PROGNOSTIC FACTORS IN RENAL CELL CARCINOMA

A retrospective population based study focusing on the clear cell type

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CONTENTS

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2. LIST OF PAPERS

I Zubac DP, Bostad L, Gestblom C, Kihl B, Seidal T, Wentzel-Larsen T, Bakke AM. Renal cell carcinoma: A clinicopathological follow-up study after radical nephrectomy. Scand J Urol Nephrol 2007; 41:191-197.

II Zubac DP, Bostad L, Kihl B, Eide J, Wentzel-Larsen T, Haukaas SA. Organ confined clear cell renal cell carcinoma. The prognostic impact of microvascular invasion, nuclear grade and tumor size. APMIS 2008; 116: 1027-1033

III Zubac DP, Bostad L, Seidal T, Wetzel-Larsen T, Haukaas SA. The prognostic relevance of interactions between venous invasion, lymph node involvement and distant metastases in renal cell carcinoma after radical nephrectomy. BMC Urology 2008; 8:19 doi:10.1186/1471-2490-8-19

IV Zubac DP, Bostad L, Kihl B, Wentzel-Larsen T, Haukaas SA. The role of thrombospondin-1 in tumour angiogenesis and patient survival after radical nephrectomy for clear cell renal cell carcinoma. J Urol 2009;182: 2144-2149

3 ABBREVIATIONS

AJCC	American Joint Committee on Cancer stage grouping
CCRCC	Clear cell renal cell carcinoma
CCTS	Continuously coded tumor size
CDK	Cyclin dependent kinase
CSS	Cancer-specific survival
CT	Computer tomography
EPO	Erythropoietin
ESR	Erythrocyte sedimentation rate
ECOG PS	Eastern Cooperative Oncology Group Performance Status
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible factor
HTN	Histological tissue necrosis
HR	Hazard ratio
INFα	Interferon alfa
KPS	Karnofsky Performance Scale
LND	Lymph node dissection
LNI	Lymph node invasion
MDM	Metachronous distant metastases
MET	MET Oncogene
mRCC	Metastatic RCC
MVI	Microvascular invasion
MVD	Microvessel density
NG	Nuclear grade
PDGF	Platelet-derived growth factor
PI	Proliferation index
RCC	Renal cell carcinoma
RN	Radical nephrectomy
SDM	Synchronous distant metastases
TGF	Transforming growth factor
TSC	Tumor suppressor gene
TSP-1	Thrombospondin-1
TNM	Tumor Node Metastasis
VCI	Vena cava inferior
VEGF	Vascular endothelial growth factor
VHL	von Hippel-Lindau tumor suppressor gene
VI	Venous invasion

4 GENERAL INTRODUCTION

4.1 *Epidemiology*

Approximately 3% of all malignant tumors in adults arise in the kidney. Renal cell carcinoma (RCC) is a heterogeneous group of sporadic or hereditary cancers that develop from kidney cells. Its frequency is next to prostate and bladder cancer, but it is the most lethal of these malignancies. The incidence of RCC has been reported to be relatively high in North America, Scandinavia and Australia compared to other countries.[1] The incidence is steadily increasing at a rate of 2-3% per year.[2-3] In the Swedish County of Värmland age standardized incidence rates for RCC in the period 1985 to 1994 were 6.7 (cases per person-year) for females and 9.9 for males, compared to 9.3 and 15.9, respectively for Sweden and 5.7 and 9.6, globally.[4] It is estimated that approximately 20-30% of the patients present with metastatic disease.[5-6] It is also well known that low T-stage tumors can occur with advanced overall TNM stage and in some studies they account for 25% of widely metastatic, stage IV disease.[7]

4.2 *Age, gender, and geographical distribution*

Most commonly RCC occurs in the fourth to sixth decades of life, but both sporadic and in particular hereditary tumors have been reported in children.

RCC has male to female preponderance of 1.5:1.[8-10] It is more common in Scandinavians and white North Americans than in those of Asian or African descent.

4.3 *Etiology, pathogenesis and risk factors*

At least 5 hereditary syndromes associated with renal cell carcinoma are recognized: von Hippel-Lindau (VHL) syndrome, hereditary papillary renal carcinoma, Birt-Hogg-Dube' syndrome, hereditary leiomyomatosis and renal cell carcinoma syndrome and renal cell tumors associated with tuberous sclerosis. Defects of either tumor suppressor genes (VHL, TSC) or oncogenes (MET) in families at high risk may result in tumor formation.

The tissue of origin for clear cell renal cell carcinoma (CCRCC) is the renal proximal tubular epithelium. It occurs in both a sporadic (nonhereditary) and a hereditary form. Both are associated with structural alterations of the short arm of chromosome 3 (Figures 1 and 2). About 70%-80% of sporadic CCRCC have inactivation of the *VHL* gene because of a combination of allelic deletion and mutation or hypermethylation.[11-12]

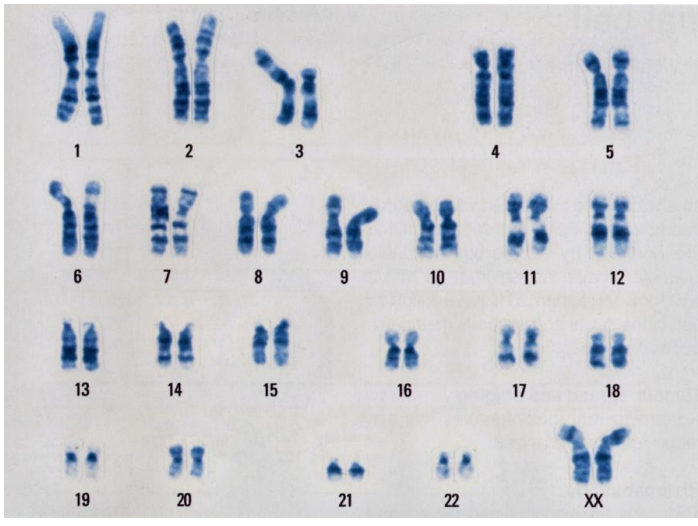


Figure 1. Clear cell RCC. Note deletion of 3p as the only karyotype change. (WHO classification of tumors. 2004)

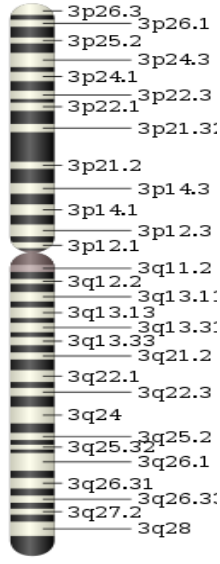


Figure 2. Chromosome 3 (human)

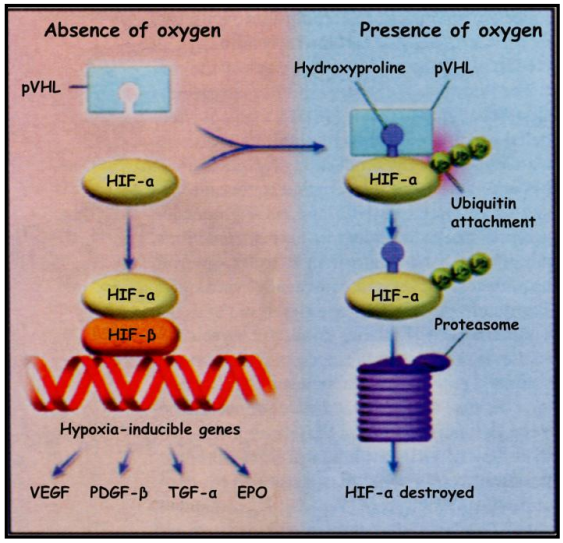


Figure 3. Control of Hypoxia-inducible factor (HIF) by the product of the von Hippel-Lindau gene (*pVHL*). From D.J. George and W.G. Kaelin Jr. (855). Copyright 2003 Massachusetts Medical Society.

Inactivation of *VHL* gene in CCRCC leads to increased level of hypoxia-inducible factor 1 (HIF1), activating a number of genes involved in angiogenesis and overexpression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor alfa (TGFα), and erythropoietin (EPO) (Figure 3). In papillary RCC a number of the cases have mutation of the *MET* Oncogene. The gene codes for the MET protein, which is receptor for hepatocyte growth factor (HGF). This molecular mechanism corresponds to the tyrosine kinase model for carcinogenesis.

A number of environmental factors have been studied as possible risk factors for RCC.[3 13-14] Cigarette smoking doubles the risk of renal cell carcinoma and contributes to as many as one third of all cases.[15] Obesity is known particularly in females to

have a linear relationship with increasing risk. However, data on the prognostic impact of overweight in RCC is still conflicting.[16] Hypertension, tuberous sclerosis, acquired renal cystic disease and regular use of analgesics may be associated with increased incidence of RCC.[17-18]

4.4 *Clinical presentation*

According to the mode of detection RCCs are classified in two groups: symptomatic and incidental. The classic clinical triad of pain, hematuria and flank mass is less frequent than previously seen and is indicative of advanced disease.

The typical tumor is characterized by lack of early warning signs and may remain clinically occult for most of its course. The majority of RCCs are now diagnosed incidentally during investigations of unrelated complaints and due to the increasing use of imaging procedures, such as ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI).[19-20] The tumors are often small and of significantly lower stage and grade.[21-22 23] RCC remains an unique and challenging tumor because of its paraneoplastic manifestations including hypercalcaemia, erythrocytosis, increased erythrocyte sedimentation rate, and non-metastatic hepatic dysfunction. Most common presentations are hematuria (40%), flank pain (40%), mass in the flank or in the abdomen (25%), weight loss (33%), fever (20%), hypertension (20%), night sweats, malaise and varicocele, usually left sided, due to obstruction of the testicular vein (2% of males).

The contribution of erythrocyte sedimentation rate (ESR) in prediction of prognosis has been a matter of debate in several studies.[24-26] However, in the recent studies of Kawai et al.[27] and Magera et al.[28] preoperative ESR has been identified as a significant independent prognostic factor in patients with localized CCRCC. ESR is

also found to be an independent prognostic factor in patients with metastatic RCC (mRCC) treated with or without cytoreductive radical nephrectomy (RN) [29].

4.5 *Performance status*

Performance status (PS) measured by Karnofsky Performance Scale (KPS) or Eastern Cooperative Oncology Group performance status (ECOG PS) has been recognized as an important predictor of cancer-specific survival (CSS) in RCC.[29-32] KPS ranges from 0-100% was the most widely used assessment tool of performance status in oncology. Oken et al.1982 introduced a new simplified measuring system ECOG PS in 1982.[33] However, the usefulness of ECOG PS in prognostication of RCC has been controversial in a number of studies.[34-36] Even though ECOG PS and symptoms at presentation were of independent prognostic significance, the combination of those two variables in prognostic models did not improve the capability to predict RCC specific mortality.[35]

Table 1

Eastern Coopertive Group Performance Status (ECOG PS)

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (such as light house work, office work).
2	Ambulatory and capable of all self-care, but unable to perform any work activities. Up and about more than 50% of waking hours.

3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Deceased.

Adapted from Oken et al. (1982)

4.6 Histopathology and tumor staging

4.6.1 Histological subtypes

The histological subtypes are diagnosed according to the Heidelberg classification guidelines (Table 2). The clear cell subtype (Figure 4) is the most common variant of RCC accounting for about 80% of this kind of tumor.[37] The two other common RCC histological subtypes are papillary (Figure 5) and chromophobe (Figure 6) carcinomas. The prognostic impact of the histological subtype has been questioned in a number of reports.[8 38-39]

Some studies have shown that CCRCC has the worst prognosis compared to papillary and chromophobe, which has the best prognosis. This concerns especially organ-localized tumors. When stage and nuclear grade were included in the analyses tumor type lost independent prognostic significance.[10 40] However, the multilocular cystic variant of CCRCC and mucinous tubular and spindle cell RCC have favorable prognosis. Collecting duct and medullary carcinoma have poor prognosis. For all the major subtypes sarcomatoid dedifferentiation is associated with adverse prognosis.

Table 2

The Heidelberg classification of renal cell tumors

Malignant tumors
1. Clear cell renal cell carcinoma
2. Papillary renal cell carcinoma
3. Chromophobe renal carcinoma
4. Collecting duct carcinoma
5. Medullary carcinoma
6. Renal cell carcinoma, unclassified

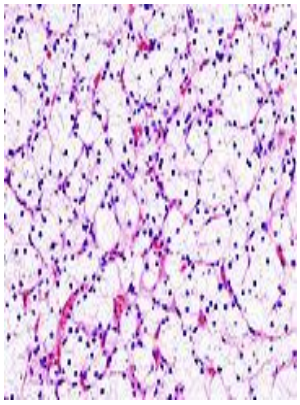


Figure 4.

Clear cell renal cell carcinoma

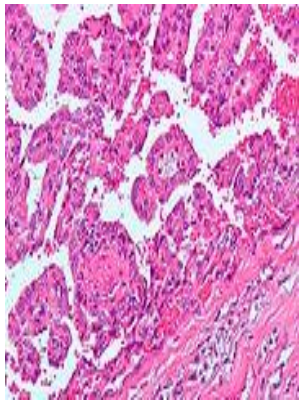


Figure 5.

Papillary renal cell carcinoma

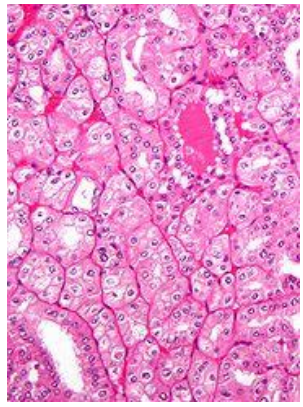


Figure 6.

Chromophobe renal cell carcinoma

4.6.2 Nuclear grade

For CCRCC the Fuhrman nuclear grading system (Table 4) has become widely used.[41] Its intra- and interobserver reproducibility, however, has turned out to be rather low.[42-44] Both how to grade and how many grades are of importance remain matters of debate.[42 45]

Table 3

Nuclear grading according Fuhrman

Grade 1	Nuclei are round, uniform, approximately 10µm with inconspicuous or absent nucleoli.
Grade 2	Nuclei are slightly irregular, approximately 15 µm with evident nucleoli.
Grade 3	The nuclei are approximately 20 µm in size and may be oval in shape with large and prominent nucleoli.
Grade 4	Nuclei are pleomorphic and multilobated with large prominent nucleoli.

4.6.3 Tumor stage

Flock and Kadesky introduced the first staging system for RCC in 1958.[46] Robson et al.[47] modified the system in 1967. Currently the most extensively used and generally recommended is the 2002 UICC TNM classification system (Table 3).[48-49] it takes into account tumor size, extent of local disease and presence of metastases when grouping patients for both prognosis and selection of treatment. However, it is still uncertain whether this version of the classification is optimal for prognostication of survival for patients with RCC. It might be changed in the future.

For many years tumors with the largest diameter less than 3cm were regarded as benign tumors/adenomas. However, in the last few years a new consensus suggests that all clear cell tumors should be considered carcinomas irrespective of size. It

remains controversial where the cut-off point lies between the different subgroups in the present TNM classification system.

Table 4

2002 TNM classification and stage grouping for renal cell carcinoma

T	**Primary tumor**
Tax	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1a	Tumor 4.0 cm or less in greatest dimension, limited to the kidney
T1b	Tumor more than 4.0 but 7.0 cm or less in greatest dimension, limited to the kidney
T2	Tumor more than 7.0 cm in greatest dimension, limited to the kidney
T3a	Tumor invades adrenal gland or perinephric tissue but not beyond Gerota fascia
T3b	Tumor grossly extends into renal vein or *vena cava* below diaphragm
T3c	Tumor grossly extends into vena cava above diaphragm
T4	Tumor invades beyond Gerota's fascia
N	**Regional lymph nodes**
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in a single regional lymph node
N2	Metastasis in more than one regional lymph node
M	**Distant metastasis**
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis

M1 Distant metastasis

Stage groupings	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
IV	T4	N0, N1	M0
	Any T	N2	M0
	Any T	Any N	M1

4.6.4 Tumor size

In patients with organ-confined disease, tumor size is related to differences in survival rates. However, the prognostic cut-off points in the TNM staging system for different stages have been subject of different reports and controversies.[32 38 50-51] A number of studies have suggested different cut-offs for the optimal T1 size for patients undergoing surgical resection for organ confined RCC. Nevertheless, all these studies confirm that primary tumor size is an important factor for prognosis.[5] Most reports suggest that the optimal cut-off size for predicting outcome is between 4 and 10 cm.[52 53-54] Lau et al. in their study suggested a cut-off point of 5 cm for pathologic stage T1 patients.[53] Frank et al. proposed that pathologic stage T2 (pT2) should be subdivided in pT2a (>7but<10cm) and pT2b ($\geq$10cm) in order to improve the prognostic accuracy of the 2002 TNM classification.[52] Furthermore, the continuously coded tumor size was reported to be more informative and to improve the predictive accuracy as compared to the categorized pT stage.[55-56]

4.6.5 Venous involvement, lymph node invasion and metastases

Stage T3 tumors are currently divided into those without vein involvement (T3a) and those with vein involvement below the diaphragm (T3b) or above the diaphragm (T3c). According to two recent studies, the presence but not the extent of venous invasion independently correlated with cancer-specific survival.[57-58]

About 25% of patients with RCC present with metastatic disease, either lymph node infiltration or simultaneous distant metastases or both.[50]

In general, distant metastases at operation have a profound adverse impact on survival after radical nephrectomy for RCC. The patients with lung or bony metastases have a worse prognosis than those with metastases limited to other organs. The lung is the most prevalent site of metastases when the tumor invades the renal vein or the *vena cava*.

One of the most important prognostic factors in RCC is lymph node invasion (LNI). However, the prognostic discrimination between pN1 and pN2 categories in the 2002 TNM system has been questioned. A recent study concludes that the percentage of positive nodes and a threshold number of four rather than one positive lymph node correlated significantly with clinical outcomes.[59]

The appropriateness of the pNx/pN0 grouping and the prognostic relevance in a multivariate setting has also been discussed.[60] The importance of extensive lymphadenectomy as a part of RN of RCC is still controversial after decades of evaluation.[61-62]

The possible impact on CSS of interactions between LNI, synchronous distant metastases (SDM) and VI have not yet been fully studied. The importance of these interactions has been discussed in recent studies.[63-64]

4.7 Tumor biology

4.7.1 Tumor biomarkers

Progression in many tumors has been found to be associated with increased cell proliferaration, cell migration, angiogenesis and decreased programmed cell death (apoptosis). Inactivation of the *VHL* gene in CCRCC increases HIF1, which activates downstream genes involved in cell proliferation and neovascularization. In this respect, several putative biomarkers associated with cell-cycle progression (Figure 7) have been identified.

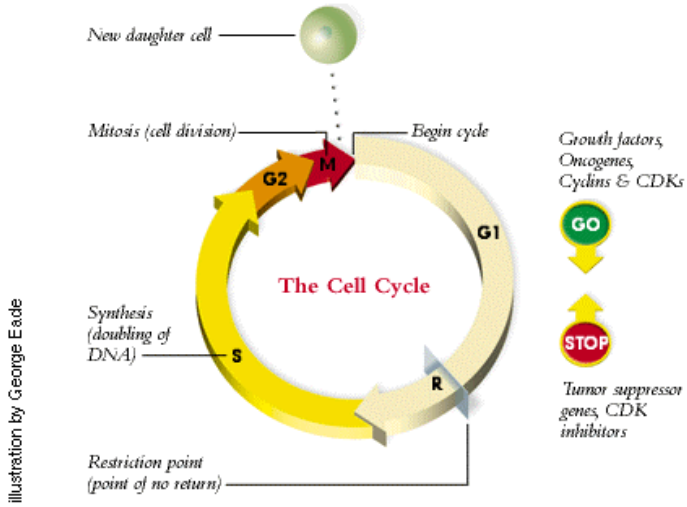


Figure 7. The cell cycle (The cell cycle & apoptosis, Sathiyaraj, 2007)

The cyclin-dependent kinase (CDK) inhibitor p21 has been investigated as an important biomarker in RCC.[65] The p21 protein is expressed in a number of tumors and normal tissues, but has specifically been associated with better clinical outcomes in patients having localized RCC. In metastatic disease, high levels of nuclear and

cytosolic p21 have been associated with reduced survival.[65] In the modified RCC cell cycle, minichromosome maintenance 2 (Mcm2), Geminin and Ki-67 define the proliferative state.[66] p27 is a member of the family of cyclin-dependent kinase inhibitors. It has been reported to be elevated in tumors compared with matched controls, and cytoplasmic mislocalization of p27 was associated with increasing tumor grade. [67] The loss of p21 expression is a risk factor for RCC progression. [68] Inactivation of the p16 gene is a common mechanism for deregulation of cell cycle control in many tumors. Expression of the cyclin-dependent kinase 4 inhibitor A (p16INK4a) tumor suppressor protein is a positive prognosticator for cancer-specific survival in patients with RCC in both uni- and multivariate analyses.[69]

Ki-67, which is expressed in all phases of the cell cycle except G0 is a sensitive and specific marker of tumor cell proliferation. However, the value of this marker in prognostication of RCC remains controversial.[70-72] Vascular cell adhesion molecule 1 has also been implicated as a predictor of survival for mRCC.[73]

p53 is a tumor suppressor gene and p53 mutations have been found in 20- 40% of CCRCC. The p53 protein is important for cellular responses involving cell cycle arrest, apoptosis and DNA repair. However, the role of p53 in RCC remains inconclusive. Some studies report that p53 overexpression is associated with sarcomatoid transformation and adverse prognosis while others could not find this association.

The role of thrombospondins (TSPs) in angiogenesis and tumor progression in different human tumors has been a matter of controversy.[74-77] TSPs are matricellular multifunctional glycoproteins secreted by most cell types and natural regulators of angiogenesis.[78-79] Since tumors and their metastases are dependent on angiogenesis, which occurs almost exclusively in the microcirculation, the role of

angiogenesis in the growth and progression of cancer has received increasing attention. In an experimental study of TSP-1 and TSP-2, it was shown that co-expression completely prevented tumor growth suggesting potential synergistic effects of these proteins. Inhibition of tumor growth by TSP-2 was not caused by direct inhibition of tumor cell proliferation.[80] Nevertheless, there are discrepancies in published literature as to what extent and in what way TSP-1 influences tumor proliferation and angiogenesis. Izumi et al.[81] reported that trastuzumab (Herceptin) is an antibody that inhibits cancer cell production of angiogenic factors such as TGF-β, angiopoietin-1, plasminogen activator inhibitor-1 and also up-regulates expression of the endogenous angiogenesis inhibitor, TSP-1. Rastinejad et al.[82] demonstrated that a nontumorigenic hamster cell line generated a truncated form of TSP-1, a negative regulator of angiogenesis. These cells became tumorigenic in association with loss of suppressor gene, consecutive decrease of TSP-1 and switch to an angiogenic phenotype.

Compelling evidence has emerged that p53 upregulates the expression of TSP-1 and that the loss of p53 function correlates with a reduction of TSP-1 protein expression and activation of angiogenic switch.[79 83-85] As normal fibroblasts and mammary epithelial cells progress toward malignancy, they switch to an angiogenic phenotype. Wild-type p53 was shown to inhibit angiogenesis in these cells through regulation of TSP-1 synthesis.[84 86] Inactivation of the p53 suppressor gene resulted in a dramatic reduction in the production of neovascularization inhibitors, while reintroduction of p53 stimulated secretion of TSP-1 and raised the antiangiogenic activity of the tumor cells.[87] Similarly the absence of TSP-1 leads to an increase of vasculature and accelerated growth of mammary tumors that arise spontaneously in neu-transgenic

mice.[87] TSP-1 blocks the ability of cultured capillary endothelial cells to organize into cords and to develop lumen formation.[88]

4.7.2 Angiogenesis, microvessel density and histological tissue necrosis

In their experimental studies of spontaneous tumors in transgenic murine models Hanahan et al.[89-90] and Kandel et al.[91] revealed that the angiogenic switch is a discrete event that develops in premalignant stages of tumorigenesis. However, most human tumors exist in situ for years then switch to an angiogenic phenotype. VEGF expression is inhibited by the VHL protein. In patients with VHL disease and in most sporadic clear cell carcinomas the VHL tumor suppressor gene is mutated which results in increased expression of VEGF. [92-93]

Microvessel density (MVD) measures the relative intensity of angiogenic activity in a majority of solid tumors and is correlated with metastasis and poorer prognosis.[94] It has become a reproducible factor for the risk of metastases. MVD may not be useful to determine efficacy of antiangiogenic therapy in solid tumors, however, it continues to be a valid prognosticator of metastasis and survival.[95-96]

Tumor necrosis has been found in 28-37% of CCRCC, most frequently in those with high nuclear grade. However, the prognostic importance of HTN is still a matter of debate.[34 36 38-39 97-98] There is no consensus yet whether HTN should be included in the histopathology report and if it has to be quantified.

4.7.3 Microvascular invasion

The importance of microvascular invasion (MVI) for the prognosis of non-metastatic RCC has been discussed in only a few earlier reports, but with divergent

conclusions.[99-105] There is also considerable variation in the reported frequencies of MVI. These discrepancies may be due to different staining methods, lack of standardized diagnostic criteria and interobserver variability. In two recent studies, however, MVI was found to be an independent prognosticator in patients with organ-confined RCC treated with RN.[106-107] Three other studies have concluded that MVI is an independent prognostic factor in all T-stages.[108-110] However, MVI is not part of the 2002 TNM classification system and it is not regularly included in the histopathology report.

4.8 Treatment of RCC

4.8.1 Radical and partial nephrectomy

Historically, the standard curative treatment for RCC has been RN.[47] Management of RCC has advanced through the development of laparoscopic approaches and nephron-sparing surgery.[111-112] Currently nephron-sparing surgery is the standard treatment for small renal tumors. Laparoscopic radical nephrectomy has been suggested as the new gold-standard[113] and has to a large extent replaced the open approach which was the standard procedure between 1985 to 1994.

4.8.2 Percutaneous cryoablation

Small renal tumors are increasingly being discovered over the last decade due to increased use of abdominal imaging performed for other purposes. A population of small renal cortical tumors (median size 4.0 cm [T1]) has emerged, comprising 70% of the renal tumors that are incidentally detected.[114]

Due to decreased morbidity, preservation of the renal function, and shorter hospital stay, ablative techniques that destroy tumor tissue have gained interest in the last

decade. Among the ablative modalities, cryoablation is the best documented procedure for treating small renal cortical tumors.[115] This minimally invasive procedure is based on freezing the tumor tissue by using nitrogen or argon. MRI or CT scans are used subsequently in order to assess the ablation. If the treatment is found to be inadequate a percutaneous biopsy, a standard resection, or retreatment with cryoablation can be performed.

4.8.3 Radiofrequency ablation

Some patients with a small RCC are unable to undergo nephron-sparing surgery due to comorbidities. In older patients with a small incidentally discovered tumor growing at a slow rate, that does not represent an immediate threat to the patients' life, watchful waiting and follow-up imaging could be appropriate.[116-118] However, CT-guided percutaneous radiofrequency ablation can be used to reliably eradicate small RCCs in patients who are unsuitable for surgery and desire a definitive treatment.[111] It can be performed in a day-hospital for selected patients.[119]

4.8.4 Observation of renal masses

According to a recent study[120], non-treated smaller renal masses diagnosed in older and comorbid patients have a low growth rate of <1cm/yr in 85% of the cases, and 100% 5-yr CSS. These findings imply that this subgroup of patients with a high risk of postoperative morbidity and mortality could safely be selected for observation and not invasively treated.

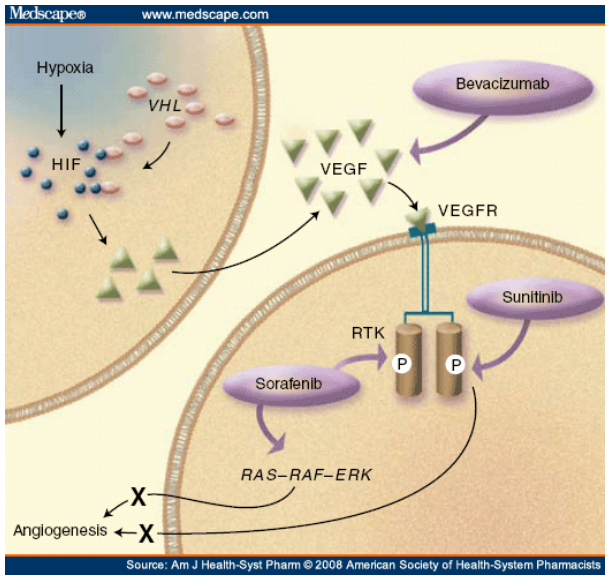
4.8.5 Radiotherapy for metastases in RCC

Historically, RCC is resistant to radiotherapy. However, this treatment option can be used for selected symptomatic patients with unresectable brain or osseous lesions.[121-122] Combined radiotherapy and zoledronate in patients with bone metastases from RCC can induce a higher response rate than radiotherapy alone.[123] In individual cases whole brain irradiation, radio surgery and/or a stereotactic radiotherapy can induce symptom palliation and improve survival.[124-126]

4.8.6 Targeted molecular therapies

A defective copy of the VHL gene is the most common cause of inherited CCRCC. Furthermore, in most patients with sporadic CCRCC, the VHL gene is inactive. Hypoxia-inducible factor accumulation due to VHL inactivation, leads to production of several growth factors including VEGF, TGFα and PDGF, which promote neo-angiogenesis and contributes to the development and progression of RCC. The PDGF and VEGF signalling pathways have been identified as targets for anticancer therapy. The targeting drugs, sorafenib, sunitinib, bevacizumab combined with INF-α (Figure 8), everolimus and temsirolimus have led to significant improvements in progression-free survival and have been approved for treatment of mRCC.[127-128] [129-131]

Figure 8. Schematic representation of the main molecular events associated with anticancer therapy by three targeting drugs: sorafenib, sunitinib and bevacizumab.



5. Aims of the thesis

5.1 Paper I

To examine the prognostic significance of **performance status**, **tumor stage**, **histological subtype**, **nuclear grade** and **histological tumor necrosis** (HTN) in a population of 196 consecutive patients subjected to radical nephrectomy for RCC.

5.2 Paper II

To evaluate the prognostic impact on CSS of **microvascular invasion**, **nuclear grade**, **tumor size** and **pT-stage** adjusted for **age** in CCRCC. A study was conducted on a complete cohort of 76 consecutive patients with pathologically organ-confined CCRCC treated with radical nephrectomy.

5.3 Paper III

To investigate the possible prognostic significance of **interactions** between **lymph node invasion**, **synchronous distant metastases,** and **venous invasion** adjusted for **mode of detection**, **Eastern Cooperative Oncology Group performance status**, **erythrocyte sedimentation rate** and **tumor size.** This study investigated 196 patients with renal cell carcinoma treated with radical nephrectomy.

5.4. Paper IV

To evaluate the possible associations between TSP-1, **p53** expression, **microvessel density, cell proliferation index**, **nuclear grade**, **tumor stage**, and **continuously coded tumor size.** A study was conducted on 160 patients with CCRCC where the significance of TSP-1 as a prognostic marker in CCRCC was examined.

6. Patients and methods

This chapter gives an overview of the patients included and the methods applied to fulfill the aims of the study. More specific details can be found in the corresponding papers.

Approval to use the biological material for research purposes was granted in 2004 by the local authority at Karlstad Central Hospital in Sweden according to Swedish regulations. In Norway the appropriate Norwegian authority, Norwegian Social Science Data Services, acknowledged this approval. The study was carried out in accordance with the standards of the World Medical Association Declaration of Helsinki as revised in 2008.

6.1 Patients and materials

6.1.1 Paper I and III

Between 1985 and 1994 a total of 203 consecutive patients underwent surgical treatment for RCC at five clinics in Värmland County, Sweden (average population 282 570). Five patients who underwent partial nephrectomy and two who had oncocytoma were excluded. The study thus comprised 196 patients treated with standard radical nephrectomy.

6.1.2 Paper II

Of 196 patients a total of 52 with AJCC stage IV and 53 with AJCC stage III were excluded. After removing 12 patients with papillary and three with chromophobe carcinoma the study group consisted of 76 patients with organ-confined CCRCC.

6.1.3 Paper IV

A total of 172 consecutive patients with CCRCC treated with radical nephrectomy during the years 1985 – 1994 were enrolled in the study. However, due to technical problems and lack of material, 12 cases without TSP-1 immunohistochemistry were excluded from the study.

6.2 Data collection

Clinical records and pathology reports were reviewed to determine stage, size and type of the primary tumor. Clinical information regarding age, sex, symptoms, preoperative evaluation, treatment, local recurrences, metastases and final disease status were extracted retrospectively from the patients' files.

The biopsy material was examined at the Department of Pathology, Central Hospital, Karlstad, and at the Department of Pathology, Haukeland University Hospital, Bergen. In all cases studied, representative formalin fixed, paraffin embedded material was available for light microscopy and immunohistochemistry.

The cause of death was determined from clinical records and death certificates. Deaths from causes other than RCC were censored. The Swedish Updated Population Register was searched. Concerning the surviving patients, local general practitioners were contacted in order to obtain the clinical status of these patients at 30 April 2004, which represented the end of follow-up. Thus patients could be assigned a date of death or identified as being alive with or without diagnosed recurrent disease.

6.3 Data elaboration

6.3.1 Tumor stage, nuclear grade and histological subtype

Tumor staging was ranked according to the 2002 TNM classification system using the American Joint Committee on Cancer (AJCC) stage grouping[1]. (**Paper I, II, III, IV**)

The *T-staging* was performed using the histopathology reports. All tumors were assigned a pT stage. The clinical *lymph node staging* was performed based on preoperative CT images of the abdomen and/or the preoperative findings. In cases with enlarged or palpable lymph nodes between the aorta and *vena cava* or other sites, additional lymph node dissection was performed. Extensive radical retroperitoneal lymph node dissection was not conducted. Only 14 of the surgical specimens had a sufficient number of negative nodes (eight) to be classified as pN0 category. Accordingly 161 of the patients were pNx (clinically N0) **(Paper III)**.

The M-status was preoperatively evaluated by a chest X-ray and kidney/abdominal ultrasound scan**,** which were done in all patients. CT of the abdomen was performed in 150 patients (77%). CT scans of the thorax and brain, bone scans, angiography and cavography were done selectively when clinically indicated.

In our study (**Paper I)** two pathologists (C.G., T.S.) examined every slide and performed the nuclear grading, which was done according to Fuhrman.[15] The grading was determined via consensus. We also investigated the prognostic relevance of changing the Fuhrman four-grade system into a two- grade system; low grade [Fuhrman nuclear grade (NG) 1 and 2] and high grade [Fuhrman NG 3 and 4].

(**Paper I, II, III, IV)**

Histological tumor subtype was diagnosed according to the Heidelberg classification guidelines by an experienced nephropathologist (L. B.). (**Paper I, II, III, IV)**

The presence of HTN was recorded (L. B.). (**Paper I)**

Performance status was scored by one of the authors (D. P. Z.) from clinical records according to the Eastern Cooperative Oncology Group performance status classification in all patients. Most of the patients in this series had performance status 0 and 1 (93.4%) and only a few (6.6%) had performance status 2, which is in line with recommendations originating from SWOG and EORTC randomized trials. [18, 19] (**Paper I, III)**

6.3.2 Microvascular and vein invasion

Microvascular invasion was diagnosed only when tumor cell aggregates were seen within lumina covered with CD31 positive cells, or when tumor cells penetrated a vessel wall. Both sinusoidal and muscular vessels within and close to the tumor were assessed. (**Paper II)**

Venous invasion was registered as no venous invasion (pV0), renal vein invasion (RVI) (pV1) or *vena cava* invasion (VCI) (pV2) and dichotomized (pV0 vs. pV1+pV2) in all except descriptive analyses for compatibility with most previous reports. RVI was diagnosed when there was invasion by tumor of major extra renal veins found microscopically in transverse slices of the vein. In patients with VCI, the tumor thrombus did not adhere to the intima of the *vena cava*. (**Paper III)**

6.3.3 Immunohistochemistry and computer assisted morphometry

The immunohistochemical staining was performed on formalin-fixed, paraffin-embedded archival tissues (4 µm sections), and conditions were optimized for each antibody; TSP-1, p53, Ki-67, Factor VIII and CD31. The DAKO inmmunostainer (TechMATE 500) was used. (**Paper II, IV)**

The proliferation index (PI) was scored. At least 1000 tumor cells in 5 different fields of view were examined under x400 magnification. Ki-67 expression was dichotomized at the value 10%. (**Paper IV)**

Microscopic screening of the Factor VIII stained slides by low-power fields (10x objective, Olympus BX51 microscope) identified tumor areas with the highest *microvessel density* (hot spots). The average MVD values were calculated in five hot spot areas including the tumor rim and the tumor core. (**Paper IV)**

A computer assisted morphometric method [AnalySIS Image Processing -Microsoft Windows NT5.0 (Build 21915) Service Pack 4] was applied. Screening for hot spots in the intratumoral or immediate peritumoral areas revealed the areas with the highest staining intensity for *TSP-1* under low power magnification (100x) (Olympus BX51 microscope). Ten representative images at x400 magnification in hot spot areas were taken by microscopic camera (Olympus U-Tvo.5xc). All immunohistochemical analyses were completed without knowledge of the clinical outcome by (D. P. Z.) supervised by (L. B.) (**Paper II, IV)**

6.3.4 Statistical analyses

Preliminary analyses included descriptive statistics and assessment of associations by cross tabulations, with exact chi square, linear by linear association or Mann-Whitney tests. Univariate and multivariate *Cox analyses* were performed for overall survival (**Paper I)** and cancer specific survival (**Paper I, II, III, IV)**.

Kaplan-Meier analysis using the log rank-test was performed for overall survival and cancer-specific survival **(Paper I),** and cancer-specific survival **(Paper II, III)**

Harrell's concordance indexes (c-indexes)[132] with 95% bootstrap BC_a confidence intervals based on 10 000 bootstrap replications were computed for estimation and comparison of predictive ability (PA) of uni- and multivariate Cox models. (**Paper I)**

The reproducibility and interobserver agreement between the two pathologists independently assessing MVI were measured using *Cohen's kappa*. (**Paper II)**

All statistical analyses were conducted using SPSS 11.0.1 **(Paper I);** SPSS 14.0 **(Paper II, III);** SPSS 15 **(Paper IV)** (SPSS Inc., Chicago, IL) and R (The R Foundation for Statistical Computing, Vienna, Austria) software.

7. Summary of the results

7.1 Paper I

Performance status, tumor stage, nuclear grade and histological tumor necrosis were found to be independent predictors for CSS in patients with RCC.

In this paper we analyzed the prognostic importance of tumor stage, nuclear grade, histological subtype, ECOG PS and histological tissue necrosis in a complete cohort of 196 patients treated with RN for RCC during 1985-1994.

By post-hoc multivariate comparisons we found a significant prognostic difference among all stages except for stages II and III. The same differences are shown in Kaplan-Meier analysis (Figure 9).

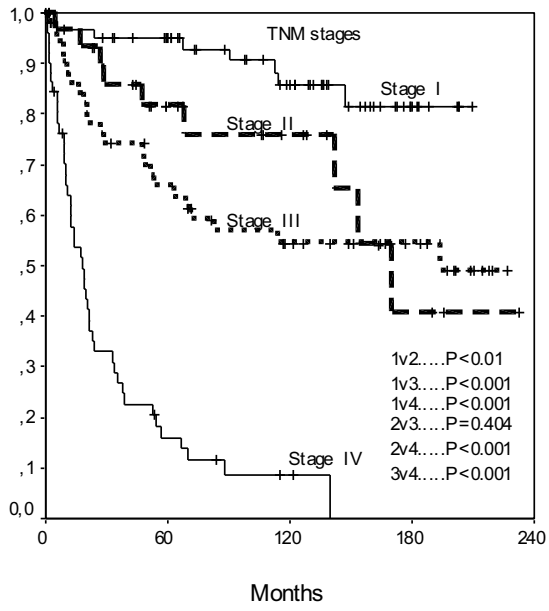


Figure 9. Kaplan-Meier survival curves for 196 patients after RN for RCC

No significant difference in CSS was found between patients with NG1 and 2 and those with NG3 and 4 tumors (Figure 10).

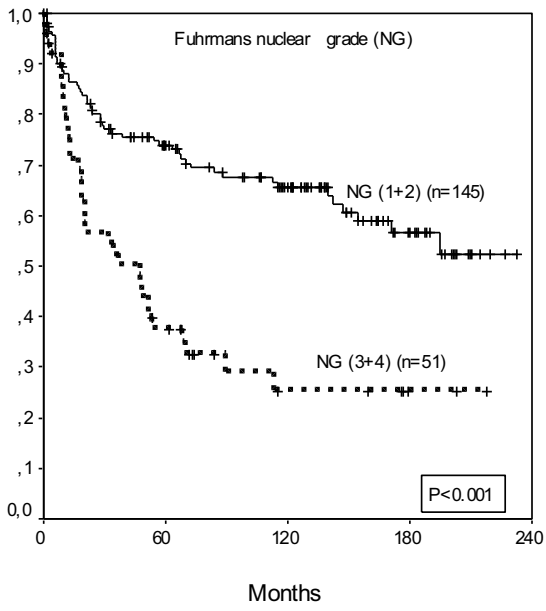


Figure 10. CSS after RN in 196 patients operated on for RCC as a function of NG.

HTN was found in 26.5% of the tumors and was shown to be an independent predictor of CSS for CCRCC ($p<0.01$), but not for papillary and chromophobe type. The clinical ECOG PS (two categories: 0 vs. >0) was found to be a reliable prognostic predictor for RCC in both univariate and multivariate analysis.

7.2 Paper II

Microvascular invasion turned out to be a strong independent predictor for CSS in patients with organ-confined CCRCC.

Of 196 patients, a total of 52 patients with AJCC stage IV and 53 with stage III were excluded. After removing 12 patients with papillary and 3 with chromophobe carcinoma, the study group consisted of 76 patients with organ-confined CCRCC.

MVI was diagnosed only when tumor cell aggregates were seen within lumina covered with CD31 positive cells (Figure 11a) or when tumor cells penetrated a vessel wall (Figure 11b). Both sinusoidal and muscular vessels within and close to the tumor were assessed. Using these criteria the interobserver agreement was very highly significant, bordering on almost perfect (Cohen′s kappa 0.75). MVI turned out to be a strong independent predictor of CSS (Figure 12).

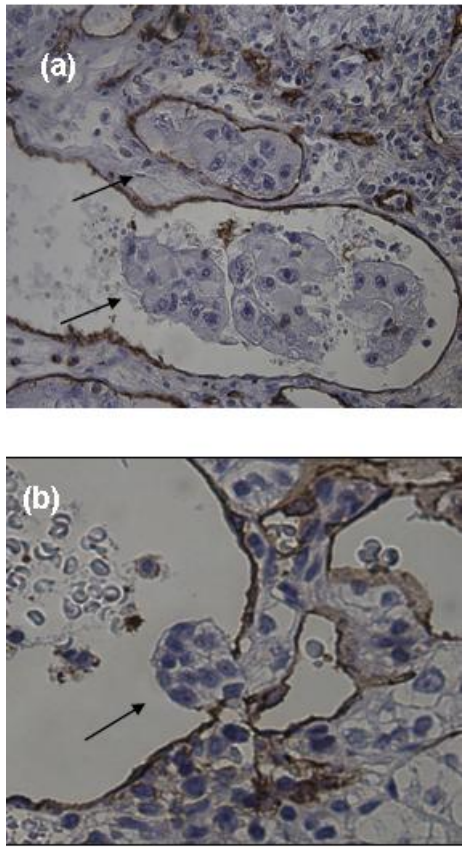


Figure. 11 Microvascular tumor x 40 obj.). Arrows point at clusters of tumor cells in two sinusoidal vessels. (a) Tumor penetrating a vessel wall (arrow) (b).

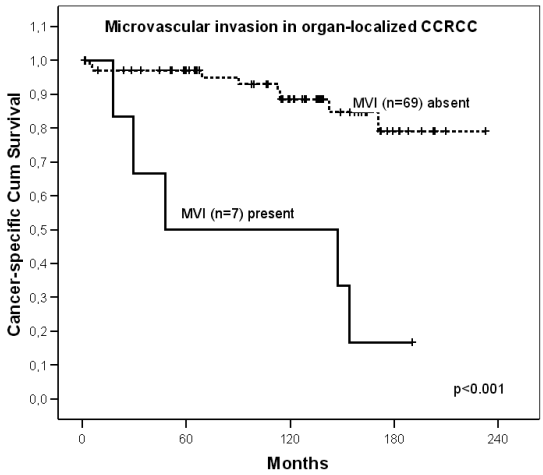


Figure 12. CSS after RN in 76 patients TNM stage pT1 and pT2 CCRCC related to MVI

By Cox multivariate analysis, tumor size coded as continuous variable, showed HR of 1.2 per cm. Patients with high-grade tumors had an estimated 5-fold higher risk of dying of CCRCC compared to patients with low-grade tumors.

7.3 Paper III

In multivariate analysis of interactions of SDM, VI and LNI on survival, LNI showed a significant impact on survival only for the patients in whom we found no distant metastases or venous invasion.

A complete cohort of 196 patients treated with RN for RCC was enrolled in this study. In multivariate Cox regression analysis only LNI, SDM, VI, and ESR remained independent prognostic factors. In patients without LNI we were able to define prognostic subgroups of patients based on multivariate analysis by including different

combinations of SMD and VI. The same relationships were observed in Kaplan-Meier analysis by combinations of LNI, SDM and VI (Figure 13).

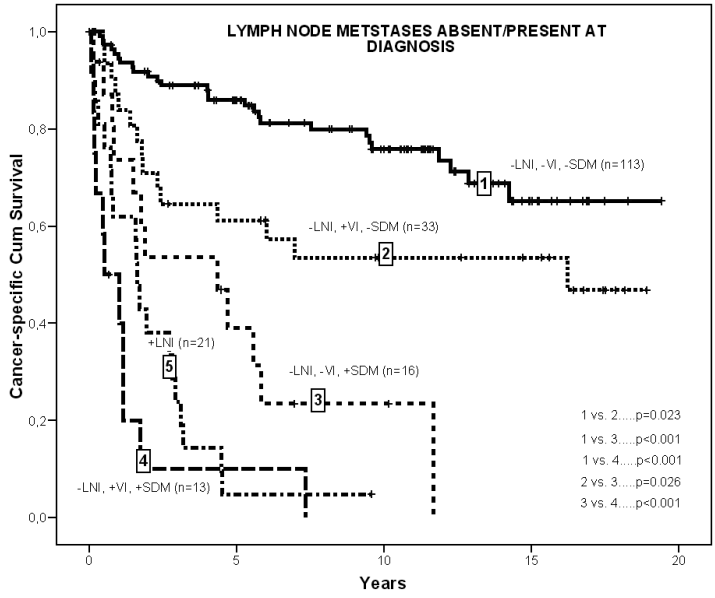


Figure 13. Kaplan-Meier analysis of CSS in 175 lymph node negative renal cell carcinoma patients by different combinations of synchronous distant metastases (SDM) and venous invasion (VI) and in 21 patients with lymph node positive renal cell carcinoma.

7.4 Paper IV

TSP-1 expression was found to be significantly associated with prognostic tumor features and was an independent prognostic factor for CSS.

A total of 172 consecutive patients with CCRCC treated with radical nephrectomy were initially enrolled in the study. Due to technical reasons and lack of material, 12 patients could not be tested for TSP-1 expression and were excluded.

TSP-1 expression (Figure 14a), p53 status (Figure 14 b), Ki-67 PI (Figure 14 c), MVD (Figure 14d), NG and tumor stage were significant prognosticators for CSS. Multivariate analysis revealed that TSP-1, tumor stage (P=0.003), p53 status (P=0.002), Ki-67 PI (P=0.010) and MVD (P=0.025) were independently significant predictive factors for CSS. Our findings reveal a significant inverse correlation between p53 status and TSP-1 expression in CCRCC.

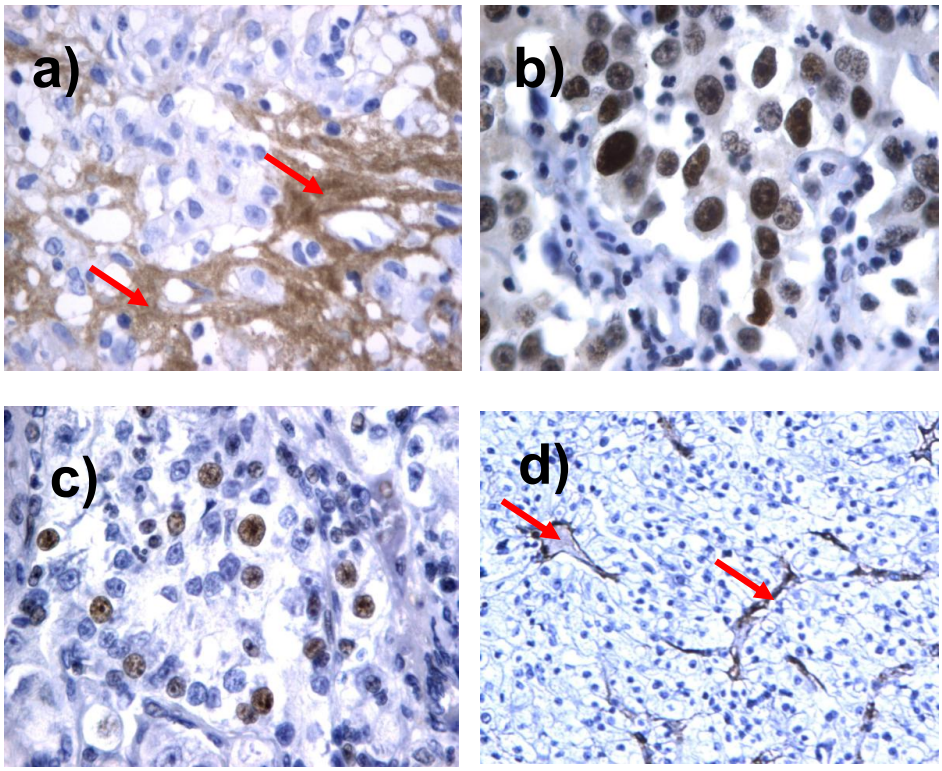


Figure 14. (a) Intratumoral intercellular TSP-1 staining (arrows) (x 40 obj.); (b) p53 staining; (c) Proliferation marker (Ki-67) staining; (d) Intratumoral micro vessel density (arrows) (Factor VIII staining x 20 obj. 868.9μm x 656.6 μm)

8. GENERAL DISCUSSION

8. Patients and methods

This study is one of the longest follow up studies on RCC conducted so far. It is based on a well defined population of more than 280 000 individuals, and includes all consecutive patients with RCC treated with RN during a 10 years period at five clinics in Värmland County, Sweden.

Although the total number of patients in the study is small, it consists of a complete cohort of RCC cases since very few patients were referred out of the region for operation. The limitation of the study is the retrospective view. The strength of the study is its long-term follow-up and the high quality of follow-up data that were obtained from institutions providing health service, local GPs or population registers.

8.2 Tumor classification and histology (Papers I-IV)

8.2.1 Tumor stage

Tumor stage has traditionally been considered the most useful tool in prognostication of RCC. Historically, RCC has been staged according to anatomical staging systems such as the TNM classification system. Tumor staging was done according to the 2002 TNM classification system of the AJCC stage grouping.[48] Stage pT1 has been subclassified in pT1a and pT1b in order to improve the precision of prognostication in this group of patients. In our study **(Paper I)** we found a significant CSS difference between these two substages in line with several other studies.[49 133-135] The critical cut-off point of 7 cm for organ confined tumors, as recommended by the TNM classification, correlated significantly with survival in our population-based study. This result is in agreement with some previous reports.[55 136] However, it has not been

confirmed in a number of other studies.[38 51 54 137-138] A possible explanation for this divergence might be differences in follow-up periods and inconsistencies in the studied populations. Test analysis of our data with a follow-up period of more than10 years revealed no difference in CSS between pT1 and pT2 tumors, supporting our hypothesis that a long follow-up period is of critical importance in analyzing prognostic factors for organ-confined RCC. Our results are consistent with Frank's report where a cut-off point at 10 cm is suggested for subclassifying patients in stage pT2 into pT2a and pT2b.

8.2.2 Nuclear grade

Fuhrman's nuclear four-grade system is the most widely used but its reproducibility and prognostic significance has been questioned.[9 39 42 45 139-140] Both how to grade and the prognostic relevance of the four grades remain matters of debate.[43-45] In our study the prognostic cut-off lies between grade 2 and 3 **(Papers I-IV)**. Thus, patients with low NG tumors had a significantly improved CSS compared with high grade tumor patients. This is in accordance with a number of earlier reports.[7 23 43 45 141-143] Our findings support the recommendations given by Bretheau et al.[45] to reduce the number of grades in order to increase the prognostic significance of grading. This 2-tiered Fuhrman classification reduces the degrees of freedom and improves the efficiency of statistical analyses.[144]

The data in the current analysis suggest that NG can be used to predict survival for patients with non-metastatic tumors. It follows that high NG may reflect a profound change in biological behavior of the tumor when the size of tumor mass has reached a critical point.[78] A significant survival difference between low and high grade tumors was revealed for TNM stage II, which is consistent with Minervini's report.[142]

However, Lang et al.[141] and Zisman et al.[32] in their studies concluded that the original Fuhrman grading system possesses significant independent prognostic value and collapsing the system leads to a loss of information.

8.2.3 Histological tumor necrosis

There are studies concluding that HTN may be an informative prognostic factor in RCC.[34 39 42 98 145-148] It has even been included in recommended guidelines.[149] In agreement with this we found HTN to be associated with an increased risk of death from RCC that even persisted after multivariate adjustment for ECOG PC, TNM stage, tumor size and NG (hazard ratio 1.75; 95% C.I. 1.09 – 2.80; $p<0.05$) **(Paper I)**. While Klatte et al.[150] and Isbarn et al.[151] previously confirmed that HTN is an adverse predictor of survival in CCRCC, they did not find it to have independent statistical significance. The possible reasons for the discrepancies reported could be different methods of assessment of necrosis, different histological RCC subtypes of the populations studied, and absence of a uniform definition of necrosis. Consistent with other studies.[34 38 145] we found the presence of tumor necrosis in papillary RCC to be of little prognostic significance.

8.3 Immunohistochemical methods and use of partly computerized morphometry

8.3.1 Immunohistochemistry

Immunohistochemistry was performed using the automated TechMATE system (DAKO, Carpentaria, CA, USA). The sections (4 µm) from the formalin-fixed, paraffin-embedded archival tissues were first deparaffinized and then hydrated through graded alcohols and water. Antigen retrieval was achieved by microwaving

the slides in the retrieval buffer for 10 minutes to boiling point, followed by heating in retrieval buffer. Peroxidase was blocked for 5 minutes; slides were then incubated with the primary antibodies (Table 1). Detection was performed using the Envision-HRP kit (DAKO K4061). Hematoxylin was used as a counter stain. Appropriate negative and positive controls were used **(Papers II, IV)**.

8.3.2 Partly computerized morphometry

A computer assisted morphometric method [AnalySIS Image Processing -Microsoft Windows NT5.0 (Build 21915) Service Pack 4] was applied. Prominent hyalinized, necrotic, and hemorrhagic areas were excluded from the analysis. As recommended by Grossfeld et al.[152] the areas examined were chosen based on extracellular reactivity for TSP-1. Screening for hot spots in the intratumoral or immediate peritumoral areas revealed areas with the highest staining intensity for TSP-1 under low power magnification (100x) (Olympus BX51 microscope). Ten representative images at x400 magnification in hot spot areas were taken by microscope mounted camera (Olympus U-Tvo.5xc). All positively stained tumor stroma areas were identified and the mean value of area (μm^2) was computed as a percentage of the surface area in the image. The average area value for each tumor was calculated from ten hot spot areas. The staining intensity level was defined and classified as: no = 0, low = 1, moderate = 2, and high level = 3. The intensity levels were defined as phase fractions by setting a color threshold (cut off value) manually for each level and were quantified by computing their percentage of the representatively stained surface area in the image. The mean value for each phase fraction (intensity level) in ten images (HPF; x400) was calculated. The highest value determined the staining intensity level of the tumor. Tumor sections were classified as having low TSP-1

expression when they showed no or negligible/equivocal reactivity (score 0-1). Tumors with detectable TSP-1 immunoreactivity were considered to have moderate or high TSP-1 expression (score 2-3). TSP-1 expression was dichotomized into none to low vs. moderate to high **(Paper IV)**.

8.4 Comments on main results

8.4.1 Clinical presentation and performance status

The proportion of incidentally detected RCC in the present study is similar to contemporary series.[14, 15]

The ECOG PS is a recognized predictor for survival in patients with RCC. Most of the patients in this series had performance status 0 and 1 (93.4%) and only few had performance status 2 (6.6%) which is in line with recommendations originating from SWOG and EORTC randomized trials.[153-154] ECOG PS was shown to be an independent predictor of survival in our patients with RCC which is in accordance with other studes.[35 155] In a number of studies however, its prognostic power has not been confirmed.[34 42]

(Papers I and III)

8.4.2 Microvascular invasion

The frequency of MVI found in our study is comparable with some of those reported previously.[103-104 156-157] There is, however, a considerable variation in reported frequencies of MVI. These discrepancies may be due to different staining methods, tissue sampling (the number of tissue slides), interobserver variability, different inclusion criteria for tumor thrombus/embolus, and differences in study populations. In the study of Sevinç et al., which was limited by the small population studied, CSS

was not found to be significantly related to MVI.[103] In our study **(Paper II)** however, MVI turned out to be a strong independent prognosticator for organ -confined CCRCC after RN, a finding in accordance with most of the previous reports on this issue. [99 104 106-107 156] Our patients with MVI positive tumors experienced a nearly 7-fold higher risk of dying from CCRCC compared with patients in whom the tumor did not invade the microvasculature. To our best knowledge there are no other published studies reporting on the extent of interobserver agreement between two pathologists independently assessing MVI in RCC **(Paper II)**. We established criteria on MVI which were strictly followed by the two pathologists. The interobserver agreement was substantial, on the borderline to almost perfect (Cohen's kappa: 0.75).
Contrary to the findings of Lang et al.[101 102] when analyzing the MVI data in patients followed up shorter than 10 years and even shorter than 5 years, we found that MVI had a significant independent impact on CSS ($p<0.01$).

8.4.3 Interactions of SDM, VI and LNI

Our data showed a significant interaction between LNI and SDM ($p=0.008$). SDM turned out to be an independent prognostic marker and had the strongest impact without simultaneous LNI. However, in some other studies[158-159] distant metastatic disease did not significantly alter the prognosis in patients with N0VI+ disease.
Lymph node invasion has been shown to convey a sinister prognosis for RCC patients.[158-162] The reported incidence of LNI among patients treated with radical nephrectomy and lymph node dissection varies from 2% to 14.2%[162-164] depending on the study population and the time period. In our series **(Paper III),** 21 patients (10.7%) had lymph node metastases. The relatively high incidence of positive nodes reflects the patient selection as 45 patients had distant metastases when undergoing

RN. Three quarters of the node positive patients had synchronous distant metastases, in line with the findings of an autopsy study.[165]

Only few studies have analyzed survival by comparing N1/N2M1 and N0M1 disease; most patients with M1 disease are grouped together regardless of lymph node status. In the absence of LNI however, we found that patients with both SDM and VI had a significantly (p=0.012) shorter survival compared with those who had SDM only. The CSS difference between these two groups was not observed if LNI was present. In accordance with other reports, the impact of VI on survival was highest for patients free from nodal and distant metastases, and was insignificant in patients with both LNI and SDM.[160-161 166]

Our findings underline the prognostic importance of the status of the lymph nodes in metastatic RCC. A recently published study[64] on RCC in patients treated with cytoreductive nephrectomy supports our findings.

In the multivariate analysis of interactions of SDM, VI and LNI on survival, LNI showed a significant impact on survival only for the patients in whom we found no distant metastases or venous invasion. This finding is also in accordance with a recently published large multi-institutional European study[63] which showed that presence of nodal metastases in non-metastatic RCC had the strongest impact on cancer specific mortality of patients with T1 RCC, and intermediate effect for patients with T2-T3 RCC.

These findings imply that once RCC has spread to the lymphatic system the risk of hematogenous spread to other regions is high and it is likely that few patients would benefit from extensive lymph node dissection.

8.4.4 Thrombospondin-1 and p53

Both a promotive and an inhibitory role for TSP-1 in cancer cell proliferation and metastasis have been suggested.[79 167] Our study **(Paper IV),** however, demonstrated that TSP-1 expression was significantly associated with prognostic tumor features. We found a significant correlation between p53 status and TSP-1 expression that to our knowledge has not been reported previously. Interestingly, a relatively high percent (53 %) of the tumors was p53 positive which might imply upregulation of wild type protein in some cases. This is supported by the report of Chemeris et al[168] which demonstrated an adverse impact on prognosis in CCRCC of upregulation of the wild type p53 protein. Post-translational changes of the wild type p53 protein might account for the non-functionality of the protein. In multivariate analyses TSP-1 was an independent prognosticator of CSS. Absent or low expression of TSP-1 conveyed a 5.85 hazard rate of dying from CCRCC compared with moderate or high TSP-1. In accordance with our results Arai et al[169] reported frequent hypermethylation of the CpG island of the TSP-1 gene in CCRCC associated with adverse prognosis.
In our study TSP-1 and MVD were inversely associated, and high expression was detected in areas with stroma fibrosis and in the tumor pseudo capsule. The hot spots of MVD and TSP-1 were selected independently. It would be of interest to see whether high TSP-1 activity corresponded with low MVD in the same areas. We postulate such association which opens up for further investigations on the relationship between TSP-1, angiogenesis and fibrosis.

The inhibitory impact of TSP-1 on angiogenesis observed in CCRCC will most likely be utilized therapeutically. In fact, a peptide analogue of an angiogenic sequence of TSP-1 has shown some effect on survival in phase 1 and 2 trials of untreated metastatic RCC. The development of new agents that mimic the antiangiogenic properties of TSP-1 warrants further clinical investigations.

8.4.5 Proliferation index and TSP-1 expression

A high PI (Ki-67) was found in 34% of the tumors in our study **(Paper IV)**, which is in accordance with the frequencies reported in previous studies. The PI was significantly associated with p53 expression, which is in agreement with the results of Kankuri et al. and Zigeuner et al.[72 170] Our findings demonstrate a high proliferation index is significantly associated with no/low TSP-1 expression, confirming the results of Ren et al. and Miyanaga et al.[79 171] Therefore, the TSP-1 protein may have a direct effect on the proliferation of tumor cells. Like Kramer et al. and Kankuri et al.[71 72] we found the PI to be a significant predictor of CSS in univariate analysis. However, in contrast to the findings of Kramer et al.[71] we were able to prove an independent significance of this prognosticator in multivariate analyses, which exhibited a high HR of 2.37 (p=0.010).

9. Conclusions

The current thesis consists of four papers addressing different prognostic factors in renal cell carcinoma.

Paper I

- Performance status, tumor stage, nuclear grade and tumor necrosis were found to be independent prognostic factors for survival in patients with RCC.
- Our findings support the use of a two-grade system to discriminate between low- (Fuhrman NG1 and 2) and high-grade (Fuhrman NG 3 and 4) tumors.

Paper II

- Both tumor size and nuclear grade showed independent prognostic significance together with MVI for patients with organ confined CCRCC.
- Our findings indicate that it may prove to be of clinical importance to include MVI together with nuclear grade and tumor size in the histopathology report of organ-confined clear cell renal cell carcinoma.

Paper III

- Interactions between LNI, VI and SDM were analyzed in patients with CCRCC. LNI provided the strongest prognostic information for patients without SDM or VI whereas SDM and VI had strongest impact on survival when there was no nodal involvement.
- These findings imply that once RCC has spread to the lymphatic system the risk of haematogenous spread to other regions is high and it is likely that few patients would benefit from extensive lymph node dissection.

Paper IV

- A new molecular prognostic factor, TSP-1, was found to be an independent prognostic factor for cancer specific survival and significantly associated with reduced tumor angiogenesis, proliferation and aggressiveness.
- Our findings reveal a significant correlation between p53 status and TSP-1 expression in CCRCC.

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10. Future perspectives

Tumor grade and stage are well known prognostic indicators for patients with RCC while MVI is not as well established. Our findings support the use of a two-graded nuclear grading system for CCRCC that potentially could reduce inter- and intraobserver inconsistencies and improve the prognostic significance of grading. This system of grading should be externally validated in a larger patient series to confirm its prognostic significance.

According to our analyses, the presence of MVI in the tumor appeared to portend a significantly worse prognosis for patients with low stage CCRCC. Consequently, patients with MVI and otherwise considered to be at low risk for progression, should be followed more closely than low risk patients without MVI. However, there is a need for caution when assessing our results because wide differences in the number of tumors found to have MVI have been reported. Despite the considerable variation in reported frequencies of MVI, it does appear to be a promising prognostic marker and

our findings justify further larger prospective multicentric studies in order to test its prognostic significance in conjunction with established prognostic factors. By confirming its reproducibility as a prognostic factor in RCC, MVI might be a valuable addition to prognostic models.

The novel finding that TSP-1 is independently associated with cancer-specific survival in a population based cohort of patients with CCRCC should be corroborated in a larger prospective study. Interestingly, TSP-1 and MVD were inversely associated, and high expression was detected in areas with stroma fibrosis and in the tumor pseudo capsule. Therefore it would be of interest to see whether high TSP-1 activity corresponds with low MVD in the same areas. We postulate such an association which opens up for further investigations on the relationship between TSP-1, angiogenesis and fibrosis. The inhibitory impact of TSP-1 on angiogenesis observed in the current study will most likely be used therapeutically. New angiogenesis inhibitors that mimic the angiogenetic properties of TSP-1 warrant further clinical investigation.

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