

## **ORIGINAL ARTICLE Urologic Oncology: Seminars and Original Investigations**

### **Resistin and interleukin-6 as predictive factors for recurrence and long-term prognosis in renal cell cancer**

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## **Abstract**

### *Objectives*

The aim of the present study was to investigate prognostic factors for long term outcome of renal cell cancer (RCC) in a cohort of patients treated before new antiangiogenic therapy modalities were introduced. Our specific aim was to explore resistin and interleukin-6 (IL-6) levels in order to find out cytokines potential to predict recurrence and survival in patients with RCC.

### *Materials and methods*

Our prospective study population consisted of 91 patients who underwent radical nephrectomy or partial resection for RCC at Tampere University Hospital between 1994 and 2001. At the time of surgery, 25 patients were diagnosed to have an advanced and 66 patients a local tumour; 34 patients in the latter group developed a recurrence during the follow up of 15 years. Serum samples were collected preoperatively and at 3, 9 and 15 months and at 2, 3, 4 and 5 years postoperatively. IL-6 and resistin levels in serum were measured by immunoassay.

### *Results*

Preoperative values of IL-6 ( $p=0.006$ ) and resistin ( $p<0.001$ ) were higher in patients with advanced RCC, when compared to patients with local tumours. Patients with local RCC who developed a recurrence during the follow-up period had higher preoperative IL-6 values ( $p=0.003$ ) than patients without a recurrence. Based on trajectory analysis of IL-6 and resistin levels, the patients were divided into three trajectory groups, respectively. According to multivariate analysis, the patients in the trajectory class of the increasing resistin values during the follow-up had a statistically significantly higher risk for RCC progression (HR: 3.73, 95% CI: 1.52-9.13) and for poor survival (HR: 4.93, 95% CI: 1.79-13.6) than the patients in the lowest trajectory class. Also, the patients in the trajectory class of the highest IL-6 values showed a significantly elevated risk for RCC progression (HR: 7.03, 95% CI: 3.00-16.5) and for RCC-related poor survival (HR: 6.09, 95% CI: 2.53-14.7), when compared to the patients in the trajectory class of the lowest IL-6 values.

### *Conclusion*

In RCC patients, the elevated preoperative resistin levels associated strongly with an advanced disease. Based on Cox regression models and trajectory analysis, resistin values were associated with disease progression and a poor survival.

Key words: renal cell cancer, adipokines, resistin, IL-6, prognosis

## Introduction

Renal cell cancer (RCC) has a marked challenge among urological malignancies having the highest mortality, up to 50% in a long-term follow-up. Its clinical behaviour is very variable and it can recurrence in an unpredictable manner even after several years since the primary treatment [1,2]. Clinical stage and histological grade continue to be the most powerful independent prognostic factors, even though newer candidates for prognostic and predictive factors have been vigorously investigated [1,2]. Markers for cell proliferation, apoptosis, invasion and angiogenesis have shown prognostic value in earlier studies [3-6].

Immunological regulation has a major role in the behaviour of RCC and immunotherapy was the main treatment strategy in a metastatic RCC before newer treatment modalities like antiangiogenic agents and mTOR-inhibitors became available [7]. High IL-6 levels have been associated with poor prognosis in RCC [8]. In addition, today new type of immunomodulation with PD-3 (ipilimumab) and PD-L1 monoclonal antibodies via immunoregulatory cells seems to be a new promising treatment modality for the metastatic RCC [9,10].

Obesity has consistently been associated with elevated risk of RCC, but the mechanisms through which obesity acts to increase RCC risk remain unclear [11]. Adipose tissue has several endocrine functions. Its hormones are collectively called as adipokines or adipocytokines. These proteins have an effect on a wide variety of complex, often proneoplastic processes such as inflammation, insulin resistance, cell growth, proliferation, and vascular and blood pressure regulation [12]. Some of these adipokines have demonstrated promising results as predictors of risk and/or progression in obesity-related cancers: breast cancer [13], colorectal cancer [14], endometrial cancer [15], and prostate cancer [16]. In renal cell cancer it has been shown that low adiponectin levels are associated with an elevated risk [17] and high adiponectin levels with a reduced risk of RCC [18]. Resistin is an adipocytokine shown to have a role in many inflammatory conditions associated with oxidative / nitrosative stress. Resistin is produced primarily by macrophages and it activates TLR4 and inflammatory signalling cascades in various cell types including tumour cells. Resistin has been shown to activate tumour cell proliferation and migration *in vitro* and it may promote cancer dissemination through increasing the expression of matrix metalloproteases, adhesion molecules and pro-angiogenic factors. [19] However, its impact in RCC has not been investigated.

In the present study, we aimed to explore RCC patients both pre- and postoperatively in order to find out cytokines potential to predict recurrence and survival. Our main interest was focused on one of the adipocytokines, namely resistin because of its potential role in tumour progression.

## **Material and methods**

Our prospective study population consisted of 91 patients who underwent radical nephrectomy or partial resection for RCC at Tampere University Hospital between 1994 and 2001. Patients with other malignant diseases or chronic inflammatory diseases were excluded. Three groups of patients were identified: patients with local tumours without recurrence during the follow-up (n=32), patients with local tumours with recurrence during the follow-up (n=34) and patients with advanced tumours at the time of diagnosis (n=25). Serum samples were collected preoperatively and at 3, 9 and 15 months and at 2, 3, 4 and 5 years postoperatively and stored at -70 °C until analysed. Clinical follow-up and data collection were performed at our university clinic up to 5 years, after which the follow-up was done by general practitioners at patient's home town. Individual survival status, date of death, and clinical diagnoses were collected from the patient's records up to 15 years.

Clinical stage was assigned using the 1997 TNM classification of malignant tumours. Because of the restricted number of patients in the study, TNM-classes were divided into two groups for the statistical analysis: local (T1-T3N0M0) and advanced (T4 and/or N1-2 and/or M1). Histology of tumours was classified according to the Heidelberg classification and graded according to the WHO classification. Eighty out of 91 were clear cell tumours, six papillary, three chromophobe and two collecting duct carcinomas. Progression of the disease was considered to happen when tumour mass was recognized in the follow-up imaging studies after surgery (local tumours) or when existing tumour masses were enlarging and/or developing new focus (advanced tumours) [20].

IL-6 and resistin concentrations were determined by enzyme-linked immunoassay by using the reagents from eBioscience Inc. (San Diego, CA, USA) and R&D Systems Europe Ltd (Abingdon, U.K.), respectively. The detection limit and inter-assay coefficient of variation were 0.2 pg/ml and 4.2% for IL-6 and 15.6 pg/ml and 3.4% for resistin.

## **Ethics**

The research plan was approved by the Ethical Committee of Tampere University Hospital (code R94144) and an informed consent was obtained from every patient.

## **Statistics**

Resistin and IL-6 values were clustered by trajectory analysis originally presented by Nagin 2005 [21]. Trajectory groups are clusters of individuals following similar trajectories on an outcome over time [22]. The trajectories were created according to all measurements of resistin or IL-6 in each

patient as a continuous outcome measure. Resistin and IL-6 were modelled separately. The trajectories are presented in Fig. 1. The analyses undertaken were latent class mixture models of quadratic trajectories including a random intercept and concomitant variables. Models were fitted by using flexmix package [23] of the statistical program R, version 2.13.0, from the R Foundation for Statistical Computing [24]. Relative goodness of fit was assessed using Bayesian information Criteria (BIC).

Differences ( $p$ ) between groups were tested by Mann-Whitney test, Pearson chi-square test or Fisher's exact test. Association of explanatory factors (age, gender, tumour stage, histological grade of the tumour, tumour diameter, resistin trajectories and IL-6 trajectories) with progression and survival were examined by univariate (survival as age-adjusted) and multivariate Cox regression proportionally hazards models (Table 2). The analyses were performed by IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Released 2011.  $P$ -values  $<0.05$  were considered statistically significant.

## Results

*The preoperative patient characteristics:* The median (Md) age of the patients at the time of operation was 65 years (interquartile range(IQR) 55-73, range 33-85) and the median tumour diameter was 7cm (range 2.3-25cm). As expected, there was a clear difference in survival between patients with a local or an advanced disease, Md 9.2 years (IQR 4.5-13.7; range 0.9-17.9) and Md 1.1 years (IQR 0.54-2.68; range 0-15.7), respectively ( $p<0.001$ ).

*Interleukin-6:* Preoperative values of IL-6 were higher in patients with an advanced RCC than in patients with a local tumour, Md 6.5 (IQR 2.8-18.6; range 0.5-39.5) pg/ml and Md 2.2 (IQR 1.1-6.1; range 0.5-36.9) pg/ml, respectively ( $p=0.006$ , Mann-Whitney U-test). In addition, the patients with a local tumour who developed a recurrence during the follow-up period had higher preoperative IL-6 values than patients without a recurrence, Md 4.9 (IQR 1.5-7.7; range 0.5-36.9) pg/ml and Md 1.4 (IQR 0.9-2.8; range 0.6-29.0) pg/ml, respectively ( $p=0.003$ ) (Table 1).

Trajectory analysis divided the patients into three groups based on the level of IL-6 values (Fig. 1A). According to the multivariate analysis, the patients in the trajectories 2 ( $p=0.004$ ) and 3 ( $p<0.001$ ) had poorer survival than patients in the trajectory 1 (i.e. patients with the lowest IL-6 values) (Fig. 2 and Table 2).

*Resistin:* Preoperative values of resistin were significantly higher in patients with an advanced RCC than in patients with a local tumour, Md 21.7 (IQR 17.9-27.5; range 9.7-80.9) ng/ml and Md 14.8 (IQR 11.3-18.6; range 7.0-34.9) ng/ml, respectively ( $p<0.001$ , Mann-Whitney U-test). In contrast to IL-6, the preoperative resistin values did not differ between local RCC patients with or without recurrence, Md 14.7 (IQR 11.3-17.6; range 8.7-34.9) ng/ml and Md 15.0 (IQR 11.3-18.9; range 7.0-31.1) ng/ml, respectively ( $p=0.647$ ) (Table 1).

Based on trajectory analysis of resistin values over time the patients were divided into three groups (Fig. 1B). According to the multivariate analysis, the patients in the resistin trajectories 2 (0.047) and 3 ( $p=0.002$ ) had poorer survival than patients in the trajectory 1 (Fig.3 and Table 2).

*Progression:* Table 2 shows univariate and multivariate analysis of risk factors for RCC progression. In multivariate analysis, an advanced disease (HR: 6.32, 95% CI: 2.90-13.8), a high histological grade (2.30, 1.28-4.12), a high tumour diameter (3.58, 1.90-6.74), a higher IL-6 trajectory class (class 2: 3.02, 1.47-6.21; class 3: 7.03, 3.00-16.5) and resistin trajectory class 3, (3.73, 1.52-9.13) were statistically significant prognostic factors for disease progression.

*Survival:* Risk factors, based on univariate and multivariate analysis, for RCC-specific survival are shown in Table 2. In the multivariate analysis, an advanced disease (HR: 10.7, 95% CI: 4.60-24.9), a high histological grade (2.79, 1.55-5.04), a high tumour diameter (3.64, 1.86-7.12), IL-6 trajectory class 2 (3.10, 1.44-6.66) and class 3 (6.09, 2.53-14.7), and resistin trajectory classes 2 and 3 (class 2: 2.93, 1.01-8.45; class 3: 4.93, 1.79-13.6) were independent prognostic factors for poor survival.

## **Discussion**

To the best of our knowledge, this is the first prospective study to evaluate resistin as a prognostic factor in a clinical population of RCC patients. The results showed that elevated preoperative resistin levels were strongly associated with an advanced disease. Furthermore, based on Cox regression models and trajectory analysis, resistin values were associated with disease progression and poor survival. In addition, as reported also earlier [25], higher IL-6 levels showed a clear association with disseminating disease and poor survival. Interestingly, preoperative IL-6 levels were higher not only in the patients with advanced / metastatic disease but also in those patients who were originally diagnosed to have a local disease but developed a recurrence during the follow-up.

Obesity has been associated with elevated risk of RCC. Findings in the large US cohort study by Adams et al. suggest complexity in the relation between weight history and renal cell cancer [11]. Especially adulthood (midlife) weight gain increased markedly RCC risk. Waist-to-hip ratio (abdominal adiposity) was associated with an elevated risk of RCC, especially in women. Factors associated with excessive body mass index (BMI) can damage the kidneys in different ways and at the same time possibly raise the risk for RCC or for RCC progression e.g. via oxidative stress [26], hypertension-induced injury to the renal tubules [27], renal atherosclerosis [28] and altered circulating concentrations of estrogen and other hormones [29].

Resistin is an adipocytokine which has been shown to have a role in several pathophysiological conditions. In arthritis elevated levels of resistin have been observed [30] and it has been suggested to play a role in the pathogenesis of osteoarthritis [31]. Circulating resistin levels have been shown to be upregulated in inflammatory bowel diseases [32], chronic kidney disease [33] and in asthma [34]. Resistin has been proposed as a biomarker for steroid-sensitive asthma [35] and as a predictive marker for the degree of ischemia-reperfusion injury during cardiac surgery [36].

Resistin is expressed in adipocytes in rodents, whereas in humans it is primarily synthesized by macrophages [37, 38] and it is not readily associated with obesity and BMI [39]. Several tissues and cell types are responsive to resistin including adipose tissue, liver, muscle, vascular endothelium and leukocytes. The main biological effects of resistin are associated with insulin

resistance in mice, and enhancement of inflammation in humans [37, 38]. Resistin has been shown to mediate its pro-inflammatory properties e.g. through promoting the expression of TNF and IL-6 in human mononuclear cells [40] and by inducing the expression of adhesion molecules in vascular endothelial cells [41].

Specific receptors for resistin have not been identified, but it has been shown that resistin acts as an endogenous ligand of Toll-like receptor 4 (TLR4) [42]. TLR4 triggers major inflammatory pathways and is also activated by bacterial products such as lipopolysaccharides. Intracellular signalling cascades for resistin include NF- $\kappa$ B and PI3K-Akt pathways as well as mitogen-activated protein kinase cascades [37]. Elevated levels of resistin have been reported in patients with breast cancer [43], colorectal cancer [44] and in non-small cell lung cancer [45]. Resistin has been shown to activate tumour cell proliferation and migration *in vitro* and it has been suggested to promote cancer progression, invasion and metastasis through increasing the expression of matrix metalloproteases, adhesion molecules and pro-angiogenic factors [19,46-49]. The present study extends the previous knowledge of the pathophysiological roles of resistin by showing that elevated resistin levels in RCC patients are associated with an advanced / metastatic disease, and with disease progression and poor survival.

Limitations of the present study include the limited number of patients in each group. For the first five years the follow-up was performed according to predefined schedule at the Tampere University Hospital, and after that the data is based on the registry collected from the patients' visits to the general practitioners. Imaging techniques have developed since 1994 and nowadays recurrence and progression are likely to be observed earlier.

In conclusion, the present study shows that resistin levels in RCC patients are associated with disseminated disease, tumour progression and poor survival. Our findings offer an interesting introduction to possible mechanisms of progression and metastasis of RCC. This is an intriguing topic for further research, which may result in clinical implications of resistin as a biomarker. Furthermore, the knowledge of the exact mechanism of action of resistin may also offer therapeutic possibilities in the future.

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Table legend:

Table 1a

Preoperative serum values of IL-6 and resistin in renal cell carcinoma (RCC) patients, advanced vs. local RCC

Table 1b

Preoperative serum values of IL-6 and resistin in local RCC, patients with recurrence vs. without recurrence

Table 1a. Preoperative serum values of IL-6 and resistin in renal cell carcinoma (RCC) patients, advanced vs. local RCC

	Advanced (n=20)	Local (n=62)	p
	Median (IQR; Range)	Median (IQR; Range)	
IL-6 (pg/ml)	6.5 (2.8-18.6; 0.5-39.5)	2.2 (1.1-6.1; 0.5-36.9)	<b>0.006</b>
Resistin (ng/ml)	21.7 (17.9-27.5; 9.7-80.9)	14.8 (11.3-18.6; 7.0-34.9)	<b>&lt;0.001</b>

Differences (p) between groups were tested by Independent Samples Mann-Whitney U test; IQR= interquartile range (25%-75%)

Table 1b. Preoperative serum values of IL-6 and resistin in local RCC, patients with recurrence vs. without recurrence

	Local – no recurrence (n=31)	Local – with recurrence (n=31)	p
	Median (IQR; Range)	Median (IQR; Range)	
IL-6 (pg/ml)	1.4 (0.9-2.8; 0.6-29.0)	4.9 (1.5-7.7; 0.5-36.9)	<b>0.003</b>
Resistin (ng/ml)	14.7 (11.3-17.6; 8.7-34.9)	15.0 (11.3-18.9; 7.0-31.1)	0.647

Differences (p) between groups were tested by Independent Samples Mann-Whitney U test; IQR= interquartile range (25%-75%)

Table legend:

Table 2

Risk factors for RCC progression and 15 years' survival

Table 2. Risk factors for RCC progression and 15 years' survival

	N	Progression						Survival					
		Age-adjusted univariate			Multivariate			Age-adjusted univariate			Multivariate		
		HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p	HR	(95% CI)	p
Age	91	1.01	(0.99-1.04)	0.395	1.01	(0.97-1.04)	0.599	1.02	(0.99-1.05)	0.184	1.04	(1.00-1.08)	0.039
Gender				0.734			0.135						0.037
Male	62	1.11	(0.62-1.97)		1.73	(0.84-3.55)		1.15	(0.62-2.13)	0.646	2.31	(1.05-5.07)	
Female	29	1.00						1.00			1.00		
Stage				<0.01			<0.001						<0.001
Local	68	1.00			1.00			1.00			1.00		
Advanced	23	6.61	(3.75-11.6)		<b>6.32</b>	<b>(2.90-13.8)</b>		7.58	(4.22-13.6)	<0.001	<b>10.7</b>	<b>(4.60-24.9)</b>	
Grade				0.005			0.005						0.001
1-2	58	1.00			1.00			1.00			1.00		
3	33	2.14	(1.26-3.61)		<b>2.30</b>	<b>(1.28-4.12)</b>		2.11	(1.22-3.66)	0.008	<b>2.79</b>	<b>(1.55-5.04)</b>	
Diameter				<0.001			<0.001						<0.001
0-7	51	1.00			1.00			1.00			1.00		
>7	40	2.71	(1.59-4.60)		<b>3.58</b>	<b>(1.90-6.74)</b>		2.34	(1.36-4.02)	0.002	<b>3.64</b>	<b>(1.86-7.12)</b>	
Resistin trajectories				0.106			0.011			0.174			0.007
1	14	1.00			1.00			1.00			1.00		
2	37	0.76	(0.33-1.73)	0.513	2.31	(0.85-6.26)	0.101	0.78	(0.33-1.84)	0.575	2.93	(1.01-8.45)	0.047
3	40	1.41	(0.65-3.05)	0.383	<b>3.73</b>	<b>(1.52-9.13)</b>	0.004	1.38	(0.61-3.13)	0.435	<b>4.93</b>	<b>(1.79-13.6)</b>	0.002
IL6 trajectories				<0.001			<0.001			<0.001			<0.001
1	39	1.00			1.00			1.00			1.00		
2	37	3.08	(1.56-6.07)	0.001	<b>3.02</b>	<b>(1.47-6.21)</b>	0.003	2.95	(1.44-6.05)	0.003	<b>3.10</b>	<b>(1.44-6.66)</b>	0.004
3	15	7.68	(3.68-16.0)	<0.001	<b>7.03</b>	<b>(3.00-16.5)</b>	<0.001	7.60	(3.54-16.3)	<0.001	<b>6.09</b>	<b>(2.53-14.7)</b>	<0.001

Analyses were performed using Cox regression models, showing results by hazard ratios (HR) with 95% confidence intervals (CI).

Figure legend:

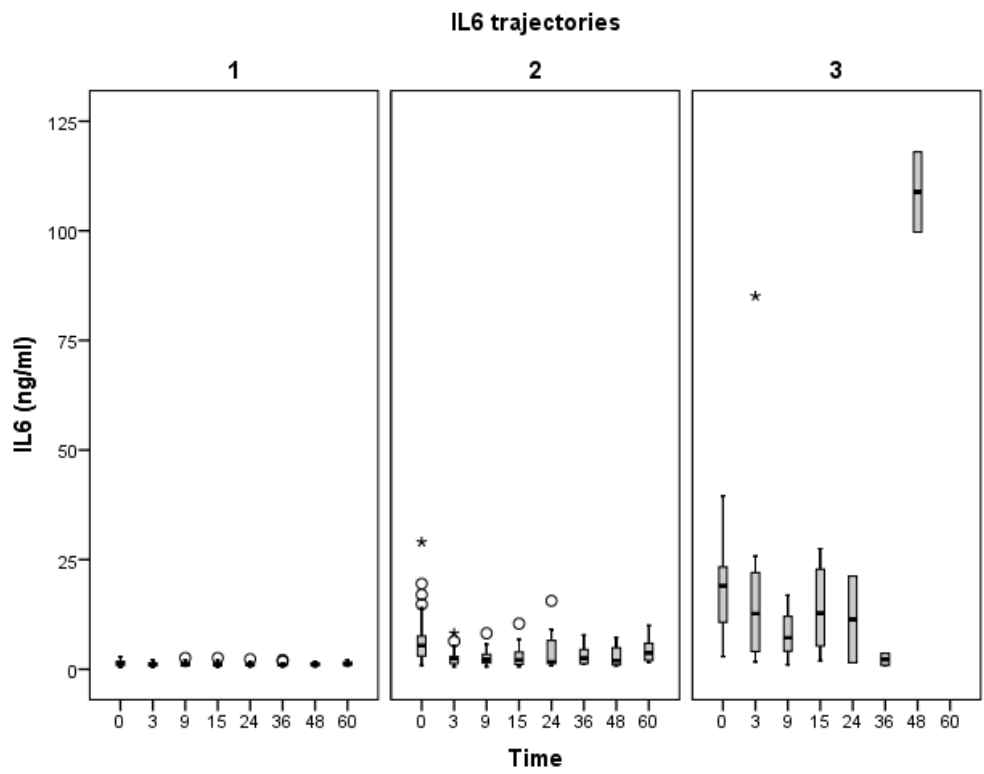
Figure 1

**A** IL-6 trajectory analysis groups of renal cell carcinoma patients

**B** Resistin trajectory analysis groups of renal cell carcinoma patients

Distributions of IL-6 and resistin are shown by medians (black line), interquartile ranges (box) and ranges (line bar) in patients with renal cell carcinoma. Outlier and extreme cases are expressed as dots or asterisks.

Figure 1  
A



B

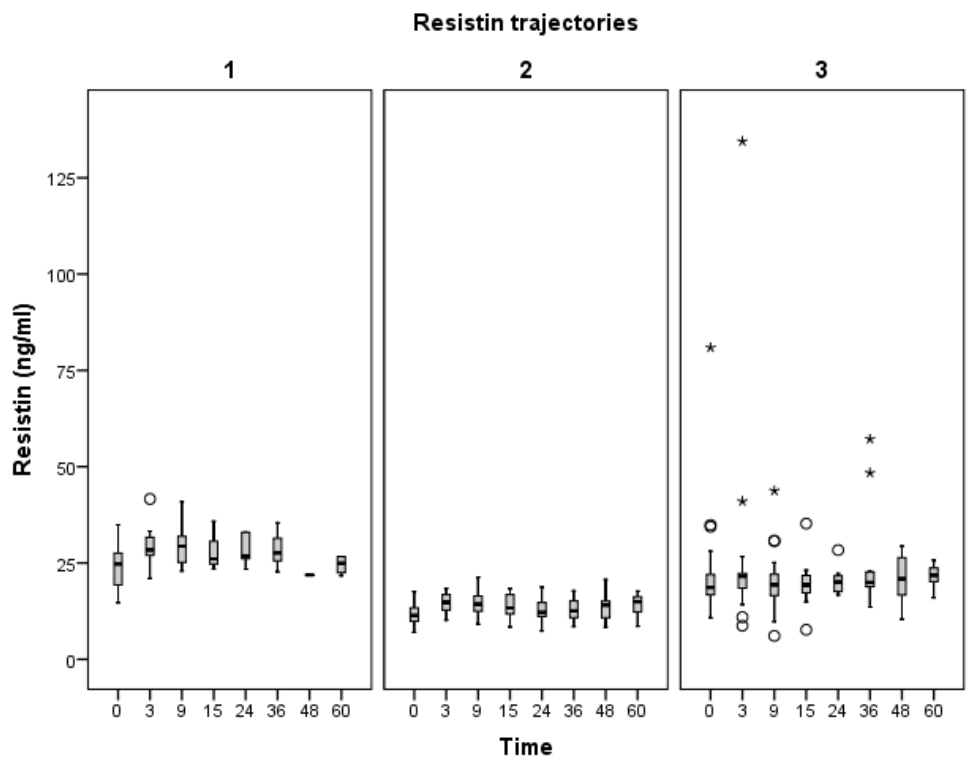




Figure legend:

Figure 2

Survival curves from Cox hazard regression multivariate models for renal cell carcinoma patients in different IL-6 trajectory analysis groups

Figure 2

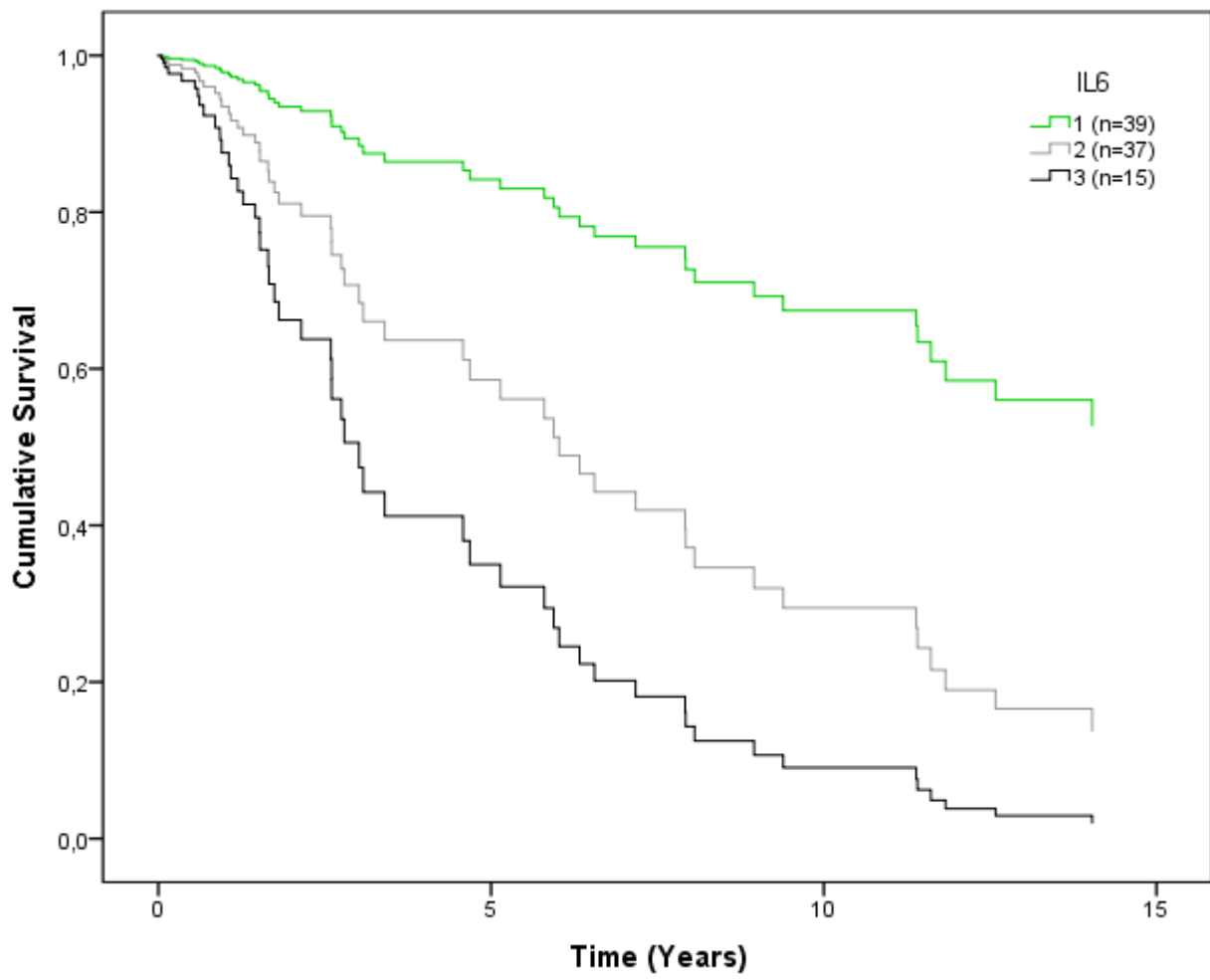


Figure legend:

Figure 3

Survival curves from Cox hazard regression multivariate models for renal cell carcinoma patients in different resistin trajectory analysis groups

Figure 3

