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ORIGINAL ARTICLE

Natural history of renal cell carcinoma: An immunohistochemical analysis of growth rate in patients with delayed treatment

Lei Zhang [a,c], Lin Yao [a,c], Xuesong Li [a,*], Michael A.S. Jewett [b], Zhisong He [a], Liqun Zhou [a,*]

[a] *Department of Urology, Peking University First Hospital, Institute of Urology, Peking University, National Urological Cancer Center, Beijing, China*
[b] *Division of Urology and Department of Surgical Oncology, Princess Margaret Hospital, University of Toronto, Toronto, Canada*

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Background/purpose: To investigate the natural history of renal cell carcinoma (RCC) with delayed treatment and to immunohistochemically analyze the correlation between some biomarkers and the growth rate of RCC.
Methods: We reviewed our institutional databases to identify renal tumors which were confirmed to be RCC by delayed surgical treatment after at least 12 months of active surveillance (AS). Growth rate was defined as the average growth rate of the maximal diameter on computed tomography or magnetic resonance imaging. The clinicopathological characteristics and immunohistochemical biomarkers (Ki-67, p53, bcl-2, and vascular endothelial growth factor) were analyzed the correlation with the growth rate of RCC.
Results: We identified 45 RCCs from 45 patients. The mean patient age was 54 years (range, 26–78 years). The mean tumor size increased from 2.39 cm (range, 0.10–6.70 cm) at presentation to 4.54 cm (range, 1.40–11.80 cm) after a mean time of 45.4 months (range, 12–155 months) of AS. The mean growth rate was 0.79 cm/y (range, 0.10–4.74 cm), and 36 (80.0%) tumors presented a growth rate $\leq$ 1.00 cm/y. Clear cell RCC had a trend of growing faster than other histological subtypes. Pathological grade was significantly correlated with the growth rate of RCC ($p = 0.043$). High positive ratio of Ki-67 ($r = 0.351$, $p = 0.018$) and being p53 positive ($p = 0.019$) were significantly correlated to the fast growth rate of RCC.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding authors. Department of Urology, Peking University First Hospital, Institute of Urology, Peking University, National Urological Cancer Center, Number 8 Xishiku Street, Xicheng District, Beijing 100034, China.
E-mail addresses: pineneedle@sina.com (X. Li), zhouliqunmail@sina.com (L. Zhou).
[c] These authors contributed equally.

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Conclusion: In general, RCCs under AS are slow growing with a wide variation of growth rate, with a portion of RCCs presenting rapid growth kinetics. RCC with rapid growth during AS is characterized by a high histological grade, high positive ratio of Ki-67, and being p53 positive.

Introduction

A dramatic increase of incidentally found small renal masses has been observed with the widespread use of modern imaging techniques.[1] Surgical excision is still the standard treatment for these localized renal tumors. However, a number of patients with a high risk of morbidity and mortality or limited life expectancy choose active surveillance (AS) instead of immediate surgeries. AS provides a unique opportunity to observe the natural history of the renal cell carcinoma (RCC), as most tumors are surgically excised soon after diagnosis. Previous studies concerning AS demonstrated that small renal tumors grew slowly and seldom metastasized.[2–23] In most of these studies, lack of pathological diagnosis was a common limitation, even a considerable portion of the tumors with pathological results were not RCC. Hence, the growth kinetics and natural history of RCC have not been well characterized.

To understand the growth kinetics of RCC fully, a few studies have evaluated the correlation between the immunohistochemical biomarkers and the growth rate of RCCs.[3–5] However, all the available researches included small sample sizes and short follow-up periods, so no consensus has been reached. The investigations regarding the correlation between immunohistochemical biomarkers and the growth rate of RCC are far from sufficient.

Our previous study demonstrated that RCCs were found to be slow growing in patients with delayed treatment, however, progression in stages was presented in some RCCs.[2] In the current study, we expanded the sample size to further examine the growth kinetics of RCC and its correlation with clinical and pathological characteristics. In addition, we selected four biomarkers, Ki-67, p53, vascular endothelial growth factor (VEGF), and bcl-2, which were considered prognostic factors of RCCs in previous studies,[24–26] and immunohistochemically analyzed whether they were involved in the growth of RCCs with delayed treatment.

Materials and methods

We reviewed the kidney cancer databases at the Institute of Urology, Peking University First Hospital, Beijing, China to identify renal tumors for which AS was performed for at least 12 months between January 1990 and July 2012. A total of 60 patients with renal tumors under AS for $>$ 12 months were identified from 2180 renal tumor cases. Patients who did not receive delayed surgical treatment were excluded. Finally, 45 renal tumors from 45 patients were enrolled. During the period of AS, computed tomography (CT) or magnetic resonance imaging (MRI) was performed every 6 months or less. Where possible, the measurement was performed based on the same modality. Because of tumor growth, obvious enhancing on CT, or metastatic lesions, delayed surgical intervention was performed on all patients at Peking University First Hospital after a mean duration of 45 months of AS. The pathological results confirmed RCC for all tumors. Growth rate was defined as the average growth rate of the maximal diameter on a series of 2-dimensional images. Histological classification was determined using the Heidelberg typing system.[27] Tumor stage was assessed according to the 2002 American Joint Committee on Cancer TNM staging system.[28] Tumor grading was performed according to the Fuhrman grade system.[29]

Paraffin-embedded sections were stained using a ChemMate EnVision Detection Kit (Genetic Technology, Shanghai, China). The antibodies (Genetic Technology, Shanghai, China) used in this study included bcl-2 and p53, VEGF, and Ki-67. Paraffin sections were deparaffinized and then dipped into phosphate buffer solution for 5 minutes three times. Then, sections were incubated in primary antibody for 30 minutes at room temperature and another 4 hours at 4°C. The Envision method was used for immunohistochemical staining. Slides were exposed to diaminobenzidine for 5 minutes three times. After immunostaining, the sections were counterstained with hematoxylin, coverslipped, and sealed. Phosphate buffer solution was used as a negative control of the first antibody for each group.

The expression levels of bcl-2 and VEGF were detected based on the intensity of staining and the percentage of positive cells. The intensity of staining was scored as follows: unstained $=$ 0 points; light brown color $=$ 1 point; brown $=$ 2 points; and deep brown color $=$ 3 points. The percentage of positive cells was scored as follows: $<$ 5% $=$ 0 points; 5–10% $=$ 1 point; 10–50% $=$ 2 points; and $>$ 50% $=$ 3 points. The sum of the two items was scored as follows: 0 points $=$ negative; 1–3 points $=$ weakly positive; and 4–6 points $=$ strongly positive. More than 10% of the nucleus stained was the positive standard for p53. Ki-67 was recorded as the Ki-67 labeling index, which was defined as the proportion of Ki-67-positive cells per 1000 cells in 10 representative Ki-67-positive fields. All sections were separately reviewed by two urological pathologists who were blinded to the patients' personal data. If the opinions were inconsistent, the sections were reviewed by the two pathologists together to reach an agreement.

For better knowledge of the natural history and growth kinetics of renal masses, we also reviewed published series regarding AS of renal masses and made a pooled analysis. In addition, through the pooled analysis, we wanted to know the metastatic rate during AS and the rate of pathologically

confirmed RCC among the renal masses that underwent AS. As all the cases were proven RCC in the current study, through comparing the results of AS from this study with the results of the pooled analysis, we might further understand the characteristics of RCCs natural history.

Statistical analysis

The Chi-square test was used to test the distribution of categorical variables. The correlations between growth rate and Ki-67, and between initial size and growth rate were assessed by calculating Pearson's correlation coefficient. The Mann—Whitney *U* test or the Kruskal-Wallis H test was used for continuous variables. SPSS version14.0 software package (SPSS Inc., Chicago, IL, USA) was used for data processing. A p value < 0.05 was considered statistically significant.

Results

Patient characteristics and outcome of AS

We identified 45 renal tumors from 45 patients who were treated by delayed intervention after at least 12 months of AS. Most tumors (40/45, 88.9%) were asymptomatic and diagnosed incidentally during the imaging procedures for physical examination; the other cases were diagnosed with complaint of flank pain or occasional hematuria. The reasons for no immediate intervention included patient preference (31/45, 68.9%), considered benign diagnosis at presentation (10/45, 22.2%), existence of bilateral disease (2/45, 4.4%), and concomitant malignancy (2/45, 6.3%). Patient demographics, pathological features, and growth rates of tumors are summarized in Table 1. The majority of patients were men (37/45, 82.2%). The mean patient age was 54 years (range, 26—78 years). The mean tumor size increased from 2.39 cm (range, 0.10—6.70 cm) at presentation to 4.54 cm (range, 1.40—11.80 cm) after a mean duration of 45.4 months (range, 12—155 months) AS. Most of the tumors (38/45, 84.4%) were $\leq$ 4 cm at presentation. Stage progression was documented in 16 tumors: 11 tumors progressed from T1a to T1b, four tumors progressed from T1a to T2, and one tumor progressed from T1b to T2. pT stage was in concordance with cT stage at operation for all tumors. During AS, only one patient presented with a biopsy-proven metastasis RCC in the lung at the 155th month of AS, the primary tumor was 1.6 cm in diameter at presentation. Interestingly, this tumor did not grow fast, the average growth rate was 0.20 cm/y.

Surgical intervention was performed because of tumor growth, presence of obvious enhancing on CT, or metastatic lesion (palliative excision of primary lesion). Twenty-three tumors (51.1%) were treated with radical nephrectomy, while the other 22 tumors (48.9%) were treated with partial nephrectomy. The pathological results confirmed RCC in all 45 cases. Thirty-nine tumors (86.7%) were clear cell carcinoma, four tumors (8.9%) were papillary cell carcinoma, one tumor (2.2%) was mucinous tubular and spindle cell carcinoma, and one tumor (2.2%) was granular cell carcinoma. Nine tumors (20%) were Grade 1, 29 tumors (64.4%) were Grade 2, and seven tumors (15.6%) were Grade 3.

Table 1 Patient demographics and tumor characteristics.

Variables	*n* (%)
Sex	
Women	8 (17.8)
Men	37 (82.2)
Age, y	
Median	54
Mean	54
Range	26—78
Initial tumor size	
Maximal diameter, cm	
Median	2.00
Mean	2.39
Range	0.10—6.70
Final tumor size	
Maximal diameter, cm	
Median	4.00
Mean	4.54
Range	1.40—11.80
Duration of AS, mo	
Median	28
Mean	45.4
Range	12—155
Growth rate, cm/y	
Median	0.50
Mean	0.79
Range	0.10—4.74
Pathological type	
ccRCC	39 (86.7)
pRCC	4 (8.9)
MTSCCa	1 (2.2)
gCC	1 (2.2)
Grade	
1	9 (20.0)
2	29 (64.4)
3	7 (15.6)
Stage	
T1a	23
T1b	17
T2	4
T3	1

ccRCC = clear cell renal cell carcinoma; gCC = granular cell carcinoma; MTSCCa = mucinous tubular and spindle cell carcinoma; pRCC = papillary renal cell carcinoma.

Growth kinetics of RCC

The mean growth rate was 0.79 cm/y (range, 0.10—4.74 cm). Most of these tumors grew slowly, 36 (80.0%) tumors presented a growth rate $\leq$ 1.00 cm/y, while nine (20.0%) tumors had a growth rate $>$ 1.00 cm/y. Initial size, sex, and age were not correlated to the growth rate of RCC. The results of growth rate based on pathological characteristics were summarized in Table 2. Given the limitation of the sample size, we did not make a comparison between different histological subtypes. However, clear cell RCC (ccRCC), with a mean growth rate of 0.82 cm/y, had a trend of growing faster than other histological subtypes. For all tumors, tumors with high grades

Table 2 Growth rate of RCC according to pathological results.

	Growth rate (cm/y) mean/median (range)	*p*
Histologic subtype		
Clear cell carcinoma ($n = 39$)	0.82/0.60 (0.07 −4.44)	
Papillary cell carcinoma ($n = 4$)	0.68/0.36 (0.20 −1.80)	—
Granular cell carcinoma ($n = 1$)	0.22	
Mucinous tubular and spindle cell carcinoma ($n = 1$)	0.34	
Histological grade of all cases		
G1 ($n = 9$)	0.34/0.30 (0.10 −0.63)	
G2 ($n = 29$)	0.74/0.63 (0.11 −2.31)	0.043*
G3 ($n = 7$)	1.56/0.60 (0.20 −4.74)	
Histological grade of clear cell RCC		
G1 ($n = 8$)	0.34/0.25 (0.10 −0.63)	
G2 ($n = 26$)	0.71/0.63 (0.11 −2.31)	0.030*
G3 ($n = 5$)	1.79/0.75 (0.26 −4.74)	

*The significance is $p<0.05$.

grew faster than tumors with low grades ($p = 0.043$). After rerunning the data by focusing on ccRCC, it was also confirmed that high grade tumors grew faster than low grade tumors and the difference was more significant ($p = 0.030$).

Pooled analysis of published series on the natural history of renal masses

The pooled analysis revealed a total of 1171 patients with 1271 renal tumors (Table 3). Of the 1271 renal tumors, only 444 (34.9%) had pathological results, and 380 (29.9%) were RCC. Collectively, the mean age was 69.5 years (range, 52.2—80.4 years), the mean growth rate was 0.33 cm/y (0.06—0.8 cm), and the mean duration of AS was 34.6 months (range, 12.6—91.5 months). Nineteen (1.6%) patients developed metastatic disease during AS.

Immunohistochemical analysis according to growth rate, tumor initial size, and histological grade

Representative immunostaining for Ki-67, p53, bcl-2, and VEGF are shown in Figure 1. The Ki-67 labeling index (Figure 2), which ranged from 0 to 70, was correlated with the growth rate of RCC ($r = 0.351$, $p = 0.018$). RCCs that were p53 positive had a faster growth pattern than RCCs that were p53 negative (0.97 cm/y vs. 0.41 cm/y, $p = 0.019$). No negative staining for bcl-2 or VEGF was observed. However, bcl-2 and VEGF were not correlated with the growth rate of RCC. No correlation was observed between initial tumor size and the expression of Ki-67, p53, bcl-2, and VEGF. Tumors of a high grade had more chance of being p53 positive compared with tumors of a low grade ($p = 0.006$), while the expression of Ki-67, bcl-2, and VEGF were not correlated to tumor grade. There was no significant correlation between any two of the biomarkers bcl-2, VEGF, p53, and Ki-67.

Discussion

For renal masses, AS with delayed treatment until progression is now gradually accepted especially for patients with a high risk of surgery and limited life-expectancy. A number of published series on AS of small renal masses provide the unique opportunity to assess the growth of RCC. A meta-analysis with 234 renal tumors revealed a mean growth rate of 0.28 cm/y, the tumors pathologically confirmed RCC had a higher growth rate of 0.4 cm/y.[6] RCC may have relatively aggressive growth potential compared with other benign renal tumors. In addition, there is no curative salvage therapy for metastatic RCCs at present. Compared with assessing the growth of renal masses in general, it will be more beneficial to focus on the growth kinetics of RCC.

We reviewed published series regarding AS of renal masses and made a pooled analysis (Table 3). The pooled analysis showed renal tumors during AS had slow growth kinetics and a low rate of metastasis. Of the 1271 analyzable lesions, only 444 (34.9%) had pathological results, and 380 (29.9%) were RCC. Apparently, the most common limitation of these reports was lack of pathological results. The kinetics of RCC could not be correctly reflected by the pooled analysis.

In the current study, all the tumors finally received delayed surgical excision after a mean duration of 45 months of AS, and were pathologically confirmed as RCC. Compared with other series, these tumors had a similar initial tumor size (mean, 2.39 cm), a relatively longer period of AS, and a similar metastasis rate during AS (2.2%). Our results also revealed most of these RCCs were slow growing, 36 of the 45 tumors (80%) had a growth rate under 1.00 cm/y. However, a wide variation of the growth rate was observed; the fastest growth rate was 47 times faster than the lowest one. In comparison with other series, this cohort had a relative rapid growth rate. We explain the phenomenon through the presence of non-RCC pathologies, elder age, and the follow up period not being long enough until delayed treatment in the other series. Jewett et al[7] demonstrated that there was no difference of the average growth rate between the biopsy-proven RCCs and benign tumors. However, most of these RCCs were not followed up long enough until delayed treatment. Our previous study indicated the growth of RCCs might accelerate along with the continuation of AS.[30] So the growth kinetics and natural history of RCC might be misjudged. We speculate that the growth rate of RCC is slow and even comparable to the growth rate of benign tumors at the

Table 3 Published series on the natural history of renal masses.

	Y	No. of lesions	Mean age (y)	Mean initial MTD (cm)	Mean follow-up (mo)	Mean LGR (cm/y)	Progression to metastasis, *n* (%)	Pathologic RCC
Fujimoto et al[3]	1995	6/6	59.7	2.47	24	0.47	0 (0)	5/5
Bosniak et al[9]	1995	37/40	65.5	1.73	39	0.36	0 (0)	22/26
Oda et al[4]	2003	16/16	54*	2.0*	25	0.54*	0 (0)	16/16
Volpe et al[10]	2004	29/32	71*	2.48	27.9	0.1	0 (0)	8/9
Wehle et al[11]	2004	29/29	70	1.83	32	0.12	0 (0)	3/4
Kato et al[5]	2004	18/18	56.5	2.0	27	0.42	0 (0)	18/18
Lamb et al[12]	2004	36/36	76.1	7.2	27.7	0.39	1 (2.8)	23/23
Chawla et al[6]	2006	49/61	71	2.97	36	0.2	1 (1.6)	16/21
Abou Youssif et al[13]	2007	35/44	71.8	2.2	47.6	0.21	2 (5.7)	6/8
Kouba et al[8]	2007	43/46	67	2.92	32.8	0.7	0 (0)	12/14
Siu et al[14]	2007	41/47	68	2.0	29	0.27	1 (2.4)	10/16
Fernando et al[15]	2007	13/13	80.4	5.01	38.38	0.17	1 (7.7)	0
Matsuzaki et al[16]	2007	15/15	67	2.2	38	0.06	0 (0)	3/3
Lee et al[17]	2008	30/30	65.5	2.6	12.6	0.59	3 (10.0)	30/30
Beisland et al[18]	2009	63/65	76.3	4.3	33	0.66	2 (3.2)	15/18
Crispen et al[19]	2009	154/173	69	2.45	31	0.285	2 (1.3)	52/61
Rosales et al[20]	2010	212/223	71*	2.8*	35*	0.34*	4 (1.9)	32/40
Hwang et al[21]	2010	56/58	64.3	2.1	22	0.21	0 (0)	10/15
Jewett et al[7]	2011	127/151	73	2.1	28	0.13	1 (0.7)	37/46
Li et al[2]	2012	32/32	52.2	2.14	46	0.8	0 (0)	32/32
Mehrazin et al[22]	2014	68/72	68.9	5.3	38.9	0.44	0 (0)	16/23
Brunocilla et al[23]	2014	62/64	75	2.0	91.5	0.4	1 (1.6)	14/16
Total		1171/1271	69.5	2.82	34.6	0.33	19 (1.6)	380/444

* Median.
MTD = *maximal tumor diameter*; RCC = renal cell carcinoma.

early stage of natural history, but along with the continuation of AS, it is not surprising that the malignancy of RCC in terms of growth rate will show up finally. Our results might more correctly reflect the potential malignancy of RCC in the aspect of growth rate.

Up to now, there is no definite prognostics factor of progression of renal tumors under AS. Previous studies demonstrated potential predictors of subsequent tumor growth including initial tumor size, sex, age, and pathological characteristics.[8,9] By contrast, the initial tumor size, sex, and age were not correlated with the growth rate of RCC in this cohort. The current study indicated that ccRCC had a trend of growing faster than other histological subtypes. More non-ccRCC histological subtypes of RCC are needed to examine the trend in further studies. RCCs with a high grade had a trend of growing faster than those with a low grade ($p = 0.043$). When focusing the histological type on ccRCC, we did find that ccRCCs with a high grade grew faster than ccRCCs with a low grade and the difference was more significant ($p = 0.030$). It might make more sense to discuss the correlation between grade and the growth rate of RCC based on a certain histological subtype.

The growth rate of renal tumors under AS is various and not well predicted by clinical and radiographic factors, while immunohistochemistry has an advantage of exploring the nature of tumor growth through assessing the expression of biomarkers that reflect cell proliferation, apoptosis, angiogenesis, and so on. To the best of our knowledge, only three studies made a immunohistochemical analysis using surgical samples of RCCs which were treated by a period of AS initially, and evaluated the correlation between the expression of immunohistochemical biomarkers and the growth rate of RCCs.[3–5] As the immunohistochemical analysis was not performed at the beginning of AS, so what they did may not reflect the prediction role of the biomarkers in the aspect of growth rate. However, they still provided valuable features of immunohistochemical biomarkers for RCCs with aggressive growth rates. In these studies, no consensus has been reached. The small sample size and short follow up period limited the strength of these studies. In the current study, we present an immunohistochemical analysis with a relatively larger sample size and longer follow up of AS compared with previous studies.

Ki-67 is considered a reliable marker of active cell proliferation. The correlation between Ki-67 and the growth rate of RCC is controversial. Kato et al[5] found the growth rate of RCC and the Ki-67 positive ratio were not correlated. Oda et al[4] discovered the Ki-67 labeling index tended to increase as the growth rate of RCC increased, but the correlation was not significant. In contrast with previous studies, a significant correlation between the Ki-67 labeling index and the growth rate of RCC was found in the current study. RCCs with rapid growth rate might present higher Ki-67 expression than RCCs with a slow growth rate. This discrepancy could be explained by the small sample size and short period of follow up in these studies.

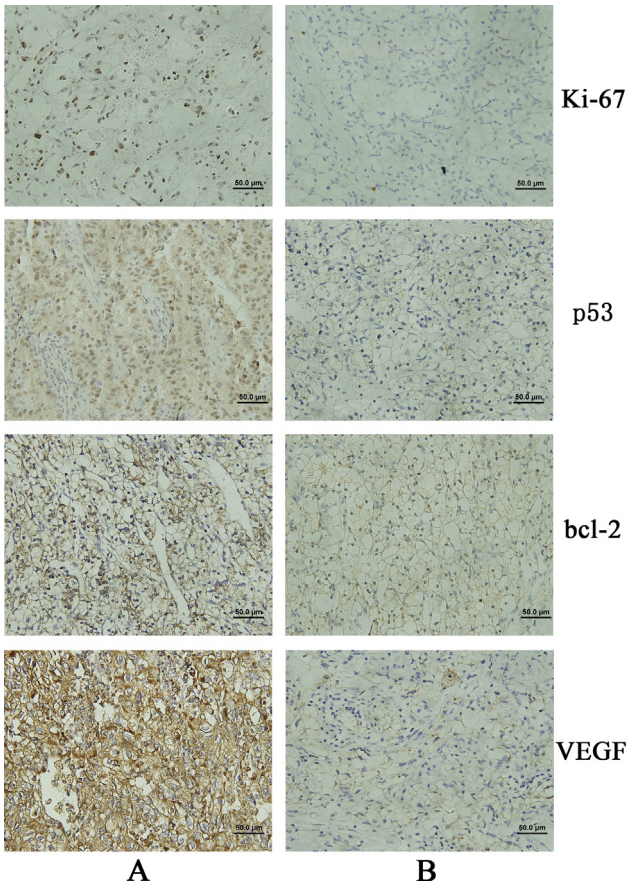

Figure 1 Immunostaining of markers in renal cell carcinoma. Original magnification: × 400. (A) High-level staining for biomarkers; (B) negative (Ki-67, p53) or low-level [bcl-2, vascular endothelial growth factor (VEGF)] immunostaining for biomarkers.

p53, bcl-2, and VEGF were deemed to have a correlation with the survival of RCC.[25,26] To our knowledge, our experience provided initial evaluation of the correlation between these biomarkers and the growth rate of RCC. Our results showed that p53 was correlated with the growth rate of RCC, while bcl-2 and VEGF were not. RCCs with rapid growth have more chance of being p53 positive than RCCs with a slow growth rate. As the limitation of retrospective design and sample size, prospective studies with a larger sample size are required to examine these results in the current study.

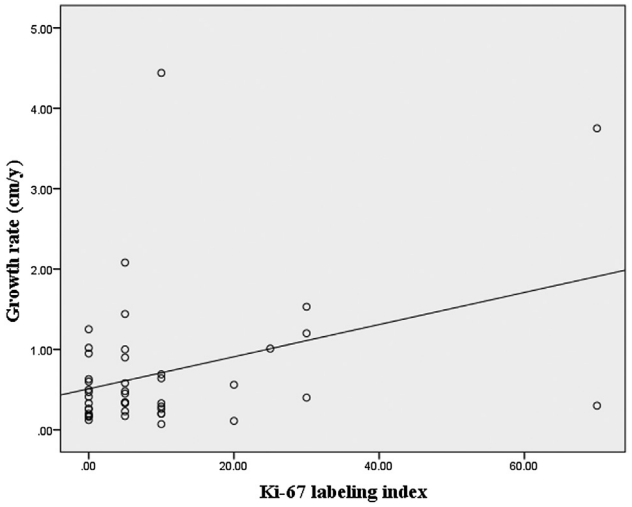

Figure 2 The growth rates of renal cell carcinoma and Ki-67 labeling index. The correlation coefficient was 0.351 ($p = 0.018$).

Conclusion

In general, RCCs are slow growing with a wide variation of growth rate, some of them present rapid growth rates, which should be considered before AS. RCC with rapid growth during AS is characterized by a high histological grade, high positive ratio of Ki-67, and is p53 positive. This is helpful for selecting optimal RCCs for AS. Before the appearance of definite predictors of the progression of RCC, more attention should be given to the natural history of RCC.

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