Diplomarbeit

Evaluation of the potential prognostic value of the preoperatively assessed basophil granulocyte count regarding clinical outcomes in non-metastatic clear cell renal cell carcinoma patients

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Graz, am 23.10.2018 *David Duckenfield eh*

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Abstract (English)

Objective: Renal cell carcinoma (RCC) represents the most common malignant tumour of the adult kidney. Inflammatory parameters like the haemoglobin concentration, platelet counts and absolute leukocyte counts have been demonstrated to influence tumour activity and thus might play an important role as prognosticators of survival in various types of cancer. We investigated the potential prognostic significance of the pre-treatment basophil granulocytes count in a European cohort of patients with non-metastatic clear cell RCC.
Materials and Methods: Clinico-pathological data from 677 consecutive, non-metastatic clear cell RCC patients, operated between 2000 and 2010 at a single tertiary academic centre, were evaluated retrospectively. Pre-treatment laboratory parameters were assessed within one week before surgery. Patients were categorized using a cut-off value of 100/µL (basophil granulocytes) according to a calculation by receiver-operating curve (ROC) analysis. Overall survival (OS) and metastasis-free survival (MFS) were assessed using the Kaplan-Meier method. To evaluate the potential prognostic significance of the pre-treatment basophil granulocytes count, a multivariate Cox regression model was performed.
Results: In multivariate analysis, age at operation (<65 *vs.* ≥65yrs., HR=1.038 [95%CI=1.023-1.052], $p<0.001$), as well as pathologic T-stage (T1 *vs.* T3+T4, HR=1.381 [95%CI=1.012-1.885], $p=0.042$) were independent predictors regarding OS, whereas tumour grade (G1+G2 *vs.* G3+G4, HR=1.277 [95%CI=1.000-1.632], $p=0.050$) was borderline significant and an elevated pre-treatment basophil granulocyte count (<100/µL *vs.* ≥100/µL, HR=1.332 [95%CI=0.927-1.914], $p=0.121$) failed to achieve independent predictor status regarding OS.
Conclusion: In the cohort studied, an elevated pre-treatment basophil granulocytes count was not confirmed as an independent predictor of OS in the non-metastatic clear cell RCC setting.

Abstract (Deutsch)

Zielsetzung: Das Nierenzellkarzinom ist der häufigste maligne Tumor der adulten Niere. Bei Entzündungsparametern wie Hämoglobinkonzentration, Plättchenzahl und absolute Leukozytenzahl konnte ein Einfluss auf die Tumoraktivität nachgewiesen werden und diese könnten daher eine wichtige prognostische Rolle im Bezug auf das Überleben in unterschiedlichen Krebsformen spielen. Es wurde untersucht, ob eine potenzielle prognostische Signifikanz in der prätherapeutischen Anzahl an basophilen Granulozyten in einer europäischen Kohorte von PatientInnen besteht, die am nicht metastasiertem klarzelligen Nierenzellkarzinom erkrankt sind.

Material und Methoden: Die klinisch-pathologischen Daten von 677 PatientInnen, die an klarzelligem Nierenzellkarzinom erkrankt sind und zwischen 2000 und 2010 in einem einzigen tertiärem Zentrum operativ behandelt wurden, wurden retrospektiv evaluiert.

Prätherapeutische Laborparameter wurden innerhalb einer Woche vor der operativen Intervention erhoben. Mit Hilfe der “receiver-operating-curve” (ROC) Analyse, konnten die PatientInnen anhand eines “cut-off” Wertes von 100/µL (basophile Granulozyten) kategorisiert werden.

Gesamtüberleben (GÜ) und metastasenfreies Überleben (MFÜ) wurden anhand der Kaplan-Meier Methode untersucht. Um eine potenzielle prognostische Signifikanz der basophilen Granulozyten zu erheben, wurde ein “Cox regression” Modell durchgeführt.

Ergebnisse: In multivariaten Analysen stellten sich sowohl das Operationsalter (<65 vs. >65à, HR=1.038 [95%CI=1.023-1.052], p<0.001) als auch das pathologische T-Stadium (T1 vs. T3+T4, HR=1.381 [95%CI=1.012-1.885], p=0.042) als unabhängige prognostische Werte im Bezug auf das GÜ heraus. Tumor Grad G1+G2 vs. G3+G4, HR=1.277 [95%CI=1.000-1.632], p=0.050) stellte sich im selben Bezug als grenzwertig dar, während sich ein erhöhter prätherapeutischer Blutwert der basophilen Granulozyten (<100/µL vs. >100/µL, HR=1.332 [95%CI=0.927-1.914], p=0.121) nicht als unabhängiger prognostischer Wert qualifizieren konnte.

Konklusion: In der untersuchten Kohorte konnte, zusammenhängend mit dem klarzelligem Nierenzellkarzinom, eine erhöhte prätherapeutische basophile Granulozyten-Zahl, als unabhängiger prognostischer Wert im Bezug auf das Gesamtüberleben nicht bestätigt werden.

Table of Contents

Abbreviations and acronyms

ACE	Angiotensin converting enzyme
AJCC	American Joint Committee on Cancer
ALP	alkaline phosphatase
BMI	body mass index
CA9/CAIX	carboanhydrase 9
ccRCC	clear cell renal cell carcinoma
chRCC	chromophobe renal cell carcinoma
CNS	central nervous system
CT	computed tomography
CSS	cancer-specific survival
ESR	erythrocyte filtration rate
GFR	glomerular filtration rate
HIF	hypoxia-inducible factor
HR	hazard ratio
HU	Houndsfield Units
IgE	immunoglobulin E
IMDC	International Metastatic Renal Cell Carcinoma Database Consortium
LH	lactate dehydrogenase
LLN	lower limit of normal
MFS	metastasis-free survival
MRI	magnetic resonance imaging
MSKCC	Memorial Sloan Kettering Cancer Center
mTOR	mechanistic target of rapamycin
OS	overall survival
PERC	perchloroethylene/tetrachloroethylene
PFS	progression-freesurvival
PS	performance status
pRCC	papillary renal cell carcinoma
ROC	receiver operating curve

RCC	renal cell carcinoma
SAA	serum amyloid A
SD	standard deviation
SRE	skeletal related events
SSIGN	Mayo Clinic stage, size, grade, and necrosis score
TKI	tyrosine kinase inhibitor
TNM	tumour, (lymph) nodes, metastasis
UCLA	University of California, Los Angeles
UICC	Union Internationale Contre le Cancer
UISS	UCLA Integrated Scoring System
ULN	upper limit of normal
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau

1 Introduction

Renal Cell Carcinoma (RCC) represents a tumourous disease of the urinary tract that takes its origin from different sections of the renal tubular epithelium [1]. It currently has reached up to 2-3% of all malignant tumourous diseases [2].

1.1 Epidemiology

Currently, RCC represents the most common primary tumour of the kidney (80-85%) and the third most common of the urinary tract after prostate cancer and bladder cancer [1-3].Over the past decades the incidence of RCC has risen by about two percent in Europe, as well as worldwide [4].
In 2018, approximately 1,735,350 new cancer cases and 609,640 cancer-related deaths are projected to occur in the United States alone [5]. Regarding cancer of the kidney and renal pelvis, the estimated 2018 rates in the US population are 65,340 new cases and 14,970 kidney cancer-related deaths [5].
Depending on geographical region, gender and ethnic groups, the incidence rates and mortality of RCC over the last years have changed, with an incidence increase being more rapid among black males than whites and a shift from a predominance among whites to blacks in the US [6]. Moreover, it has been demonstrated over the last decades that RCCs are worldwide being diagnosed at an earlier stage, and among localised tumours, a clear shift towards smaller tumour sizes was observed [7].
Various factors could have contributed in part to a recent levelling of RCC-mortality rates in the US, as well as in many European countries. Whether biological RCC behaviour has changed over time towards less aggressive variants, which might explain increased survival rates, remains speculative. Additionally, improved RCC cancer-specific survival (CSS) rates in the industrialized world have been heavily influenced by the introduction of novel targeted therapies, which improved progression-free- survival (PFS) and OS rates of metastatic RCC patients [8–10]. Men are 1,5 times more likely to suffer from RCC

than women. There is also an age-peak between 60 and 70 years, where the likelihood to receive the diagnosis of RCC is the highest [11].
For Austria, a decrease of the incidence and the mortality between the years of 1990 and 2015 can be observed in the following graphic.

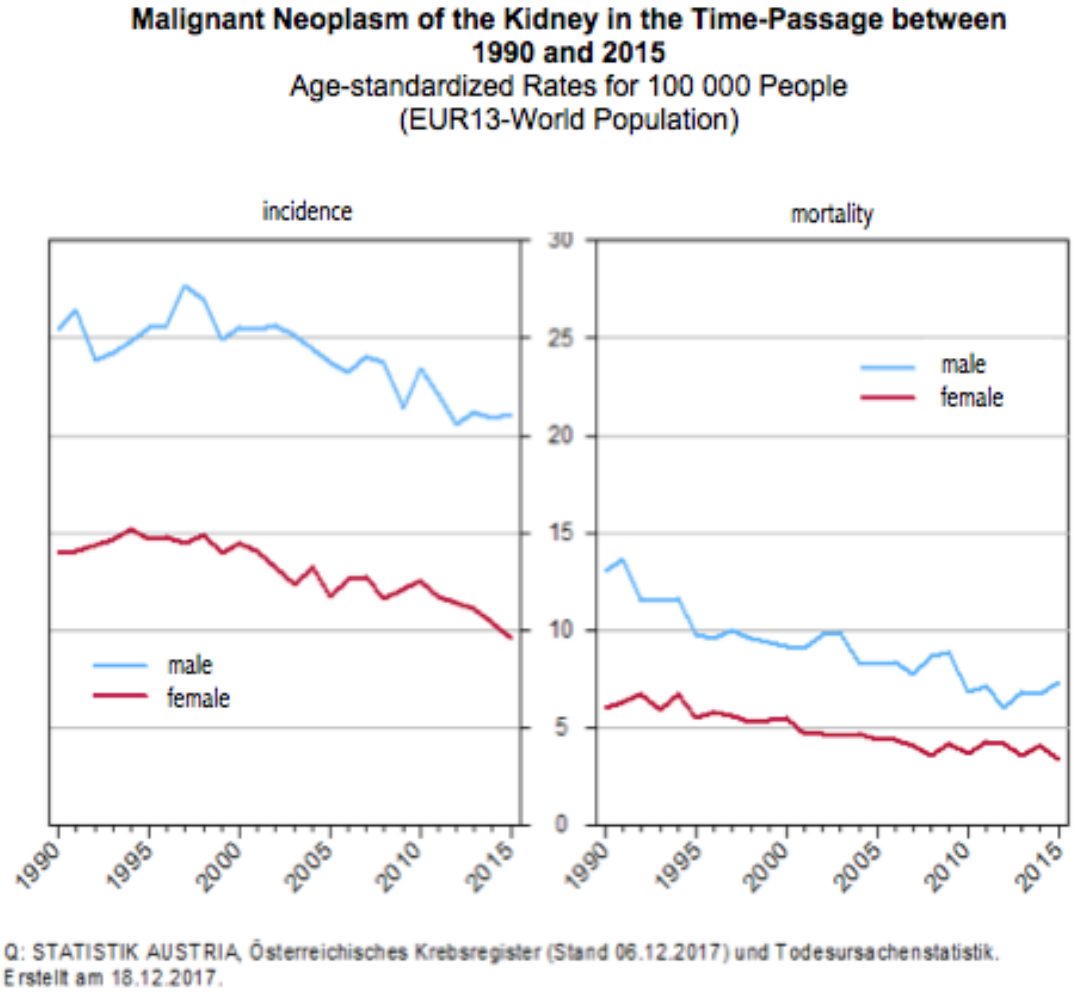

Fig. 1: Incidence and mortality for cancer of the kidney in Austria [12].

Fig. 1 shows that there were about 15 new cases and about 5 deaths per 100.000 people in Austria. Moreover, as evidenced by Fig. 1, the incidence and mortality rates for men and for women are on a steady decline since 1990 in Austria. It therefore follows a trend observed in the entire European Union [12,13].

1.2 Aetiology

The main risk factors for the development of RCC are lifestyle-related causes, including smoking, obesity and arterial hypertension[14]. Combined, these factors serve as the main cause for over 50% of all RCC cases worldwide [15]. Other risk factors can be industrially used chemicals or elements as stated in the paragraph below [16,17].

It is a proven fact that the major risk-factor for RCC is the smoke from cigarettes and that doesn't only concern active smokers but also people who are exposed to smoke passively [18]. Male smokers have a relative risk that is increased by 54%, females, after all, by 22%, in comparison to male and female non-smokers [19]. In addition, the risk of the disease increases even more with long-time smokers that are exposed to the drug more than ten years. And even non-smoking people that are exposed passively to smoky environments over a time period of 20 years or longer, show a two to four times higher risk of harbouring RCC [20].

The suspected cause for that is, amongst others, a chronic hypoxia of tissue, caused by chronic carbon dioxide-intoxication. In addition to the significantly higher risk of falling ill to the disease, smoking also has a negative effect on morbidity, mortality and hence the prognosis of RCC. This is attributed to a significantly higher p53-mutation-rate, as well as other negatively impacted clinical-pathological factors caused by smoking [21].

Another risk factor for RCC development, not to be taken lightly, is obesity [14]. An increased body mass index (BMI) implies an increased risk in men and women [22,23], while an increased waist-to-hip-ratio also proves a correlation in increased incidence rates of RCC [24].

Nevertheless, tumours developing due to obesity, may be more indolent. Obese and overweight patients were amongst others, less likely to present with advanced-stages of the disease compared with normal-weight patients, as well as differences in the gene expression of metabolic and fatty acid genes, including fatty acid synthase. This phenomenon is known as so called 'obesity paradox' [25].

Furthermore, arterial hypertension has been consistently shown to represent one of the major three- lifestyle risk factors [14] for RCC. Several studies show a correlation between these two diseases [26,27]
Men with a diastolic blood pressure above 90 mmHg over a long period of time were prone more than twice as highly to the disease than men whose long-time diastolic blood pressure didn't exceed 70mmHG [26]. Moreover, an elevated blood pressure might also show a connection to a higher disease risk in women [27].
Carcinogenic toxins, such as trichloroethylene (an industrial solvent [28]), PERC or tetrachloroethylene (commonly used as a dry cleaning fluid [28]) , asbestos and other aromatic carbohydrates are highly suspected to be able to cause cancers of the kidney. Until now, only trichloroethylene has been demonstrated to evidently represent a causing-factor for RCC [16].

Amongst carcinogenic metals, such as arsenic, cadmium chrome, lead, and nickel, only lead could prove a direct correlation up to now. Further connections were described between fiberglass, mineral-wool-fibre and brick-dust [17,29].

Hereditary forms of RCC represent a relatively small fraction of renal tumours with less than three percent [30].
Their most commonly known representative is the Von-Hippel-Lindau disease; an autosomal dominantly inherited syndrome with a high penetrance that predisposes to the development of a variety of highly vascularized tumours (model of tumoural angiogenesis) [31]. Main manifestations include the central nervous system (CNS) and retinal haemangioblastomas, endolymphatic sac tumours, clear-cell renal cell carcinomas (ccRCC), phaeochromocytomas, as well as pancreatic neuroendocrine tumours [31].

1.3 Symptoms and Diagnostics

1.3.1 Clinical Symptoms

Due to modern and non-invasive imaging methods, the classical trias of symptoms for RCC: haematuria, flank pain, and a palpable mass in the abdomen, nowadays only occur in about one percent or less of patients. This implies that the great majority of all renal tumours are found incidentally and symptoms will typically occur as late-onset-symptoms [32].
In about 30% of RCC patients paraneoplastic syndromes can be found. These include hypoalbuminemia, weight loss, anorexia and malaise high blood pressure, fever, neuromyopathia, hypercalcaemia and polycythaemia and are also linked to a bad prognosis [33,34].

1.3.2 Clinical Diagnostics

As previously stated, the clinical symptoms often occur in rather later onsets of the disease. Hence, the importance of the physical examination is comparably low towards other diagnostic methods. The majority of renal masses is being diagnosed through non-invasive imaging methods, such as medical ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI), which are mainly being performed for the diagnosis of other ailments.
If there is any sign suspecting a renal tumour, medical imaging, if not already done, should be used as well as laboratory analysis. Parameters like serum creatinine, glomerular filtration rate (GFR), complete blood cell count, liver function study, erythrocyte sedimentation rate (ESR), alkaline phosphatase (ALP), lactate dehydrogenase (LH) and serum calcium might show pathological signs.
If large central renal masses abutting or invading the collecting system are found in the imaging, urinary cytology and endoscopic assessment should be considered in order to exclude urothelial cancer, which has a different biological behaviour, as well as different therapeutic approaches compared with RCC [3].

1.3.3 Imaging

Most renal tumours detected by abdominal sonography or CT are described as solid or cystic masses [32].

For a detailed examination of a suspected lesion (solid or cystic renal mass) a medical imaging should be made via CT or MRI in combination with a contrast agent, whenever feasible. The reason for this is to recognise a so-called contrast media enhancement. An alteration of ≥15 Houndsfield Units (HU) can drastically harden the radiographic suspicion on a potential malignancy.

The imaging should be performed before and after the application of contrast agent and furthermore images should be made during the nephrographic phase and considered during the assessment [35,36].

In the vast majority of all cases it is possible to verify a RCC using contrast enhanced CT. Unfortunately though, it might be difficult to differentiate between benign lesions, such as oncocytomas, as well as angiomyolipomas (containing minimal fatty tissue) and malignant renal tumours [37,38].

In such cases MRI can deliver crucial additional information and is generally indicated when the patient has an intolerance towards the contrast agent or is pregnant [39].

To eliminate or confirm the suspicion of the presence of pulmonary metastases, a CT of the thorax should be made [40]. A bone scintigraphy and/or a cranial CT/MRI can be undertaken additionally, if specific symptoms or inconsistencies with laboratory parameters are observed [41–43].

1.3.4 Bosniak-Classification

The radiological classification of renal cysts into complicated and uncomplicated cysts is being done via the so-called 'Bosniak'-classification [44].

Category	Results	Consequences
I: uncomplicated cyst		none
II-IV: complicated cyst	• hyperdencies within the cyst (>20 HU) • septa • calcifications	
II: cyst, probably non-malignant	• natively homogenous • no contrast-agent enhancement • <3cm diameter • <1/3 is located extra-renally with smooth contours • anamnestic infection (optional)	Surveillance after 3, 6 and 12 months
III: complicated cyst, potentially malignant	• one of the first four criteria of category II does not apply	resection (except for high risk patients)
IV: complicated cyst, probably malignant	• verification of a solid portion with contrast-agent enhancement • irregular, indistinct contours	resection

Tab. 1 Bosniak-classification of renal cysts [44]

1.4 RCC-Classification systems

1.4.1 Histological Classification

The 2004 World Health organisation histological classification system for RCC combines morphological, as well as molecular genetical traits and also has an influence on the prognosis of the disease [1,3,45].

Histological-Subtype	Frequency
➢ Clear cell renal cell carcinoma (ccRCC)*	83%
➢ Papillary renal cell carcinoma (pRCC) • basophil type (type I) • eosinophil type (type II)	11%
➢ Chromophobe renal cell carcinoma (chRCC)	5%
➢ Ductus-Bellini-Carcinoma	<1%
➢ Unclassified carcinoma	<1%

Tab. 2: Histological Subtypes according to WHO [1,3,45]

*) Due to reasons of specificity we only considered patients with clear cell renal cell carcinoma in our study.

1.4.2 TNM-Classification according to UICC

The TNM-Classification is being done according to the 'Union Internationale Contre le Cancer' (UICC). The current edition is version 7 and has been the guideline since 2009[1,3,46].

- **T** describes the size of the original (primary) tumour and whether it has invaded nearby tissue,
- **N** describes nearby (regional) lymph nodes that are involved,
- **M** describes distant metastasis (spread of cancer from one part of the body to another).

T	
TX	tumour cannot be assessed
T0	no evidence of tumour
T1	tumour ≤7 cm in it's longest diameter; restricted to the kidney
T1a	tumour ≤4 in it's longest diameter
T1b	tumour 4-7 in it's longest diameter
T2	tumour >7 cm in it's longest diameter; restricted to the kidney
T2a	tumour 7-10 cm in it's longest diameter
T2b	tumour >10cm in it's longest diameter
T3	tumour spreading into the larger veins, infiltrates perirenal fatty tissue, or the adrenal gland, expansion does not exceeds Gerota's fascia (renal fascia)
T3a	tumour spreading into the vena renalis, it's segmental branches, or infiltration of perirenal fatty tissue
T3b	tumour spreading into the vena cava below the diaphragm
T3c	tumour spreading into the vena cava above the diaphragm, or infestation of the vein walls
T4	tumour exceeds Gerota's fascia
N	
NX	lymph nodes cannot be assessed
N0	no regional lymph nodes metastasis
N1	1 regional lymph node metastasis present
N2	more than 1 regional lymph node metastasis present
M	
MX	distant metastasis cannot be assessed
M0	no distant metastasis
M1	metastasis to distant organs (beyond regional lymph nodes)

Tab. 3: TNM-Classification system for RCC according to UICC [1,3,46]

1.4.3 Fuhrman Grading

The Fuhrmann Grading system is a histo-pathological grading system that differentiates between four stages of nuclear differentiation. Three factors are taken into consideration: nuclear size, nuclear contours and the presence/size of nucleoli. RCC patients show a significant correlation between grading and CSS. Grade 1 is the most favourable, grade 4 the least [47].

Grade	Nuclear Size	Nuclear Contours	Nucleoli
1	<10 µm	round, uniformous	none
2	15 µm	slightly irregular	visible at 400x microscopic enhancement
3	20 µm	very irregular	visible at 100x microscopic enhancement
4	>20 µm	pleomorphic, often lobuluar	macro-nucleoli

Tab. 4: Fuhrman Grading system

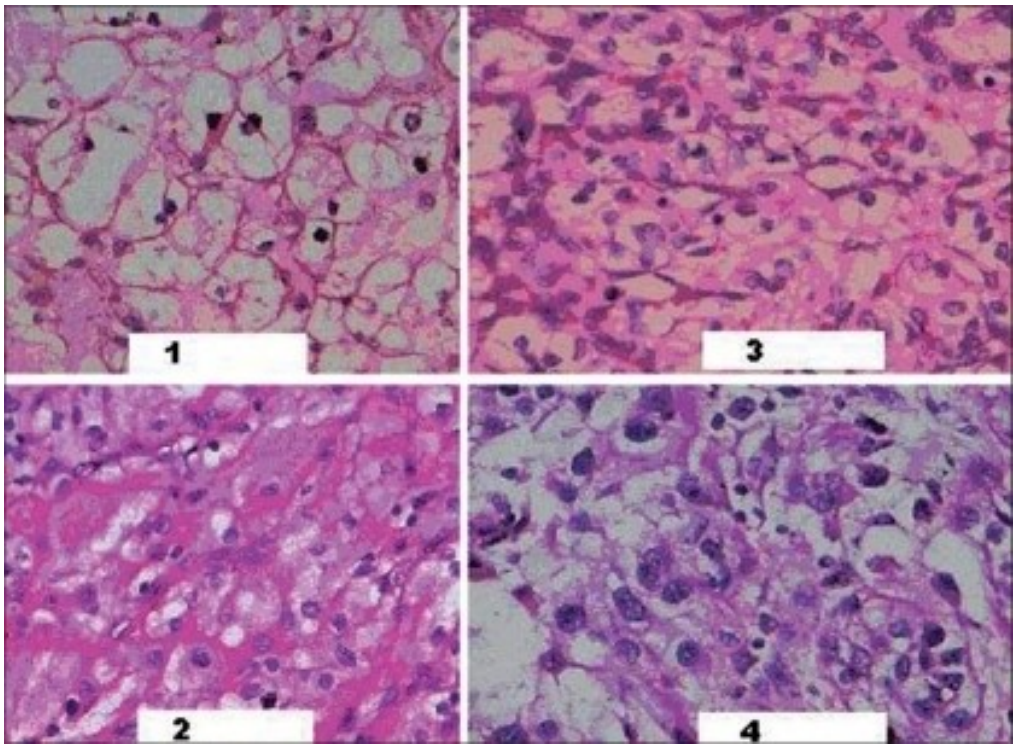

Fig. 2: Fuhrman nuclear grades 1 to 4 [48]

1.5 Prognosis

The prognosis of RCC is dependent on anatomical, histological, clinical, as well as molecular factors [46].
The five year OS rate of all histological RCC subtypes in combination is estimated at ~49%. This is signalling an improvement towards 2006 which is possible due to the increase of incidentally found RCCs in early stages of the disease, as well as the introduction of systemic medical treatment modalities, such as tyrosine kinase inhibitors (TKIs)[49].

1.5.1 Anatomical Factors

Anatomical prognostic factors are primarily compiled in the TNM-Classification system and contain, as mentioned above, tumour size, venous invasion, renal capsular invasion, adrenal involvement, and nodal, as well as distant metastases [46].

1.5.2 Histological Factors

Fuhrman_Grading, the WHO-Classification of RCC and it's subtypes, histological tumour necrosis, micro-vascular tumour invasion and invasion of the urinary system are included into the histological prognostic evaluation of the disease.
The three most common histological subtypes, namely ccRCC, pRCC, and chRCC, all do have a different prognosis. The histological subtype with the best prognosis regarding survival is chRCC, followed by pRCC and at last, with the worst prognosis, the most common subtype, namely ccRCC [1,3,49].

1.5.3 Clinical Factors

Patients clinical performance status (PS), localised symptoms, cachexia, anaemia, platelet count, neutrophil count, and neutrophil-to-lymphocyte ratio, do all represent important clinical parameters regarding an individual patients prognosis [33,50,51].

1.5.4 Molecular Factors

Molecular factors or potential RCC biomarkers can be found in tissue samples, as well as in the peripheral blood of patients.

Tissue Sample	Blood Serum
• von Hippel-Lindau-Gene (VHL) • hypoxia-inducible factor (HIF) • vascular endothelial growth factor (VEGF) • Carboanhydrase 9 (CA9/CAIX) • mechanistic target of rapamycin (mTOR) • tumour suppressor protein p53 (p53)	• thrombocytosis • neutrophilia • vascular endothelial growth factor (VEGF) • serum amyloid A (SAA) • carbonic anhydrase 9 (CA9/CAIX) • antigene Ki-67 • tumour suppressor protein p53 (p53)

Tab. 5: Prognostic biomarkers listed by their origin: tissue sample or blood serum

The correlation between RCC-specific biomarkers and the OS-rate could potentially substantiate an independent predictive value for e.g.: Ki-67, CA9 and thrombocytosis.
Because a prospective validation of these biomarkers, as well as of several prognostic models for RCC patients is difficult, they have not yet made it into the clinical day-to-day routine in the treatment of RCC [52].

1.5.5 MSKCC-Score ('Motzer'-Criteria)

At the Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City, a dedicated medical oncologist and expert on RCC, Robert J. Motzer, developed a model in 1999 where different risk factors for patients with metastatic RCC were combined to give a prognosis about their survival. It was dubbed the Motzer-Score[53].
Three years later, in 2002, the score was extended to the so called Motzer-Criteria by validating the score on RCC patients that were treated with interferon-α [54].

Five risk factors are included into this score. For each confirmed risk factor one point is given. According to the amount of points, patients can be categorised into three different risk groups.

The risk factors include:

- low Karnofsky PS(<80%)
- high serum lactate dehydrogenase (>1.5 times upper limit of normal [ULN])
- low haemoglobin (< lower limit of normal [LLN])
- high 'corrected' serum calcium (>10 mg/dL)
- time from initial RCC diagnosis to start of interferon-alpha therapy of less than one year

With zero points patients are placed into the 'favourable-risk' group with a median survival time of 30 months. Patients with one to two risk factors are placed into the 'intermediate-risk group with a median life expectancy of 14 months. At last, patients that have three or more points, are classified to be in the least favourable 'poor-risk-_ group, with only five months of a median survival time [53,54].

1.5.6 IMDC prognostic model ('Heng'-Criteria)

The International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) prognostic model was introduced since VEGF-targeted therapies started to play a growing role in the treatment of RCC. Hence it became necessary to create a model that is applicable to all patients with metastatic RCC undergoing such treatments [55].

A team around Daniel YC Heng from Calgary, Alberta, Canada, gathered data on prognostic factors regarding the OS of RCC-patients receiving VEGF-targeted therapies, which included sunitinib [8], sorafenib [10], bevacizumab [56,57], pazopanib [58], and axitinib [59].

Including haemoglobin less than the LLN, serum corrected calcium greater than the ULN, Karnofsky PS below 80% and the time for the initial diagnosis to initiation less than one year, this model would validate four out of the five adverse

prognostic factors of the MSKCC-Score. Additionally, an absolute neutrophil count greater than ULN and platelets greater than ULN were introduced because they were also found to be independent adverse prognostic factors.
Along the lines of the MSKCC-Score, RCC-patients are segregated into three risk groups: favourable (no risk factors), intermediate (one or two risk factors), and poor (more than three risk factors)[55].
In 2013 an external validation study, conducted on 1028 patients from 13 international cancer centres came to following conclusion: the favourable risk group had a median OS of 43.2 months, the intermediate risk group of 22.5 months and the poor risk group of 7.8 months [60].

1.5.7 Mayo Clinic Scoring System (Leibovich-Prognosis-Score)

At the Mayo Clinic in Rochester, Minnesota, a urologist named Bradley C. Leibovich developed a prognostic scoring algorithm for surgically treated, non-metastatic RCC patients in 2003. It is based on features (shown in table 6) that can be used to predict progression to metastatic disease after patients have undergone radical nephrectomy for clinically localised ccRCC [61].

Parameters		Score-Points
tumour-stage	T1a	0
	T1b	2
	T2	3
	T3a	4
	T3b	4
	T3c	4
	T4	4
regional lymph node status	NX	0
	N0	0
	N1	2
	N2	2
tumour size	<10 cm	0
	>10 cm	1
nuclear grade	G1	0
	G2	0
	G3	1
	G4	3
histologic tumour necrosis	no	0
	yes	1

Tab. 6: Parameters with associated score-points used for the Mayo Clinic Scoring System

Maximum eleven points can be achieved and as in the MSKCC-Score, patients will be categorized into three different risk groups. Patients achieving zero to two points are therefore in the low risk category for disease progression. Three to five points indicate an intermediate risk and from six to eleven points there is a high risk of developing metastases over time.

In 2011, Martin Pichler, Georg C. Hutterer and colleagues published an external validation of the Leibovich-Prognosis-Score, using a European RCC-patient-cohort. The same team achieved an enhancement of the accuracy of this score, supplementing the factor of vascular invasion, in the following year [61,62].

1.6 Disease Management

Importantly, it has to be assessed whether the disease represents a local/loco-regional tumour or if it is already metastatic [63].

1.6.1 Treatment of local/loco-regional RCC

The treatment of choice for locally restricted tumours is principally surgical, with the extent of the procedure depending on the pathological tumour stage.
In pT-1(tumour-diameter <7cm) tumours, an organ-saving partial nephrectomy is recommended [64].
Alternatively, radiofrequency ablation or cryo-ablation can be used. These treatments are suitable especially for small cortical, hereditary RCCs und multiple bilateral tumours [65]. In elderly RCC-patients that are frail and have many comorbidities or a low life expectancy, with solid tumours that do not exceed 4cms in size, active surveillance might represent an alternative option [66].
At pT-2 stage (tumour-diameter >7cm) the laparoscopic radical nephrectomy is considered to represent the method of choice.
For locally advanced RCCs stages pT-3 and pT-4, surgically open radical nephrectomy is the state-of-the-art treatment at the moment. Unfortunately, up to now, there is no standardized indication outside controlled clinical studies for adjuvant or neoadjuvant therapy approaches [63].

1.6.2 Treatment of metastatic RCC

The generally recommended surgical therapeutic option in metastatic RCC is a debulking operation on patients with large primary tumours and good prognostic score. Recently, a European, randomized phase III non-inferiority trial (CARMENA) was published that demonstrated non-inferiority in metastatic MSKCC-intermediate- and poor-risk RCC patients who were treated with the TKI Sunitinib vs. the combination therapy of Sunitinib plus cytoreductive nephrectomy. Additionally, the clinical benefit was higher in the Sunitinib-treated patient cohort

vs. the combination therapy [67]. Thus, the paradigm of cytoreductive nephrectomy representing the gold standard in the metastatic RCC setting is questioned in the era of targeted therapies.
Nevertheless, patients with solitary, easily accessible lung metastases, solitary, resectable intra-abdominal metastases or only partly responding metastases on immunotherapy/targeted therapy, should undergo a metastasectomy [63] when surgically feasible.

Only if all tumour deposits can be completely excised, the treatment can have a curative purpose. This also counts for patients with primary tumours in place and single or oligo- metastatic resectable cancer. In most cases of metastatic disease, cytoreductive nephrectomy remains palliative and systemic treatment becomes necessary [68].

The principle treatment of metastatic RCC is systemic therapy, especially in connection with ccRCC. The fast development and availability of novel agents in metastatic RCC lead to new guideline-updates every couple of months [69], whereby in particular novel immuno-therapeutic combinations and approaches start to enter the large field of systemic treatment options in RCC [70].

Furthermore, a retrospective analysis of over 4000 RCC-patients was able to prove a significant improvement of prognosis in patients using angiotensin converting enzyme
(ACE)-inhibitors [71].

Radiotherapy plays a minor role in the primary treatment of RCC, nevertheless it is being used in certain clinical situations such as: [63]

- non-resectable tumours or patients that are unable to undergo surgery
- in palliative care of symptomatic metastases or to avoid metastatic progression in critical locations (brain, bones)
- treatment of bone-marrow compression by metastases
- treatment of cerebral metastases

Furthermore it is recommended that bone metastases caused by RCC should be treated with skeleto-protective bisphosphonates, such as Zoledronic acid or Denosumab to reduce the frequency of skeletal-related events (SREs), like pathologic fractures, spinal cord compression, radiation therapy, or surgery to the bones [72,73].

2 Materials and Methods

This retrospective analysis included data from 677 consecutive, non-metastatic ccRCC-patients who underwent curative radical or partial nephrectomy at the Department of Urology at the Medical University of Graz between 2000 and 2010. The cohort of patients consisted of 404 men (59.7%) and 273 women (40.3%) with a median age of 65 years (minimum: 9 years, maximum: 8 years, with 50% of all patients aged between 57 and 76 years). All clinico-pathological data were retrieved from medical records from the Department of Urology, as well as from pathology reports from the Institute of Pathology at the same institution. Pathologic T-stages were uniformly adjusted according to the 7th edition of the TNM classification system [46]. Other documented parameters included the presence of histological tumour necrosis, sarcomatoid transformation, histological RCC subtype, tumour grade, as well as patients' age and gender. All pre-treatment laboratory data were obtained within one week before surgical intervention. Patients' post-operative surveillance included routine clinical and laboratory examination; regarding imaging methods, X-rays of the chest and abdominal ultrasound were predominantly used, especially in patients with a low relapse risk (pT-1, G1-2), whereas CT or MRI was performed in all other patients as previously reported [61]. Follow-up evaluations were performed every six months for the first five years and annually thereafter for locally advanced tumours. In organ-confined cancers, imaging was performed twice in the first year after surgery and annually thereafter. Dates of death were obtained from the central registry of the Austrian Bureau of Statistics. This study was approved by the ethical committee of the Medical University of Graz (29-150 ex 16/17).

2.1 Statistical analysis

Co-primary endpoints of this study were OS (the time between diagnosis and patients' death of any cause) and MFS [the time between diagnosis and occurrence of distant metastases]. Patients were categorized using a cut-off value of 100/µL (basophil granulocytes) according to a calculation by ROC analysis. The ideal cut-off value for the continuously coded laboratory parameter (basophil

granulocytes) was calculated by testing all possible cut-offs that would discriminate between survival and cancer-related death by Cox proportional analyses. The ideal cut-off value was then rounded to clinically relevant values as previously reported [74]. The relationship between the preoperatively assessed basophil granulocytes and clinico-pathological parameters was studied by non-parametric tests (χ2- and Mann-Whitney's U-test). Patients' OS and MFS were assessed using the Kaplan-Meier method and compared by the log-rank test. Backward stepwise multivariate Cox proportion analysis was performed. Hazard ratios (HR's) estimated from the Cox analysis are reported as relative risks with corresponding 95% confidence intervals (CI's). All statistical analyses were performed using the Statistical Package for Social Sciences version 18.0 (SPSS Inc., Chicago, IL, USA). A two-sided $p<0.05$ was considered statistically significant.

3 Results

Overall, a total of 677 patients with non-metastatic ccRCC were included into this study. Descriptive clinico-pathological parameters of the study cohort are shown in Table 7.

Parameters	No. (%)
Age at operation (yrs.)	
mean ± SD	63.8 ± 12.0
median	65.0
range	9.0-88.0
Gender	
Male	404 (59.7)
Female	273 (40.3)
pathologic T-stage (TNM 2010)	
pT1a	334 (49.3)
pT1b	117 (17.3)
pT2a	32 (4.7)
pT2b	5 (0.7)
pT3a	169 (25.0)
pT3b	16 (2.4)
pT3c	2 (0.3)
pT4	2 (0.3)
Tumor grade	
G1	170 (25.1)
G2	410 (60.6)
G3	92 (13.6)
G4	5 (0.7)
Presence of histologic tumor necrosis	
No	512 (75.6)
Yes	165 (24.4)
Sarcomatoid transformation	
No	653 (96.5)
Yes	24 (3.5)
Basophil granulocytes	
mean ± SD	0.0162 ± 0.03810
median	0.0000
<100/µL	570 (84.2)
≥100/µL	107 (15.8)

Tab. 7: Descriptive clinico-pathological parameters of the study cohort comprising of patients with non-metastatic clear cell renal cell carcinoma (n=677).

Applying ROC-analysis as mentioned above, the value regarding the preoperative basophil granulocyte counts was 100 cells/µL. 570 (84,2%) of the 677 patients were measured with less than100 basophils/µL before the surgical intervention, while 107 (15,8%) patients were measured with more.

As shown in Fig. 3, the Kaplan Meier curves indicate that there are no statistically significant differences in OS between those two groups below and above the cut-off value. This was confirmed by the log-rank test (p=0.249).

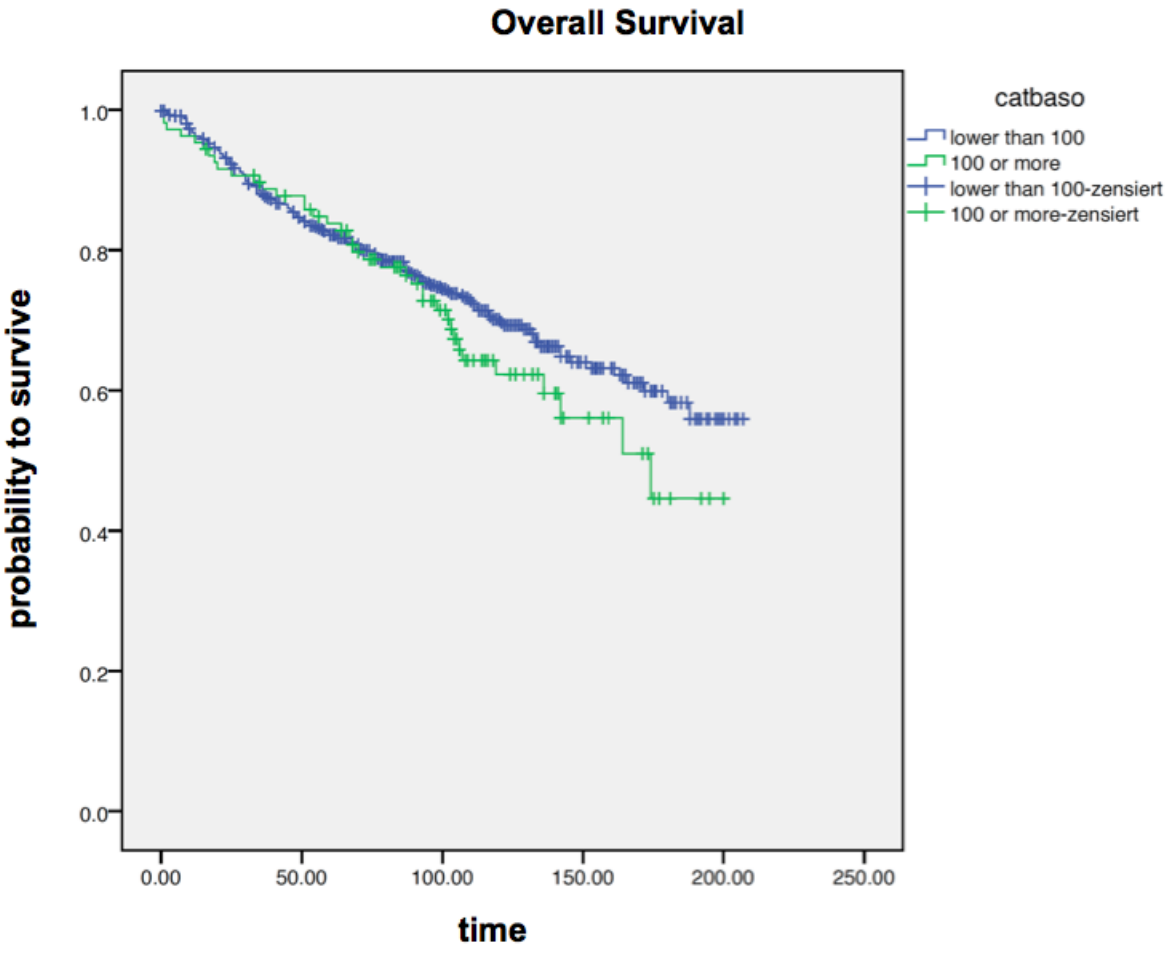

Fig. 3: Kaplan-Meier curves for the preoperative basophil granulocyte count regarding OS.

The associations between the preoperative basophil granulocyte count with clinico-pathological parameters, as well as the multivariate analysis of clinico-pathological parameters for the prediction of OS at 10 years are depicted in Table 8.

Parameters	OS	
	HR (95%CI)	*p*-value
Gender		
Male	1 (reference)	0.152
Female	0.808 (0.603-1.082)	
Age at operation (yrs.)		
<65	1 (reference)	<0.001
≥65	1.038 (1.032-1.052)	
Tumor grade		
G1+G2	1 (reference)	0.050
G3+G4	1.277 (1.000-1.632)	
pathologic T-stage		
pT1	1 (reference)	
pT2	1.365 (0.721-2.583)	0.339
pT3+pT4	1.381 (1.012-1.885)	0.042
Presence of tumor necrosis		
No	1 (reference)	0.669
Yes	1.074 (0.773-1.493	
Basophil granulocytes		
<100/µL	1 (reference)	0.121
≥100/µL	1.332 (0.927-31.914)	

Tab. 8: Multivariate analysis of clinico-pathological parameters for the prediction of overall survival (OS) at 10 years in patients with non-metastatic clear cell renal cell carcinoma (n=677).

It reveals that patients' age at operation (<65 *vs.* ≥65yrs., HR=1.038 [95%CI=1.023-1.052], *p*<0.001), as well as pathologic T-stage (T1 *vs.* T3+T4, HR=1.381 [95%CI=1.012-1.885], *p*=0.042) were independent predictors regarding OS, whereas tumour grade (G1+G2 *vs.* G3+G4, HR=1.277 [95%CI=1.000-1.632], *p*=0.050) was borderline significant and an elevated pre-treatment basophil granulocyte count (<100/µL *vs.* ≥100/µL, HR=1.332 [95%CI=0.927-1.914], *p*=0.121) failed to achieve independent predictor status regarding OS (Fig. 3).

Fig. 4 shows the Kaplan-Meier curves for the preoperative basophil granulocyte count regarding MFS, which equally failed to achieve statistical significance (p=0.314).

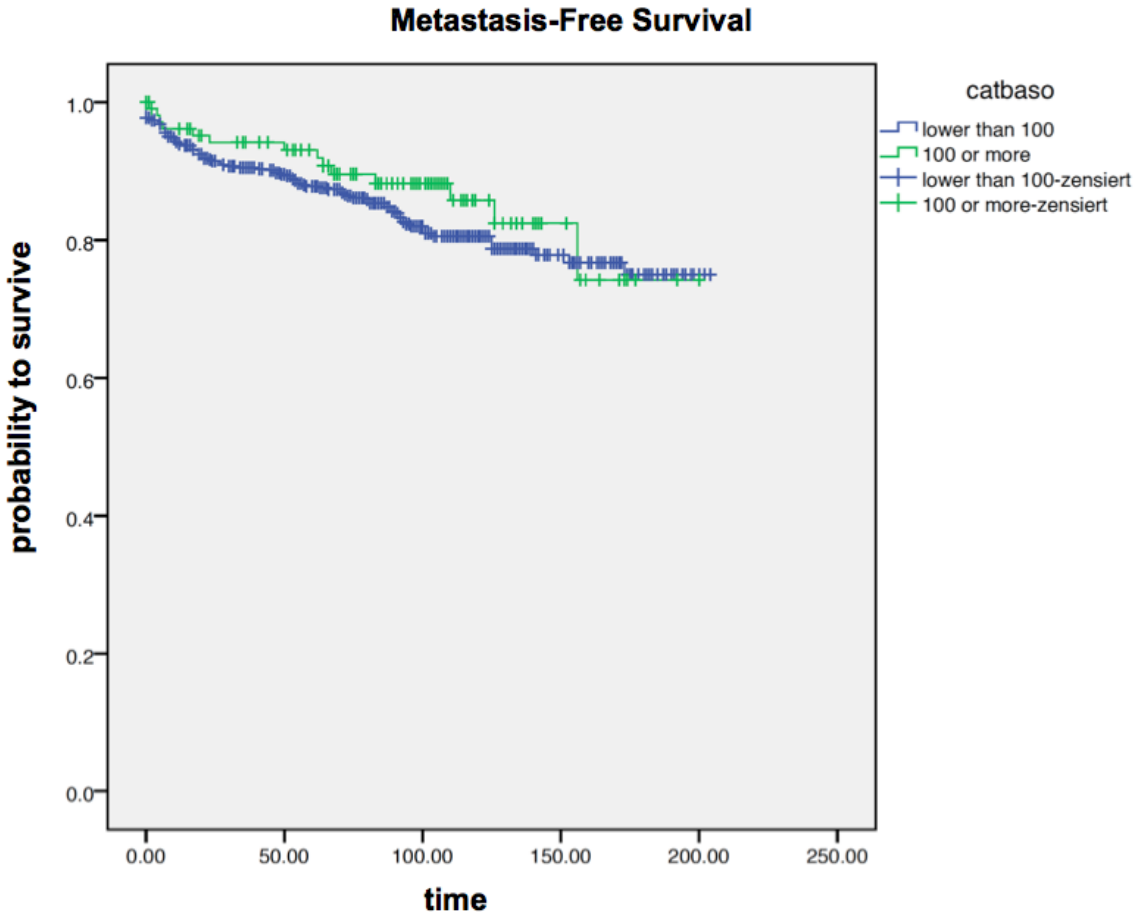

Fig. 4: Kaplan-Meier curves for the preoperative basophil granulocyte count regarding MFS

4 Discussion

Renal cell carcinoma represents a relatively rare disease that accounts for up to 2-3% of all malignant tumourous diseases. After prostate cancer and bladder cancer it is the 3rd most common malignant disease of the urinary tract. It is the most common cancerous disease of the kidney and with 83% the ccRCC is by far the most frequent histological subtype of RCC. With an estimated five year OS rate of ~49% RCC is a relatively deadly disease with ccRCC being not only the most common, but also the most dangerous of all histological subtypes.

In recent years, an enormous research effort was undertaken regarding various biomarkers and it was found that certain markers might have a positive predictive value on the outcome of RCC-patients. Several clinico-pathologic scoring systems have been published, such as the 2002 American Joint Committee on Cancer (AJCC) TNM stage groupings, the UCLA Integrated Scoring System (UISS), postoperative prognostic nomograms, the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score, the Karakiewicz nomogram, and the Leibovich prognosis score, to name some prominent ones.[75–77]
Naturally, various problems arise, regarding the comparability of medical scoring systems in general: 1) They are each generated in different patient cohorts with distinct clinico-pathologic features. 2) They concern different timelines, using various methodological approaches, as well as endpoints. 3) Some major scoring systems were externally validated, while others were not. 4) They often lack biomarker-driven information and data, which would help to better understand underlying molecular pathways and explains, why several groups of researchers do focus on detecting and/or integrating biomarkers (mostly blood-based), as well as clinico-pathologic patient features into combined models.

Our retrospective study includes a total of 677 Patients and is therefore relatively large compared to other studies concerning this topic[78]

Regarding OS results of the multivariate cox proportional analysis, patients' age at operation (p<0.001), as well as pathologic T-stage (p=0.042) emerged as

independent predictors, whereas tumour grade (p=0.050) turned out to represent a borderline significant predictor and the preoperatively assessed basophil granulocyte count (p=0.121) failed to achieve independent predictor status.

Up to date, to the best of our knowledge, evidence demonstrating a robust link between the preoperative basophil count and RCC-patients' survival has not been published so far, whereas blood parameters, such as preoperative haemoglobin levels, neutrophil counts and blood platelet size have proven to do so and therefore have shown a potential to be integrated into clinically applicable models for survival prediction purposes in RCC-patients.

Basophil granulocytes are a part of the white blood cell spectrum, they perform phagocytosis and are responsible for inflammatory reactions during the immune response [79(p85),80(p180)]. Moreover, they are also known to be contributing to allergies by having protein receptors on their cell surface that binds immunoglobulin E (IgE). It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens [81].
Particularly their involvement in allergic reactions might awaken research interests, since there is the hypothesis of a general inverse association between cancer diagnosis and a predisposition towards allergies with evidence to actually support it. For example, in 2016, a large retrospective study was performed by Kozłowska R et al., who found that the overall incidence of allergies, particularly allergic rhinitis, was lower in patients with some types of cancer [82].

After all, the observed missing association between the preoperative basophil count and patients' survival in our study cohort remains elusive. Acknowledging important limitations of our study, such as the retrospective nature of data assessment, as well as multiple surgeons involved and the lack of a central pathology review, we do believe that our finding might be of importance, if tested and reproduced in other independent RCC-patient cohorts.

4.1 Conclusion

In the cohort studied, an elevated pre-treatment basophil granulocytes count was not confirmed as an independent predictor of OS in the non-metastatic clear cell RCC setting.

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