Diplomarbeit

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

eingereicht von:

Martin Breiteneder

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr.med. univ)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Urologie

unter der Anleitung von

Assoz.-Prof. Priv.-Doz.Dr.med.univ. Georg C. Hutterer

und

Assoz.-Prof.Priv.-Doz.Mag.rer.nat.Dr.med.univ. Martin Pichler

Alberndorf in der Riedmark, February 5, 2019

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe

Alberndorf in der Riedmark, February 5, 2019 Martin Breiteneder eh.

Contents

List of Abbreviations

RCC Renal cell carcinoma

HPRCC Hereditary papillary renal cell carcinoma

BMI Body mass index

PY Pack years

GFR Glomerular filtration rate

CT Computed tomography

MRI Magnetic resonance imaging

TNM Tumour, node, metastases

WHO World Health Organisation

ISUP International Society of Uropathology

UISS UCLA Integrated Staging System

ECOG- PS Eastern Cooperative Oncology Group Performance status

OS Overall survival

CSS Cancer- specific survival

PFS Progression- free survival

INF Interferone

VEGF Vascular endothelial growth factor

MSKCC Memorial Sloan-Kettering Cancer Center

EAU European Association of Urology

RFA Radio frequency ablation

List of Figures

List of Tables

Abstract Deutsch

Einleitung

Das Nierenzellkarzinom repräsentiert den häufigsten malignen Tumor in der Niere des Erwachsenen. Inflammatorische Parameter wie die Hämoglobinkonzentration, die Anzahl der Thrombozyten oder die absolute Anzahl von Leukozyten können die Tumoraktivität beeinflussen und so eine wichtige prognostische Rolle in Bezug auf das Überleben bei Patientinnen und Patienten mit unterschiedlichen Tumorerkrankungen spielen. Wir untersuchten die potentielle prognostische Aussagekraft der präoperativ erhobenen eosinophilen Granulozyten in einer grossen europäischen Kohorte von Patientinnen und Patienten mit nicht metastasiertem, klarzelligem Nierenzellkarzinom.

Methoden

Klinisch- pathologische Daten von 677 Patientinnen und Patienten mit nicht metastasiertem, klarzelligem Nierenzellkarzinom die im Zeitraum von 2000-2010 in einem akademischen Zentrum operiert wurden, wurden retrospektiv ausgewertet. Eine Woche vor der Operation wurden die Laborparameter erhoben und die Patientinnen und Patienten sodann in zwei Gruppen kategorisiert (Grenzwert 200 eosinophile Granulozyten/μl) Gesamtüberleben (OS) wurde mittels Kaplan- Meier Methode erfasst. Um die potentielle Signifikanz des präoperativen Eosinophilenwerts zu evaluieren wurde eine multivariate COX- Regression durchgeführt.

Ergebnisse

Multivariat zeigten sich Alter bei Operation (<65 vs. 65yrs., HR=1.037 [95% CI=1.023-1.052], p<0.001), Tumorgrad (G1+G2 vs. G3+G4, HR=1.329 [95%CI=1.036-1.704], p=0.025), pathologisches Tumorstadium (T1 vs. T3+T4, HR=1.387 [95%CI=1.017-1.891], p=0.039), sowie der präoperative eosinophile Granulozyten Wert (<200/μl vs. >200/μl,HR=1.472 [95%CI=1.103-1.964], p=0.009) als unabhängige Prädiktoren in Bezug auf das Gesamtüberleben.

Schlussfolgerung

In der untersuchten Kohorte zeigte sich der präoperativ erhobene Wert der eosinophilen Granulozyten als unabhängiger Prädiktor für das Gesamtüberleben bei Patientinnen und Patienten mit nicht metastasiertem, klarzelligem Nierenzellkarzinom.

Abstract English

Objective

Renal cell carcinoma (RCC) represents the most common malignant tumour of the adult kidney. Distinguishable 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 pretreatment eosinophil granulocytes count in a large European cohort of patients with non-metastatic clear cell RCC.

Material and Methods

Clinico-pathological data from 677 consecutive, non-metastatic clear cell RCC patients, operated between 2000 and 2010 at a single tertiary academic center, were evaluated retrospectively. Pretreatment laboratory parameters were assessed within one week before surgery. Patients were categorized using a eosinophil granulucytes-cutoff value of $200/\mu$l according to a calculation by receiver-operating curve (ROC) analysis. Overall survival (OS) was assessed using the Kaplan-Meier method. To evaluate the potential prognostic significance of the pretreatment eosinophil granulocytes count, a multivariate Cox regression model was performed.

Results

In multivariate analysis, age at operation (<65 vs. ≥ 65yrs., HR=1.037 [95% CI=1.023-1.052], $p<0.001$), tumour grade (G1+G2 vs. G3+G4, HR=1.329 [95%CI=1.036-1.704], p=0.025), pathologic T-stage (T1 vs. T3+T4, HR=1.387 [95%CI=1.017-1.891], p=0.039), as well as an elevated pretreatment eosinophil granulocytes count ($<200/\mu$l vs. $>200/\mu$l, HR=1.472 [95%CI=1.103-1.964], p=0.009) achieved independent predictor status regarding OS, respectively.

Conclusion

In the cohort studied, an elevated pretreatment eosinophil granulocytes count was confirmed as an independent predictor of OS in the non-metastatic clear cell RCC setting.

1 Introduction to renal cell carcinoma

1.1 Epidemiology

In the year 2014, 1254 patients in Austria have been diagnosed with ICD C64, which includes all malignant kidney diseases except urothelial carcinoma of the kidney basin [1]. Over 90 % of those patients suffer from renal cell carcinoma (RCC), which makes it the most prevalent malign kidney tumour in the adult population [2].

The incidence rates of RCC diversify all over the world with a higher incidence in well developed countries like Northern America and Europe, especially in the east of Europe, and significantly lower incidence rates in Africa and Asia. The incidence varies from about 1 per 100.000 residents (Africa) to a maximum of 22 per 100.000 in the Czech Republic (Fig.: 1.1) [2].

The incidence rates are reported to increase worldwide but seem to stabilise in well developed countries [2].

For Austria, a relatively stable incidence rate until 2030 is predicted, with a total increase of 7 % compared to 2009. Incidence rates for Austria are prognosted to diversify, with a decrease for the female gender of 18% against an increase of 25% for the male gender. Although the incidence rate of RCC in Austria increases, mortality rates are decreasing (34% for females, 29% for males) (Fig.: 1.2) [4].

Renal cell carcinoma represents a disease of the older population. The average age at diagnosis is 67 years in males and 72 years in females. Not only are there differences between the genders, in males, RCC is with 3.5 % of all new diagnosed malignant neoplasms more prevalent compared to females with 2.5 % of all malignant neoplasms [5].

One reason for the rising incidence- and falling RCC mortality rates might be more frequently performed imaging, often leading to an incidental, as well as an earlier detection of renal masses [2].

Figure 1.1: Wordwide incidence rates of kidney cancer [3]

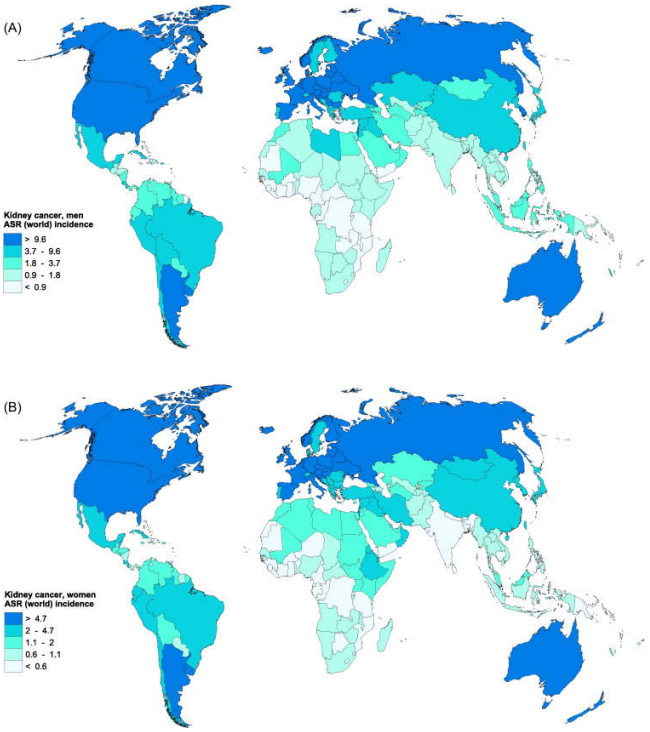
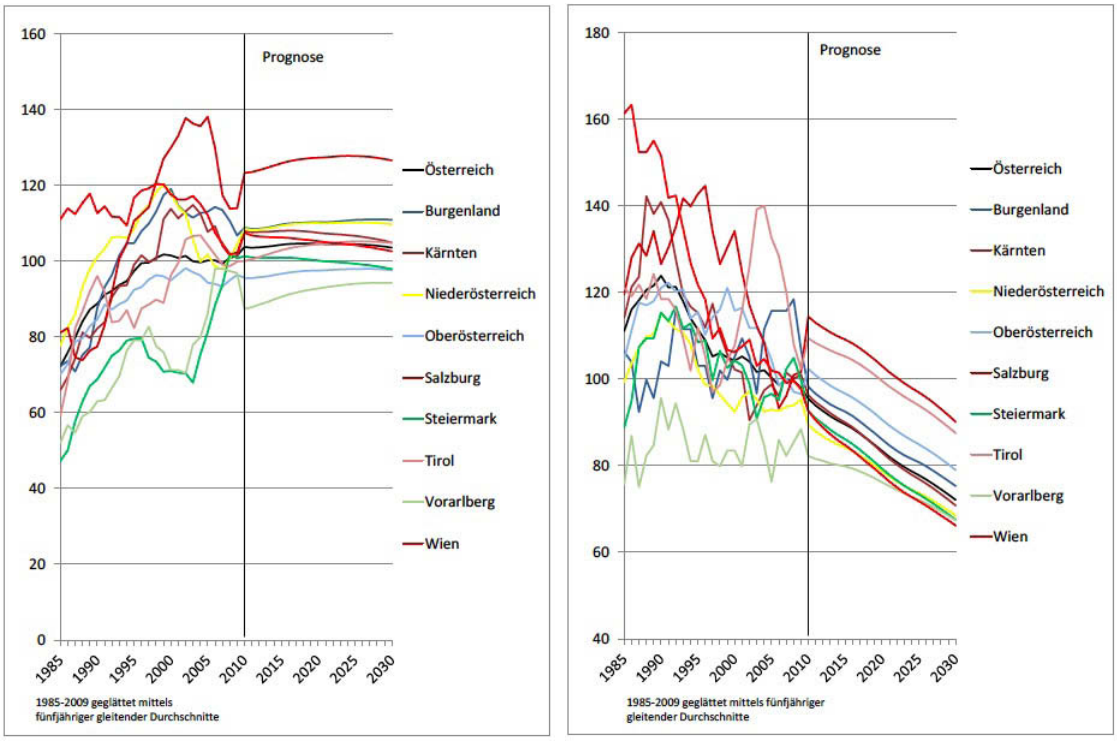


Figure 1.2: Index of disease (left) and mortality (right) in Austria till 2030 [4]



1.2 Etiology

Risk factors for the development of RCC can be divided in modifiable and non- modifiable ones.

1.2.1 Non- modifiable risk factors

In particular, end- stage renal disease with dialysis, as well as hereditary variants of RCC are regarded to represent non- modifiable risk factors.
Patients with end-stage renal disease have shown to develop 4- 5 times more often renal tumours (besides other malignant diseases) [6].
Up to date, hereditary RCC variations can be found as part of at least ten distinct genetic syndromes. The most important are:

- von Hippel-Lindau syndrom
- Birt-Hogg-Dubé-Syndrom
- Hereditary papillar RCC (HPRCC)
- Hereditary Leiomyomatosis

In 3 % to 5 % of all RCCs germ- cell mutations can be detected. First and second grade relatives of such patients also show a higher risk for a development of RCC. Patients with hereditary forms of RCC are of younger age when they are diagnosed and often show multifocal lesions in both kidneys [7].

1.2.2 Modifiable risk factors

Risk factors can best be summarized as lifestyle- based and contain tobacco smoking, overweight, high blood pressure and poor nutrition patterns [8, 9].
Tobacco smoking is a well documented risk factor for RCC. The risk is 54 % elevated for males and 22 % for females. Also the packyears (PY), defining how much someone smokes in his life, matter and elevate the risk for the development of RCC [8].
Overweight, which means higher body mass index (BMI) than recommended, was shown to significantly highten the risk for several types of malign diseases.
Especially for RCC, studys have shown, that people with elevated BMI have a higher risk for development of RCC. Weight gain in younger years (<50 years) has been shown to increase the risk for RCC, gaining weight later on doesnt seem to have influence [10].

For people with a BMI of over 27, studies were able to show that the risk for developing RCC is almost twice as high as for the population with a BMI of 20. Even a slighty higher bodyweight is associated with a higher risk for the development of RCC [11].
High blood pressure represents another well established risk factor for RCC. It seems to have influence if the systolic or the diastolic blood pressure is out of the normal range. A higher systolic blood pressure (130 mmHg - 149 mmHg) leads to a 1.7 times higher risk for RCC (compared to <130 mmHg), while a systolic blood pressure of over 150 mmHg doubles the risk. Additionally, for diastolic blood pressure (normal <85 mmHg) higher than 105 mmHg an 1.6 times elevated risk for RCC could been identified [12].
Patients who maintain a normal blood- pressure under anti- hypertensive medication do not show an elevated risk for RCC, which means that the usage of those substances can lower the risk for RCC and is not an additional risk factor [9].
All the described risk factors have been investigated separately, are independent from each other and lead to a higher possibility of developing RCC.

1.3 Symptoms and diagnosis of RCC

As all other malignant kidney diseases, RCC represents an often late and incidentally diagnosed tumour. Clinical symptoms can be very rare and unspecific. Initial symptoms, which are often linked to a higher progression of the disease, can be macro- or microhaematuria, wheras pain in the flank or a palpable renal mass typically represent late symptoms and can be combined with general signs of a malignant disease like unintended weight loss, anaemia, subfebrile temperature and general weakness [13, 14].
The above mentioned classical trias (palpable tumour, painless macro- haematuria, flank pain) is nowadays rarely seen and typically affects patients with large tumours, distant metastases, higher pathological tumour stage and invasion in other organs more often compared to patients with incidentally detected RCCs, whose overall survival (OS) is significantly higher [15].

Associated with metastatic stages of RCC, paraneoplastic symptoms, like pathological fractures and bone pain, hypercalcaemia due to dysregulation of parathormone, as well as polycythaemia as a result of pathological erythropoetin levels can occur. Stauffer-syndrome, which consists of an elevation of liver enzymes and coagulation problems (hepatic dysfunction) can also be found as part of paraneoplastic symptoms, which usually disappear in most cases after removement of the tumour [16].

A full blood count, kidney- (serum creatinine, glomerular filtration rate (GFR)), liver function- and coagulation- study should be taken from all patients [13].

1.3.1 Sonography

Sonography represents a powerful and non- invasive imaging method to detect cysts or other, potentially tumorous lesions in the kidney. Sonography is an interdisciplinary used imaging tool and ubiquitarily available, increasing the detection rate of asymptomatic renal masses nowadays [17].
Small renal masses are in general more difficult to stage and evaluate sonographically. Moreover, an inter-observer variability can be observed, and thus the sensitivity of sonographic evaluations strongly depend on how skilled and experienced the examiner is. Because of that, contrast enhanced computed tomography (CT) has been established as method in detecting and characterizing RCC [14, 18].

1.3.2 CT- and MRI imaging

For a more detailed characterization of renal masses, CT or in some cases, MRI are used. Abdominal CT shows the morphology and function of the contralateral kidney, gives information about the primary tumour and its expansion and is also used to find venous involvement, enlargement of locoregional lymph nodes and the condition of other solid organs which represent important prognostic factors [18].
With a contrast enhanced CT most malign masses can be differentiated. Contrast enhancement is a strong indicator for RCC and therefore preferredly used for diagnosis [14].
Renal cysts are radiologically classified via the Bosniak classification (Table 1.1). This classification system is based on the contrast enhanced CT image and to determine whether a cyst is malign [19].
A commonly used examination protocol for contrast enhanced CT consists of native phase (CT before intravenous application of contrast fluid), arterial phase, venous phase and urographic phase. The native phase is important to find concrements which could later on be mistaken for tumour cells uptaking contrast fluid [14].
MRI can be used to gain additional information or when there are contraindications for CT imaging like pregnancy or allergic reactions against contrast fluids in a patients past medical history [18].
CT- and MRI imaging can also be used to help at percutanous biopsy and makes it

Table 1.1: Bosniak classification of renal cysts [19]

Bosniak	Features	Work up
I	Simple benign cyst with a hairline-thin wall without septa, calcification, or solid components. Same density as water and does not enhance with contrast medium.	Benign
II	Benign cyst that may contain a few hairline-thin septa. Fine calcification may be present in the wall or septa. Uniformly high-attenuation lesions < 3 cm in size, with sharp margins without enhancement.	Benign
III	These are indeterminate cystic masses with thickened irregular walls or septa with enhancement.	Surgery
IV	Clearly malignant containing enhancing soft-tissue components.	Surgery

more safe and precise. According to the newest guidelines of the European Association of Urology (EAU), percutanous biopsy should only be performed when it has a therapeutical consequence, for example if the renal mass cannot be clearly identified or when metastases are found and a histological examination for targeted therapy is needed. If radiological findings show clearly that there is an indication for surgery or for frailty patients which are only suitable for conservative treatment a biopsy is not indicated [13, 14].
Biopsy represents a low risk for the patient (significant bleeding rarely exists and is in most cases self limiting), the risk for seeding of tumour cells is very low. Especially for older or unfit patients, a biopsy clearly tells the dignity of a renal mass and can prevent unnecessary therapy which can have severe side effects. For non diagnostic biopsies, a repeated biopsy should be performed [20, 21].

1.4 Staging, Grading and Prognosis

Renal cell carcinoma is classified via the TNM classification system for solid tumours, which includes various factors like tumour size, venous invasion, renal capsular invasion, adrenal involvement, lymph node and distant metastasis. Staging is done from stage I to stage IV and depends on findings from the TNM classification (Fig.: 1.3) [22].
Grading of RCC is done based on histological findings. Depending on how far the tumouros tissue corresponds to the original tissue, the tumour is sorted into grades (I to IV). A lower grade means the tumour is nearly as configurated as the healthy tissue which means better prognosis for the patient. Higher grading means the tumour cells

Figure 1.3: TNM classification of RCC [13, 22]

T - Primary Tumour		
TX	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
T1	Tumour ≤ 7 cm or less in greatest dimension, limited to the kidney	
	T1a	Tumour ≤ 4 cm or less
	T1b	Tumour > 4 cm but ≤ 7 cm
T2	Tumour > 7 cm in greatest dimension, limited to the kidney	
	T2a	Tumour > 7 cm but ≤ 10 cm
	T2b	Tumours > 10 cm, limited to the kidney
T3	Tumour extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota fascia	
	T3a	Tumour grossly extends into the renal vein or its segmental (muscle-containing) branches, or tumour invades perirenal and/or renal sinus fat (peripelvic fat), but not beyond Gerota fascia
	T3b	Tumour grossly extends into the vena cava below diaphragm
	T3c	Tumour grossly extends into vena cava above the diaphragm or invades the wall of the vena cava
T4	Tumour invades beyond Gerota fascia (including contiguous extension into the ipsilateral adrenal gland)	

N - Regional Lymph Nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)

M - Distant Metastasis	
M0	No distant metastasis
M1	Distant metastasis

TNM stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

Table 1.2: Fuhrmann- Grading [23]

Grade	Nuclear size	Nuclear quality	Nucleoli
I	10μm	small, round and uniform	absent to inconspicuous
II	15μm	larger, crinkly nuclear membrane	inconspicuous
III	20μm	irregular nuclear membranes	conspicuous
IV	>20μm	irregular nuclear membrane, pleomorphic, multi-lobed with clumped chromatin; +/- sarcomatoid spindle cells	very prominent

vary widely from the original ones, causing a worse prognosis.

Until 2015, grading has been done depending on cellbased criteria, so called Fuhrman-Grading (table 1.2). Fuhrman Grading has been established in 1982 and has been critized because of the missing validation of new RCC subtypes in the past years. Also, for an accurate prediction of the patients prognosis, Fuhrman Grading hasn't been a reliable system [23, 24].

Since 2015, ISUP Grading (table 1.3) is recommended as standard [24, 25].

RCC patients prognosis highly depends on the initial tumour stadium and is because of that, better when diagnosed early. 75 % of all diagnosed males and 77 % of females survive for at least 5 years [5].

In Stadium I 97%, in stadium II 87% and in stadium III 69% of patients with RCC survive for at least 5 years, compared to that, in stadium IV the 5 year survival only lies at 14% [26].

To improve RCC patients individual prognosis, tumour related biological factors, as well as the histological type are important and were identified in the past years. Clear cell carcinoma is often diagnosed at a lower tumour stadium (28% with T3 or higher) compared to sarcomatoid (82% with T3 or higher at diagnosis). Papillary- (17.6%) and chromophobe types (16.9%) show an advanced tumour stadium (T3 or higher) at a much lower frequency [27].

In addition to TNM and other grading scores, molecular techniques and biomarkers can be used to give more detailed information about prognosis and survival of patients with RCC.

Table 1.3: WHO/ISUP grading [24]

Grade	Features
I	Tumour cell nucleoli invisible or small and basophilic at 400 x magnification
II	Tumour cell nucleoli conspicuous at 400 x, inconspicuous at 100 x magnification
III	Tumour cell nucleoli eosinophilic and clearly visible at 100 x magnification
IV	Tumours showing extreme nuclear pleomorphism and/or containing tumour giant cells and/or the presence of any proportion of tumour showing sarcomatoid and/or rhabdoid dedifferentiation

1.4.1 Histological subtypes of RCC

Histologically, RCC can be divided into clear-cell- and non clear-cell carcinomas. Clear cell types of RCC are the most common ones found (representing approximately 82% of all RCCs) [28].
In 80% of those types a functional inactivation of von- Hippel- Lindau gene, resulting in activation of Hypoxia-inducible Factor (HIF) 1α and 2α can be detected. This activation leads to a higher expression of genes which are responsible for proliferation and neoangiogenesis. Next to those, mutations of several genes (PBRM1, SETD2, BAP1) are often found [29].
Non clear- cell carcinomas are (the most common ones):

- papillary type I and II
- chromophobe
- collecting duct
- unclassified RCC

These histological subtypes also show different genetic mutations. Papillary type I is associated with alterations of the MET gene. Papillary type II shows mutations in different genes, which are also of prognostic interest. Papillary type I and II also differ in terms of patients clinical outcomes [30].

1.4.2 Prognostic systems

Instead of using only a single parameter, like, for example, Fuhrman nuclear grade to determine an RCC patients prognosis, complex classification systems, which include anatomical, histological and clinical factors are used for an individualised and more precise prediction of the disease.
Newer findings in RCC studies, mostly molecular ones, which also have a high prognostic value are not included. To improve their prognostic accuracy, addition of molecular factors is necessary [31].
One of these classification systems, used for localised tumour stadium is the UCLA Integrated Staging System (UISS), which includes TNM stage, ECOG PS and Fuhrman grade. The UISS has been validated in clinical use and has been shown to be appliable for the prediction of prognosis in patients with RCC [31].
Moreover, UISS is used after renal surgery and categorises patients into risk groups (low, intermediate and high risk) for OS, cancer specific survival (CSS), and progression- free survival (PFS). This model can be used for all histological subtypes of RCC [32].

Table 1.4: MSKCC prognostic system [33]

Risk factor	**limit**
low Karnofsky-Index	<80%
high LDH	>1.5 times reference
low Hb	< lowest reference
high corrected serum calcium	> 10 mg/dl
Time from nephrectomy till occurence of metastases	< 1 year

For metastatic stages of RCC, one possible prognostic model is the Memorial Sloan Kettering Cancer Center (MSKCC) prognostic system, also known as so called Motzer criteria, named after the Oncologist and kidney cancer expert Robert J. Motzer [33].
It has been developed in a cohort of RCC- patients who underwent surgery and were treated with immunotherapy (INFα). Depending on how many risk factors are existing (see table 1.4), patients are sorted into one of three groups:

- low risk (average survival 30 months)= 0 risk factors
- intermediate risk (average survival 14 months)= 1-2 risk factors
- high risk (average survival 5 months)= 3 and more risk factors [33, 34]

A newer prognostic system with a higher prediction accuracy than MSKCC are the IMDC- criterias. Moreover, the IMDC- critera have been externally validated and should, because of that, be preferred [35].

1.5 Therapy

Depending on the tumour stage, there are different possibilities of treatment.

1.5.1 Non- metastatic RCC

For RCC patients with a high amount of comorbidities or patients with small renal masses ($\leq$ 4cm - T1a), active surveillance might be offered, since those patients have a low RCC- specific mortality [36].
Active surveillance means that the size of the tumour gets observed and if it shows rapid progression, an intervention can be done later on, especially since small renal masses are often expanding very slowly and rarely metastasize, active surveillance represents a

valid treatment modality. Imaging series and ongoing care can be a better option than invasive procedures in those cases [37, 38].
The curative treatment of a localised tumour stadium consists of the surgical removal, which are partial- or radical nephrectomy [39].
Partial nephrectomy, which means only the tumour and a surrounding area of normal renal parenchyma is surgically removed, is indicated for patients with only one remaining functioning kidney (or patients with bilateral RCC), patients at high risk for renal failure, patients with hereditary kidney cancer and RCC in stadium T1. In those cases, partial nephrectomy has benefits for patients in mortality compared to radical nephrectomy [40, 41].
There are also some contraindications for partial- nephrectomy such as:

- insufficient volume of the remaining parenchyma to maintain a proper organ function
- renal vein thrombosis
- unfavourable tumour location, adherence to the renal vessels
- use of anticoagulants

in these cases the whole kidney, which contents the tumour, has to be removed [13].

Radical nephrectomy includes the surgical removal of the whole kidney with the adipose capsule, the fascia renalis and regional lymphnodes. Depending on the staging of the tumour, the ipsilateral adrenal gland also has to be removed [42].
Studies have tried to find out if extended lymph node dissection and ipsilateral adrenalectomy produce better outcomes in combination with radical nephrectomy but failed to bring clear evidence. Thus, a clear recommandation for those procedures can not be given and the decision depends on individual factors [43].
Radical and partial nephrectomy can be done both, in laparoscopic (manual or robotic assisted) or conventional open fashion, but laparoscopic surgery has been shown to be better tolerated and leads to faster recovery. But due to longer ischaemia time in laparoscopic surgery, partial nephrectomy is predominantly done conventionally in an open fashion [44].

For patients who do not want, or who are not healthy enough for renal surgery and

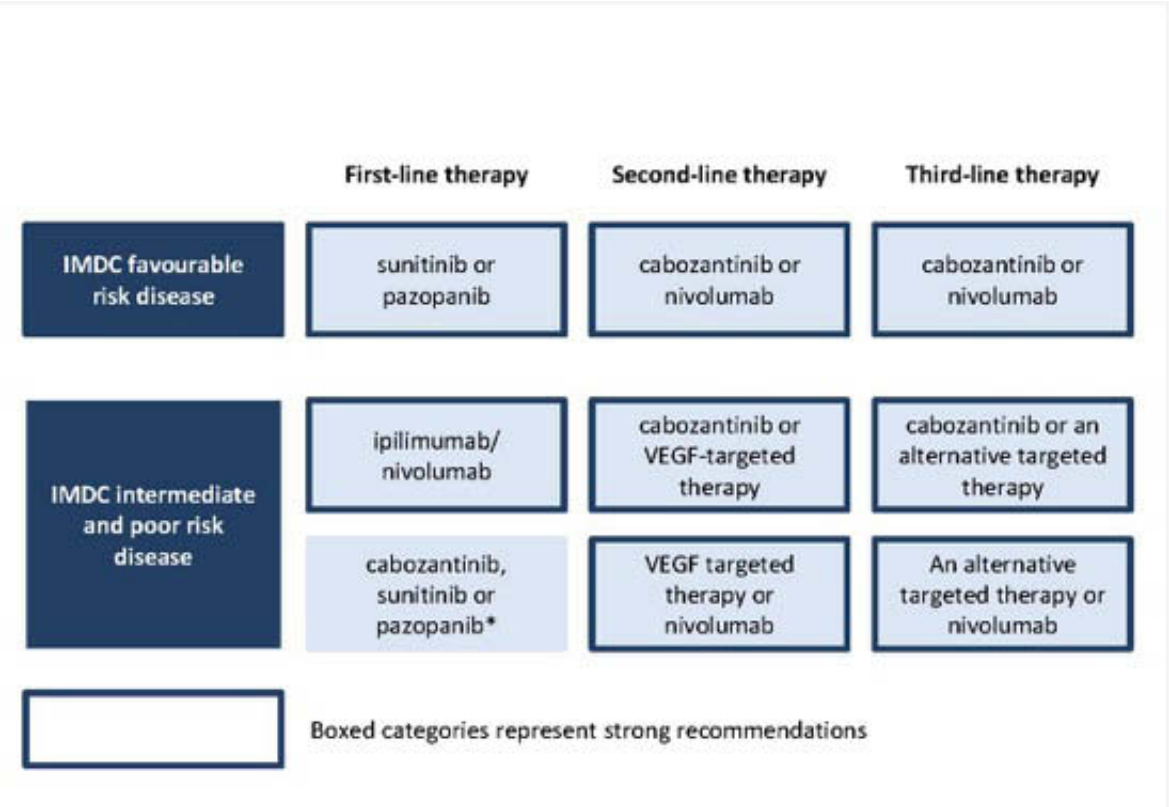
have small renal masses, local physical methods, like cryoablation or radiofrequency ablation (RFA) are possible treatment options. For these methods, renal biopsy before treatment is mandatory to confirm malignancy. Radiofrequency ablation is made percutanously under imaging control. The applicator is located in the tumour and submits electrical energy into it, which leads to a thermical coagulation and necrosis of the tumour cells. The above described local physical methods are limited to small lesions and are not efficent when there are bigger blood vessels in surrounding area (like at the renal hilus) [45].

1.5.2 Metastatic RCC

Patients with metastatic RCC do not benefit from typical cytotoxic medication. For decades, Interferon α and Interleukin 2 were used as unspecific immunotherapy exerting massive toxic side-effects, thereby only leading to very moderate survival improval. Nowadays, more often specific antibodies matched to molecular findings from the patients tumours are used [13, 46].
Also for patients with metastases, surgical treatment might be offered under certain circumstances. Nephrectomy and resection of metastases can lead to remission but is reserved for patients who do not suffer from major comorbidities. Debulking of big tumour masses can also be indicated [13].

For patients with large primary tumours, nephrectomy is recommended at the time. Because of the newly available options in the metastatic RCC setting (targeted therapies), the role and benefit of surgical treatment needs to undergo evaluation. Recently, a randomized phase III trial named CARMENA compared the outcome (OS and other parameters) of patients with confirmed metastatic clear-cell renal-cell carcinoma (MSKCC intermediate and poor risk) who received different therapies. Patients were categorized into two groups, one group received nephrectomy combined with sunitinib treatment vs. the other group of patients was treated with sunitinib alone. For the first time, this study was able to show that the survival outcome of patients with sunitinib treatment alone was non- inferior to that of patients who have undergone additional nephrectomy. Moreover, the observed clinical benefit was greater in the sunitinib treatment alone group compared to the surgically treated one [47].

Figure 1.4: Systemic therapy recommendation from EAU guidelines [13]



Systemic therapy

Because of a low responder- and 5 year OS rate, unspecific immunotherapy is getting obsolete, but for RCC patients with good prognosis a combination of INF α and the vascular endothelial growth factor (VEGF) inhibitor Bevacizumab can be considered. As a standard first line therapy, Tyrosine-kinase inhibitors like Sunitinib and Pazopanib are recommended. Tumours with a high progression risk should be treated with a mTor- inhibitor, like Temsirolimus. For non responders there are different substances from those groups available, which are then recommended as second- or higher line therapy [46, 48] (Fig.: 1.4). The fast development and availability of novel agents in metastatic RCC lead to new guideline-updates every couple of months [49], whereby in particular novel immuno-therapeutic combinations and approaches start to enter the large field of systemic treatment options in RCC [50].

1.6 Objective

The prognosis of patients with RCC depends on multiple factors, most importantly the pathological tumour stage, the presence of remote metastases or the age at diagnosis, only to name some of the discussed above.
Next to those established parameters, which can give reliable information for the patients future and prognosis and are used in a variety of pre- and postoperative scores like the UISS Modell, new risk factors for a poor long time survival are tried to be identified and are examined if they can be generalised for daily routine clinical practice in different RCC patient populations.
Therefore, blood based parameters, especially inflammatory- or haemostatic markers, are recently in the spotlight and have been investigated whether they have any significant influence on the long term survival of RCC patients.
Such immuno- or tissue based parameters, which show immune- responses, are shown to be important factors in RCC development and prognosis [51].
The pre-treatment neutrophil-lymphocyte ratio, for example, was identified as an independent risk factor for poorer outcome regarding RCC patients overall survival [52].
Several studies were able to show that blood based parameters, like a pre-treatment thrombocytosis, leucocytosis, anemia and plasma fibrinogen are independent risk factors for poorer outcomes and can be used, in addition to pathological findings, to determine an individual patients risk and prognosis. Because those laboratory values are easily determinable, they should be considered as additional risk factors [53, 54].
To the best of our knowledge, the potential prognostic significance of the pretreatment eosinophil count in non-metastatic clear cell RCC patients has not been evaluated yet.

2 Methods

This retrospective analysis included data from 677 consecutive, non-metastatic clear cell RCC patients who underwent curative radical or partial nephrectomy at the Department of Urology at the Medical University of Graz between 2000 and 2010. 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 [22]. 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 pretreatment 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 [55]. 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

The primary endpoint of this study was OS (the time between diagnosis and patients death of any cause). Patients were categorised using a cutoff value of 200/μl (eosinophil granulocytes) according to a calculation by receiver-operating curve (ROC) analysis. The ideal cutoff value for the continuously coded laboratory parameter (eosinophil granulocytes) was calculated by testing all possible cutoffs that would discriminate between survival and cancer-related death by Cox proportional analyses. The ideal cutoff value

was then rounded to clinically relevant values as previously reported [56]. The relationship between the preoperatively assessed eosinophil granulocytes and clinico-pathological parameters was studied by non-parametric tests (2- and Mann-Whitneys U-test). Patients OS was assessed using the Kaplan-Meier method and compared by the log-rank test. Backward stepwise multivariate Cox proportion analysis was performed. Hazard ratios (HRs) estimated from the Cox analysis are reported as relative risks with corresponding 95% confidence intervals (CIs). 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 clear cell RCC were included into this study. Descriptive clinico-pathological parameters of the study cohort are shown in Table 1. Applying ROC-analysis as mentioned above, the cutoff value regarding the preoperative eosinophil granulocyte counts was 200 cells/μl (s. Fig. 1). The associations between the preoperative eosinophil granulocyte counts 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 2. It reveals that patients age at operation (<65 vs. >65yrs., HR=1.037 [95%CI=1.023-1.052], p<0.001), pathologic T-stage (T1 vs. T3+T4, HR=1.387 [95%CI=1.017-1.891], p=0.039), tumour grade (G1+G2 vs. G3+G4, HR=1.329 [95%CI=1.036-1.704], p=0.025), as well as an elevated pretreatment eosinophil granulocytes count (<200/μl vs. >200/μl, HR=1.472 [95%CI=1.103-1.964], p=0.009) achieved independent predictor status regarding OS, respectively (Fig. 5).

Figure 3.1: Kaplan-Meier curves for OS

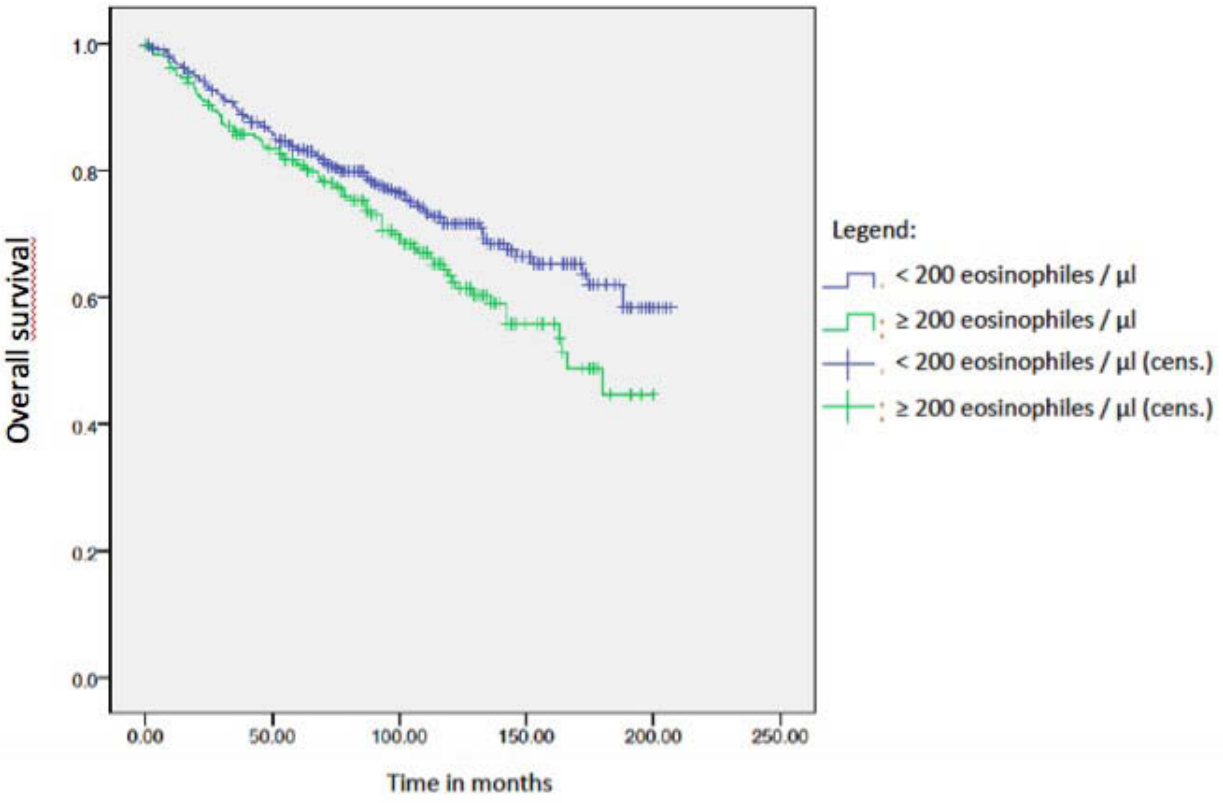


Table 3.1: Descriptive clinico-pathological parameters of the study cohort comprising of patients with non-metastatic clear cell renal cell carcinoma (n=677)

Parameter	Number (%)
Age at operation (years)	
Mean ± SD	64.0 ± 12
Median	65.0
Range	9.0 - 88.0
Gender	
Male	404 (59.7%)
Female	273 (40.3%)
pathologic T-stage (TNM 2010)	
T1a	334 (49.3%)
T1b	117 (17.3%)
T2a	32 (4.7%)
T2b	5 (0.7%)
T3a	169 (25.0%)
T3b	16 (2.4%)
T3c	2 (0.3%)
T4	2 (0.3%)
Tumour grade	
G1	170 (25.1%)
G2	410 (60.6%)
G3	92 (13.6%)
G4	5 (0.7%)
Presence of histologic tumour necrosis	
No tumour necrosis	512 (75.6%)
tumour necrosis	165 (24.4%)
Sarcomatoid transformation	
No sarcomatoid transformation	653 (96.5%)
Sarcomatoid transformation	24 (3.5%)
Eosinophil granulozytes	
$< 200/\mu$l	435
$\geq 200/\mu$l	242

Table 3.2: Multivariate analysis of clinico-pathological parameters for the prediction of overall- survival (OS) at 10 yrs. in patients with non-metastatic clear cell renal cell carcinoma (n=677)

Parameter	OS	
	HR (95% CI)	p-value
Gender		
Male	1 (reference)	
Female	0.839 (0.627 - 1.121)	0.235
Age at operation		
$<$ 65 years	1 (reference)	
$\geq$ 65 years	1.037 (1.023 - 1.052)	$<$0.001
Tumour grade		
G1 + G2	1 (reference)	
G3 + G4	1.329 (1.036 - 1.704)	0.025
pathologic T-stage		
T1	1 (reference)	
T2	1.490 (0.789 - 2.816)	0.219
T3 + T4	1.387 (1.017 - 1.891)	0.039
Presence of tumour necrosis		
No	1 (reference)	
Yes	1.082 (0.779 - 1.501)	0.639
Eosinophile granulocytes		
$<$ 200/μl	1 (reference)	
$\geq$ 200/μl	1.472 (1.103 - 1.964)	0.009

4 Discussion

Although RCC represents a relatively rare disease, it counts for about 90% of all malignant renal lesions.
A lot of potential biomarkers have been identified in the past by different research teams around the world, but none of them have been included into daily routine clinical prognostic models so far, particularly because of validation difficulties regarding the different study results [52–55, 57].
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) A lot of these models lack biomarker-driven information and data, which would help to better understand the underlying molecular pathways and explains, why several groups of researchers (including our own) focused on integrating biomarkers (mostly blood-based), as well as clinico-pathologic patient features into combined models [52–54, 57].
Nevertheless, the incorporation of non-validated biomarker-information into existing algorithms remains problematic, since it would require a revalidation of the entire algorithm.
To the best of our knowledge, the potential prognostic significance of the pretreatment eosinophil count in non-metastatic clear cell RCC patients has not been evaluated yet.
Regarding our large (n>670) non-metastatic clear cell RCC patient cohort, multivariate analysis of clinico-pathological parameters for the prediction of OS at 10 years after surgery revealed patients age at operation, pathologic T-stage, tumour grade, as well as an elevated pretreatment eosinophil granulocytes count to represent independent predictors regarding OS.

Why eosinophile granulocytes are often elevated in tumorous lesions of different entities and wheter they are active against tumour cells or only act as mediators to attract T- cells is not clear until today.

Several studies indicated that tumour infiltrating neutrophils or lymphocyte subtypes might play a meaningful role for the biological behaviour of tumour cells in RCC. For instance, Jensen et al. demonstrated that a high number of intratumoural neutrophils is a strong and independent poor prognostic factor for the clinical outcome in localised clear cell RCC [58]. In a study by Kondo et al. the authors demonstrated a favourable outcome for RCCs where high levels of lymphocytic attractant chemokines are expressed [59]. Hotta et al. postulated that the intratumoural CD45RO+ memory T-cell status has a significant independent prognostic value, indicating that the adaptive immune response is functionally critical in human RCC [60]. Cumulating data indicated that T regulatory cells (Tregs), which are detectable by different markers such as CD4CD25 positivity and intracytoplasmic-Foxp3 expression, are involved in RCC progression and RCC prognosis [61]. Carretero et. al. showed that activated eosinophiles are able to change the microenvironment of the tumour and thus to promote an infiltration of CD8+ T-cells, which might improve the immuno- response of the patient and subsequently lead to a better prognosis [62].

After all, since a direct comparison with other RCC patient cohorts is not feasible for the time being, the reason for our results remain elusive. Acknowledging important limitations of our study, such as the retrospective nature of data assessment, as well as multiple surgeons involved, we do believe that a further evaluation of the potential prognostic significance of the pretreatment eosinophil granulocytes count in independent clear cell RCC datasets is strongly warranted.

5 Conclusion

In the cohort studied, an elevated pretreatment eosinophil granulocytes count was confirmed as an independent predictor of OS in the non-metastatic clear cell RCC setting.

Bibliography

[1] STATISTIK AUSTRIA, Österreichisches Krebsregister (Stand 15.11.2016). Erstellt am 28.11.2016. Niere (C64) - Krebsinzidenz (Neuerkrankungen pro Jahr), Österreich ab 1983. *Krebsinzidenzen Österreich, Niere ICD C.64*, 2016.

[2] Ariana Znaor, Joannie Lortet-Tieulent, Mathieu Laversanne, Ahmedin Jemal, and Freddie Bray. International Variations and Trends in Renal Cell Carcinoma Incidence and Mortality. *European Urology*, 67(3):519–530, nov 2017.

[3] Ferlay Jacques, Soerjomataram Isabelle, Dikshit Rajesh, Eser Sultan, Mathers Colin, Rebelo Marise, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, 136(5):E359–E386, sep 2014.

[4] Monika Hackl, Alexander Hanika, Johannes Klotz, Barbara Leitner, and Nadine Zielonke. *Trends der Entwicklung von Krebserkrankungen in Österreich - Eine Prognose bis 2030*. 2015.

[5] Robert-Koch-Institut (Hrsg) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg). Krebs in Deutschland für 2013/2014. (11):154, 2017.

[6] F K Port, N E Ragheb, A G Schwartz, and V M Hawthorne. Neoplasms in dialysis patients: a population-based study. *American journal of kidney diseases : the official journal of the National Kidney Foundation*, 14(2):119–23, 1989.

[7] Naomi B. Haas and Katherine L. Nathanson. Hereditary Kidney Cancer Syndromes. *Advances in Chronic Kidney Disease*, 21(1):81–90, 2014.

[8] Jay D. Hunt, Olga L. Van Der Hel, Garnett P. McMillan, Paolo Boffetta, and Paul Brennan. Renal cell carcinoma in relation to cigarette smoking: Meta-analysis of 24 studies. *International Journal of Cancer*, 114(1):101–108, 2005.

[9] S Weikert, H Boeing, T Pischon, C Weikert, A Olsen, A Tjonneland, et al. Blood pressure and risk of renal cell carcinoma in the European prospective investigation into cancer and nutrition, 2008.

[10] Kenneth F. Adams, Michael F. Leitzmann, Demetrius Albanes, Victor Kipnis, Steven C. Moore, Arthur Schatzkin, et al. Body size and renal cell cancer incidence in a large US cohort study. *American Journal of Epidemiology*, 168(3):268–277, 2008.

[11] W H Chow, G Gridley, J F Fraumeni, and B Järvholm. Obesity, hypertension, and the risk of kidney cancer in men. *The New England journal of medicine*, 343(18):1305–1311, 2000.

[12] L. J. Vatten, D. Trichopoulos, J. Holmen, and T. I L Nilsen. Blood pressure and renal cancer risk: The HUNT Study in Norway. *British Journal of Cancer*, 97(1):112–114, 2007.

[13] B Ljungberg, L Albiges, K Bensalah, A Bex, R H Giles, M Hora, et al. EAU Guidelines on Renal Cell Carcinoma 2018. In *European Association of Urology Guidelines. 2018 Edition.*, volume presented at the EAU Annual Congress Copenhagen 2018. European Association of Urology Guidelines Office, Arnhem, The Netherlands, 2018.

[14] Christoph Karlo. Diagnostische Bildgebung des Nierenzellkarzinoms. *Leading Opinions Hämatologie und Onkologie*, pages 76–79, 5/2015.

[15] Jean-Jacques Patard, Emmanuelle Leray, Alejandro Rodriguez, Nathalie Rioux-Leclercq, François Guillé, and Bernard Lobel. Correlation between Symptom Graduation, Tumor Characteristics and Survival in Renal Cell Carcinoma. *European Urology*, 44(2):226–232, aug 2003.

[16] P J Gold, A Fefer, and J A Thompson. Paraneoplastic manifestations of renal cell carcinoma. *Seminars in urologic oncology*, 14(4):216–222, 1996.

[17] H G W Frohmüller, J W Grups, and V Heller. Comparative Value of Ultrasonography, Computerized Tomography, Angiography and Excretory Urography in the Staging of Renal Cell Carcinoma. *The Journal of Urology*, 138(3):482–484, 1987.

[18] Pietro Pavlica, Lorenzo Derchi, Giuseppe Martorana, Eugenio Brunocilla, Alessandro Bertaccini, Fabio Manferrari, et al. Renal Cell Carcinoma Imaging, 2006.

[19] Morton A Bosniak. The Use of the Bosniak Classification System for Renal Cysts and Cystic Tumors. *The Journal of Urology*, 157(5):1852–1853, may 1997.

[20] Michael J Leveridge, Antonio Finelli, John R Kachura, Andrew Evans, Hannah Chung, Daniel A Shiff, et al. Outcomes of Small Renal Mass Needle Core Biopsy, Nondiagnostic Percutaneous Biopsy, and the Role of Repeat Biopsy. *European Urology*, 60(3):578–584, sep 2011.

[21] Alessandro Volpe, John R Kachura, William R Geddie, Andrew J Evans, Arash Gharajeh, Arthy Saravanan, et al. Techniques, Safety and Accuracy of Sampling of Renal Tumors by Fine Needle Aspiration and Core Biopsy. *The Journal of Urology*, 178(2):379–386, aug 2007.

[22] Antonio Lopez-Beltran, Marina Scarpelli, Rodolfo Montironi, and Ziya Kirkali. 2004 WHO Classification of the Renal Tumors of the Adults. *European Urology*, 49(5):798–805, 2006.

[23] Limas C Fuhrman SA, Lasky LC. Prognostic significance of morphologic parameters in renal cell carcinoma. *American Journal of Surgical Pathology*, (4):655–664, 1982.

[24] Hemamali Samaratunga, Troy Gianduzzo, and Brett Delahunt. The ISUP system of staging, grading and classification of renal cell neoplasia. *Journal of Kidney Cancer and VHL*, 1(3):26–39, jul 2014.

[25] H Moch. WHO-ISUP-Graduierungssystem für Nierenkarzinome. *Der Pathologe*, 37(4):355–360, 2016.

[26] Christian Doehn, Viktor Grünwald, Thomas Steiner, Markus Follmann, Heidrun Rexer, and Susanne Krege. The diagnosis, treatment and follow-up of renal cell carcinoma. *Deutsches Arzteblatt International*, 113(35-36):590–596, 2016.

[27] Anand A Joshi, Abhijit J Chaudhari, Changqing Li, Joyita Dutta, Simon R Cherry, David W Shattuck, et al. NIH Public Access. 55(20):6197–6214, 2011.

[28] D. S. Finley, A. J. Pantuck, and A. S. Belldegrun. Tumor Biology and Prognostic Factors in Renal Cell Carcinoma. *The Oncologist*, 16(Supplement 2):4–13, 2011.

[29] Chad J. Creighton, Margaret Morgan, Preethi H. Gunaratne, David A. Wheeler, Richard A. Gibbs, Gordon Robertson, et al. Comprehensivemolecular characterization of clear cell renal cell carcinoma. *Nature*, 499(7456):43–49, 2013.

[30] Cancer Genome Atlas Research Network, WM Linehan, PT Spellmann, CJ Ricketts, and SS Creighton. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. *New England Journal of Medicine*, 374(2):135–145, 2016.

[31] Cindolo Luca, Patard JeanJacques, Chiodini Paolo, Schips Luigi, Ficarra Vincenzo, Tostain Jacques, et al. Comparison of predictive accuracy of four prognostic models for nonmetastatic renal cell carcinoma after nephrectomy. *Cancer*, 104(7):1362–1371, aug 2005.

[32] Amnon Zisman, Allan J Pantuck, Jeffery Wieder, Debby H Chao, Fredrick Dorey, Jonathan W Said, et al. Risk Group Assessment and Clinical Outcome Algorithm to Predict the Natural History of Patients With Surgically Resected Renal Cell Carcinoma. *Journal of Clinical Oncology*, 20(23):4559–4566, dec 2002.

[33] Robert J Motzer, Jennifer Bacik, Barbara A Murphy, Paul Russo, and Madhu Mazumdar. Interferon-Alfa as a Comparative Treatment for Clinical Trials of New Therapies Against Advanced Renal Cell Carcinoma. *Journal of Clinical Oncology*, 20(1):289–296, jan 2002.

[34] Robert J Motzer, Madhu Mazumdar, Jennifer Bacik, William Berg, Alison Amsterdam, and Joseph Ferrara. Survival and Prognostic Stratification of 670 Patients With Advanced Renal Cell Carcinoma. *Journal of Clinical Oncology*, 17(8):2530, aug 1999.

[35] Daniel Y C Heng, Wanling Xie, Meredith M Regan, Lauren C Harshman, Georg A Bjarnason, Ulka N Vaishampayan, et al. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. *The Lancet Oncology*, 14(2):141–148, feb 2013.

[36] Brian R. Lane, Robert Abouassaly, Tianming Gao, Christopher J. Weight, Adrian V. Hernandez, Benjamin T. Larson, et al. Active treatment of localized renal tumors may not impact overall survival in patients aged 75 years or older. *Cancer*, 116(13):3119–3126, 2010.

[37] Michael A.S. Jewett, Kamal Mattar, Joan Basiuk, Christopher G. Morash, Stephen E. Pautler, D. Robert Siemens, et al. Active surveillance of small renal masses: Progression patterns of early stage kidney cancer. *European Urology*, 60(1):39–44, 2011.

[38] Marc C Smaldone, Alexander Kutikov, Brian L Egleston, Daniel J Canter, Rosalia Viterbo, David Y T Chen, et al. Small Renal Masses Progressing to Metastases under Active Surveillance: A Systematic Review and Pooled Analysis. *Cancer. February*, 15(1184):997–1006, 2012.

[39] Leitlinienprogramm Onkologie. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF): Diagnostik, Therapie und Nachsorge des Nierenzellkarzinoms, Langversion 1.2, 2017, AWMF Registernummer: 043/017OL, http://leitlinienprogramm- onkologie.de/Nierenzellkarzinom.85.0.html. (April):1–219, 2017.

[40] William C Huang, Elena B Elkin, Andrew S Levey, Thomas L Jang, and Paul Russo. Small Renal Tumors : Is There a Difference in Mortality and Cardiovascular Outcomes. *The Journal of Urology*, 181(1):55–62, 2009.

[41] R Houston Thompson, Stephen A Boorjian, Christine M Lohse, Bradley C Leibovich, Eugene D Kwon, John C Cheville, et al. Radical Nephrectomy for pT1a Renal Masses May be Associated With Decreased Overall Survival Compared With Partial Nephrectomy. *The Journal of Urology*, 179(2):468–473, feb 2008.

[42] Sadhana V Deo and Dhananjay S Kelkar. Laparoscopic Right Radical Nephrectomy. *Journal of Surgical Technique and Case Report*, 3(2):106–109, 2011.

[43] Hendrika J Bekema, Steven MacLennan, Mari Imamura, Thomas B L Lam, Fiona Stewart, Neil Scott, et al. Systematic Review of Adrenalectomy and Lymph Node Dissection in Locally Advanced Renal Cell Carcinoma. *European Urology*, 64(5):799–810, nov 2013.

[44] A K Hemal, A Kumar, R Kumar, P Wadhwa, A Seth, and N P Gupta. Laparoscopic Versus Open Radical Nephrectomy for Large Renal Tumors: A Long-Term Prospective Comparison. *The Journal of Urology*, 177(3):862–866, mar 2007.

[45] Martin Schostak and Andreas Blana. *Alternative operative Therapien in der Uroonkologie*. 2016.

[46] Alejo Rodriguez-Vida, Thomas E Hutson, Joaquim Bellmunt, and Michiel H Strijbos. New treatment options for metastatic renal cell carcinoma. *ESMO Open*, 2(2):e000185, 2017.

[47] Arnaud Méjean, Alain Ravaud, Simon Thezenas, Sandra Colas, Jean-Baptiste Beauval, Karim Bensalah, et al. Sunitinib Alone or after Nephrectomy in Metastatic Renal-Cell Carcinoma. *New England Journal of Medicine*, jun 2018.

[48] Bursch Jonas, Erber Barbara, Magheli Ahmed, and Miller Kurt. Status der Therapie 2015. *Perspektiven der Urologie*, pages 4–7, 2015.

[49] Thomas Powles, Michael Staehler, Börje Ljungberg, Karim Bensalah, Steven E Canfield, Saeed Dabestani, et al. Updated EAU Guidelines for Clear Cell Renal Cancer Patients Who Fail VEGF Targeted Therapy. *European Urology*, 69(1):4–6, jan 2016.

[50] Robert J Motzer, Nizar M Tannir, David F McDermott, Osvaldo Arén Frontera, Bohuslav Melichar, Toni K Choueiri, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *New England Journal of Medicine*, 378(14):1277–1290, mar 2018.

[51] S J Clarke, W Chua, M Moore, S Kao, V Phan, C Tan, et al. Use of Inflammatory Markers to Guide Cancer Treatment. *Clinical Pharmacology & Therapeutics*, 90(3):475–478, jul 2011.

[52] M Pichler, G C Hutterer, C Stoeckigt, T F Chromecki, T Stojakovic, S Golbeck, et al. Validation of the pre-treatment neutrophillymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. *British Journal Of Cancer*, 108:901, feb 2013.

[53] Georg C Hutterer, Daniel Krieger, Edvin Mrsic, Kristof Pohlmann, Angelika Bezan, Tatjana Stojakovic, et al. Preoperative Leucocytosis, Thrombocytosis and Anemia as Potential Prognostic Factors in Non-metastatic Renal Cell Carcinoma. *Anticancer Research*, 35(6):3463–3469, jun 2015.

[54] M Pichler, G C Hutterer, T Stojakovic, S Mannweiler, K Pummer, and R Zigeuner. High plasma fibrinogen level represents an independent negative prognostic factor regarding cancer-specific, metastasis-free, as well as overall survival in a European cohort of non-metastatic renal cell carcinoma patients. *British Journal Of Cancer*, 109:1123, aug 2013.

[55] Martin Pichler, Georg C Hutterer, Thomas F Chromecki, Johanna Jesche, Karin Kampel-Kettner, Peter Rehak, et al. External Validation of the Leibovich Prognosis Score for Nonmetastatic Clear Cell Renal Cell Carcinoma at a Single European

Center Applying Routine Pathology. *The Journal of Urology*, 186(5):1773–1778, nov 2011.

[56] J Atzpodien, P Royston, T Wandert, and M Reitz. Metastatic renal carcinoma comprehensive prognostic system. *British Journal of Cancer*, 88(3):348–353, feb 2003.

[57] Angelika Bezan, Edvin Mrsic, Daniel Krieger, Tatjana Stojakovic, Karl Pummer, Richard Zigeuner, et al. The Preoperative AST/ALT (De Ritis) Ratio Represents a Poor Prognostic Factor in a Cohort of Patients with Nonmetastatic Renal Cell Carcinoma. *The Journal of Urology*, 194(1):30–35, jul 2015.

[58] Hanne Krogh Jensen, Frede Donskov, Niels Marcussen, Marianne Nordsmark, Finn Lundbeck, and Hans von der Maase. Presence of Intratumoral Neutrophils Is an Independent Prognostic Factor in Localized Renal Cell Carcinoma. *Journal of Clinical Oncology*, 27(28):4709–4717, oct 2009.

[59] Tsunenori Kondo, Fumio Ito, Hayakazu Nakazawa, Shigeru Horita, Yukinari Osaka, and Hiroshi Toma. High Expression of chemokine gene as a favorable prognostic factor in renal cell carcinoma. *The Journal of Urology*, 171(6):2171–2175, jun 2004.

[60] K Hotta, M Sho, K Fujimoto, K Shimada, I Yamato, S Anai, et al. Prognostic significance of CD45RO+ memory T cells in renal cell carcinoma. *British Journal of Cancer*, 105(8):1191–1196, oct 2011.

[61] A Jeron, S Pfoertner, D Bruder, R Geffers, P Hammerer, R Hofmann, et al. Frequency and Gene Expression Profile of Regulatory T Cells in Renal Cell Carcinoma. *Tumor Biology*, 30(3):160–170, 2009.

[62] Rafael Carretero, Ibrahim M Sektioglu, Natalio Garbi, Oscar C Salgado, Philipp Beckhove, and Günter J Hämmerling. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8+ T cells. *Nature Immunology*, 16:609, apr 2015.

,