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High YKL-40 is associated with poor survival in patients with renal cell carcinoma: novel independent prognostic marker

Running Head: YKL-40 and renal cell carcinoma

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High YKL-40 is associated with poor survival in patients with renal cell carcinoma: novel independent prognostic marker

Abstract

Objective: YKL-40 is an inflammation-associated glycoprotein supposed to have a role in cell survival and angiogenesis. RCC is characterized by varying prognosis and risk of relapse after disease free time of years. Prognostic markers are critically needed. We investigated if YKL-40 could serve as a useful biomarker in RCC patients. *Materials and Methods:* Blood samples from 82 patients with RCC were collected at the time of diagnosis and 3, 5, and 9 months and 2 and 3 years after nephrectomy. Levels of YKL-40 were determined by ELISA. Survival of patients and relapse of RCC was followed up to 15 years. *Results:* Circulating YKL-40 levels were increased in patients with metastatic RCC at the time of diagnosis (115.7, 61.0-221.6 ng/ml; median, IQR). More importantly, among patients primarily diagnosed to have nonmetastatic RCC, baseline YKL-40 levels were significantly higher in those patients who experienced a relapse during the follow-up (103.7, 59.3-242.0 ng/ml) than in patients without relapse (50.6, 33.8-97.1 ng/ml). Moreover, high baseline YKL-40 was highly associated with poor prognosis in RCC: in age-adjusted univariate analysis, YKL-40 over 120 ng/ml (highest tertile) predicted over 5-fold mortality in 5 years, and in multivariate analysis high YKL-40 stayed as a statistically significant independent risk factor for 5 and 15 years survival. *Conclusions:* Increased circulating YKL-40 levels were found to be significantly associated with poor survival in patients with RCC. The results suggest YKL-40 as a useful novel biomarker in evaluating prognosis and relapse risk in RCC, being especially beneficial in patients primarily diagnosed to have nonmetastatic RCC.

Keywords: Prognosis; Renal Cell Carcinoma; YKL-40

Introduction

Renal cell carcinoma (RCC) belongs to the ten most common malignancies worldwide and for unrevealed reasons, it is more common in men and in developed countries. Clear cell carcinoma is the prevailing subtype of RCC, the other subtypes including papillary, cromophope and ductal carcinomas. The prognosis of RCC is variable and depends on the size of the primary tumor and the dissemination at the time of diagnosis: 5-year survival of patients with local tumor with diameter under 7 cm may be 80 – 95 %, whereas the 5-year survival of patients with stage IV RCC has been reported to be less than 10 % before the development of the latest targeted drugs. Today many of the RCCs are found incidentally, when imaging is used to investigate other abdominal complaints. In patients with RCC who don't have any evidence of metastases, partial or radical nephrectomy is the treatment of choice with curative intent. However, a fourth of these patients develop later recurrence or metastases [1].

In recent years, several genetic factors associated with RCC have been recognized, e.g. mutation in von Hippel-Lindau tumor suppressor gene resulting in changes in the response to hypoxia and further, activation of genes associated with angiogenesis, cell migration, and metabolism. The primary treatment of RCC is surgery. Systemic drugs such as cytokines interleukin-2 and interferon-α, vascular endothelial growth factor (VEGF) pathway inhibitors and mammalian target of rapamycin (mTOR) inhibitors are used to treat metastatic RCC [1]. In addition, check-point inhibitors alone or in combinations have been shown promise in the most recent clinical trials [2,3].

YKL-40, known also by names human cartilage glycoprotein-39 (HcGP-39), Chitinase 3-like 1 (Chi3-l1), and breast regression protein 39 (BRP-39), is an enzymatically inactive glycoprotein belonging to the 18-glycosyl hydrolase family. In

addition to its relation to inflammation, YKL-40 is known as a factor potentially associated with malignancies [4].

It is a clinical challenge to estimate patients' prognosis after radical primary treatment of RCC. Novel prognostic tools are needed to identify patients with high-risk characteristics and to develop strategies for follow-up of this increasing patient group [1]. The most powerful known independent prognostic factors are clinical stage and histological grade even though candidates for prognostic and predictive factors have been actively investigated [1,5]. The aim of the present study was to investigate if serum levels of YKL-40 are associated with the prognosis of renal cell carcinoma.

Materials and methods

Patients

The present study included 82 patients with renal cell carcinoma diagnosed in Tampere University Hospital, Tampere, Finland. The patients were treated with a radical or palliative nephrectomy and patients with metastatic disease received oncologic treatment. Thereafter, the patients were followed up at the university clinic according to a following schedule: 3, 9 and 15 months and at 2, 3, 4 and 5 years. After 5 years, follow-up was continued by general practitioners at patient's hometown. Individual survival status (date of death, cause of death) and clinical diagnoses were collected from the patient's records up to 15 years. Endpoints were relapse (reappearance of a sign of the disease after radical surgery; this was applied for the tumours primarily diagnosed as nonmetastatic, i.e. N0M0) and disease-specific survival (death due to RCC) [6].
The 1997 TNM-classification was used to describe RCC [7]. T1-T2/N0M0 was regarded as local, T3-T4/N0M0 as advanced and N+ and/or M+ as metastatic disease. The present patient cohort did not include any T4N0M0 tumours; all cases classified as advanced tumours were T3N0M0. Histology of tumors was classified according to the Heidelberg classification [8] and graded according to the WHO classification [9]. For the statistical analysis, the TNM classification was dichotomized according to the metastatic status as nonmetastatic (N0M0) or metastatic (N+ and/or M+) and also the grade of the tumor was dichotomized to grades 1-2 and grade 3.

Blood samples and measurement of YKL-40

Blood samples were collected at baseline and postoperatively at 3, 9 and 15 months, and 2 and 3 years. Serum samples were stored at -80 °C until analyzed. YKL-40 was

measured with enzyme-linked immunosorbent assay (ELISA) using commercial reagents (R&D systems Europe ltd, Abingdon, UK, DuoSet ELISA) according to the manufacturer's instructions.

Statistics

Differences in demographics between RCC groups (nonmetastatic without or with relapse and metastatic) were analyzed by Pearson Chi-Square test or independent samples Kruskall-Wallis test when appropriate. Changes in YKL-40 levels before and after nephrectomy were studied by Related-Samples Wilcoxon Signed Rank Test. Changes in YKL-40 values according to nonmetastatic RCC with or without relapse during 3 years' follow-up were analyzed by Related samples Friedman's Two-Way Analysis of Variance by Ranks. Differences in baseline YKL-40-values between RCC-groups were calculated by Mann-Whiney test. Due to the skew distribution, the logaritmic transformation (Ln) was performed to normalize the distribution of YKL-40 for the further analyses. Transformed YKL-40 was used to model the effect of increasing YKL-40 values on RCC-survival. YKL-40 values were categorized for tertiles producing classes ≤55.0, 55.1-120.0 and >120.0 ng/ml. Those YKL-40 tertiles were used together with age, gender, metastatic status and grade to model age-adjusted univariate and multivariate 5- and 15-year survival. Survival analyses were performed using Cox proportional hazard regression models showing results by hazard ratios with 95 % confidence intervals. Survival times were illustrated by Kaplan-Meier curves with log-rank analyses by Mantel-Cox to test the equality of the survival distributions. All data was analyzed using IBM SPSS Statistics version 23 (IBM SPSS Statistics, IBM Corporation, Chicago, IL). P-values below 0.05 were considered as statistically

significant.

Results

Demographics of the patients and key variables of the cancer are presented in Table 1. At the time of diagnosis 37 patients were found to have local disease (T1-T2/N0M0), 24 patients had advanced disease (T3/N0M0), whereas 21 patients had metastatic RCC (N+ and/or M+). For the further analyses the TNM classification was dichotomized to nonmetastatic (N0M0) and metastatic (N+ and/or M+) disease. During the follow-up, RCC relapsed in 31 patients, who had primarily diagnosed to have a nonmetastatic (T1-T3) disease and treated with radical surgery. Forty-six patients died within the 15 years' follow-up, 30 of them during the first 5 years.

At the time of diagnosis, the median serum YKL-40 level was 90.9 ng/ml (IQR 41.8 – 182.0 ng/ml). YKL-40 levels were significantly higher in patients with the metastatic RCC (115.7; 61.0 – 221.6 ng/ml, n=21, p=0.006) and in nonmetastatic patients with relapsing disease (103.7; 59.3 – 242.0 ng/ml, n=31, p = 0.005) than in those with nonmetastatic RCC without relapse during the follow-up (50.6; 33.8 – 97.1 ng/ml, n=30) (Figure 1).

When YKL-40 levels were compared before and after the nephrectomy, no statistically significant change was seen in the whole group or in any of the three subgroups of the patients (Table 2). In the patients with nonmetastatic disease at the baseline, the complete series of blood samples from baseline to 3 years was available from 18 patients. There was no significant change in YKL-40 levels during this 3 years' follow-up (Table 3).

When the association of the circulating YKL-40 with the survival was investigated, the baseline YKL-40 was found to predict mortality. In the whole study population, one unit increase in the log-transformed YKL-40 increased 5-year and 15-year mortality over 80 % (5 year: HR 1.84, 95% CI 1.27 – 2.67, p = 0.001; 15-year: HR 1.85, 95% CI 1.37 – 2.51, p < 0.001). The association was even stronger in patients with metastatic RCC: one unit increase in log-transformed YKL-40 increased 5 and 15-year mortality 3-fold (5 year: HR 3.31, 95% CI 1.63 – 6.71, p = 0.001; 15-year: HR 2.98, 95% CI 1.51 – 5.85, p = 0.002). In support, in patients with metastatic disease, baseline YKL-40 correlated negatively with survival time after the surgery (Spearman's rho = -0.608, p = 0.003, n=21).

To further assess the association of YKL-40 with survival, the patients were divided into tertiles according to baseline YKL-40 levels: lowest tertile, YKL-40 < 55 ng /ml (n = 27), medium tertile, YKL-40 = 55-120 ng/ml (n=27), and highest tertile YKL-40 >120 ng/ml (n = 28). The cumulative survival between YKL-40 levels differed significantly both for 5-year survival (log-rank (Mantel-Cox) p = 0.008) and for 15-year survival (p<0.001) (Figure 2).

Moreover, in age-adjusted univariate analysis, baseline YKL-40 over 120 ng/ml (highest tertile) predicted over 5-fold mortality during 5 years period (HR 5.26, 95% CI 1.67 – 16.59, p = 0.005) and over 4-fold during 15 years (HR 4.34, 95% CI 1.87 – 10.07, p = 0.001) as compared to the lowest tertile (YKL-40 < 55 ng/ml). When the risk factors for mortality in patients with renal cell carcinoma were assessed, metastatic status (metastatic vs. nonmetastatic tumour at the time of diagnosis), tumor grade (3 vs. 1-2), and high YKL-40 (the highest tertile) were all significant risk factors for 5 and 15-year mortality. In multivariate analysis, high YKL-40 (> 120 ng/ml) stayed as a

statistically significant independent risk factor for both 5-year and 15-year survival (Table 4).

Discussion

In the present study, YKL-40 was shown to associate with the prognosis of renal cell carcinoma. Increased levels of circulating YKL-40 were found in patients with metastatic RCC at the time of diagnosis. Importantly, in patients whose disease appeared as nonmetastatic at the time of diagnosis and primary surgical treatment, baseline YKL-40 was found to be higher in those patients who subsequently experienced a relapse. High YKL-40 levels were significantly associated with poor survival; the strongest association being present in patients with metastatic RCC.

YKL-40, a glycoprotein with a molecular weight of approximately 40 kDa, is a member of a protein family of 18-glycosyl hydrolases and in contrast to the true chitinases of this protein family, YKL-40 is enzymatically inactive [10]. However, two distinct binding sites for oligosaccharides are recognized [11]. The CHI3L1-gene coding YKL-40 protein is localized to chromosome 1 and its full amino acid sequence has been reported [12].

The circulating levels of YKL-40 in patients with RCC detected in the present study were higher than those in healthy people or in patients with other diseases measured in our laboratory recently [13-15]. Increased levels of YKL-40 have been associated with diseases characterized by acute or chronic inflammation and also with cancer [4]. The detailed role of YKL-40 in malignant diseases is not known but some effects have been proposed: YKL-40 could act as a proinflammatory, antiapoptotic, or growth-promoting factor, or be involved in angiogenesis or in the extracellular matrix remodeling [16].

There are only a few previous studies evaluating YKL-40 in RCC. Zhang et al. studied YKL-40 expression in RCC tumors by immunohistochemical staining and found an intense staining of intracellular YKL-40 to associate with poor prognosis [17].

Berntsen and coworkers reported serum YKL-40 to associate with poor prognosis in patients with metastatic RCC treated with experimental dendritic cell vaccination therapy; YKL-40 levels increased significantly within the first four weeks of the treatment, with higher increase being in patients with progressive disease [18]. Although the number of the patients in the present study is limited there are several strengths. In addition to patients with metastatic disease, patients with a nonmetastatic RCC were included, the treatment was similar for all patients, the study design was longitudinal and patients were followed up to 15 years after the diagnosis. The findings of the present study are also supported by previous studies in other solid malignancies. High circulating levels of YKL-40 have been found to be associated with poor prognosis also in patients with breast cancer, gynecological carcinomas, bladder cancer, colorectal cancer, and hepatocellular carcinoma [16,19-22].

YKL-40 is expressed in a variety of cell types including inflammatory and malignant cells e.g. macrophages, neutrophils, osteosarcoma-, and glioblastoma cells [4]. The results of the present study, however, indicate that tumor cells were not the primary source of the circulating YKL-40 as the levels remained high despite the surgical removal of the tumor. Unchanged YKL-40 levels after nephrectomy rule out also the possibility that kidney function could significantly account for increased YKL-40 levels, as the operation is known to decrease glomerular filtration rate [23]. These views are supported by a recent study of vom Dorp et al, who found increased YKL-40 expression in peritumoral renal tissue and YKL-40 positive tumor tissue in some cases, but there was no correlation between circulating YKL-40 and its tissue levels or creatinine [24].

RCC tumors are associated with large amount of tumor-associated macrophages (TAMs) with a mixed M1/M2 phenotype [25]. Macrophages have different roles in

tumorigenesis depending on their phenotype. Classically activated M1 macrophages have been linked to protective role against the tumor, while M2 macrophages seem to have a tumour-supporting role [26]. Intriguingly, YKL-40 is acknowledged as a marker of alternatively activated M2 macrophages, which have been shown to suppress adaptive tumor-specific immune response and promote tumor growth, angiogenesis, invasion, metastasis and stroma remodeling [26,27]. Further, YKL-40 seems to be a pro-angiogenic factor itself as it has been shown to induce VEGF expression in U87 glioblastoma cells and angiogenesis in vitro and in vivo [27-29]. In the study by Faibish et al. blocking YKL-40 with monoclonal antibody was demonstrated to suppress tumor growth and angiogenesis [30]. In the present study, YKL-40 may have been produced by M2 type TAMs in RCC patients, as was shown with small cell lung cancer cells implanted in mice [31] and this could explain the association of YKL-40 with more aggressively progressing tumors. In addition, breast cancer cells have been reported to educate macrophages towards the M2 type phenotype, which correlates to recurrence free survival even in early breast cancer [32]. High YKL-40 levels may even hold potential as a biomarker for the disease type responding favorably to the treatment with drugs inhibiting vascular endothelial growth factor (VEGF) pathway. Based on the findings of the present study we cannot confirm these hypotheses, but the current results do indeed support YKL-40 as a prognostic biomarker in RCC.

Classical biomarkers used in algorithms for evaluating the prognosis of RCC are tumor node metastasis (TNM) and stage classifications, tumor size and necrosis, performance status of the patient, and for metastatic disease also circulating calcium, hemoglobin, lactate dehydrogenase, thrombocyte- and leucocyte counts [1]. In the present study baseline YKL-40 predicted a relapse in patients, who had a nonmetastatic disease at the time of diagnosis and primary surgical treatment. To our knowledge, this

has not been reported previously. YKL-40 was also strongly associated with poor survival, as only the metastatic status of the cancer had higher hazard ratio on 5 or 15-years mortality. More importantly, in the multivariate analysis YKL-40 was found to be as a statistically significant independent risk factor for poor survival in RCC. YKL-40 also correlated negatively with survival time in patients with metastatic RCC. There is a lack of good biomarkers in RCC, and the present results propose YKL-40 as a novel biomarker to be added into the repertoire when assessing probability of a relapse, especially in a nonmetastatic disease, and risk of mortality in RCC.

The present results propose circulating YKL-40 as a useful prognostic biomarker in RCC patients. The results encourage further confirming studies in larger patient groups. Intriguing hypotheses in mechanisms of the role of YKL-40 in cancers are discussed.

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Disclosure statement

The authors report no conflicts of interest.

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Tables

Table 1. Demographics of the study population.

	All (n=82)	Nonmetastatic RCC		Metastatic RCC (n=21)	p
		without relapse (n=30)	with relapse (n=31)		
Age, Median (range)	67 (33 – 85)	63 (33 – 84)	71 (45 – 85)	65 (45 – 74)	0.028
Gender, n (%)					
male	54 (66)	21 (70)	20 (65)	13 (62)	0.819
female	28 (34)	9 (30)	11 (35)	8 (38)	
PAD, n (%)					
Clear cell	73 (89)	27 (90)	27 (87.1)	19 (90.5)	
Chromophobe	3 (3.7)	1 (3.3)	1 (3.2)	1 (4.8)	
Papillary	5 (6.1)	2 (6.7)	3 (9.7)	0 (0)	
Collecting duct	1 (1.2)	0 (0)	0 (0)	1 (4.8)	
Stage, n (%)					
Local (T1-T2/N0M0)	37 (45)	22 (27)	15 (18)		
Advanced (T3-T4/N0M0)*	24 (29)	8 (10)	16 (19)		
Metastatatic (N+ and/or M+)	21 (26)			21 (26)	
Grade, n (%)					
1-2	54 (66)	23 (77)	20 (65)	11 (52)	0.194
3	28 (34)	7 (23)	11 (35)	10 (48)	
5-year survival, n (%)	52 (63)	30 (100)	20 (65)	2 (9)	<0.001
15-year survival, n (%)	36 (44)	30 (100)	5 (16)	1 (5)	<0.001

The difference between groups were tested by independent samples Kruskall-Wallis test or Pearson Chi-Square test.

*The present patient cohort did not include any T4N0M0 tumours; all cases classified as advanced tumours were T3N0M0.

Table 2. Serum YKL-40 levels before and after surgical in patients with metastatic or nonmetastatic renal cell carcinoma with or without relapse during a 15-years follow-up.

	YKL-40 (ng/ml)				
	Baseline		Post-surgery		p
	Md	(IQR)	Md	(IQR)	
All (n=61)	84.9	(38.0 – 172.3)	77.9	(39.4 – 144.4)	0.516
Metastatic (n=9)	91.4	(43.4 – 190.5)	63.0	(44.8 – 227.8)	0.594
Nonmetastatic with relapse (n=25)	103.7	(68.4 – 231.6)	110.0	(65.4 – 144.4)	0.427
Nonmetastatic without relapse (n= 27)	48.5	(31.1 – 90.8)	59.6	(31.1 – 154.5)	0.107

Md=Median; IQR=Interquartile range. Differences (p) between YKL-40 levels before and after nephrectomy were analyzed by Related-Samples Wilcoxon Signed Rank Test.

Table 3. YKL-40 levels (median, range) from baseline to 3 years from the diagnosis in patients with nonmetastatic RCC*

	Nonmetastatic RCC		
YKL-40	All (n=18)	with relapse (n=6)	without relapse (n=12)
Baseline	59.4 (16.1 – 285.9)	102.5 (16.1 – 123.9)	45.3 (21.6 – 285.9)
3 months	78.2 (20.8 – 226.3)	111.1 (20.8 – 171.4)	67.0 (21.5 – 226.3)
9 months	64.5 (25.3 – 329.6)	81.6 (25.3 – 329.6)	57.4 (26.1 – 209.3)
15 months	55.0 (27.7 – 209.8)	100.9 (35.3 – 209.8)	48.0 (27.7 – 202.2)
2 years	76.5 (17.6 – 494.9)	142.1 (18.3 – 494.9)	48.4 (17.6 – 275.1)
3 years	70.1 (23.6 – 833.0)	79.7 (47.1 – 217.9)	55.7 (23.6 – 833.0)
p	0.352	0.119	0.396

Change during follow-up were analysed by Related samples Friedman's Two-Way Analysis of Variance by Ranks.

*Complete series of blood samples from baseline to 3 years was available from 18 patients

Table 4. Risk factors for survival in RCC patients

		5-year survival				15-year survival			
		Age-adjusted univariate		Multivariate		Age-adjusted univariate		Multivariate	
	N	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Age	82	1.01	(0.98-1.05)	1.03	(0.99-1.08)	1.02	(0.99-1.05)	1.02	(0.99-1.06)
Gender									
Male	54	1.08	(0.50-1.37)	**2.55**	**(1.07-6.08)**	1.18	(0.62-2.24)	2.06	(0.996-4.25)
Female	28	1.00		1.00		1.00		1.00	
Metastatic status									
Nonmetastatic	61	1.00		1.00		1.00		1.00	
Metastatic	21	**12.3**	**(5.66-26.7)**	**19.2**	**(7.49-49.2)**	**7.59**	**(4.08-14.1)**	**11.8**	**(5.54-25.0)**
Grade									
1-2	54	1.00		1.00		1.00		1.00	
3	28	**2.93**	**(1.40-6.11)**	**2.51**	**(1.11-5.66)**	**2.01**	**(1.11-3.64)**	1.89	(0.997-3.57)
YKL-40 tertiles									
1 (≤55.0)	27	1.00		1.00		1.00		1.00	
2 (55.1-120.0)	27	**3.24**	**(1.01-10.4)**	2.31	(0.70-7.62)	2.21	(0.95-5.14)	1.55	(0.66-3.64)
3 (>120.0)	28	**5.26**	**(1.67-16.6)**	**3.84**	**(1.16-12.7)**	**4.34**	**(1.87-10.1)**	**3.19**	**(1.38-7.36)**

Figures

Figure 1

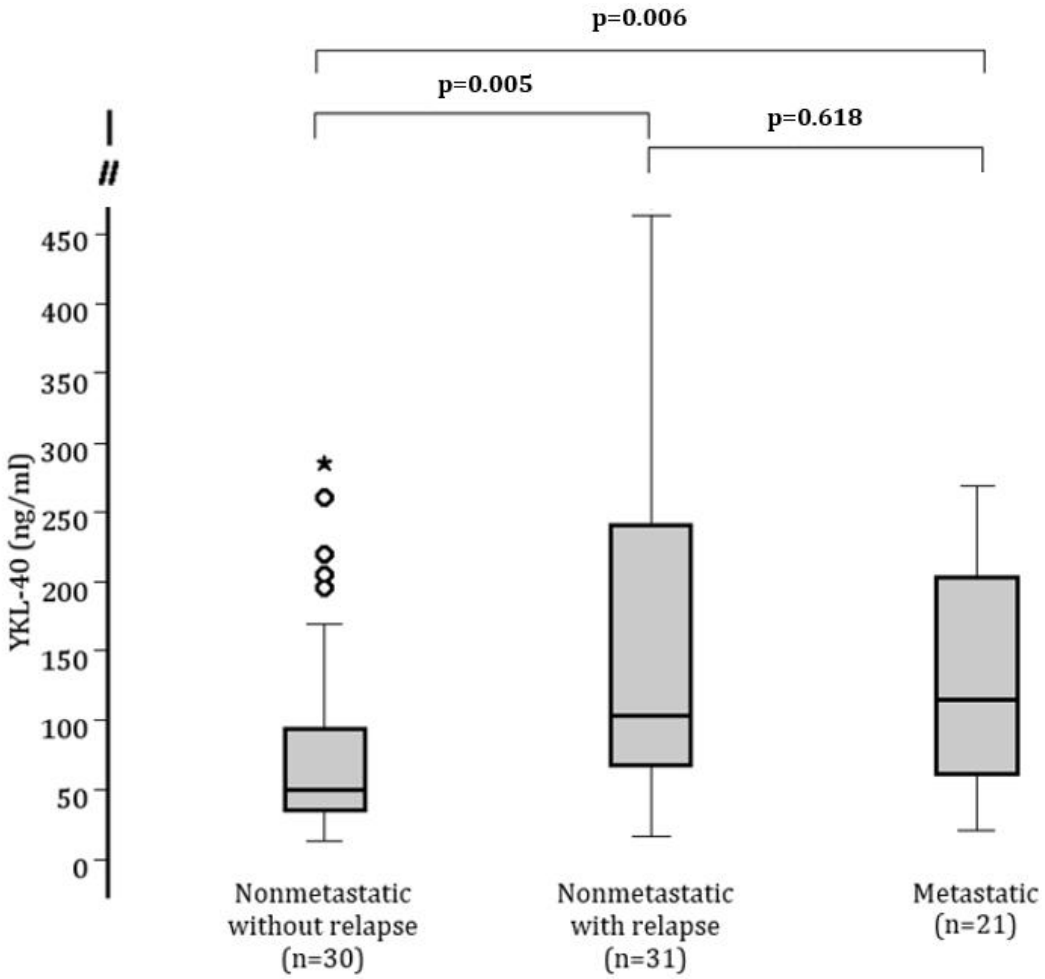

Figure 1. Serum YKL-40 levels at the time of renal cell carcinoma diagnosis. Group medians (black line), interquartile range (upper and lower border of the box) and ranges (line bar) are presented in patients with nonmetastatic carcinoma with (n=31) or without (n=30) relapse during the 15 years' follow-up and in patients with metastatic disease at the time of diagnosis (n=21). Differences between groups were calculated by Mann-Whitney test. Outliers and extreme outliers are expressed as dots or stars.

Figure 2

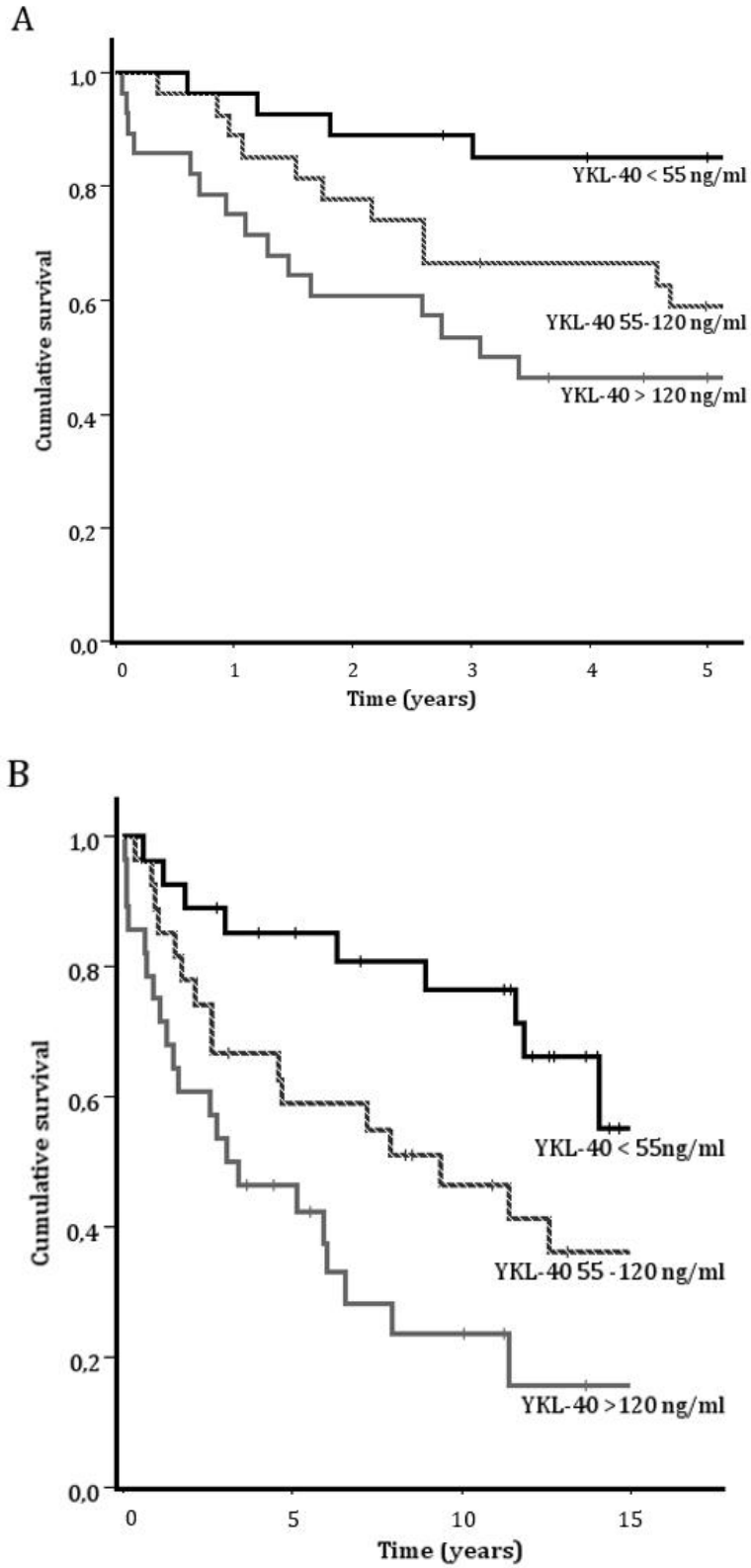

Figure 2. The cumulative 5-year (A; Log-Rank (Mantel-Cox) $p = 0.008$) and 15-year (B; Log-Rank (Mantel-Cox) $p < 0.001$) survival curves of 82 patients with renal cell carcinoma, divided into tertiles according to their baseline YKL-40 levels.