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# VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management

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ABSTRACT

Proteinuria is a dose-related side-effect occurring after inhibition of vascular endothelial growth factor (VEGF) signalling and may reflect severe glomerular damage. The inhibition of the VEGF signalling axis induces downexpression or suppression of nephrin, an important protein for the maintenance of the glomerular slit diaphragm, sometimes leading to nephritic syndrome and/or glomerular thrombotic microangiopathy, the main-associated kidney disease. A MEDLINE search was carried out using the following criteria: (1) all MEDLINE listings as of 01-01-2000 with abstracts; (2) English language; and (3) Humans. The following phrases were used to query the database: (proteinuria) AND (anti-VEGF OR VEGF inhibition OR bevacizumab OR sunitinib OR sorafenib OR VEGF Trap OR axitinib OR pazopanib OR AZ 2171). The references of each article identified were carefully reviewed for additional reference. The incidence of mild and asymptomatic proteinuria ranges from 21% up to 63%, but heavy proteinuria has been reported in up to 6.5% of renal cell carcinoma patients. Although discontinuation of anti-VEGF agent induced significant reduction, persistence of proteinuria is common. Although angiotensinconverting-enzyme inhibitors and/or angiotensin receptor blockers seem to be preferred, no specific recommendation for an antiproteinuric agent can be made in this context because there are no controlled studies addressing the subject. Periodic monitoring of urinary protein should be carried out in anti-VEGF-treated patients and patients showing proteinuria need special referral to nephrologists.



## 1. Introduction

Proteinuria and/or hypertension are shared toxic effects among all therapies targeting the vascular endothelial growth factor (VEGF) pathway. Proteinuria can be a major clue to underlying renal disease or a transient finding in those patients. The onset of urinary protein excretion is of importance because proteinuria is a prognostic marker and an independent risk factor for cardiovascular disease.[1] Whether the development of proteinuria might also serve as a surrogate marker of on-target effect (antitumour efficacy) and/or off-target effect (adverse event) is unknown. This article will deal

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with the evaluation of a treated-patient with proteinuria, what basic investigations are needed and when to refer to a nephrologist.

## 2. Proteinuria: physiology, detection and clinical evaluation

The term proteinuria is taken to mean abnormally high protein excretion in the urine. Proteinuria is the consequence of glomerular filtration of plasma proteins, their defective subsequent reabsorption by the proximal tubular cells and secretion by the tubular cells and distal urinary tract. In humans, close to 180 l of primary urine are produced each day at capillary pressures. Only 5 mg of proteins pass across the glomerular barrier per litre of primitive urine, this is about 900 mg a day. Up to 98% of these are reabsorbed in the proximal tubule. There is a further source of protein secretion from the distal loop of Henle (Tamm–Horsfall protein). Finally, urinary protein excretion in a normal adult should be less than 150 mg/day or 60 mg/m$^2$/day. Proteinuria is said to be present when the urine contains more than 300 mg protein per day (or 200 mg/l). Therefore, the quantitative and qualitative evaluation of proteinuria is important for the diagnosis of renal disease. Urine with daily protein excretion of 30–300 mg and superior to 300 mg reflects micro and macroalbuminuria, respectively (Available at: http://www.emedicine.com/ped/topic3048.htm. Accessed November 27, 2008).

There are several qualitative and quantitative tests available for the measurement of urinary protein. A urinalysis dipstick is quite sensitive for albuminuria but is insensitive to the presence of non-albumin proteins. Thus pure tubular proteinuria will not be diagnosed unless a 24-h urine sample is collected. A positive dipstick (only when protein excretion exceeds 300 mg/day) usually reflects glomerular proteinuria. They are intended to correlate as follows: + with 0.3 g/l, ++ with 1 g/l, +++ with 3 g/l and ++++ with >3 g/l. False-positive results may be obtained with concentrated or alkaline urine and with many iodinated radiocontrast agents.[2] False-negative results may be obtained with dilute or markedly acidic urine. On the other hand, there are a variety of semiquantitative dipsticks which can be used to screen for albuminuria, including microalbuminuria. Whenever proteinuria is suspected, a urine sample should be sent for laboratory quantification. The gold standard in adults is a 24-h collection so that protein excretion may be expressed per unit body surface area per day (mg/m$^2$/day). Timed urine collections are not always practical, particularly in old patients or those with deteriorating general conditions. Many clinicians prefer to use albumin excretion in relation to that of creatinine to correct for differences in urine dilution. Normally, the albumin-creatinine concentration ratio is <30 μg/mg.[3,4] There are three basic types of proteinuria: glomerular, tubular and overflow.[5] All three types are easily separated by urine protein electrophoresis. Glomerular proteinuria is due to increased filtration of macromolecules (such as albumin, molecular weight 69,000) across the glomerular capillary wall and is identified on a urine dipstick. Tubular proteinuria or low molecular weight proteins, such as ß2-microglobulin, immunoglobulin light chains, retinol-binding protein, and amino acids, can be filtered across the glomerulus and are then almost completely reabsorbed in the proximal tubule. Tubular proteinuria is often not diagnosed clinically since the urinalysis sticks do not detect proteins other than albumin. It occurs when there is failure of resorption of proteins secreted by the proximal tubule, and is indicative of proximal renal tubular damage expressed as partial or more generalised Fanconi syndrome (acidosis, hypokalaemia, reduced tubular reabsorption of phosphate and uric acid, normoglycaemic glycosuria or aminoaciduria).

Overflow proteinuria is due to immunoglobulin light chains in multiple myeloma, lysozyme, myoglobin, or haemoglobin.[6] A combination of these different patterns of proteinuria can occur. It is important to understand how to differentiate amongst "benign" transient proteinuria (e.g. only one positive test proteinuria), common causes of pathologic proteinuria (such as diabetic nephropathy, previous known glomerular disease), and molecular targeted therapy (MTT)-induced proteinuria that requires further evaluation and possible kidney biopsy (Fig. 1). In most cases of new or heavy proteinuria (>3 g/day) associated with microscopic haematuria, a kidney biopsy is generally required.

## 3. Evidence for MTT-induced proteinuria

Anti-VEGF agents are generally well tolerated. Hypertension and asymptomatic proteinuria are common dose-related side-effects that frequently occur together.[7,8] Incidence and rate of proteinuria are variable in different studies according to patients' characteristics, signals targeted and cancer type. Clinical reports suggest that many patients may have increased protein excretion during treatment with bevacizumab. Tables 1 and 2 summarise the National Cancer Institute's proteinuria gradings and findings of the available phase 2 and 3 studies concerning the proteinuria induced by VEGF-targeted therapies.[9–28] Bevacizumab therapy has been associated with the development of proteinuria in 23–38% of patients with colorectal cancer, and in up to 64% of patients with renal cell carcinoma in whom 6.5% experienced a grade 3–4 proteinuria denoting structural damage to the glomerular filtration barrier.[12,29] In the AVOREN (*Avastin* for Renal Cell Cancer) study,[13] out of the 95 patients who received bevacizumab plus interferon alfa for longer than 1 year, 6 and 3% reported grade 3/4 proteinuria and hypertension, respectively. However, in the First Bevacizumab Expanded Access Trial (BEAT) trial, grade 3–5 proteinuria related to bevacizumab was found in only 0.4% of 1903 patients. Furthermore, in the TREE (Three Regimens of Eloxatin Evaluation) trials the incidence of grade 3/4 proteinuria was low and similar in all groups (1%) including the bevacizumab group.[30] A meta-analysis of randomised controlled trials with patients receiving bevacizumab indicated a relative risk of 1.4 for proteinuria with bevacizumab at a low dose (2.5 to 7.5 g/kg) and 1.6 for a high dose (10 to 15 mg/kg)[7] suggesting a dose-dependency to bevacizumab-associated proteinuria. In a phase 1 trial of the small-molecule VEGF receptor antagonist KRN951, 14 of 15 patients developed hypertension and three patients developed dose-limiting proteinuria.[31] Patel et al. reported a preeclampsia-like syndrome characterised by

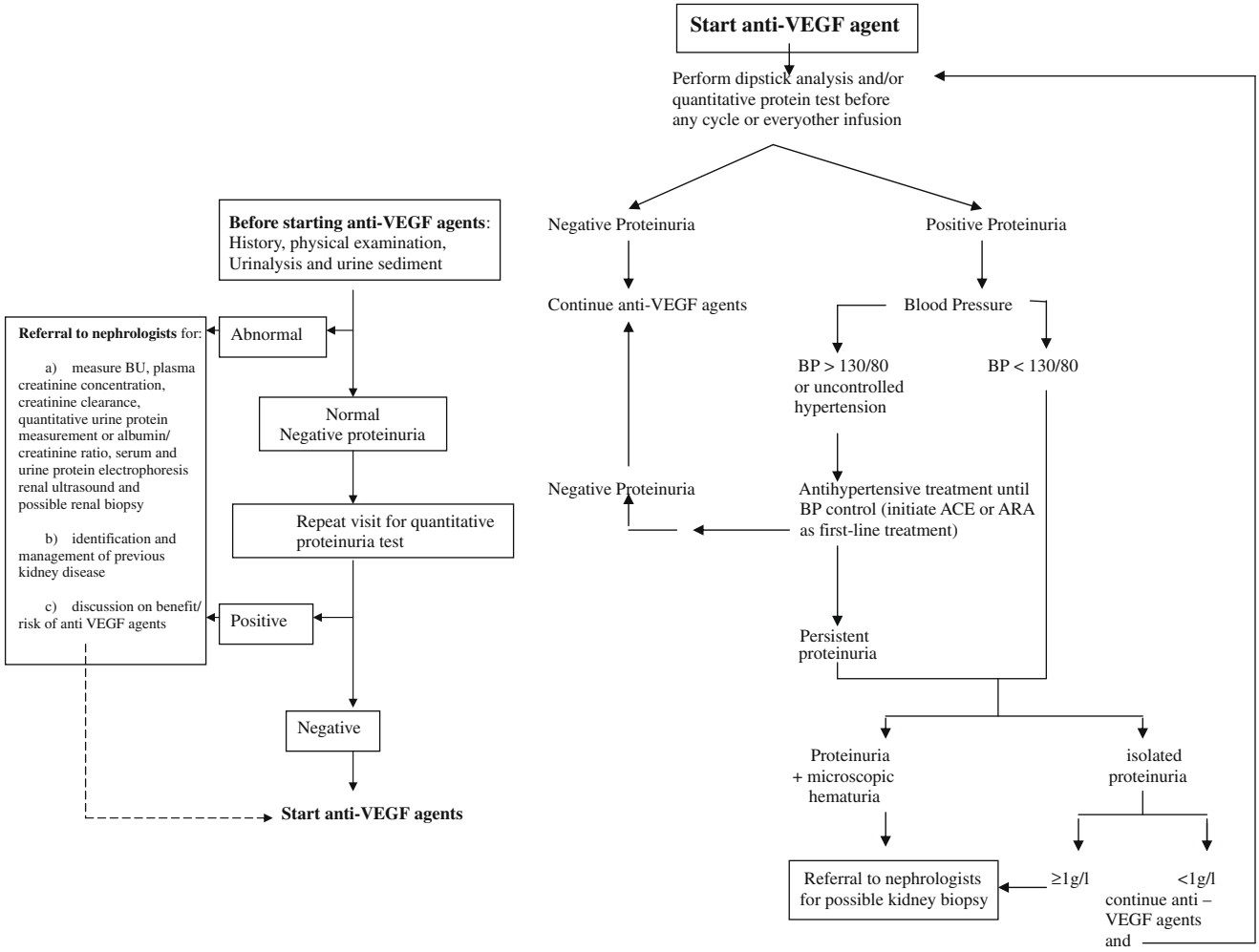


**Fig. 1 – Management of proteinuria induced by angiogenic inhibitor.**

oedema, hypertension and proteinuria in seven patients after starting therapy with the oral multitargeted kinase inhibitors sunitinib and sorafenib. All seven patients developed proteinuria (average 3.8 g/g, range 1.1–10.4 g/g), with peak urine protein excretion occurring at a median of 24 weeks. In most patients, the dose was either reduced or the drug discontinued. Subsequently, in patients with follow-up information, there was a dramatic improvement of blood pressure control and proteinuria level.[32] The renal pathological findings in anti-VEGF therapy (mainly bevacizumab)-associated proteinuria include 12 cases of thrombotic microangiopathy (TMA),[33–38] two cases of collapsing glomerulopathy,[15,37] which probably reflects podocyte toxicity due to concomitant pamidronate therapy,[39] and one case each of cryoglobulinaemic glomerulonephritis,[40] immune complex-associated focal proliferative glomerulonephritis[41] and sorafenib-induced acute interstitial nephritis[42] (Table 3). Reports of biopsy-proven renal TMA secondary to VEGF-targeted therapies highlight the possible discrepancy between the observed mild clinical manifestations (proteinuria <1 g/day, easily controlled hypertension, inconsistent microscopic haematuria and/or renal failure), inconsistent biological features of TMA (e.g. haemolytic anaemia, thrombopaenia, schistocytes) and the underlying histology. TMA is therefore likely under diagnosed. This pleads for a wider indication of renal biopsy in these patients, even in the absence of renal failure and biological features of TMA.[38] The incidence of proteinuria in patients receiving concurrent bevacizumab and pamidronate was 33.9%, compared with 18.5% in patients receiving bevacizumab without pamidronate ($p < 0.026$).[15] From October 2005 to June 2007, 40 patients with mRCC were treated with sunitinib (50 mg given daily for 4 weeks followed by 2 weeks off) either in a phase 2 trial or in the expanded access programme. Twenty-four (60%) and 6 (15%) experienced grade 1 and 3 proteinuria, respectively. Treatment was interrupted and dose decreased for proteinuria in 5 patients (12.5%). Proteinuria reverses at least partially after drug discontinuation in most cases. In some, however, treatment was resumed without further worsening of proteinuria.[43] Proteinuria is partially correlated with hypertension: 54% of patients with grade 2/3 hypertension developed proteinuria grade 2/3, and 16% of patients with grade 0/1 hypertension developed proteinuria under bevacizumab.[12] In the combination arm of the study of Miller et al., patients who developed proteinuria were more likely to become hypertensive (47.1% versus 16.9%, $p \leqslant 0.001$) than patients who did not develop proteinuria.[15] Whether there is a difference between proteinuria produced by VEGF ligand binding agents and that produced by VEGF

**Table 1 – NCI Proteinuria grading.**

| | |
|---|---|
| Grade 0 | Negative proteinuria |
| Grade 1 | 1+ on urine dipstick test or 0.1 to 1 at 24-h urine collection |
| Grade 2 | 2+ /3+ on urine dipstick test or 1 to 3.5 g at 24-h urine collection |
| Grade 3 | 4+ on urine dipstick test or more than 3.5 g at 24-h urine collection |
| Grade 4 | Nephrotic syndrome |

Common Terminology for Adverse Events (v 2.0) Grading of Hypertension in Cancer Trials [Cancer Therapy Evaluation Programme. Common terminology criteria for adverse events (version 2.0; April 30,1999). Rockville, MD: National Cancer Institute, National Institutes of Health. Available at: http://ctep.cancer.gov/reporting/ctc.html. Accessed March 28, 2006].

receptor inhibitors is not known. Data concerning the prevalence of tyrosine kinase inhibitor-induced proteinuria are lacking except for phase 2 Axitinib trials (all grades, 18 to 36% and grade 3/4, 0 to 5%) (Table 2). The relationship between duration of treatment and proteinuria and whether the development of proteinuria might also serve as a surrogate marker of antitumour efficacy remains unknown. The identification of factors that confer susceptibility to overt glomerular disease in this subgroup of patients will be important. Poor renal reserve (e.g. pre-existing renal disease, past

**Table 2 – Incidence of VEGF-targeted therapy-associated proteinuria compared to controls in selected randomised phase II/III clinical trials.**

| Disease | Author | Regimen | Patient, *n* | Proteinuria (%) | |
|---|---|---|---|---|---|
| | | | | All grades | Grade 3/4 |
| mCRC | Hurwitz et al., 2004 | Irinotecan, Fluorouracil, leucovorin | 397 | 21.7 | 0.8 |
| | | IFL + Bevacizumab 5 mg/kg | 393 | 26.5 | 0.8 |
| | Hurwitz et al., 2005 | Fluorouracil + leucovorin + Placebo | 98 | 25.1 | 0 |
| | | Fluorouracil + leucovorin + Bevacizumab 5 mg/kg | 109 | 34.9 | 1.8 |
| | Giantonio et al., 2007 | FOLFOX4 + Bevacizumab 10 mg/kg | 287 | NA | 0.7 |
| | | FOLFOX4 | 285 | NA | 0 |
| | | | 234 | NA | 0 |
| | Tang et al., 2008 | VEGF Trap 4 mg/kg every 2 weeks | 51 | 49 | 7.8 |
| mRCC | Yang et al., 2003 | Placebo | 40 | 15 | 0 |
| | | Placebo + Bevacizumab 3 mg/kg | 37 | 15 | 2 |
| | | Placebo + Bevacizumab 10 mg/kg | 39 | 25 | 3 |
| | Motzer et al., 2007 | Interferon alpha | 375 | NA | NA |
| | | Sunitinib 50 mg once daily for 4 weeks | 375 | NA | NA |
| | Escudier et al., 2007 | Placebo + Interferon alpha | 322 | 3 | 0 |
| | | Interferon alpha + Bevacizumab 10 mg/kg | 327 | 18 | 7 |
| | Escudier et al., 2007 | Placebo | 452 | NA | NA |
| | | Sorafenib 400 mg twice daily | 451 | NA | NA |
| | Rini et al., 2008 | Interferon alpha | 349 | NA | 0 |
| | | Interferon alpha + Bevacizumab 10 mg/kg | 366 | NA | 15 |
| | Hutson et al., 2007 | Placebo | 27 | NA | NA |
| | | Pazopanib 800 mg daily | | NA | NA |
| | Rixe et al., 2007 | Axitinib 5 mg twice daily | 52 | 36.7 | 0 |
| | Sridhar et al., 2007 | AZD2171 45 mg daily | 37 | NA | NA |
| NSCLC | Sandler et al., 2006 | Carboplatin + Paclitaxel | 444 | NA | 0 |
| | | Carboplatin + Paclitaxel + Bevacizumab 15 mg/kg | 434 | NA | 3.1 |
| | Massarelli et al., 2007 | VEGF Trap 4 mg/kg every 2 weeks | 33 | NA | 9 |
| mBC | Miller et al., 2005 | Capecitabine | 215 | 7.4 | 0 |
| | | Capecitabine + Bevacizumab 15 mg/kg | 247 | 25.3 | 0.9 |
| | Miller et al., 2007 | Paclitaxel | 346 | NA | 0 |
| | | Paclitaxel + Bevacizumab 10 mg/kg | 365 | NA | 3.5 |
| Ad TC | Cohen et al., 2008 | Axitinib 5 mg twice daily | 60 | 18 | 5 |
| AdPC | Spano et al., 2008 | Gemcitabine | 34 | NA | 0 |
| | | Gemcitabine + Axitinib 5 mg twice daily | 69 | NA | 0 |
| Hepatoma | Cheng et al., 2009 | Placebo | 76 | NA | NA |
| | | Sorafenib 400 mg twice daily | 150 | NA | NA |
| EOC | Tew et al., 2007 | VEGF Trap 2 or 4 mg/kg every 2 weeks | 162 | 7 | 4 |

mCRC, metastatic colorectal cancer; NSCLC, non-small cell lung cancer; ARMD, age-related macular degeneration; GIST, gastrointestinal stromal tumour; mBC, metastatic breast cancer; mRCC, metastatic renal cell carcinoma; AdTC, advanced thyroid cancer; AdPC, advanced pancreatic cancer; EOC, epithelial ovarian cancer; NA, not available.

**Table 3 – Biopsy-documented kidney diseases in anti-VEGF-treated patients.**

| Disease | Targeted therapy | | Past medical history and/or other previous or concomitant therapies | Proteinuria | | Kidney biopsy findings | Follow-up after anti-VEGF discontinuation | | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Agent | Dose | | Onset | NCI Grade (g/24 h) | | Month | Proteinuria Grade (g/day) | |
| RCC | BVZ | 10 mg/kg | Nephrectomy IFN-α | 2 weeks | Grade 3 (6g) | Glomerular TMA and IgA ICGN | 2 | Grade 2 (2.63) | 34 |
| RCC | BVZ | N/A | Nephrectomy, Diabetes IFN-α | 9 months | Grade 2 (1.8) | Glomerular TMA | 16 | Grade 2 (1.8) | 35 |
| RCC | BVZ | N/A | FN-α | 7 months | Grade 1/2 | Glomerular TMA | N/A | N/A | 35 |
| NSCLC | BVZ | 7.5 mg/kg | Carboplatin/paclitaxel | N/A | Grade 4 | Cryoglobulinemic GN | N/A | N/A | 39 |
| BC | BVZ | 15 mg/kg | CKD Capecitabine/Pamidronate | N/A | Grade 4 | Collapsing GN | N/A | N/A | 15 |
| PC | BVZ | 5 mg/kg | Gemcitabine/Capecitabine | 1 month | Grade 4 (9.6) | Proliferative ICGN | 9 | Grade 1 (0.4) | 40 |
| HC | BVZ | 7.5 mg/kg | Unremarkable | 9 months | Grade 2 (3.4) | Glomerular TMA | 9 | Grade 2 (1.7) | 36 |
| HC | BVZ | 7.5 mg/kg | Unremarkable | 3 months | Grade 2 (2.7) | Glomerular TMA | 3 | Grade 0–1 (0.03) | 36 |
| BAC | BVZ | 15 mg/kg | Gemcitabine/Cisplatine | N/A | Grade 1 (0.16) | Glomerular TMA | N/A | N/A | 36 |
| SLC | BVZ | 10 mg/kg | CKD, Diabetes Cisplatin/Docetaxel | 3 months | Grade 1 (0.5) | Glomerular TMA | 2 | Resolved | 36 |
| PC | BVZ | 10 mg/kg | Gemcitabine, Erlotinib | 5 months | Grade 4 (4.6) | Glomerular TMA | N/A | N/A | 36 |
| OC | BVZ | 15 mg/kg | Unremarkable | 9 months | Grade 1 (0.8) | Glomerular TMA | N/A | N/A | 36 |
| MDA | BVZ | 10 mg/kg | Paclitaxel/Pamidronate | 3 months | Grade 4 (3.6) | Glomerular TMAand Collapsing GN | 6 | Grade 1 (0.99) | 37 |
| OC | VEGF Trap | 4 mg/kg | Gemcitabine LV5FU2/CPT11 | 1 week | Grade 4 (16.6) | Glomerular TMA | 2 | Grade 1 (0.3) | 33 |
| RCC | Sorafenib | 400 mg/d | Nephrectomy Sunitinib | 10 days | Grade 2 (2.4) | AIN | N/A | N/A | 42 |
| SH | Sunitinib | 37.5 mg/d | Radiotherapy Taxol, adriamycine | 2 weeks | Grade 2 (1) | Glomerular TMA | 3 | Undetectable | 38 |

NCI, National Cancer Institute; VEGF, vascular endothelial growth factor; RCC, renal cell carcinoma; BVZ, bevacizumab; IFN-, interferon-alpha; TMA, thrombotic microangiopathy; ICGN, immune complex glomerulonephritis; N/A, not available; CKD, chronic kidney disease; NSCLC, non-small-cell lung cancer; BC, breast cancer; PC, pancreatic cancer; HC, hepatocarcinoma; BAC bronchoalveolar carcinoma; SLC small-cell lung carcinoma; OC, ovarian cancer; MDA: mammary ductal adenocarcinoma; LV5FU2, LV5FU2-CPT11, leucovorin5-fluorouracil/capecitabine; AIN, Acute interstitial nephritis; SH, skin hydradenoma.

medical history of hypertension, Afro–American origin and renal cell carcinoma...) may be a predisposing factor.[44,45]

## 4. Potential mechanisms and significance of MTT-induced proteinuria

### 4.1. *Common proteinuria*

The causes of common proteinuria are attacks to the filtration barrier in the glomeruli of the kidney cortex. This barrier has three layers: the fenestrated endothelium, the glomerular basement membrane, and the podocytes with the slit diaphragm. The fenestrated endothelium would constitute a physical barrier for macromolecules in the plasma. The glomerular basement membrane provides structural support for the capillary wall. How these two layers contribute to macromolecular filtration is not clearly understood. The podocyte slit diaphragm, however, has an important and direct role in glomerular filtration. Some of its protein components are involved in the mechanism of proteinuria including nephrin, Neph, FAT, podocin, CD2-associated protein (CD2AP) and others. Inactivation of these protein genes in mice causes massive proteinuria, and sometimes absence of a slit diaphragm and death. Recent studies in genetically modified mice suggest that podocyte-derived VEGF has a major role in the development of the endothelium and the maintenance of its fenestrations.[46]

### 4.2. *Proteinuria related to angiogenesis inhibition*

The pathogenesis of proteinuria in patients receiving anti-VEGF therapy likely relates to multiple pathways.

#### 4.2.1. *Post exercise proteinuria-like syndrome*

It has been suggested that the elevation in blood pressure leads to proteinuria and glomerular disease in bevacizumab-treated patients.[7,8,47] Indeed, in some patients, both hypertension and proteinuria uniformly decreased after cessation of anti-VEGF therapy suggesting a possible haemodynamic mechanism similar to the temporary proteinuria related to nitric oxide (NO) blockage inducing renal haemodynamic alteration occurring after exercise.[48] As hypertension associated with VEGF inhibition is, for an important part, thought to involve decreased vascular production of NO, anti-VEGF therapy looks like physical exercise in some respects. Hence, in some patients, the causality relationship between hypertension, proteinuria and angiogenesis inhibitor introduction and their regression upon withholding the treatment would be coincident to an initial decrease in NO expression followed by a resuming of its normal levels of production, respectively. However, in their murine model, Eremina et al. found that glomerular injury preceded hypertension, indicating that elevated blood pressure cannot be the only trigger for proteinuria in anti-VEGF treated patients.[36]

#### 4.2.2. *Perturbation of podocyte-endothelial VEGF axis signalling*

VEGF is constitutively expressed by podocytes, and VEGF receptors are present on normal glomerular capillary endothelial cells.[49] The common occurrence of proteinuria after inhibition of VEGF signalling reflects the importance of VEGF in normal renal function.[49,50] VEGF, expressed by podocytes, activates VEGFR-2 on glomerular capillary endothelial cells. Pharmacological inhibition[51] or targeted heterozygous deletion of VEGF in podocytes[49,50] results in renal pathology manifested by loss of endothelial fenestrations in glomerular capillaries, proliferation of glomerular endothelial cells (endotheliosis), loss of podocytes, and proteinuria in mice. Interestingly, in human preeclampsia, fenestrated endothelium is especially vulnerable to injury, and the placenta overproduces a soluble VEGF receptor that acts as a VEGF antagonist[52,53]; conversely, podocyte-specific overexpression of VEGF leads to collapsing glomerulopathy.[54] These studies suggest that neutralisation of physiologic levels of VEGF, a key endothelial survival factor, may lead to proteinuria.

#### 4.2.3. *Podocyte protein junction down-regulation*

It has been demonstrated, in both mice[55,56] and humans,[57] that anti-VEGF antibodies and fms-like tyrosine kinase-1 (sFlt-1) cause rapid glomerular endothelial cell detachment and hypertrophy, in association with down-regulation of nephrin but not podocin, CD2AP, actin and α- Actinin-4.[55] However, the significance of these proteins (Neph, FAT, ...) has not been analysed in this particular context.

#### 4.2.4. *Subacute glomerular thrombotic microangiopathy*

In cases with biopsy-proven glomerular disease, most patients experienced features of subacute glomerular thrombotic microangiopathy, predominantly endotheliosis and membranoproliferative changes, similar to the pathology of preeclampsia/eclampsia suggesting that glomerular endothelium may be particularly susceptible to the toxicity of anti-VEGF agents.

#### 4.2.5. *Additional pathogenic factors*

Proteinuria has been reported with several anti-VEGF agents, including bevacizumab, sunitinib, and VEGF Trap, suggesting a drug class effect.[8] The relatively high incidence of proteinuria in bevacizumab-treated renal cell carcinoma patients[12] suggests a possible role for adaptive hyperfiltration response to nephrectomy. On the other hand, the relative lack of renal parenchymatous damage in anti-VEGF-treated patients suggests a role for additional pathogenesis factors, including hypertensive injury or concomitant use of other nephrotoxic agents, such as IFN-α, gemcitabine, and pamidronate.[15] Concerning nephrotoxic associations with anti-VEGF agents, none are contraindicated to start with. In case of renal side-effects, renal histology permits identification of the causative ones.

## 5. Management of VEGF inhibitor-induced proteinuria

As cancer patients live longer, thanks to the development of newer antineoplastic agents, oncologists need to be aware of their adverse effects including proteinuria. While proteinuria is a cardiovascular risk factor and clearly plays a pathogenic role in loss of renal function, to our knowledge, no study has as yet linked it to the progression of renal

impairment in this context. This is due to the fact that treatment was consistently stopped after the appearance of the proteinuria.

Control of proteinuria is seen as critical to delaying disease progression, even in normotensive patients. It is therefore identified as a target for treatment in kidney diseases in general. Reduction of proteinuria by >30% of baseline within the first 6 to 12 months of treatment has been shown to predict long-term renal and cardiovascular (CV) outcomes.[58] One of the early clinical trials supporting the concept of proteinuria as an independent risk factor for renal disease progression was the Modification of Diet in Renal Disease (MDRD) trial.[59] Multiple studies that demonstrated renoprotection with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin 2 receptor antagonist (ARA) therapy also reported a reduction in proteinuria. A 50% decrease in proteinuria during the first 6 months of losartan or placebo treatment was associated with a 36% reduction in risk for the composite renal end point, a 45% reduction in risk for end stage renal disease (ESRD), and an 18% reduction in risk for CV events during subsequent follow-up.[60] Analysis of irbesartan's effects identified a similar pattern. For every 50% reduction in proteinuria in the first 12 months of ARA therapy, risk for the combined renal outcome (doubling of baseline serum creatinine, serum creatinine, 6.0 mg/dl, or development of ESRD) fell by more than half (hazard ratio 0.44; 95% confidence interval [CI] 0.40 to 0.49; $p < 0.001$).[61] Data from the AASK study demonstrate this relationship in patients without diabetes. Change in proteinuria at 6 months predicted subsequent risk for ESRD. This relationship extended to patients with baseline urinary protein excretion <300 mg/d. A 50% reduction in proteinuria at 6 months was associated with a 72% reduction in risk for ESRD at 5 yrs.[62] In the Ramipril Efficacy in Nephropathy (REIN) study, ramipril therapy prevented the need for dialysis when used for 3 to 4 years in patients with proteinuria and chronic kidney disease. Ramipril also reduced the risk for a combined secondary end point (doubling of baseline serum creatinine or development of ESRD) in this stratum of patients ($p = 0.02$). This relationship remained significant after adjustment for changes in blood pressure ($p = 0.04$), suggesting a mechanism other than blood pressure lowering.[63]

More than 50% of patients who developed TMA, the severest renal adverse event induced by VEGF-targeted therapies, had the lesser grade 1/2 proteinuria (Table 3) suggesting the absence of correlation between abundance of proteinuria and the severity of histological damage. Indeed, all patients at the start of an anti-VEGF drug should be assessed for existing kidney disease with a screening urine analysis for proteinuria, blood pressure and a calculated estimate of renal function. If there is no evidence of proteinuria at initial evaluation, patients should undergo repeat screening before any cycle or every other infusion. If proteinuria of grade $\geqslant$1+ (which roughly correlates to a protein level of 30 mg/dL or a protein-to-creatinine ratio >300 mg/g) is present on screening urine analysis, then quantifying urine protein excretion using a spot urine albumin-to-creatinine ratio provides information relevant to both type and activity of renal disease. Referral to a nephrologist is recommended for additional evaluations (quantification of proteinuria, renal ultrasound, and potentially renal biopsy) of etiological results and/or the treatment of chronic kidney disease. The timing of referral should be made on a case-by-case basis. Because the degree of proteinuria may not predict histological diagnosis in those patients and because treatment options and prognosis may be influenced by the actual histological diagnosis, renal biopsy is recommended whenever feasible. Renal biopsy is indicated in patients with metastatic cancer and proteinuria, evidence of progressive kidney disease, unexplained acute renal failure or subacute renal failure, or an acute nephritic syndrome (e.g. haematuria, proteinuria or hypertension with renal insufficiency), depending on life expectancy and therapeutic options. Indeed, paraneoplastic glomerulopathy (a rare manifestation of neoplastic disease and mainly expressed as nephrotic syndrome) should be distinguished from iatrogenic renal damage. Neoplasia most commonly associated with paraneoplastic glomerular disease are carcinomas of the lung and the gastrointestinal tract. There is no evidence to suggest that cancer patients experience risk related to biopsy that is different from that experienced by patients with chronic kidney disease who are not cancer affected. Suggestion for screening is found in Fig. 1. Interrupting such vital treatments is always subject to benefit/risk ratio analysis. However, we are more inclined to withhold such treatment when faced by a thrombotic microangiopathy or a nephrotic range proteinuria.

Because no interventional study has been performed in regard to anti-VEGF agent-induced proteinuria, and because the mechanisms underlying its development are far from being well understood, evidence-based recommendations cannot be made and most treatments are nonspecific. As a result, many patients with proteinuria under anti-VEGF agents are given conservative treatments such as antihypertensive ACEIs or ARA drugs. ACE inhibitors or ARA have been shown to reduce proteinuria in patients treated by mTOR inhibitors, and their renoprotective effects might be useful in patients with mild proteinuria.[64] Indeed, it has been proposed that utilising ACEIs is more rational on the basis of the proposed mechanisms of angiogenesis-induced hypertension acting through nitric oxide, which is affected by ACEIs and not calcium channel blockers.[65] Moreover, it has been demonstrated that ACEIs induced re-expression of nephrin in diabetic nephropathy[66] and improved endothelial function and microcirculatory density.[67] Blockade of the renin-angiotensin system may have specific benefits in those hypertensive patients with proteinuria. So, it is reasonable to initiate ACEIs or ARA as first-line therapy for anti-VEGF-treated patients with hypertension and proteinuria, as per K/DOQI recommendations,[68] although this remains to be validated in randomised, controlled trials. The optimal intensity of therapy and the specific benefits of ACEIs on anti-VEGF-related kidney diseases are unknown. Furthermore, no recommendations can be made regarding their use among patients with anti-VEGF-related proteinuria that is not associated with hypertension. Nonpharmacologic strategies, especially salt restriction, should be encouraged. Recently, Faul et al.[69] provided a significant step forward towards understanding the pathophysiology of proteinuria based on a tightly regulated actin cytoskeleton by synaptopodin, a key stabiliser of the actin cytoskeleton in podocytes. The authors found that increasing the level of degradation-resistant synaptopodin in podocytes protects against proteinuria

and that expression of activated degradation of synaptopodin in podocytes leads to proteinuria. These findings strongly suggest a reassessment of whether agents that stabilise the podocyte cytoskeleton such as glucocorticosteroids[70] are a logical choice for treating proteinuric patients, although this remains to be validated.

In chronic renal failure, there is no recommended adaptation of anti-VEGF doses to renal clearance, i.e. they can be used regardless of baseline creatinine clearance. On the other hand, it is clear that if a patient with advanced baseline renal impairment develops a serious renal side-effect such as TMA, he will be at risk of a more severe dysfunction. The problem is that this cannot be predicted beforehand and this is radically different from the classic toxic chemotherapeutic agents.

## 6. Conclusion

Targeted therapies may induce proteinuria, glomerular endothelial cell detachment and suppression of nephrin, sometimes leading to glomerulopathy and/or renal thrombotic microangiopathy. Periodic monitoring of urinary protein should be necessary in patients on anti-VEGF agents and those showing nephrotoxicity need special referral to nephrologists. Prospective studies are warranted to better define optimal antiproteinuric regimens with concomitant VEGF signalling inhibition and to determine whether targeting a therapeutic proteinuria level is a reasonable strategy to identify patients who may derive better antitumour effects with such therapy.

## Conflict of interest statement

None declared.

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