# Review Article

# Proteinuria and Hypertension in Patients Treated with Inhibitors of the VEGF Signalling Pathway – Incidence, Mechanisms and Management

(VEGF inhibition / proteinuria / hypertension / bevacizumab / sunitinib / sorafenib)

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**Abstract. Anti-VEGF therapy dramatically improved the outcome of patients with renal cancer and other advanced malignancies, but may be complicated by proteinuria and hypertension. VEGF is indispensable for the normal development of glomerulus and preservation of glomerular filtration barrier. Interference with its action may result in damage to glomerular endothelial cells and (in severe cases) in renal thrombotic microangiopathy. Blood pressure and proteinuria (using dipstick) should be assessed in all patients before starting anti-VEGF therapy and regularly monitored during the treatment. Patients with severe proteinuria and/or impaired renal function should be referred to the nephrologist for further work-up. Hypertension caused by anti-VEGF therapy can be effectively treated; progression of proteinuria and/or renal dysfunction may require tapering, or even withdrawal of anti-VEGF treatment.**

## Introduction

Inhibition of the vascular endothelial factor (VEGF) signalling pathway is used in the treatment of advanced renal cell cancer, but also in the treatment of advanced stages of some other malignancies, e.g. metastatic colorectal cancer, lung cancer and hepatocellular cancer. The VEGF pathway may be inhibited either by a monoclonal antibody binding circulating VEGF (bevacizumab), or by the small orally active tyrosine kinase inhibitors (sunitinib, sorafenib, pazopanib, axitinib, vandetanib) that block the intracellular domain of the VEGF receptor. VEGF inhibition is aimed at restricting the tumour blood supply and limiting the tumour growth (Izzedine et al., 2007).

Although VEGF inhibitors are generally quite well tolerated, patients treated with anti-VEGF therapy may relatively frequently (up to 50 % of patients, especially during the first course of the treatment) complain of fatigue and oral toxicity (dry mouth, mucosal sensitivity, taste change – usually under the heading of mucositis/stomatitis). Skin toxicity (hand-foot syndrome/acral erythema) may occur later in the course of the treatment, but also rather frequently (10–30 %). Hypothyroidism is much less frequent (Kollmannsberger et al., 2011; Poprach et al., 2012). VEGF inhibition may also be complicated by proteinuria and hypertension. In this review we will concentrate on the incidence, mechanisms, management and outcome of these relatively frequent complications.

## Role of VEGF in the normal development and maintenance of glomerular filtration barrier

The glomerular fitration barrier (glomerular capillary wall) consists of three layers (fenestrated glomerular endothelial cells, glomerular basement membrane, and podocytes interconnected with slit diaphragm – Fig. 1) and its permeability for serum proteins is normally very low (less than 5 mg/l of glomerular filtrate).

Vascular endothelial factor A (VEGF-A) is a member of the family of growth factors including VEGF-B, -C, -D and -E, placental growth factor (PlGF) and platelet-derived growth factor (PDGF), of which VEGF-A plays the major role in both regulating angiogenesis and vascular permeability (Eremina et al., 2007). There are multiple splice variants of *VEGFA* which may have both

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Abbreviations: HIF-α – hypoxia inducible factor α, NO – nitric oxide, PDGF – platelet-derived growth factor, PIGF – placental growth factor, VEGF – vascular endothelial factor, VEGF-A – vascular endothelial factor A, VEGFR – VEGF receptor.

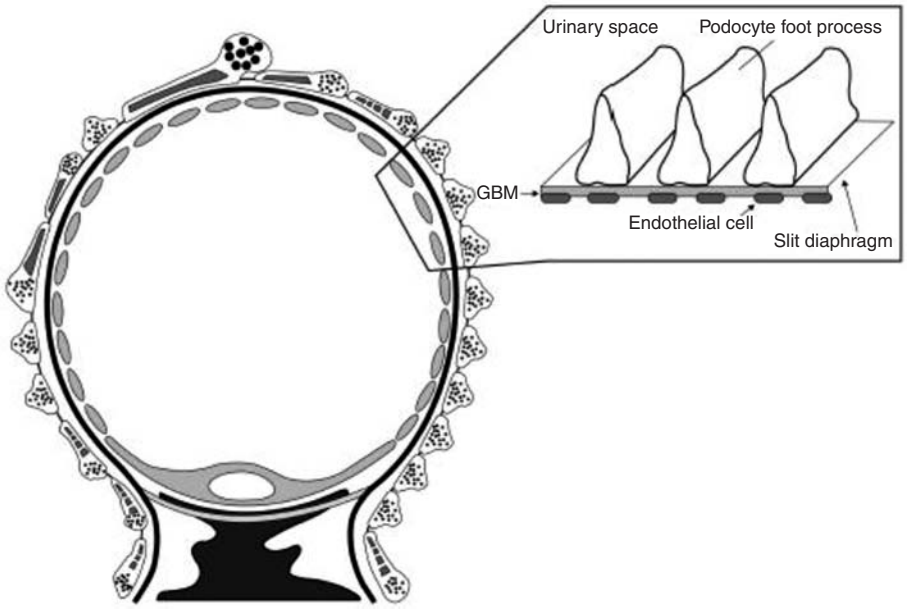


*Fig.1.* Schematic presentation of the glomeral capillary and (in detail) glomerular capillary wall

pro-angiogenic and anti-angiogenic properties. Out of the three major isoforms (VEGF120, VEGF164 and VEGF188), VEGF164 is indispensable (and possibly sufficient) for the normal vascular development, although mice expressing only VEGF188 present retinal abnormalities but normal glomerular development (Eremina et al., 2007).

VEGF-A is a potent pro-angiogenic factor essential for mitosis, migration and survival of endothelial cells (Leung et al., 1989; Ferrara et al., 2003) and plays an important role not only in tumour angiogenesis, but also in would healing, diabetes (Lindenmeyer et al., 2007) and arthritis. VEGF-A signalling depends mainly on VEGF receptor 2 (VEGFR2, Flk1) with neuropilin 1 and neuropilin 2 as co-receptors; VEGFR1 (Flt1) serves as a decoy receptor. VEGF-A is essential for the normal function of the glomerular filtration barrier. Genetically induced lack of VEGF-A in the endothelium results in swelling of the glomerular endothelium, endothelial degeneration and vascular thrombosis (Lee et al., 2007).

Podocytes are (except for the distal tubule and collecting duct) the major source of local production of VEGF in the kidney (Schrijvers et al., 2004). Podocytes differ from many other cells producing VEGF in that they produce VEGF-A not only during development, but also (albeit at a lower rate) as fully differentiated cells. *In vitro* podocytes express several VEGF receptors including VEGFR1, VEGFR3, neuropilin 1 and neuropilin 2, but until recently there was some uncertainty concerning the podocyte expression of VEGFR2. VEGF is produced by glomerular podocytes, but the regulation of podocyte VEGF-A expression is unclear. Generally, VEGF expression is regulated by hypoxia inducible factor α (HIF-α), but as healthy adult glomeruli are not hypoxic, integrins were suggested as non-hypoxic mediators of constitutive VEGF podocyte expression (Datta et al., 2004). Angiotensin II may also stimulate VEGF-A production, possibly by stimulation of HIF-α (Kang et al., 2006).

Inhibition of podocyte VEGF receptors may result in decreased podocyte survival (Foster et al., 2003, 2005). Autocrine VEGF secretion may regulate podocin and its interaction with CD2AP, with potential impact on the normal function of slit diaphragm (Guan et al., 2006), the ultimate barrier preventing the urinary loss of protein. Nephrin is not only the essential component of the slit diaphragm, but it also signals inside the podocyte, regulating the actin cytoskeleton and thereby the podocyte shape. VEGFR2 interacts with the cytoplasmic domain of nephrin and the adapting proteins Nck and actin. VEGF-A may in this way decrease podocyte cell size and regulate the foot process structure and glomerular filter integrity (Bertuccio et al., 2011). VEGF may also be important for the survival and integrity of the podocyte (Guan et al., 2006). The role of the autocrine stimulation of podocytes by the podocyte-derived VEGF has not been unequivocally confirmed and according to some reports podocyte-derived VEGF does not seem to be indispensable in the autocrine fashion for the normal development and function of podocytes (Sison et al., 2010).

More importantly, VEGF released from the podocytes exerts a paracrine effect on the glomerular endothelial cells which express both VEGFR1 (Flt1) and VEGFR2 (Flk1) receptors (Fig. 2). Most effects of podocyte-derived VEGF on glomerular endothelial cells are probably mediated by VEGFR2. During glomerular development VEGF secretion by podocytes attracts the VEGFR2-expressing endothelial cells to form the glomerular capillary tuft. VEGF-A is absolutely indispensable for the normal development of glomerular filtration barrier; in mice *VEGFA* knockout is lethal, VEGF inhibition during embryonic life impairs normal glomerular vascularization and results in smaller glomeruli

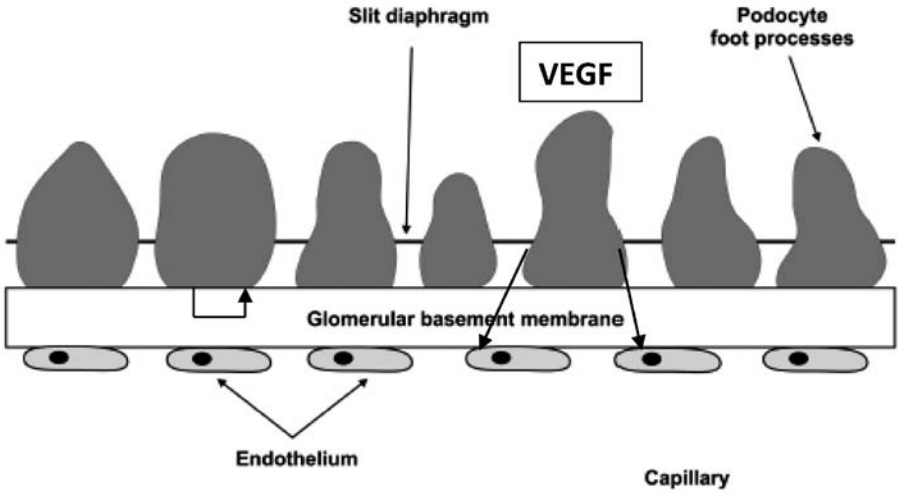


*Fig. 2.* Glomerular filtration barrier consisting of fenestrated glomerular endothelial cells, glomerular basement membrane and slit diaphragm between podocytes. VEGF produced by podocytes may stimulate itself in the autocrine manner, or (probably more importantly) stimulate endothelial cells in the paracrine manner

with less capillary loops (Eremina et al., 2003, 2007). The degree of glomerular damage is related to the degree of the lack of VEGF: podocyte-selective knockout results in perinatal lethality and renal failure; loss of a single *VEGFA* allele in podocytes results in the renal lesion similar to thrombotic microangiopathies (Eremina et al., 2003).

Specific deletion of VEGF from podocytes causes glomerular changes characterized namely by profound endothelial cell injury, demonstrating that the paracrine (not autocrine) VEGF-VEGFR2 signalling is crucial for the normal structure and function of the glomerular filtration barrier. In this model, podocyte-specific deletion of VEGFR2 did not have any demonstrable effect on glomerular development and function; deletion of VEGFR2 in endothelial cells led, however, to widespread defects in the glomerular vasculature (Sison et al., 2010).

In adult mice podocyte or endothelial cell *VEGFA* knockout may cause thrombotic microangiopathy (Eremina et al., 2008). Inducible, podocyte-specific *VEGFA* knockout in mice (Veron et al., 2012) results in reducing glomerular VEGF-A to less than 20 % of control value, with subsequent proteinuria and renal failure with associated mesangiolysis and microaneurysms, endothelial swelling, lamination of glomerular basement membrane and podocyte effacement. At the molecular level inducible *VEGFA* knockout is characterized by a decreased podocyte fibronectin level and decreased expression of $\alpha_v\beta_3$ integrin in the glomerular endothelial cells. Interaction with $\alpha_v\beta_3$ integrin and neuropilin 1 is indispensable for the normal action of the VEGFR2 receptor. $\alpha_v\beta_3$ integrin is normally expressed in endothelial and mesangial cells and podocytes both in rodents and humans (Hafdi et al., 2000) and $\beta_3$ integrin-deficient mice suffer from Glanzmann thrombastenia with fatal haemorrhages and glomerular lesions (Hodivala-Dilke et al., 1999). $\alpha_v\beta_3$ integrin is activated by soluble plasminogen activator receptor of urokinase type (suPAR), which was recently suggested as a permeability factor causing proteinuria in patients with focal segmental glomerulosclerosis (Wei et al, 2008, 2011).

Hypertension and thrombotic microangiopathy in preeclampsia is caused by the abundance of soluble VEGFR1 (sFlt1) produced by placenta (Koga et al., 2003; Maynard et al., 2003; Eremina et al., 2008). In preeclampsia the circulating levels of sFlt1 are inversely related to the serum levels of free VEGF-A and directly correlate with the severity of preeclampsia (Levine et al., 2004). Although in preeclampsia circulating sFlt may titrate circulating VEGF, it has been suggested that it may also cross the glomerular filtration barrier and titrate VEGF locally produced by the podocyte (Eremina et al., 2007).

VEGF podocyte production must be tightly regulated as not only lack, but also surplus of VEGF may damage the glomerular filtration barrier. Over-expression of the major isoform of VEGF-A (VEGF164) leads to the collapse of the glomerular capillary tuft, proteinuria, renal failure and neonatal death (Eremina et al., 2003). More moderate VEGF over-expression induces different glomerular diseases during development (congenital nephrotic syndrome and minimal change disease (Eremina et al., 2003; Veron et al., 2010)) and in adult mice (lesions indistinguishable from early diabetic nephropathy – Eremina et al., 2003; Veron et al., 2010a).

Excessive VEGF-A expression in podocytes was reported in diabetic nephropathy. Podocyte-specific overexpression of VEGF164 in adult transgenic mice led to glomerulomegaly, thickening of glomerular basement membrane, mesangial expansion, podocyte effacement and loss of slit diaphragms with down-regulation of nephrin expression. These changes similar to murine diabetic nephropathy were mediated by VEGFR2 expressed by both podocytes and glomerular endothelial cells (Veron et al., 2010b). Podocyte-specific, doxycycline inducible over-expression of soluble VEGFR1 (sFlt1) significantly ameliorated diabetic nephropathy

in transgenic mice (Ku et al., 2008). Podocyte-specific, doxycycline-inducible VEGF-A over-expression in adult transgenic mice with streptozotocin-induced diabetes dramatically worsen diabetic nephropathy with nodular glomerulosclerosis, glomerular membrane thickening and severe proteinuria with increased VEGFR2 and decreased nephrin expresion in podocytes (Veron et al., 2011).

In conclusion, the main source of VEGF in the glomeruli are podocytes and the main target od podocyte-derived VEGF are glomerular endothelial cells. Podocyte-derived VEGF is indispensable for the normal development and integrity of the glomerular capillary wall and normal function of glomerular filtration barrier (Eremina et al., 2008); on the other hand, glomerular over-expression of VEGF may result in other types of glomerular damage, e.g. collapsing glomerulopathy, or contribute to the development or aggravate diabetic nephropathy. Podocyte secretion of VEGF should thus be tightly regulated and both its under-expression and over-expression may result in proteinuria. Unfortunately, the mechanisms of this regulation are still incompletely understood.

## Incidence and severity of proteinuria in patients treated with VEGF inhibition

Asymptomatic albuminuria/proteinuria is common in patients treated with VEGF inhibition (Izzedine et al., 2010), but is rarely nephrotic (Costero et al., 2010; Okuno et al., 2011). In some patients nephrotic syndrome may be accompanied by renal dysfunction (Takahashi et al., 2012). Proteinuria is frequently accompanied by hypertension. In patients treated with bevacizumab mild asymptomatic proteinuria is very frequent and ranges betweeen 21 to 63 %, but high grade (3+ on dipstick evaluation) or nephrotic (> 3.5 g/24 h) proteinuria probably occurs in less than 2 % of patients (Yeh et al., 2010). However, in patients with renal cell cancer treated with small orally active tyrosine kinase inhibitors heavy proteinuria may affect as many as 6.5 % of patients (Izzedine et al., 2010).

Bevacizumab therapy was associated with the development of proteinuria in 23–38 % of patients with colorectal cancer and as many as 64 % of patients with renal cell cancer (Yang et al., 2003; Gordon and Cunningham, 2005). According to the meta-analysis of randomized controlled trials with bevacizumab (Zhu et al., 2007), the relative risk of proteinuria was 1.4 in patients on a low dose (2.5 to 7.5 mg/kg) and 1.6 in patients on a high dose (10 to 15 mg/kg), so the appearance of proteinuria with bevacizumab seems to be dose dependent.

A recently published systematic review and meta-analysis of 16 randomized controlled trials with bevacizumab comprising 12,268 patients (Wu et al., 2010) confirmed low incidence of grade 3 and grade 4 proteinuria – only 2.2 % , but still the relative risk of high-grade proteinuria and nephrotic syndrome with bevacizumab compared to chemotherapy alone was 4.79 and 7.78, respectively. The highest risk was in patients with renal cell carcinoma (cumulative incidence 10.2 %). The relative risk was dose dependent, higher in patients on higher (5 mg/kg/week) than on lower (2.5 mg/kg/week) dose of bevacizumab (compared to chemotherapy only – lower dose 2.62, higher dose 8.56). All-grade proteinuria occurred in 13.3 % of bevacizumab-treated patients. Importantly, overall and progression-free survival of patients with high-grade proteinuria did not differ from other bevacizumab-treated patients, suggesting that even high-grade proteinuria may not have significant impact on the improved outcome of the bevacizumab-treated patients. The exact incidence of proteinuria in patients treated with small orally active VEGF tyrosine kinase inhibitors is less clear. In trials with newer VEGF tyrosine kinase inhibitor, axitinibe, all-grade proteinuria (from + dipstick to nephrotic proteinuria) occurred in 18–36 % of patients and high-grade proteinuria (4 + on dipstick, or more than 3.5 g/24 h urine collection) in only 5 % of patients (Izzedine et al., 2010).

Treatment with sunitinib or sorafenib may also result in preeclampsia-like syndrome with hypertension, moderate to nephrotic proteinuria and oedema (Patel et al., 2008), usually after several months of treatment (median time of peak protein excretion was 24 weeks in seven reported patients). Reduction of the dose of the drug or its withdrawal was followed by significant improvement of the control of hypertension and decrease of proteinuria. Damage to glomerular endothelial cells with anti-VEGF therapy in tumours is characterized by the disappearance of endothelial cells with preserved capillary basement membranes (Mancuso et al., 2006). Renal biopsy findings available in only several patients with severe renal involvement most frequently demonstrated thrombotic microangiopathy (Izzedine et al., 2007; Eremina et al., 2008); case reports of cryoglobulinemic membranoproliferative glomerulonephritis, focal proliferative glomerulonephritis may only be a coincidence, and collapsing glomerulopathy could have been caused by the concomitant treatment with pamidronate. One case report of sorafenib-induced interstitial nephritis has also been published (Izzedine et al., 2010).

Thrombotic microangiopathy in patients treated with VEGF inhibition may be under-diagnosed, as the histological findings in a limited number of biopsied patients were not related to the degree of proteinuria or haematuria and were not regularly accompanied by severe hypertension, renal failure, haemolytic anaemia and/or thrombocytopenia. Renal biopsy should thus be possibly considered even in patients treated with VEGF inhibition with relatively mild urinary abnormalities and in the absence of renal failure (Bolée et al., 2009). Not surprisingly, proteinuria is usually aggravated by concomitant hypertension. In patients treated by bevacizumab proteinuria was present in 54 % of patients with grade 2/3 hypertension and only in 16 % of patients with grade 0/1 hypertension (Yang et al., 2003). Patients with bevacizumab-induced proteinuria are also more commonly hypertensive (47.1 % vs. 16.9 %, Miller et al., 2005).

Proteinuria is also more common in patients treated concomitantly by bevacizumab and pamidronate (Miller et al., 2005), which may cause another type of glomerular damage, collapsing glomerulopathy. The factors associated with the occurrence and severity of proteinuria remain poorly understood, but pre-existing renal disease with higher proteinuria and hypertension (Zhu et al., 2007, 2009), African-American ethnicity (Zhu et al., 2009) and renal cell cancer compared to other cancers (Tomita et al., 2011) were identified as putative predisposing factors. Proteinuria in patients treated with bevacizumab may be dose dependent (see above, Zhu et al., 2007). On the other hand, duration of the treatment with VEGF inhibitors is probably not related to proteinuria and the occurrence of proteinuria does not seem to be related to the efficacy (to be a surrogate marker of efficacy) of the treatment with VEGF inhibitors (Izzedine et al., 2010).

## Hypertension in patients treated by VEGF inhibition

Hypertension is a frequent side effect of the systemic inhibition of VEGF. Its incidence and severity is related not only to the type of the drug used, dose of the drug and dosing schedule, but also to the age of the patients, pre-existing hypertension and coexisting cardiovascular disease (Izzedine et al., 2009a). In large studies in advanced and metastatic renal cancer (Hurwitz et al., 2005; Escudier et al., 2007; Motzer et al., 2007) and metastatic colorectal cancer (Yang et al., 2003), hypertension may newly occur (worsen) in 17–35 % of patients treated with VEGF inhibition compared to only in 1–8 % of patients in the placebo limb, and severe (grade 3/4) hypertension occurred in 4–20 % of VEGF inhibitor-treated patients compared to only 0–3 % in the placebo limb (same trials). Life-threatening hypertensive crisis is rare and may occur in less than 1 % of patients treated with VEGF inhibition. Higher incidence of intracerebral haemorrhage in patients with metastatic renal cancer and brain metastasis treated with VEGF inhibitors may also be at least partly related to poorly controlled hypertension (Poussel and Culine, 2008). A recent meta-analysis suggested that the incidence of hypertension in patients treated by angiogenesis inhibitors is 22.5–57.7 % (Wu et al., 2008) and the relative risk of developing hypertension is 7.5, 6.1 and 3.9 with bevacizumab, sorafenib and sunitinib, respectively (Wu et al., 2008; Izzedine et al., 2009b).

The mechanisms of "isolated" hypertension in patients treated with VEGF inhibitors is unclear, but could be related to increased systemic vascular resistance, possibly due to endothelial dysfunction with a decrease of nitric oxide (NO) production (VEGF normally stimulates NO production – Hood et al., 1998) and oxidative stress, or vascular rarefaction (decrease in the density of microvessels). In patients with metastatic breast cancer vandetanib, VEGFR2 and 3 tyrosine kinase inhibitor increased blood pressure and decreased plasma systemic nitrate/nitrite levels and endothelial cell nitrite levels (Mayer et al., 2011), although the eNOS membrane concentration doubled. Apparently, the exact mechanism by which inhibition of VEGF action results in the increase of blood pressure remains to be elucidated.

Recent studies (Kappers et al., 2011) demonstrated in both patients and rats that hypertension, proteinuria and renal dysfunction are associated with the activation of the endothelin system. In rats on sunitinib glomerular endotheliosis was accompanied by the increase of urinary excretion of endothelin 1 and diminished excretion of NO metabolites. Sunitinib-induced increase of blood pressure, but not renal histological abnormalities and increase of serum creatinine, could have been prevented by endothelin receptor antagonist macitentan. In swine sunitinib-induced hypertension, non-selective endothelin antagonist tezosentan completely reversed blood pressure to pre-sunitinib values (Kappers et al., 2012). The role of decreased NO bioavailability and oxidative stress, if any, is much less important than the apparent activation of the endothelin system. Proteinuria and renal function should be assessed in all patients with VEGF inhibition-related hypertension (or better reassessed as it should have been evaluated before the start of the treatment). Patients with concomitant proteinuria and renal dysfunction should be referred to the nephrologist to exclude glomerular disease and especially thrombotic microangiopathy.

Patients with hypertension induced by VEGF inhibitors should be treated in a similar way as other patients with hypertension, including lifestyle modifications and antihypertensive drugs, taking into consideration the putative interaction of some antihypertensive drug with the metabolism of VEGF tyrosine kinase inhibitors and the impact of the antihypertensive drugs on VEGF production. In most patients hypertension can be controlled by the available medication, and reduction of the dose of VEGF inhibitor or even temporary or permanent withdrawal of the drug should be considered in only exceptional cases of patients with persistent or severe hypertension. ACE inhibitors, beta-blockers, diuretics and calcium antagonists have been the most common drugs used in the treatment of VEGF inhibition-induced hypertension. As small orally active VEGF tyrosine kinase inhibitors are metabolized by cytochrome P450, CYP3A4 inhibitors verapamil and diltiazem should be avoided and as nifedipine was shown to stimulate vascular VEGF secretion (Miura et al., 2005), felodipine and amlodipine should be the preferred calcium channel blockers.

Long-acting oral nitrates may also quickly normalize blood pressure in patients with VEGF inhibition-induced hypertension (Dirix et al., 2007). Similarly effective could be phosphodiesterase inhibitors, e.g. sildenafil (Oliver et al., 2006) or nebivolol, which also increase NO concentration in the vascular wall. Very recently, endogenous inhibitor of angiogenesis with anti-tumour activity which also increases endothelial production of NO, endostatin, was shown to prevent hypertension induced by anti-VEGF antibodies in mice (Sunshine et al., 2012).

## VEGF inhibition in patients with reduced renal function

Small inhibitors of VEGF receptor-dependent tyrosine kinases are partly excreted by the kidneys. According to some recommendations these orally active inhibitors may be safely given to patients with decreased glomerular filtration rate, but close monitoring and dose adjustment is necessary (Khan et al., 2010; Gupta et al., 2011). There may be, however, worsening of renal function in more than 50 % of patients with renal insufficiency treated by sunitinib or sorafenib (Khan et al., 2010).

Glomerular injury may be aggravated during anti-VEGF treatment in patients with pre-existing renal disease and decreased renal function. More frequent proteinuria in patients with renal cell cancer compared to e.g. colorectal cancer may be related in some of them to concomitant hyperfiltration in residual nephrons if the patients underwent nephrectomy. Concomitant use of other nephrotoxic drugs (e.g. pamidronate, gemcitabine, or interferon α) should also be taken into consideration.

In the remnant kidney model induced in rats by 5/6 renal ablation (Machado et al., 2012) VEGF inhibition by sunitinib had no effect on the already present expansion of renal cortical interstitium and rarefaction of peritubular capillaries (demonstrating little antiangiogenic effect) and induced neither hypertension nor proteinuria, but aggravated glomerulosclerosis, possibly as a result of capillary microthrombosis. No effect of sunitinib in control rats demonstrates that already existing renal damage increases the risk of nephrotoxicity of VEGF inhibition.

Although according to the manufacturer's labelling for sorafenib no dose adjustment should be necessary even for patients with severe renal insufficiency not undergoing dialysis according to the phase 1 study (Miller et al., 2009), the sorafenib dose should be preferably reduced to 200 mg twice daily in patients with creatinine clearance 20 to 30 ml/min and to only 200 mg once daily in patients on haemodialysis. The optimal initial dosing of sorafenib for patients with creatinine clearance < 20 ml/min not yet on dialysis is currently unclear. When the patients undergoing haemodialysis were treated by higher doses of sorafenib (200 mg twice daily), both the higher (Kennoki et al., 2011) and the same (Masini et al., 2012) rate of adverse events was reported. Some patients on haemodialysis may apparently tolerate the doses as high as 400 mg twice daily (Rey and Villavicencio, 2008).

There is only a limited number of reports (in a small number of patients) on the efficacy and safety of sunitinib in patients with reduced renal function (Izzedine et al., 2009a; Khosravan et al., 2010; Josephs et al., 2011; Masini et al., 2012). A single dose of 50 mg of sunitinib appeared to be well tolerated in patients with severe renal insufficiency (creatinine clearance < 30 ml/min) and patients with end-stage renal disease on haemodialysis (Khosravan et al., 2010). Pharmacokinetics of sunitinib and its major metabolite (SU12662) seemed to be similar for patients with normal renal function, and the plasma levels of sunitinib were even lower in patients with end-stage renal failure treated by haemodialysis. In another study (Izzedine et al., 2009) pharmacokinetics of sunitinib in two patients with end-stage renal failure on haemodialysis seemed to be similar for patients with normal renal function. Starting doses from 25 to 50 mg of sunitinib daily for four out of six weeks were used in another study in patients with severe renal impairment (creatinine clearance < 30 ml/min), 10 of them already on haemodialysis, with similar efficacy as in normal subjects. Dose reduction due to toxicity was necessary in eight patients, but only in one of them permanent discontinuation was necessary (Josephs et al., 2011). Out of the adverse events only diarrhoea, hand-foot skin syndrome and neutropenia were more common in patients on dialysis compared to non-dialysis patients (Josephs et al., 2011). In conclusion, sunitinib at a dose of 25 to 50 mg daily seems to be well tolerated even in patients with severe renal insufficiency or end-stage renal disease on dialysis.

## Mechanisms of proteinuria in VEGF inhibition

As already stressed, VEGF is indispensable for normal glomerular development and maintenance of normal fenestrated endothelium (Eremina and Quaggin, 2004) and VEGF inhibition may not only cause proteinuria and hypertension (Izzedine et al., 2007, 2010; Kappers et al., 2009), but may even induce renal thrombotic microangiopathy (Eremina et al., 2008).

There are several putative pathogenic types of the proteinuria (Table 1) induced by the use of VEGF inhibitors: 1. Haemodynamically mediated glomerular in-

*Table 1. Putative mechanisms of proteinuria induced by anti-VEGF treatment*

| Type of glomerular injury | Mechanisms |
|---|---|
| Haemodynamically mediated glomerular injury | High systemic blood pressure and high glomerular pressure, possibly related to decreased production of NO |
| Podocyte and slit diaphragm damage | Down-regulation of some podocyte proteins (e.g. nephrin) important for the normal function of the slit diaphragm, or podocyte apoptosis (probably uncommon) |
| Endothelial damage | Damage to glomerular endothelial cells (glomerular endotheliosis) caused by the decreased production of VEGF by podocytes |
| Thrombotic microangiopathy | More severe damage to glomerular (and systemic) endothelial cells with associated glomerular thrombosis due to low circulating and/or podocyte-derived VEGF |

jury. 2. Damage to glomerular endothelial cells related to the interference with their stimulation by podocyte-derived VEGF. 3. Podocyte and more specifically slit diaphragm damage caused by down-regulation of some podocyte proteins. 4. Subacute thrombotic microangiopathy (Izzedine et al., 2010).

Proteinuria could be the result of systemic and glomerular hypertension, as suggested by the concomitant decrease of both hypertension and proteinuria on anti-VEGF therapy withdrawal (Advani et al., 2007; Izzedine et al., 2007; Zhu et al., 2007). It has been suggested that the mechanism of proteinuria in patients treated by anti-VEGF therapy may be similar as in post exercise proteinuria (Gündüuz et al., 2003; Izzedine et al., 2010), as the mechanism of both hypertension and proteinuria in these settings may be related to the decreased production of NO. In the murine model (Eremina et al., 2008), however, glomerular injury preceded hypertension, suggesting that in patients treated by anti-VEGF therapy hypertension may not be the only cause of proteinuria.

Podocyte-derived VEGF stimulates VEGFR2 on glomerular endothelial cells (Eremina et al., 2003). Inhibition of VEGF (Kamba et al., 2006) or tageted deletion of the *VEGF* gene in podocytes (Eremina et al., 2003) results in the loss of fenestra of glomerular endothelial cells, proliferation of endothelial cells (endotheliosis) and proteinuria. These changes are very similar to glomerular damage in preeclampsia, where the overproduction of soluble VEGF receptor (sFlt1) in placenta blocks the circulating and possibly also podocyte-derived VEGF (Eremina et al., 2008), suggesting that the damage to glomerular endothelial cells could be the major cause of proteinuria in patients on anti-VEGF therapy. More severe changes with the same pathogenesis may result in thrombotic microangiopathy.

Anti-VEGF treatment may also possibly cause direct damage to the podocytes with down-regulation of the major protein of the slit diaphragm nephrin (Sugimoto et al., 2003). The relative contribution of endothelial cell and podocyte damage to proteinuria is uncertain, but currently the injury to glomerular endothelial cells is supposed to be more important. The exact mechanisms of glomerular toxicity of VEGF remain to be fully elucidated and may be different in different patients. Tissue hypoxia caused by the reduced angiogenic activity in renal tissue with resultant interstitial inflammation (Tanaka and Nangaku, 2010) and the direct damage to the glomerular endothelium resulting in thrombotic microangiopathy (Eremina et al., 2008) are the leading hypotheses, although a direct toxic effect of VEGF inhibition on the podocytes (Eremina et al., 2007) cannot be excluded, either.

## Management of proteinuria in patients treated with VEGF-inhibiting drugs

All patients considered for treatment with VEGF-inhibiting drugs should have assessed blood pressure, at least dipstick examination of proteinuria and calculated glomerural filtraton rate before starting the treatment. In patients with normal blood pressure, normal renal function and negative proteinuria, repeated screening should be done before each cycle of treatment or every other infusion. Patients with positive dipstick proteinuria (more than +) should have their proteinuria quantified using the albumin/creatinine ratio, and referral to the nephrologist for further work-up (quantitative proteinuria, haematuria, renal ultrasound, potentially even renal biopsy) should be considered.

It should be stressed that as there is no correlation between the degree of proteinuria and the severity of renal damage (half of the patients with biopsy finding of thrombotic microangiopathy may have only + to ++ proteinuria on dipstick evaluation), renal biopsy should be considered in all patients with unexplained (before the start of the treatment) and persistent or progressive (on treatment) proteinuria, and especially in patients with unexplained acute or progressive decline of renal function. In patients with metastatic cancer, life expectancy of the patient and available therapeutic options should always be taken into consideration. Anti-VEGF therapy dramatically improved the outcome of patients especially with metastatic renal cell cancer, and it is therefore always difficult to withdraw the effective treatment because of laboratory findings only. Although there is no general recommendation, treatment should probably be withdrawn in patients with nephrotic proteinuria, progressive decline of renal function, or histologic finding of thrombotic microangiopathy on renal biopsy.

Patients with proteinuria associated with VEGF-inhibiting treatment should be given antihypertensive treatment (if hypertensive) and ACE inhibitors or angiotensin receptor blockers to reduce proteinuria (Dincer and Altundag, 2006), although their targets and recommended doses are unclear, especially in normotensive subjects. Discontinuation of treatment usually results in the reduction of proteinuria, but persistence of some proteinuria even after the withdrawal of the drug is common (Patel et al., 2008), even the persistence of nephrotic syndrome (Okuno et al., 2011) or renal dysfunction (Takahashi et al., 2012). Manufacturer's labelling for bevacizumab recommends intermittent monitoring for proteinuria and temporary withdrawal of the drug for patients with proteinuria > 2 g/24 h and permanent withdrawal for patients with nephrotic syndrome. In clinical practice, the presence of proteinuria may impact the clinical decision of oncologists in only 2 % of bevacizumab-treated patients (Yeh et al., 2010). For small orally active tyrosine kinase inhibitors the only recommendation is withdrawal of the drugs in patients who develop nephrotic syndrome.

It is currently unclear whether all patients with anti-VEGF treatment-induced thrombotic microangiopathy require intensive treatment with corticosteroids and plasma infusion/exchange after anti-VEGF treatment withdrawal. In a small pilot prospective study (Izzedine et al., 2011) intensive treatment (maximally tolerated

antihypertensive treatment + corticosteroids + fresh plasma infusion +/- plasma exchange) was not superior to conservative treatment (only maximally tolerated antihypertensive treatment including ACEI or ARB to achieve blood pressure under 130/80 mm Hg) in terms of blood pressure control, decrease of proteinuria and preservation of renal fuction in patients with biopsy-proven anti-VEGF treatment-induced thrombotic microangiopathy after anti-VEGF agent withdrawal. Patients in the intensive limb, however, had higher baseline proteinuria and lower platelets, and intensive treatment was successful in normalizing haemoglobin, platelets and lactate dehydrogenase. Apparently, intensive treatment need not necessarily be used in all patients with anti-VEGF treatment-induced thrombotic microangiopathy, but still should be considered in patients with nephrotic proteinuria, impaired renal function and low platelets.

In patients with angiogenesis inhibitor-induced proteinuria the use of anti-proteinuric drugs, e.g. ACE inhibitors and/or angiotensin receptor blockers, may be preferable, although no specific recommendation exists and controlled trials demonstrating their antiproteinuric efficacy in this specific setting are available.

## Conclusions

The importance of VEGF in the maintenance of integrity of the glomerular filtration barrier has direct implications for the use of VEGF inhibitors in patients with cancer. Anti-VEGF therapy may induce proteinuria and hypertension. Damage to the glomerular endothelial cells with imminent thrombotic microangiopathy is probably the most important mechanism of the derangement of glomerular filtration barrier.

Oncologists should be aware of the potential renal toxicity of VEGF inhibitors, and baseline proteinuria and renal function should be assessed in all patients before the treatment and regularly checked during the treatment. Referral to the nephrologist should also be considered.

Optimal use of the available antiproteinuric regimens (ACE inhibitor or angiotensin receptor blockers) should be studied prospectively. Withdrawal of anti-VEGF treatment should be considered in patients with nephrotic proteinuria and progressive decline of the renal function.

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