THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PH.D.)

Compression of the hepatoduodenal ligament affecting the results of hepatic surgery. Experimental and clinical data.

by
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1. INTRODUCTION, REVIEW OF THE LITERATURE

The segmental anatomy of the liver elaborated by Couinaud nearly 50 years ago laid the foundation of modern hepatic surgery. The first reports about segmental resections were published in the 1960s. Surgical treatment may be the only cure in several liver diseases including focal changes, and primary and secondary tumours. Although oncology and intervention radiology have significantly developed over the past 15-20 years, surgery is still the first line choice in treating the liver. Due to the introduction of new technologies and possibilities provided by modern anaesthesiology and intensive therapy, and, simultaneously with the dramatic improvement of the surgical arsenal, liver operations can be performed safely on patients chosen with due circumspection and prepared thoroughly. In combination with significant decrease of risk over the past 30 years, the radical development of hepatosurgery has resulted in surgical mortality rates reduced to approximately 5%.

The introduction of several new devices and **modern surgical techniques** into **liver-saving** surgery has been aimed at minimizing blood loss, decreasing the demand in transfused blood, preserving enough functional liver parenchyma, minimizing the size of parahepatic necrotic zones and providing tumour-free resection edges. (According to the latest literature, it is no longer required to ensure the R_0 safety zone.) The above contribute to a decrease in perioperative morbidity and mortality parameters. The prevention of postoperative bile leak and abscess formation, ensuring accurate drainage and decreasing the duration of the operation are equally important indeed.

It is an established fact that the intermittent compression of the structures of the hepatoduodenal ligament for a certain period of time can contribute to the safety of surgical interventions. At the same time, however, the efficacy of liver operations is greatly influenced by damage associated with ischaemia-reperfusion in close correlation with the duration of hypoxia during surgery. Therefore the length of exclusion may also play a role in the development of complications.

In 1876, Jónás Báron was the first Hungarian surgeon to suggest the temporary atraumatic compression of the hepatoduodenal ligament in order to decrease blood loss, a technique known as **Báron's manoeuvre.** In the international literature, the compression of the hepatoduodenal ligament was later associated with Pringle's activities who published his method known as Pringle's manoeuvre in 1908. At that time, this technique was basically used in acute traumatological treatment. The compression period was suggested to be 10-15 minutes followed by 5-10 minutes' intermittent reperfusion periods.

The development of hepatic surgery has allowed the use of resection techniques in which the hepatic circulation is temporarily switched off applying the Báron/Pringle manoeuvre, but exclusion has also been used in liver transplantation. The intermittent compression of the structures of the hepatoduodenal ligament for a certain period of time ensures safer surgical interventions. Temporary exclusion of the structures at the porta hepatis (vascular exclusion) is done using atraumatic forceps, but even this technique may require that the surgeon dissects the tunica intima. Selective afferent vascular exclusion is an alternative, which means that, in addition to the exclusion of the portal vein, either the right or left hepatic artery is excluded. It should be noted, however, that any condition in which the intra-abdominal pressure rises (external compression, blunt force trauma of the abdomen, pregnancy, laparoscopic surgery, ascites, etc.) can also cause indirect portal compression.

After vascular compression is released, the reperfusion of the ischaemic tissue may, paradoxically, cause further and even greater damage than the one resulting from ischaemia itself, a phenomenon known as **ischaemia-reperfusion** or **I/R damage**.

In hepatic surgery, the exact knowledge of I/R damage and its signs is a prerequisite to improve the safety of such operations, and prevent and treat pathologic conditions of the liver. This organ does not cope with ischaemia easily and our knowledge of the duration of "safe ischaemia" is very poor. The quantity of free radicals generated by ischaemia-reperfusion may exceed the capacity of antioxidant defence mechanisms, leading to a greater damage to biological systems. The free radicals of oxygen alter the behaviour of blood cells, which leads to perfusion disturbances at the level of microcirculation. Being the number one mediator in hepatocellular damage and participating in a potent defence mechanism, nitrogen monoxide (NO), a pluripotent free radical, may play a double role. NO synthetase action, among others, can contribute to controlling portal pressure. Due to its double blood supply by the hepatic artery and portal vein, perfusion is individually controlled in the liver.

Direct and leukocyte-induced damage to the endothelium as well as the release of inflammatory mediators interfere with capillary permeability resulting in exudation in the interstitium and haemoconcentration and stasis in the vessels. Interstitital oedema causes the compression of venules and arterioles, damages their endothelium, further aggravating the damage resulting from ischaemia-reperfusion, and maintains the vicious circle of inflammatory cascade. It can be concluded that there lies a complex pathomechanism in the background of tissue damage and disturbed microcirculation during ischaemia-reperfusion. This pathomechanism affects the perfusion profile of the specific tissue via physical, haemodynamic and microrheological changes, and is dependent on time considering its extent and dynamics.

Several attempts have been made to determine the optimal duration for compressing the hepatoduodenal ligament. According to current information, the aforementioned intermittent compression/release period should not exceed 120 minutes. In comparison, the duration of cumulative ischaemic periods should be less than 70-80 minutes in order to prevent irreversible damage. Beyond keeping loss of blood to the minimum, this manoeuvre is analogous to ischaemic preconditioning which, influencing the release of free radicals, can make hepatosurgical interventions even safer. Several alterations such as changes in local metabolism, bodily fluid (re)distribution and pathophysiology occur not only in local hepatic but also mesenteric, even systemic circulation.

Alterations of the **haemorheological parameters** associated with ischaemia and reperfusion periods are also observed, parallel to changes in temperature. *Whole blood viscosity* is affected collectively by plasma viscosity, haematocrit, erythrocyte aggregation, blood cell deformability, fibrinogen and globulin levels as well as leukocyte and thrombocyte counts exceeding a certain level.

The rheological properties of suspensions greatly depend on the concentration of particles in them. Since the proportion of erythrocytes among the formed elements of the blood is by magnitudes higher than that of the other cells, erythrocyte count is regarded to basically determine haematocrit and the viscosity of the blood. Parameters describing *erythrocyte deformability* (capability to passively change shape) play an important role among haemorheological factors. In addition to affecting the lifespan of cells, adequate deformability is an indispensable condition in microcirculation as erythrocytes should be able to pass along microcapillaries without difficulty. The rheological importance of leukocytes is determined by their larger size, rigidity and activity. Their condition of flow is substantially influenced by the membrane and cytoplasm, the latter exhibiting significant viscosity; besides passive motility, active processes have to be considered, too. *Thrombocytes* in an activated state or in the form of aggregates are capable of mounting great resistance. *Erythrocyte aggregation* describes the transitory and reversible adhesion of red blood corpuscles, affected by plasmatic and cellular factors.

During ischaemia, congested blood in the excluded region, and local, metabolic, physical and pathophysiological processes – at the onset of reperfusion – exert their influence on haemorheological parameters in a complex manner.

The extent and dynamics of these changes, their local and systemic differences depend on several factors, the duration of ischaemia playing a special role among them. Therefore it is of utmost importance to determine the optimal duration and repeatability of vascular compressions and decompressions which are inevitable in such surgical interventions. In our opinion, investigations into the haemorheological parameters may help with determining the optimal duration and repeatability of compressions, therefore animal trials can provide important data for clinicians in their attempt to improve the safety of surgical interventions.

2. GOALS

2.1. ANIMAL TRIALS

Investigation of the Báron/Pringle manoeuvre and damage caused by ischaemia-reperfusion in animal models

1. Elaboration of an animal model to analyse the local and systemic haemorheological effects of the Báron/Pringle manoeuvre (repeated three times) in hepatic surgery.
2. Investigation of samples obtained from the systemic venous circulation for changes in whole blood and plasma viscosity, erythrocyte deformability and erythrocyte aggregation index as well as general changes in coagulation time using an experimental hepatosurgical model.
3. Analysis of the haematological parameters and erythrocyte aggregation index to compare possible local and systemic differences in samples from systemic arteries and veins and the portal and hepatic veins taken prior to and after the individual Báron/Pringle manoeuvres.
4. Investigation of hepatic enzymes to judge hepatic function in samples from systemic circulation.
5. Histological studies supplemented by morphometric investigations of samples taken from the liver, small intestines and pancreas.

2.2. CLINICAL INVESTIGATIONS

Analysis of major operations on the liver at the 2nd Department of Surgery (currently known as the Institute of Surgery), Medical and Health Science Center, University of Debrecen, performed between 1 January 2004 and 31 December 2008

1. General overview of results obtained after surgery due to primary hepatic cancers and metastases.
2. Effects of the Báron/Pringle manoeuvre on intraoperative loss of blood, and morbidity and mortality rates in the intraoperative and early postoperative periods.
3. Introduction of modern principles applied at our department in order to preserve organs and further improve surgical safety.

3. MATERIAL AND METHODS

3.1. ANIMAL TRIALS IN BEAGLE DOG MODEL

Pilot investigations were performed on two mongrel dogs. Next, twelve beagles of both sexes (11.57 ± 2.09 kg) were included and divided into two groups:

Báron/Pringle group: Having prepared and cannulated the left femoral artery and the external jugular vein, we performed median laparotomy to explore the hepatoduodenal ligament. The hepatic portal system was cannulated via a thin mesenteric ramus and, along the inferior vena cava, another cannula was introduced to the level of the opening of the hepatic veins. Next, the Báron/Pringle manoeuvre was performed as follows: using atraumatic forceps, we gently compressed the hepatoduodenal ligament for 3x15 minutes, including intermittent reperfusion periods for 5 minutes each. After the third compression, the last samplings were performed following reperfusion for 30 minutes. During the compressions, we observed the mesenteric region macroscopically in order to assess congestion and took samples for histological investigations.

Control group: Except for median laparotomy and cannulation of vessels similarly to the other group, no other interventions or the Báron/Pringle manoeuvre were performed.

After collecting samples according to schedule and performing the third Báron/Pringle manoeuvre followed by 30 minutes of reperfusion, the dogs in both groups were exterminated in accordance with the European guidelines for the use of laboratory animals.

Comparative laboratory studies

In order to investigate the possible systemic and local differences, blood samples of 0.5 ml were collected (K_3-EDTA, 7.5%, 0.04 ml, BD Vacutainer, Belliver Industrial Estate, UK) in both groups, from the cannulated external jugular vein, femoral artery, hepatic vein and portal system following surgical preparation, and before and after each compression as well as at the end of the 30-minute-reperfusion period, according to the experimental protocol. The samples were used to determine the haematological parameters and erythrocyte aggregation.

Before the first and after the third Báron/Pringle manoeuvre and the subsequent 30-minute reperfusion period, blood samples were collected from the systemic circulation (external jugular vein) for complex haemorheological and coagulation tests. To perform filtrometric investigations into erythrocyte deformability, whole blood and plasma viscosity and erythrocyte aggregation we used vacutainer tubes pretreated with Na-heparin (143 IU, BD Vacutainer, Belliver Industrial Estate, UK) and coagulation tests were carried out in Na-citrate tubes (109 mg/ml, BD Vacutainer, Belliver Industrial Estate, UK). Liver necroenzymes were determined from blood samples collected in plain vacutainer tubes (BD Vacutainer, Belliver Industrial Estate, UK).

Haematological parameters were determined using an automatic Sysmex F-800 Microcell Counter (TOA Medical Electronics Co., Ltd., Japan).

During the **haemorheological investigations**, *whole blood viscosity* (HBV [mPa.s]) and plasma viscosity (PV [mPa.s]) were measured using a Hevimet-40 capillary viscosimeter (Hemorex Kft., Hungary). In order to characterize HBV, we applied values measured at the 90 s^{-1} velocity gradient. The data were processed according to the recommendations by Mátrai et al in 1987, using the mathematical formula below, which was corrected for 40% haematocrit (HBV40%), considering the plasma viscosity and haematocrit (Htc) of the sample:

$$\frac{TVV_{40\%}}{PV} = \left(\frac{TVV_{Htc}}{PV}\right)^{\frac{40\%}{Htc}}$$

Erythrocyte deformability was measured using a Carat FT-1 filtrometer (Carat Kft., Hungary) determining the parameters of erythrocyte suspension passing through the filter. In an attempt to keep to the standards set for such procedures, the measurements were carried out within two hours of sampling, at 22 ± 1°C. The erythrocyte suspension was made to pass through a polycarbonate filter with an average pore diameter of 5 μm (Nuclepore®, Whatman Inc.) at constant (negative) pressure (4 cmH_2O). The initial relative filtration rate (IRFR) and, in the possession of haematocrit values of the suspension, relative cell transfusion time (RCTT) were determined.

Using aggregation indices determined at different gradient rates, a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was applied to describe erythrocyte aggregation capacity. The aggregation index (AI) was calculated from light intensity measured in the state of aggregation and disaggregation, and its increase indicated the extent of aggregation.

Coagulation parameters – prothrombin time (PT) [s], activated partial thromboplastin time (APTT) [s], thrombin time (TT) [s] and fibrinogen concentration (g/l) – were measured in systemic venous blood samples using an automatic Sysmex CA-550 coagulometer (TOA Medical Electronics Corp., Japan).

During the trial, we drew systemic venous blood at intervals described earlier and we checked the changes of liver **necroenzymes** (GOT, GPT, LDH). The enzymes were determined by spectrophotometry (37 °C, 340 nm, using GOT-DCC 020075, GPT-DCC 010075 and LDH- DCC 220300 reagents, Diagon Kft.).

For **morphological investigations**, liver biopsies were performed at the beginning of the operation, at the end of reperfusions (5 minutes each) following the first and second Báron/Pringle manoeuvres, after the last Báron/Pringle manoeuvre and, also, at the end of the successive 30-minute reperfusion period. At the end of the investigations, additional samples were taken from the wall of the small intestines, pancreas and areas draining the portal vein.

In order to interpret statistical data, we designed a multifocal analysis using SigmaStat for Windows software (Jandel Scientific Co., Germany). One Way ANOVA (Dunnett and Bonferroni tests), and Wilcoxon/Paired t-test were applied to follow up the effects of surgery. The level of significance was set at $p<0.05$.

Licensing and financing research: We performed the experiments in accordance with Act XXVIII , 1998, about "Protecting and sparing animals" and governmental decree 1999/253 and in the possession of a license issued by the Committee of Animal Research,

University of Debrecen (11/2003 and 6/2006 DE MÁB). The topic of the dissertation was part of the research project originally submitted by the Department of Operative Techniques and Surgical Research, Medical and Health Science Center, University of Debrecen (ETT 6003/16 [2001-2003] and OTKA K-67779 [2007-2011], in which I have been an active participant.

3.2. CLINICAL INVESTIGATIONS

I have processed the data of operations on the liver performed at the 2nd Department of Surgery (currently known as the Institute of Surgery) at UDMHSC in the five-year period between **1 January 2004 and 31 December 2008**. The data included important information about

- **primary liver cancers** (hepatocellular carcinoma, cholangiocellular carcinoma)
- **non-colorectal liver metastases**
- and **colorectal liver metastases**.

I have paid special attention to the

- effects of the Báron/Pringle manoeuvre on the results of hepatic surgery,
- extent of intraoperative loss of blood,
- enforcement of modern surgical principles in order to preserve the organ, aimed at improving surgical safety,
- use of new surgical techniques at our department (anterior hemihepatectomy, mesohepatectomy).

The **Báron/Pringle manoeuvre** was performed in cases when prolonged intervention, substantial blood loss and relatively great loss of the parenchyma was expected. In our practice, 15-minute compression periods were followed by 5-minute decompressions under strict anaesthesiological surveillance. If necessary, compressions could be repeated. In the vast majority of the cases, resections were performed using a CUSA-knife (Cavitron Ultra Sound Aspirator). The resection surfaces were coagulated using an argon plasma coagulator and the surgical area was drained in each case.

The **evaluation of the surgical results** in primary and secondary hepatic carcinomas was made on the basis of the following parameters:

- distribution of patients according to gender and age;
- surgical technique – number of resected segments,
- tumour size;
- demand in transfused blood (erythrocyte and thrombocyte concentrate);
- possible complications;
- histological evaluation of the resected tumour;
- duration of the mean nursing period;
- and details of perioperative morbidity and mortality.

Statistical data procession was based on discharge reports, medical documentation, surgical protocols and anaesthesiology documents; the findings were compared with the relevant results in the literature. The statistical analysis of these clinical parameters included the use of descriptive statistics, χ^2 and Fisher exact tests, as well as double-t test. We also used a non-parametric statistical hypothesis test, also known as the Mann-Whitney U test. The Shapiro-Wilk test for normality for continuous data was also included among the tests. Survival was determined using the method by Kaplan-Meier. The value of the level of significance was established at $p<0.05$. The analysis was performed using SPSS 16.0 and EpiInfo statistic programs.

4. RESULTS

4.1. RESULTS OF ANIMAL TRIALS

Changes in certain parameters of systemic blood samples

There was an increase in **whole blood viscosity** after the third compression and, compared to the initial level (p=0.028) and control group (p=0.018), it became significant following the 30-minute reperfusion period. In the control animals, decreased haematocrit values were observed. The viscosity of whole blood also decreased in the Báron/Pringle-group, but it exceeded the initial level after 30-minute-reperfusion. In the Báron/Pringle-group, significant decrease was detected in the haematocrit/viscosity ratio compared to the initial value (p=0.048) and control (p=0.043), and it remained at a lower level until the end of 30 minutes' reperfusion (p=0.01) when it was compared to the initial value. Blood viscosity values corrected for 40% haematocrit value showed mild increase in the control group. Compared to the control (p=0.028) as well as the initial value (p=0.026), whole blood viscosity was significantly elevated after the Báron/Pringle manoeuvres.

No significant change in **plasma viscosity** was detected, although the Báron/Pringle-group exhibited elevation at the end of the trial.

The **aggregation index** (parameter M) measured at 0 s^{-1} velocity gradient decreased by approximately 30% after Báron manoeuvres, but by the end of the experiment its values had become normal in both groups. There was an increase of 34% in the **aggregation index** after the Báron/Pringle compressions, while the control group exhibited a decrease of 15%. Compared to the initial level, there was minimal increase in the control group after 30 minutes' reperfusion. In the Báron group, the M1 aggregation index was still higher (by 19.5%) than the initial value.

With regard to erythrocyte deformability, relative cell transit time (RCTT) did not change markedly in the control group, while in the Báron group it had increased significantly by the end of the 30-minute reperfusion period, compared to the initial values (p=0.004). The initial relative filtration rate changed in a reverse correlation with RCTT. There was nothing worth highlighting in this case.

As far as the **coagulation times** were considered, prothrombin time had grown steadily in the Báron group during the whole experimental period; compared to the initial values, the findings were significantly higher after the compressions and at the end of the 30 minutes' reperfusion period (p=0.021 vs p=0.013). Activated partial thromboplastin time was significantly elongated in the Báron group by the end of the 30 minutes' reperfusion period and was significantly higher than the initial value or that of the control group (p=0.007 and p=0.014, respectively).

Routine liver function tests: After the Báron/Pringle manoeuvres, there was an increase in all of the three enzyme activities we tested, GPT values having shown the greatest increase compared to the control group. In comparison, the ratio of the Báron group versus control was as follows: GOT=1.77, GPT=3.15 and LDH=1.61. Further increase in the samples' enzyme activity was detected at the end of the 30-minute-reperfusion period following the Báron/Pringle manoeuvres. It was especially expressed in GOT and LDH values (the Báron/control group ratio was found to be as follows: GOT=7.62, GPT=2.55 and LDH=8.57.) Compared with the initial levels, the above values represented 17.58-fold, 12.48-fold and 14.39-fold increase for GOT, GPT and LDH, respectively.

Changes in individual parameters in local and systemic blood samples

Leukocyte counts appeared lower in the portal samples after Báron manoeuvres. The first Báron/Pringle manoeuvre was followed by slight decrease in leukocytes in the portal blood sample and the second one was significantly lower compared to the initial value ($p=0.007$). After the third manoeuvre, the leukocyte count was significantly lower ($p<0.001$) than the initial value ($p=0.002$) or the values of the hepatic venous samples.

Hematocrit values (Hct [%]) of Control Group did not show marked changes except for a diminished increase (within 5%) by the end of experiment. Otherwise, after the 2nd and 3rd Pringle (Baron) maneuver significantly elevated hematocrit values were found in portal venous blood (from a base of 45.85±3.73 % to 56.42±9.09 %, $p=0.006$; and to 57.21±7.95 %, $p=0.004$; respectively).

Aggregation index M (shear rate: 0 s^{-1} at 5 sec) of Control Group showed decreasing tendency in each type. In the terminating phase of the experiment the decrease was marked in arterial samples, and it was found significant compared to base values (Control Group: 6.25±0.49 vs. 4.56±1.21, $p=0.015$; Pringle Group: 6.7±0.47 vs. 4.25±1.28, $p<0.001$). In Pringle (Baron) Group after the 1st maneuver aggregation index M declined significantly in hepatic venous blood (5.9±0.49 base vs 3.88±1.32, $p=0.001$), and it started to increase by the end of 5-minute interpolated reperfusion phase. After the 2nd Pringle maneuver lower aggregation indexes were found in arterial, systemic venous and hepatic venous samples, while in portal venous blood aggregation index increased (6.28±1.89). By the end of the interpolated reperfusion phase these values moved toward the base values, however arterial and systemic venous values were significantly lower. After the 3rd Pringle maneuver aggregation index of portal blood raised again (5.66±2.27). By the end of the experiment each blood sample type showed almost the same values.

Aggregation index M1 (shear rate: 3 s^{-1} at 5 sec) expressed more apparent alterations. In Control Group a slight elevation and a following decrease were observed. The lowest values could be found in arterial samples in the last two sampling of the experiment (5.07±0.4 and 5.62±0.85 vs. base 7.01±1.19, $p<0.05$). After the 1st Pringle maneuver aggregation index increased in each blood sample types, which elevation was significant in systemic venous and hepatic venous blood compared to their initial values (V: 5.91±0.35 base vs 9.01±0.9, $p<0.001$; and H: 6.18±1.86 base vs. 8.01±2.2, $p=0.013$; respectively). Also the increase in aggregation index was significant in systemic venous blood compared to the parallel control (7.96±0.83, $p=0.018$). By the end of the interpolated 5-minute reperfusion aggregation index slightly decreased in systemic venous blood, while parallel arterial samples showed further elevation of aggregation index, significantly compared to its base (8.42±0.89 vs. 6.2±2.06, $p=0.006$). After the 3rd Pringle maneuver arterial values were near to the base, while systemic venous, portal and hepatic venous blood showed increased aggregation index: apparently and significantly in systemic venous (8.71±1.74) and portal venous (7.52±1.59) samples, comparing to their base and parallel control values. By the end of the 30-minute reperfusion period the previously elevated values started to decrease

Conducting the **morphological investigations** we found several necrotic cells, apoptosis, expressed lymphocytic infiltration, congestion and vacuolated hepatic cells. Centrilobular disorganization of the hepatic trabecula could also be detected. The small intestines exhibited mild congestion, the apices of some villi showed signs of the formation of subepithelial space and focal denudation, and explicit destruction and exulceration of the villi in some regions were also noticed.

4.2. RESULTS OF CLINICAL INVESTIGATIONS

4.2.1. Evaluation of the results of surgical interventions for primary liver cancer

In the five-year period of this study, major liver operations were performed in **21 cases**. The mean age of the ten females (47.6%) and eleven males (52.4%) was 64.7±10.9 years at the time of the operation. Histological findings justified cholangiocellular carcinoma (CCC), hepatocellular carcinoma (HCC) and combined HCC-CCC in five cases (23.8%), fifteen cases (71.4%) and one case (4.8%), respectively. The size of the primary hepatic tumours was 8.9±3.3 cm on average, in the range between 2 cm and 15 cm. Tumour sizes were also analyzed according to the type of the tumour; the mean values were as follows: cholangiocellular carcinoma 9.2 ± 1.7 cm (7-11cm), hepatocellular carcinoma 9.2 ± 3.7 cm (2-15 cm) and combined type 5 cm. In the patients who did not undergo the Báron/Pringle manoeuvre, the size of the excised tumour was 8.7 ± 3.4 cm. If the resection was supplemented with the Báron/Pringle manoeuvre, the mean tumour size was found to be 9.6± 3.2 cm. Therefore, there was no significant difference between the two groups in this respect (p=0.96). Analyzing tumour sizes according to the gender of the patients we could see that "male" tumours were 10.2 ± 3.2 cm whereas the "female" ones were 7.5 ± 2.98 cm in size on average. The differences between the two groups were noticeable but not significant (p=0.18).

According to the type of operations: in 1-1 cases we performed left or right hemihepatectomy, in 12 cases two or more segmental resections were done. In 7 cases atypical resection could be done. **Báron/Pringle manoeuvre** in 5 cases were used (35,7%) to minimize intraoperative blood-loss, in those cases where greater resection was planned. Using the manoeuvre 2-2 hemihepatectomies and one mesohepatectomy and 1 trisegmentectomy were done. The overall exclusion time was 21 minutes.

Among 21 patients 9 needed transfusion (42,9%). These patients received average 2,5±1,1 units. In 12 cases (57,1%) we could carry out transfusionless operations, 3 of them underwent Báron/Pringle manouvre. The average blood need were 0,8 unit of this group, while the other group blood consumption was 2,1 units, the difference was not significant (p=0,64). There were 2 (9,52%) major and 5 (23%) minor complications, but none of them from the Báron group.

The average nursing time was 13,5±7,3 days, the shortest was 3 in a case of laparoscopic operation, the longest was 34 days.

4.2.2. Evaluation of the results of operations performed for non-colorectal hepatic metastases

Fifteen patients had to undergo operation for non-colorectal hepatic metastases in the aforementioned period.

The **site of the primary tumour** and the number of affected patients were as follows:

- neuroendocrine tumour	3 patients
- breast cancer	4 patients
- gastric cancer	1 patient
- tumours of the reproductive organs	3 patients
- renal cancer	1 patient
- pulmonary cancer	1 patient
- melanoma	1 patient
- pancreatic carcinoma	1 patient

3 patients were operated for *neuroendicrine* originated liver metastases. In 1-1 cases the primary tumour was located in the adrenal gland, in the lung and 1 was unknown origin.

In 4 cases the liver resection were done for late metastases of breast cancer. In 3 cases the breast cancer were located in the right side. By my previous study we revealed 17 breast cancer patients' data for hepatosurgery of metastases in an earlier period. In one patient 2 liver metastasectomies were performed for gastric cancer origin, between the hepatosurgeries 12 months passed. For genital originated liver metastsases 3 patients underwent operations. In

1-1 cases the primary tumor was located in the kidney (clarocellular cc), in the lung (planocellular cc), and in the pancreas and 1 was originated from malignant melanoma.

10 female (66%) and 6 male (33%) were the subjects, **average age** 59±11,7 years). The **tumour size** of noncolorectal liver metastases was 6,5±4,1 (1-15) cms.

According to the operative techniques in 1 case the adrenalectomy and the liver metastasectomy were done in one stage by laparoscope. In 2 cases mesohepatectomies, in 1 case trisegmentectomy and in the other 12 cases simple metastasectomies were performed. In addition 7 patient underwent **Báron/Pringle manoeuvre** of the 15.

8 of the 16 subjects needed blood **transfusion**, 2 of them had Báron/Pringle manoeuvre. The blood consumption was tendently less but not significant (p=0,15).

The **complication rate** was less (p=0,4) but not significant.

The **average nursing period** was near the same in the 2 groups (10±2,7 and 10±3 days, respectively, p=0,94).

4.2.3. Evaluation of the results of operations performed for colorectal hepatic metastases

75 patients underwent liver resections for colorectal metastases in the above mentioned period. The disease free interval was 25,2 (4-168) months. Accorging to gender the **interval period** was significantly longer in male (31,6 and 18,3 months, respectively p=0,011). There were 40 male and 35 female patients with the **average age** 58,1±7,2 years. The average **tumour size** was 6,6±3,2 cms, in the Báron/Pringle manoeuvre group it was 7,9±3,7 vs. control group 5,6±2,5 cms.

Preoperatively in 11 cases radiofrequentic ablation were performed but because of early recurrence we decided by resection. In 11 cases the colorectal tumour resections and the liver metastasectomies were done in one stage. In the other 64 cases the diseases free interval was 25,3 months. In 9 cases two times repeated metastasectomies were performed. In all cases the operations seemed to be curable.

In 29 (38,6%) cases **Báron/Pringle manoeuvre** were used, 9 of them required **transfusion**. In the control group 26 patients received transfusion. The difference was significant between the two groups (p=0,0026). The need of transfusion was also significantly less (0,6 and 1,8 units, respectively, p= 0,0053).

In 1 cases severe *intraoperative* **complication** happened, ie. The tumourous caval vein was ruptured and haemorrhagic shock developed. In 78,7 % of the cases early postoperative complication were not found. In 1 case subphrenic abscess developed which healed for CT-guided drainage. Haematoma and wound healing problems occurred in 1-1 cases. Fever were in 10,3% and 25,3%, respectively, p=0,31). The complication rate was tendentiously less in Báron/Pringle group.

The **average hospitalization time** was nearly the same with no significant difference (10,1±4,7 and 10±4,7 days, p=0,53).

5. DISCUSSION

5.1. DISCUSSION OF EXPERIMENTAL RESULTS

Due to the effects of the Báron/Pringle manoeuvre repeated three times, local white blood counts, haematocrit and erythrocyte aggregation indices show different changes depending on the number of repetition, especially in the hepatic portal system.

During ischaemia-reperfusion, leukocytes may adhere to the endothelium as a result of chemotaxis and the endothelium-leukocyte interaction triggered by inflammatory processes, which can partly explain the lower values measured in portal samples, since significant congestion can be observed in the mesenteric venous system during the Báron/Pringle manoeuvre. The increase in local haematocrit also supports the above finding.

The rheological properties of the blood in the excluded region show major changes during ischaemia. Decreased pH, accumulation of anaerobic metabolites, changes in local fluid distribution, haemoconcentration, changes in the osmotic environment all contribute to the worsening of erythrocyte deformability and may increase their aggregation capacity.

Systemic effects may manifest themselves in a relative increase in the blood's viscosity and the deterioration of erythrocyte deformability, as it has been observed in samples from the external jugular vein. Prothrombin time and activated partial thromboplastin times have also exhibited a tendency of decline by the end of the experimental period. No such changes have been found in the control group. In clinical practice it means that prolonged exclusion increases the risk of bleeding complications.

In the experimental animal models, compression on three occasions proved to be long (Subsequent multiple samplings may also have affected the results). These findings show how important the time factor is in applying this technique and, also, in pathologies associated with compression.

The most dramatic changes have emerged in the portal system, basically due to stasis. This, however, may be suggestive of a subsequent functional lesion of the organs draining the portal vein. Prolonged compression may lead to multi-organic failure and disseminated intravascular coagulation which affects mortality parameters. Being aware of the changes of those parameters may also contribute to the proper choice of surgical techniques.

5.2. DISCUSSION OF CLINICAL RESULTS

In our practice, the **Báron/Pringle manoeuvre** was applied in cases in which prolonged operations and substantial loss of blood were expected, the latter being quite common during operations involving relatively significant loss of the parenchyma.

The application of the Báron/Prinlge manoeuvre in performing the resection of **primary hepatic tumours** reduced the demand in transfused blood and duration of hospitalization. A significantly lower rate of early postoperative complications was noted in the group "using" the Báron/Prinlge manoeuvre.

In the resection of hepatic metastases from **non-colorectal tumours**, the use of the Báron/Pringle manoeuvre decreased the mean demand in transfused blood. Although the incidence of early postoperative complications was lower, the duration of hospitalization was similar in this group too.

In the resection of hepatic metastases from **colorectal tumours**, the use of the Báron/Pringle manoeuvre significantly decreased the mean demand in transfused blood. The size of the resected tumour was also significantly smaller and the incidence of early postoperative complications was lower. It is interesting to remark that there was a significant shift toward men regarding the elapsed time between the removal of colon tumours and hepatic interventions.

As neovascularisation does not always keep abreast with the growth of the tumour (development of central necroses), the tumour itself is much more sensitive to hypoxia, i.e. ischaemic episodes may interfere with the viability of possible micrometastases. The application of adequate compression techniques may result in fewer relapses, which is sometimes shown by the length of survival. The protocol, however, should always be individualized since a dramatic drop in blood pressure can be expected due to inhibited venous reflux which may precipitating cardiac depression. Hepatic enzymes become normal quickly with subsequent fast recovery.

The results suggests that this technique is effective to apply even in the treatment of hepatic malignancies; its protective effects manifest themselves in lower complication rates, decreased demand in transfused blood and shorter periods of hospital care.

It is the surgical team's joint responsibility to find an ideal solution to reduce the loss of blood. The Báron/Pringle manoeuvre can become one of such solutions with due consideration to the limitations posed by the duration of compression and number of repetitions.

Harmonically functioning oncoteams and theatre staff selecting patients and operating on them with great circumspection and, of course, adequate surgical treatment all contribute to the patient's recovery or at least the improvement in his quality of life even in severe cases when patients are treated for hepatic metastases.

6. SUMMARY OF EXPERIMENTAL RESULTS AND CLINICAL CONCLUSIONS

1. We designed a new *hepatosurgical animal model* using beagle dogs, which, in addition to ensuring the atraumatic compression of the hepatorenal ligament, involved the cannulation of the hepatic portal system and the hepatic vein via the mesenteric vein and vena cava inferior, respectively, and allowed us to analyze the systemic and local haemorheological effects of the Báron/Prinlge manoeuvre.

2. We were the first to conclude that the *application of three Báron/Pringle manoeuvres* (i.e. compression of the hepatoduodenal ligament for fifteen minutes three times including five-minute reperfusion periods between the compressions) *was too long to perform* in an animal model because

- Coagulation time parameters had become worse in the systemic circulation by the end of the third compression. Applied in clinical practice, a long-term compression may increase the risk of bleeding complications.

- As a result of haemostasis, the most significant changes occurred locally, mainly in the portal system. It can also indicate subsequent lesion in the organs draining the hepatic portal vein. Prolonged compression may even lead to multi-organ failure (MOF) and disseminated intravascular coagulation (DIC), reflected by mortality parameters. These signs are of importance in clinical practice; they show how influential time parameters are in the Báron/Pringle manoeuvre and pathologies caused by various types of compression (e.g. trauma, pregnancy).

3. Experimental trials proved the advantages of the Báron/Pringle manoeuvre (if they were adapted to the actual period of time). Advantages included

- reduction in bleeding resulting in decreased demand in blood transfusions

- reduction in the number of complications,

- decrease in the mean duration of hospitalization.

4. By *introducing new haemorheological measuring techniques, never used before in hepatosurgery*, we confirmed the standpoint in the literature according to which the Báron/Pringle manoeuvre is still a choice in expansive liver resections in modern surgery since the use of this manoeuvre once or twice has a preventive effect.

5. We have come to a new conclusion – which was supported experimentally – that long-term rheological, haemostaseological and morphological changes (e.g. Báron/Pringle manoeuvre used three times) result in an increased risk for early complications (also suggested by clinical trials) although statistic tests did not confirm significant differences.

6. In the Institute of Surgery at UDMHSC, we have managed to introduce new hepatosurgical techniques into our daily routine soon after they were published in the literature. Since then, anterior hemihepatectomy and mesohepatectomy have been applied routinely in interventions affecting the liver. The use of CUSA liver dissector, ultrasonographic cutting device (Ultracision) and Ligasure is part of the daily routine, inspired by an urge to make hepatic surgery safer, with due consideration to the modern principles of organ preservation.

7. Based on the results, we could confirm modern literature's standpoint that in several liver diseases such as focal changes, primary and secondary tumours – *despite remarkable development in oncology and intervention radiology – surgical treatment is the only option to cure the patient*. Surgery still has an important role in treatment.

Applying the correct approach and adequate knowledge, oncoteams should select patients who can really profit from the surgical intervention.

7. PUBLICATIONS

7.1. In extenso publications associated with the dissertation

1. **Furka A**., Halász L., Szentkereszty Zs., Pósán J., András Cs., Sápy P.: Surgical treatment of liver metastases from breast cancer.
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   (Impact factor: 0.608)

2. **Furka A**., Németh N., Gulyás A., Bráth E., Pető K., Takács E.I., Furka I., Sápy P., Mikó I.: Hemorheological changes caused by intermittent Pringle (Baron) manoeuvre in experimental beagle canine model.
   Clin. Hemorheol. Microcirc. 2008. 40: 177-189.
   (Impact factor: 1.814)

Total impact factor: 2.422

7.2. Other in extenso publications associated with the topic of the dissertation

3. **Furka A.,** Záborszky Z., Ács G., Vigh G., Nagy A., Roman E.: Causes of death of patients with multiple injuries in Debrecen, Hungary.
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Total impact factor: 4.554

7.3. Other publications

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List of publications related to the dissertation

1. **Furka, A.**, Halász, L., Szentkereszty, Z., Pósán, J., András, C., Sápy, P.: Surgical treatment of liver metastases from breast cancer.
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