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Determination of pyruvate kinase type tumor M2 in human renal cell carcinoma: a suitable tumor marker?

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Abstract Renal cell carcinoma (RCC) expresses an isoform of the glycolytic enzyme pyruvate kinase (type M2). The dimeric form (TuM2-PK) is over expressed in tumor cells and is detectable in blood with a sensitive enzyme-linked immunosorbent assay (ELISA). The aim of the present study was to evaluate the clinical value of TuM2-PK as a tumor marker for RCC. The TuM2-PK concentration in EDTA-plasma was determined quantitatively and immunologically using an ELISA. We measured the TuM2-PK plasma levels of 83 patients before and after surgery. Ninety-seven patients with various non-malignant diseases were also recruited as a control group. The control group displayed mean levels of 11.37 U/ml of TuM2-PK. Values were elevated in patients with RCC prior to surgery (mean 21.88 U/ml). The plasma levels increased after surgery until day 5 (mean 53.97 U/ml). At day 10, marker levels started to decrease without reaching preoperative values (mean 43.5 U/ml). Plasma levels in the renal vein (obtained during surgery) were not different from those in the peripheral blood. Follow-ups after 2–6 months showed a decrease to below preoperative levels (mean 16.3 U/ml). A significant difference was obtained by comparing the patients according to their Robson score. We found a significant difference ($P < 0.01$, Wilcoxon's two-sample test) in TuM2-PK levels between patients with RCC and the control group. Nevertheless, using the manufacturer's recommended cut-off value (15 U/ml), sensitivity was only 50.6% and specificity was 80.4%. Our results suggest that TuM2-PK is not a suitable tumor marker for RCC.

Keywords Renal cell carcinoma · Tumor marker · Pyruvate kinase · TuM2-PK

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Introduction

Renal cell cancer (RCC) is the third most common urological tumor after prostate and bladder cancer and accounts for 3% of all solid tumors. It is diagnosed in approximately 29,900 Americans per year causing over 11,000 deaths [1]. The aetiology of RCC is still unknown andthere is no reliable method for early detection. Therefore, at the time of diagnosis about 30% of patients present with metastasis, and 5-year over-all survival at this stage decreases to between 0 and 15% [2, 9, 10, 14]. So far potential tumor markers for this disease are still lacking. Although a variety of different tumor markers have been described, none are used in clinical routine due to their low sensitivity [3, 11, 15, 21].

In comparison to physiological proliferation, tumor cells exhibit higher levels of glycolytic metabolites. One of these metabolic enzymes is pyruvate kinase (PK), which exists in tissue-specific isoforms (type L-PK, R-PK, M1-PK, M2-PK) [8]. In anaerobic glycolysis, PK catalyses the transformation of phosphoenolpyruvate to pyruvate. Only the tetrameric form is enzymatically active. In tumor cells the isoenzyme M2-PK exists predominantly in its dimeric form, which has a lower affinity to phosphoenolpyruvate [4] and leads to high intracellular levels of these phosphometabolites. Therefore, the tumor-isoform of M2-PK is referred to as TuM2-PK. Due to high cell turnover and necrosis, TuM2-PK can readily be detected in body fluids using a sandwich ELISA with monoclonal antibodies which are specific for the tumor isoforms. As previously shown, high levels of

Tu-M2 PK exist in the majority of human tumors [5, 13, 17]. The immunohistochemistry of RCC tissue showed positive staining for TuM2-PK [6]. Detection in serum was unsuccessful due to the instability of the marker [7].

The aim of this study was to evaluate the clinical value of TuM2-PK as a tumor marker for the diagnosis and postoperative follow-up of RCC.

Materials and methods

Peripheral blood samples were collected in EDTA-supplemented tubes. After centrifugation (2,000 *g*, 10 min) and removal of the supernatant plasma, the samples were kept at –20°C.

Quantitative determination of TuM2-PK in EDTA-plasma was performed by using a commercially available sandwich enzyme-linked immunosorbant assay (ScheBo Tech, Germany) based on two monoclonal antibodies specific for TuM2-PK. The kit requires 10 µl EDTA-plasma per sample and was performed according to the manufacturer's instructions.

To determine the value of TuM2-PK in the diagnosis of RCC, we measured the plasma levels of 83 patients (36 females, 47 males, mean age 60.2 years) with RCC before therapy (radical nephrectomy, tumor enucleation). TuM2-PK was also measured on days 1 ($n=69$), 5 ($n=66$), 10 ($n=58$) and 2–6 months ($n=44$) after surgery. During surgery, blood was obtained both from the renal and a peripheral vein of ten patients. Ninety-seven patients (20 female, 77 male, mean age 54 years) with various non-malignant diseases were recruited as a control group. Out of this group we also determined TuM2-PK plasma levels on days 1, 5 and 10 after intervention in ten patients who underwent renal surgery for oncocytoma or angiomyolipoma. SPSS for personal computers was used to perform the statistical analyses.

Results

The intra-assay variance was evaluated by 20-fold determination of one sample (16.4–23.4 U/ml). The average coefficient of variance was 6.5%. The inter-assay variance was calculated from eight samples which were tested on ten different days. The mean coefficient of variance was 17.5%.

In patients suffering from non-malignant urological diseases, the TuM2-PK concentration was in the range of < 5–37.8 U/ml with a mean of 11.37 U/ml (SEM 6.85). In contrast, the range of TuM2-PK concentrations in patients with RCC was < 5–100 U/ml with a mean of 21.88 U/ml (SEM 25.44 U/ml) (Fig. 1). Wilcoxon's two-sample test showed a significant difference with $P=0.009$. We found no significant sex-specific differences in either group. The TuM2-PK plasma levels obtained from all 83 patients with RCC were separated into four groups according to the patients' Robson stages [16]. Fifty-six patients were diagnosed as Robson stage I, 3 as stage II, 15 stage III and 9 as stage IV. Because of the small number of patients in Robson stages II and IV we pooled stages I and II as well as stages III and IV. The mean TuM2-PK plasma levels in Robson stage I/II was 16.55 U/ml (SEM 0.27 U/ml) and in stage III/IV was 35.05 U/ml (SEM 1.58 U/ml) (Fig. 2). Using the Mann-Whitney U-test we found a significant ($P=0.003$) difference between these groups.

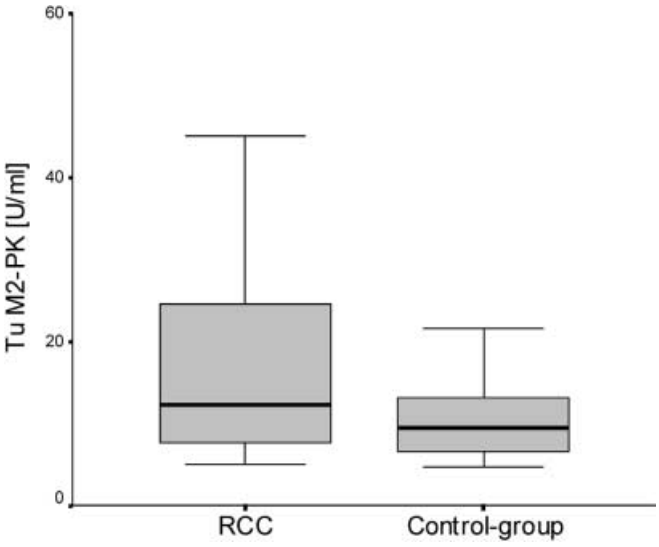

Fig. 1 Concentration of TuM2-PK in patients with renal cell carcinoma ($n=83$) and in patients with various non-malignant urological diseases ($n=97$). TuM2-PK levels between patients with RCC (mean 21.88 U/ml) and the control group (mean 11.37 U/ml) were significantly different ($P<0.01$)

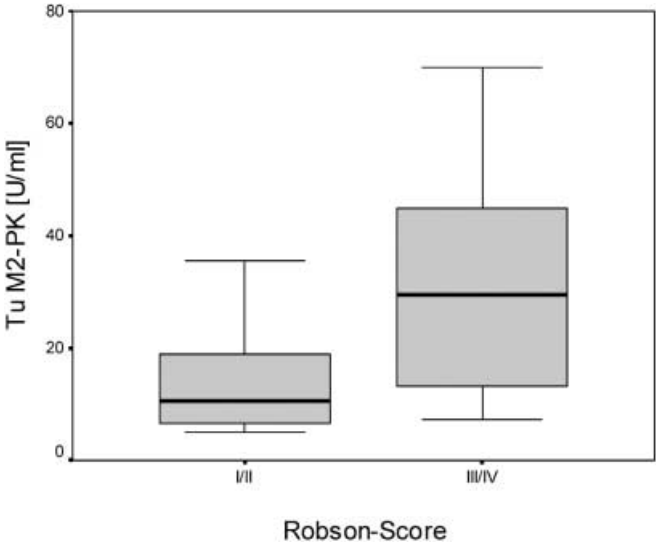

Fig. 2 Mean TuM2-PK plasma levels in Robson stage I/II was 16.55 U/ml and in stage III/IV was 35.05 U/ml ($P=0.003$)

The plasma levels increased after surgery until day 5 (mean 53.97 U/ml). At day 10, marker levels started to decrease without reaching preoperative values (mean 43.5 U/ml). In controls between 2 and 6 months after treatment we found a decrease in the mean TuM2-PK plasma levels (mean 16.3 U/ml) below the starting value (Fig. 3). The postoperative increase of TuM2-PK was also compared with blood taken during surgery from the renal and a peripheral vein on the side of the tumor. No significant differences of TuM2-PK levels were noted, when comparing peripheral to renal blood. There was also no difference between pre- and perioperative levels.

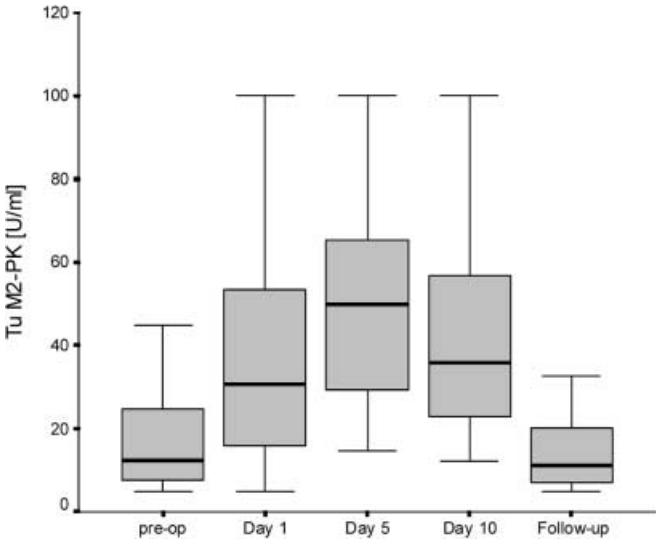

Fig. 3 TuM2-PK levels preoperatively and in the follow-up after surgery

Ten patients who underwent surgery because of non-malignant disease showed preoperative TuM2-PK plasma levels with a mean of 8.06 U/ml. We also noticed a postoperative increase of TuM2-PK on days 1 (mean 16.98 U/ml), 5 (mean 51.42 U/ml) and 10 (mean 44.76 U/ml) as was the case in patients from the RCC group.

Discussion

The present study was initiated to evaluate TuM2-PK as a tumor marker for RCC in plasma.

We found a significant difference in TuM2-PK levels between patients with RCC and the control group, as well as between Robson stages I/II and III/IV. Nevertheless, discrimination between localized and metastasised RCC using TuM2-PK is not possible. Postoperatively, TuM2-PK levels increased reaching a maximum on day 5. From day 10, the levels started to decrease and returned to normal within 2 months after successful therapy. Considering the plasma levels from the tumor sided renal vein and the postoperative TuM2-PK course in patients with benign kidney tumors, it seems highly unlikely that the immediate postoperative increase in the TuM2-PK level is due to RCC. The data point rather towards a cross reaction between isoenzymes (e.g. muscle-PK) of pyruvate kinase and the monoclonal antibodies. The postoperative increase, as well as the decrease of TuM2-PK levels that we observed in all patients, has not been described previously. Oremek et al. [12] examined 116 patients with RCC and also found a significant difference in the TuM2-PK level between tumor patients and patients suffering from nephritis. In a previous publication [18], specificity was shown to be dependent on the histopathological grading, and ranged from 46.5% (G1) to 79.8% (G3). However,

Table 1 Using a cut off value of 15 U/ml (manufacturer's recommendation) a sensitivity of 50.6%, a specificity of 80.41% and a positive predictive value of 68.9% was found in patients with renal cell carcinoma

TuM2PK ≥15 U/ml	RCC	Control	Total
Positive	42	19	61
Negative	41	78	119
Total	83	97	180

we concluded that the determination of TuM2-PK concentration in EDTA-plasma might close a long standing gap in the diagnosis of renal tumors. Wechsel et al. [18, 19] analysed the TuM2-PK in 48 patients with RCC and found a sensitivity of 50% and a specificity of 74% using a cut off value of 15 U/ml (manufacturer's recommendation). In their opinion TuM2-PK also appears to be a useful marker for RCC detection and follow-up. However, they also found a high variability in the intra-assay and inter-assay coefficients of variance, similar to our data [20]. We found a specificity of 80.4% and a sensitivity of 50.6% (15 U/ml cut off value) (Table 1). Only about 5% of renal tumors cannot be classified by radiological and ultrasonographic examinations. A tumor marker with a sensitivity of 50% however, cannot give additional information. In the face of the high variability of the intra-assay and inter-assay coefficients of variance, the supposed cross-reaction of the antibodies and the low sensitivity, we conclude that the TuM2-PK-test is unsuitable for routine diagnostics and that TuM2-PK does not seem to be a new tumor marker for RCC.

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