Improvements of surgery and prognosis of organ confined kidney tumours

Ph.D. thesis

by

Csaba Pusztai M.D.

University of Pécs, Medical School

Department of Urology

Pécs, 2020

Head of the PhD school: Lajos Bogár M.D., Ph.D., D.Sc.

Program leader: Attila Miseta M.D., Ph.D., D.Sc.

Supervisors: Gyula Kovács M.D., Ph.D., D.Sc., FRCPath

Árpád Szántó M.D., Ph.D.

Introduction

In clinical practice, in the broadest sense the term „kidney tumour" refers to a solid or atypical cystic mass of the kidney, usually detected by imaging. It is mentioned as a small renal mass (SRM), if the largest diameter of the lesion does not exceed 4 cm.

At least three-quarters of SRMs, and even higher portion of the larger lesions are proved as malignant tumor on histopathological examination. With the exception of angiomyolipoma, there are no definite radiological signs that would predict the histological type with acceptable accuracy.

Renal cell cancer (RCC) represents 95% of all malignant kidney tumours. The most common subtypes include conventional (also known as clear cell), chromophobe, and papillary RCC. Renal cell carcinoma is one of the ten most common solid malignancies in both sexes and its incidence is increasing. Although advances have been reached in the last decade in the drug treatment of metastatic disease, complete healing can only be achieved in organ confined disease with perfect surgical excision.

With the advent and widespread use of sonography (UH) and computed tomography (CT), renal mass has become a frequent diagnosis. The altered clinical presentation of renal cancer required changes in the surgical approach, too. In case of large, locally advanced renal tumours the classical „**ROBSON** principles" of radical removal of the kidneys were necessary in the 1960s to achieve appropriate local tumour control, but in case of small tumours incidentally recognized nowadays, its value is questionable.

In the EORTC 30904 study, it was demonstrated that the oncological efficacy of partial renal nephrectomy for local tumour control is similar to that of radical nephrectomy in renal cell cancer smaller than 5 cm. Meanwhile, in the first decade of the 2000s, minimally invasive

surgery was inceasingly used, and laparoscopic radical nephrectomy became one of the most commonly performed urological surgeries.

Minimally invasive surgery and organ preservation initially seemed mutually exclusive. Laparoscopic radical nephrectomy is a well-standardized procedure and with help of an experienced mentor, the learning curve is quite steep. In contrast, laparoscopic partial nephrectomy according to **GILL** is "hundred different operation". Surgery can vary greatly depending on tumour (size, position, endophytic nature) and patient (e.g. obesity, vascular malformations) factors. This procedure requires advanced laparoscopic skills such as fast and safe suturing and knotting, often with bleeding that interferes with orientation and not always in the most favourable trocar position. Despite the challenges, **WINFIELD**, **JANETSCHEK**, **GILL** and other outstanding surgeons have developed safe surgical techniques for laparoscopic partial nephrectomy. In Hungary, **FLASKÓ** and **BAGHERI** performed the first laparoscopic partial nephrectomies. Adapting the surgical procedure studied abroad, we developed the "Pécs method", and with this technique we have removed more than 700 tumours in 15 years.

Aims

The main goals of research summarized in the dissertation were:

- To determine and describe epidemiological and statistical characteristics of the renal cancer patient population, with the aim of comparing them with the cohorts reported in the literature.
- To evaluate methods and innovations used in the introduction and development of laparoscopic radical and partial nephrectomies.
- To examine whether organ preservation is advantageous for the patient in elective indication compared to radical nephrectomy.
- To evaluate and validate different scoring systems used to describe tumour anatomical features (e.g. PADUA, RENAL, C-index).

- To demonstrate that the new surgical procedure (laparoscopic partial nephrectomy) is as feasible and at least as safe as the reference method (open partial nephrectomy).
- To demonstrate that laparoscopic partial nephrectomy with intact circulation ("zero ischemia" LPN) is feasible and beneficial for the patient.
- Biomarker research, in a narrower sense, the study of histopathological risk factors predicting the recurrence and progression of the disease.

Clinical studies

Methods

With the permission of the Regional Research Ethics Committee, in accordance with the current data protection laws (GDPR) and the relevant regulations of the University of Pécs (PTE), we established a clinical research database to analyse the course and prognosis of patients operated on for kidney tumour at the Urology Department of University of Pécs. The data collection covered the period from January 2004 to December 2019 and in some cases even earlier. For the research, we used electronic and paper-based patient documentation, images in DICOM format, and video recordings of surgeries. Data collection covered patients' demographic and physiological parameters (age, gender, height, weight and blood type), health status, major comorbidities (e.g. diabetes, hypertension, other cancers, heart disease and kidney disease), tumour characteristics and surgical data. Parameters characterizing the general condition of the patients (ECOG, ASA) and laboratory results including haemoglobin, haematocrit, creatinine and CRP were collected and recorded. Data regarding surgical interventions included surgical time, length of renal ischemia, estimated blood loss and adverse events. Renal function was estimated by the eGFR value calculated with the CKD-EPI formula, and tumour complexity was characterized by the PADUA score. Perioperative complications were classified according to the **CLAVIEN-DINDO** system. Patients were regularly controlled according to current

guidelines and the visits included regular imaging examinations for early detection of recurrence of the disease and laboratory tests to monitor renal function. Data were stored using a Microsoft Access (MS Jet) database application developed especially for this purpose by the author, and data were collected, pre-processed, and pre-evaluated using Microsoft Excel spreadsheet software (both from Microsoft Corporation, Redmond, WA, USA; version: 16.0). For statistical tests and graphical plotting, the SPSS software package (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY, USA) and the R environment (R Foundation for Statistical Computing, Vienna, Austria; URL: https://www.R-project.org/; version: 3.6.1) were used. In case-control studies, the "propensity score matching" (PSM) method was applied to eliminate inclusion bias. For the testing of statistical hypotheses conventional values of $\alpha = 0.05$ and $\beta = 0.20$ were used.

Results

Tumour size

Tumour size is the main determinant of clinical T-stage classification and highly influences the choice of surgical method. Other common factors influencing T-stage (e.g. sinus fat or perirenal fat infiltration, limited vascular invasion) can be poorly assessed by imaging studies, and they are usually revealed during histopathological examination. By analysing the size of more than 2,100 kidney tumours, we found that tumour diameter was less than 70 mm in 78% of the cases and there was a 43% chance that the tumour is smaller than 4 cm (T_{1a}). The median tumour size was 45 mm, the IQR (interquartile range; 25–75 percentile: middle 50% of cases) is 31–67 mm, and 90% of the tumours ranged between 19 and 120 mm. Dividing the 15-year period from 2004 to 2018 into three equal periods, the mean size and standard deviation of T_1 tumours were 42.1 ± 14.9 mm, 40.0 ± 14.0 mm, and 38.3 ± 14.9 mm, respectively. The difference between the last two five-year periods was significant ($p = 0.047$).

Mortality of patients after kidney tumour surgery

KAPLAN-MEIER analysis was performed based on follow-up and survival data of patients operated on for renal cancer between 2004 and 2009 (n = 544). Our results show that overall survival and tumour-specific survival do not differ at all in metastatic cases and are not significant in T_2 or higher T-stage (T2+) tumours because in these cases CSM is predominant. In contrast, for benign and T_1 tumours, the ACM is significantly higher than the CSM. This observation draws attention to the fact that it is not enough to focus on oncological efficacy in the treatment of T_1 kidney cancer, but it is necessary to eliminate any potential side effects of the surgery that may affect OS (such as CKD).

Group	Mark	Cases	CSM	ACM	CSM/ACM	Median OS
Benign	BEN	49	0	8	0,00	n.a.
Organ confined T_1	T1	297	22	64	0,34	n.a.
Locally advanced ($T_{2\text{-}4}N_0M_0$)	T2+	129	40	49	0,82	108,9 mts
Metastatic ($T_XN_{1\text{-}3}M_X$ or $T_XN_XM_1$)	N+/M+	69	53	54	0,98	20,5 mts
Total		544	115	175	0,66	

Renal function following radical and partial nephrectomy

The study group consisted of patients who underwent partial nephrectomy for renal tumour less than 7 cm in diameter. Those who underwent radical nephrectomy for the same reason served as controls. Only cases where both preoperative and one-year postoperative eGFRs were available were analysed. Thus, we could analyse 367 patients with partial nephrectomy and 144 cases of radical nephrectomy. Preoperative eGFR did not differ between the two groups (means were 84.6 and 84.9 ml/min/1.73m^2, respectively; $p = 0.87$). One year after surgery, the mean eGFRs were 73.1 and 52.9 ml/min/1.73m^2, respectively, and the difference was strongly significant ($p < 0.001$). Thus, for T_1 tumours, the mean decrease in eGFR was 32 ml/min after radical nephrectomy, and it was 11 ml/min following nephron-sparing surgery.

Zero ischaemia laparoscopic partial nephrectomy

The conventional technique of organ-preserving kidney-tumour-excision is accomplished by identification and isolation of renal hilum, and compressing the vessels or only the renal artery in order to achieve a bloodless field for tumour excision and parenchymal reconstruction. The disadvantage of this procedure is that the healthy parenchyma may be damaged by ischemia-reperfusion injury. In 2011, **GILL** et al. published a novel method, called „zero ischemia" laparoscopic partial nephrectomy. We adopted this technique in order to avoid renal ischemia during LPN surgery. For study cases of zero ischemia (ZI), control cases were selected from the patients undergoing ischemic LPN using the PSM method. The cases and controls did not differ significantly in terms of patients' age, gender, body mass index and comorbidities, as well as tumour characteristics (size, PADUA score, location).

Variable	All LPN* n = 731	ZI* n = 150	Control* n = 150	p
Operative time (min)	164 (53)	**127** (42)	**171** (45)	**< 0.001**
Renal ischemia (min)	19.5 (6.3)	0.0	19.6 (6.3)	< 0.001
Change in hemoglobin (g/l)	-24.2 (13.7)	-25.6 (12.3)	-24.4 (13.8)	0.488
Transfusion (n; %)	47 (6.4%)	10 (6.7%)	10 (6.7%)	1.000
Length of hospital stay (day)	4.3 (1.9)	4.1 (2.8)	4.4 (1.1)	0.383
Positive surgical margin (n; %)	47 (6,4 %)	9 (6,0 %)	9 (6,0 %)	1.000
Serious complication (Clavien 3+)	40 (5.9 %)	3 (2.1 %)	7 (5.0 %)	0.335
Follow-up (months)	29.6 (33.1)	20.3 (26.1)	34.5 (35.0)	< 0.001
eGFR postop. @ 1 year (ml/min)	74.4 (19.0)	**77.2** (18.4)	**70.1** (17.8)	**< 0.050**
eGFR change @ 1 year (ml/min)	-9.7 (13.2)	**-4.4** (14.5)	**-13.7** (11.8)	**< 0.001**
Relative eGFR change @ 1 year	-10.9 % (17.6)	**-5.7 %** (17.6)	**-16.3 %** (14.8)	**< 0.001**

** Mean values, standard deviation in parentheses*

There was no significant difference in eGFR between the study groups (ZI: 83.6 vs. Control: 86.3 ml/min; $t = -1.26$, $p = 0.208$). However, one year after surgery, there was a significant difference in the mean eGFR of the two groups (77.2 vs. 70.1 ml/min; $t = 2.10$, $p < 0.05$) and in the absolute and relative change in eGFR.

Biomarker research

Introduction

In some patients with apparent organ confined renal cancer, there are clinically undetectable distant metastases at the time of surgery, which is a source of tumour recurrence in the course of disease. The majority of prognostic models have been developed for clinically advanced renal tumours and the $T_1N_0M_0$ tumours that are the subject of the present dissertation belong to the "good" prognostic group with an expected 85% - 95% five-year tumour-specific survival. However, the 5 to 15% recurrence affects a significant patient population, as the majority (60–80%) of newly diagnosed renal cancers belong to this stage. Most common recurrences occur in the form of lung metastases, retroperitoneal lymph node metastases or local (kidney, kidney bed) recurrence and it is difficult to detect them early. The sooner it is detected the treatment of recurrence is more effective. Patients with apparent localized renal tumours should also be monitored regularly after surgery for the detection of recurrence in time. There are various recommendations for the intensity of follow-up that have in common:

- significant resources on a regular basis are required (imaging, human resources, loss of patient time)
- the currently available tests are not sensitive and specific enough (many false-positive cases, on the other hand, late detection due to a false-negative result)
- the recurrence detected with intensive follow-up is not suitable for curative care in all cases
- the duration of the required follow-up cannot be determined

Our working group led by Professor Gyula **KOVÁCS** M.D., D.Sc. is making significant efforts to find biomarkers that may be suitable for distinguishing between favourable and unfavourable prognosis of renal tumours confined to the kidney at the time of surgery.

Methods

Gene expression assays were performed in the Molecular Biology Laboratory of Ruprecht-Karls University in Heidelberg. Based on histological examination and genetic profiling, representative tumour samples were taken, from twelve conventional renal cell cancers leading to death of patients within three years and from another twelve tumours without progression at least eight years of follow up. RNA was isolated from tissue samples and gene expression analysis was performed using an Affymetrix Human Genome U133 Plus 2.0 array suitable for the identification of 54675 human DNA sequences. Gene expression was visualized using the freely available public licensed JAVA-based GSEA-P (Gene Set Enhancement Analysis) version 2.0 software (http://www.broad.mit.edu/GSEA). On the expression map, we searched for genes whose expression was significantly different in conventional renal cell carcinomas with aggressive and indolent behaviour. In this study, the protein products of the genes identified in progressive tumours were examined by immunohistochemistry in tissue multi-array (TMA) sections prepared from histological blocks of renal tumours removed in the Department of Urology Pécs. After dewaxing and rehydration, the 4-µm thick sections were subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0) or in a higher pH solution (Envision FLEX Target Retrieval Solution, DAKO). Endogenous peroxidase was blocked with the Envision FLEX Peroxidase-Blocking Reagent (DAKO) for 10 min at room temperature. After a brief wash in buffer rinse, sections were incubated for 1 hour at room temperature with primary antibody diluted in REAL Antibody Diluent (DAKO). After repeated short rinses, they were incubated with Envision FLEX / HRP (horseradish peroxidase conjugated) secondary antibody. After further rinsing, sections were covered with AEC (3-amino-9-ethylcarbazole) substrate (DAKO) and the reaction was stopped after microscopic examination. Nuclear and background staining were performed with **MAYER**'s Haematoxylin (Lillie's modification) (DAKO) and sections were covered with Glycelgel Mounting Medium (DAKO). Normal adult

kidney included in the TMA was used as positive control. For the negative control, the primary antibody was omitted. Sections were evaluated with a Leitz Laborlux S microscope. The intracellular localization of the immune response was observed.

Results

Transmembrane protein 27 (TMEM27)

Tissue samples extracted from paraffin blocks of 486 renal cancer patients and integrated into TMA were used for the study. In the immunohistological examination, variable intensity membrane-associated TMEM27 labelling was detected in 356 tumours, while 130 cases were negative. Compared with clinical data, our results show that lack of TMEM27 expression is an independent risk factor for tumour progression. Based on our studies, we found that the risk of progression of TMEM27 negative cases was almost three times higher than that of positive cases (HR: 2.95; 95% CI: 1.81-4.80; $p < 0.001$).

Lipopolysaccharide binding protein (LBP)

The Affymetrix array detected increased expression of the lipopolysaccharide binding protein (LBP) of inflammatory origin only in conventional renal tumours showing progression. In immunohistological examination, LBP protein expression was found in foetal and adult renal proximal tubule cells. No positive reaction was obtained with the LBP antibody in 588 (85%) conventional kidney cancers, while 103 (15%) tumours showed different strengths of LBP positivity. **KAPLAN-MEIER** analysis using a log-rank test showed significantly shorter tumour-specific survival in tumours positive for LBP antibody staining. In univariate analysis, both T-stage, tumour grade, tumour stage, and tumour size were significantly correlated with LBP positivity (all $p < 0.001$). In multivariate analysis, LBP expression was found to be an independent prognostic factor in addition to the nuclear grade (RR = -2.37; 95% CI: -1.50 - -3.73; $p < 0.001$).

β-catenin

This cohort contained cases with metastatic disease at the time of surgery, however, 427 patients with localized disease were evaluated also separately. According to univariate analysis, known parameters associated with progression, such as tumour grade, local extent (T-stage), disease stage, as well as β-catenin expression were significant ($p < 0.001$). After multivariate analysis of data from 427 patients, only T-stage ($p < 0.001$) and β-catenin positivity showed significant association with tumour progression. Cytoplasmic expression of β catenin posed a 4-fold increased risk of disease progression (RR = 4.017; 95% CI: 2.489-6.482; $p < 0.001$). β-catenin may be an excellent biomarker for the identification of patients with renal cancer who are at high risk for postoperative tumour progression and tumour mortality.

M2 macrophage chitinase (CHI3L2)

Based on Affymetrix array analysis, overexpression of CHI3L2 occurred only in progressive tumours. To confirm this, CHI3L2 was examined by immunohistochemistry in a group of 634 non-metastatic conventional kidney cancers.

A strong membrane attenuated and submembranous or cytoplasmic expression of CHI3L2 protein was seen in at last one of the core biopsies of 132 (20,8%) tumours. During the follow-up 100 patients (15,8%) developed metastasis or died of cancer.

Progression-free survival (PFS) of CHI3L2-negative and positive patients differed significantly. Five-year PFS was 95.4% and 59.0% for CHI3L2 negative and positive tumours, respectively (log-rank test: $p < 0.001$). In a multivariate analysis, CHI3L2 positivity was significantly correlated with G2 and G3 tumour grades. Furthermore, CHI3L2 positivity was shown to be a strong, independent risk factor for tumour progression (RR = -3.49; 95% CI: -2.21 to -5.51).

Variable	Group	Cases (634)	CHI3L2 expression		Cramer V	p
			negative (502)	positive (132)		
Gender					0,093	0,02
	male	371	282	89		
	female	263	220	43		
State					0,450	<0,001
	Free of disease	534	465	69		
	Progression	100	37	63		
Tumour size					0,210	<0,001
	< 4 cm	251	222	29		
	4-7 cm	251	193	58		
	> 7 cm	132	87	45		
T-stage					0,294	<0,001
	pT_1	477	405	72		
	pT_2	87	64	23		
	pT_3	70	33	37		
Grade					0,420	<0,001
	G1	430	386	44		
	G2	158	101	57		
	G3	46	15	31		

Discussion

Working up the database created during my research, we gained important information about the cohort of patients with kidney cancer, and this valuable source of information can be the basis for further research.

Analyzing the tumor size, we showed that 78 percent of newly diagnosed renal cancer patients have a lesion smaller than 7 cm and are potentially suitable for organ-preserving surgery. Our study confirmed the observation reported by others that within ten years only a small portion of deaths of patients with $pT_1N_0M_0$ stage kidney cancer are caused by cancer recurrence, most of them die from other causes, so it is not enough to focus on oncological efficiency in the treatment. One of the important factors negatively influencing life expectancy, which can be altered by the choice of the type of surgery and the surgical technique, is the development of chronic renal failure. Supporting the results of others, we demonstrated that the decrease in renal function after organ-sparing surgery of T_1 tumors is significantly less than after radical nephrectomy. Following modern international trends, the use of "zero ischemia" has further improved the functional outcome of laparoscopic partial nephrectomy without increase of positive surgical margin, surgical risk, and in the transfusion rate.

In biomarker research, we searched for histopathological risk factors that are associated with renal tumor progression. Based on the results of a previous genetic analysis, our team has identified several substances that can be detected by immunohistochemistry. In my work, we examined M2 macrophage chitinase (CHI3L2), a chitinase-like protein previously observed in stimulated macrophages. We demonstrated that CHI3L2 expression in conventional kidney cancer poses more than a threefold risk for subsequent progression. CH3L2 immunohistochemical staining, especially in combination with other biomarkers and clinic-pathological features, can be a valuable tool for the identification of organ-confined but later progressive renal tumours at the time of surgery.

Summary of original findings

1. I collected data for scientific purposes to investigate the course and prognosis of kidney tumour patients treated with surgeries. The database application developed for this goal is my own intellectual product and provides efficient information technology tools for retrospective and prospective clinical trials.
2. With my colleagues, we continuously refined the surgical technique of laparoscopic partial nephrectomy. I adapted the methods I learned abroad to our technical environment. I presented our method in live surgeries, publications and I helped others to learn it. Our method is efficient, safe and cost-effective.
3. I have demonstrated that in clinical T_1 tumours, organ-sparing surgery causes significantly less decrease in renal function than radical nephrectomy. I have quantified the average eGFR gain.
4. I have advocated for the use of nephrometric scoring when planning renal tumour surgeries. I drew attention to the fact that the application of PADUA scoring makes the decision between organ preservation and organ removal more objective.
5. I introduced and propagated the "zero ischemia" laparoscopic partial nephrectomy. I have demonstrated that the decrease of renal function caused by this kind of surgery is significantly less compared to same intervention with ischemia.
6. Our team identified several promising biomarkers that are associated with poor prognosis of kidney cancer. I have demonstrated with my colleagues that positive immunohistochemistry staining with CHI3L2 can be an independent risk factor for the progression of conventional kidney cancer and can be a valuable biomarker in the identification of patients with high risk for recurrence.

Acknowledgements

First of all, I am grateful to Professor László Farkas who set me up and supported me in my professional and research career, and provided me with the opportunity to learn laparoscopy.

I would like to thank Professor Gyula Kovács for introducing me to the beauties of molecular pathology research and for his valuable guidance, experience, encouragement and helpful work in achieving my goals.

Head of the Urology Department, Árpád Szántó made it possible for me to continue and complete the work I had started. Kinga Villányi provided valuable assistance in the preparation of scientific publications and helped to find the right direction with her critical proofreading and advice. All employees of the Urology Department took part in the treatment of the group of patients included in the work, so in some way, all contributed to present work. Special thanks should go to Mrs. Viktória Szentai Nagyné, who helped me in follow up of patients with kidney cancer, and to Mrs. Erzsébet Óbert Hargitainé who contributed to my research work with her valuable help during the surgeries.

The biomarker research described in this dissertation would not have been possible without the preliminary work of the staff of the Molecular Oncology Laboratory of the University of Heidelberg and the staff of the Institute of Pathology of the University of Pécs.

Finally, I thank my loving partner, Márti, for her love, patience and support.

To my Parents and to my Children.