Change of attitude in the treatment of colorectal liver metastases, is R1 resection no longer a survival disadvantage? The possible role of “spray” diathermy

Doctoral (Ph.D.) thesis

András Palkovics MD

Clinical Medical Sciences (D94)

Surgery and its border fields B-1/2008

Head of the Doctoral School: Lajos Bogár MD, PhD, DSc

Program leader: András Vereczkei MD, Phd

Tutor: András Papp MD, Phd and András Vereczkei MD, Phd

University of Pécs

OGYDHT

Pécs

2020

1. Introduction

According to the latest WHO data, the worldwide incidence of colorectal cancer (CRC) is over 1.4 million and it is the third most frequently occurring cancer type and the third most common cause of cancer related deaths in Europe.

More than 50 percent of patients suffering from CRC develop liver metastases (CRLM). In 25 percent of the patients, synchronous hepatic metastases are present at the time of CRC diagnosis. On the other hand, about 40-50 percent of the patients develop metachronous liver metastases after resection of the primary CRC.

According to epidemiological data a new or recrudescent hepatic metastasis is likely to develop in 50-75 percent of the patients even after an acceptable liver resection. However, only one-fifth of these metastases are resecable. In patients with untreated colorectal liver metastases the estimated median survival reaches only 5-8 months. Currently, the most effective therapies for patients with CRLM are: multi-modal chemotherapy, multiple ablation modalities and hepatic resection.

Regarding surgical success, the current opinion is that patients with macroscopically-positive surgical margin (R2) have worse survival than patients with microscopically positive surgical margin (R1) and microscopically-negative surgical margin (R0). However, the results of the studies investigating the effect of R1 and R0 on survival are controversial.

Recently, the surgical margin and liver parenchyma-sparing technique has become an issue among hepatic surgeons. In the case of CRLM the consensus – since the 1980s – was that the optimal surgical margin is more than 1 cm. In contrast, in the past two decades, several publications revealed that a subcentimeter free resection margin has no significant effect on survival. In 2008, Haas et al. publicated that in the era of modern customized multimodal therapy and modern surgical devices, R1 resection has no negative effect on the survival of patients with CRLM.

Due to improvement in surgical and oncological therapies 5-year survival rate for CLRM patients is increasing, thus the need for repeated surgery becomes higher and higher. On the other hand, in numerous cases, due to the chemotherapy-induced liver parenchyma injury and the

effects of the currently used surgical techniques, patients do not have enough liver volume to provide sufficient liver function. Thus, a repeated resection is frequently contraindicated. These ideas form the basis for the reduction of the surgical margin and parenchymal-sparing non-anatomical resection.

According to a systematic review investigating 2,500 patients with CRLM, the safety and efficacy profile of parenchymalsparing surgery are comparable to those of anatomical resection with an acceptable oncological outcome. The resection margin status is defined by the resected specimen, but R1 does not necessarily mean that the surface of the remnant organ itself is microscopically positive because there is a distance between the surface of the resected specimen and the resection surface of the remnant organ. This distance is determined by the surgical technique used.

The “Kelly-clamp crashing technique” is a gold-standard liver transection technique to expose and isolate small vessels and the biliary duct, which can be ligated or cut with bipolar electrocautery or modern vessel-sealing devices. During the “Kelly-clamp crashing technique”, the parenchyma is transected with a mosquito clamp and the

surgeon is capable of crushing 2-4 mm parenchyma in the resection line.

Nowadays, this technique can also be executed with sealing devices [e.g. Harmonic Scalpel (Ethicon Endo-Surgery, Cincinnati, OH, USA) or The Ligasure Vessel Sealing System (Covidien, Mansfield, MA, USA)]. After using one of these tools, 2-4 mm of the parenchyma is destroyed on the resection line too.

During the resection, the vascular inflow (Pringle maneuver, extra-hepatic selective clamping and intraglissonian clamping) and/or outflow (hepatic vascular exclusion, total vascular exclusion), can be blocked to control the bleeding.

Finally, the resected surface can be coagulated with a monopolar electrocautery device in “spray” mode to prevent bleeding. This procedure can increase the distance between the two surfaces on the resection line, providing a higher tumor clearance. According to an ex vivo animal investigation performed on a sheep liver, spray diathermia causes 3-4 mm deep tissue destruction.

2. Aims

2.1. Survival of patients with colorectal liver metastases

The aim of our follow-up study was to investigate the prognostic value of the surgical margin and parenchymasparing on survival in patients who underwent resection of hepatic CRLM between January 2005 and December 2014, at the Surgery Clinic of the University of Pécs, Pécs, Hungary.

2.2. Effect of “spray” diathermy on the resection surface

The aim of our study was to set up a surviving animal model to demonstrate and investigate the effect of monopolar electrocautery in “spray” mode, which is routinely used at our Clinic.

3. Material and methods

3.1. Survival of patients with colorectal liver metastases

The present retrospective analytical study was based on the data of patients with colorectal liver metastases who underwent hepatic surgery between January 2005 and December 2014. All surgeries were performed at the Surgery Clinic of the University of Pécs, Pécs, Hungary. Data collection was performed by using the hospital's medical database. No patients' rights were violated while acquiring data. Patients with non-CRC metastases, and patients with any other malignancies were excluded.

The final database contained the following parameters: age, gender, T and N stadium of the primary tumor, type of liver resection, time of diagnosis of liver metastases, resection of liver metastases, diameter of the largest resected liver metastasis and the time of death.

All patients have been curatively treated for the primary colon neoplasm and did not have any surgical/oncological treatment for extrahepatic metastases.

The resection types were: classic lobectomy or parenchyma sparing segmentectomy or non-anatomical

metastasectomy. To perform liver parenchymal transection,

“Kelly clamp-crush technique”, ultrasonic dissector and electrosurgical pencil were used. In the few cases of major bleeding, intermitted Pringle maneuver was used to reduce hemorrhage. The cutting surface was coagulated with electrosurgical pencil in “spray mode” to prevent postoperative bleeding in all cases.

All resected specimens underwent histopathological examination. During this, the number, the size, the histological type of metastases, and the resection margin were documented.

Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM Corporation, Armonk, NY, USA). Kaplan–Meier method, log rank test, ANOVA regression test and Cox multiple regression model were used for comparing survival to possible prognostic factors. All p-values lower than 0.05 were considered as statistically significant.

3.2. Effect of “spray” diathermy on the resection surface

Eighteen male Wistar rats (Charles River Breeding Laboratories, Isaszeg, Hungary) weighing between 510 and 690 g were used in our study. The present study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the local Institutional Committee on Animal Research of the University of Pécs (BA02/2000-29/2001).

The animals were anesthetized and through a median laparotomy, the right and left medial lobes of the liver were isolated. Following standardized resections, the left medial lobe was coagulated without Pringle maneuver in all the animals, whereas the right medial lobe was coagulated with Pringle maneuver (the blockage of the hepatic artery and portal vein). During the coagulation, the resection surface was coagulated with monopolar electrocautery in “spray” mode with 120 W energy settings.

The rats were divided into three groups with six rats in each group. In the first group, liver resection surface

samples were collected immediately after the operation, in the second group they were collected 1 week after the operation, and in the third group, samples were collected 3 weeks after the operation.

The aim of the histological examination was to detect histological changes induced by the thermal effect and to compare these changes between the groups. Ten histological slices were created from every model.

To evaluate the histological slices, Pannoramic Viewer software was used and a magnification of 200× was applied to measured the thickness of the coagulation, the necrotic and the different fibrotic zones.

Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM, Armonk, NY, USA). Differences during the follow up were investigated by paired-sample t-test. Independent sample t-test was used to compare parameters between groups. All differences with p-values lower than 0.05 were considered statistically significant.

4. Result

4.1. Survival of patients with colorectal liver metastases

Between January 2005 and December 2014, 528 patients underwent hepatic surgery for secondary liver cancer at our Regional Surgery Center. A total of 209 patients were excluded, because of the following: primary cancer was not CRC, synchronous extra-hepatic metastases were present and onset of other type of previous malignancies. Finally, we analyzed the data of 319 patients [123 (36.6%) female and 196 (61.4%) male] who had colorectal cancer and underwent a surgery to treat colorectal liver metastases. Fifty-three (16.7%) of these patients underwent a second liver surgery and eight (2.5%) patients had a third liver surgery. The median follow-up was 47 months.

The mean overall survival from the first liver surgery was 51.5 months, with a median of 39 months. The 1-, 3-, and 5-year overall survival rates from the date of hepatic surgery were 85.9%, 51.2%, and 29%, respectively.

The mean age of the patients at the time of the operation of the primary tumor was 60±0.67 years (range=27-84

years) and 62±0.57 years (range=28-85 years) at the time of the liver resection.

Mean time between the primary tumor resection and the liver operation was 18.63±0.97 months (range=1-104 months). The origin of the primary tumor was the right side of the colon in 72 cases (22.6%). The left side of the colon was found to be the origin in 114 cases (35.7%). In the remaining 133 cases (41.7%) the rectum was identified as the origin of the primary tumor.

Overall, 167 patients (52.4%) were over 60 years at the time of diagnosis of the primary tumor. A total of 106 patients (33.2%) had synchronous and 213 patients (66.8%) had metachronous liver metastases.

Overall, 228 patients (71.5%) received neoadjuvant chemotherapy before the first liver surgery. 96 (30.1%) of these patients received biological therapy and 152 (64.7%) patients received adjuvant chemotherapy after the first liver surgery, including the 51 (16%) patients who received biological therapy.

The most commonly used resection type was the nonanatomic resection (43%) and during this surgery sometimes 5-6 metastases were removed. One segment resection was performed in 19.7% of the cases and two or

more segment resections in 27.1 percent. In the remaining 10.2 percent of the cases hepatolobectomy was performed. No significant differences were found in survival between the gender (p=0.667), age (p=0.241), location of the primary tumor (p=0.566), time of diagnosis of liver metastases (p=0.298) and type of resection (p=0.257). Patients with unresectable CRC liver metastases had significantly worse (p<0.001) survival. Patients with a large tumor number and size of resected liver metastases had significantly shorter (p=0.016) survival.

In the case of 207 (70.2%) patients, the first liver resection margin was microscopically negative (R0), 54 (18.3%) patients' resection was microscopically positive (R1) while the remaining 34 (11.5%) patients' surgery showed macroscopically positive (R2) results. There was no significant difference (p=0.490) in survival between the R0 and R1 groups regarding the interval between hepatic resection and the date of death. Comparing R0 to R1, the 1-year survival was 90.5% vs. 87.9%, the 2- years survival was 58.4% vs. 56.4% and the 5-years survival was 35.8% vs. 23.2%. However, comparing the survival of R1 to R2 (p=0.037) and R0 to R2 groups (p=0.002) significant differences were found.

According to the multivariate analysis there were no significant differences (p=0.473) between the R0 and R1 resection surface in survival. On the other hand, R0 and R2 (p=0.031) and R1 and R2 (p=0.042) resections showed significant differences in survival.

4.2. Effect of "spray" diathermy on the resection surface

Group 1 – day zero. As a direct thermal effect, a coagulation zone appeared on the liver resection surface. In the coagulation zone, the whole liver tissue had been destroyed. The mean depth of these zones was significantly ($p<0.001$) higher in the Pringle right lobe compared to that of the non-Pringle left lobe.

Group 2 – 1 week after resection. In group two, 1 week after the operation, three different zones were found in both non-Pringle and Pringle cases. The coagulation zone in which the whole liver tissue was destroyed was the first zone. This zone was significantly ($p<0.001$) wider in Pringle cases than in non-Pringle cases. Moreover, the coagulation zone was significantly ($p<0.001$) wider 1 week after the operation compared to measurements

performed immediately after the operation for both forms of resection.

The second zone was a necrotic zone. In this zone, uncompleted coagulatory necrosis was found. The physiological microscopic structure was recognizable, but the contours gradually became blurred. This zone was significantly ($p=0.034$) wider in Pringle cases than in non-Pringle cases.

The third zone identified was a reparative, fibrotic zone. In this zone, there were newly developed tissue elements, hypercellular connective tissue, and granulation tissue. This fibrotic zone was also significantly ($p=0.003$) wider in Pringle cases than in non-Pringle cases.

Finally, considering the three zones, in the case of the Pringle maneuver, the total damage induced by the thermal effect was significantly ($p<0.001$) thicker compared to that in the non-Pringle cases. Under these three zones, normal structured, living liver tissue was identified.

Group 3 – 3 weeks after resection. Three weeks after the operation, three different zones were identified. However, these zones were different from the zones described in the previous two groups.

The first zone was a young fibrotic a hypercellular zone with low collagen content. This zone was significantly ($p<0.001$) wider in Pringle cases than in non-Pringle cases. The second zone was a necrotic zone which was thickly encircled by the young granulation tissue. Deep in this zone, necrotic areas with disintegrated liver structures were seen. This zone was significantly ($p<0.001$) wider in Pringle cases than in non-Pringle cases. However, these zones were significantly ($p<0.001$) narrower after 3 weeks than after 1 week.

The third zone was an old fibrotic zone, a hypercellular zone with high collagen content with newly formed bile ducts, vessels, and mild chronic inflammatory infiltration. This was significantly ($p=0.034$) wider in non-Pringle cases than in Pringle cases. After 3 weeks, these zones were significantly ($p<0.001$) wider than those after 1 week in both non-Pringle and Pringle cases.

5. Discussion

According to previous outcome studies, liver resection is the best treatment option for patients suffering from CRC with hepatic metastases. More than 50 percent of these patients suffering from CRC develop liver metastase and approximately 50-75 percent of these patients develop new or recurrent CRLM after curative liver resection. Only one-fifth of these metastases can be treated surgically. Several studies were performed to investigate the optimal surgical margin of hepatic resection.

In 1986 Ekberg et al. suggested that the optical surgical margin should be more than 1 cm to achieve significantly higher survival. Twelve years later Elias et al. found that the sub-centimeter resection distance is significantly sufficient. In the early 2000s both Kokudo et al. and Pawlik et al. found that even 2-5 mm and 1-4 mm is enough to improve survival. In 2008 Haas et al. were one of the first to publish a follow-up study including nearly 500 CRLM resected patients who did not show significant 5-year disease-free survival differences between R1 and R0 resected CRLM patients.

In line with Haas et al., this study showed that patients who underwent R1 liver resection did not have significantly worse 5-year survival compared to patients who had R0 resection.

This can be explained by the following factors:

1. Multimodal neoadjuvant chemotherapy and adjuvant chemotherapy after hepatic metastasectomy: It has been shown that preoperative chemotherapy does not only convert the initially unresectable disease to resectable (downstaging-downsizing), but is also used to improve the complete resection rate, and increase disease-free survival for resectable CRC. There is clear evidence that the adjuvant therapy after hepatic-metastasectomy is capable of reducing cancer recurrence and increasing survival.

2. "Kelly-clamp crashing technique": with clamp transection of the liver parenchyma (with a mosquito clamp) the surgeon is capable of crushing 2-4 mm parenchyma in the resection line.

3. Ultrasonic dissectors and other modern parenchyma dissectors (such as harmonic scalpels, or CUSA) which are nowadays used, "dissolves" hepatic tissue on the resection surface as well.

4. We routinely coagulated the resection surface in "spray

mode" with maximum energy to prevent bleeding.

After a comprehensive search, we were able to find only one article which investigated the effect of spray diathermy on the liver resection surface. In 2005, Gananadha et al. found spray diathermy caused 3 to 4 mm deep liver tissue destruction in an ex vivo model.

The main result of our study is that we also found spray diathermy can cause 3 to 4 mm deep liver tissue destruction in an in vivo model and this destruction was significantly deeper in the case of resection under Pringle maneuver. As an immediate effect, one coagulation zone appeared on the liver resection surface due to the thermal effect. In the chronic model, after a few days, a wide necrotic zone developed below the coagulation zone, which was significantly deeper when the Pringle maneuver was performed.

As a result of the Pringle maneuver, the intermittent and reversible blockage of the portal vein and hepatic artery reduces bleeding during parenchymal transection. The Pringle maneuver has another positive effect, as it reduces the so called 'heat-sink' effect. The pathophysiological background of this cooling is perfusion-mediated cooling provided by local blood vessels. This is the same effect as

that which reduces the effectivity of thermal ablation of liver tumors. If the heat-sink effect is blocked, spray diathermy can increase tumor clearance during parenchymalsparing liver resection.

On the other hand, it must be noted that approximately 50-75% of patients with CRLM develop new or recurrent metastases after curative liver resection but only one-fifth of these metastases are suitable for further resection, often because there would not be enough liver parenchyma left after the resection.

Although liver tissue can regenerate, functional regeneration is not as extensive as volume regeneration. Factors that can attenuate the regeneration are surgical time, intraoperative blood loss, blood flow blocking time, the patient's own pathological status, and finally, one of the most important factors is chemotherapy.

The expected survival of patients with CRLM is increasing, so too is the chance of having a second surgery. Consequently, parenchymal-sparing techniques are becoming more and more important. Many publications concluded that parenchymal sparing liver resection is not an oncological compromise, and while there may be no

difference in 5-year overall survival, it may give the opportunity for repeated resection.

Based on the previous findings and our investigation, using spray diathermy for resection surface coagulation can increase the oncologically acceptable tumor clearance during parenchymal-sparing liver resection. In agreement with these findings, in our investigation/follow-up study the type of resection (non-anatomic liver resections, segment resection, or lobectomy) had no significant effect on the 5-year survival.

In short, the present study showed that in the era of modern chemotherapeutics and developed surgical devices the 5-year survival is similar for a patient with R0 and R1 resection, and there seems to be no significant difference between anatomic and non-anatomic liver resection.

6. Novel findings

- In the surviving animal model, that we used, the thermal damage caused by the “spray” diathermy as well as the necrosis induced by it can be well studied
- When spray diathermy is used on the resection surface, a significant (approximately 3 mm) zone of damage develops from the resection line to the intact parenchyma.
- Our study, as well as the analysis performed on our patient material, confirm the trend that seems to overturn the dogma that only R0 resection is the only acceptable solution for survival in the surgical treatment of colorectal liver metastases.
- R0 resection should be preferred, however according to the literature and our study the occurrence of R1 resection during colorectal liver metastases resection, does not represent a significant survival disadvantage in addition to modern surgical instruments and chemotherapeutics.

7. List of publication

Publications related to the Theses

1. Palkovics A, Vereczkei A, Kalmár KN, Fincsur A, Kiss I, Németh B and Papp A: The Issue of Survival After Colorectal Liver Metastasis Surgery: Parenchyma Sparing vs. Radicality. **Anticancer Research** 2018 38(11):6431-6438. **Q2, IF:1,935**
2. Palkovics A, Vereczkei A, Fincsur A, Kiss I, Németh B, Takács I, Papp A: Short- and Long-term Histological Changes in Liver Parenchyma After Different Resection Methods and Their Potential Role in Treatment of Colorectal Liver Metastasis. **Anticancer Research** 2020 40(3):1359-1365. **Q2, IF:1,935**

Abstracts and oral presentations related to the Theses

1. Palkovics A, Kalmár NK, Horváth ÖP, Vereczkei A, Fincsur A,Takács I, Papp A: Impact of margin status on survival after liver resection for colorectal liver metastasis. **38th Congress of European Society of Surgical Oncology** Budapest, Magyarország, 2018

oktober 12-18. **European Journal of Surgical Oncology** 2019 45(2), Pe130-e131. **Q1, IF: 3,349**

2. Palkovics A, Kalmár NK, Horváth ÖP, Vereczkei A, Fincsur A, Papp A: Prediktív faktorok colorectalis májmetasztázisok miatt végzett műtétek során: az R1 reszekció már nem jelent hátrányt? **A Magyar Sebész Társaság Sebészeti Onkológiai Szekciójának 1. Országos Kongresszusa**, Szeged, Magyarország, 2017. március 23–25. **Magyar Sebészet** 2017 70(2), pp. 159–194.
3. Palkovics A, Kalmár NK, Horváth ÖP, Vereczkei A, Papp A: Prediktív faktorok vizsgálata colorectalis májmetasztázisok miatt végzett műtétek során: az R1 reszekció már nem jelent hátrányt? **A Magyar Sebész Társaság 2016. évi Kongresszusa**, Budapest, Magyarország, 2016. június 02-04.
4. Palkovics A, Vereczkei A, Kalmár NK, Fincsur A,Takács I, Papp A: Kolorektális májmetasztázisok miatt végzett műtétek során az R1 reszekció már nem jelent túlélésbeli hátrányt? A „spray" diatermia lehetséges szerepe a májreszekciók során. **A Magyar Sebész Társaság 2018. évi Kongresszusa**, Debrecen, Magyarorzág, 2018. május. 24-26.

5. Palkovics A, Papp A, Fincsur A, Kustán P, Ferencz S, Jancsó G, Vereczkei A, Takács I: termikus szövetkárosodás vizsgálata szövettani modellben. **A Magyar Sebész Társaság Kísérletes Sebészeti Szekciójának XXVI. Kongresszusa** Herceghalom, Magyarország, 2017. szeptember 28–30. **Magyar Sebészet** 2017 70(3), pp. 255–291.
6. Palkovics A, Vereczkei A, Kalmár NK, Takács I, Fincsur A, Papp A: A „spray" diatermia szerepe a májreszekciók során – az R1 reszekció már nem jelent túlélésbeli hátrányt? **A Magyar Sebész Társaság Sebészeti Onkológiai Szekciójának II. Kongresszusa**, Szeged, Magyarország, 2019. május 24–25. **Magyar Sebészet** 2019 72(3), pp. 115–140.
7. Palkovics A, Vereczkei A, Horváth ÖP, Kalmár NK, Ferencz S, Jancsó G, Takács I, Fincsur A, Kustán P, Papp A: Az R1 reszekció már nem jelent hátrányt? Prediktív faktorok vizsgálata colorectalis májmetasztázisok miatt végzett műtétek során. **Doktoranduszok a Klinikai Kutatásokban** Pécs, Magyarország 2017. október 28. **DKK17-Doktoranduszok a Klinikai Kutatásokban absztraktkötet** 2017 pp. 46.

Other abstracts and oral presentations not related to the Theses

1. Palkovics A, Papp A: Hogyan befolyásolja a sebészeti gyakorlatban alkalmazott vágó-coaguláló eszközök hatékonyságát a thrombocyta aggregatio gátló kezelés? PTE ÁOK Tudományos Diákköri Konferencia 2014, Pécs, Magyarország, 2014.04.03.-2014.04.04 (Operatív klinikai orvostudomány I.)
2. Palkovics A, Papp A: Hogyan befolyásolja a sebészeti gyakorlatban alkalmazott vágó-coaguláló eszközök hatékonyságát a thrombocyta aggregatio gátló kezelés? Semmelweis Egyetem XXXII. Országos Tudományos Diákköri Konferencia, Orvos- és Egészségtudományi Szekció 2015. Budapest, Magyarország, 2015.03.31.-2015.04.03. A Graduális és Posztgraduális képzés folyóirata 2015 90(2), pp. 506)
3. Palkovics A, Papp A, Vereczkei A: A phaeochromocytoma ritka esete. A Magyar Sebész Társaság Fiatal Sebészek Szekciójának IV. Kongresszusa, Balatonalmádi, Magyarország, 2016. április 15–16. Magyar Sebészet 2016 69(2), pp. 61–80.

4. Palkovics A, Szemes K, Kelemen D, Vereczkei A: gastrointestinalis vérzések interdiszciplináris ellátása – a sebész szemszögéből. A Magyar Sebész Társaság Fiatal Sebészek Szekciójának VI. Kongresszusa, Balatonalmádi, Magyarország, 2018. március 23–25. Magyar Sebészet 2018 71(2), pp. 63–93
5. Palkovics A, Ember Á, Kövér E, Tornóczki T, Fincsur A, Hertelendy Á, Dr. Bálint M, Hernádi G: Ritka mesenchymalis daganat szokatlan lokalizációban. PTE ÁOK Tanulságos esetek Fóruma, Pécs, Magyarország, 2017. március 20.
6. Palkovics A, Papp A, Hegedűs I, Farkas P: Egy gyakori hasi kórkép diagnosztikai nehézsége a várandósság következtében megváltozott anatómiai viszonyok miatt. PTE ÁOK Tanulságos esetek Fóruma, Pécs, Magyarország, 2019. februára 04.

Cumulative impact factor of publications related to the Theses: 3,57
Cumulative impact factor of abstracts related to the Theses: 3,349
Cumulative impact factor of all publications (2020): 6,919

8. Acknowledgement

I would like to express my sincere gratitude to my supervisors and mentors Dr. András Papp for the continuous support and for his patience, motivation, and immense knowledge. I am grateful to the staff of the Department of Surgery, Medical School, University of Pécs, especially Prof. Dr. András Vereczkei, who provided the time and guidance necessary for the research and the writing of the dissertation. Special thanks to Dr. András Fincsur for the preparation of histological specimens and their contribution to their analysis, and to Dr. Károly Berényi for his assistance in the statistical analysis. I would like to thank Dr. Balázs Németh and Mihály Köntös for their help in writing the publications and proofreading them in a foreign language.

I would also like to thank Dr. Ildikó Takács and the staff of the Department of Surgical Research and Techniques, Medical School, University of Pécs for their help in carrying out the experiments.

Last, but not least, I am grateful to my family who supported me in completing this work.