Urol Res (2003) 31: 358–362
DOI 10.1007/s00240-003-0331-4

ORIGINAL PAPER

J. Roigas · S. Deger · J. Schroeder · A. Wille · I. Turk
B. Brux · K. Jung · D. Schnorr · S. A. Loening

Tumor type M2 pyruvate kinase expression in metastatic renal cell carcinoma

Received: 22 October 2002 / Accepted: 25 April 2003 / Published online: 26 September 2003

Abstract The M2 isoenzyme of pyruvate kinase (M2-PK) is specifically expressed in tumor cells (TuM2-PK) and has been detected in the peripheral blood of patients with renal cell carcinoma (RCC). TuM2-PK is not useful as a biological marker in localized RCC. We analysed TuM2-PK in 68 patients with metastatic RCC after initial surgery and prior to or during chemoimmunotherapy of metastases. In 50 patients, the levels of TuM2-PK were measured during chemoimmunotherapy with interleukin-2, interferon-α2a and 5-fluorouracil for up to 8 months and were correlated to response as assessed by radiological imaging techniques. TuM2-PK was quantified with a commercially available enzyme linked immunosorbent assay kit using a cut off of 15 kU/l. In 48 of 68 patients (71%), TuM2-PK was elevated above the cut-off. TuM2-PK was significantly higher in G3 tumors than in G2 tumors. In 34 of 50 patients (68%) undergoing chemoimmunotherapy, a positive correlation between TuM2-PK values and response to treatment was observed. Based on these data, we would not recommend the routine clinical use of TuM2-PK in metastatic RCC at this point.

Keywords Tumor type M2 pyruvate kinase · Biological marker · Metastatic renal cell carcinoma

J. Roigas (✉) · S. Deger · J. Schroeder · A. Wille · I. Turk
K. Jung · D. Schnorr · S. A. Loening
Department of Urology, University Hospital Charité, Humboldt University of Berlin, Schumannstrasse 20/21, 10117 Berlin Germany
Tel.: +49-30-450515052
Fax: +49-30-450515915

B. Brux
Institute of Pathobiochemistry and Laboratory Medicine, University Hospital Charité, Humboldt University of Berlin, 10117 Berlin, Germany

Introduction

The clinical management of metastatic renal cell carcinoma (RCC) often includes surgery of the primary tumor and subsequent immunotherapy to treat metastases [3]. Recent multicenter trials have supported this strategy by showing a prolonged survival in these aggressively treated patients [5, 9]. Thus, an extensive follow-up, based mainly on imaging techniques such as computer tomography (CT), bone scan or magnetic resonance imaging (MRI), is necessary and one of the consequences of this treatment strategy.

The presence of a reliable biological marker for RCC such as prostate specific antigen (PSA) for prostate cancer or alpha-fetoprotein (AFP) and ß-human chorionic gonadotropin (ß-HCG) for germ cell tumors would be very helpful for monitoring patients under therapy or during follow-up.

It has recently been shown that a specific carbohydrate metabolism enzyme, tumor type M2 pyruvate kinase (TuM2-PK), is significantly enhanced in tumor tissue and the peripheral blood of patients with malignant tumors such as colon carcinoma, pancreatic cancer, renal cell cancer and lung cancer [1, 2, 4, 10, 11, 14, 15].

The expression of TuM2-PK is related to the increased rate of aerobic glycolysis in tumor cells compared to normal cells. This tumor specific metabolic state requires changes in glycolytic enzyme capacities and isoenzyme pattern [8, 13]. Pyruvate kinase is a carbohydrate metabolism enzyme that varies with its isoform expression (L-PK, R-PK, M1-PK, M2-PK) tissue specifically. In normal cells, these isoforms can be found as enzymatically active tetramers. However, in tumor tissue the inactivated dimeric or monomeric M2 isoenzyme is overexpressed, contributing to aerobic glycolysis via reduced phosphoenolpyruvate affinity [8].

Preliminary studies of TuM2-PK in RCC suggest that this marker is not of clinical significance for the early diagnosis of small renal neoplasms but may function as a biological marker in advanced disease [12, 14, 16].

In this study, we investigated whether TuM2-PK can act as a useful biomarker in patients with metastatic RCC after tumor nephrectomy and prior to or during the treatment of established metastatic disease with interleukin-2 and interferon-α2a based chemoimmunotherapy.

Materials and methods

Patient characteristics

TuM2-PK was only analysed in patients with metastatic renal carcinomas. Data from a total of 68 patients were obtained (nine female patients, 59 male patients; median age 61 years, range 36–77 years). In all patients, tumor nephrectomy was performed and RCC was histologically proven. In 17 patients, the primary tumor was limited to the kidney (pT1/2), in 42 patients a locally advanced tumor (pT3/4) was present and in nine patients the T category could not be assessed. In 11 patients, metastatic lymph nodes (pN1/2) were primarily diagnosed. Pathology reports revealed two highly differentiated tumors (G1), 36 patients with G2 carcinomas and 18 patients with poorly differentiated G3 carcinomas. There was no grading possible in 12 cases.

One organ system in 17 patients, two organ systems in 26 other patients and three or more in 25 patients were involved in the metastatic process. Metastases occurred most often in the lungs (56 patients, 82%), followed by retroperitoneal or hiliac lymph nodes (30 patients, 44%) and the liver (22 patients, 32%). Bone metastases occurred in 19 patients (28%), and local recurrences were diagnosed in seven patients (10%). Other metastatic sites were the pancreas, contralateral kidney, brain or skin.

Of the 68 patients, 24 had metastatic disease at the time of diagnosis of the primary tumor (synchronous disease). In these patients, TuM2-PK was measured after a median time of 82 days (range 18–144 days) after initial surgery, usually directly prior to the start of immunotherapy. In the other 44 patients, metastases were diagnosed during follow-up after a median of 23 months (range 6–156 months, metachronous disease).

Treatment of patients

Out of 68 patients, 50 were treated with chemoimmunotherapy consisting of subsequent cycles of interleukin-2, interferon-α2a and 5-fluorouracil according to a protocol of a German multicenter trial given at an 8 weekly schedule per cycle. Of the 18 untreated patients, eight were not treated due to rapid disease progression, two discontinued therapy due to side effects and eight further patients were lost to follow-up. TuM2-PK values could not be assessed in these 18 patients during follow-up.

In all 50 patients, follow-up was at least 2.5–3 months which equals one cycle of chemoimmunotherapy and a recovery period. In 30 patients, the follow-up was 5 months for two therapy cycles. TuM2-PK data were analysed during follow-up and the response of the patients was estimated for the same period of time. Restaging was performed every 2.5 months by CT, bone scan, chest-X-ray or MRI. Response was estimated according to the objective response criteria of the UICC. Chemoimmunotherapy was discontinued when progressive disease was revealed by restaging techniques.

Measurement of TuM2-PK

TuM2-PK was determined in patients with synchronous metastases after surgery of the primary tumor and prior to chemoimmunotherapy. In patients with metachronous disease, TuM2-PK was measured when progress was revealed by radiological imaging or routinely during clinical follow-up. In patients treated with chemoimmunotherapy, TuM2-PK was determined during therapy on an outpatient basis.

For analysis, patients' samples were collected as EDTA plasma, centrifuged for 15 min at 1500 *g* and then stored until analysis at −80°C. TuM2-PK was determined using a commercially available sandwich enzyme linked immunosorbent assay (ELISA, Schebo Tech, Giessen, Germany) that does not cross react with the other pyruvate kinase isoenzymes (L-, R-, M1-, M2-PK). A cut-off of 15 kU/l was used according to the manufacturer's instructions. The intra-assay variance was 6.5%. The inter-assay variance, which was calculated on the basis of 20 control samples that were run over four periods of 4–5 months each, was 6.5%, 8.8%, 7.8% and 8.4% for the different periods. For the estimation of the correlation between TuM2-PK and response to therapy, the follow-up levels were related to initial TuM2-PK values.

Statistical analysis

The different groups of nonparametric data were statistically analysed using the Mann-Whitney U-test and the nonparametric Kruskal-Wallis test. GraphPad 3.0L (PRISM, San Diego, USA) was used for statistical calculations.

Results

The TuM2-PK levels from a total of 68 patients with metastatic RCC were obtained after tumor nephrectomy and prior to the treatment of metastases. In this typical oncological situation, TuM2-PK data were analysed according to the number of positive values, the differences between TuM2-PK in patients with metastases present at the time of diagnosis of the primary tumor and after a disease free interval as well as the tumor burden, the number of organ systems involved, the histological subtype, the grading of the tumor, the relationship of TuM2-PK levels with the radiologically assessed tumor burden and the gender of the patients.

The median value of TuM2-PK was 22.9 kU/l (mean 32.4 ± 35.5 kU/l). The enzyme levels were positive (higher than the recommended cut-off of 15 kU/l) in 48 patients (sensitivity 71%), thus biochemically detecting metastatic disease (Fig. 1). A significant difference was

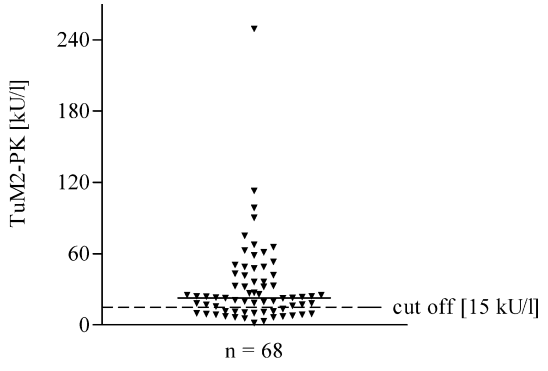

Fig. 1 Demonstration of all 68 TuM2-PK values in patients with metastatic RCC after surgery of the primary tumor and prior to immunotherapy. Median (22.9 kU/l) and individual values are given. Using the cut off of 15 kU/l, the sensitivity of the test was 71% (48 patients with positive values)

observed in relation to the cellular differentiation of the primary tumor when G2 and G3 carcinomas were analysed (Fig. 2). G2 tumors showed lower TuM2-PK values compared to G3 renal cancers (median 21.7 kU/l vs 37.0 kU/l, mean $28.2 \pm 24.1.8$ kU/l vs 52.8 ± 55.2 kU/l, $P = 0.0198$, Mann-Whitney U-test).

There was no significant difference between the 24 patients with synchronous metastatic disease and the 44 patients with metachronously occurring metastatic lesions (median 23.7 kU/l vs 22.7 kU/l, mean 45.1 ± 51.8 kU/l vs 25.5 ± 19.7 kU/l, $P = 0.2404$, Mann-Whitney U-test). We found elevated TuM2-PK levels at the time of diagnosis of metastases in 30 out of the 44 patients (68%) with metachronous disease. The difference between small ($n = 22$) and large ($n = 46$) metastatic tumor burdens was only apparent as a trend (median 21.4 kU/l vs 23.7 kU/l, mean 21.0 ± 12.8 kU/l vs 37.9 ± 41.3 kU/l, $P = 0.1550$, Mann-Whitney U-test). The number of organ systems involved, the histological subtype of the primary tumor and the gender of the patients had no significant influence on TuM2-PK levels (data not shown).

In Table 1, the sensitivities of the different subgroups based on the recommended cut off of 15 kU/l are summarized. Overall sensitivity of the test was 71%. The sensitivity was highest in untreated patients (94%) or in patients relapsing following chemoimmunotherapy (100%) (Table 1).

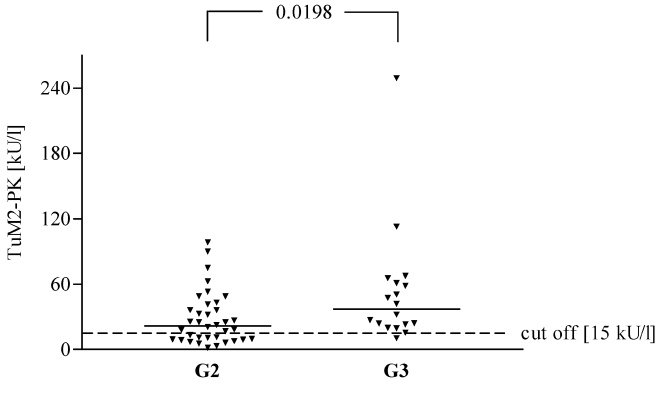

Fig. 2 Renal carcinoma patients with a moderate differentiation (G2, $n = 36$) had significantly lower TuM2-PK levels compared to undifferentiated carcinomas (G3, $n = 18$), median 21.7 kU/l vs 37.0 kU/l ($P = 0.0198$, Mann Whitney U-test)

Table 1 Sensitivity of TuM2-PK in patients with metastatic renal cell carcinoma after surgery of the primary tumor and prior to the treatment of metastases with chemoimmunotherapy. Overall sensitivity was 71%. Sensitivity was highest in patients with progression following therapy or in patients who could not be treated. In both groups, a high tumor load and a reduced performance status were characteristic

	n	Sensitivity
All patients	68	71%
Metachronous patients	44	68%
Synchronous patients	24	75%
Untreated patients	18	94%
Treated patients	50	62%
Progression post therapy	9	100%

The analysis of TuM2-PK in relation to the response to chemoimmunotherapy was also performed. Routine clinical and radiological restaging during follow-up revealed that of the 50 patients, four responded with complete remission (CR) and eight with partial remission (PR). We observed 26 patients who remained stable (stable disease, SD) and 12 patients who did not respond to chemoimmunotherapy (progressive disease, PD).

Of the four patients with CR, three were negative for TuM2-PK prior to therapy and remained negative during treatment (data not shown). The TuM2-PK levels of the eight patients with PR are shown in Fig. 3a. Although there was a tendency towards decreasing TuM2-PK levels, the changes in the biomarker were not significant over the entire observation period of 8 months. In SD patients, with unchanged radiological results of metastatic sites, no significant differences occurred between TuM2-PK values over the 8 months of therapy (Fig. 3b). TuM2-PK values of progressive patients are shown in Fig. 3c. No significant changes occurred over a period of 5 months.

In nine patients with tumor progression after chemoimmunotherapy, TuM2-PK was measured when progression was documented by radiological imaging. In all of these patients the TuM2-PK levels were higher than the cut-off of 15 kU/l (median 31.0 kU/l, mean 53.2 ± 36.3 kU/l).

The positive predictive value of TuM2-PK was expressed as the relation between the number of correct positive values during follow-up and the number of all values during follow-up including false positive values. False positive values were defined as values of TuM2-PK that did not correspond to the response evaluated by restaging techniques. For all patients, the positive predictive value of TuM2-PK for follow-up was 0.68.

Discussion

In this study, we examined the role of TuM2-PK as a biological serum marker in patients with metastatic RCC after tumor nephrectomy and prior to the chemoimmunotherapeutic treatment of metastases.

From the clinical point of view, a reliable biological tumor marker reflecting both the actual presence of metastatic disease and the response to treatment is of the greatest interest in this situation. To our knowledge, there is no information in the literature on the importance of TuM2-PK during the follow-up of patients with metastatic RCC who are undergoing therapy. Recent studies have suggested that TuM2-PK may not be reliable for localized, non-metastatic RCC [12, 14, 16]. However, our own results on metastatic RCC revealed that TuM2-PK had a sensitivity of 66% and was

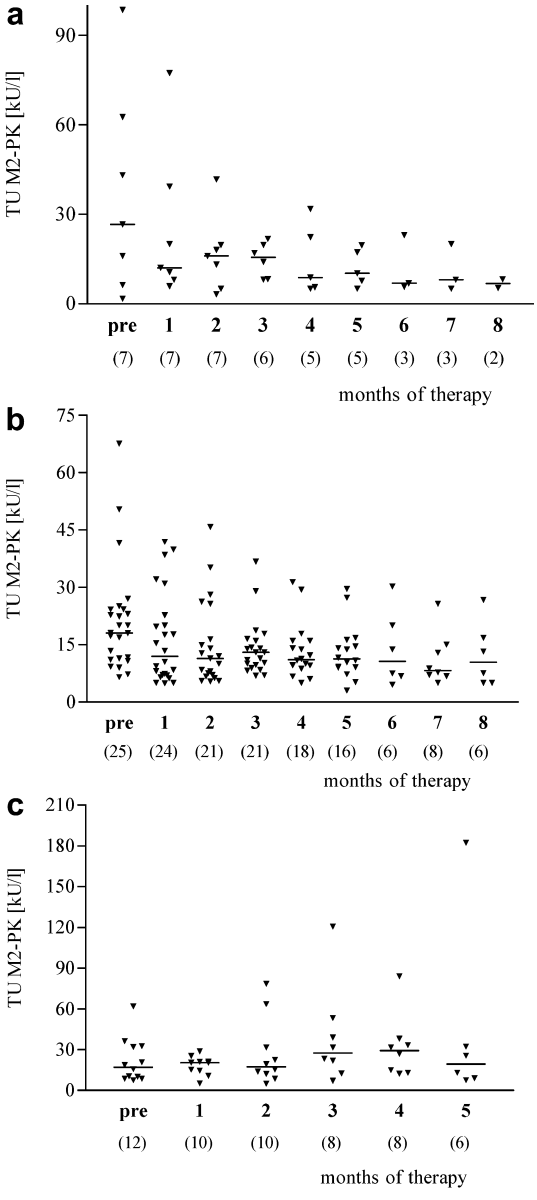

Fig. 3 Analysis of TuM2-PK in patients responding with **a** partial remission or **b** stable disease to chemoimmunotherapy. Data in **c** present patients who remained in progressive disease. No statistical differences in levels of TuM2-PK during therapy of the patients could be assessed in any group using the Kruskal-Wallis test. Median and individual values are given. *Numbers in brackets* represent the numbers of patients at each time point

significantly higher than in a normal control group and when compared to patients with localized RCC [12].

In our current group of patients with metastatic RCC, TuM2-PK was increased above the cut off of 15 kU/l in 48 out of 68 patients (Fig. 1). Poorly differentiated G3 neoplasms had significantly higher TUM2-PK levels than G2 cancers (Fig. 2). There was no significant difference between histological subtype, tumor mass, organ systems, gender and the time of occurrence of metastases. Wechsel et al. also found higher TuM2-PK levels in renal cancers with a higher grading in 40 RCC patients, but no correlation between the histological subtype of the primary tumor and TuM2-PK levels [14]. In contrast, Oremek et al. reported no relationship between TuM2-PK and the grading of the primary carcinoma in 64 RCC patients [11]. In our study, the relationship between tumor size and TuM2-PK elevation was not significant. However, Varga et al., as well as both of the studies mentioned above, reported a positive correlation between Robson stage, which also indirectly represents tumor size, and levels of the biomarker [11, 14, 16].

Varga et al. also observed an elevation of TuM2-PK levels after tumor nephrectomy with a maximum at postoperative day 5 and a decrease starting at postoperative day 10 [16]. In our study, the time of TuM2-PK measurements in patients with synchronous metastatic disease occurred after a median of 82 days (range 18–144 days), usually directly prior to the start of chemoimmunotherapy. Therefore, an influence of postoperative TuM2-PK elevation on the levels obtained at the later time is unlikely and TuM2-PK elevation should only represent metastases.

Only preliminary information is available on the course of TuM2-PK during therapy of patients with metastatic disease. A positive correlation between TuM2-PK and clinical follow-up of the disease has been shown casuistically for lung cancer and in five untreated patients with metastatic RCC [8, 14]. Luftner et al. analysed 38 patients for TuM2-PK suffering from advanced breast cancer during 45 courses of chemotherapy [7]. In 13 out of 19 courses (68.4%) of progressing patients, increasing values of TuM2-PK were found. In 17 out of 20 courses with stable disease, the biomarker was initially negative or dropped below normal within 4 weeks of treatment. In another study, TuM2-PK was determined in 22 patients with pancreatic cancer and four further patients with colorectal or gastric cancer. These data were compared to CA 19–9 levels [6]. Only seven out of 22 patients (32%) had elevated levels of the marker (>15 kU/l). In 14 patients, TuM2-PK was normal, including three patients with CR and six other patients with disease progression. Similar to these observations, we found negative TuM2-PK levels in three out of four patients (75%) responding with CR to therapy, whereas only 25, 35 and 42% of the patients with PR, SD and PD, respectively, had primarily negative levels of the biomarker.

There was a positive correlation between clinical and marker follow-up in 34 out of 50 patients (68%), but when the values were analysed for all groups, no significant differences were found (Fig. 3). We suggest that this observation is related to the high individual variability of TuM2-PK, which might be at least partially influenced by tumor mass. The sensitivity of TuM2-PK was highest in patients with a relapse following immunotherapy (100%) or in untreated patients (94%, Table 1). A high tumor load associated with a reduced general performance status and a poor prognosis was characteristic for these patients with advanced disease. TuM2-PK is informative for subgroups of patients

representing disease burden, tumor activity and a poor prognosis. At this point, TuM2-PK has only a limited impact on treatment decisions or follow-up. Thus, we would currently not recommend the routine clinical use of TuM2-PK in patients with metastatic RCC.

References

1. Brink U, Eigenbrodt E, Oehmke M, Mazurek S, Fischer G (1994) L- and M2- pyruvate kinase expression in renal cell carcinomas and their metastases. Virchows Arch 424: 177
2. Eigenbrodt E, Basenau D, Holthusen S, Mazurek S, Fischer G (1997) Quantification of tumor type M2 pyruvate kinase (TUM2-PK) in human carcinomas. Anticancer Res 17: 3153
3. Figlin RA (1999) Renal cell carcinoma: management of advanced disease. J Urol 161: 381
4. Fischer G, Holzrichter S, Reinacher M, Heinrichs M, Dembowski J, Eigenbrodt E (1989) Immunohistochemical demonstration of L- and M2-pyruvate kinase in primary renal cell carcinomas and their metastases. Verh Dtsch Ges Pathol 73: 422
5. Flanigan RC, Salmon SE, Blumenstein BA, Bearman SI, Roy V, McGrath PC, Caton JRJr, Munshi N, Crawford ED (2001) Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. N Engl J Med 345: 1655
6. Klapdor R, Bahlo M, Fenner C (1999) Tumormarker M2-PK in the follow up of pancreatic and colorectal cancer disease. Abstract, 10th International Hamburg Symposium on Tumor Markers, Hamburg, Germany, 05—07.12.1999
7. Luftner D, Mesterharm J, Akrivakis C, Geppert R, Petrides PE, Wernecke KD, Possinger K (2000) Tumor type M2 pyruvate kinase expression in advanced breast cancer. Anticancer Res 20: 5077
8. Mazurek S, Scheefers-Borchel U, Scheefers H, Michel A, Basenau D, Fischer G, Dahlmann N, Laumen R, Eigenbrodt E (1993) Die Bedeutung der Pyruvatkinase in der Onkologie. notabene medici 3: 97
9. Mickisch GH, Garin A, Van Poppel H, De Prijck L, Sylvester R (2001) Radical nephrectomy plus interferon-alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. Lancet 358: 966
10. Oremek GM, Eigenbrodt E, Rädle J, Zeuzem ST, Seiffert UB (1997) Value of the serum levels of the tumor marker TUM2-PK in pancreatic cancer. Anticancer Res 17: 3031
11. Oremek GM, Teigelkamp S, Kramer W, Eigenbrodt E, Usadel K-H (1999) The pyruvate kinase isoenzyme tumor M2 (TUM2-PK) as a tumor marker for renal cell carcinoma. Anticancer Res 19: 2599
12. Roigas J, Schulze G, Raytarowski S, Jung K, Schnorr D, Loening SA (2001) Tumor M2-pyruvate kinase (TUM2-PK) in plasma of patients with urological tumors. Tum Biol 22: 282
13. Steinberg P, Storkel S, Oesch F, Thoenes W (1992) Carbohydrate metabolism in human renal clear cell carcinomas. Lab Invest 67: 506
14. Wechsel HW, Petri E, Bichler KH, Feil G (1999) Marker for renal cell carcinoma (RCC): the dimeric form of pyruvate kinase type M2 (TUM2-PK). Anticancer Res 19: 2583
15. Wechsel HW, Petri E, Feil G, Nelde H, Bichler K (1999) Renal cell carcinoma. Immunohistological study to the expression of the inactive form of the pyruvate kinase. Urologe A 38: 583
16. Varga Z, Hegele A, Stief T, Heidenreich, Hofmann R (2002) Determination of pyruvate kinase type tumor M2 in human renal cell carcinoma: a suitable tumor marker. Urol Res 30: 122