

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

**Clinical experiences and experimental hemorheological
and morphological investigations
of peripheral vascular graft implanting**

by Csaba Zsigmond Tóth, MD

Supervisor:

Norbert Németh, MD, PhD



**UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE
DEBRECEN, 2015**

**Clinical experiences and experimental hemorheological and morphological
investigations of peripheral vascular graft implanting**

By Csaba Zsigmond Tóth, MD

Supervisor: Norbert Nemeth, MD, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Examination Committee:** Prof. András Berta, MD, PhD, DSc
Members of the Examination Committee: Prof. Béla Fülesdi, MD, PhD, DSc
Prof. Lajos Kollár, MD, PhD

The Examination takes place at the library of the Ophthalmology Clinic, Faculty of
Medicine, University of Debrecen, 18 May 2015, at 11 a.m.

Head of the **Defense Committee:** Prof. András Berta, MD, PhD, DSc
Reviewers: Prof. Pál Soltész, MD, PhD, DSc
Endre Arató, MD, PhD
Members of the Defense Committee: Prof. Béla Fülesdi, MD, PhD, DSc
Prof. Lajos Kollár, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal
Medicine, Faculty of Medicine, University of Debrecen, 18 May 2015, at 1 p.m.

1. INTRODUCTION

Open surgical procedures, such as bypass operations still have an important role in today's vascular surgery. For the bypass implants, the patient's superficial vein or artificial vascular graft is used, what can be made of polyethylene terephthalate (PTE, Dacron[®]) or polytetrafluoroethylene (PTFE). However, bypass surgeries made with the patients' own veins are statistically proved to have twice as more patency rates than the artificial grafts.

During the last 10-15 years a reduction in the number of infrainguinal bypass operations can be observed. The reasons are not well known but risk factor reduction, modification, early referral and the improvement of the endovascular techniques (even for TASC C,D lesions) could be a reason for that. However, by surgeons' opinion the open surgery remains the first choice for TASC D lesions. The greater saphenous vein (GSV) is the gold standard for infrainguinal bypasses at any level. If the GSV is of poor quality or has been removed (for example CABG or varicectomy was performed), the use of the contralateral GSV has to be considered, rather than arm veins, which have lower patency rates. In case of the surgeries above the knee the implantation of an artificial graft is chosen since with progression of the underlying disease it might be the necessary to do surgery below the knee, where veins are preferred for the bypass. In absence of vein a prosthetic graft should be used. In this case a vein cuff recommended at the distal anastomosis. The Joint Vascular Research Group RCT of Miller vein cuff versus non-cuff for femoro-distal PTFE grafts demonstrated significantly higher patency rates for prosthetic graft with vein segment at P III level. The number of prosthetic grafts, used for intermittent claudication/critical limb ischemia has fallen. Poor patency rate and the concerns about graft infections are the main reasons for that.

The first couple of postoperative days are always critical. The problem of early thrombosis in case of small-diameter artificial vascular conduits still means a serious question in vascular surgery. The wall of the artificial graft is

more rigid, the arterial three-phased blood flow pattern cannot be observed. After the implantation of an artificial vascular graft, we may see several early and late complications. Early: suture insufficiency, hemorrhage, graft infection, wound infection, vascular and nerve injuries, early obstruction of the graft. Late: pseudoaneurysm formation due to suture insufficiency, obstruction of the graft, stenosis caused by neointima formation or occlusion, graft infection. The blood flow characteristics change at the anastomoses, the cells may suffer mechanical injury - here the formation of deposits usually leads to another operation. Although it is not completely clarified that from which point the flow properties of the altered vascular geometry can lead to thrombotic complications later.

Although biocomparability and hemostasis issues are the most important factors concerning the implantation of various synthetic vascular grafts, the continuous mechanical shear on circulating red blood cells means another unquestionable hemorheological aspect, mostly in small-caliber grafts sutured into peripheral vascular regions. Geometry, length, diameter, possible torquation/torsion of the connections (vessel-graft anastomoses), narrowing by neointima formation and proliferation, and/or thrombus formation (e.g., early thrombosis in the prosthetic grafts) all may have impact as alteration in the mechanical shear force profile. After implanting artificial small-caliber grafts the ratio of early failure is still high in the clinical practice.

We hypothesized that micro-rheological variables, including red blood cell deformability and membrane stability parameters, may show deterioration in the early postoperative period, and may have an importance to predict the possible complications or to accompany them.

3. OBJECTIVES

1. Our purpose was to investigate the effect of presence of small-caliber PTFE vascular graft implanted in the femoral artery on haematological, blood coagulation time and haemorheological parameters, in special consideration of red blood cell aggregation, deformability and membrane stability over a 2-week follow-up period on beagle dogs.
2. Using red blood cell membrane (mechanical) stability test we aimed to investigate the effect of various shear stress magnitude and exposition time combinations on the samples, focusing on demonstrability of the sublethal blood cell trauma.
3. Geometrical and histomorphological analyses of the vessel parts involving the graft at the end of the 2-week follow-up period.

4. MATERIALS AND METHODS

4.1. Experimental animals and operative protocol

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 20/2011. UD CAR), in accordance with the relevant Hungarian Animal Protection Act (Law XVIII/1998) and EU directives.

All the surgical interventions were performed under general anesthesia (10 mg/kg ketamin + 0.1 mg/kg xylazin, i.m.)

In the *Grafted group* (n=5): the left femoral artery was gently exposed and atraumatically clamped proximally and distally. A 3.5 cm long segment was excised and replaced with a polytetrafluoroethylene (PTFE) graft (diameter = 3 mm, Atrium Co.) of the same length (3.52 ± 0.48 cm) using end-to-end anastomoses (continuously suture line, 6/0 polypropylene). The time for the necessary clamping of the vessel was 25 ± 3.1 minutes. In the Control group (n=4) only anesthesia was induced and for a 2-hour-period animals were laid on the operative table under the same circumstances as in the Grafted group.

Animals received 1000 IU sodium-heparin intravenously at the beginning of the operation. Postoperatively, on the 1st and 3rd days 500 IU Clexan was given subcutaneously. Intramuscularly 50 µg/kg sodium-metamizole (Algopyrin 1 g/2 ml ampule) was administered for analgesia just after the operation and on the 1st postoperative day.

Via puncturing the cephalic vein, blood samples were collected before the operation, on the 1st, 3rd, 5th, 7th and on the 14th postoperative days using Vacutainer[®] system. At the end of the experimental period, on the 14th postoperative day, the animals of the Grafted group were over-anesthetized and the grafts with intact femoral artery parts were excised for further histomorphological examinations.

4.2. Postoperative physical examinations

Skin temperature was measured at the end of the operation, on the 1st, 3rd, 5th, 7th and 14th postoperative days both on the operated and non-operated (control side) extremities. Besides the absolute values, the ratios of operated / non-operated extremities' skin temperature values have been analysed. Observation for possible pain, swelling and circulatory problem were carried out during daily wound control and walkings.

4.3. Laboratory examinations

For testing lactate, blood pH, haematological and haemorheological parameters samples anticoagulated with K₃-EDTA (1.8 mg/ml, Vacutainer[®]), for determining coagulational parameters and fibrinogen concentration samples anticoagulated with Sodium-citrate (0.129 M, Vacutainer[®]) were used.

4.3.1. Lactate concentration and blood pH

Lactate concentration [mmol/l] and blood pH values were determined by an automated blood gase analyser (ABL555 Radiometer Copenhagen, Denmark). We avoided samples from direct contact with air.

4.3.2. Coagulational time parameters and fibrinogen concentration

Blood coagulation time parameters, such as prothrombin time (PT [s]), activated partial thromboplastin time (APTT [s]), as well as fibrinogen concentration (Fbg [g/dl]) were determined by a Sysmex CA-500 automated coagulometer (TOA Medical Electronics Co. Ltd., Japan).

4.3.3. Haematological parameters

A Sysmex F-800 microcell counter (TOA Medical Electronics Co. Ltd., Japan) was used to determine the general quantitative and qualitative haematological parameters.

4.3.4. Red blood cell deformability and mechanical stability test

For red blood cell deformability measurements, a LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used.

For *regular red blood cell deformability tests* 5 μ l blood sample was gently mixed in 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 26 mPa.s, osmolality = 290-300 mOsm/kg ; pH ~ 7.3). The suspension was injected into the bob-cup system of the device, which generated shear stress (SS) (range: 0.3-30 Pa), while the laser diffraction pattern has been analyzed. The software calculated elongation index (EI) values. EI increases with red blood cell deformability. The tests were carried out at constant temperature of 37 °C. For data reduction and comparison, calculated maximal elongation index at infinitive shear stress (EI_{max}) and the shear stress values at half of it ($SS_{1/2}$ [Pa]) were used, according to the Lineweaver-Burk analyses: $1/EI = SS_{1/2}/EI_{max} \times 1/SS + 1/EI_{max}$, in a SS range of 0.95-30 Pa.

During *membrane stability test*, the protocol started with a regular deformability measurement, then a shearing period has been applied on the samples (60 Pa for 300 seconds or 100 Pa for 300 seconds). After the shearing, a second deformability test was carried out. Since the mechanical force is expected to cause deterioration of deformability values, the EI-SS curves obtained before and after the shearing have been analyzed, and the ratio of the values after/before the mechanical stress has been also evaluated.

For the *osmotic gradient ektacytometry (osmoscan)* measurements 250 μ l blood was gently mixed in 5 ml PVP solution. A constant shear stress of 30 Pa was applied, while the osmolality of the sample was changing, since the device was continuously aspirating the samples together with 0 or 500 mOsmol/kg PVP solutions. So the EI values were registered in the function of osmolality. The tested parameters included maximal EI, minimal EI, EI values at half maximal

EI, and the osmolality values at these EI points, as well as the area under the EI-osmolality curves (area under curve, AUC).

4.3.5. Red blood cell aggregation

A Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used to test red blood cell aggregation based on Schmid-Schönbein's light-transmission method. For the tests 20 μl blood is needed. During the measurements the device generates 600 s^{-1} shear rate to disaggregate the blood cells. Then suddenly the shear rate drops to 0 (M mode) or to 3 s^{-1} (M1 mode). At stasis or at low shear rate the red blood cells start to aggregate while the light-transmittance changes (disaggregation: low, aggregating erythrocytes: increasing light-transmission). The device calculates the light-transmission index at the 5th or at the 10th seconds of the process. When aggregation is enhanced, these index parameters (M 5s, M1 5s, M 10s, M1 10s) increase.

4.4. Histological examinations

On the 14th postoperative day under general anesthesia the grafts with intact vessel parts over the anastomoses and the contralateral, intact femoral arteries were excised. The specimens were fixed in 10% formalin before the regular dehydration and embedding protocol, and microtomed into 5 μm sections. Standard hematoxylin-eosin (H&E) staining, as well as immunohistochemistry for CD31 was carried out on the specimens.

4.5. Statistical analyses

Data are presented as means \pm standard deviation (S.D.). Although the case number was low, for inter-group comparison student t-test or Mann-Whitney RS test were used, and one-way ANOVA tests (Dunn's or Bonferroni method) were carried out for intra-group comparisons, depending on the data distribution, with a level of significance of $p < 0.05$.

5. RESULTS

5.1. Postoperative physical examinations, skin temperature

All experimental animals survived the operations and there was no death during the 2-week postoperative follow-up period. No surgical complication - neither early, nor late- was detected. The motion of the animals were normal during the 2-week follow-up period, there was no sign for hind limb circulatory problem. The skin temperature values of the non-operated and operated legs were identical.

5.2. Lactate concentration and blood pH

Lactate concentration of the Control group slightly increased on the 1st and 3rd postoperative days. By the end of the second week decreased ($p=0.029$ vs. base). In the Grafted group lactate concentration expressedly decreased by the 1st day, and remained low over the follow-up period, showing significant difference compared to the Control group (1st day: $p<0.001$, 3rd day: $p=0.023$, 5th day: $p=0.035$). Blood pH did not show important changes in either group, except for a slight decrease on the 1st and 3rd day in the controls.

5.3. Haematological parameters

White blood cell count (total leukocyte count) increased by the 1st postoperative day ($p<0.001$ in both groups), in a larger magnitude in the Grafted group ($p=0.002$ vs. Control). The cell count normalized in the Control group, but in the grafted it remained elevated until the end of the first postoperative week (compared to base: $p=0.019$ on the 3rd day, $p=0.005$ on the 5th day and $p=0.016$ on the 7th day.) The monocyte-granulocyte ratio remained between 60-70%, except for the 5th and 7th day, when the values were $81.73 \pm 3.78 \%$ and $74.3 \pm 3.98 \%$, respectively.

Platelet count of the Grafted group continuously increased over the experimental period. The rise was significant from the 3rd postoperative day

compared to the base values (5th day: p=0.015; 7th day: p=0.001; 14th day: p<0.001) and versus the Control group, too (3rd day: p=0.03; 5th day: p=0.046; 14th day: p=0.003).

The red blood cell count slightly decreased over the 2-week follow-up period in the Control group (versus base values: p=0.002 on the 1st, p=0.018 on the 7th and p=0.003 on the 14th postoperative day). The Grafted group showed the same tendency (versus base values: p=0.021 on the 3rd, p=0.033 on the 5th, p<0.001 on the 7th, and p=0.038 on the 14th day), and expressed moderately lower values compared to the Control group (p=0.046 on the 3rd, and p=0.049 on the 7th day). By the 7th day a definitive decrease in the red blood cell count was observed, being significant compared to the base values.

Hematocrit values decreased over the follow-up period in both groups (in Control group versus base values: p<0.001 on the 1st day, p=0.006 on the 3rd day, p=0.011 on the 7th day and p=0.002 on the 14th day; in the Grafted group versus base values: p=0.016 on the 1st day, p=0.029 on the 3rd day, p=0.036 on the 5th day and p<0.001 on the 7th day). However, in Grafted group the values were markedly lower (versus the Control group: p=0.014 on the 3rd day, p=0.018 on the 5th day and p=0.029 on the 7th day).

MCV values did not show any important alterations except for the fact that the Grafted group showed minimally lower values during the 1st postoperative week (versus Control group p=0.017 on the 3rd day, and p=0.083 on the 5th day).

Hemoglobin values of the Control group moderately decreased till the end of the follow-up period (versus base values: p=0.015 on the 7th day and p=0.003 on the 14th day). In the Grafted group, the decrease showed similar tendency (versus base values: p=0.003 both on the 5th and 7th days, and p=0.028 on the 14th day). The values were lower compared to the Control group (p=0.004 on the 3rd, p=0.002 on the 5th and p=0.016 on the 7th day).

MCH values did not show important changes, except for the numerically significant inter-group difference on the 5th day ($p=0.05$) and on the 14th day ($p=0.003$).

MCHC of the Grafted group decreased by the end of the follow-up period ($p=0.054$ vs. base and $p=0.007$ vs. Control).

5.4. Coagulational time parameters and fibrinogen concentration

Prothrombin time did not show important changes, however, activated partial thromboplastin time rose twice in the Grafted group: on the 3rd day ($p=0.048$ vs. base) and on the 7th day ($p=0.012$ vs. base). Fibrinogen concentration rose by the 1st day and gradually decreased by the end of the follow-up period. Although it remained in physiological manner, there were significant differences (on the 1st day: $p<0.001$ vs. base and vs. Control; on the 3rd day: $p=0.023$ vs. base and $p=0.002$ vs. Control; on the 5th day: $p=0.043$ vs. base and $p=0.002$ vs. Control).

5.5. Red blood cell deformability and membrane stability

When testing the red blood cell deformability with normal, regular ektacytometry test (elongation index in the function of shear stress), the calculated parameter EI_{max} showed a continuous decrease over the experimental period. In the Grafted group it reached the lowest values by the 7th postoperative day ($p<0.001$ vs. base; $p<0.001$ vs. Control). The calculated $SS_{1/2}$ [Pa] values were significantly higher in the Grafted group on the 3rd postoperative day ($p=0.029$ vs. Control), then interestingly, it was the lowest on the 7th day ($p=0.25$ vs. base and $p=0.042$ vs. Control), and increased again by the 14th day ($p=0.011$ vs. Control).

From the osmoscan data, the maximal EI were lower in the Grafted group, as it was also reflected during the normal ektacytometrial tests. The minimal EI, i.e. the point where the cells ruptures at hypoosmolar environment, were slightly

and non-significantly higher on the 1st, 3rd, 5th and 14th day in Grafted group, while the osmolality values at these points were moderately higher or lower, or even identical compared to the Control group, without any significant difference (data not shown). The area under EI-osmolality curve (AUC) expressed significant decrease mostly in the Grafted group, showing the lowest values by the 7th postoperative day (vs. base values $p=0.056$ on the 7th day; vs. Control group $p=0.005$ on the 3rd day, $p=0.017$ on the 5th day, and $p=0.061$ on the 7th day).

We had investigated the membrane stability using two different protocols (shearing with 60 Pa for 300 s or with 100 Pa for 300 s) in all samples. Obviously, the elongation index values that tested just after the shearing were always significantly lower ($p<0.001$, in the range of 0.95-30 Pa) compared to the initial values, in both Control and Grafted groups. However, the absolute EI values and the difference existed between the values before and after the shearing period were completely different in the two groups. The diversity was much more expressed using the shearing protocol at 100 Pa. The cell membrane stability investigation showed a lower erythrocyte deformability profile for the Grafted group and a narrowed alteration in the deformability curves due to mechanical stress (the ratio of ‘after/before’ values were smaller). The morphology of the EI-SS curves also altered in the Grafted group: under 0.95 Pa the curve was ‘put-up’ at the lowest shear stress range (EI values were higher at 0.3 - 0.5 Pa).

In ektacytometrial tests the largest changes have been observed on the 7th postoperative day. Further analyzing the mechanical stability test results, the most expressed alterations appeared in these samples.

5.6. Red blood cell aggregation

The aggregation index (AI [arbitrary unit]) represent the magnitude of aggregation over the tested 120-second period, the amplitude shows the heights

of the syllectogram curve compared to the initial values (Amp [au]), while $t_{1/2}$ [s] shows the time point when the aggregation process reaches the half of the total aggregation index values. AI values were moderately higher in the Grafted group on the 1st, 3rd and 5th postoperative day, together with increased Amp values (at the 7th day it was significant: $p=0.008$ vs. Control), while the $t_{1/2}$ values alluded to a faster aggregation.

Erythrocyte aggregation index *M 5 s* and *M 10 s* values (tested with Myrenne aggregometer) represent the magnitude of the aggregation at the 5th and 10th second of the process measured at stasis. In the Grafted group these values showed significant increase during the 1st postoperative week, peaking on the 1st and 3rd postoperative days.

The *M 5 s* values (at 5th second of the aggregation process) increased on the 1st ($p=0.011$ vs. base, $p=0.03$ vs. Control), 3rd ($p<0.001$ vs. base and vs. Control) 5th ($p=0.021$ vs. base) and 7th day ($p<0.001$ vs. base and vs. Control). The values of *M 10 s* (at 10th second of the aggregation process) resulted in larger values and more prominent differences between the experimental groups (on the 1st day: $p=0.029$ vs. base; on the 3rd day: $p<0.001$ vs. base and vs. Control; on the 5th day: $p=0.006$ vs. base and $p<0.001$ vs., Control; and on the 7th day: $p=0.006$ vs. base).

5.7. Histological examinations

On the 14th day during the biopsy taking, the diameter of the control-side femoral artery was 3.56 ± 0.13 mm, the graft's one was 3.62 ± 0.17 , while the artery segment just above the graft was 3.5 ± 0.41 mm, but below, it was 2.75 ± 0.28 mm in diameter ($p<0.001$ vs. graft, $p=0.024$ vs. above graft and $p=0.016$ vs. control-side artery).

Histologically we found matured thrombus at the anastomoses narrowing the lumen. Imbedded capillary network lined with endothelium was observed in the fibrin web and connective tissue filled with various sized and shaped red

blood cells. It seemed to be fixed to the inner side of the arterial intimal layer. At site of the proximal anastomoses the grafts were observed in the scared thickening of the adventitia. The fixture of thrombus inside the grafts was not obvious, here we could see “free” inner layer. Freshly formed thrombotic layers were seen towards the distal segment. At the site of the distal anastomoses we observed scar tissue with foreign body giant cells and mixed inflammatory cells around the surgical suture material in the adventitia, which was present as continuity along a short section of the graft.

The widening of the internal elastic lamina was observed, but the endothelium lining was not always present. The observed thrombi seem to be the continuity of this widening. In the reticulate wall of the thrombi red blood cells and inflammatory cells were apparent. The endothelialization did not occur during the 2-week period, which was confirmed by the CD31 immunohistochemical examination. However, pseudointima formations made up from thrombotic elements are visible in certain segments. The arterial sections from the control side show regular, intact histological structure.

6. DISCUSSION

Small-caliber vascular conduits are still an important tool in the surgical management of peripheral vascular diseases. However, the problem of early failure is a serious clinical problem. Since not only the problem of small-caliber vascular conduits must be considered, but the question of biomechanical tissue remodeling is also a key factor with all the shear stress-, stretching-, fluid biomechanical and mechanical micro-environmental relations. The healing of arterial graft is a complex and long process, depending on numberless factors.

In this model we focused on the changes over the first two postoperative weeks. We found that the majority of the blood coagulation and red blood cell aggregation changes happened during the 1st week. Inflammatory processes, acute phase reactions can be associated with alterations with hemorheological

parameters. We saw that fibrinogen concentration closed very quickly and the 2-peak elongation of coagulation time parameters with a continuously increasing platelet count might suggest that a thrombotic event could happen in the period of 3rd - 7th postoperative day.

Failure of the graft can be due to complex reasons that include the hemodynamic effect of the small-caliber tube, the surface properties, activation of hemostatic cascades and mechanical damage of blood cells, among others. Along with the injury of the intima, the inflammatory events induced by the sutures of the anastomoses may contribute to the development of thrombus. Here it may come into play that the vessels incidentally bend and refract above the graft after surgery while positioning the lower limb back into natural position and during the normal everyday movement of the animal. There were no sign of circulatory problems on the operated side, no change in the movement and behavior of the animal, and the skin temperatures were normal; it showed virtually similar values with the contralateral non-operated side. The anastomoses originated from the gluteal region might compensate the circulation of the limb.

Besides the general hematological and blood coagulation time parameters' changes, we observed an early increase in erythrocyte aggregation values, too. Red blood cell aggregation is determined by cellular (cell morphology, deformability, membrane mechanical properties, composition of the surface glycocalyx) and plasmatic factors (e.g. fibrinogen concentration). Free radicals deliberating during ischemia-reperfusion and inflammatory processes, mechanical cell damage, changes in red blood cell deformability, alteration in fibrinogen concentration, as well as micro-environmental conditions (pH, osmolality), all may result in altered red blood cell aggregation.

Furthermore, the mechanical properties of the cell membrane also play an important role. Mechanism of erythrocyte mechanical cell damage includes overstretching or fragmentation of the cells (hemolysis) resulting in free

hemoglobin in the plasma, release of microparticles and the sublethal trauma to the cells with increase in red blood cell aggregation and decrease in deformability. The hemoglobin released to plasma is bound to haptoglobin, which complex is known to be removed from the circulation continuously by the reticuloendothelial system. It is known that a moderate mechanical stress can even improve the erythrocytes' micro-rheological characteristics, however, a more significant mechanical stress causes deterioration in the deformability. The key factor is the relation of exposure time and the magnitude of shear stress. When red blood cells are still not disrupted yet, but their deformability and aggregation definitely impaired due to the mechanical stress of any origin, we call it sublethal trauma.

It is supposed that thrombus formation along the graft wall -by its narrowing effect- could also increase or at least modulate the shearing forces. At the end of the experiment the grafts were excised and narrowing longitudinal thrombi were found in the grafts. We assume that the early thrombus formation could be occurred around the 7th day when we could observe the most expressed micro-rheological changes, too. In the Grafted group the deformability decreased and during the membrane stability test a smaller difference was observed between the states before and after the shearing, expressing a narrowed alteration range in the deformability against the mechanical stress applied.

The PTFE graft implantation for the replacement of the resected femoral arterial segment caused changes in the coagulation parameters and hemorheological properties. Better clarifying the factors leading to early thrombosis of the small-caliber grafts is a very important issue. Further studies are needed for revealing the optimal conditions on geometry, length, position, hemodynamic and hemorheological factors, moving relations or even impregnated grafts that may decrease the chance for thrombus formation.

7. SUMMARY OF THE FINDINGS AND MAIN CONCLUSIONS

1. We demonstrated in a 2-week follow-up animal experiment that after supplementation of a femoral artery part (length: 3.5 cm) by an equal-length PTFE graft (diameter: 3 mm) most of the significant alterations in haematological, red blood cell aggregation and blood coagulation time parameters pass off by the end of the first postoperative week. The highest rise was observed in partial thromboplastin time on the 3rd and 5th postoperative days.
2. In the Grafted group red blood cell aggregation significantly increased on the 1st – 3rd postoperative days. Compared to the Control group red blood cell deformability showed significant deterioration on the 3rd, 5th and mostly on the 7th postoperative days after implanting the PTFE graft.
3. Red blood cell mechanical stability test showed well-demonstrable differences on those days: in the Grafted group a smaller difference was observed between the states before and after the shearing, expressing a narrowed alteration range in the deformability against the mechanical stress applied.
4. The worst deformability parameters found on the 7th postoperative day might be coincided with the development of thrombus that could narrow or occlude the graft. The presence of matured thrombi has been confirmed by histological examinations in the biopsies taken on the 14th day. It may call the attention for the importance of red blood cell deformability and membrane (mechanical) stability tests when monitoring the early postoperative days after implanting a small-caliber graft. Thus micro-rheological examinations can be recommended in following-up of vascular graft implants of various size and geometry.



Registry number:
Subject:

DEENK/57/2015.PL
Ph.D. List of Publications

Candidate: Csaba Tóth
Neptun ID: XLQEQ1
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

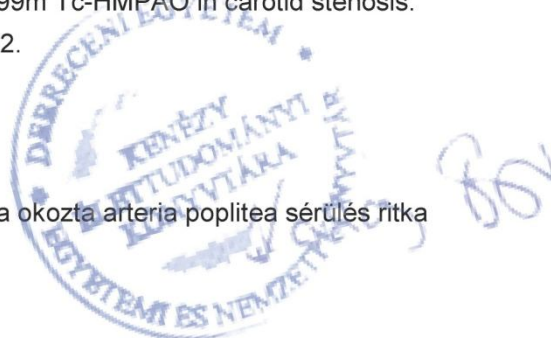
1. **Tóth, C.**, Klárik, Z., Kiss, F., Tóth, E., Hargitai, Z., Németh, N.: Early postoperative changes in hematological, erythrocyte aggregation and blood coagulation parameters after unilateral implantation of polytetrafluoroethylene vascular graft in the femoral artery of beagle dogs. *Acta Cir. Bras.* 29 (5), 320-327, 2014.
DOI: <http://dx.doi.org/10.1590/S0102-86502014000500006>
IF:0.57 (2013)
2. **Tóth, C.**, Kiss, F., Klárik, Z., Gergely, E., Tóth, E., Pető, K., Ványolos, E., Mikó, I., Németh, N.: Following-up changes in red blood cell deformability and membrane stability in the presence of PTFE graft implanted into the femoral artery in a canine model. *Korea-Aust. Rheol. J.* 26 (2), 209-215, 2014.
DOI: <http://dx.doi.org/10.1007/s13367-014-0023-3>
IF:0.632 (2013)





List of other publications

3. Daróczy J., Kovács L.A., **Tóth C.**, Szolnok G., Csernus M., Hunyadi J., Sugár I., Bihari I., Mecsek L., Szokoly M.: A nem gyógyuló (krónikus) bőrsebek ellátásának irányelve II. rész. *Érbetegségek*. 21 (4), 123-135, 2014.
4. **Tóth C.**, Lőrincz Á., Olvasztó S., Garai I., Kálvin B.: A 18-Fluorodeoxyglucose PET szerepe a grafftuppuratio pontosabb diagnózisában. *Érbetegségek*. 16 (3), 79-84, 2009.
5. Garai I., **Tóth C.**, Szabados L., Sóvágó J., Tóth L., Csiki Z.: A metabolikus képalkotás - PET, PET/CT - onkosebészeti vonatkozásai =Metabolic imaging - PET, PET/CT - in oncology. *Magyar Seb.* 61 (4), 217-224, 2008.
DOI: <http://dx.doi.org/10.1556/maseb.61.2008.4.2>
6. Papp L., Litauszky K., Bodnár F., Olvasztó S., Kosztyu L., **Tóth C.**: A rupturált abdominalis aorta aneurysma (RAAA) kezelési elveiről: Szükséges-e a szemléletváltozás az endovascularis érában. *Anaesthesiol. Intenziv Ther.* 37 (1), 8-16, 2007.
7. Litauszky K., **Tóth C.**, Bodnár F., Magyar T., Papp L.: Az arteria carotis extracranialis szakaszának aneurysmáiról-electiv és rupturált aneurysma-esetünk kapcsán. *Érbetegségek*. 14 (4), 139-144, 2007.
8. Garai I., Varga J., Szomják E., **Tóth C.**, Csiki Z., Olvasztó S., Galuska L.: Az agyi vérátfolyás rezerv kapacitásának mérése 99mTc HMPAO-val carotis angioplasztikán átesett betegeknél. *Érbetegségek*. 10 (3), 63-68, 2003.
9. Garai I., Varga J., Szomják E., **Tóth C.**, Bánk J., Ficzer A., Olvasztó S., Galuska L.: Quantitative assessment of blood flow reserve using 99m Tc-HMPAO in carotid stenosis. *Eur. J. Nucl. Med. Mol. Imaging*. 29 (2), 216-220, 2002.
DOI: <http://dx.doi.org/10.1007/s00259-001-0688-y>
IF:3.568
10. **Tóth C.**, Olvasztó S., Dinya T.: Femurcondylus exostosis okozta arteria poplitea sérülés ritka esete:(Esetismertetés). *Magyar Seb.* 54, 115-117, 2001.





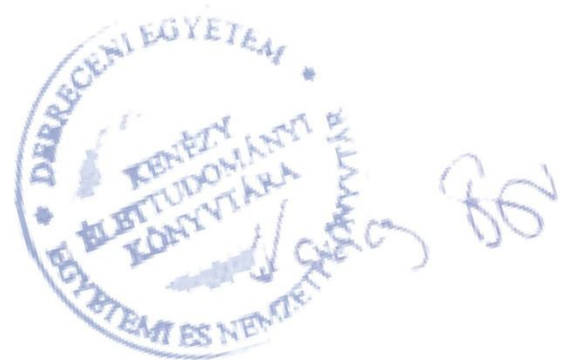
11. Olvasztó S., Litauszky K., Rácz T., Kosztyu L., **Tóth C.**, Antek C.: Fejlődés és szemléletváltás az arteria carotis sebészetében Debrecenben.
Magyar Seb. 54, 105-109, 2001.
12. Garai I., Szomják E., **Tóth C.**, Varga J., Bánk J., Ficzer A., Olvasztó S., Csiki Z., Galuska L.:
Kvantitatív agyi vérátfolyás rezerv vizsgálatok 99mTc-HMPAO SPECT-tel nyaki verőér szűkületben.
Érbetegségek. 8 (3), 75-79, 2001.
13. Litauszky K., Olvasztó S., Rácz T., Kosztyu L., **Tóth C.**: Leriche syndroma és zsigeri arteriális kompresszió együttes kezelési stratégiája..
Érbetegségek. 7 (4), 123-126, 2000.

Total IF of journals (all publications): 4,77

Total IF of journals (publications related to the dissertation): 1,202

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

06 March, 2015



Acknowledgements

First of all, I would like to thank my supervisor, associate professor Norbert Németh, head of the Department of Operative Techniques and Surgical Research, Institute of Surgery, Faculty of Medicine, University of Debrecen, for letting me carry out the research work the thesis is based upon. He was a great help throughout, and his outstanding expertise in the field of haemorheology and his ideas made it possible to finish my thesis and the related publications.

I wish to express my sincere thanks to Dr. Zsolt Lampé, general director and Dr. László Mikó, medical director of the Kenézy Gyula County Hospital, for their help and support.

I am also grateful to Professor László Damjanovich, director of the Institute of Surgery, Faculty of Medicine, University of Debrecen, for his guidance and suggestions.

My special thanks should go to Sándor Olvasztó, consultant surgeon of the Division of Vascular Surgery of the Institute of Surgery, Faculty of Medicine, University of Debrecen, from whom I learnt most what I know of vascular surgery. I can rely on his expertise and assistance for more than twenty years now. His excellent personal and professional qualities are unquestionable.

I express my gratitude to the whole staff of the Hand and Limb Surgery Unit of the Kenézy Gyula County Hospital, the Division of Vascular Surgery and Department of Operative Techniques and Surgical Research of the Institute of Surgery, University of Debrecen for their continuous support.

Last but not least, I am most grateful to my Family for everything I was given!