

Serum Thymidine Kinase 1 Activity Following Nephrectomy for Renal Cell Carcinoma and Radiofrequency Ablation of Metastases to Lung and Liver

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Abstract. *Background/Aim:* Thymidine kinase 1 (TK1) is involved in DNA synthesis and is considered a reliable and sensitive marker of cell proliferation. The aim of this study was to investigate the prognostic value of measurements of serum TK1 activity following tumor ablation. *Patients and Methods:* This study was performed on 32 patients with renal cell carcinoma (RCC) who had undergone nephrectomy and 35 patients with cancer of different histology with metastases to the liver (n=28) and lung (n=7) treated with radiofrequency ablation (RFA). The TK1 activity was measured with DiviTum (Biovica) immunoassay. *Results:* In patients with RCC with no evidence of disease during their observation, a significant decrease of the TK1 activity was observed on the day following nephrectomy ($p < 0.0001$). The mean calculated half-life \pm SEM was 10.8 ± 1.2 h. Taking into account the short half-life, measurements of TK1 were performed 24 h after nephrectomy or RFA of metastases. It was found that elevated TK1 activity (>60 Du/l) on the day after nephrectomy independently predicted poor recurrence-free survival (hazard ratio=5.0, $p=0.040$), after adjustment for T-stage, age and pretreatment TK1. Patients scheduled for RFA averaged 1.4 lesions and an average lesion diameter of 2.2 cm. Multivariate Cox's regression model demonstrated the significant association of any increase of TK1 activity or decrease not reaching ≤ 60 Du/l on the day after ablation with poor progression-free survival (hazard ratio=4.6, $p=0.001$), after adjustment for the type of primary tumor, the

number and size of metastases. *Conclusion:* The half-life for serum TK1 activity is 10.8 ± 1.2 h. The measurements of TK1 activity following nephrectomy or RFA of metastases could be an important tool in prognostic evaluation.

Tumor proliferation is one of the most commonly used variables in evaluating tumor progression and prognosis (1). The metabolic enzyme thymidine kinase 1 (TK1) is involved in DNA synthesis and found only in dividing cells (2). It is considered a reliable marker of cell proliferation. Elevated serum TK1 activity was reported as an important risk factor indicating a high proliferative potential of tumors at the time of excision (3) and predicting poor recurrence-free survival (4, 5).

In this study, we analyzed the kinetics of serum TK1 activity following nephrectomy of renal cell carcinoma (RCC) and radiofrequency ablation (RFA) of metastases to liver or lung in patients with cancer of different histologies. We hypothesized that TK1 activity, measured after tumor ablation, may have a prognostic significance and this predefined the aims of our present study.

Patients and Methods

This study was performed on 32 patients with RCC who had undergone nephrectomy, and on 35 patients treated with RFA for metastatic cancer to the liver and lung. These patients were continuously enrolled at the Urology and Oncology Departments of Hadassah and Hebrew University Medical Centre in Jerusalem. Three hundred healthy individuals were used as a control.

The patients scheduled for nephrectomy (either radical or nephron-sparing) were more than 18 years of age, with disease at a clinical stage T3cN0M0 or less, pathology of clear cell type renal cell carcinoma and no previous known malignancy.

The preoperative imaging included computed tomography of chest, abdomen, and pelvis. Tumor stage was determined according to the TNM stage classification system (6).

RFA was performed to treat metastases in liver and lung. Computed tomography and ultrasonography were used in needle placement and accurate tumor targeting. Ablation success was

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Key Words: TK1 activity, half-life, nephrectomy, radiofrequency ablation, prognosis.

Table I. Distribution of preoperative serum thymidine kinase 1 (TK1) activity in patients with renal cell carcinoma and healthy controls.

| Characteristic | n | Preoperative TK1 (Du/l) mean, median (IQR) | p-Value |
|-------------------------|-----|---|---------|
| Healthy | 300 | 41, 19 (10-39) | |
| Renal cell carcinoma | 32 | 119, 51 (27-183) | <0.001 |
| Age, years | | | |
| ≤65 | 17 | 96, 38 (23-128) | |
| >65 | 15 | 146, 53 (31-187) | 0.355 |
| Gender | | | |
| Female | 11 | 123, 47 (30-188) | |
| Male | 21 | 118, 52 (20-180) | 0.876 |
| Stage, pT | | | |
| 1-2 | 20 | 112, 49 (27-171) | |
| 3 | 12 | 131, 51 (22-186) | 0.938 |
| Tumor ablation approach | | | |
| Open | 24 | 137, 58 (36-187) | |
| Laparoscopic | 8 | 67, 28 (14-84) | <0.05 |

IQR, Interquartile range.

defined in terms of lack of abnormal contrast enhancement at 1 month post-treatment imaging. Enhancement at the border of earlier site of ablation was termed local tumor recurrence.

To determine parameters at the time of RFA that might predict survival, a number of variables were analyzed, including age, sex, type of primary tumor, the number and the size of the metastases.

The study was approved by the Institutional Ethical Review Board (0441-08-HMO) and all participants provided their informed consent. Serum samples were obtained from all patients, aliquoted, and stored at -80°C until analysis. The TK1 activity was measured with DiviTum highly sensitive kit provided by Biovica/Ronnerbol (Uppsala, Sweden). The activity of TK1 is expressed in Divitum units/l (Du/l). All steps in the DiviTum assays were performed according to the manufacturer's instructions.

Statistical analysis. Three groups of participants were analyzed, namely healthy individuals, patients with RCC, and patients with different histology of cancer metastatic to the liver and lung.

For numeric variables, the Mann-Whitney and Wilcoxon tests were performed. For categorical variables, Fisher's exact test was applied. Logistic regression was used for prediction.

Calculation of half-life for serum TK1 activity was performed by applying the equation $t_{1/2} = t \times \ln(2) / \ln(N_0/N_t)$, where t was the time elapsed after tumor resection, N_0 was TK1 activity before tumor resection and N_t was TK1 activity after time t .

The endpoints were recurrence-free survival (RFS) as measured from the date of nephrectomy to the last follow-up evaluation or disease recurrence, and progression-free survival (PFS) defined as the time elapsed between RFA until tumor progression or death from any cause, with censoring of patients before any new anticancer treatment or at loss to follow-up. A survival analysis was performed by the Kaplan-Meier method and the log-rank test. Cox regression analysis was used to assess the impact of multiple covariates (7). Statistical calculations were performed using SPSS for Windows, Version 10 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

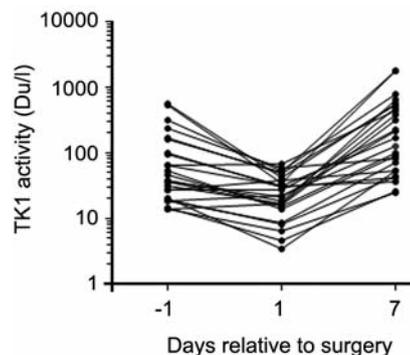


Figure 1. Logarithmic plot of individual preoperative serum thymidine kinase 1 (TK1) activity in patients with renal cell carcinoma who remained without evidence of disease during follow-up.

Results

TK1 activity kinetics in patients with RCC at nephrectomy.

In the population of 300 healthy individuals, there was no association of TK1 activity with gender ($p=0.14$) nor any correlation with age ($r=0.02$). The mean serum TK1 activity was 41 Du/l.

The measurements of TK1 activity in patients with RCC were performed preoperatively and on days 1, 7, 30 and 180 after nephrectomy.

Significantly high preoperative TK1 activity was found in patients with RCC compared to healthy individuals (median, 51 vs. 19 Du/l, $p < 0.001$, Table I). In patients with RCC, preoperative TK1 activity weakly correlated with tumor size ($r=0.28$, $p=0.03$) and was associated with the type of tumor ablation approach, being higher at open than at laparoscopic surgery (median, 58 vs. 28 Du/l, $p < 0.05$, Table I).

After nephrectomy, 23 out of the 32 patients remained without evidence of disease (NED) during observation (60-174 months). In these patients, a significant decrease of TK1 activity was observed on the day after surgery (median, 38 vs. 19 Du/l, $p < 0.0001$; Figure 1, Table II). The ratio of preoperative TK1 to 1-TK1 (day after surgery) was 3.1 ± 0.5 (mean \pm SEM).

The calculation of half-life for serum TK1 activity was performed on 10 patients with mean preoperative TK1 of 252 ± 52 Du/l and 1-TK1 of 42 ± 4 Du/l, equivalent to the mean TK1 in the healthy cohort (Table I). The calculated mean half-life for TK1 corresponded to 10.8 ± 1.2 h.

The initial decrease of TK1 activity was followed by a strong increase (median, 19 vs. 129 Du/l, $p < 0.0001$, Figure 1, Table II) with a maximum at day 7 (7-TK1). The extent of this increase correlated with tumor size ($r=0.55$, $p=0.02$) and the severity of surgical intervention, tending to be higher at open than at laparoscopic resection (median, 7.4 vs. 4.7 Du/l, $p=0.05$).

Table II. Serum thymidine kinase 1 (TK1) activity in patients with renal cell carcinoma who remained without evidence of disease (NED) or developed recurrence during follow-up after nephrectomy.

| Time relative to surgery | NED mean, median (IQR) | Recurrence mean, median (IQR) | p-Value ^a |
|---|---------------------------|----------------------------------|----------------------|
| Before | 87, 38 (19-95) Du/l | 202, 186 (48-330) Du/l | 0.022 |
| 1 day after | 25, 19 (8-41) Du/l | 68, 50 (18-98) Du/l | 0.041 |
| p-value ^b (1 day after vs. before) | <0.0001 | 0.008 | |
| 7 days after | 303, 129 (45-447) Du/l | 415, 256 (164-830) Du/l | 0.120 |
| p-value (7 days after vs. 1 day after) | 0.0001 | 0.018 | |
| 30 days after | 94, 52 (19-202) Du/l | 466, 160 (90-268) Du/l | 0.181 |
| p-value (30 days after vs. 7 days after) | 0.028 | 0.24 | |

^ap-values derived from Mann-Whitney test. ^bp-values derived from Wilcoxon test. IQR, Interquartile range.

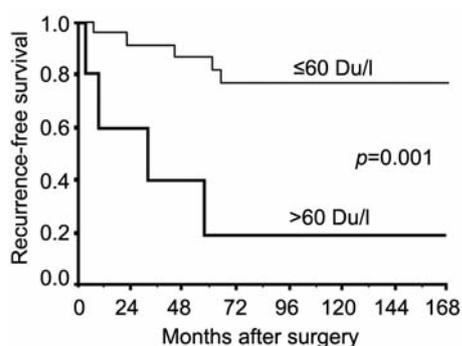


Figure 2. Kaplan–Meier estimates according to the thymidine kinase 1 (TK1) activity measured on the day after nephrectomy.

Nine patients with RCC developed recurrence after nephrectomy, that occurred within 4–65 months. Significantly high preoperative TK1 values were found in patients who developed tumor recurrence after surgery compared to those who did not (median, 186 vs. 38, Du/l, $p=0.022$ Table II). 1-TK1 activity in patients with recurrence was also higher than in patients with NED (median, 50 vs. 19; $p=0.041$). Normalization of the marker level in the NED and recurrence groups was observed only 6 months after surgery (median, 36 vs. 48; $p=0.56$). Although the TK1 activity in patients with RCC at the time of recurrence was higher than in those that remained with NED, the difference did not reach statistical significance (median, 103 vs. 36 Du/l; $p=0.08$).

Two cut-offs were used in this study. The cut-off of 170 Du/l for preoperative TK1 activity was extracted from the receiver operating characteristic (ROC) analysis of patients with RCC preoperatively (5). The ROC analysis exploring 1-TK1 activity was used to estimate the optimal cut-off predicting recurrence. Defined as the maximal Younden's index, it was found to be 60 Du/l.

Both elevated preoperative TK1 activity (*i.e.* >170 Du/l), and elevated 1-TK1 activity (*i.e.* >60 Du/l) were significantly

Table III. Multivariate Cox's regression: hazard ratio for patients with renal cell carcinoma ($n=32$).

| Characteristic | Hazard ratio | 95% CI | p-Value |
|----------------------------------|--------------|----------|---------|
| Age (>65 years vs. ≤65 years) | 3.0 | 0.4-21.0 | 0.259 |
| T-Stage (3 vs. 1-2) | 7.5 | 1.5-38.6 | 0.016 |
| PreTK1 (>170 Du/l vs. ≤170 Du/l) | 6.4 | 0.9-45.1 | 0.064 |
| 1-TK1 (>60 Du/l vs. ≤60 Du/l) | 5.0 | 1.1-23.0 | 0.040 |

PreTK1: Preoperative thymidine kinase 1; 1-TK1: thymidine kinase activity on the day after nephrectomy, CI: confidence interval.

associated with disease recurrence [odds ratio (OR)=13.3, 95% confidence interval (CI)=2.1-84.1, $p=0.006$, and OR=17.6, 95% CI=1.6-193.4, $p=0.019$; respectively].

Kaplan-Meier estimates demonstrated a significant difference in RFS for patients with normal *versus* elevated (>60 Du/l) activity of 1-TK1 (Figure 2).

Univariate Cox proportional hazards analysis for 32 patients with RCC demonstrated that T stage 3, age >65 years, preoperative TK1 >170 Du/l and 1-TK1 >60 Du/l were all significantly associated with poor RFS ($p<0.05$, $p=0.008$ and $p=0.005$, respectively). However, only stage and elevated 1-TK1 activity retained significance in multivariate analysis (Table III).

TK1 activity following RFA of metastases. Thirty-five patients including those with colorectal cancer ($n=16$), non-small cell lung carcinoma ($n=4$), hepatocellular carcinoma ($n=4$), neuroendocrine pancreatic carcinoma ($n=3$), melanoma ($n=3$), breast carcinoma ($n=2$), endometrial carcinoma ($n=1$), esophageal carcinoma ($n=1$) and sarcoma ($n=1$) were treated with RFA for metastases to the liver ($n=28$) and the lung ($n=7$). Five patients remained during follow-up (35–66 months) with NED.

In this group of patients there was no association of preoperative TK1 with age, gender, tumor histology,

Table IV. Distribution of serum preoperative thymidine kinase 1 (TK1) activity in patients that underwent radio-frequency ablation of metastases.

| Characteristic | n | Preoperative TK1 (Du/l) mean, median, (IQR) | p-Value |
|------------------------------|-----|--|---------|
| Healthy | 300 | 41, 19 (10-39) | |
| All patients with metastases | 35 | 166, 60 (35-125) | <0.001 |
| Age, years | | | |
| ≤65 | 17 | 93, 37 (32-79) | |
| >65 | 18 | 222, 87 (35-197) | 0.117 |
| Gender | | | |
| Female | 15 | 111, 38 (24-146) | |
| Male | 20 | 208, 62 (36-122) | 0.458 |
| No. of metastases | | | |
| 1 | 27 | 189, 53 (35-147) | |
| >1 | 8 | 91, 72 (29-116) | 0.954 |
| Size | | | |
| ≤3 cm | 26 | 191, 45 (34-163) | |
| >3 cm | 9 | 95, 84 (37-106) | 0.725 |
| Metastases | | | |
| Liver | 28 | 119, 62 (36-122) | |
| Lung | 7 | 336, 32 (24-294) | 0.505 |
| Histology | | | |
| Colorectal | 16 | 134, 59 (37-108) | |
| Other | 19 | 193, 38 (32-146) | 0.612 |

IQR, Interquartile range.

metastatic site (liver or lung), number of metastases or size (Table IV). Patients averaged 1.4 lesions (range=1-3) and an average lesion diameter of 2.2 cm (range=0.8-4.2 cm). Recurrence according to the pattern was classified namely into those in the ablation zone (n=4), only in the treated organ (liver, n=11 or lung, n=1), only distant metastases (n=6), and mixed (n=8).

Significantly increased preoperative TK1 and 1-TK1 activity was found in patients with recurrence at the site of ablation compared to the other patients (median, preoperative TK1: 308 vs. 46 Du/l, $p=0.032$ and 1-TK1: 350 vs. 40 Du/l, $p=0.025$).

Elevated (>170 Du/l) preoperative TK1 activity was found to be a significant predictor of recurrence in the ablation zone (OR=20.2, 95% CI=2-245, $p=0.018$).

Three patterns of change of TK1 activity following RFA were observed (Figure 3): (A) any increase, (B) decrease not reaching normal 1-TK1 (*i.e.* still >60 Du/l) and (C) decrease, with 1-TK1 below 60 Du/l. The median PFS for patients in these three categories was: 1.3 months, 1.5 months and 11.5 months, respectively. Since the first two categories did not differ in prognosis they were considered together *versus* the latter.

For patients with any increase or decrease not reaching normal TK1 activity after ablation (≤60 Du/l), the median PFS was significantly shorter compared to patients with decrease and 1-TK1 levels below 60 Du/l (Figure 4).

Table V. Cox proportional hazards analyses for disease progression-free survival for 35 patients who underwent radiofrequency ablation (RFA) of metastases (n=32).

| Characteristics | Hazard ratio | 95% CI | p-Value |
|---|--------------|----------|---------|
| Histology (colorectal vs. other) | 2.5 | 0.9-6.3 | 0.060 |
| Size (>3.0 cm vs. ≤ 3.0 cm) | 1.1 | 0.4-3.1 | 0.933 |
| Lesions (≥2 vs. 1) | 1.5 | 0.6-3.5 | 0.351 |
| Increase or 1-TK1 >60 Du/l vs. decrease, 1-TK1 ≤60 Du/l | 4.6 | 1.9-11.3 | 0.001 |

1-TK1: Thymidine kinase 1 on the day after RFA of metastases; CI: confidence interval.

In multivariate Cox's regression model (Table V), any increase or decrease not reaching normal TK1 activity (≤60 Du/l), being adjusted for the type of primary tumor, the number and size of metastases, independently predicted poor PFS (HR=4.6, 95% CI=1.9-11.3, $p=0.001$).

When serum 1-TK1 activity after logarithmic transformation was considered as a continuous variable in a Cox model, the HR for disease progression was 1.4 (95% CI=1.1-1.9, $p=0.02$), after adjustment for the type of primary tumor, and the number and size of metastases.

Discussion

TK1 is the key enzyme in the salvage pathway for deoxythymidine monophosphate synthesis. The expression of TK1 is cell cycle-dependent (8). Dividing cells release TK1 during mitotic exit and intracellular degradation of TK1 is mediated *via* a ubiquitin-proteasome pathway (9). In serum of patients with cancer, TK1 is confined to the stable high molecular weight complex (10), probably originating from disrupted proliferating tumor cells. TK1 may also be released by normal proliferating tissues. In our cohort of 300 healthy individuals, the mean serum TK1 activity was 41 Du/l. This activity appears to reflect the normal physiological proliferative background in the human body.

The preoperative TK1 activity in the two groups of patients, namely those with non-metastatic RCC and those with different types of cancer metastatic to liver or lung, was significantly higher than in the healthy group.

Since TK1 is produced in normal conditions; it cannot be a precise parameter for evaluation of the radicality of tumor resection, as for example prostatic-specific antigen (11). Meanwhile, we suggested that TK1 activity measured after tumor ablation may have a prognostic significance.

According to the results of this study, surgical ablation of RCC was followed by the strong decrease of TK1 activity, with mean preoperative TK1/1-TK1 ratio of 3.1. This suggests that tumor seems to be the main origin of TK1 in

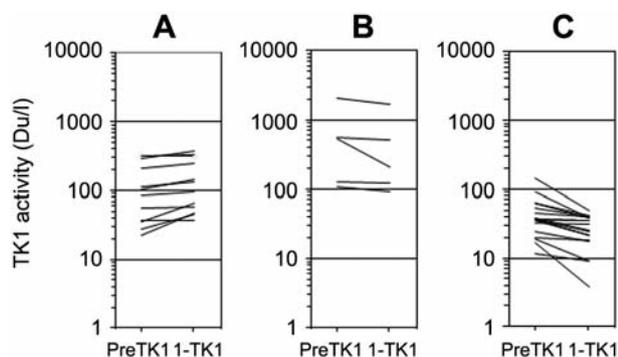


Figure 3. Change of thymidine kinase 1 (TK1) activity following radiofrequency ablation (RFA) of metastases assigned into three categories: A: any increase; B: decrease not reaching normal 1-TK1 (>60 Du/l); C: decrease, with 1-TK1 below 60 Du/l. PreTK1: Preoperative TK1; 1-TK1: TK1 activity on the day after RFA of metastases.

serum from patients with RCC. The calculated half-life for serum TK1 activity was 10.8 ± 1.2 h. To our knowledge, there are no existing studies analyzing the half-life of TK1 activity in patients following surgical tumor resection. Early, in a study on proliferating synchronized HeLa cells released from G₂/M arrest, the half-life of human cytosolic TK1 protein was reported to be longer than 9 h (9). However, these data could be more indicative of the stability of cytosolic TK1 protein. Released TK1 was reported to form high-molecular weight aggregates in the blood (10) and our data appears to describe a half-life of enzymatic activity of these aggregated forms of TK1.

We found that an initial decrease of TK1 activity after nephrectomy was followed by its strong increase. This rise of TK1 activity appears to coincide with the second proliferative phase of wound healing (12). During this phase, immune cells and fibroblasts secrete different growth factors and cytokines engaged in promoting angiogenesis and in repair of tissue injury (13). In line with data indicating dependence of proliferative events after surgery on the severity of injury (14), we found that increase of TK1 activity in open nephrectomy was stronger than in laparoscopic nephrectomy.

TK1 demonstrated normalization only 180 days after surgery. From a clinical point of view, when using the TK1 assay after surgery, the increase in TKI activity should be taken into account.

Two different cut-offs were used for pre-treatment and post-treatment evaluation of serum TK1 activity: 170 Du/l and 60 Du/l, respectively. They were defined by the Younden's index. Confirming our previous results we showed the association of higher preoperative TK1 level (>170 Du/l) with poor RFS (5). The results of this study showed that after nephrectomy 1-TK1 activity was higher in patients with recurrence compared to those without recurrence during long follow-up. Furthermore,

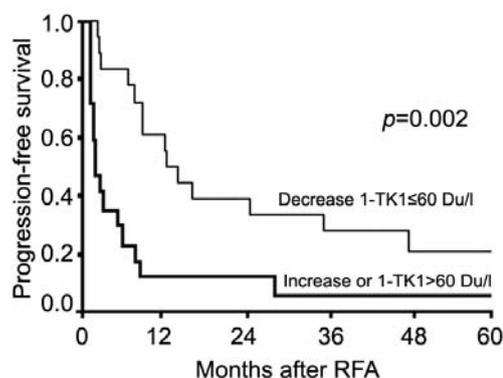


Figure 4. Kaplan–Meier estimates according to the change of thymidine kinase 1 (TK1) activity following radiofrequency ablation (RFA) of metastases. 1-TK1: TK1 activity on the day after RFA of metastases.

TK1 >60 Du/l on the day after surgery was significantly associated with disease recurrence (OR=13.3, $p=0.019$). In multivariate Cox's regression analysis, elevated 1-TK1 and T stage were retained as independent predictors of poor RFS. A high preTK1 (>170 Du/l) when controlled for the aforementioned variables was no longer significant. These results suggest that 1-TK1 measured on the day after nephrectomy outperforms preoperative TK1 as prognostic factor in terms of RFS.

Elevated TK1 activity (>60 Du/l) was found in 44% of patients at the time of recurrence. These results suggest that longitudinal measurements of TK1 in patients with RCC may be useful for the detection of disease recurrence.

In recent years, RFA has been developed as a new tool in the treatment of metastases in liver and lung (15). RFA eradicates metastases by thermal degradation of tumor cells, inducing cellular destruction and necrosis. It offers an additional curative treatment option for inoperable metastases or for patients who are not candidates for surgery.

Local tumor progression after RFA remains an important limitation, with a relatively short local PFS in a substantial percentage of patients. In our study, 11.4% patients had a recurrence at the site of ablation. These patients had significantly high pretreatment serum TK1 activity compared to the remaining patients. Logistic regression analysis showed that preoperative TK1 >170 Du/l was a significant predictor of recurrence in the ablation zone (HR=20.2, $p=0.018$).

We observed in some patients an increase of TK1 activity on the day after RFA, suggesting the stimulating effect of surgery on the proliferative processes. These patients demonstrated poor median PFS (1.3 months).

Mounting clinical and experimental evidence has indicated that RFA may cause rapid growth of residual tumor cells (16–18). In the murine model, incomplete thermal ablation stimulated

proliferation of residual renal carcinoma cells (19). A two-fold higher proliferation rate was observed at the border of the ablated region from 2 h after RFA. Insufficient RFA may accelerate the growth of residual hepatocellular carcinoma (20). Residual RCC cells after incomplete RFA may exhibit a more aggressive phenotype (21). It was suggested that growth activation may be caused by stimulatory factors such as hypoxia, heat-shock proteins and inflammatory cells (22). The growth-promoting effect of RFA on residual hepatocellular carcinoma was related to stimulation of VEGF overexpression (20).

Taken together these data suggest that an increase of TK1 activity after RFA (first category of changes) may be related to activation of residual disease in the ablation zone or occult metastases.

The second category of changes in TK1 after RFA included variation at high levels (>60 Du/l), that may indicate active disease beyond the lesion supposedly ablated. The median PFS for these patients was also poor (1.5 months).

These data justified combining the two categories into one group, including patients with any increase and those with a decrease not reaching normal TK1 activity after ablation. This pattern of changes observed on the day after RFA of metastases was shown to be an independent predictor of poor PFS (HR=4.6). The patients with a decrease and 1-TK1 levels below 60 Du/l after RFA demonstrated a better prognosis with a PFS of 11.5 months.

1-TK1 activity was also considered as a continuous variable. Each rise of 1 unit of logarithmically transformed 1-TK1 activity was associated with an increase of disease progression risk of 1.4 ($p=0.02$).

In conclusion, the results of dynamic measurement of serum TK1 activity showed a strong decrease of TK1 activity following surgical ablation of RCC. The half-life for serum TK1 activity was found to be 10.8 ± 1.2 hours. The measurements of TK1 activity following nephrectomy and RFA of metastases were associated with disease recurrence and disease progression, respectively and might be important for prognosis in terms of RFS and PFS, respectively.

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Conflicts of Interest

The Authors state that there are no conflicts of interest regarding the publication of this article.

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