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Predictive value of baseline serum vascular endothelial growth factor and neutrophil gelatinase-associated lipocalin in advanced kidney cancer patients receiving sunitinib

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To identify factors that might predict response to sunitinib in patients with renal cell carcinoma, we measured serum vascular endothelial growth factor (VEGF) and neutrophil gelatinase-associated lipocalin (NGAL) levels. A total of 85 patients were selected and, using the Motzer classification, 46 were assigned to the good- and 38 to the intermediate-risk groups. With univariate Cox analysis, both baseline serum VEGF and NGAL titers, determined by enzyme-linked immunosorbent assay, significantly predicted progression-free survival. For each biomarker, a threshold value was identified, which proved useful to classify patients into groups having titers above or below the thresholds. We then stratified patients according to the two dichotomous variables into good-, intermediate-, and poor-risk groups, and found significantly different progression-free survival rates ranging from 3.5 to 11.6 months. Both VEGF and NGAL maintained their predictive significance at bivariate analysis. Our study shows that serum levels of VEGF and NGAL are significant predictors of progression-free survival in patients with renal cell carcinoma treated with sunitinib.

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Despite the recent therapeutic improvements obtained with the use of molecularly targeted agents,[1] metastatic renal cell carcinoma (RCC) should still be considered incurable.

This lack of curative treatment options, together with the peculiar natural history of metastatic RCC and the highly perceived problem of the costs of the newer agents, highlight the need for identifying both prognostic and predictive factors in these patients.[2]

Despite decades of clinical research,[3] Motzer (or MSKCC) criteria remain the most reliable prognostic factors available for advanced RCC patients.

Motzer *et al.*[4] studied 670 advanced RCC patients treated with immunotherapy or chemotherapy and identified, using multivariate analysis, five pre-treatment features that were significantly associated with shorter survival, namely, low Karnofsky performance status, high lactate dehydrogenase levels, low hemoglobin levels, high corrected calcium levels, and no nephrectomy. Using these factors, they stratified patients into three groups (good-, intermediate-, and poor-risk groups) that had a completely different prognosis, that is, from 20 months for the good prognosis group to just 4 months for the poor prognosis group.[4]

A similar analysis was then applied to 400 patients treated with interferon-α as first-line systemic therapy; this analysis reduced the heterogeneity caused by multiple treatments and highlighted the essential role of cytoreductive nephrectomy in metastatic patients with a good performance status. Still, the prognostic model remained the same, except that time from diagnosis to treatment with interferon-α <1, year was replaced with absence of previous nephrectomy.[5]

More recently, the same group reviewed clinical and laboratory data relative to 137 patients enrolled into clinical trials with more modern treatments (from 1990 onward[6]) and evidenced the following independent predictors of worse survival, namely, poor Karnofsky performance status, low hemoglobin, and elevated corrected serum calcium. Again, the number of poor prognostic variables stratified patients

into favorable- (no risk factors), intermediate- (one risk factor), and poor-risk (two or three risk factors) groups, which had overall 1- and 3-year survival rates of 76 and 25%, 49 and 11%, and 11 and 0%, respectively.

Despite the fact that the above prognostic models and predictors of outcome were identified in patients who mainly received immunotherapy, hormonal therapy, and/or chemotherapy, the applicability of the MSKCC criteria in the era of molecularly targeted agents has been confirmed by subgroup analysis of both registrative trials and large expanded access programmes.

With the advent of molecularly targeted therapies, molecular tumor markers have the potential to considerably improve attempts to individualize patient prognosis and treatment strategies.[1]

We investigated the potential predictive factors of sunitinib malate antitumor activity through the assessment of serum levels of soluble vascular endothelial growth factor (VEGF) and of neutrophil gelatinase-associated lipocalin (NGAL).

Vascular endothelial growth factor, the most important pro-angiogenic cytokine, is particularly relevant in kidney cancer owing to the peculiar pathogenesis of this neoplasm (that is, the deletion/mutation of the Von Hippel-Lindau (VHL) protein, leading to an overproduction of pro-angiogenic cytokines through hypoxia-inducible factor-1 (HIF-1) stabilization),[7] whereas NGAL is a protein typically upregulated in cells under 'stress', for example, in the presence of a tumor,[8] and is tightly correlated with matrix metalloproteinase-9 (MMP-9),[9] another key protein involved in the degradation of the extracellular matrix, and thus in invasion and metastasis. Furthermore, NGAL can protect the kidney against acute ischemic injury,[10] has a role in self-survival, and has been observed to be elevated in a number of human cancers, in which its role seems to be different depending on different tumor types.[11]

RESULTS

Patients' characteristics and sunitinib efficacy in terms of progression-free survival

In total, 85 patients received sunitinib; the average age of our patient population was 59.77 years (median: 60; range: 35–77 years); 60 patients had a pure clear-cell RCC, whereas 12 had a predominantly clear-cell mixed histotype and 13 a pure non-clear-cell histotype. The Motzer score[4] only detected favorable ($n=46$) and intermediate ($n=38$) prognosis patients, with no poor-risk patients. The Motzer score was evaluated in all but one patient because some requested biochemical tests were missing on enrollment.

Patients' characteristics are reported in Table 1.

At the time of analysis, average progression-free survival (PFS) was 10.4 months (median: 7.5, interquartile (IQ) range: 2.9–15.4; range: 0.76–30.02 months).

As far as compliance to sunitinib treatment, a dose reduction to either 37.5 or 25 mg, due to toxicity, was carried out in 35 patients (41%). Unexpected toxicities were not recorded.

Table 1 | Patients' characteristics

Patients' characteristics	(*n*=85)
Median age, years (range)	60 (35–77)
Male/female	72/13
ECOG performance status	
0	67
1–2	18
Histology	
Clear cell	60
Mixed	12
Others	13
Motzer's score	
Good	46
Intermediate	38
Poor	0
Not available	1
Previous therapy lines (n)	
0	1
1–3	74
>3	10
Median number of metastatic sites (range)	3.2 (1–7)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Baseline serum VEGF and NGAL titers

Of the 85 patients assessed, 52 (61%) had serum VEGF values within the normal range (0–707 pg/ml), with a median baseline value of 560.24 (IQ range: 347.25–958.18; range: 44.16–4522.41).

Neutrophil gelatinase-associated lipocalin baseline value was within the normal range (42–177 ng/ml) in 56 patients (66%). Low and high values were detected in 12 and 17 patients, respectively. The median baseline value was 83.2 ng/ml (IQ range: 62.2–140.4; range: 2.8–363.4).

The VEGF and NGAL normal ranges used for the purpose of this study were those indicated by the manufacturers of the commercial kits we used.

No correlation was found between either glomerular filtration rate or creatinine and NGAL values (data not shown).

Univariate analysis of the predictive role of Motzer score, VEGF, and NGAL basal titers

All predictive relationships were investigated in 84 (of 85) patients, in which all the three variables investigated (that is, Motzer score, VEGF and NGAL basal titers) were available.

Using the univariate Cox analysis (Table 2), in our case series the Motzer score was not predictive of PFS. Figure 1 shows the PFS curves for favorable and intermediate Motzer scores: the score is predictive of the outcome only in the short term (up to about 1 year), whereas in a longer term, the two curves overlap.

As for VEGF and NGAL, their baseline values were significant predictors of PFS; as shown in Table 2, the relative risk (RR) for a unitary increase of these parameters is

Table 2 | Univariate Cox proportional hazard model for PFS analysis

Parameter	Coefficient	Relative risk (95% CI)	*P*-value
Motzer's score	0.168	1.18 (0.73–1.90)	0.49
VEGF	0.0004	1.0004 (1.00037–1.00042)	0.004
NGAL	0.004	1.004 (1.00045–1.00734)	0.02

Abbreviations: CI, confidence interval; NGAL, neutrophil gelatinase-associated lipocalin; PFS, progression-free survival; VEGF, vascular endothelial growth factor.

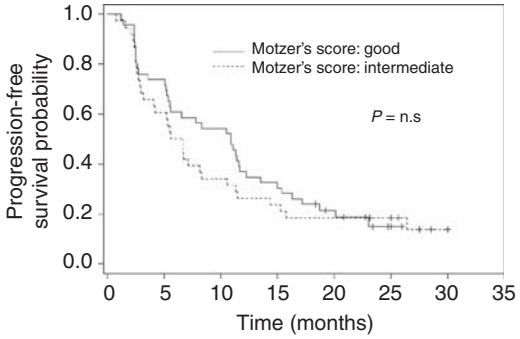

Figure 1 | Survival curves of patients with favorable (=0) or intermediate (=1) Motzer score. Although different up to about 1 year, the two groups come to a similar prognosis, the resulting overall difference being not significant.

1.0004 and 1.004, respectively, meaning that if we consider two generic patients, P_1 with a VGEF value equal to v_1, and P_2 with VGEF value equal to $v_1 + 100$, the RR of P_2 with respect to P_1 is 4%. The same holds true for two patients, in whom P_1 has an NGAL value equal to n_1 and P_2 an NGAL value equal to $n_1 + 10$.

To better interpret the predictive value of VEGF and NGAL, that is, the RR values found by the Cox regression model, threshold values were identified that better correlated with PFS. Thus, patients were subdivided into two groups according to whether their VEGF and NGAL values were above or below the threshold.

As for VEGF, the histogram shown in Figure 2a does not show a clear bimodal distribution; however, we chose a threshold value corresponding to the upper value of the normal range (707 pg/ml); patients with a baseline VEGF titer above this threshold had a RR of progressing of 2.14 (95%CI: 1.324–3.459), relative to patients with a value below the threshold. Thus, this threshold value allowed us to discriminate two patient groups with significantly different PFS, as shown in Figure 2b. Different from Figure 1, in which the Motzer score was considered, groups distinguished by this VEGF threshold maintain a different prognosis up to the longest follow-up.

As for NGAL, the histogram reported in Figure 3a shows a bimodal distribution, with the separation line close to the upper value of the normal range (177 ng/ml). Patients with a baseline titer above this threshold had a RR of progressing of 1.86 (95% CI: 1.142–3.019), relative to patients with values below the threshold.

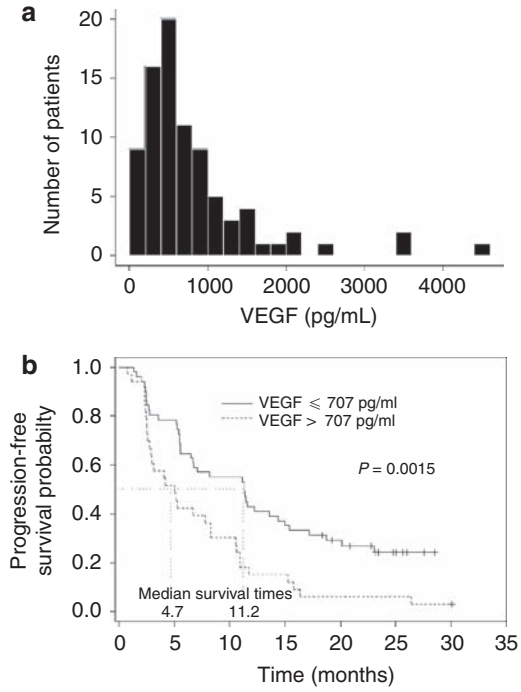

Figure 2 | VEGF value distribution and relationship to PFS. (**a**) Histogram showing vascular endothelial growth factor (VEGF) value distribution. (**b**) Survival curves of patients with VEGF values below and above the threshold.

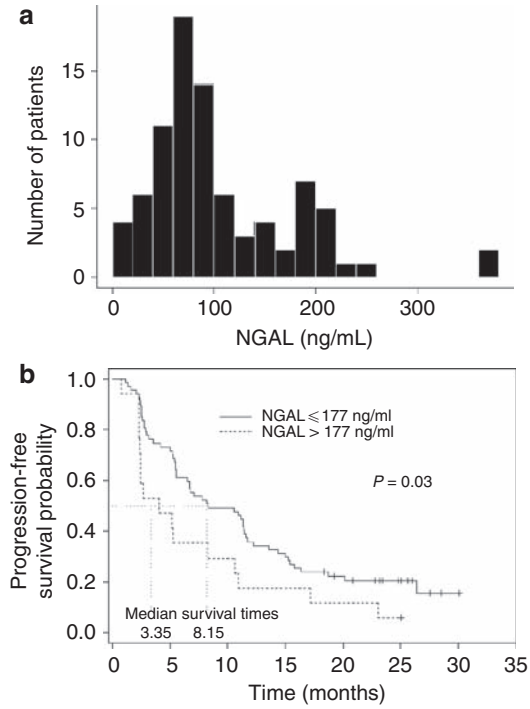

Figure 3 | NGAL value distribution and relationship to PFS. (**a**) Histogram showing neutrophil gelatinase-associated lipocalin (NGAL) value distribution. (**b**) Survival curves of patients with NGAL values below and above the threshold.

Considering this threshold, the resulting PFS survival curves are those reported in Figure 3b. Even if less separated than those of VEGF, the two curves maintain their difference in time.

Table 3 | Counting tables correlating progression of disease with basal values under/over the threshold

	VEGF ⩽ 707 pg/ml	VEGF > 707 pg/ml	NGAL ⩽ 177 ng/ml	NGAL > 177 ng/ml	Motzer score=good	Motzer score=intermediate
Not progressed	13	1	11	3	8	6
Progressed	39	32	44	27	38	32

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; VEGF, vascular endothelial growth factor.

Table 4 | Number of patients at risk and percent PFS at 3, 6, 12, 18, and 24 months

	3 months, percentage (*n*)	6 months, percentage (*n*)	1 year, percentage (*n*)	18 months, percentage (*n*)	2 years, percentage (*n*)
VEGF < 707 pg/ml	80 (42)	65 (35)	43 (23)	31 (17)	24 (10)
VEGF ⩾ 707 pg/ml	61 (21)	42 (15)	15 (6)	6 (3)	6 (3)
NGAL < 177 ng/ml	78 (53)	60 (43)	35 (25)	23 (17)	17 (13)
NGAL ⩾ 177 ng/ml	50 (10)	34 (7)	17 (4)	11 (3)	5 (1)

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; VEGF, vascular endothelial growth factor.

For purely descriptive purposes (the statistical inference was done by means of survival analysis), Table 3 shows the absolute number of progressing and non-progressing patients according to dichotomized variables, that is, Motzer favorable and intermediate groups and normal or above-threshold VEGF and NGAL titers.

The probability of being progression free at 3 months, 6 months, 1 year, 18 months and 2 years according to the baseline VEGF and NGAL titers are reported in Table 4.

Furthermore, patients with above-threshold VEGF titers have a median PFS of 4.7 months (95% CI: 2.8–8.3), whereas patients with VEGF titers below the threshold have a median PFS of 11.2 months (95% CI: 6.5–15). Patients with above-threshold NGAL titers have a median PFS of 3.35 months (95% CI: 2.3–10.9), whereas patients with NGAL titers below the threshold have a median PFS of 8.15 months (95% CI: 5.5–11.6).

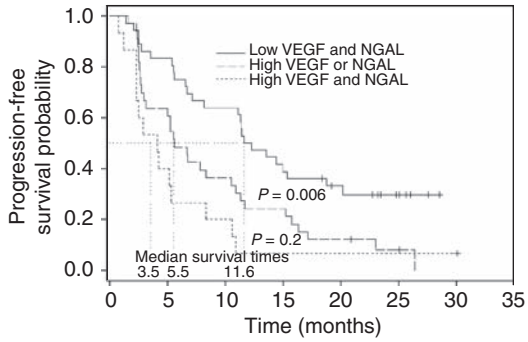

Figure 4 | Survival curves of patients with both vascular endothelial growth factor (VEGF) and neutrophil gelatinase-associated lipocalin (NGAL) titers below the threshold, with both VEGF and NGAL titers above the threshold, and with either value above the threshold and the other one below.

Bivariate analysis

The predictive value of the two considered parameters (that is, VEGF and NGAL), already evidenced as statistically significant at univariate analysis, was then studied in bivariate analysis; both VEGF and NGAL maintained their significance, once NGAL threshold for high values was set to 110 instead of 177 ($P = 0.0048$ and $P = 0.034$, respectively). RRs for VEGF and NGAL and their 95% CI were 2.04 (1.236–3.267) and 1.65 (1.041–2.775), respectively.

We then grouped patients according to VEGF and NGAL values into three groups, namely, patients with both titers below the threshold, patients with both titers over the threshold, and patients with either titer above the threshold and the other one below. The resulting PFS curves for the three groups are reported in Figure 4.

Multivariate analysis

As a possible confounding role of other known prognostic factors could be postulated, a multivariate analysis that also included age and histology (clear-cell or non-clear-cell) was performed; previous treatments were not included in this analysis, as only one patient from this series was treatment naive. Once again, both VEGF and NGAL remained significant, whereas age and histology were not; indeed, the RR for VEGF and NGAL were 1.96 (95% CI: 1.47–2.45) and 1.91 (95% CI: 1.39–2.42), respectively.

DISCUSSION

Until recently, therapeutic options for patients with advanced RCC were limited, but things have changed dramatically over the past few years. Improved understanding of the biology of RCC has permitted the development of novel targeted therapeutic agents that have altered the natural history of this disease.[12] In particular, sunitinib has become one of the two reference first-line treatments available in RCC and likely the most commonly and widely used drug in this setting.[13]

Nevertheless, all the treatments developed so far do not benefit every single patient and the disease itself remains uncurable. Identifying both prognostic and predictive factors in these patients has thus become a priority.

An impressive number of prognostic and predictive factors have been proposed in Medical Oncology for virtually all tumor histotypes, but they usually failed to prove to be more reliable than the best clinical predictive/prognostic factors available and thus they often lacked any practical relevance.

As for RCC, despite all the efforts these factors are still clinical, namely, the so-called Motzer criteria.

We have found that, in an unselected population of advanced RCC patients treated with sunitinib malate, two circulating cytokines, VEGF and NGAL, are more reliable predictors of PFS than the classical Motzer criteria.

Our RCC patients with a baseline VEGF titer above a given threshold had a RR of progressing more than twofold higher than the patients with a value below the threshold, and VEGF maintained its predictive value up to the longest follow-up.

As for NGAL, patients with a baseline titer above the threshold had a RR of progressing of 1.86 relative to patients with a value below the threshold.

The predictive value of the VEGF and NGAL still remained significant also when analyzed with bivariate analysis.

On the contrary, Motzer criteria proved to be reliable predictors of outcome only in the short term (up to about 1 year), whereas in a longer term the two PFS curves overlap.

As for VEGF, its predictive importance in RCC is quite obvious. RCC is often characterized, from a molecular viewpoint, by the loss of the VHL tumor suppressor gene, which results in the dysregulation of one of the key mechanisms responsible for cellular response to hypoxia.[14] Indeed, the VHL protein is involved in the degradation of the α-subunit of HIF-1, a heterodimeric transcription factor that regulates a program of gene expression engaged in facilitating adaptation to tissue hypoxia. Unlike normal cells, cells deficient in VHL inappropriately accumulate HIF-α under conditions of normoxia, and overexpress HIF-regulated genes, ultimately resulting in the overproduction of several pro-angiogenic factors, the most important of which is VEGF.[14,15]

As for NGAL, its involvement in RCC pathogenesis is more subtle.

Neutrophil gelatinase-associated lipocalin belongs to the lipocalin family of proteins, typically small secreted proteins characterized by their ability to bind small, hydrophobic molecules in a structurally conserved pocket formed by β-pleated sheet, to bind to specific cell-surface receptors, and to form macromolecular complexes. In neutrophils (neutrophilic polymorphonuclear leukocytes) and urine, it occurs as a monomer, with a small percentage of dimers and trimers, and also in complex with 92-kDa human neutrophil type IV collagenase, also called gelatinase B or MMP-9.[9] NGAL was originally isolated from the supernatant of activated human neutrophils,[8] but it is also expressed at a low level in other human tissues including the kidney, prostate, and epithelia of the respiratory and alimentary tracts;[16] it is strongly expressed in inflammatory, pre-tumoral and neoplastic lesions.[11,17–20]

Although its functions are not fully understood, NGAL seems to be upregulated in cells under 'stress', for example, from infection, inflammation, ischemia or neoplastic transformation, or in tissues undergoing involution. Its complex formation with MMP-9 seems to protect MMP-9 enzymatic activity from degradation.[9] The upregulation of NGAL in involuting tissues suggests that it may have a role in apoptosis, but in fact NGAL seems more likely to be associated with a survival response.[11] This seems to be the case in the kidney, in which NGAL siderophore–iron complexes protect the mouse kidney from ischemic injury.[10]

In particular, the above tight relationship of NGAL with MMP-9 suggests a role for NGAL in angiogenesis also; indeed, much experimental evidence supports the direct involvement of MMP-9 (which we did not considered in this study) in angiogenesis.[21,22]

As VEGF and NGAL significantly correlated one with the other, as NGAL could also be considered a marker of kidney injury, and as the vast majority of our patients were nephrectomized, we also checked whether there was any relationship between NGAL and both serum creatinine levels and glomerular filtration rate, but no significant correlation between the two parameters was evidenced in our case series.

Theoretically, the observed increase in VEGF and NGAL may derive from sites other than the kidney, hence, diluting the impact of our findings; however, both VEGF and NGAL expression by neoplastic or stressed kidneys have been well documented in the literature.

In conclusion, our study showed that both circulating VEGF and NGAL titers are predictive of a longer PFS in kidney cancer patients treated with sunitinib malate and that both factors performed better than the best clinical factors available so far, that is, the Motzer score.

These parameters, which are easy to evaluate and are reproducible in routine clinical practice also, could thus be included in newer scoring systems/prognostic or predictive nomograms to be routinely applied in clinical practice.

MATERIALS AND METHODS

Patients

A total of 85 patients with advanced RCC were studied. Patients had mainly (but not exclusively) clear-cell histology, age $\geqslant$18 years, an ECOG (Eastern Cooperative Oncology Group) performance status $\leqslant$2, measurable metastatic disease, and adequate hematological, hepatic, renal, and cardiac functions. They had also been treated with non-molecularly targeted agents.

All patients voluntarily consented to participate in both the clinical and the biological study.

Treatment schedule and disease assessment

Patients were treated with sunitinib malate at the starting dose of 50 mg/day, with the classical 4 weeks on/2 weeks off schedule, within the global expanded access program (EAP, NCT00130897).[23]

Sunitinib was self-administered orally once daily irrespective of meals. Dose reduction for toxicity to 37.5 mg/day and then to 25 mg/day was allowed depending on the severity of the side effects reported. The above treatment was approved by the institutional

review board of our Hospital and performed in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Tumor assessment was performed at every two cycles (that is, 12 weeks) according to the Response Evaluation Criteria in Solid Tumors (RECIST).[24]

Treatment end point

For the purpose of this paper, the primary end point was PFS, defined as the time from enrollment to the first observation of disease progression or death. Usually, PFS is an important surrogate of overall survival but, as it could reflect patient benefit by prolonging the interval to disease progression, it may also be important as an end point in itself.[25]

At the time of this analysis (the data lock for this study was 30th March 2008), the median observation period for the whole patient population was 25.82 months, with 13 patients still under active treatment.

Collection of biological samples

Blood samples for VEGF and NGAL were drawn early in the morning after overnight fasting, immediately before treatment start. A blood aliquot was then collected into a whole blood (non heparin) tube and allowed to separate at room temperature for 30 min before being centrifuged at 1000 *g* for 15 min. The serum thus obtained was immediately frozen at -80°C for subsequent assaying.

Serum human VEGF and lipocalin-2/NGAL assays

The two assays use the quantitative sandwich enzyme immunoassay (enzyme-linked immunosorbent assay; ELISA) technique (R&D Systems, Milan, Italy). Briefly, a monoclonal antibody specific for VEGF or NGAL is pre-coated into a microplate; standards and samples are then pipetted into the wells and any VEGF present is therefore bound by the immobilized antibody. After washing away any unbound substances, an enzyme-liked polyclonal antibody specific for VEGF or NGAL is added to the wells. Following another wash to remove any unbound antibody–enzyme reagent, a substrate solution is added to the well and color develops proportionally to the amount of VEGF or NGAL bound in the initial step. Color development is stopped and color intensity is measured. Sensitivity is expressed as pg/ml for VEGF and ng/ml for NGAL. The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard and calculating the corresponding concentration.

Sensitivity is expressed as pg/ml for VEGF and ng/ml for NGAL. The MDD of VEGF is typically <5.0 pg/ml and the mean MDD for NGAL is 0.012 ng/ml. Inter and intra-assay coefficients of variation are 7.3 and 5.4%, respectively, for VEGF, and 6.5 and 3.7%, respectively, for NGAL. The range of values is 62–707 pg/ml for VEGF and 42–177 ng/ml for NGAL.

As NGAL could also be considered a marker of kidney injury, we also checked a possible correlation between NGAL and both serum creatinine as well as GFR.

Data analysis and statistics

Vascular endothelial growth factor and NGAL serum concentrations were analyzed and correlated with PFS and the original Motzer score.[4]

Descriptive statistics are reported as median, IQ range, and whole range. We preferred non-parametric statistics because of the non-normal distribution of variables. Logarithmic transformation was applied to improve normality of VEGF distribution. Histograms of variable distributions were used to detect possible thresholds, suitable for classifying patients in low/high variable value groups; both continuous and dichotomized values were tested in statistical models. Survival curves were drawn according to Kaplan–Meier estimate and log-rank test was used to test the difference between two or more survival curves. Cox's proportional hazards model, both uni- and multivariate, was used to test the statistical significance of potential PFS predictors. RR associated to a prognostic factor was computed as the antilogarithm of the corresponding coefficient in the Cox regression model.

Boxplots were drawn to visualize a variable difference between two groups. They show the median, IQ (25th and 75th percentile), and whiskers extending to the most extreme data point, which is no more than 1.5 times the IQ range from the box. Individual outliers are also shown beyond whiskers. Correlation between two continuous variables was tested through the Pearson or Spearman coefficient.

The statistical package S-PLUS (TIBCO Software, Palo Alto, CA, USA) was used for all the statistics.[26]

DISCLOSURES

C Porta acted as a paid consultant and/or speaker for Pfizer Oncology, Bayer-Schering Pharma, Hoffman La Roche, Novartis Pharma, and Wyeth Pharmaceuticals; he also received research support from Bayer-Schering Pharma and Novartis Pharma.
C Paglino and I Imarisio acted as paid speakers for Bayer-Schering Pharma.

REFERENCES

1. Hutson TE, Figlin RA. Evolving role of novel targeted agents in renal cell carcinoma. *Oncology (Williston Park)* 2007; **21**: 1175–1180.
2. Park WH, Eisen T. Prognostic factors in renal cell cancer. *BJU Int* 2007; **99**: 1277–1281.
3. Porta C, Imarisio I, Paglino C *et al.* Prognostic factors in advanced renal cell cancer. *Eur J Cancer* 2008; **6**(Suppl.): 35–37.
4. Motzer RJ, Mazumdar M, Bacik J *et al.* Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol* 1999; **17**: 2530–2540.
5. Motzer RJ, Mazumdar M, Bacik J *et al.* Effect of cytokine therapy on survival for patients with advanced renal cell carcinoma. *J Clin Oncol* 2000; **18**: 1928–1935.
6. Motzer RJ, Bacik J, Schwartz LH *et al.* Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. *J Clin Oncol* 2004; **22**: 454–463.
7. Pantuck AJ, Zeng G, Belldegrun AS *et al.* Pathobiology, prognosis and targeted therapy for renal cell carcinoma: exploiting the hypoxia-induced pathway. *Clin Cancer Res* 2003; **9**: 4641–4652.
8. Kjeldsen L, Johnsen AH, Sengelov H *et al.* Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993; **268**: 10425–10432.
9. Matthaeus T, Schulze-Lohoff E, Ichimura T *et al.* Co-regulation of neutrophil gelatinase-associated lipocalin and matrix metalloproteinase-9 in the postischemic rat kidney. *J Am Soc Nephrol* 2001; **12**: 787A.
10. Mori K, Lee HT, Rapoport D *et al.* Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; **115**: 610–621.
11. Tong Z, Wu X, Ovcharenko D *et al.* Neutrophil gelatinase associated lipocalin as a survival factor. *Biochem J* 2005; **391**: 441–448.
12. Hutson TE, Figlin RA. Evolving role of novel targeted agents in renal cell carcinoma. *Oncology (Willinston Park)* 2007; **21**: 1175–1180.
13. O'Brien MF, Russo P, Motzer RJ. Sunitinib therapy in renal cell carcinoma. *BJU Int* 2008; **101**: 1339–1342.
14. Kaelin Jr WG. The Von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin Cancer Res* 2007; **13**: 680s–684s.
15. Iliopoulos O. Molecular biology of renal cell cancer and the identification of therapeutic targets. *J Clin Oncol* 2006; **24**: 5593–5600.
16. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. *Genomics* 1997; **45**: 17–23.

17. Friedl A, Stoesz SP, Buckley P *et al.* Neutrophil gelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression. *Histochem* 1999; **31**: 433–441.
18. Nielsen BS, Borregaard N, Bundgaard JR *et al.* Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. *Gut* 1999; **38**: 414–420.
19. Stoesz SP, Friedl A, Haag JD *et al.* Heterogeneous expression of the lipocalin NGAL in primary breast cancers. *Int J Cancer* 1998; **79**: 565–572.
20. Monier F, Surla A, Guillot M *et al.* Gelatinase isoforms in urine from bladder cancer patients. *Clin Chim Acta* 2000; **299**: 11–23.
21. Hawinkels LJ, Zuidwijk K, Verspaget HW *et al.* VEGF release by MMP-9 mediated heparan sulphate cleavage induces colorectal cancer angiogenesis. *Eur J Cancer* 2008; **44**: 1904–1913.
22. Ardi VC, Kupriyanova TA, Deryugina EI *et al.* Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. *Proc Natl Acad Sci USA* 2007; **104**: 20262–20267.
23. Gore ME, Szczylik C, Porta C *et al.* Safety and efficacy of sunitinib for metastatic renal-cell carcinoma: an expanded-access trial. *Lancet Oncol* 2009; **10**: 757–763.
24. Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. *J Nat Cancer Inst* 2000; **92**: 205–216.
25. Niimi M, Yamamoto S, Fukuda H *et al.* The Influence of handling censored data on estimating progression-free survival in cancer clinical trials (JCOG9913-A). *Jpn J Clin Oncol* 2002; **32**: 19–26.
26. Venables WN, Ripley BD. *Modern Applied Statistics with S-PLUS*, 3rd edn. New York: Springer, 1999.