All anti-vascular endothelial growth factor drugs can induce ‘pre-eclampsia-like syndrome’: a RARe study*

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ABSTRACT

Background. Specific therapies that target vascular endothelial growth factor (VEGF) and its receptors have improved the survival of patients with metastatic cancers, but can induce side effects. Renal side effects (proteinuria, hypertension and renal failure) are underestimated.

Methods. The French RARe (Reins sous traitement Anti-VEGF Registre) study collects data on patients with cancer who had a renal biopsy because of major renal side effects during treatment with anti-VEGF drugs.

Results. We collected 22 renal biopsies performed 16.2 ± 10.6 months after the beginning of treatment; of which 21 had hypertension, mean proteinuria was 2.97 ± 2.00 g/day and mean serum creatinine, 134 ± 117 μmol/L. Thrombotic microangiopathy (TMA) was observed in 21 biopsy specimens, sometimes associated with acute tubular necrosis (ATN; n = 4). TMA histological lesions were more important than the biological signs of TMA could suggest. Patients with ATN of >20% had higher serum creatinine levels than those with only TMA (231 versus 95 μmol/L). Nephrin, podocin and synaptopodin were variably down-regulated in all renal biopsies. VEGF was down-regulated in all glomeruli.

Conclusion. This study underlines the importance of regular clinical and biological cardiovascular and renal checking during all anti-VEGF therapies for cancer for early detection of renal dysfunction. Collaboration between oncologists and nephrologists is essential. In such cases, renal biopsy might help in appreciating the severity of the renal lesions and after multidisciplinary discussion whether or not it is safe to continue the treatment.

Keywords: anti-VEGF, drug side effects, kidney biopsy, pre-eclampsia

INTRODUCTION

Angiogenesis plays a key role in tumour progression and metastasis spreading. Tumour neovascularization is regulated by a number of molecules, particularly vascular endothelial growth factor (VEGF) that stimulates endothelial cell proliferation and vessel formation. New specific therapies that target VEGF and its receptors have improved the survival of patients with metastatic clear cell renal cell carcinoma [1–9], liver carcinoma, gastrointestinal stromal tumours (GIST) [10] and colorectal carcinoma [11, 12]. Moreover, these drugs are sometimes administered before surgery to decrease tumour size even in the...
absence of metastases (neoadjuvant therapy) [13–15]. Bevacizumab (humanized monoclonal antibody against VEGF-A) and VEGF-Trap (decoy VEGF receptor) block the activity of circulating VEGF, whereas sunitinib, sunitinib and brivanib are multi-target tyrosine kinase inhibitors (MTKIs) of the VEGF signalling pathway (especially the VEGF receptors).

All these anti-VEGF drugs share similar side effects, most frequently gastrointestinal disturbance, skin toxicity and hypertension. Renal side effects may have been underestimated and their exact frequency is not known [9, 16]. For example, the sunitinib licensing trial (375 patients) did not report any renal toxicity [17]. Recent studies described a small number of patients with proteinuria or acute renal failure after therapy with bevacizumab or sunitinib [18]. Moreover, a few patients treated with sunitinib or sorafenib developed a ‘pre-eclampsia-like syndrome’ characterized by severe hypertension, proteinuria and oedema, in a dose-dependent manner, sometimes associated with renal failure or thrombotic microangiopathy (TMA) [16]. Usually, these symptoms disappeared or disappeared following arrest of the treatment. Renal biopsies, which were not systematically performed, revealed the presence of TMA, endotheliosis and effacement of foot processes, mostly following treatment with bevacizumab [12, 19–23]. Acute nephritic syndrome with renal failure has also been reported 2 or 3 weeks after the beginning of treatment with MTKIs. Renal biopsies showed the presence of acute tubular necrosis (ATN), interstitial inflammatory infiltration and sometimes C3 and/or IgA deposition or focal segmental glomerulosclerosis (FSGS) [24–27]. TMA and FSGS could also be associated [28].

In normal kidney, VEGF is produced by podocytes and VEGF-R expressed by endothelial cells [20, 29]. VEGF acts as a paracrine signal to regulate the slit diaphragm integrity and function. During pre-eclampsia, expression of the soluble form of the VEGF receptor (sFLT-1) increases and circulating VEGF decreases. Similar variations were reported after treatment with bevacizumab or MTKIs [30–37]. Moreover, injection of anti-VEGF antibodies in wild-type mice or targeted deletion of VEGF-A in the podocytes in adult mice resulted in a ‘pre-eclampsia-like syndrome’ with endotheliosis, TMA and decreased expression of nephrin (one of the key slit diaphragm proteins) [20, 30, 38], similarly to what has been observed in severe forms of pre-eclampsia [39, 40].

Based on the comparable clinical and histopathological features of pre-eclampsia and the renal side effects of anti-VEGF therapies, we hypothesized that such pre-eclampsia-like syndrome might occur during treatment with all types of anti-VEGF drugs. We describe here the results of the histological and immunohistochemical analysis of kidney biopsies from a series of patients with cancer who were treated with different anti-VEGF drugs (anti-VEGF monoclonal antibodies, VEGF-Trap and tyrosine kinase inhibitors) and experienced major renal side effects.

**Materials and Methods**

**Patients**

The RARe study (‘Reins sous traitement Anti-VEGF Registre’) has been established in 2009 for collecting data on renal side effects during treatment with anti-VEGF drugs. Publicity for the study was made through the French Nephrology Society website. The study includes patients from all nephropathy centres in France who had a renal biopsy for proteinuria or renal failure following adjuvant treatment with anti-VEGF drugs for different types of cancer. In addition, samples from patients who received neoadjuvant anti-VEGF therapy for renal carcinoma in Rennes and did not report proteinuria were collected as controls as normal kidneys (10 samples).

Biopsies were not performed specifically for the study, but were decided by the patient’s nephrologist in order to exclude other renal diseases and to help in forming a diagnosis and choosing the most appropriate therapy.

All patients were informed about the constitution of this cohort. This study has been approved by the Rennes University Hospital Ethics Committee, the CCTIRS (Comité Consultatif sur le Traitement de l’Information en matière de Recherche dans le domaine de la Santé) and the CNIL (Commission Nationale de l’Informatique et des Libertés). The procedures were in accordance with the Helsinki declaration of 1975.

**Renal biopsies**

Renal biopsies were fixed in alcoholic Bouin’s fixative or in 10% formalin, paraffin-embedded and 3-μm thick sections stained with periodic acid–Schiff (PAS), haematoxylin–eosin–safran (HES), Masson’s trichrome and silver stain for light microscopy. Immunofluorescence analysis using anti-fibrin, -C1q, -C3, -IgA, -IgG, -IgM, -Kappa and -Lambda antibodies was also carried out. The initial results of each biopsy analysis were sent to the Rennes University Hospital (the RARe coordinating centre) together with a few kidney sections for further immunohistochemical investigations (see below). All data were reviewed by the same pathologist (N.R.L.). At least five glomeruli were considered an adequate sample for diagnosis and immunostaining evaluation.

**Immunohistochemistry**

Paraffin-embedded kidney sections were dewaxed using xylol and rehydrated through decreasing ethanol concentrations. Sections were then heated in hot 0.01 mol/L citrate buffer pH 8 at 100°C for 40 min (antigen retrieval), left to cool down for 20 min and then transferred into phosphate-buffered saline (PBS)/0.1% Tween (PBS-T) buffer for 5 min. Endogenous peroxidase activity was quenched in 3% hydrogen peroxide for 10 min, and slides were again transferred into PBS-T for 5 min. After pre-incubation in 2.5% normal horse serum blocking solution (ready to use) for 20 min (for podocin and nephrin detection), slides were incubated with the rabbit polyclonal anti-podocin antibody (1:400) (H-130;sc-21009, Santa-Cruz®) at 4°C overnight, with the rabbit polyclonal anti-nephrin antibody (1:500) (ab 58968, Abcam®) at room temperature for 20 min, with the rabbit polyclonal anti-VEGF A antibody (1:100) (Santa-Cruz®) at 4°C overnight or with re-diluted anti-mouse synaptopodin (BM 5086, Acris-Interchim®) for 20 min (without blocking solution). Negative controls (sections incubated in the dilution solution but without the primary antibody) were concomitantly carried.
out. Sections were rinsed in PBS-T buffer and incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) polymers (Dako® EnVision™ + Dual Link System-HRP for anti-mouse primary antibodies or ImmPRESS™ reagent, Vector Laboratories®, for anti-rabbit primary antibodies). Antibody reactions were detected with diaminobenzidine in the presence of hydrogen peroxide. Slides were counterstained with haematoxylin.

VEGF and podocin stainings were evaluated in all glomeruli as 0 (no staining), +/- weak (staining observed in rare podocytes) or + strong (diffuse staining in numerous podocytes).

For synaptopodin, and nephrin immunostaining, the expression was localized along the glomerular basement membrane. The morphology of synaptopodin, and nephrin expression, was either absent (0), weak with a segmental pattern and staining interruption (+) or intense, continuous and linear along the glomerular basement membrane (++).

RESULTS

Patients’ clinical characteristics

Twenty-two kidney biopsies from patients (12 males and 10 females, mean age 59 ± 11 year old) who received adjuvant anti-VEGF-targeted therapy and five renal tissue specimens from patients with renal carcinoma (normal part) who had neoadjuvant anti-VEGF therapy and 10 tumour-free renal resection samples were included in the RARe study between 2009 and 2011.

Table 1 and 2 summarize the clinical and biological characteristics of the patients who had a kidney biopsy (n = 22). The used adjuvant anti-VEGF treatments included anti-VEGF monoclonal antibodies, VEGF-Trap and different tyrosine kinase inhibitors. Renal biopsies were performed 16.2 ± 10.6 months (range, 1–35 months) after the beginning of treatment. Patients were treated with standard doses of anti-VEGF drugs for 13.0 ± 8.7 months. All but one patient had hypertension at the time of the biopsy and 20 of 22 were treated with one or two renin–angiotensin system (RAS) blockers in order to control hypertension and/or decrease proteinuria (Table 1). There were mild or no biological signs of TMA (Table 2). The mean proteinuria in the 22 patients was 2.97 ± 2.0 g/day, and the mean serum creatinine, 134 ± 117 μmol/L. Serum creatinine was higher in patients with >20% ATN than in those with only glomerular TMA (231 versus 95 μmol/L). Patients with higher proteinuria had FSGS as well. Because of the small number of patients, no statistical analysis could be performed.

None of the five patients who received neoadjuvant anti-VEGF therapy (sunitinib) had hypertension and only one reported low proteinuria (0.58 g/day) at the time of unilateral nephrectomy. Normal kidney samples were coming from the tumour-free renal resection in patients without any anti-angiogenic treatment before surgery and any abnormality on urinary dipstick.

Analysis of renal biopsies

Silver and Masson’s trichrome staining of kidney sections from the 22 biopsies showed in all glomeruli some glomerular
microangiopathy lesions. TMA lesions are focally recent in four biopsies with endothelial swelling, mesangiolysis, widening of the subendothelial space and fibrin thrombi (Figure 1E) or former in 20 biopsies with, in some capillary loops, duplication of glomerular basement membranes, segmental or global collapse of underlying glomerular basement membranes (Figure 1A–E). More than 20% ATN was seen in four samples (Figure 1F). One patient presented membrano-proliferative glomerulonephritis.

All the kidney tissue samples from patients who received neoadjuvant anti-VEGF therapy showed ATN with no or rare inflammatory cells due to surgical clamping, but no glomerular disease (data not shown).

A mild to strong nephrin, podocin and synaptopodin down-regulation were observed in all renal biopsies following adjuvant anti-VEGF treatment compared with ‘normal kidneys’ (Figure 2: panels C, G and K for the strongest reduction; panels D, H and L for mild variation). Treatment with RAS blockers did not seem to influence the expression of these proteins, and no correlation between their expression level and the presence/severity of ATN was found (Table 3).

All samples (adjuvant and neoadjuvant kidneys) showed the decrease or absence of VEGF expression in glomeruli, confirming that all the drugs act through VEGF inhibition.

All 10 normal kidneys (tumour-free renal resections) showed positive staining for synaptopodin (++), nephrin (+), podocin (+) and VEGF (++) expression. Finally, the levels of nephrin and synaptopodin expression (assessed by immunohistochemistry) (Figure 2 and Table 4) were normal in renal specimens from patients who received neoadjuvant anti-VEGF therapy (Figure 2B and J), whereas podocin expression was lower despite the absence of proteinuria (Figure 2E and F).

Electron microscopy has been performed for five biopsies showing always TMA lesions at different stages. Figure 3 shows the ultrastructure of one of this case from Poitiers Hospital, France.

**Clinical outcome**

Among the four patients with ATN, different outcomes were recorded after interruption of the adjuvant anti-VEGF treatment. Patient #8 stayed on dialysis, patients #4 and #11 kept chronic renal failure (around 30 mL/min/1.73 m²) and only patient #13 recovered normal renal function but died 4 months after biopsy. In the other patients, proteinuria decreased and renal function improved only following the definitive interruption of the anti-VEGF drug. Eight patients died within a few months (the cause was not recorded).

**DISCUSSION**

This study presents the results of the analysis of the largest series of renal biopsies from patients who reported renal side effects during treatment with different anti-VEGF drugs (anti-VEGF monoclonal antibodies, VEGF-Trap and tyrosine kinase inhibitors). The main finding is the association of ATN with the use of adjuvant and neoadjuvant anti-VEGF therapy, which was confirmed by the down-regulation of nephrin, podocin and synaptopodin expression, and the absence of VEGF expression in glomeruli. These results suggest that the mechanism of ATN is related to the inhibition of VEGF, which is consistent with the results of previous studies showing that VEGF plays a role in the maintenance of glomerular filtration. The clinical outcome of the patients with ATN was variable, with some patients recovering normal renal function and others remaining on dialysis. The use of RAS blockers did not seem to influence the expression of nephrin, podocin and synaptopodin, which suggests that these proteins are involved in the pathogenesis of ATN independently of RAS activity. Further studies are needed to elucidate the mechanisms underlying ATN and to develop strategies to prevent and treat this complication of anti-VEGF therapy.
kinase inhibitors). Previous works focused mainly on the renal side effects secondary to treatment with bevacizumab or sunitinib [9, 19, 21, 22], which have been the first and most used anti-VEGF drugs in oncology since 2005. Our findings confirm that the use of anti-VEGF drugs is associated with the development of renal side effects that require constant monitoring.

**FIGURE 1:** Histological analysis of kidney biopsies from patients who received adjuvant anti-angiogenic treatment for metastatic cancer. (A) Silver staining: glomerulus with multiple double contours of capillary basement membrane (×400). (B–E) Masson’s trichrome staining (×400); (B) enlarged glomerulus showing chronic TMA with duplication of the glomerular basement membranes and widening of the subendothelial spaces. Hypertrophic podocytes containing cytoplasmic droplets with segmental collapse of the underlying glomerular basement membranes are also visible. (C) Mildly enlarged glomerulus with acute and chronic TMA characterized by focal capillary thrombosis, focal subendothelial widening and rare cellular interposition. (D) Small arteries with prominent endothelial swelling (arrow). No vasculitis. (E) Acute microangiopathy characterized by focal duplication of the glomerular basement membranes, with endothelial swelling, widening of the subendothelial space, mesangiolysis and podocytosis. (F) Masson’s trichrome staining (×100). Severe and diffuse ATN with no interstitial inflammatory cells.

**FIGURE 2:** Analysis of nephrin (A–D), podocin (E–H) and synaptopodin (I–L) expression in normal (A, E and I) and normal kidney (B, F and J) sections from patients who received neoadjuvant anti-VEGF treatment (nephrectomy) and from kidney biopsy specimens from patients with proteinuria or renal failure during adjuvant anti-VEGF therapy (×400).
surveillance. Moreover, the glomerular pathology is not secondary to the use of monoclonal antibodies as suggested for bevacizumab, but is a specific side effect of VEGF blockage within the glomerulus [20].

Mild to severe ATN, in addition to TMA, was observed in some patients without any evident link between the treatment (dose and length) and the severity of the lesions, whereas interstitial nephritis was not reported possibly due to the long mean interval between the beginning of treatment and the renal biopsy (16.2 months). Indeed, interstitial nephritis, which has been described in case reports, is probably due to an immunoallergic reaction, leading to renal failure soon after starting the treatment. TMA and ATN could be the result of direct kidney toxicity of the anti-VEGF drug. Specifically, ATN may be caused by direct tubular injuries as reported for other chemotherapeutic agents. In addition, all the ATN cases were seen in patients who did not have other nephrotoxic drug, which could have explained the ATN. Our results indicate that the clinical presentation of ATN is quite different from that of TMA, with rapid renal failure in addition to the ‘pre-eclampsia-like syndrome’. In the presence of ATN, the treatment should be immediately stopped, while in those with only TMA, the anti-VEGF therapy continuation has to be multidisciplinary discussed and might be continued under close monitoring of the renal function in the absence of other available therapeutics. Indeed, despite the small number of patients, our series show that the condition might become severe leading to transitory or permanent dialysis. As all patients had proteinuria and, with the exception of serum creatinine, no other clinical or biological sign could be used to distinguish TMA from ATN, histological evaluation of a renal biopsy is still required for early diagnosis and optimal therapeutic decision making.

Analysis of the renal biopsy results indicated that the histological TMA lesions were generally more severe than what could be expected from the biological data (no or very few signs of haemolysis), as previously reported [19–21]. Because of these few biological signs, most of the patients did not have any additional extensive exploration in order to find another TMA aetiology, especially no extensive exploration of the complement. As our series included only patients with renal biopsies, patients with severe biological TMA might have had a too low platelet count to authorize renal biopsy and were thus not included. However, only eight patients of more than 1000 in the French TMA register with severe TMA required plasmatic exchanges after anti-VEGF therapy (unpublished data from P. Coppo). Thus, severe clinical TMA after anti-VEGF therapy is probably a rare event, which real incidence impossible to access and it might also be related to other associated therapies or to the cancer itself.

These observations strongly suggest that renal function must be carefully monitored in all patients treated with anti-VEGF drugs at least once a month even in the absence of clinical signs. We think that renal biopsy should be discussed in the presence of proteinuria or of serum creatinine variations in order to precisely evaluate the extent of TMA and ATN. The presence of ATN lesions must urge to stop the anti-VEGF treatment at least until the level of serum creatinine decreases, whereas isolated TMA should authorize its continuation under regular surveillance, in the absence of alternative therapeutic and after multidisciplinary discussion.

Immunohistochemical analysis can also help to understand the mechanisms of proteinuria secondary to anti-VEGF therapy. Decreased VEGF availability within the glomerulus may have detrimental effects on kidney function as the glomerular filtration barrier might be particularly susceptible to

### Table 3. Clinical and biological characteristics of the five patients with neoadjuvant therapy (all renal carcinoma and sunitinib treatment)

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Number of sunitinib cycles</th>
<th>RAS blocker</th>
<th>Serum creatinine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>35</td>
<td>2</td>
<td>ARB</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>61</td>
<td>3</td>
<td>ACEI</td>
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<tr>
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<td>F</td>
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<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>M</td>
<td>41</td>
<td>3</td>
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<td>79</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>66</td>
<td>2</td>
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<tr>
<td>Mean</td>
<td>4 M/1 F</td>
<td>51.8 ± 13.2</td>
<td></td>
<td>2/5</td>
<td>103 ± 19</td>
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</table>

### Table 4. Expression of podocin, nephrin, synaptopodin, and VEGF (by immunochemistry), number of glomeruli per section and percentage of acute tubular necrosis in renal biopsies from the 22 patients with proteinuria and/or renal failure during adjuvant anti-angiogenic treatment

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>Number of glomeruli</th>
<th>Podocin</th>
<th>Nephrin</th>
<th>Synaptopodin</th>
<th>VEGF</th>
<th>ATN (%)</th>
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<tbody>
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<tr>
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<td>++</td>
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<tr>
<td>3</td>
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<td>&gt;50</td>
</tr>
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</tr>
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<td>0/+</td>
<td>&lt;10</td>
</tr>
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</table>

Score: 0, no expression; 1, mild expression and 2, strong expression. The number of analysed glomerular cross-sections is reported on the second column. The percentage of ATN in renal biopsy sections is reported in the right column. NA, not available.
toxicity from VEGF inhibitors [41]. We found that, as in pre-eclampsia, synaptopodin and nephrin expression levels were decreased in kidney biopsy specimens from patients who received adjuvant therapy. Moreover, podocin expression also was reduced in almost all samples and also in the kidney specimens from patients who had neoadjuvant therapy and without proteinuria. This, in addition to VEGF decrease in all glomeruli, confirms that deregulation of slit diaphragm proteins is a direct effect of anti-VEGF drugs and not a consequence of the proteinuria.

RAS blockers, which were prescribed to control hypertension and proteinuria, did not seem to have any effect on the expression of these proteins. As VEGF blockage induces renin secretion via tissue ischaemia, RAS blockers might help manage the cardiovascular and renal side effects of anti-VEGF drugs [42]. Indeed, in some patients, they reduced the proteinuria secondary to the anti-VEGF treatment [19]. However, despite the absence of a control group without RAS blockers, our data suggest that these drugs might not be sufficient for controlling hypertension (most patients needed other antihypertensive drugs) or proteinuria (the mean proteinuria was 2.97 g/day). More experimental data are needed to understand the role of the RAS system in the renal and cardiovascular toxicity of anti-VEGF drugs.

In summary, this large series of renal biopsies underlines the importance of regular (at least once a month) monitoring of blood pressure and serum creatinine as well as urinary dipstick analysis for all patients treated with anti-VEGF drugs for cancer. This simple surveillance might allow the early detection of TMA biological signs. In such cases, the option of carrying out a renal biopsy should be discussed between oncologists and nephrologists in order to determine the severity of the renal lesions and whether it is safe to continue the anti-VEGF therapy. We also show that nephrin, synaptopodin, podocin and VEGF expression decreased in glomeruli after VEGF blockage.

Although these drugs have now demonstrated their efficacy in several cancers, their renal and cardiovascular consequences should not be underestimated.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

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