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# TGF-$\beta$1 in patients with renal cell carcinoma

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**Abstract** Up to now, clinical tumor-markers for renal cell carcinoma (RCC) have been lacking. Increased plasma levels of transforming growth factor-$\beta$1 (TGF-$\beta$1) were described as a tumor-marker and prognostic factor in RCC. The aim of this study was to test the clinical suitability of plasma TGF-$\beta$1 as a tumor-marker for RCC. The concentrations of active and latent TGF-$\beta$1 were determined in plasma of patients with localized ($n = 39$) and metastasised ($n = 17$) RCC. A newly developed, highly sensitive ELISA, which is specific for the isoform $\beta$1, was used. Active TGF was directly measured in the EDTA plasma. To determine the amount of latent TGF-$\beta$1, which is bound predominantly at $\alpha$2-macroglobulin, an optimized activation procedure was applied. Patients with localized RCC showed median concentrations of 16,700 ng/l (6,200–54,800 ng/l) for latent TGF-$\beta$1. A total of 94 patients with various non-malignant urological diseases were recruited as a control group. In comparison, this group had median concentrations of 19,900 ng/l (2,640–52,300 ng/l) for latent TGF-$\beta$1. There was no significant difference (nonparametric Kruskal-Wallis ANOVA) between these groups. Patients with metastatic RCC showed median concentrations of 34,500 ng/l (6,800–48,960 ng/l) for latent TGF-$\beta$1. In comparison to the localized RCC group, a statistically significant difference was found. Plasma levels after operative therapy (days 1, 5 and 10) and during follow-up without evidence of disease (2–6 months) showed no significant differences. Contrary to other study groups, our results suggest that TGF-$\beta$1 is not a suitable tumor-marker for the diagnosis of localized RCC. In the face of higher TGF-$\beta$1 plasma levels in metastatic disease, TGF-$\beta$1 may be useful in the early detection of RCC recurrence or to control the success of immunochemotherapy.

**Keywords** Renal cell carcinoma · TGF-$\beta$1 · Plasma level

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## Introduction

Renal cell carcinoma (RCC) is a common urological tumor with an incidence of nine newly diagnosed cases per 100,000 inhabitants per year in Germany. About 30% of patients initially present with metastatic disease and another 20% will develop systemic disease during the future clinical course. To date only a few tumor markers for RCC have been evaluated [4, 5, 10, 13, 19]. However, due to comparatively low sensitivity levels, these markers are not applicable for routine diagnosis. Usually only detailed histopathological examination and the analysis of the primary tumor is available for limited prognostic information, with the pT-stage being the most useful parameter. However, plasma/serum tumor markers with high sensitivity and specificity would be useful in order to provide adequate monitoring of the patients following radical nephrectomy or immunochemotherapy and for the early detection of metastatic disease.

Recently, Philipps et al. reported various different immunomodulators to be produced by tubular and renal cancer cells [15]. One of these immunomodulators, transforming growth factor-$\beta$1 (TGF-$\beta$1), an isoform of the dimeric 25-kDa polypeptide TGF-$\beta$, has been suggested to be a tumor-specific marker for RCC by Wunderlich et al. [20]. TGF-$\beta$1 is mainly synthesized in bone matrix and the $\alpha$-granules of platelets [17]. The immunomodulator represents a cytokine with multiple functions such as the regulation of cell proliferation and differentiation, promotion of wound healing and suppression of the immune system by interacting with a

number of different binding proteins and receptors [11]. After secretion, latent TGF-$\beta$1 is bound to a propeptide called latency-associated peptide (LAP). Acidification is necessary to activate this latent form for detection.

In addition, TGF-$\beta$1 as a cytokine can modulate lymphocyte activation and may therefore locally influence an immune response in RCC by suppressing immunological effects against the tumor and by the promotion of metastases [11, 16].

The determination of TGF-$\beta$1 in patients with RCC may prove to be of clinical value. Various different immunoassays for its detection have been described, but reference values, as well as sample handling procedures, differ greatly [12, 14]. For measuring TGF-$\beta$1 levels, we have developed a new, sensitive enzyme linked-immunosorbent assay (ELISA), which is specific for TGF-$\beta$1. This assay, which applies an optimized activation procedure for TGF-$\beta$1, has been described in connection with other commercially available assays [9]. The aim of the present study was to introduce the new detection assay and to evaluate the clinical value of TGF-$\beta$1 as a tumor marker for the diagnosis and postoperative follow-up of patients suffering from RCC.

## Materials and methods

Peripheral blood samples were collected in EDTA-supplemented tubes and processed for plasma within 3 h. After centrifugation (2,000 rpm, 10 min) and removal of supernatant plasma the samples were stored at –70°C until measurement for a maximum of 3 months.

To evaluate the usefulness of TGF-$\beta$1 in the diagnosis of RCC, we measured the plasma levels of 39 patients (19 females, 20 males, mean age 61.5 years) with localized RCC prior to surgical therapy (radical nephrectomy, tumor enucleation). Physical examination, chest X-ray, intravenous urography, abdominal sonography, CT/MRI were used for clinical staging. TGF-$\beta$1 was also measured on days 1 ($n=31$), 5 ($n=31$), 10 ($n=21$) and 3–6 months (n = *18*) after surgical treatment. During surgery ($n=10$) blood was obtained from the renal vein and a distant peripheral vein in order to test for any tumor specific secretion of TGF-$\beta$1. We also measured the TGF-$\beta$1 plasma levels of 17 patients with metastatic disease (2 females, 15 males, mean age 63 years). Of this group, 11 patients presented with multiple pulmonary metastases, four with pulmonary and bone metastases, one with bone and cerebral metastases and one with pulmonary and thyroid gland metastases. A total of 93 patients (20 females, 73 males, mean age 57.2 years) with various benign diseases were recruited as a control group.

Measurement of the plasma samples was always performed by the same person.

Statistical analysis was performed using the nonparametric Kruskal-Wallis ANOVA test (SPSS software). A $P$-value $<0.05$ was accepted as statistically significant.

## Results

Patients with localized RCC showed median preoperative plasma concentrations of 16,700 ng/l (6,200–54,800 ng/l) for latent TGF-$\beta$1 and 455 ng/l (66–1,610 ng/l) for active TGF-$\beta$1. In comparison, the control group had median concentrations of 19,900 ng/l (2,640–52,300 ng/l) for latent TGF-$\beta$1 and 455 ng/l (56–1,650 ng/l) for active TGF-$\beta$1. The differences between these two groups were not statistically significant (Fig. 1). No significant differences between the pathological subgroups was found after stratifying the patients using the UICC TNM-system (1998) and nuclear grading (Table 1).

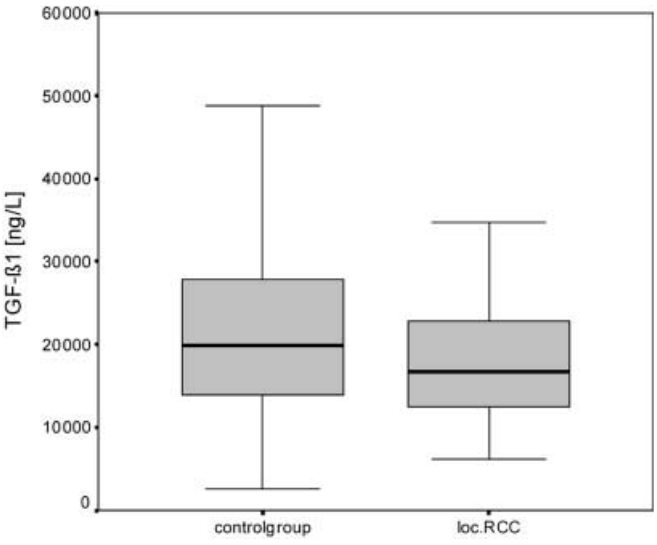


**Fig. 1.** Concentration of TGF-$\beta$1 in patients with localized RCC ($n=39$) and in patients with various benign urological disorders ($n=93$). No significant difference (nonparametric Kruskal-Wallis ANOVA) between the groups was found

**Table 1.** Stratifying the TGF-$\beta$1 levels to the T-system and nuclear grading we identified no significant differences in these subgroups

| T system | *n* | TGF-$\beta$1 ± SEM (ng/l) |
|---|---|---|
| *pT1* ($n=21$) | 21 | 19,316 ± 594 |
| *pT2* ($n=7$) | 7 | 18,281 ± 721 |
| *pT3* ($n=9$) | 9 | 22,994 ± 1,002 |
| *pT4* ($n=2$) | 2 | 19,421 ± 1,701 |
| Nuclear grading | | |
| *G1* (n = 13) | 13 | 18,861 ± 847 |
| *G2* ($n=23$) | 23 | 19,809 ± 489 |
| *G3* ($n=3$) | 3 | 20,665 ± 1,197 |

Patients with metastatic disease showed median plasma concentrations of 34,500 ng/l (6,800–48,960 ng/l) for latent TGF-$\beta$1 and 720 ng/l (320–1,120 ng/l) for active TGF-$\beta$1. Comparing these TGF-$\beta$1 plasma levels with the localized RCC group, a statistically significant difference ($P<0$, 01) was found (Fig. 2). No difference was found between the different locations of the metastases. Comparing TGF-$\beta$1 and latent TGF-$\beta$1 plasma concentrations of the peripheral blood versus the renal vein blood, no significant differences in TGF-$\beta$1 levels were observed. Plasma levels after surgical therapy (days 1, 5 and 10) and during follow-up without evidence of disease (2–6 months) also showed no distinct differences.

## Discussion

The aim of the present study was to evaluate the clinical utility of TGF-$\beta$1 plasma-levels in patients suffering

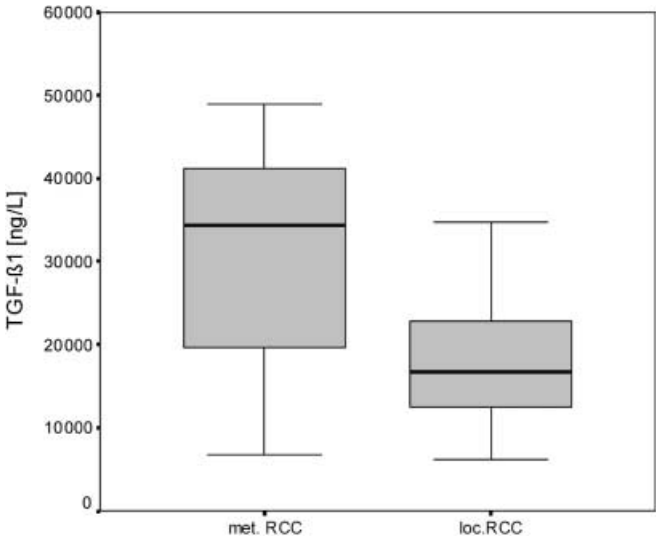


**Fig. 2.** Concentration of TGF-$\beta$1 in patients with localized RCC ($n = 39$) and in patients with metastatic RCC ($n = 17$). A statistically significant difference between the groups was found ($P < 0.01$)

from RCC in order to obtain a prognostic reference and to facilitate postoperative monitoring. To determine the amount of TGF-$\beta$1 an optimized activation procedure was applied. The applied assay has been thoroughly tested and the advantages of the new test in comparison with other assays has recently been published [9]. In our institution, this new assay is used in clinical routine for the detection of TGF-$\beta$1 in various other diseases. We are aware that our results are in contrast to those reported by others [7, 8, 20, 21]. Wunderlich et al. described increased TGF-$\beta$1 levels in RCC [20]. In contrast to our investigation in which TGF-$\beta$1 was detected in plasma, Wunderlich and co-workers first measured TGF-$\beta$1 levels in serum. Measuring serum-levels may produce false positive values as TGF-$\beta$1 is released by platelets [1] during the clotting process. This topic has been addressed extensively by Wakefield et al. [18]. Additionally, paraneoplastic, IL-6 induced thrombocytosis occurs in some cases of RCC [2] and may be responsible for higher serum-levels of TGF-$\beta$1, which are not tumor specific. Wakefield strongly recommended the measurement of TGF-$\beta$1 in plasma. In 1998, Wunderlich et al. determined TGF-$\beta$1 plasma concentrations and described elevated levels in 20 patients suffering from RCC by using an ELISA developed and published by Danielpour [3, 21]. In our opinion the different results could be caused by the assay used. Especially with TGF-$\beta$1, the problems of comparability between the different analytical methods should be stressed [9]. Junker et al. showed TGF-$\beta$1 production in culture supernatants as well as TGF-$\beta$1 mRNA expression in tumor samples [8]. These results underline the important but still unclear role of TGF-$\beta$1 in RCC. We first describe significantly higher plasma levels of TGF-$\beta$1 in metastasised RCC in comparison to localized disease. This aspect is of great interest for the follow-up after surgical therapy and the response to subsequent immunochemotherapies in metastatic disease. Although, there is no doubt that TGF-$\beta$1 plays an important role in the neovascularization of RCC [6] our results demonstrate that the evaluation of TGF-$\beta$1 plasma levels in patients with localized RCC has no diagnostic value. We therefore conclude that the tumor cells are not the predominant source of TGF-$\beta$1 within the organism and that tumor cells also do not stimulate TGF-$\beta$1 output by other tissues. We propose that TGF-$\beta$1 in plasma is not a suitable tumor marker for the diagnosis of localized RCC. Whether TGF-$\beta$1 might be useful for the early detection of RCC recurrence, to control the success of immunochemotherapy in metastatic disease or as a prognostic parameter in the face of higher plasma levels in metastatic disease has to be proved in further investigations.

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