Venous perfusion and intravenous dissection for fixation, evaluation and staging of renal tumours in nephrectomy specimens

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Abstract

Invasion of renal cell carcinoma into the renal vein has a detrimental effect on the prognosis as this is an important tumour dissemination route. Determination of renal vein invasion is vital to accurate pathological staging. The purpose of this study is to determine if perfusing with formalin and probing the veins of radical nephrectomy specimens allows for easier visualization of the veins and an improved diagnosis of vascular invasion. In this study, 28 radical nephrectomy specimens were examined using renal vein probing and perfusion techniques. The tumours were segregated based on size, Fuhrman grade and tumour type. Comparison of the study tumours versus renal tumours examined in 2009 that were not perfused and probed were based on these groupings. There was a trend to identifying more renal vein invasion, especially for tumours 4.1 to 7.0 cm in diameter, but this did not result in statistical significance in this small study group.

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Introduction

Epidemiology

Renal cell carcinoma, also known as adenocarcinoma of the kidney, is a cancer that arises from the renal parenchyma and derives from renal tubular epithelium. The incidence of renal cell carcinoma has steadily increased during the past 30 years and this increase in incidence has been more rapid in those of African descent and in females in general (Lipworth *et al*, 2006), even though there is still a 2-3:1 male to female predominance (Kumar *et al,* 2010). The incidence of renal cell carcinoma increases steadily after 40 years of age (Eble *et al*, 2004), with the average age at diagnosis being 66 years and the average age of death being 70 years (Ng *et al,* 2008). Renal cell carcinoma tends to have a higher incidence in Western and Eastern Europe, North America and Australia. Asia and Africa have the lowest rates (Lipworth *et al,* 2006). These trends are attributable to increased medical imaging in Western countries. In 2008, It was estimated that 54 390 new cases of renal cancer would be diagnosed in the US, and that 13 010 people would die from this disease (Jemal *et al,* 2008). There are an increasing number of asymptomatic tumours being discovered incidentally during radiologic studies, such as MRI or CT scan, which are performed for non-renal reasons (Chow *et al,* 1999).

Etiology

Cigarette smoking is the most significant risk factor for renal cell carcinoma. Cigarette smokers have twice the incidence of renal cell carcinoma compared to non-smokers (Kumar *et al*, 2010). The risk of renal cell carcinoma increases steadily as body mass index increases (Eble *et al*, 2004). The increase may be partly explained by a rise in obesity (Lipworth *et al*, 2006). There is also a significant increase of renal cell carcinoma in those with a history of hypertension

(Eble *et al,* 2004). Additional factors that are thought to increase risk of renal cell carcinoma are unopposed estrogen therapy; exposure to asbestos, petroleum products, and heavy metals; chronic renal failure with acquired renal cystic disease and tuberous sclerosis (Kumar *et al,* 2010).

In general, the occurrence of renal cell carcinoma is sporadic. However, about 4% of renal cancers are autosomal dominant familial cancers (Kumar *et al,* 2010). Some tumours are associated with the Von Hippel-Lindau (VHL) syndrome. These are mostly of the clear cell type and are associated with germline mutations of the *VHL* gene located on chromosome 3p. Somatic VHL mutations have also been observed in about 50% of sporadic cases of renal cell carcinoma. Hereditary papillary carcinoma is another autosomal dominant form and is related to germline mutations and activation of the MET proto-oncogene located on chromosome 7p (Moore and Wilson, 2005).

Clinical Features

The majority of renal cell carcinomas will be detected incidentally. Renal cell carcinoma can present with a classic triad of features, which include costovertebral flank pain, palpable mass and hematuria. Some patients present with one of these symptoms, but the combination of all three is only seen in 10% of cases (Kumar *et al,* 2010). Others present with systemic symptoms such as weight loss, abdominal pain, anorexia and fever (Eble *et al,* 2004). All of the symptoms are generally indicative of more advanced disease as there is a tendency with renal cell carcinoma to remain silent, or asymptomatic, until the tumour has attained a larger size, sometimes reaching a diameter of 10 cm or greater, or metastasized. Even then, the symptoms that present may not be directly related to the kidney, resulting in renal cell carcinoma being

considered a disease of mimicry. Very rarely, renal cell carcinoma can result in multiple disorders related to abnormal hormone production or a paraneoplastic syndrome. Some renal cell carcinoma patients will have widely metastatic disease before becoming symptomatic.

Gross Pathology

The gross appearance of renal cell carcinomas can vary depending on the type of tumour (Figure 1). Clear cell carcinomas are more likely to arise from the proximal tubular epithelium and there is usually only a solitary unilateral lesion. Papillary tumours usually arise

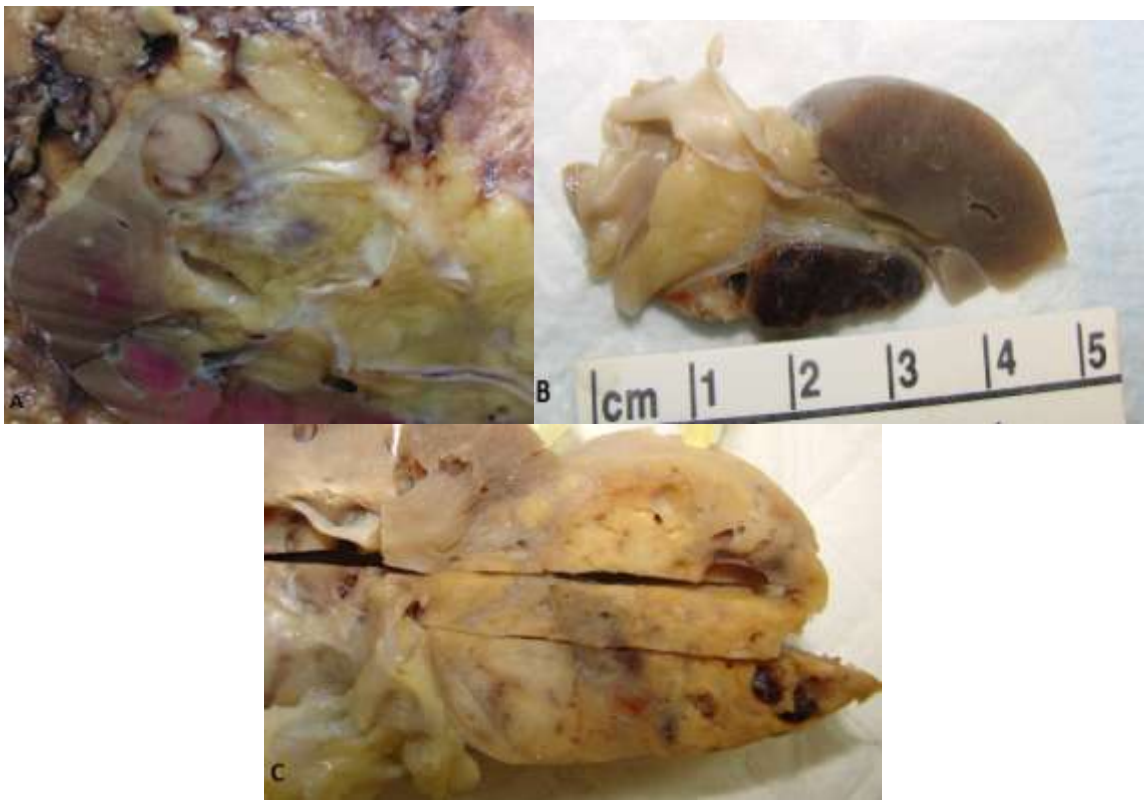

Figure 1*: Gross pictures of renal cell carcinoma*. A-C) Gross pictures of clear cell type renal cell carcinomas from radical nephrectomy specimens without venous invasion identified.

from the distal convoluted tubules, and can be multifocal and bilateral (Kumar *et al,* 2010). Renal cell carcinoma tumours often appear as encapsulated, spherical masses of varying sizes. The consistency of the tumours may be solid, cystic or mixed, with areas of calcification, fibrosis, necrosis and hemorrhage (Ng *et al*, 2008). Overall, tumours usually have a yellow-orange appearance due to the accumulation of lipids in the tumour cells (Kumar *et al,* 2010).

Histological Sub-Types

Histologically there are four major types of tumour associated with renal cell carcinoma. Clear cell carcinoma is the most common type. It accounts for 70% to 80% of renal cell cancers and can be familial but is sporadic 95% of the time. The tumour cells form packets and typically have clear or granular, eosinophilic cytoplasm. The second most common type, accounting for 10% to 15% of renal cell cancers, is papillary carcinoma. It too can be familial or sporadic, and is characterized by a papillary growth pattern with vascular stromal cores covered by neoplastic epithelium. Accounting for 5% of renal cell tumours is chromophobe renal carcinoma. It is composed of cells with prominent cell membranes and pale eosinophilic cytoplasm, usually with a halo around the nucleus. The least common renal cell tumour is collecting duct, or Bellini duct carcinoma, which accounts for 1% or less of renal cell carcinomas. These tumours arise from collecting duct cells in the medulla (Kumar *et al,* 2010) and typically have an invasive poorly differentiated glandular pattern.

Fuhrman Nuclear Grading

One prognostic predictor for renal cell carcinoma is histologic nuclear grade. In North America, the Fuhrman grading system is most commonly used. Created in 1982 by Fuhrman *et al*, the system is based solely on nuclear features of tumour cells in renal cell carcinoma. A

Fuhrman grade 1 tumour would have tumour cells that have small (approximately 10 μm), round, uniform nuclei without nucleoli. A Fuhrman grade 2 would have tumour cells with larger nuclei (approximately 15 μm) with irregularities in outline and visible nucleoli when examined under higher power (400X). Tumour cells with even larger nuclei (approximately 20 μm) with an obvious irregular outline and prominent larger nudeoli even at low power (100X) would be classified as a Fuhrman grade 3. The highest grade, a Fuhrman grade 4, would have tumour cells with bizarre, multilobed nuclei and heavy clumps of chromatin. Grade 1 tumours have a lower metastatic risk than tumours that are grades 2 to 4, and are associated with a greater 5-year survival rate (64% for a grade 1 tumour versus 10% for a grade 4 tumour) (Novara *et al.*, 2007).

TNM Staging

The Union for International Cancer Control (UICC) first published recommendations for the clinical stage classification of cancers in 1958. The general technique of classification was based on the anatomical extent of the disease using the Tumour, Node, Metastasis (TNM) system (Sobin *et al,* 2010). According to the seventh edition TNM staging handbook (Sobin *et al,* 2010), which is the most current version, renal cell carcinoma tumours are staged based on size and extent of invasion beyond the kidney. A T1 tumour is any tumour less than or equal to 7 cm in diameter and confined to the kidney. Within this stage, a tumour can be considered to be T1a, which is any tumour less than or equal to 4 cm, or T1b, which is any tumour between 4.1 cm and 7.0 cm. For a tumour be considered T2, it must be confined to the kidney and greater than 7 cm. A T2a tumour is any tumour that is 7.1 cm to 10.0 cm and a T2b tumour is any tumour greater than 10 cm. If a tumour extends into any major veins or perinephric tissues but not into the adrenal gland or beyond the Gerota fascia, it is considered to be a T3 tumour. A T3a tumour is one that extends grossly into the renal vein or its segmental, muscle-containing branches, or

one that invades perinephric and/or renal sinus fat (Sobin *et al,* 2010). That is, there is cellular invasion of tumour cells into fat without a surrounding pseudocapsule (Fleming and Griffiths, 2004). A T3b tumour extends grossly into the vena cava below the diaphragm and a T3c tumour extends grossly into the vena cava above the diaphragm or invades the wall of the vena cava. Finally, a T4 tumour invades beyond Gerota's fascia, which can include contiguous extension into the ipsilateral adrenal gland (Sobin *et al,* 2010). It is important to note that most of the criteria of the TNM staging are derived from macroscopic observation from the primary specimen dissection rather than histology. Assessing the status of the lymph nodes, invasion into renal sinus including microvenous invasion, invasion of segmental veins, and micro-invasion outside of the kidney are assessed histologically (Fleming and Griffiths, 2004).

Prognosis

There is a correlation with prognosis and staging severity, with lower stage disease being associated with longer survival rates and a higher stage disease being associated with a worse prognosis (Ng *et al,* 2008). The 5-year survival rate, on average, for an individual with renal cell carcinoma is approximately 45% but can be as high 70% if there are no distant metastases (Kumar *et al,* 2010). Specifically, a person with a T1a tumour has a 5-year survival rate of 90% to 100%, whereas it is 80% to 90% if they have a T1b tumour. The 10-year survival rate of a T1 tumour is about 91%. As tumour size increases above 7 cm, the 5-year survival rate decreases to 70% to 80% and the 10-year survival rate to 70% (Ng *et al,* 2008). Invasion into renal vein or perinephric fat has a detrimental effect on the prognosis, resulting in an approximate 5-year survival rate of 15% to 20% (Kumar *et al,* 2010). For any T4 tumours, lymphatic involvement or systemic metastases imparts a 0% to 20% 5-year survival rate (Ng *et*

al, 2008). The occurrence of metastasis with renal cell carcinoma of any size has a negative effect on prognosis.

Treatments

Current treatment options are mostly limited to surgery as renal cell carcinoma is unresponsive to most conventional chemotherapeutic options. If the renal cell carcinoma tumour is localized, surgical resection is often curative and the only known effective treatment (Curti, 2004; Rini *et al.*, 2008). Radical nephrectomy involves complete removal of Gerota's fascia and its contents, including the kidney and the adrenal gland (Karumanchi *et al,* 2002). Partial nephrectomy is the standard procedure for suitably located, smaller tumours whereby the renal tumour is excised from the kidney and is done in the hopes of preserving renal function (Kumar *et al,* 2010). It is important to determine by imaging techniques the tumour size as well as the extent of the tumour by distinguishing if the tumour is limited to the renal parenchyma or extending into the renal veins (Ojemakinde *et al.*, 2011 and Zhao *et al.*, 2011). The surgical implications of a T4 tumour, which would be one that has spread beyond Gerota's fascia, are that the tumour may not be completely excised and that there would be residual retroperitoneal disease (Fleming and Griffiths, 2004). Residual tumour would mean that the patient had not been cured and the prognosis would be poor. A T3 tumour that has extended into the renal vein results in the patient doing almost as poorly as if they had a T4 tumour, even though it is theoretically more easily resected.

Implications of a T3 Tumour

Anatomically, there is no fibrous barrier between the renal cortex and the renal sinus fat, the way that there is between the renal cortex and the perinephric fat. The renal capsule,

which separates the renal cortex from the perinephric fat, terminates just inside the hilus of the kidney. The renal sinus contains lymphatics and many large thin-walled tributaries of the main renal vein. It has been observed that a tumour can directly invade venous structures from sinus fat (Bonsib *et al,* 2000). It has also been observed in some tumours that venous involvement can occur without identified extension into the sinus fat. This may mean that sinus fat invasion represents tumour extension from initially involved sinus veins, rather than direct extension from the main cortical tumour (Bonsib, 2004).

Invasion into the renal sinus is fairly common, but is more common in high-grade tumours than in low-grade tumours and is correlated to tumour type (Bonsib, 2004). Renal vein extension occurs in approximately 20% to 35% of patients, and into the inferior vena cava 4% to 10% of the time. It is also more common for there to be a tumour thrombus in the inferior vena cava coming from the right side as the renal vein is shorter on this side (Ng *et al,* 2008). It has been suggested that an important route for dissemination in renal cell carcinoma is through renal sinus fat invasion, and particularly invasion into the renal sinus veins (Bonsib, 2004). If venous invasion is accepted as being a precursor to hematogenous metastases then it is important to recognize venous invasion during the initial dissection of the nephrectomy specimen (Fleming and Griffiths, 2004).

At the time of diagnosis, approximately 25% to 30% of patients have metastatic disease, and more than 95% of these have multiple metastases. Even in those patients with localized disease, 20% to 30% develop metastases after nephrectomy (Curti, 2004). If early dissemination of renal cell carcinoma occurs in tumours that invade the renal sinus fat and renal veins then those patients that were diagnosed as pT1 or pT2 that subsequently develop metastases and/or die from the disease may, in fact, have unrecognized renal sinus and renal vein invasion

(Thompson *et al,* 2007). This early dissemination could account for T3 tumours doing almost as poorly as T4 tumours, as tumour cells have already metastasized at the time of surgery.

It is important to recognize disease extension into the veins of the renal sinus as it may identify patients who are at risk for metastatic disease even when the tumour is otherwise confined to the kidney (Mejean *et al,* 2003). Proper sampling of the renal sinus should be included in appropriate assessment of nephrectomy specimens (Thompson *et al,* 2007). Recent studies using venous perfusion to fix radical nephrectomy specimens and rigorous probing of the renal vein branches to aid in intravenous dissection have suggested that this gives better visualization of the veins, and the ability to recognize more subtle venous extension was improved (Bhalodia and Bonsib, 2010; Bonsib and Bhalodia, 2010; Bonsib, 2007). Those patients that do have venous invasion identified would have more frequent post-operative follow-up than those patients without venous invasion. Patients are usually seen every 3 to 6 months postoperatively for the first year, with a chest x-ray, blood work, and urinalysis. Renal imaging is generally done at a year post-operation. They are then seen every 6 to 12 months thereafter, with the same studies being done.

Objective

The purpose of this study is to determine if perfusing the veins with formalin of radical nephrectomy specimens and probing the renal veins prior to slicing open the kidney allows for easier visualization of the veins and therefore an improvement in the frequency of the diagnosis of venous invasion of renal cell carcinoma. If the venous system is the primary route of dissemination of renal cell carcinoma, then there are important negative implications on the prognosis of the patient. The survival outcome decreases as the T-stage increases from a 90% 5-

year survival rate for a T1 tumour and a 70% 5-year survival rate for a T2 tumour, to 20% for a T3 tumour (Ng *et al*, 2008). Our study is based on the previous works of Bonsib and Bhalodia (Bhalodia and Bonsib, 2010; Bonsib and Bhalodia, 2010) and is a validation of their method.

Materials and Methods

In this study, 28 radical nephrectomy specimens containing renal cell carcinoma were examined using renal vein probing and perfusion techniques. Specimens were obtained from those sent to the surgical pathology cutting room at the Health Sciences Centre (HSC) and the Grace General Hospital (GGH) (Figure 2). At HSC, 22 specimens were perfused via the renal vein with formalin for several minutes until inflated (Figure 4 C-D). The specimens were then placed in formalin intact overnight. The following day, the specimens were probed along the renal vein

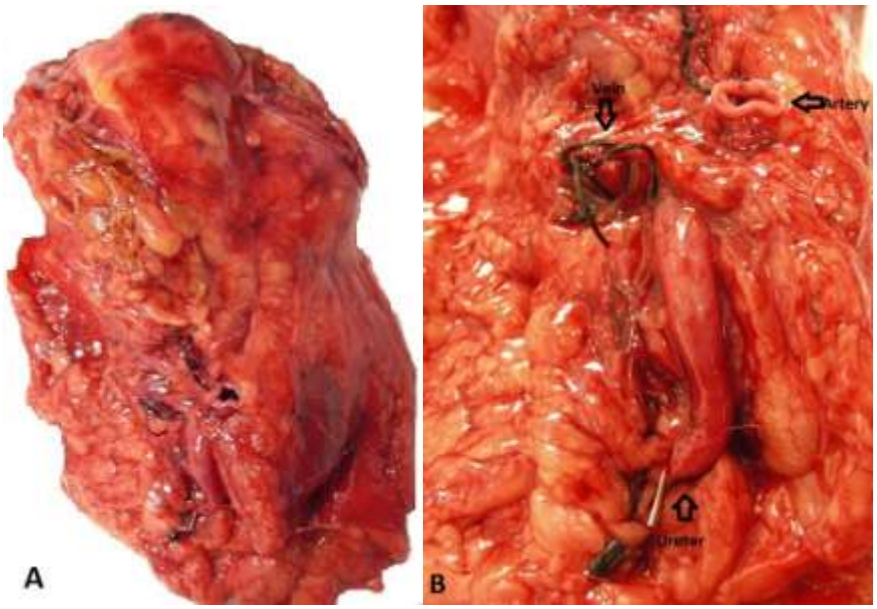

Figure 2: *Radical nephrectomy specimens*. A) A radical nephrectomy specimen sent fresh to the cutting room at HSC. B) Ureter is clipped and renal artery and vein are sutured.

using 2-4 probes and bisected (Figure 3). Specimens were otherwise grossed as per the standard protocol for tumour nephrectomies. At GGH, 6 specimens were perfused via the renal vein with 60cc of formalin (Figure 4 A-B). The specimens were then placed in formalin intact for

approximately an hour. They were then probed along the renal vein using 2-4 probes and bisected, and placed back in formalin overnight. The specimens were grossed as per the standard protocol for tumour nephrectomies the following day.

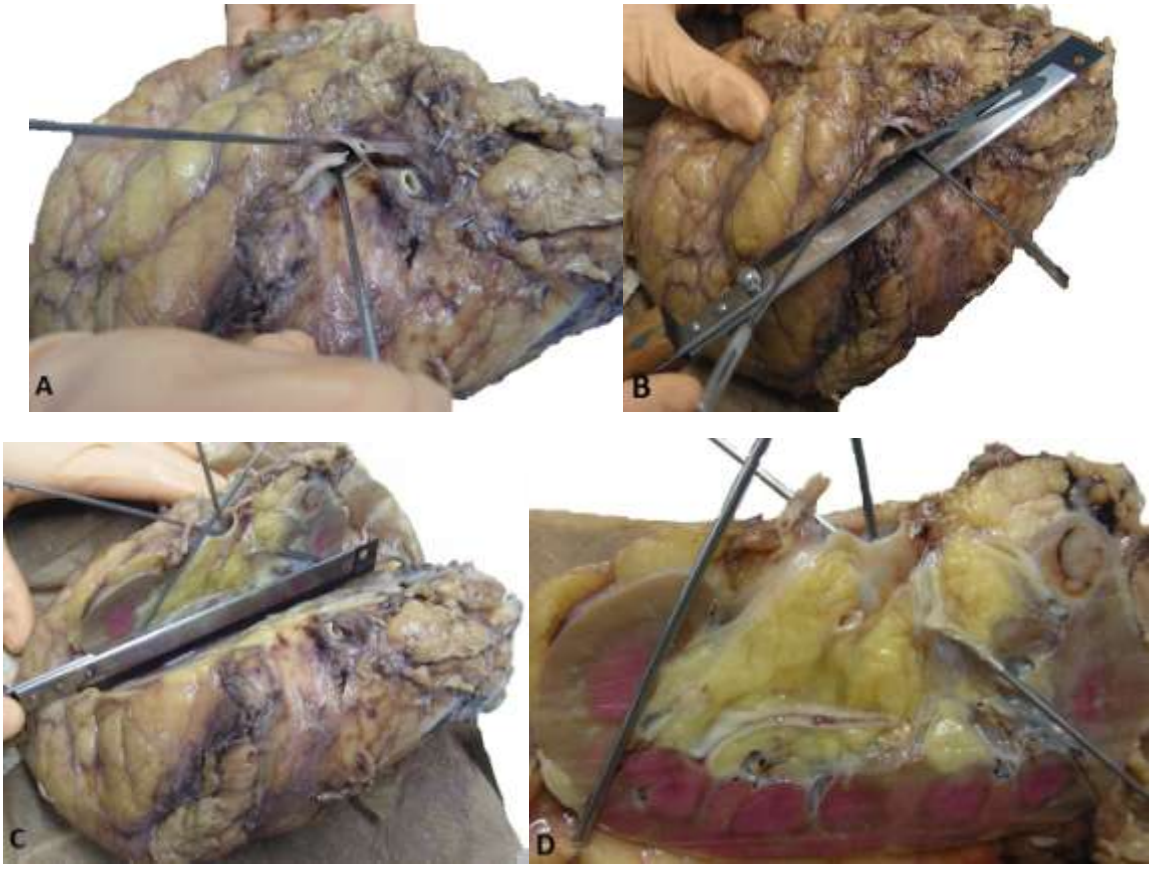

Figure 3: *Venous probing and bisection.* A) Specimen is probed along the branches of the renal vein. B and C) Specimen is bisected along the probes within renal veins. D) Specimen is completely bisected with renal vein probes in place.

This study was conducted from May 2010 to February 2011. The incidence of renal vein invasion in cases during the study period was then compared to the incidence of renal vein invasion in those radical nephrectomy specimens examined without specific renal vein probing

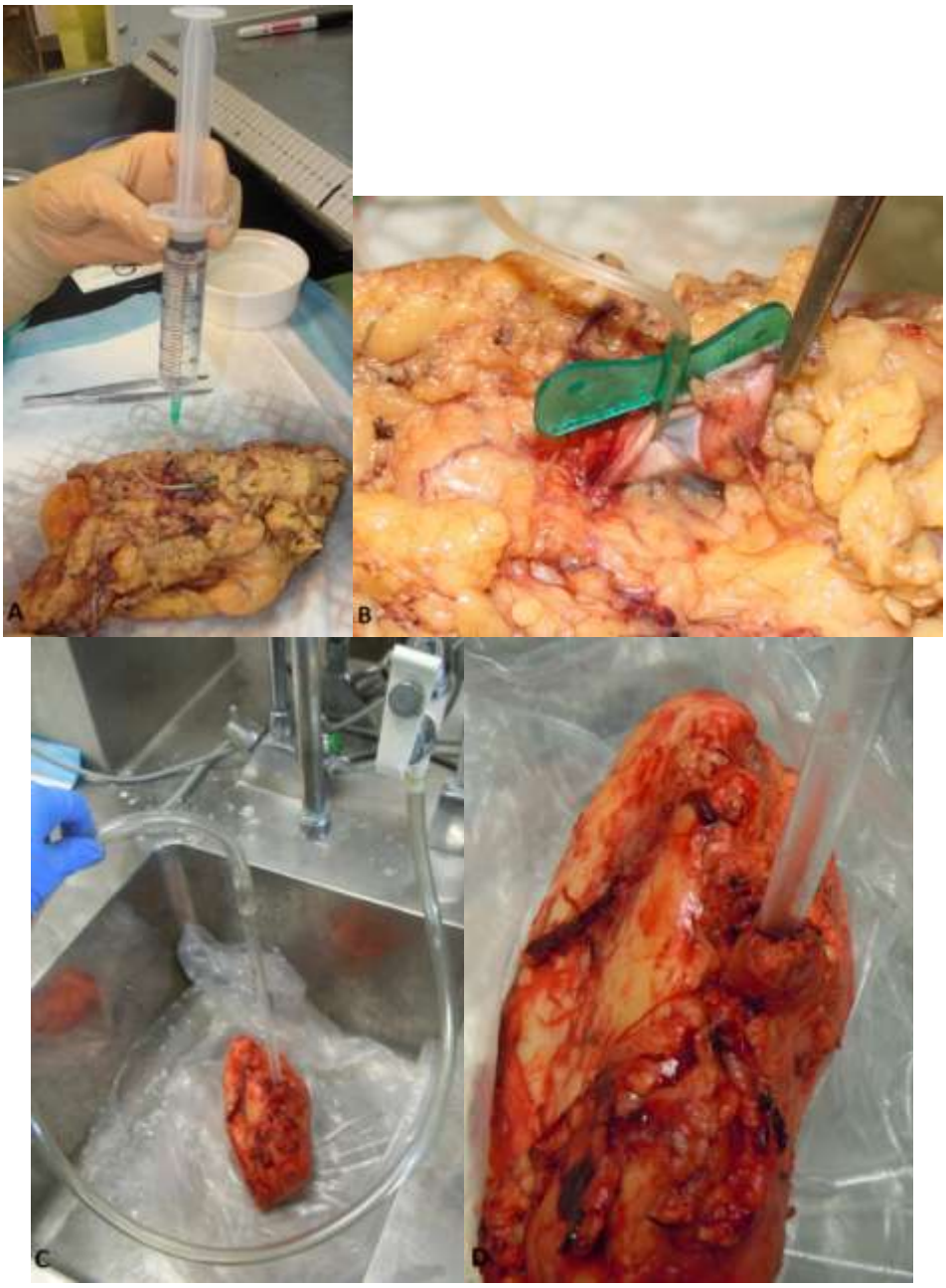

Figure 4: *Renal vein perfusion at GGH and HSC.* A-B) Renal vein perfusion at GGH with approximately 60cc of formalin with a syringe and butterfly needle. C-D) Renal vein perfusion at HSC with a pipette hooked up to the formalin tap for approximately 10 minutes until kidney was inflated.

and perfusion techniques in 2009. Pathology reports were found using SNOMED (systemized nomenclature of medicine) using the search parameters "kidney OR kidneys" for both locations, Health Sciences Centre and Grace General Hospital. Each report was then manually reviewed and only those kidneys that were radical nephrectomies for renal cell carcinoma were included in this study. There were a total of 42 reports of radical nephrectomies for renal cell carcinoma from 2009.

Pearson Chi-Square analysis or Fisher's Exact tests were used to compare the project kidneys to the nephrectomy specimens from 2009 as applicable. Fisher's Exact test was used when the sample size was too small to be utilized in the Pearson Chi-Square analysis. The renal tumours were segregated into groups based on size, Fuhrman grade and tumour type, and compared on these basis. A logistic regression model was also applied to the data based on size, Furhman grade and venous invasion.

Results

2009 Nephrectomies

There were a total of 42 kidneys removed for renal cell carcinoma with radical nephrectomy in 2009 from HSC and GGH (Table 1). Thirty-two kidneys were removed at HSC and ten were removed at GGH. Only eight kidneys were found to have venous invasion. All eight were grossed and diagnosed at HSC. Thirty-six of the kidneys were of the clear cell type, including all eight with venous invasion. The kidneys were also categorized by Fuhrman grade, with two kidneys having a Fuhrman grade 1, twenty-three were Fuhrman grade 2, sixteen were Fuhrman grade 3 and one was Fuhrman grade 4 (Table 2).

Table 1: *Total number of radical nephrectomies for renal cell carcinoma from 2009 at HSC and GGH.* The numbers in brackets indicate those of clear cell type.

Tumour size (cm)	Total Number of Kidneys (clear cell type)	Number of Kidneys w/ venous invasion (clear cell type)
Less than or equal to 4.0	7 (5)	0 (0)
4.1 - 7.0	23 (20)	3 (3)
7.1 - 10.0	8 (8)	4 (4)
Greater than 10.0	4 (3)	1 (1)
All Kidneys	42 (36)	8 (8)

The first case with venous invasion from the 2009 group had a tumour with Fuhrman grade 2 and a maximum dimension of 5.0 cm. On gross inspection the tumour extended into the renal sinus and on histological inspection there was focal invasion by the tumour through the muscle wall of a moderate-sized vein within the renal sinus. The second case had a Fuhrman grade of 2 and a maximum dimension of 9.5 cm. The tumour extended to the renal sinus but did not definitively invade it grossly. The tumour invaded a segmental renal vein when examined

under the microscope. The third case had a tumour with a Fuhrman grade of 3 and a maximum dimension of 4.5 cm. Grossly, the tumour invaded the renal sinus. Histologically, there was

Table 2: *Total number of kidneys from 2009 categorized based on Fuhrman grade.* The numbers in brackets indicate those of clear cell type.

Fuhrman Grade	Total Number of Kidneys (clear cell type)	Number of Kidneys w/ venous invasion (clear cell type)
1	2 (2)	0 (0)
2	23 (19)	3 (3)
3	16 (14)	4 (4)
4	1 (1)	1 (1)
Total	42 (36)	8 (8)

venous invasion into a segmental renal vein branch. Case #4 had a tumour with Fuhrman grade 3 and a maximum dimension of 7.5 cm. The tumour grossly and histologically invaded the renal vein and renal sinus. Case #5 had a Fuhrman grade of 2 with a maximum dimension of 9.0 cm. The renal vein was compressed by the tumour upon gross inspection, but did not definitively invade it. Under the miscropscope, the tumour was noted focally in the renal vein and one of its segmental branches. The sixth case had a tumour with Fuhrman grade 4 and a maximum dimension of 8.0 cm. The tumour extended to and involved the renal sinus grossly. Histologically there was a focus of invasion into a muscle-containing segmental branch of renal vein. The seventh case, with a Fuhrman grade of 3 and a maximum dimension of 4.6 cm, did not definitely involve the renal sinus. The tumour ran adjacent to the renal vein but did not grossly appear to involve it. Histological examination showed that the tumour was positive for venous invasion. The eighth and final case with venous invasion was a tumour with Fuhrman grade 3 and a

maximum dimension of 13.0 cm. The tumour appeared to grossly extend to the renal sinus but did not definitely involve it. Microscopically the tumour infiltrated the renal vein.

Table 3: *Total number of radical nephrectomies for renal cell carcinoma that were from the study group at HSC and GGH.* The number in brackets is the total number of tumours that are of clear cell type.

Tumour size (cm)	Total Number of Kidneys (clear cell type)	Number of Kidneys w/ venous invasion (clear cell type)
Less than or equal to 4.0	8 (5)	0 (0)
4.1 - 7.0	14 (13)	5 (5)
7.1 - 10.0	2 (2)	1 (1)
Greater than 10.0	4 (2)	2 (2)
All Kidneys	28 (22)	8 (8)

2010 Study Nephrectomies

There were a total of 28 kidneys removed for renal cell carcinoma with radical nephrectomy from HSC and GGH included in the study group (Table 3). Twenty-two kidneys were removed at HSC and six were removed at GGH. Eight kidneys were found to have venous invasion. Of these eight, seven were grossed and diagnosed at HSC, with the eighth grossed and

Table 4: *Total number of kidneys from the study group categorized based on Fuhrman grade.* The numbers in brackets indicate those of clear cell type.

Fuhrman Grade	Total Number of Kidneys (clear cell type)	Number of Kidneys w/ venous invasion (clear cell type)
1	1 (0)	0 (0)
2	7 (7)	2 (2)
3	13 (11)	3 (3)
4	5 (3)	3 (3)
Total	26 (21)	8 (8)

diagnosed at GGH. Twenty-two of these kidneys were of the clear cell type, including all eight with venous invasion. When categorized based on Fuhrman grade, one was Fuhrman grade 1, seven were Fuhrman grade 2, thirteen were Fuhrman grade 3 and five were Fuhrman grade 4 (Table 4).

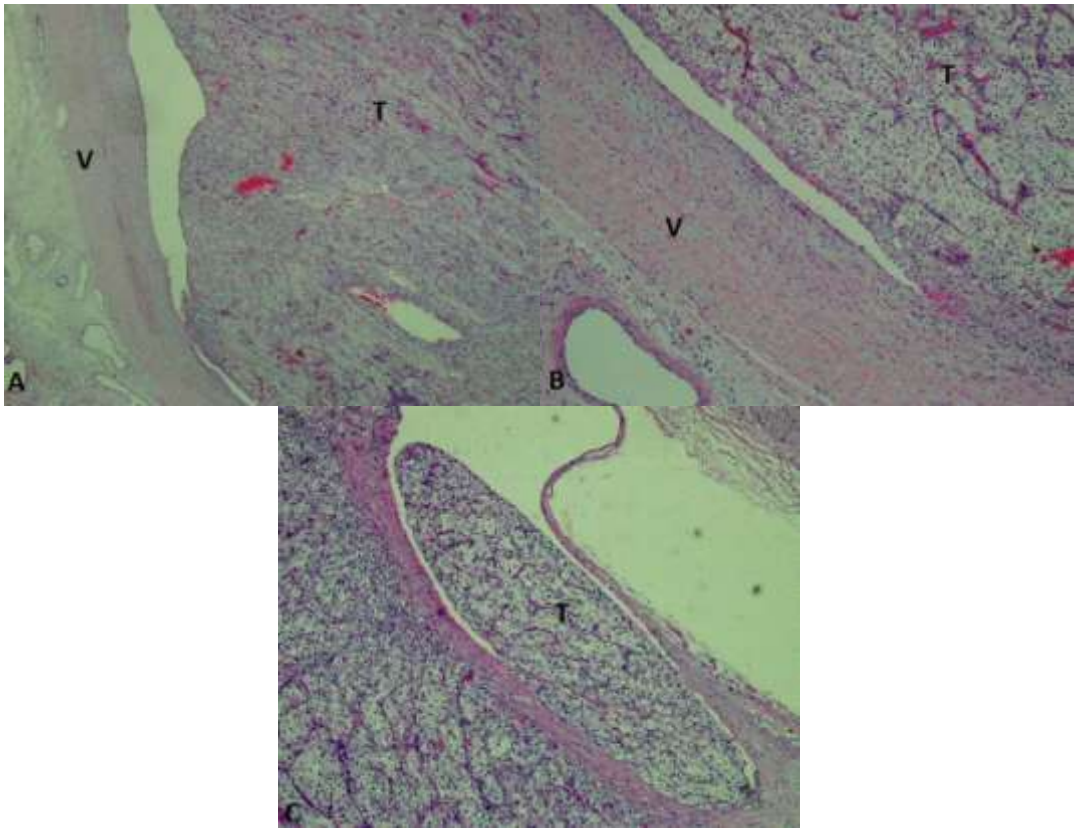

Figure 5: *Histological renal vein invasion in case #1 from the study group*. A) Tumour thrombus (T) in a large segmental branch of renal vein (V). B) Tumour thrombus (T) lined by endothelial cells invading into the wall of renal vein (V). C) Tumour thrombus (T) in a segmental branch of renal vein.

The first case with venous invasion from the study group had a Fuhrman grade of 3 and a maximum dimension of 5.7 cm. The tumour grossly appeared to involve a branch of the main

renal vein and was 0.8 cm from the venous line of resection. Histologically, the tumour invaded into a larger segmental branch of the renal vein (Figure 5). The second case with venous invasion had a Fuhrman grade of 3 and a maximum dimension of 5.0 cm. On gross examination the tumour did not appear to invade the renal sinus fat or any branches of renal vein. On histological examination, however, there was a focus of invasion into a thin-walled muscle-containing segmental branch of the renal vein (Figure 6). In case #3, the Fuhrman grade was 4

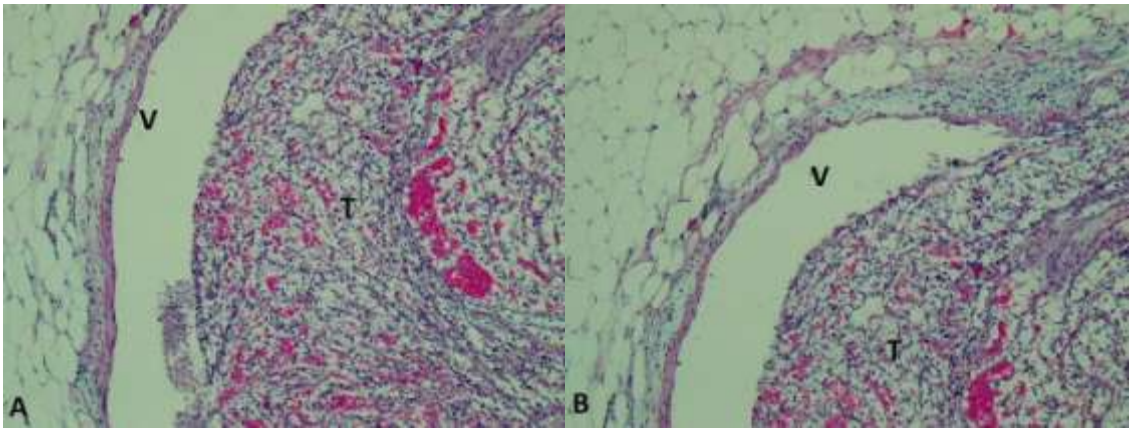

Figure 6: *Histological renal vein invasion in case #2 from the study group*. A) Tumour thrombus (T) in segmental branch of renal vein (V). B) Tumour thrombus (T) invading into the wall of a segmental branch of renal vein (V).

and the maximum dimension was 4.5 cm. The tumour was noted to grossly extend into the renal sinus and bulge into the renal vein. Under the microscope the tumour is seen to invade into large, muscle-containing segmental branches of renal vein within the renal sinus (Figure 7). Case #4 had a tumour with a Fuhrman grade of 4, and a maximum dimension of 5.9 cm. The tumour did not definitely invade the renal sinus or segmental branches of the renal vein on gross inspection. On histological inspection there was focal tumour invasion into a segmental muscle-containing branch of the renal vein (Figure 8). The fifth case had a tumour with a Fuhrman grade of 2 and a maximum dimension of 7.1 cm. Grossly, the tumour appeared to involve the renal

sinus and histologically it infiltrated into a muscle-containing segmental branch of renal vein in the renal sinus (Figure 9). Case #6, grossed at GGH, had a tumour with a Fuhrman grade 2 and a maximum dimension of 4.5 cm. The tumour appeared to grossly bulge into the renal sinus but did not definitely invade it. The tumour invaded into the renal vein upon histological examination (Figure 10). Case #7 had a tumour with a Fuhrman grade of 4 and a maximum dimension of 10.2 cm. Grossly, the tumour extended to the renal sinus but did not definitely

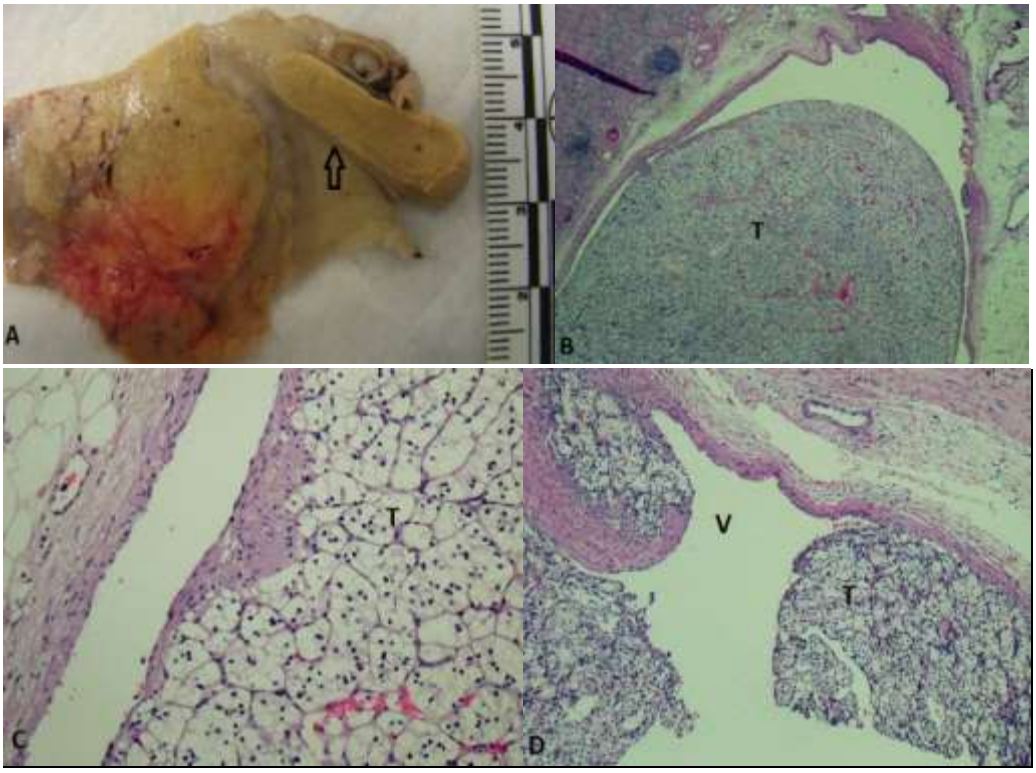

Figure 7: *Gross and histological renal vein invasion in case #3 from the study group*. A) Gross picture of venous invasion (arrow) in a radical nephrectomy specimen. B) Tumour (T) within renal vein. C) Tumour thrombus (T) lined with endothelial cells. D) Tumour (T) invading the wall of segmental branches of the renal vein (V).

involve it, while under the microscope there was invasion into segmental branches of the renal vein (Figure 11). The eighth and last case with venous invasion had a tumour with Fuhrman

grade 3 and a maximum dimension of 10.3 cm. Upon gross inspection, a tumour thrombus could be seen within the renal vein at the hilum line of resection. The tumour also appeared to extend into the renal sinus. Histologically, the vein line of resection was negative for tumour invading into the wall but there was tumour present in the main renal vein and its segmental branches (Figure 12).

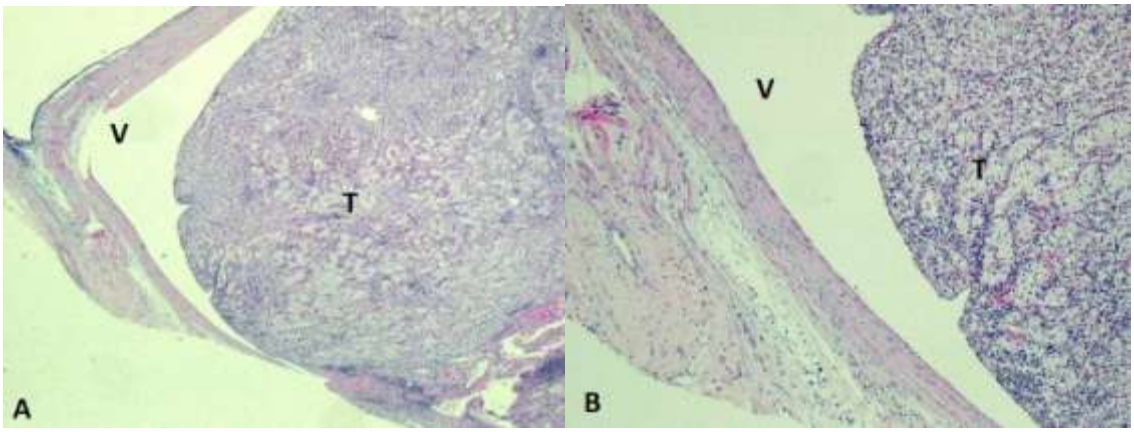

Figure 8: *Histological renal vein invasion in case #4 from the study group.* A) Tumour (T) invading into the wall of a segmental branch of the renal vein (V). B) Tumour (T) thrombus within the renal vein (V).

To compare the 2009 data to the study group a Pearson chi-squared analysis or a Fisher's exact test was conducted, depending upon the sample size and the variables being compared (Table 6). First, all the kidneys from 2009 were compared to all the kidneys from the study group, regardless of tumour size, cell type or Fuhrman grade. Next only the clear cell type tumours from the two groups were compared, regardless of tumour size or Fuhrman grade. Then the kidneys were broken down into groups based on size. The size categories correspond to the T-stage parameters from the UICC TNM guidelines (Sobin *et al.*, 2010). All the kidneys were compared based on these variables, and then only those kidneys that were of clear cell

type were compared. Comparisons between all of the kidneys and then only those of clear cell type were also done based on Fuhrman grade. In all of the categories, the $p > 0.05$ which means that none of the findings were statistically significant.

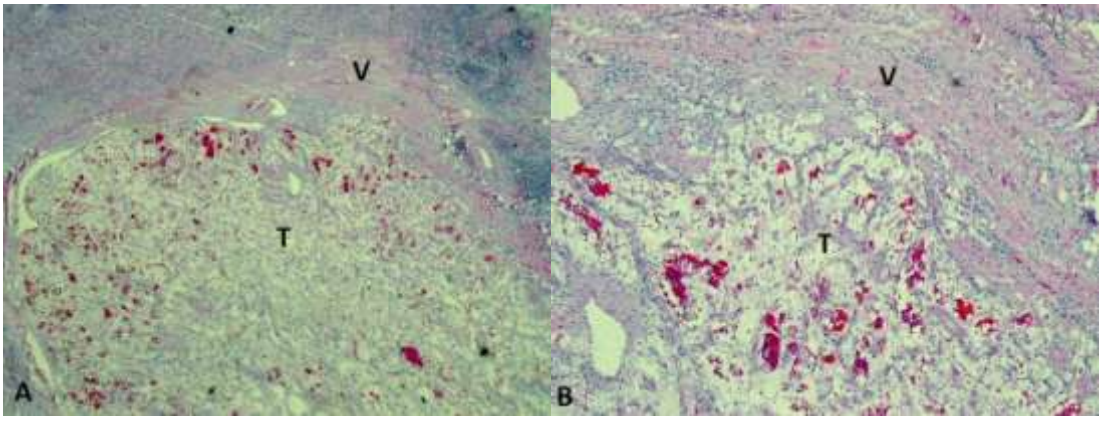

Figure 9: *Histological renal vein invasion in case #5 from the study group*. A-B) Tumour infiltrates into a muscle containing segmental branch of renal vein in the renal sinus disrupting the wall of the vein.

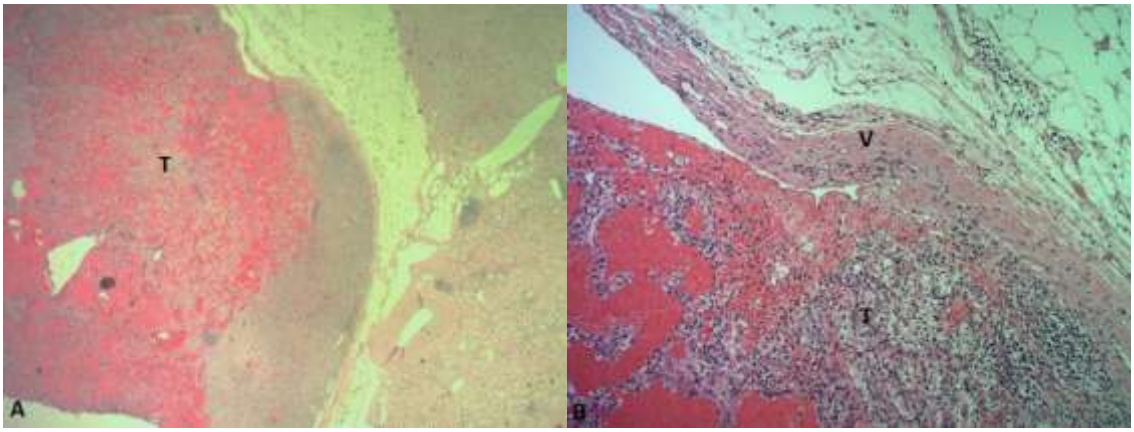

Figure 10: *Histological renal vein invasion in case #6 from the study group.* A) Tumour thrombus (T) within the renal vein. B) Tumour (T) invading the wall of the renal vein (V).

There were some trends that emerged, especially in those clear cell tumours that were 4.1 cm to 7.0 cm. In the study group tumours, five out of thirteen (38%) had venous invasion (Table 3), whereas only three out of 20 (15%) 2009 clear cell tumours had venous invasion (Table 1).

The gross identification of venous invasion in the 2009 cases was compared to that seen in the 2010 study cases, overall as well as in the clear cell histological type (Table 5 and Table 7). One out of forty-two cases (2.4%) in 2009 has venous invasion grossly identified overall. Considering only the clear cell cases from 2009, there was only one out of thirty-six cases (2.8%). In the 2010 study cases four out of twenty-eight cases (14.3%) overall and four out of twenty-one clear cell cases (19.0%) had gross venous invasion identified. Again the $p > 0.05$ and therefore not statistically significant, however the p-value of the clear cell cases when comparing the 2009 cases versus the 2010 study cases approached significance ($p = 0.0563$).

A logistic regression model was also done for the sake of interest and used to assess whether the probability of invasion was a function of group, size and Fuhrman grade (Table 8). Again these findings were not statistically significant ($p > 0.05$).

Table 5: *Gross identification of venous invasion in 2009 cases and in 2010 study cases overall.*

Gross Identification	2009 Cases	2010 Study Cases
	1 (2.4%)	4 (14.3%)
Total # of Cases	42	28

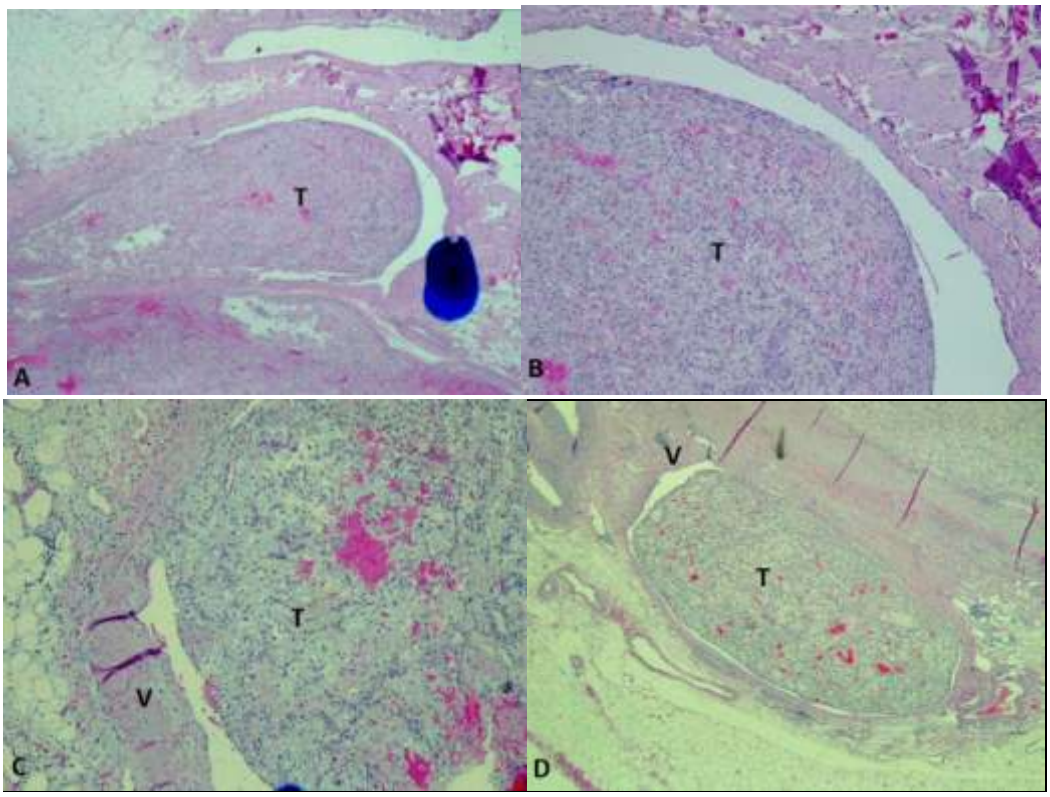

Figure 11: *Histological renal vein invasion in case #7 from the study group.* A-B) Tumour thrombi (T) within segmental branches of renal vein (dot in A). C-D) Tumour (T) invading into the wall of segmental branches of the renal vein (V).

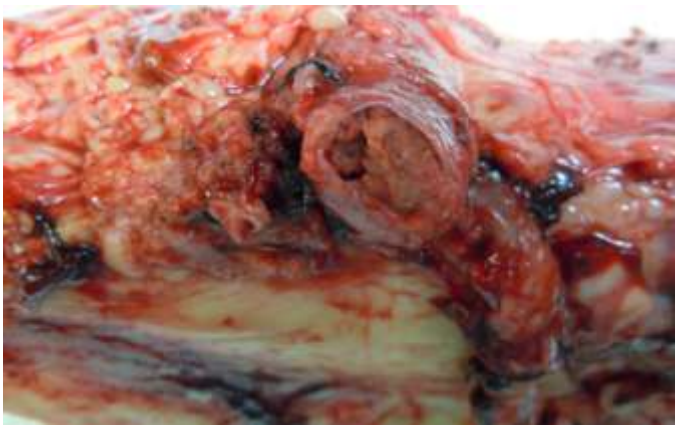

Figure 12: *Gross renal vein invasion in case #8 from the study group*. A tumour thrombus can be seen within the renal vein at the hilum line of resection.

Table 6: *Comparison of 2009 kidneys to the study group kidneys based on tumour size, type and Fuhrman grade.*

Comparison	Probability	Statistical Analysis
All kidneys, irrespective of tumour size, type or Fuhrman grade	0.3526	Pearson Chi-Square
All clear cell kidneys, irrespective of size, or Fuhrman grade	0.2423	Pearson Chi-Square
All kidneys, 4.1 - 7.0 cm	0.2152	Fisher's Exact Test
All kidneys, 7.1 - 10.0 cm	1.0000	Fisher's Exact Test
All kidneys, >10.0 cm	1.0000	Fisher's Exact Test
Clear cell kidneys, 4.1 - 7.0 cm	0.2134	Fisher's Exact Test
Clear cell kidneys, 7.1 - 10.0 cm	1.0000	Fisher's Exact Test
Clear cell kidneys, > 10.0 cm	0.4000	Fisher's Exact Test
All kidneys, Fuhrman grade 2	0.565	Fisher's Exact Test
All kidneys, Fuhrman grade 3	1.0000	Fisher's Exact Test
All kidneys, Fuhrman grade 4	1.0000	Fisher's Exact Test
Clear cell kidneys, Fuhrman grade 2	0.5875	Fisher's Exact Test
Clear cell kidneys, Fuhrman grade 3	1.0000	Fisher's Exact Test

Table 7: *Gross identification of venous invasion in 2009 clear cell and in 2010 study clear cell cases.*

Gross Identification	2009 Clear Cell Cases	Study Clear Cell Cases
	1 (2.8%)	4 (19%)
Total # of Clear Cell Cases	36	21

Table 8: *Results of the logistic regression model.*

Effect	Probability
Group (study vs 2009)	0.3348
Size	0.3796
Fuhrman grade	0.6022

Discussion

Renal cell carcinoma is staged based on size and extent beyond the kidney. Even a smaller tumour, if it extends beyond the capsule into the perinephric fat, or into the renal sinus and veins is upstaged accordingly. It is important to accurately identify and diagnose any extension as approximately 30% of patients treated with nephrectomy for seemingly localized renal cell carcinoma will develop metastatic disease (Thompson *et al.*, 2007). This is because the renal sinus and the renal sinus veins in particular, provide an important route for dissemination of the tumour (Bonsib, 2004).

This study was conducted as a validation of the previous works of Bonsib and Bhalodia (Bhalodia and Bonsib, 2010; Bonsib and Bhalodia, 2010). They found that venous perfusion "markedly enhances the direct inspection of the venous system for intravenous tumour," as well as resulting in "markedly improved histology compared to immersion fixed" and it "facilitates gross recognition of important sinus vein staging features in nephrectomy specimens" (Bhalodia and Bonsib, 2010). They also found that intravenous probing and dissection "permitted optimum visualization of renal sinus veins and intravenous tumour by fixing the veins in an open position" (Bonsib and Bhalodia, 2010).

In this study venous perfusion, probing and bisecting along the veins was done to determine if there is an improved ability to visualize the renal sinus and veins and a resultant increase in venous invasion being diagnosed. Although none of the findings were statistically significant, this may have been due to too small a sample size. The study could be continued until larger numbers are accrued. As well, the comparison group from 2009 could also be extended further back in time. Even though the findings are not statistically significant there

appear to be some trends that were emerging, especially in the smaller tumour sizes. In those clear cell tumours that were 4.1 cm to 7.0 cm, five out of thirteen (38%) study group tumours had venous invasion diagnosed compared to three out of twenty (15%) 2009 tumours. This suggests an improved sensitivity in detecting venous invasion in smaller sized tumours. An extended investigation with a larger sample size is warranted.

Another finding of interest was that in 2009 the only cases of venous invasion being diagnosed were at HSC. In the study group there was one case diagnosed with venous invasion at GGH, but the remaining seven cases were again diagnosed at HSC. It would be interesting to see if a trend would emerge if the study was continued and the comparison group went back further.

In previous research, renal vein invasion has been reported to occur in 20% to 35% of patients (Ng *et al.*, 2008). In 2009, venous invasion was diagnosed in eight out of forty-two cases, which is 19%. These findings fall just below the low end of the range reported in Ng *et al.,* 2008. Venous invasion was diagnosed in eight out of the twenty-eight nephrectomies from the study group (28%) and is well within the expected range based on the previous research. It would be interesting to see if further investigation supported this trend where venous perfusion and probing increased the overall rate of identifying the incidence of venous invasion.

It should be noted that according to the TNM staging handbook (Sobin *et al.*, 2010) the tumour staging criteria for a T3a tumour states that the "tumour **grossly** extends into the renal vein or its segmental (musde containing) branches." For the purposes of this study, even tumours that only had venous invasion identified histologically were still considered T3a. In the 42 nephrectomies from 2009, only one gross description (2.4%) mentioned renal vein

involvement, whereas four cases from the study group of 28 nephrectomies (14.3%) identified gross renal vein involvement. This clearly suggests that renal vein invasion is more readily identified with gross examination after renal vein perfusion and probing techniques.

It should also be noted that there were some changes in the staging parameters between the study group and the 2009 group. Specifically, a T3a tumour in the study group is one that extends into the renal vein or its segmental branches. This was considered to be a T3b tumour in the 2009 group. However not all of the pathologists explicitly say the stage in their microscopic description. For the purposes of this study any mention in the microscopic description of tumour invasion into the main renal vein or its segmental, muscle-containing branches was considered as venous invasion.

The methods used in this study varied slightly between HSC and GGH. The nephrectomies grossed at HSC were perfused until inflated, placed intact into formalin overnight and then probed and bisected along the renal vein the following day and the nephrectomies at GGH were perfused with a syringe of 60cc of formalin, placed intact into formalin for approximately an hour and then probed and bisected along the renal vein to finish fixing in formalin overnight. Although the methods did vary between location it is not likely that one method would affect the incidence of identifying venous invasion, grossly or histologically, over the other method. Both methods showed an increase in the number of venous invasion identified with HSC increasing from eight out of forty-two (19%) tumours having venous invasion to seven out of twenty-eight tumours (25%). At GGH, venous invasion was not identified in any of the 2009 cases, whereas one case out of twenty-eight (3.5%) from the study group had venous invasion.

The standard of care for primary renal cell carcinoma is nephrectomy, radical or partial (Curti, 2004; Rini *et al.*, 2008) and at the time of diagnosis 20% to 30% already have metastatic disease (Stadler *et al.*, 2010). As the renal vein is thought to be a primary route of dissemination any patient with extension of tumour into the renal vein or its segmental branches will have increased follow-up to monitor for recurrence and/or metastatic disease. As the understanding of the biological mechanisms of carcinogenesis and signal transduction pathways for renal cell carcinoma has advanced, new therapies are being developed that can target metastatic renal cell carcinoma, as these tumours tend to be resistant to traditional chemotherapies and radiation therapies. These targets include von Hippel Lindau (VHL)-mediated pathways, venous endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), mammalian target of rapamycin (mTOR), platelet derived growth factor (PDGF), and non-VHL mediated pathways (Ansari *et al.*, 2010).

Venous endothelial growth factor (VEGF) plays an essential role in renal cell carcinoma pathogenesis. This recognition led to testing angiogenesis inhibitors, such as Sorafenib, which is a strong multikinase inhibitor of VEGF receptors and PDGF receptors (Stadler *et al.*, 2010). Sunitinib, pazopanib, and bevacizumab are also multitargeted kinase inhibitors that predominantly target VEGF. These drugs are only effective in controlling the disease for a limited time, however, before progression occurs (Powels *et al.*, 2011).

Drugs that inhibit mTOR work by binding to FKBP-12, an intracellular protein, to form a complex which acts to inhibit mTOR signalling, causing the cell cycle to halt at the G1 phase in tumour cells. Temsirolimus, everolimus, and sirolimus are all mTOR inhibiting drugs (Ansari *et al.*, 2010). Both everolimus and temsirolimus have been investigated and are widely used as a metastatic renal cell carcinoma treatment (Powels *et al.*, 2011).

Currently under investigation is Tivozinib, which is a potent VEGF tyrosine kinase inhibitor. Dovitinib is another tyrosine kinase inhibitor that targets FGF-2, as well as VEGF that is showing promise as a treatment option (Powels *et al.*, 2011). Other tyrosine kinase inhibitors currently being investigated include axitinib, pazopanib, cediranib, ABT-869 and perifosine (Ansari *et al.*, 2010). Besides these drugs, a logical next step is to investigate drugs in combination with one another to see if the outcome is potentially enhanced. Possible disadvantages though could include additional toxicity, cost and perhaps the potential for developing multidrug resistance (Powels *et al.*, 2011).

Developing a method that enhances the visualization of renal cell carcinoma invading into the venous system could have implications for patient treatment. As those patients diagnosed with venous invasion are more closely followed and are therefore more likely to be treated sooner in the case of recurrence or metastasis, it is therefore important that the patient is accurately diagnosed and staged. Although the findings in this study were not statistically significant, there were some trends emerging, especially with the smaller tumour sizes. It is possible that, if statistically significant differences were seen with larger sample sizes, this method would help identify patients that would benefit from the currently available and developing therapies for metastatic renal cell carcinoma.

References

1. Ansari, J., Glaholm, J., McMenemin, R., James, N.D., and Hussain, S.A. 2010. Recent advances and future directions in the management of metastatic renal cell carcinoma. *Anti-Cancer Agents in Medicinal Chemistry* **10**: 225-235.

2. Bhalodia, A. and Bonsib, S.M. 2010. Venous perfusion optimizes the fixation and evaluation of the renal vasculature and parenchyma in nephrectomy specimens. *Modern Pathology* **23**: 178A-179A.

3. Bonsib, S.M. 2007. Renal veins and venous extension in clear cell renal carcinoma. *Modern Pathology* **20**: 44-53.

4. Bonsib, S.M. 2004. The renal sinus is the principal invasive pathway: a prospective study of 100 renal cell carcinomas. *The American Journal of Surgical Pathology* **28**: 1594-1600.

5. Bonsib, S.M. and Bhalodia, A. 2010. Intravenous dissection, the optimum strategy for staging of renal cell carcinoma. *Modern Pathology* **23**: 180A.

6. Bonsib, S.M., Gibson, D., Mhoon, M., and Greene, G.F. 2000. Renal sinus involvement in renal cell carcinomas. *The American Journal of Surgical Pathology* **24**: 451-458.

7. Chow, W.H., Devesa, S.S., Warren, J.L., and Fraumeni, Jr., J.F. 1999. Rising incidence of renal cell carcinoma in the United States. *Journal of the American Medical Association* **281**: 1628-1631.

8. Curti, B.D. 2004. Renal cell carcinoma. *Journal of the American Medical Association* **292**: 97-100.

9. Eble, J.N., Togashi, K., and Pisani, P. 2004. WHO Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. IARC Press, Lyon. p. 12-43.

10. Fleming, S., and Griffiths, D.F.R. 2004. Nephrectomy for renal tumour; dissection guide and dataset. *The Journal of Clinical Pathology* **58**: 7-14.

11. Fuhrman, S.A., Lasky, L.C., and Limas, C. 1982. Prognostic significance of morphologic parameters in renal cell carcinoma. *American Journal of Surgical Pathology* **6**: 655-663.

12. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., and Thun,. M.J. 2008. Cancer statistics, 2008. *CA: A Cancer Journal for Clinicians* **58**: 71-96.

13. Karumanchi,S.A., Merchan, J., and Sukhatme,V.P.2002. Renal cancer: molecular mechanisms and newer therapeutic options. *Current Opinion in Nephrology and Hypertension* **11**: 37-42.

14. Kumar, V., Abbas, A.K., Fausto, N., and Aster, J.C. 2010. Robbins and Cotran Pathologic Basis of Disease. Saunders Elsevier, Philadelphia. p. 964-697.

15. Lipworth, L., Tarone, R.E., and Mclaughlin, J.K. 2006. The epidemiology of renal cell carcinoma. *The Journal of Urology* **176**: 2353-2358.

16. Mejean, A., Oudard, S., and Thiounn, N. 2003. Prognostic factors of renal cell carcinoma. *The Journal of Urology* **169**: 821-827.

17. Moore, L.E., and Wilson, R.T. 2005. Lifestyle factors, exposures, genetic susceptibility, and renal cell cancer risk: a review. *Cancer Investigation* **23**: 240-255.

18. Ng, C.S., Wood, C.G., Silverman, P.M., Tannir, N.M., Tamboli, P., and Sandler, C.M. 2008. Renal cell carcinoma: diagnosis, staging, and surveillance. *American Journal of Roentgenology* **191**: 1220-1232.

19. Novara, G., Martignoni, G., Artibani, W., and Ficarra, V. 2007. Grading systems in renal cell carcinoma. *The Journal of Urology* **177**: 430-436.

20. Ojemakinde, K.O., Zhao, C.X., Bhalodia, A., and Bonsib, S.M. 2011. Intravenous pT3 renal cell carcinoma: The challenge of gross recognition. *Modern Pathology* **24**: 214A.

21. Powels, T., Chowdhury, S., Jones, R., Mantle, M., Nathans, P., Bex, A., Lim, L, and Hutson, T. 2011. Sunitinib and other targeted therapies for renal cell carcinoma. *British Journal of Cancer* **104**: 741-745.

22. Rini, B.I., Rathmell, W.K., and Godley, P. 2008. Renal cell carcinoma. *Current Opinion in Oncology* **20**: 300-306.

23. Sobin, L., Godpodarowicz, M., and Wittekind, C. 2010. TNM Classification of Malignant Tumours. Wiley-Blackwell, Oxford. p. 255-257.

24. Stadler, W.M., Figlin, R.A., McDermott, D.F., Dutcher, J.P., Knox, J.J., Miller, W.H., Hainsworth, J.D., Henderson, C.A., George, J.R., Hajdenberg, J., Kindwall-Keller, T.L., Ernstoff, M.S., Drabkin, H.A., Curti, B.D., Chu, L., Ryan, C.W., Hotte, S.J., Xia, C., Cupit, L., Bukowski, R.M. 2010. Safety and efficacy results of the advanced renal cell carcinoma sorafenib expanded access program in North America. *Cancer* **116**: 1272-1280.

25. Thompson, R.H., Blute, M.L., Krambeck, A.E., Lohse, C.M., Mager, J.S., Leibovich, B.C., Kwon, E.D., Frank, I., and Cheville, J.C. 2007. Patients with pT1 renal cell carcinoma who die from

disease after nephrectomy may have unrecognized renal sinus fat invasion. *The American Journal of Surgical Pathology* **31**: 1089-1093.

26. Zhao, C.X., Ojemakinde, O.J., Bhalodia, A., Heldmann, M., Elmajian, D., Bonsib, S.M. 2011. Venous invasion in renal cell carcinoma: Preoperative imaging-gross correlation. *Modern Pathology* **24**: 233A.