Reduction in skin microvascular density and changes in vessel morphology in patients treated with sunitinib

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Hypertension is a common side effect in cancer patients treated with inhibitors of vascular endothelial growth factor/vascular endothelial growth factor receptor-2 signaling and may represent a marker of clinical benefit. Functional rarefaction (a decrease in perfused microvessels) or structural rarefaction (a reduction in anatomic capillary density) may play an important role in the development of hypertension. We investigated whether sunitinib caused impairment of microvascular function and/or reduction of capillary density in patients with metastatic renal cell cancer (mRCC). Sixteen mRCC patients were treated with sunitinib (50 mg/day). Assessments of 24-h ambulatory blood pressure, microvascular endothelial function by laser Doppler fluxmetry, and capillary density by capillary microscopy were performed at baseline and days 14 and 28. Median blood pressure had increased on day 14 (systolic 10 mmHg, $P<0.01$ and diastolic blood pressure 8 mmHg, $P<0.01$). Capillary density had decreased from 69 to 61 capillaries/mm^2 ($P<0.01$). This decrease was related to the increase in systolic and diastolic blood pressure ($r=-0.57$, $P<0.05$ and $r=-0.68$, $P<0.01$, respectively). A more pronounced decrease in capillary density was associated with increased visibility of the subpapillary plexus ($P=0.041$). Preliminary findings indicated that median progression-free survival was significantly prolonged in patients with a greater than 6 capillaries/mm^2 decrease in density as compared with patients with a less pronounced decrease ($P=0.044$). In conclusion, reduction in skin capillary density is associated with a rise in blood pressure during sunitinib therapy and, by itself, might be useful as a predictive marker of clinical outcome. ***Anti-Cancer Drugs*** **21:439–446 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The central role of angiogenesis in promoting tumor progression and metastases formation is now well appreciated and is governed by a balance between stimulators and inhibitors of angiogenesis, a process by which new capillaries are formed from preexisting blood vessels [1]. Vascular endothelial growth factor (VEGF)/VEGF receptor-2 (VEGFR-2) signaling constitutes the predominant regulatory pathway for developmental and tumor angiogenesis. Therapeutic targeting of VEGF/VEGFR-2 signaling with bevacizumab, an anti-VEGF monoclonal antibody, or sunitinib and sorafenib, both receptor tyrosine kinase inhibitors targeting VEGFR-2, have provided survival benefit in specified cancer patients [1].

Arterial hypertension is a commonly reported side effect in trials with inhibitors of VEGF/VEGFR-2 signaling, such as bevacizumab and sunitinib [2,3]. Although conflicting data have been reported [4], the occurrence of antiangiogenic treatment-related hypertension seems to be a predictive marker for clinical outcome [5–8]. As an example, bevacizumab-induced hypertension was associated with favorable progression-free survival (PFS) in advanced colorectal cancer patients [8]. In addition, the occurrence of hypertension, particularly grade 3, was associated with better treatment response to sunitinib in metastatic renal cell cancer (mRCC) [7]. These findings suggest that the increase in blood pressure (BP) or underlying causative mechanisms may represent clinical biomarkers of treatment efficacy.

The mechanisms by which antiangiogenic therapy can increase BP are not yet fully understood. Proposed mechanisms include reduced formation of nitric oxide by endothelial cells, a reduced responsiveness of vascular smooth muscle cells to nitric oxide, an increased production of or reaction to vasoconstricting stimuli, and a reduction in microvascular density (rarefaction) [9–11]. Microvessels, that is, arterioles and capillaries, are a major contributor to total peripheral vascular resistance. Functional rarefaction (a decrease in perfused

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microvessels) or structural rarefaction (a reduction in anatomic capillary density) may play an important role in the development of hypertension [12,13].

We investigated in mRCC patients whether sunitinib, a small molecule tyrosine kinase inhibitor of VEGFR-1, 2 and 3, platelet-derived growth factor receptor-α and β, c-KIT, and FLT3, impairs microvascular function and induces capillary rarefaction, which in turn might be associated with BP rise and clinical outcome in patients with mRCC.

Methods

Patients, treatment, and evaluation

Patients treated with sunitinib for mRCC from March 2006 to October 2007 were enrolled. Most patients had been included in an expanded access program until September 2006 [14] after which sunitinib was registered and available on doctor's prescription. Each participant signed an institutional review board-approved protocol-specific informed consent in accordance with national and institutional guidelines. Sunitinib was administered orally at a dose of 50 mg daily, consisting of 4 weeks of treatment followed by a 2-week rest period in cycles of 6 weeks.

Computed tomography was performed at baseline and for every two to three cycles of treatment to assess clinical response according to Response Evaluation Criteria in Solid Tumors (RECIST) [15]. PFS was the time between the first day of sunitinib and the date of progressive disease on computed tomography, or clear clinical evidence of progressive disease. Overall survival (OS) was the time between the first day of treatment and the date of death or the date at which patients were last known to be alive.

Blood pressure measurements

At baseline, and days 14 and 28 ambulatory BP monitoring (Spacelabs 90207, Redmond, Washington, District of Columbia, USA) was performed to obtain 24-h recordings of BP and heart rate [16]. Measurements were made at the nondominant arm with appropriately sized cuffs. The monitors measured BP and heart rate every 20 min from 7:00 to 22:00 h and every 30 min from 22:00 to 7:00 h. Hypertension was graded according to National Cancer Institute-Common Toxicity Criteria Version 3.0.

In patients with preexisting antihypertensive medication, the dose and schedule of antihypertensive treatment were not changed during the first 28 days of sunitinib treatment unless the patients developed grade 3 hypertension requiring antihypertensive treatment. The latter precluded patients from subsequent skin microvascular measurements.

Skin microvascular measurements

Baseline, day-14 and day-28 skin microvascular measurements were made as described earlier [16]. Microvascular studies were carried out in the morning in a temperature-controlled room (median: 22.9°C, range: 21.3–25.1°C) after 30 min of acclimatization. Patients had abstained from caffeine, alcohol, smoking, and meals overnight.

Nail fold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope. Capillary density was defined as the number of erythrocyte-perfused capillaries per mm^2. Baseline capillary density represented the number of functional capillaries. The number of capillaries was counted off-line by an experienced investigator (MPdB.) from a videotape. The investigator counting the capillaries was blinded to patients' history and clinical outcome. The day-to-day coefficient of variation of baseline capillary density was 2.3 ± 1.8% [17].

After baseline measurements, venous occlusion was applied with the digital cuff inflated to 60 mmHg for 60 s, to expose a maximal number of perfused capillaries. Venous occlusion is supposed to reflect structural capillary density.

Using the same visual fields as those used during baseline measurements, the capillaries were counted in the 60-s recordings. The day-to-day coefficient of variation of peak capillary density during venous congestion was 9.5 ± 7.1% [12]. Venous occlusion at 60 mmHg for 120 instead of 60 s did not further increase the number of visible capillaries [12].

Endothelium-dependent and endothelium-independent vasodilatation of finger skin microcirculation were evaluated with laser Doppler fluxmetry combined with iontophoresis of acetylcholine (Ach, for endothelium-dependent vasodilatation) and sodium nitroprusside (SNP, for endothelium-independent vasodilatation) as described earlier [16]. ACh (1%, Miochol; Novartis, Basel, Switzerland) mediates vasodilatation through the generation of nitric oxide and prostanoids in the endothelium. SNP (0.1%, Nipride; Roche, Basel, Switzerland) is a nitric oxide donor, acting directly on smooth muscle cells to induce relaxation. Laser Doppler flux was measured on the middle phalanx of the second and fourth digits with the Periflux 4000 system (Perimed, Stockholm, Sweden) and expressed as arbitrary perfusion units. A protocol of multiple fixed doses (current intensity × delivery time) was used, resulting in an incremental dose–response curve. Skin temperature was monitored. Day-to-day reproducibility for iontophoresis of ACh and SNP was 15.9 ± 8.4 and 13.9 ± 9.0%, respectively [16].

Statistical analysis

Data are expressed as median with range. Statistical analysis was performed using SPSS software (SPSS for Windows 15.0; SPSS, Inc., Chicago, Illinois, USA). The Wilcoxon signed-rank test was used to compare BP and microvascular variables on days 14 and 28 with baseline. The relationship between changes in these variables was

assessed by the Spearman correlation test. In addition, Fisher's exact test was performed to determine associations between categorical variables. The association between BP rise and microvascular rarefaction on the one hand and PFS and OS on the other were calculated with the Kaplan–Meier method. For survival analysis, data collection was closed on 1 March 2009. For Fisher's exact and Kaplan–Meier analyses, changes in vascular parameters on day 14 were dichotomized by median splitting. A two-tailed probability value of P less than 0.05 was considered significant.

Results

Patients' characteristics and treatment effects

Patients' characteristics and treatment effects are summarized in Table 1.

Table 1 Patient characteristics

Characteristic	$N=16$ (%)	Median (range)
Male	9 (56)	
Female	7 (44)	
Age, years	60 (47–84)	
Tumor type		
Clear cell	15 (94)	
Other	1 (6)	
MSKCC risk groups[a]		
0 (favorable)	3 (19)	
1–2 (intermediate)	10 (63)	
≥ 3 (poor)	3 (19)	
Prior treatment		
First-line	7 (44)	
Second-line	9 (56)	
Best response according to RECIST		
Partial response	3 (19)	
Stable disease	8 (50)	
Progressive disease	4 (25)	
Not evaluable	1 (6)[b]	
Median follow-up (months)		17 (2–35)
Median progression-free survival (months)		9 (1–29)
Median overall survival (months)		18 (2–35)
Cardiovascular related		
Weight (kg)		83 (44–96)
BMI (kg/m^2)		25 (16–30)
Fasting glucose (mmol/l)		5.3 (4.4–5.6)
Fasting serum total cholesterol (mmol/l)		4.3 (3.1–6.6)
Fasting LDL cholesterol (mmol/l)		2.7 (0.7–4.4)
Fasting HDL cholesterol (mmol/l)		1.1 (0.65–2.31)
Fasting serum triglycerides (mmol/l)		1.3 (0.7–3.9)
Preexistent cardiovascular risk factors		
Hypertension	3 (19)	
Diabetes mellitus	0 (0)	
Smoker	4 (25)	
Cardiovascular event	2 (12)[c]	
Concomitant medication		
Antihypertensive medication	4 (25)[d]	
Cholesterol-lowering medication	3 (19)	

BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; RECIST, Response Evaluation Criteria in Solid Tumors.
[a]Risk groups according to Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria [based on the five risk factors: low Karnofsky performance status (<80%), high LDH (>1.5 times the upper limit of normal), low serum hemoglobin, high corrected serum calcium (>10 mg/dl), and time from initial diagnosis to treatment of less than 1 year] [18].
[b]Because of early termination of sunitinib owing to sunitinib-related adverse events.
[c]Myocardial infarction and coronary artery disease requiring percutaneous transluminal coronary angioplasty, respectively 7 and 16 years before this study.
[d]One patient without preexistent hypertension was treated with an angiotensin-converting enzyme-inhibitor for nephropathy.

During the first cycle, all 16 patients received 50 mg sunitinib daily. All patients underwent measurements of BP and microvascular function at baseline and on day 14, whereas 11 patients also had measurements on day 28. Reasons for dropout were a temporary ($n=3$) or permanent discontinuation ($n=1$) of treatment owing to sunitinib-related adverse events. Another patient was excluded from the analysis after day 14 because of grade 3 hypertension requiring antihypertensive medication.

Blood pressure increases during sunitinib treatment

During sunitinib treatment 24-h systolic BP (SBP), diastolic BP (DBP) and mean arterial BP (MAP) significantly increased on days 14 and 28, whereas the 24-h heart rate showed a significant decrease (Table 2). On day 14, the median increase in SBP, DBP and MAP were 10 mmHg (range −8 to +33 mmHg), 8 mmHg (range −7 to +24 mmHg) and 9 mmHg (range −6 to +27 mmHg), respectively. Only one patient developed grade 3 hypertension (170/107 mmHg) and another two patients had grade 2 hypertension (both 24-h DBP increase by >20 mmHg). On day 28, SBP, DBP and MAP had further increased with a median increase of 13 mmHg (range, −9 to +27 mmHg), 10 mmHg (range −7 to +22 mmHg) and 12 mmHg (range −7 to +24 mmHg), respectively.

Capillary density decreases during sunitinib therapy

During sunitinib treatment, baseline capillary density decreased on days 14 and 28 (Table 2). Capillary density during venous occlusion significantly decreased on days 14 and 28 ($P=0.005$ and 0.003, respectively). Endothelium-dependent (Ach mediated) and endothelium-independent (SNP mediated) vasodilatation did not change during treatment.

Changes in blood pressure are related to changes in capillary density

On day 14, there were significant inverse correlations between changes in BP and in capillary density at baseline and during venous occlusion (Fig. 1) whereas this was not the case for changes in microvascular endothelium-dependent and endothelium-independent vasodilatation. Analyses on day 28 were not substantially different, although differences were not statistically significant.

Changes in vessel morphology

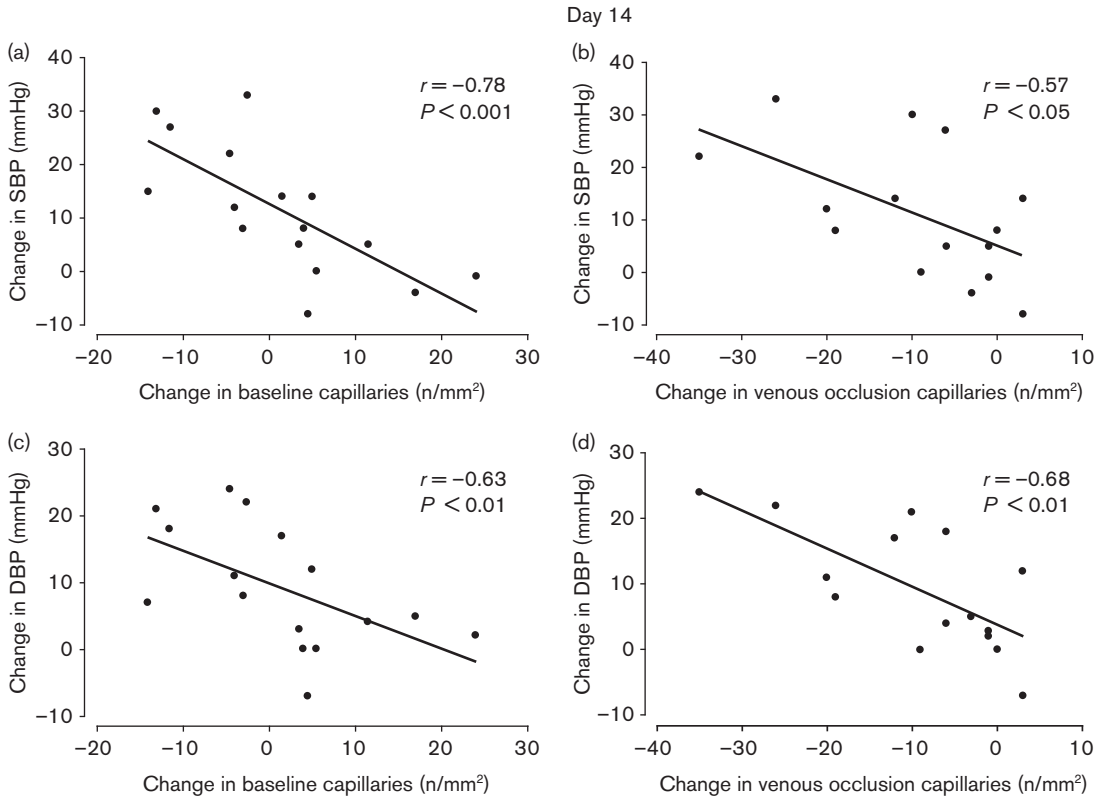
An unexpected finding on day 14 was that capillary microscopy revealed prominent visibility of the subpapillary plexus in eight out of 16 patients (Fig. 2). In all patients, the subpapillary plexus was not visible at baseline. Remarkably, seven out of eight patients with a visible subpapillary plexus had a more pronounced sunitinib-induced decrease in capillary density during venous occlusion. The association between the increased visibility of the subpapillary plexus and the more

Table 2 Blood pressure and microvascular measurements before and during sunitinib 50 mg/day

		During sunitinib	
	Baseline median (range) $N=16$	Day 14 median (range) $N=16$	Day 28 median (range) $N=11$
Blood pressure			
24-h SBP (mmHg)	118 (98–141)	123 (108–170)**	123 (107–157)
24-h DBP (mmHg)	68 (55–86)	75 (63–107)**	79 (65–91)*
24-h MAP (mmHg)	87 (75–105)	93 (79–128)**	93 (80–113)*
24-h heart rate (bpm)	84 (55–98)	75 (56–97)*	77 (63–92)*
Capillary density			
Baseline (capillaries/mm^2)	50 (36–59)	46 (38–77)	43 (23–54)
Venous occlusion (capillaries/mm^2)[a]	69 (51–99)	61 (44–88)**	65 (47–85)**
Temperature (°C)	31.5 (30.5–32.5)	30.2 (29.9–32.5)	30.4 (29.3–33.3)
ACh-mediated vasodilatation			
Baseline skin perfusion (PU)	14 (5–34)	13 (4–35)	9 (6–27)
Plateau (PU)	59 (14–191)	60 (12–192)	73 (12–160)
Percentage increase (%)	295 (71–1032)	326 (1–1230)	448 (44–1803)
Temperature (°C)	31.2 (29.9–31.4)	30.8 (29.8–32.4)	30.4 (30.0–33.4)
SNP-mediated vasodilatation			
Baseline skin perfusion (PU)	13 (4–25)	8 (5–87)	10 (3–28)
Plateau (PU)	63 (40–106)	69 (28–122)	68 (16–185)
Percentage increase (%)	468 (92–1838)	475 (34–1280)	483 (79–3482)
Temperature (°C)	31.1 (30.0–32.0)	31.2 (30.1–32.5)	30.2 (30.0–32.2)

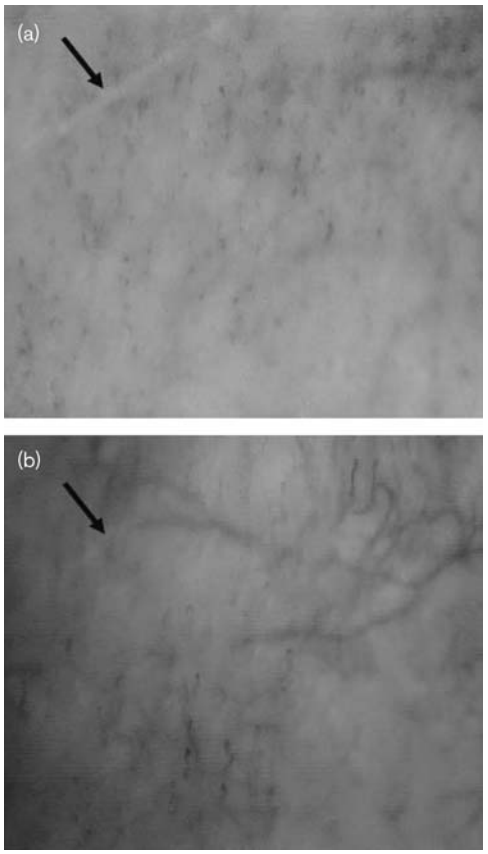
ACh, acetylcholine; bpm, beats per minute; DBP, diastolic blood pressure; MAP, mean arterial pressure; PU, arbitrary perfusion unit; SBP, systolic blood pressure; SNP, sodium nitroprusside.
[a]One patient was excluded because of missing data.
*$P<0.05$, compared with baseline value by the Wilcoxon signed-rank test.
**$P<0.01$, compared with baseline value by the Wilcoxon signed-rank test.

Fig. 1



Relation between changes in blood pressure and changes in capillary density at baseline (a and c) and during venous occlusion (b and d) on day 14 of sunitinib 50 mg/day. DBP, diastolic blood pressure; SBP, systolic blood pressure.

Fig. 2



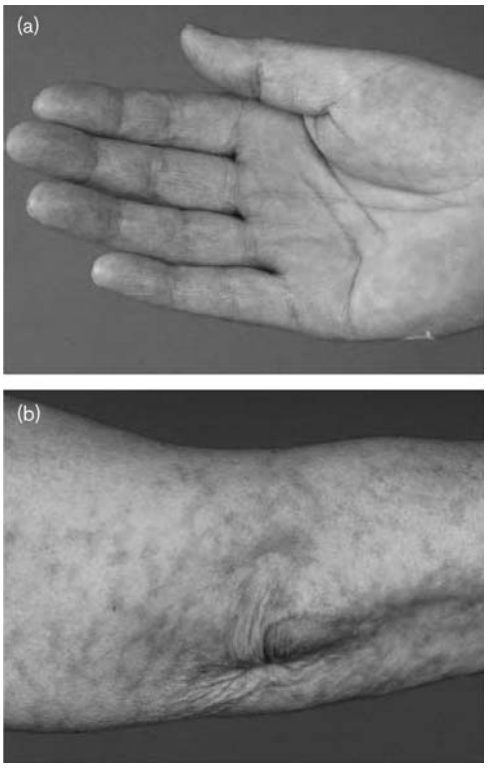
Skin capillaroscopy at baseline (a) and on day 14 (b) showing increased visibility of the subpapillary plexus after 2 weeks of sunitinib 50 mg/day. The skin fold in the left upper part of the figure (arrow) indicates that the same capillary field was visualized.

pronounced decrease in capillary density during venous occlusion was significant (Fisher's exact, $P = 0.041$). At later time points, a livedo reticularis-like disorder on the fingers and arms could be observed in patients with an increased visibility of the subpapillary plexus (Fig. 3).

Changes in capillary density and vessel morphology predict clinical outcome

In our RCC patients treated with sunitinib we carried out a preliminary analysis to know whether the vascular changes were related to clinical outcome. On day 14, changes in BP did not have a significant predictive value for PFS and OS (SBP, $P = 0.87$ and 0.34, respectively; DBP, $P = 0.50$ and 0.22, respectively). Patients with a decrease in the number of capillaries greater than median (2 capillaries/mm^2) had a prolonged OS ($P = 0.033$). Patients with a decrease in the number of capillaries during venous occlusion greater than median (6 capillaries/mm^2) had a prolonged PFS and OS ($P = 0.044$ and 0.008, respectively) (Fig. 4). In these patients median PFS and OS were 11 and 32 months whereas these values were 3 and 11 months for patients with

Fig. 3



Patient showing a reticular pattern on fingers (a) and arms (b) during sunitinib treatment.

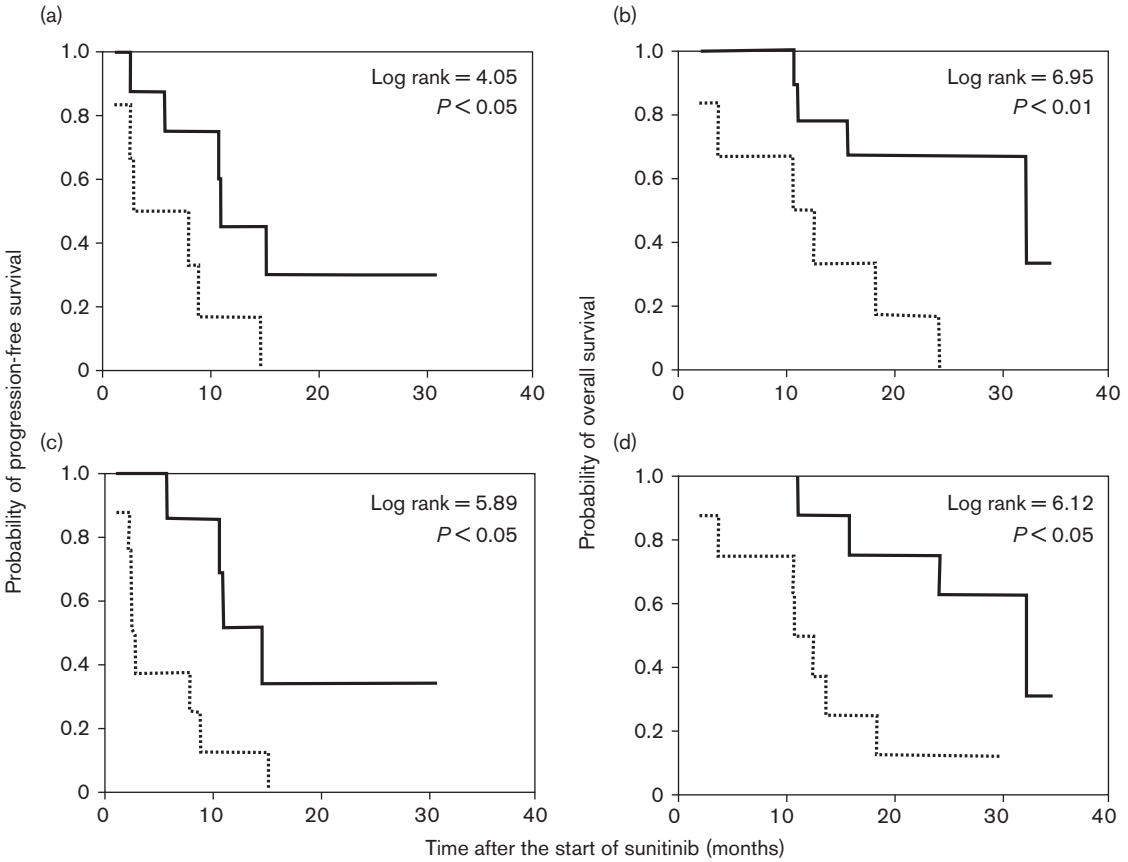
capillary rarefaction ≤ 6/mm^2. In addition, patients with an increased visibility of the subpapillary plexus on day 14 had a prolonged median PFS and OS compared with patients without this microscopic skin pattern ($P = 0.015$ and 0.013, respectively) (Fig. 4). There were no associations between changes in BP and microvascular function with tumor response according to RECIST.

Discussion

In this study we tested the hypothesis that the BP rise, known to be induced by sunitinib, is associated with a reduction in skin microvascular density. We report four novel observations. First, sunitinib treatment is indeed associated with capillary rarefaction, which in turn is directly related to an increase in BP. Second, sunitinib treatment is not associated with impaired microvascular endothelium-dependent and endothelium-independent vasodilatation. Third, the visibility of the subpapillary plexus increased during sunitinib, which was associated with a decrease in capillary density. Fourth, although preliminary, sunitinib-induced capillary rarefaction is predictive for PFS and OS.

Our study has several strengths. All measurements were performed at relatively early time points (day 14). Twenty-four-hour BP monitoring greatly improved the

Fig. 4



Kaplan–Meier curves for progression-free survival and overall survival of metastatic renal cell cancer patients treated with sunitinib 50 mg/day according to microvascular changes on day 14: high (—) and low (- - - -) decrease in capillary density during venous occlusion (a and b) as well as the presence (—) and absence (- - - -) of visibility of the subpapillary plexus (c and d).

reliability of BP recordings. In addition, our patient population was homogenous with regard to tumor type and drug dose (50 mg sunitinib per day), and we excluded patients requiring anti-hypertensive treatment during the study. Finally, the long follow-up enabled us to evaluate possible associations between clinical outcome, BP and microvascular function.

A rise in BP can be found in most patients treated with sunitinib [19] with overt hypertension arising in approximately 20% of the patients [20]. Although sunitinib targets several receptor tyrosine kinases, VEGF/VEGFR-2 signaling seems to be essential for the rise in BP. The role for VEGF/VEGFR-2 in BP regulation is illustrated by the extremely high prevalence of hypertension (92%) during treatment with a combination of sunitinib and bevacizumab [21]. Two recent studies have suggested microvascular rarefaction as a mechanism leading to high BP when abrogating VEGF/VEGFR-2 signaling, but a possible relationship was not examined [10,11]. Microvascular rarefaction was described to occur in the skin of patients with advanced colorectal cancer receiving bevacizumab [10] and in the mucosal surface of the inner lip of patients with advanced solid tumors receiving telatinib, a small molecule tyrosine kinase inhibitor of VEGFR-2 and 3, platelet-derived growth factor receptor-β, and c-KIT [11]. Here, we not only measured the development of capillary rarefaction during the inhibition of VEGF/VEGFR-2 signaling, but we were also able to show the presence of a direct association between the decrease in capillary density and the rise in BP.

Although the cause-and-effect relationships of rarefaction and hypertension are still under debate [9,13], mathematical modeling of in-vivo microvascular networks predicts an exponential relation between capillary and arteriolar number and vascular resistance. Total vessel rarefaction up to 42% can increase tissue vascular resistance by 21% [22]. Indirect evidence suggests that microvascular rarefaction, by affecting peripheral vascular resistance, may indeed initiate the pathogenic sequence in sunitinib-induced BP rise. Hypertension caused by an increase in vascular resistance is characterized by a slight decrease in circulating plasma volume, a decrease in

cardiac output and reduced sympathetic activity. In accordance, hematocrit values and erythrocyte numbers are increased in sunitinib-treated patients [19], possibly reflecting a decreased circulating plasma volume. Simultaneously, cardiac output is decreased [23] and heart rate is depressed ([2], this study), possibly reflecting decreased sympathetic activity. Humoral factors, such as catecholamines, endothelin-1 and urotensin-II, seem to play a minor role in arterial hypertension during inhibition of VEGF/VEGFR-2 signaling [24]. Renal microvascular dysfunction, accompanied by a shift in the renal pressure–natriuresis relationship, is probably necessary to maintain the initial elevation of BP [25].

Capillary nonperfusion may merely represent the downstream consequence of impaired nitric oxide synthesis leading to reduced vasodilatation at the precapillary arteriolar level. Indeed, Mourad *et al.* [10] have reported a decrease in endothelium-dependent vasodilatation after 6 months of bevacizumab treatment. Endothelium-independent vasodilatation, however, was not assessed and, therefore, reduced formation of nitric oxide by endothelial cells could not be distinguished from a decreased responsiveness of vascular smooth muscle cells to nitric oxide. Moreover, these findings may be secondary to a chronic increase in BP instead of the cause of a rapid rise in BP [13]. In this study both microvascular endothelium-dependent and endothelium-independent vasodilatation were unaffected by sunitinib therapy. It is important to realize, however, that dermal vasodilatation in response to iontophoresis of Ach is mediated not only by nitric oxide, but also by prostanoids [26], which are unaffected by antiangiogenic therapy [27]. Nevertheless, the responsiveness of microvascular smooth muscle cells to nitric oxide seems to be intact. Our methodology does not preclude impaired endothelium-dependent and endothelium-independent vasodilatation at the level of the resistance vessels or conduit arteries. Impaired endothelium-independent vasodilatation in conduit arteries has been shown during telatinib therapy [11].

An unexpected, but related finding was the visibility of the subpapillary plexus during sunitinib treatment. The subpapillary plexus, which consists of an anastomosing network of arterioles and venules and is located at a depth of 400–500 μm from the surface, is not visible in most healthy individuals (> 80%) [28]. At later time points during sunitinib treatment, a livedo reticularis pattern could be observed macroscopically on the fingers and arms of the patients with a visible subpapillary plexus. The visibility of the subpapillary plexus was associated with a more marked decrease in capillary density and, in addition, predictive of favorable clinical outcome. In general, a reticular pattern can be caused by several underlying factors that increase the visibility of the venous network in the skin [29]. Venous stasis of blood owing to slow flow in the draining veins secondary to reduced arterial inflow is a hallmark feature of vasospastic livedo reticularis [30] and may have caused the change in vessel morphology during sunitinib treatment. In addition, the increase in hematocrit and erythrocyte numbers during sunitinib treatment [19,31] may have contributed to the observed livedo reticularis pattern, because this phenomenon is also associated with polycythemia vera [29].

Capillary density, as a possible direct biomarker of the antiangiogenic and BP increasing potential of sunitinib, might be useful as a biomarker of efficacy. Although preliminary, median PFS and OS were 11 and 32 months for patients showing sunitinib-induced capillary rarefaction greater than 6/mm^2 whereas these values were 3 and 11 months for patients with a capillary rarefaction $\leq$ 6/mm^2. As expected, three patients who developed $\geq$ grade 2 hypertension showed capillary rarefaction greater than 6/mm^2 associated with a better clinical outcome. Whether sunitinib-induced capillary rarefaction may indeed constitute an early indicator of antitumor activity needs to be confirmed in a larger series of mRCC patients. In addition, it needs to be investigated whether sunitinib-induced capillary rarefaction in the skin is indicative of changes in microvascular perfusion at the site of the tumor. In conclusion, early measurements of microvascular parameters in mRCC patients treated with the VEGFR-2 inhibitor, sunitinib, showed a reduction in capillary density whereas endothelium-dependent and endothelium-independent vasodilatations were unaffected. Reduction in skin capillary density was directly related to a rise in BP. Patients with a marked decrease in capillary density and/or increased visibility of the subpapillary plexus seemed to have a significantly prolonged PFS and OS, suggesting that these microvascular parameters may constitute an early indicator of antitumor activity.

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References

1 Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008; **358**:2039–2049.
2 Azizi M, Chedid A, Oudard S. Home blood-pressure monitoring in patients receiving sunitinib. *N Engl J Med* 2008; **358**:95–97.
3 Sica DA. Angiogenesis inhibitors and hypertension: an emerging issue. *J Clin Oncol* 2006; **24**:1329–1331.
4 Chan A, Vanlemmens L, Conte PF, Beith J, Ten Bokkel Huinink D, Samonigg H, *et al.* Efficacy of bevacizumab plus docetaxel does not correlate with hypertension or G-CSF use in patients with locally recurrent or metastatic breast cancer in the AVADO phase III study. *San Antonio Breast Cancer Symposium* 2008; abstract 1027.
5 Bono P, Elfving H, Utriainen T, Osterlund P, Saarto T, Alanko T, *et al.* Hypertension and clinical benefit of bevacizumab in the treatment of advanced renal cell carcinoma. *Ann Oncol* 2009; **20**:393–394.
6 Ravaud A, Sire M. Arterial hypertension and clinical benefit of sunitinib, sorafenib and bevacizumab in first and second-line treatment of metastatic renal cell cancer. *Ann Oncol* 2009; **20**:966–967.

7 Rixe O, Billemont B, Izzedine H. Hypertension as a predictive factor of sunitinib activity. *Ann Oncol* 2007; **18**:1117.
8 Scartozzi M, Galizia E, Chiorrini S, Giampieri R, Berardi R, Pierantoni C, *et al*. Arterial hypertension correlates with clinical outcome in colorectal cancer patients treated with first-line bevacizumab. *Ann Oncol* 2009; **20**:227–230.
9 Levy BI. Blood pressure as a potential biomarker of the efficacy angiogenesis inhibitor. *Ann Oncol* 2009; **20**:200–203.
10 Mourad JJ, Des Guetz G, Debbabi H, Levy BI. Blood pressure rise following angiogenesis inhibition by bevacizumab. A crucial role for microcirculation. *Ann Oncol* 2008; **19**:927–934.
11 Steeghs N, Gelderblom H, Roodt JO, Christensen O, Rajagopalan P, Hovens M, *et al*. Hypertension and rarefaction during treatment with telatinib, a small molecule angiogenesis inhibitor. *Clin Cancer Res* 2008; **14**:3470–3476.
12 Serne EH, Gans RO, Ter Maaten JC, Tangelder GJ, Donker AJ, Stehouwer CD. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension* 2001; **38**:238–242.
13 Serne EH, De Jongh RT, Eringa EC, Ijzerman RG, Stehouwer CD. Microvascular dysfunction: a potential pathophysiological role in the metabolic syndrome. *Hypertension* 2007; **50**:204–211.
14 Van der Veldt AA, Boven E, Helgason HH, Van Wouwe M, Berkhof J, De Gast G, *et al*. Predictive factors for severe toxicity of sunitinib in unselected patients with advanced renal cell cancer. *Br J Cancer* 2008; **99**:259–265.
15 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, *et al*. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000; **92**:205–216.
16 Serne EH, Stehouwer CD, Ter Maaten JC, Ter Wee PM, Rauwerda JA, Donker AJ, *et al*. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 1999; **99**:896–902.
17 Serne EH, Ijzerman RG, Gans RO, Nijveldt R, De Vries G, Evertz R, *et al*. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. *Diabetes* 2002; **51**:1515–1522.
18 Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol* 2002; **20**:289–296.
19 Van der Veldt AA, Boven E, Vroling L, Broxterman HJ, Van den Eertwegh AJ, Haanen JG. Sunitinib-induced hemoglobin changes are related to the dosing schedule. *J Clin Oncol* 2009; **27**:1339–1340.
20 Zhu X, Stergiopoulos K, Wu S. Risk of hypertension and renal dysfunction with an angiogenesis inhibitor sunitinib: systematic review and meta-analysis. *Acta Oncol* 2009; **48**:9–17.
21 Feldman DR, Baum MS, Ginsberg MS, Hassoun H, Flombaum CD, Velasco S, *et al*. Phase I trial of bevacizumab plus escalated doses of sunitinib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**:1432–1439.
22 Greene AS, Tonellato PJ, Lui J, Lombard JH, Cowley AW Jr. Microvascular rarefaction and tissue vascular resistance in hypertension. *Am J Physiol* 1989; **256**:H126–H131.
23 Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, *et al*. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet* 2007; **370**:2011–2019.
24 Veronese ML, Mosenkis A, Flaherty KT, Gallagher M, Stevenson JP, Townsend RR, *et al*. Mechanisms of hypertension associated with BAY 43-9006. *J Clin Oncol* 2006; **24**:1363–1369.
25 De Jongh RT, Serne EH, Ijzerman RG, Stehouwer CD. Microvascular function: a potential link between salt sensitivity, insulin resistance and hypertension. *J Hypertens* 2007; **25**:1887–1893.
26 Noon JP, Walker BR, Hand MF, Webb DJ. Studies with iontophoretic administration of drugs to human dermal vessels in vivo: cholinergic vasodilatation is mediated by dilator prostanoids rather than nitric oxide. *Br J Clin Pharmacol* 1998; **45**:545–550.
27 Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM. Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. *Hypertension* 2009; **54**:652–658.
28 Kabasakal Y, Elvins DM, Ring EF, McHugh NJ. Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls. *Ann Rheum Dis* 1996; **55**:507–512.
29 Gibbs MB, English JC III, Zirwas MJ. Livedo reticularis: an update. *J Am Acad Dermatol* 2005; **52**:1009–1019.
30 Parsi K. Dermatological manifestations of venous disease. Part II: reticulate eruptions. *Aust NZ J Phleb* 2008; **11**:11–46.
31 Vroling L, Van der Veldt AA, De Haas RR, Haanen JB, Schuurhuis GJ, Kuik DJ, *et al*. Increased numbers of small circulating endothelial cells in renal cell cancer patients treated with sunitinib. *Angiogenesis* 2009; **12**:69–79.