Diagnostic and prognostic value of placental growth factor serum concentration in clear cell renal cell carcinoma

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Background and Aim: Placental Growth Factor (PIGF) plays a crucial role in angiogenesis and was identified as a potential prognostic biomarker in various types of cancer. Therefore, we evaluated the diagnostic accuracy and prognostic value of PIGF serum concentration in patients with clear cell renal cell carcinoma (ccRCC).

Patients and Methods: A total of 49 patients subjected to partial or radical nephrectomy for ccRCC [localized without relapse (lccRCC; n=31), localized with later relapse (rccRCC; n=8), primary metastatic cancer (mccRCC; n=10); median of follow-up 4.4 years] were enrolled in a prospective study to assess the significance of PIGF serum concentration. PIGF was measured prior to surgery and 3 months postoperatively. Our control group consisted of 38 healthy subjects. **Results:** PIGF serum concentration was significantly higher in ccRCC compared to controls (P=0.002). The cut-off value of PIGF concentration for the risk of ccRCC was determined at 12.71 pg/mL (AUC=0.729; P=0.0001). Prior to surgery, among ccRCC subgroups, significantly higher PIGF concentration was detected in mccRCC compared to lccRCC (P=0.002). Postoperatively, we observed a tendency to higher PIGF serum concentration in rccRCC compared to lccRCC subgroup, however without significance (P=0.17). The cut-off value for the risk of relapse was 11.41 pg/mL (AUC=0.792; P=0.0003). In subjects with localized ccRCC with PIGF concentration below 11.41 pg/mL 3-years cancer specific survival was 93% compared to 61% in subject with concentration above the cut-off value (P=0.018).

Conclusion: Based on our findings, PIGF serum concentration seems to be a useful biomarker in diagnostics and prediction of prognosis in ccRCC.

Key words: placental growth factor, PIGF, clear cell renal cell carcinoma, biomarker, diagnosis, prognosis

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INTRODUCTION

Worldwide, renal cell carcinoma (RCC) is the sixth most frequently diagnosed cancer in men and eighth in women, accounting for 5% respectively 3% of all carcinomas with the highest incidence in the Western countries¹. In 2018 in Europe new cases of RCC were estimated to occur in 136,500 patients resulting in 54,700 deaths².

Although most detected RCCs are incidentally diagnosed small tumours, locally advanced disease is still found in a significant proportion of patients with up to one third presenting with distant metastasis at the time of diagnosis³. Moreover, about 20-40% patients experience recurrence after surgical treatment of localized disease⁴. The introduction of targeted agents has considerably improved the prognosis of patients with metastatic disease, yet, median survival is beyond two years⁵.

Despite improvements in diagnostics and treatment, RCC still remains among urological malignancy with the highest mortality. Although several risk models for prediction of recurrence have been introduced, the individual course of diseases is difficult to predict. Biomarkers can improve the diagnostics of RCC, and even more can pro-

vide additional predictive accuracy in identifying patients with higher risk of recurrence which would enable treatment individualization and improvement of patients prognosis. Therefore, investigation of potential biomarkers is needed.

Cancer-related angiogenesis is crucial for tumour growth and progression, and it is regulated by various tumour cell produced growth factors⁶. Placental growth factor (PIGF) belongs to vascular endothelial growth factor (VEGF) family, which is the most important trigger of angiogenesis and endothelial cell growth. PIGF stimulates angiogenesis specifically targeting VEGF receptor 1 (Flt-1) and co-receptor neuropilin (NRP1). Originally PIGF was described in human placenta and it is highly expressed throughout pregnancy⁷. In healthy subjects PIGF serum levels are typically low but increase under pathological conditions such as ischemia, inflammation or tumour growth^{8,9}. Overexpression of PIGF has been described in several types of carcinomas. Furthermore, PIGF was identified as a potential prognostic biomarker of cancer progression as its higher expression correlates with advanced tumour stage, hypervascularity, presence of metastasis and shorter patient survival¹⁰⁻¹⁵. However, there

is only a limited number of studies assessing the role and significance of PIGF in RCC (ref. 16,17).

The present study aimed to evaluate PIGF serum concentration in patients suffering from clear cell renal cell carcinoma (ccRCC), and furthermore, to explore its diagnostic accuracy and prognostic value.

PATIENTS AND METHODS

Subjects

Into this prospective study we included 49 individuals (30 men, 19 women, mean age 62.9±10.6 years) who were subjected to radical or partial nephrectomy due to ccRCC from June 2011 to June 2013. In all resected tumour specimens negative surgical margins were described. For further comparison ccRCC subjects were classified in three subgroups: subjects with localized ccRCC without further relapse (lccRCC, n=31), subjects with localized ccRCC with relapse 6 to 18 months following surgical treatment (rccRCC, n=8) and subjects with primary metastatic carcinoma (mccRCC, n=10). The staging of ccRCC was performed following the 2009 TNM classification system, Fuhrman Nuclear Grading System was used for evaluation of the tumour grade. Surveillance after partial or radical nephrectomy was performed in adherence to the EAU Guidelines for Renal Cell Carcinoma¹⁸. Median of follow-up of our study group was 4.4 years. During the follow-up relapse occurred in 8 subjects (rccRCC subgroup) and 17 subjects died. Table 1 describes demographic and clinicopathological parameters of ccRCC cases.

Thirty-eight matched for age and sex healthy subjects with no history of any malignant disease formed the control group. In all, basic uro-oncological screening including ultrasound, urine cytology and measurement of PSA levels in men was performed.

The study was conducted in adherence to ethical guidelines and approved by the institutional review board and ethics committee. A written informed consent was obtained from all participants prior to entering the study.

Table 1. Parameters of ccRCC patients.

Number of patients (men/women)	49 (30/19)
Age (years)	62.9±10.6
Stage	
I	23 (46.9%)
II	1 (2%)
III	15 (30.6%)
IV	10 (20.4%)
Grade	
G1	10 (20.4%)
G2	31 (63.3%)
G3	5 (10.2%)
G4	3 (6.1%)

ccRCC: clear cell renal cell carcinoma

Blood samples

In all individuals PIGF serum concentration was measured prior to surgical treatment. 3 months postoperatively blood samples were collected from patients with localized disease (lccRCC and rccRCC subgroups). Blood samples were obtained after overnight fast by puncture *via* cubital vein, at the same time with blood collection for routine control examinations. For PIGF analysis blood was centrifuged for 10 min at 3000 rpm and serum was stored at -80 °C until analysis.

Biomarker analysis

PIGF serum concentration was assessed with the enzyme-linked immunosorbent assay (ELISA) using standard kits (R&D Systems, Minneapolis, MN, USA) following the manufacturers' instructions. Results are expressed in picograms per millilitre (pg/mL).

Statistical analysis

Data are presented as the means ± standard deviation (SD) for continuous variables and percentages for categorical variables. For continuous variables the difference between subgroups was analysed by one-way ANOVA or Mann-Whitney U test, as appropriate. The repeated measures ANOVA test was used for the analysis of continuous variables over time. The Receiver Operating Characteristics (ROC) curves were created and relative potential of PIGF to identify ccRCC was specified with calculation of area under the ROC curve (AUC). Kaplan-Meier method was used for survival analysis and longrank test was performed to compare survival between subgroups. Univariate Cox proportional hazards regression analyses were applied for assessment of PIGF serum concentration as a potential predictor of cancer specific and overall survival. Statistical significance was set at P<0.05. Data analyses were carried out using MedCalc for Windows, version 13.0 (MedCalc Software, Ostend, Belgium).

RESULTS

Diagnostic accuracy of PIGF in ccRCC

PIGF serum concentration in ccRCC subjects (16.1±10.5 pg/mL) was significantly higher compared to healthy controls (10.5±2.4 pg/mL) (*P*=0.002). The cut-off value of PIGF serum concentration for the risk of ccRCC was determined at 12.71 pg/mL with AUC 0.729, providing the specificity of 84.21% and sensitivity of 61.22% (*P*=0.0001) (Fig 1). Prior to surgical treatment, a significant difference in PIGF serum concentration was found between lccRCC (13.1±5.1 pg/mL) and mccRCC (25.8±18.1 pg/mL) subgroups (*P*=0.002). However, we found no statistically significant difference between rccRCC (15.9±6.9 pg/mL) and lccRCC or mccRCC subgroups, respectively. PIGF serum concentration was not found to correlate with tumour stage (T1-T2 vs T3-T4, *P*=0.76) or Fuhrman's grade (G1-G2 vs G3-G4, *P*=0.07).

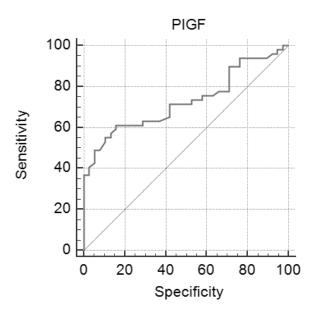


Fig. 1. Cut-off value of PIGF serum concentration for the risk of ccRCC.

Cut-off value of PIGF serum concentration for the risk of ccRCC >12.71 pg/mL (AUC 0.729, specificity 84.21%, sensitivity 61.22%, *P*=0.0001).

PIGF: placental growth factor, ccRCC: clear cell renal cell carcinoma, AUC: area under the curve

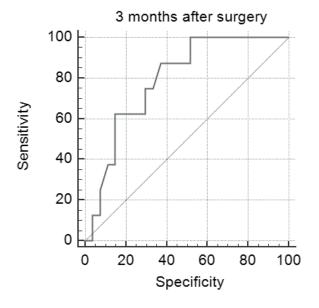


Fig. 2. Cut-off value of PIGF serum concentration for the risk of ccRCC recurrence.

Cut-off value of PIGF serum concentration for the risk of ccRCC recurrence >11.41 pg/mL (AUC 0.792, specificity 62.96%, sensitivity 87.5%, *P*=0.0003).

PLGF: placental growth factor, ccRCC: clear cell renal cell carcinoma, AUC: area under the curve

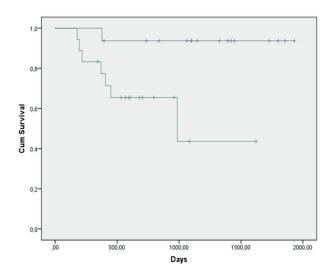


Fig. 3. Kaplan-Maier curves of 3-years cancer specific survival (CSS).

Kaplan-Maier curves of 3-years cancer specific survival (CSS). Upper line – patients with PIGF serum concentration <11.41 pg/mL 3-years CSS 93%, lower line – patients with PIGF serum concentration >11.41 pg/ml 3-years CSS 61%.

PIGF: placental growth factor, CSS: cancer specific survival.

Prognostic value of PIGF in ccRCC

Three months postoperatively, higher PIGF serum concentration in rccRCC (14.87 \pm 2.96 pg/mL) compared to lccRCC (12.1 \pm 5.4 pg/mL) subgroup was found, but the difference was not statistically significant (P=0.17). Table 2 depicts all PIGF serum concentrations.

However, the cut-off value for the risk of relapse was determined at 11.41 pg/mL with AUC 0.792, providing the specificity of 62.96% and sensitivity of 87.5% (P=0.0003) (Fig 2).

3-years cancer specific survival in localized ccRCC subjects (lccRCC and rccRCC subgroup) with PIGF serum concentration below the cut-off (<11.41 pg/mL) was 93% compared to 61% in subjects with PIGF serum concentration above the established cut-off for the risk of relapse (>11.41 pg/mL) (HR 7.9967, CI 1.9938 to 32.0733, P=0.018) (Fig 3).

DISCUSSION

Angiogenesis is essential for tumour growth and is assumed to be a key factor of cancer progression and development of metastasis⁶. Among various angiogenic factors VEGF is the most powerful and has been extensively studied over the last years. PIGF is a member of VEGF family and its overexpression is associated predominantly with pathological angiogenesis. Increased PIGF concentrations have been described in several conditions including carcinogenesis. Additionally, several studies imply that higher circulating levels of PIGF correlate with cancer aggressiveness. Therefore, PIGF was identified to be a prognostic marker of cancer progression of many

Table 2. PIGF serum concentrations.

PIGF (pg/mL)		ccRCC		Controls	P
Preoperative		16.1±10.5		10.5±2.4	P=0.002
Subgroups of ccRCC					
	1ccRCC	rccRCC	mccRCC		
Preoperative	13.1±5.1	15.9±6.9	25.8±18.1		lccRCC vs mccRCC P=0.002*
3 months after surgery	12.1±5.4	14.87±2.96			P=0.17

^{*}Preoperatively, PIGF serum concentration significantly higher in mccRCC compared to lccRCC (P=0.002). No statistically significant difference between lccRCC vs rccRCC and rccRCC vs mccRCC.

PIGF: placental growth factor, ccRCC: clear cell renal cell carcinoma, lccRCC: localized ccRCC without further relapse, rccRCC: localized ccRCC with later relapse, mccRCC: metastatic ccRCC.

tumours including breast, ovarian, gastric, colorectal, hepatocellular, lung carcinoma or malignant pleural mesothelioma^{10-15,19-22}.

Although the role of PIGF in diagnostics and estimation of prognosis has been studied in various tumours, little in known regarding this angiogenic factor in RCC. Since, hypervascularization is a common feature among various solid tumours as well as RCC, production of angiogenic factors in kidney cancer cells have been presumed. Takahashi et al. (ref.¹⁶) as first proved elevated expression of VEGF and PIGF in hypervascular RCC tissue with no detection of PIGF in normal kidney tissue. Matsumoto et al. (ref.¹⁷) analysed PIGF serum levels and its correlation with clinical features of RCC and described association between PIGF concentration and histological grade and tumour vascularity. Furthermore, proposed a prognostic significance of PIGF in RCC.

The aim of the present study was to explore the significance of PIGF serum concentration in patients with ccRCC, the predominant histological subtype of RCC. In the first place, we evaluated the diagnostic significance of PIGF serum concentration. Consistent with previous studies we confirmed significantly higher PIGF serum concentration in ccRCC patients compared to healthy controls. The cut-off value for PIGF serum concentration for the risk of ccRCC was established at 12.71 pg/mL, reaching the specificity of 84.21% and sensitivity of 61.22% with AUC 0.729. Based on our results, PIGF serum concentration can be considered as a diagnostic biomarker for detection of ccRCC. However, with respect to AUC most probably as a component of multi-marker diagnostic test rather than a single-marker test.

In our study group PIGF serum concentration was not found to correlate with tumour stage and Fuhrman's grade. Although, a trend towards higher PIGF serum concentration was observed in ccRCC with more aggressive grade, the results were not significant (G1-G2 vs G3-G4, P=0.07). This could be explained by the small number of patients and a higher proportion of lower stage and grade tumours.

Secondly, we assessed the potential value of PIGF serum concentration in prediction of relapse and prognosis in patients with localized disease. Postoperatively, we observed a tendency to higher PIGF serum concentration in rccRCC compared to lccRCC. The cut-off value of PIGF serum concentration for the risk of relapse was determined at 11.41 pg/mL and provided the specificity of

62.96% and sensitivity of 87.5% with AUC 0.792. 3-years cancer specific survival in patients with localized disease with PIGF serum concentration below this cut-off was 93% compared to only to 61% with concentration above the cut-off. According to these findings, we presume PIGF serum concentration can be useful in identifying patients with higher risk of recurrence. Even more, PIGF serum concentration can be considered as a potential prognostic biomarker in ccRCC.

Based on previously published studies we assumed higher PIGF serum concentrations in ccRCC patients compared to healthy individuals. Still, the primary goal of our study was to assess whether higher serum concentrations are of clinical importance in diagnosis and estimation of prognosis in ccRCC. Therefore, we did not examine expression of PIGF in tumour tissue. Main limit of our study was a small number of patients in each subgroup. PIGF was shown to have a significant position in gynaecological non-oncological testing (early evaluation of the risk of pre-eclampsia in pregnant women), resulting in broadening and availability of diagnostic technologies of the marker. Combined with the fact, that ccRCC does not have a suitable biomarker, PIGF could, based on our results, show to be a promising one. Though, further studies on larger patient cohorts are necessary to confirm this premise.

CONCLUSION

We can conclude that PIGF can be a useful biomarker in diagnostics and prediction of prognosis in subjects with ccRCC. Our results show higher PIGF serum concentration in ccRCC patients compared to healthy controls with the cut-off value for the risk of ccRCC at 12.71 pg/mL. Concomitantly PIGF seems to be a predictor of relapse and prognosis in patients with localized disease with the cut-off value at 11.41 pg/mL. In these patients with PIGF serum level below the cut-off, 3-years cancer specific survival was 93% in comparison to 61% for those with level above the cut-off.

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