 Pa shear stress. In humans, sheep and cats, the morphology of the EI-SS curves showed a large variation compared to the osmoscan curves for the other species studied. Interspecies variation was also observed in the elongation index values obtained from osmotic gradient ectocytometry measurements. There are significant and diverse differences in the deformability of red blood cells between animal species, which are influenced by a number of factors including cell structure, membrane composition and physiological adaptations. Recent studies have shed light on these differences, providing a clearer picture of how red blood cells of different species behave under mechanical stress. The research shows that the deformability of red blood cells differs significantly between mammals. For example, red blood cells in horses show a high degree of deformability, with EI values ranging from 0.047 at low shear stress to 0.541 at high shear stress. In comparison, dog red blood cells show EI values between 0.035 and 0.595 under similar conditions. However, sheep (ovine) red blood cells show significantly lower deformability, with EI values ranging from 0.005 to 0.400, suggesting that their red blood cells are less elastic when subjected to similar shear stress. The point defined by EI min and O min in the osmoscan curves can be partly related to classical osmotic fragility studies. Information on the wide interspecific variation in osmotic fragility data is available in the literature. During osmotic gradient ectocytometry measurements, cells start to fragment, but the size and number of fragmented cells cannot be correctly determined by this tool. Therefore, a clear comparison with the osmotic fragility test is not possible. However, the part of the curve to the left of the maximum EI point reflects the elastic properties of the cells as they swell with decreasing osmolality. As the surface to volume ratio and shape of the cells change, their deformability decreases.

In terms of interspecific differences, cell volume, cell shape, membrane viscosity and elastic properties, as well as cell membrane permeability, can be classified as species-specific. For O (EI max), red blood cells are allowed to deform to the maximum at a given shear stress, which is the optimum osmolar state for the cells. As osmolality increases, cell volume decreases with an increase in intracellular viscosity and density and a change in the cell surface-to-volume ratio. The result is again reduced elongation and deteriorating deformability. Since MCHC and MCV show high variability in animals, this parameter also provides an important explanation for osmotic differences. It is also important to note that the osmotic data and even the shape of

the elongation index-osmolality curves are shear stress-dependent. As the applied shear stress decreases, the osmoscan curves move to the left, and their shape depresses and decreases in the hyperosmolality range. The magnitude of this phenomenon also varies between species.

The differences in red blood cell deformability between species are not only of scientific interest - they have important physiological and clinical implications. Species have evolved different adaptations in response to environmental demands, such as the level of physical exertion or exposure to environmental stressors. These adaptations are often manifested in increased deformability of red blood cells, allowing for more efficient oxygen and nutrient transport in animals with higher metabolic demands. From a clinical perspective, understanding the differences between these species is vital for veterinary science and comparative physiology. Understanding the deformability of red blood cells can help improve the diagnosis and treatment strategies for blood-related disorders in different species. Our results may also help to extrapolate and compare data towards clinical studies, as the study of red blood cells with different sizes, morphologies and different biomechanical nuclei properties may also provide useful information for the micro-rheological aspects of abnormal cells in haematological disorders.

5.2. LOCAL AND SYSTEMIC MICRO-RHEOLOGICAL CHANGES DURING INTESTINAL ANASTOMOSIS OPERATION: A METABOLIC DEPENDENCE IN AN EXPERIMENTAL MODEL

Changes in haemorheological parameters in vivo are very difficult to study. Measurements are made on ex vivo samples, although many factors influence macro- and micro-rheological parameters. The sampling site and the dynamics of changes are particularly important issues in experimental or clinical studies of many pathological processes. In surgical pathophysiological processes, the effects that necessarily accompany interventions, anaesthesia, immobilisation, haemorrhage, sub-macro-fluid therapy, anticoagulants, and many other factors combine to shape the picture. All these need to be considered in their complexity.

In our work, we analysed how abdominal surgery can affect macro- and micro-rheological parameters and whether these factors differ when sampling from different blood collection points at different stages of surgery. In recent decades, the use of modern measurement techniques has led to a significant increase in knowledge of general haemorheological properties. The data also provide insights into local and systemic changes in red blood cell deformability and aggregation under different pathophysiological conditions such as inflammatory pathologies, sepsis, ischaemia-reperfusion and surgical interventions involving the vascular system. The use of minimal sample sizes and advanced methods has

opened up the possibility of in-depth investigation of micro-rheological differences between arteries and veins.

Standardisation of blood collection techniques and sites is equally important for experimental surgical and microsurgical research models. Before blood collection, we need to take into account race, genetic background, weight, gender, age, how often and how much blood we want to take, and which blood collection points to use. The amount of blood circulating depends on race and weight. Depending on the breed, pigs have between 56 and 69 ml of circulating blood per kg body weight. In general, 10% of the circulating blood volume can be removed without complications. By following these criteria, the parameters we studied reliably describe the effect of the intervention: the animals used in our study had about 1100 mL of circulating blood, of which only about 28 mL were withdrawn during the study.

Examining haematological parameters, we found that after surgery, the leukocyte count was lower at all blood sampling points, while the red blood cell count, mean corpuscular volume and haematocrit were elevated. The most significant changes were seen at portal and renal vein sampling sites. Our study revealed a similar pattern to previous studies, with lower white blood cell counts observed in arterial blood. It can be assumed that this difference is due to the distribution of leukocytes, their distribution in the circulation or due to the order of blood sampling (the last sampling site was the abdominal aorta). However, we also observed higher red blood cell count, haematocrit and platelet count in systemic venous and portal venous blood samples.

Furthermore, osmoscan measurements allow the exploration of the relationship between red blood cell deformability and key parameters such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). Overall, osmoscan is a valuable tool for the comprehensive study of the association of red blood cell deformability with physiological and pathological conditions.

In this study, clear differences were observed between arterial, systemic and portal venous blood in terms of red blood cell deformability and osmotic gradient deformability. It is noteworthy that arterial blood showed the lowest elongation index values, while systemic venous blood showed the highest. Portal blood, on the other hand, had EI values in between. Furthermore, the operated group showed a significantly greater reduction after anastomosis than the control group. The largest changes in this case were observed in the arterial and portal samples. Several interrelated factors may contribute to the reduction of red blood cell deformability, potentially affecting tissue perfusion. Inflammation is a key part of the healing process, but may also affect the micro-rheological properties of red blood cells, leading to

reduced deformability. With blood loss, the introduction of older or damaged red blood cells during a possible transfusion may further contribute to reduced deformability. Haemodilution during surgery to maintain blood pressure and fluid balance due to the administration of large volumes of intravenous fluids may also affect red blood cell deformability. The acute phase reactions associated with anaesthesia and surgery add to this, in addition to the oxygenation, acid-base and metabolic effects. Possible hypoxia may also impair red blood cell deformability. Anaesthetics, drugs, contrast agents used during surgery may also directly or indirectly affect red blood cell deformability, which may contribute to the observed changes.

Data on the aggregation time of red blood cells showed marked differences. However, the differences in the four variables (M and M1 index values at 5 s and 10 s) did not show a similar pattern. However, aggregation index values were significantly higher in all cases after completion of intestinal anastomosis than in the control group. The most significant differences were observed in portal and renal samples. In addition to the effects detailed above, the effect of changes in the flow profile and mechanical stress on the cells cannot be excluded.

Factors such as cellular oxygenation, blood pH and lactate concentration are crucial to reveal local and systemic changes in red blood cell deformability and aggregation. The rate of rouleaux formation is positively correlated with increasing pH, with the lowest rate observed in oxygen/nitrogen and nitrogen/carbon dioxide incubations and the highest rate observed in air and nitrogen incubations in vitro. Uyuklu et al. examined the effect of oxygenation or deoxygenation on the aggregation and deformability of red blood cells and found that oxygenated samples had lower aggregation and better deformability than deoxygenated samples. These results are essential for refining laboratory measurement techniques and standardising sampling and handling conditions.

In terms of our results, as expected from physiological considerations, arterial blood samples showed the highest pO_2 values, while lower values were observed in renal and systemic venous blood samples. The lowest pCO_2 values were recorded in arterial blood and the highest in venous blood, with portal blood samples falling in between. Blood pH showed similarities between systemic and portal venous blood; however, both glucose and lactate concentrations were lower in systemic venous and arterial blood samples. These associations shed light on the complex relationship between haemorheological factors and metabolic status. The results suggest that changes in blood pH and lactate levels may affect the micro-rheological properties of red blood cells, which in turn affects tissue perfusion.

6. MAIN FINDINGS AND CONCLUSIONS

1. We have investigated the osmotic gradient deformability of red blood cells in a broad species comparison. We are the first in the literature to report red blood cell osmotic data comparing human and seven mammalian species, showing the variability of these parameters, with characteristic elongation index - osmolality curves.
2. We confirmed with new data that red blood cell osmotic gradient deformability parameters correlate well with mean corpuscular volume (MCV) in the sub-optimal osmolality range and with mean corpuscular haemoglobin concentration (MCHC) in the higher osmolality range.
3. We have shown that micro-rheological parameters, in addition to arterio-venous differences, also differ in comparisons of portal and renal venous blood, with differences influenced mainly by oxygenation level, pH and lactate concentration. Intestinal anastomosis surgery caused immediate micro-rheological changes, with portal venous dominance in our experiment.
4. Changes in pH and lactate concentration correlated with changes in red blood cell deformability and aggregation. Our data show that as pH decreases and lactate concentration increases, erythrocyte deformability deteriorates and aggregability increases.

6.1. List of publications

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/546/2024.PL
Subject: PhD Publication List

Candidate: Ádám Varga
Doctoral School: Doctoral School of Clinical Medicine
MTMT ID: 10076783

List of publications related to the dissertation

1. **Varga, Á.**, Mátrai, Á. A., Baráth, B., Fazekas, L., Brasil, F. S., Mehta, A., Ványolos, E., Deák, Á., Lesznyák, T., Pető, K., Németh, N.: Local and Systemic Micro-Rheological Changes during Intestinal Anastomosis Operation: a Metabolic Dependence in an Experimental Model.
   Metabolites. 14 (8), 2-15, 2024.
   DOI: http://dx.doi.org/10.3390/metabo14080458
   IF: 3.4 (2023)
2. **Varga, Á.**, Mátrai, Á. A., Baráth, B., Deák, Á., Horváth, L., Németh, N.: Interspecies Diversity of Osmotic Gradient Deformability of Red Blood Cells in Human and Seven Vertebrate Animal Species.
   Cells. 11 (8), 1-15, 2022.
   DOI: http://dx.doi.org/10.3390/cells11081351
   IF: 6

List of other publications

3. **Varga, Á.**, Mátrai, Á. A., Fazekas, L., Al-Khafaji, M. Q., Ványolos, E., Deák, Á., Szentkereszty, Z., Pető, K., Németh, N.: Changes in microcirculation of small intestine end-to-end anastomoses in an experimental model.
   Microvasc. Res. 156, 1-8, 2024.
   DOI: http://dx.doi.org/10.1016/j.mvr.2024.104731
   IF: 2.9 (2023)
4. Mátrai, Á. A., **Varga, Á.**, Fazekas, L., Baráth, B., Nellamkuzhi, N. J., Nghi, T. B., Németh, N., Deák, Á.: Effect of Bile on Hemodynamics and Blood Micro-Rheological Parameters in Experimental Models of Bilhemia.
   Metabolites. 14 (4), 1-13, 2024.
   DOI: http://dx.doi.org/10.3390/metabo14040211
   IF: 3.4 (2023)

5. Mátrai, Á. A., **Varga, Á.**, Baráth, B., Ványolos, E., Orbán-Kálmándi, R. A., Lóczi, L., Bagoly, Z., Jouppila, A., Lassila, R., Németh, N., Deák, Á.: Heparin-like effect of a dual antiplatelet and anticoagulant (APAC) agent on red blood cell deformability and aggregation in an experimental model.
J Thromb Thrombolysis. [Epub ahead of print], 2024.
DOI: http://dx.doi.org/10.1007/s11239-024-03040-8
IF: 2.3 (2023)

6. Al-Smadi, M. W., Fazekas, L., **Varga, Á.**, Mátrai, Á. A., Aslan, S., Beqain, A., Al-Khafaji, M. Q. M., Baráth, B., Novák, L., Németh, N.: Minor micro-rheological alterations in the presence of an artificial saphenous arteriovenous shunt, as an arteriovenous malformation model in the rat.
Clin. Hemorheol. Microcirc. 87 (1), 27-37, 2024.
DOI: http://dx.doi.org/10.3233/CH-231825
IF: 2.1 (2023)

7. Fazekas, L., Szabó, B., Szegeczki, V., Fillér, C., **Varga, Á.**, Godó, Z., Tóth, G., Reglődi, D., Juhász, T., Németh, N.: Impact Assessment of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) and Hemostatic Sponge on Vascular Anastomosis Regeneration in Rats.
Int. J. Mol. Sci. 24, 1-24, 2023.
DOI: http://dx.doi.org/10.3390/ijms242316695
IF: 4.9

8. Baráth, B., **Varga, Á.**, Mátrai, Á. A., Pocsai, K., Németh, N., Deák, Á.: Estradiol Valerate Affects Hematological and Hemorheological Parameters in Rats.
Metabolites. 12 (7), 602, 2022.
DOI: http://dx.doi.org/10.3390/metabo12070602
IF: 4.1

9. Szabó, B., Gasz, B., Fazekas, L., **Varga, Á.**, Kiss-Pápai, L., Matolay, O., Rezsabek, Z., Al-Smadi, M. W., Németh, N.: Heterogeneous Maturation of Arterio-Venous Fistulas and Loop-Shaped Venous Interposition Grafts: a Histological and 3D Flow Simulation Comparison.
Biomedicines. 10, 1-14, 2022.
DOI: http://dx.doi.org/10.3390/biomedicines10071508
IF: 4.7

10. Gál, K., Veres, K., Halmi, S., Bozoki-Beke, K., Fekete, K., Homoki, J., Gálné Remenyik, J., Baráth, B., **Varga, Á.**, Németh, N., Soltész, P.: The effect of rheopheresis treatment on the cytokine profile in diabetic foot syndrome with hyperviscosity in the aspect of clinical changes: a preliminary study.
Clin. Hemorheol. Microcirc. 80 (2), 117-125, 2022.
DOI: http://dx.doi.org/10.3233/CH-211188
IF: 2.1

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

11. Szántó, S., Módy, T., Némethné Gyurcsik, Z., Babják, L. B., Somogyi, V., Baráth, B., **Varga, Á.**, Mátrai, Á. A., Németh, N.: Alterations of Selected Hemorheological and Metabolic Parameters Induced by Physical Activity in Untrained Men and Sportsmen.
Metabolites. 11, 1-12, 2021.
DOI: http://dx.doi.org/10.3390/metabo11120870
IF: 5.581

12. Baráth, B., Somogyi, V., Tánczos, B., **Varga, Á.**, Bereczky, Z., Németh, N., Deák, Á.: Examination of the relation between red blood cell aggregation and hematocrit in human and various experimental animals.
Clin. Hemorheol. Microcirc. 78 (2), 187-198, 2021.
DOI: http://dx.doi.org/10.3233/CH-211109
IF: 2.411

13. Körei, C., Szabó, B., **Varga, Á.**, Baráth, B., Deák, Á., Ványolos, E., Hargitai, Z., Kovács, I., Németh, N., Pető, K.: Hematological, Micro-Rheological, and Metabolic Changes Modulated by Local Ischemic Pre- and Post-Conditioning in Rat Limb Ischemia-Reperfusion.
Metabolites. 11 (11), 776, 2021.
DOI: http://dx.doi.org/10.3390/metabo11110776
IF: 5.581

14. Mátrai, Á. A., Varga, G., Tánczos, B., Baráth, B., **Varga, Á.**, Horváth, L., Bereczky, Z., Deák, Á., Németh, N.: In vitro effects of temperature on red blood cell deformability and membrane stability in human and various vertebrate species.
Clin. Hemorheol. Microcirc. 78 (3), 291-300, 2021.
DOI: https://doi.org/10.3233/CH-211118
IF: 2.411

15. Szabó, B., Tánczos, B., **Varga, Á.**, Baráth, B., Ghanem, S., Rezsabek, Z., Al-Smadi, M. W., Németh, N.: Micro-rheological changes of red blood cells in the presence of an arterio-venous fistula or a loop-shaped venous graft in the rat.
Front. Physiol. 11, 1-12, 2020.
DOI: http://dx.doi.org/10.3389/fphys.2020.616528
IF: 4.566

Total IF of journals (all publications): 56,45
Total IF of journals (publications related to the dissertation): 9,4

DEBRECENI EGYETEM * Egyetemi és Nemzeti Könyvtár *

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

04 November, 2024

7. ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor Prof. **Dr. Norbert Németh**, who has supported and encouraged me as my supervisor since the beginning of my PhD thesis in 2019. I thank him for giving me the opportunity to carry out my research and for his professional support. Thank you for guiding me through my PhD work.

I would like to thank **Dr. Viktória Somogyi**, Assistant Professor, who, as a co-supervisor, has set an example and helped me to advance my scientific work. Thank you for the opportunity to work with her, she was a great motivation for me.

I would like to thank **Prof. Dr. Irén Mikó, Dr. Katalin Pető, Dr. Erzsébet Ványolos** and **Dr. Ádám Deák** for their professional advice and suggestions.

I owe a huge thank you to my current and former colleagues and good friends **Ádám Attila Mátrai, Róbert Füzesi, Dr. Balázs Szabó** and **Dr. László Ádám Fazekas**, who have always supported me in everything. Thank you for the time spent together and for your support in difficult times.

I would like to thank my former colleague, **Dr. Barbara Bedőcs-Baráth** for the help she gave me in writing my dissertation.

I would also like to thank all the staff of the Department of Surgical Surgery for the friendly environment in which I was able to spend my everyday life and carry out my work.

To my family and ***friends*** for their love and selfless support over these long years. I owe an ocean of thanks and gratitude to ***my Parents***, ***Sister*** and ***Fiancée*** who have supported my studies, provided a peaceful and intimate background and stood by me through every trial, believing in me and giving me strength when I needed it.

Financial support:

NKFI-1 “OTKA” K-139184 and ÚNKP-22-3-II-DE-308.