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Authors

Hanna, Ramy M
Ahdoot, Rebecaa S
Kim, Matthew S
et al.

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Intravitreal vascular endothelial growth factors hypertension, proteinuria, and renal injury: a concise review

Ramy M. Hanna[a], *Rebecaa S. Ahdoot*[a], *Matthew S. Kim*[a]
Kenar D. Jhaveri[b,c], *Kamyar Kalantar-Zadeh*[a], *and Ira B. Kurtz*[d]

Purpose of review
Nearly 20 years ago, vascular endothelial growth factor (VEGF)inhibitors (VEGFi) were adapted from systemic use from antiangiogenesis roles to intravitreal uses. Initially bevacizumab a murine immunoglobulin was injected 'off label' as a treatment for diabetic macular edema and age-related macular degeneration. Throughout the following decade aflibercept and finally ranibizumab were adapted and obtained Food and Drug Administration approval for intravitreal use. Initially systemic absorption was thought to be quite low after intravitreal injections and was quoted as being 200-fold lower than levels postulated to induce significant VEGF inhibition. Pharmacodynamic studies obtained in 2014 and again in 2017 revealed significant systemic absorption and detectable VEGF inhibition, this has since been confirmed in multiple subsequent studies.

Recent findings
A few case reports of renal dysfunction and glomerular disease related to VEGFi were initially identified. Mixed findings on effects on blood pressure were noted in studies. More recently, 32 cases of de-novo glomerular disease and/or proteinuria exacerbation were identified. New studies have corroborated increased blood pressure, proteinuria exacerbation in patients with pre-existing nephrotic syndrome, and systemic VEGF depletion. Further, the most common lesion of systemic VEGFi nephrotoxicity, thrombotic microangiopathy, has recently been reported by our group.

Summary
We will review the pharmacokinetic, translational, and epidemiological data that year upon year establish the finite-yet real risk of intravitreal VEGFi.

Keywords
diabetic retinopathy, hypertension, nephrotic syndrome, TMA, VEGF blockade

INTRODUCTION

Angiogenesis inhibition became a top priority for adjunctive chemotherapy in the 1990s and early 2000s [1]. These agents were used in solid organ cancer including previously highly resistant malignancies like glioblastoma multiforme (GBM). Vascular endothelial growth factor (VEGF), is a powerful pro angiogenic signaling molecule that performs a plethora of functions from angiogenesis, platelet derived growth factor, and lymph angiogenesis [2–4] (Supplemental Figure 1, http://links.lww.com/CONH/A35).

As VEGF inhibitors (VEGFi) were developed they were recognized as powerful antiangiogenesis tools that developed into extremely useful forms of adjunctive chemotherapy [3]. Initially, their promise was tempered by the recognition of various forms of systemic toxicity and nephrotoxicity observed with their use. It was noted that VEGFi result in hypertension, worsening proteinuria, risk of increased venous thromboembolism, and arterial clotting [5,6]. This was noted to occur in a high

[a]UC Irvine, Department of Medicine, Division of Nephrology, Irvine, California, [b]Division of Kidney Diseases and Hypertension, Department of Medicine, North Shore University Hospital and Long Island Jewish Medical Center, Hofstra North Shore-LIJ School of Medicine, [c]Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Great Neck, New York and [d]Brain Research Institute, Westwood, Los Angeles, California, USA

Correspondence to Ramy M. Hanna, MD, 333 The City Drive, Suite #400, Orange, CA 92868, USA.
E-mail: Ramyh1@uci.edu, rhannamd81@yahoo.com

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KEY POINTS

- VEGF blockade can be associated with worsening hypertension, proteinuria, and less frequently renal limited thrombotic microangiopathy in both systemic and intravitreal administrations.
- Absorption of VEGF inhibitors is measurable in serum and can be clinically significant.
- Patients with worse pre-existing renal disease and hypertension are at higher risk in a manner similar to how pre-eclampsia affects patients with more severe renal comorbidities.
- Lower potency VEGF inhibitors like ranibizumab maybe safer in patient with comorbid conditions.

proportion of patients and the burgeoning field of onconephrology was quick to recognize these complications and address them. The most common agents used for systemic VEGF blockade were bevacizumab (Avastin ©) and later z-aflibercept (Zaltrap ©) [7–9].

During the early 2000's, bevacizumab (Avastin ©) was first used off label in patients with retinal neovascularization [7]. This was eventually expanded to three main indications, age-related macular degeneration (AMD), Diabetic Macular Edema (DME), and Central Retinal Vein Obstruction (CRVO). Eventually aflibercept (trade name Eylea ©) for intravitreal use was added to the US Food and Drug Administration (FDA) list of approved agents in 2007 [9]. Finally, Ranibizumab (trade name Lucentis ©) was approved in 2011[8]. In both cases, the FDA reported that serum levels of these agents were 200-fold lower than levels expected to cause 'significant' VEGF inhibition [8,9]. This was not defined as the IC50 but rather a serum level of drug that would cause 50% inhibition of 'total body' VEGF rather than vascular circulating VEGF [8,9,10▪▪,11▪,12▪▪,13,14▪,15▪▪].

MOLECULAR BIOLOGY

VEGF is an essential growth and cellular integrity signal for the endothelium globally. Since the glomerulus is an extremely vascular organ it follows that VEGF signaling (predominantly VEGF-A) to VEGF Receptor 2 (VEGFR2) is important to kidney health [11▪,12▪▪,13,14▪,15▪▪,16]. It has been well described that VEGF signaling is necessary for nitric oxide production, endothelial cell survival, and regulation of clotting via di-acyl-glycerol kinase epsilon (DAGK-E) [13]. More recently well recognized is the necessary autocrine/paracrine role of VEGF signaling in podocyte health. VEGF signaling involves downstream Fyn and N-WASP kinase signaling, the first important downstream target is Akt which signals for podocyte survival [10▪▪]. The downstream mediators also signal C2DAP which regulates the actin cytoskeleton and nephrin which affects the integrity of the podocyte actin filament network [15▪▪]. This results in the podocyte's ability to maintain the structural integrity of the glomerular basement membrane. Finally, Rel-A and C-MIP interact to regulate NFKB which regulates RAAS and downstream mediators of inflammation [14▪,15▪▪] (Fig. 1).

PHARMACOKINETICS OF SYSTEMIC ABSORPTION AFTER INTRAVITREAL VASCULAR ENDOTHELIAL GROWTH FACTORS INJECTION

In 2014 Avery *et al.* demonstrated that in patients with age related macular degeneration that VEGFi are absorbed at a point near and above the IC50 (50% inhibitor concentration) [17]. These findings indicated that levels higher than those needed to inhibit 50% of circulating vascular VEGF were achieved after intravitreal injection [17]. These results were in AMD patients [17] but were replicated again in 2017 in DME and CRVO patients [18]. Subsequent studies by multiple authors [19,20,21] continued to confirm the observation that pharmacokinetically certain VEGFi led to very powerful depletion of vascular VEGFi. The typical order of inhibition seen was bevacizumab was associated with significant but moderate length durations of VEGF inhibition, aflibercept was associated with profound and also longer durations of VEGF inhibition [17,18,19,20–23]. Ranibizumab was associated with the least time of VEGFi at concentrations greater than the IC50 and for the shortest duration of time [17,18,19,20–23]. This body of pharmacological worked was also observed in at least one simian study showing binding of VEGFi in simian glomeruli (Tschulakow et. al.) [24] (Supplemental Figure 2, http://links.lww.com/CONH/A36). Supplemental Table 1, http://links.lww.com/CONH/A37 summaries the data in the literature regarding intravitreal VEGFi and evidence of systemic VEGF depletion after injection.

CLINICAL EFFECTS OF INTRAVITREAL VASCULAR ENDOTHELIAL GROWTH FACTORS INHIBITORS: HYPERTENSION EXACERBATION

Studies of intravitreal VEGFi and subsequent hypertension have been mixed [25–29]. The earliest three

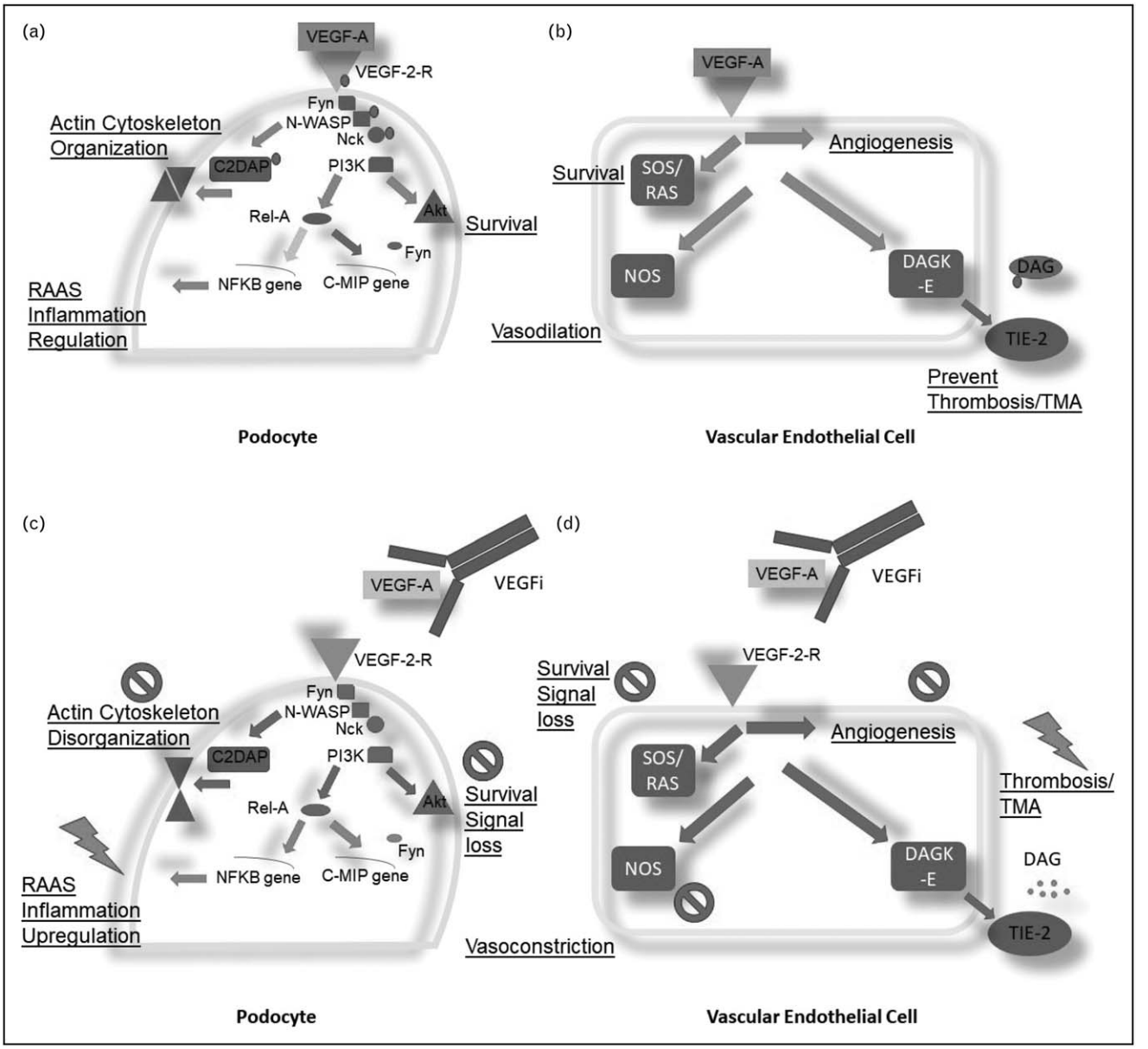

FIGURE 1. VEGF renal molecular physiology and pathophysiology. Molecular physiology of VEGF signaling in podocytes and endothelial cells and renal pathophysiology that ensues with VEGF blockade. (a,b) Molecular physiology and (c,d) pathophysiology with VEGF blockade. VEGF-A signaling to renal podocytes maybe paracrine or mediated through VEGF-2 receptors. Akt, protein kinase B (PKB); CD2AP, CD2-associated protein; C-MIP, C-Maf-inducing protein; DAG, diacyl glycerol; DGKE, diglyceride kinase epsilon; F-Act, F-actin; Fyn, proto-oncogene tyrosine-protein kinase fyn; GN, glomerulonephritis; GS, glomerulosclerosis; Nck, NCK tyrosine kinase; NFkB, nuclear factor kappa light chain enhancer of activated B cells; NP1, neuronal pentraxin 1; N-WASP, Neural Wiskott–Aldrich syndrome protein; PI3K, phosphatidylinositol-4, 5-bisphosphate 3-kinase; RAS, rat sarcoma protein; Red P, phosphoryl group; RelA, v-rel avian reticuloendotheliosis viral oncogene homolog A; SOS, son of sevenless; sVEG2R, soluble VEGF receptor 2; VEGF-A, VEGF receptor A; VEGFR2, VEGF receptor 2; Tie2, tyrosine-protein kinase receptor TIE-2. Twin nucleic acid strands = messenger RNA. Red circle is representative of a phosphorous group placed by the kinase proximate to the reaction in the figure. Adapted from: -Shye M, Hanna RM, Patel SS, *et al.* Worsening Proteinuria and Renal Function after Intravitreal Vascular Endothelial Growth Factor Blockade for Diabetic Proliferative Retinopathy. Clin Kidney J. 2020. -Hanna RM, Lopez E, Hasnain H, et. al. Three patients with injection of intravitreal vascular endothelial growth facto-inhibitors and subsequent exacerbation of chronic proteinuria and hypertension. Clin Kidney J. 2018. -Phadke G, Hanna RM, Ferrey AJ, *et al.* Review of intravitreal vascular endothelial growth factor inhibitor toxicity, and report of collapsing focal and segmental sclerosis with thrombotic microangiopathy in a patient with age-related macular degeneration. Clin Kidney J. 2021. (With attribution under open access license).

studies were Rasier *et al*. which showed a rise in systolic and diastolic hypertension with intravitreal VEGFi [28]. Risimic and Lee published mostly negative studies (Lee did show a short-term elevation in diastolic blood pressure) [27,29]. More recently Bagheri *et al*. and Anjali showed positive correlations with a single and multiple intravitreal VEGFi and a rise in hypertension [25,26] (Table 1).

CLINICAL EFFECTS OF INTRAVITREAL VASCULAR ENDOTHELIAL GROWTH FACTORS INHIBITORS: ACUTE KIDNEY INJURY

At this time there have been multiple clinical case reports of renal injury, glomerular disease, and even renal failure requiring renal replacement therapy after VEGFi given intravitreally. This is different

Table 1. Literature review: systemic effects of intravitreal anti-VEGF injection

Systemic Effect/Pathology	Study Type	Study name/Reference
A. Drug Absorption and Systemic VEGF inhibition		
Absorption in AMD, dec. systemic VEGF (Bev, Aflb) > Ran	Prospective observational study	Avery [17]
Absorption in AMD/DME/CRVO, dec. systemic VEGF (Bev, Aflib) > Ran	Prospective observational study	Avery [18]
Dec. systemic VEGF (Bev, Aflib) > Ran	Prospective randomized clinical study	Jampol [19]
Absorption of drug in AMD, dec systemic VEGF	Retrospective study of RCT data	Rogers [20]
Dec. systemic VEGF (Bev, Aflib)	Prospective randomized observational study	Zehetner [21]
Bev > Ran dec. in systemic VEGF	Prospective observational study	Yoon [61]
Dec. systemic VEGF (Bev, Aflib)	Prospective non- randomized clinical study	Hirano [62]
B. Animal studies		
Absorption of drug, binding at glomerulus	Animal (Simian) study	Tschulakow [24]
C. Effects on hypertension after intravitreal injection		
Higher blood pressure linked to need for more VEGFi	Retrospective study	Anjali [25]
Limited short term rise in blood pressure at 1 h	Prospective Observational study	Lee [27]
Long and short term rise in systolic blood pressure	Observational study	Rasier [28]
No significant change in blood pressure	Observational study	Risimic [29]
D. Trial Data		
Increased proteinuria 45% of patients (Not statistically significant)	Prospective Observational study	Bagheri [26]
Significant rise in diastolic blood pressure		
Significant rise in hemoglobin and platelets		
No change in eGFR 7–30 days after injection (Bev, Aflib, Ran)	Retrospective observational study	Kameda [30]
4% of patients with AKI and elevated UPCR after VEGFi	Retrospective observational study	Jalalonmuhali [31]
No long-term change in HTN or category of albuminuria	Planned retrospective analysis of trial	Glassman [32]
No association with # VEGFi injections and proteinuria	Retrospective observational study	O'Neill [33▪]
Significant rise in UPCR in patients with preexisting proteinuria	Prospective observational study	Chung [34▪▪]
E. Population studies showing increased morbidity and mortality		
Increased risk of CVA in DME patients	Meta-analysis	Avery [23]
Increased A.C. mortality in AMD patients	Retrospective observational study	Hanhart [35]
Increased risk of mortality after MI in AMD patients	Retrospective observational study	Hanhart [36]
Increased risk of mortality after CVA in AMD patients	Retrospective observational study	Hanhart [37]
Increased risk of Thrombotic events	Clinical trial database retrospective	Schmid [38]
No finding of CVA, MI, A.C. mortality in AMD patients	Retrospective observational study	Dalvin [39]
No finding of increased CVA in DME patients	Retrospective observational study	Starr [40]
Ran versus Bev same number of reported SAE	Clinical trial database retrospective	Ran/Bev Trial N [41]

#, number of (injections); A.C., all-cause mortality; Aflib., aflibercept; AMD, age related macular degeneration; Bev., bevacizumab; CRVO, central retinal vein obstruction; CVA, cerebrovascular accident; dec., decreased; DME, diabetic macular edema; eGFR, estimated glomerular filtration rate; HTN, hypertension; MI, myocardial Infarction; n, number of study subjects; Ran, ranibizumab; Ran/Bev Trial N, Ranibizumab Bevacizumab Trial Network; RT, randomized trial; SAE, serious adverse event; VEGF, vascular endothelial growth factor; VEGFi, vascular endothelial growth factor inhibitors. Adapted from Shye, Phadke *et al*. CKJ (under open access license with attribution) [14▪,15▪▪].

from the observed data with systemic VEGFi where worsening proteinuria, AKI, and thrombotic microangiopathy were commonly noted. Kameda et. al. did not find any significant change in eGFR after VEGFi given intravitreally [30]. Maisirah *et al*. noted 4% instance of AKI and proteinuria after VEGFi but without a control group in patients with diabetes mellitus, diabetic nephropathy, and diabetic retinopathy [31] (Table 1).

CLINICAL EFFECTS OF INTRAVITREAL VASCULAR ENDOTHELIAL GROWTH FACTORS INHIBITORS: PROTEINURIA EXACERBATION

Intravitreal VEGFi studies did not initially reveal a rise in proteinuria after VEGFi [32]. More nuanced findings are now being noted challenging the notion that there is no effect. This is congruent with the pharmacokinetic findings, mechanistic insights, and multiple reproducible clinical observations of severe renal function decline and glomerular injury after intravitreal VEGFi [12▪▪,22]. Glassman et. al. and O'Neill et. al. reported no proteinuria exacerbation post intravitreal VEGFi injections [32,33▪]. Then Maisarah et. al. noted a 4% incidence of AKI and elevated urine protein to creatinine ratio (UPCR) [31], and Bagheri *et al*. noted that nearly half of its patients had an elevated UPCR after injection, though this was not statistically significant [26]. Finally, Chung *et al*. reported a very interesting finding that potentially explains the admixture of results noted [34▪▪]. Specifically, a significant correlation of worsening UPCR was noted in patients who were already near nephrotic range proteinuria. As such, it appears that as in preeclampsia, VEGFi tends to induce renal dysfunction disproportionately in patients who already have renal pathology [12▪▪,13] (Table 1).

CLINICAL EFFECTS OF INTRAVITREAL VASCULAR ENDOTHELIAL GROWTH FACTORS INHIBITORS: EPIDEMIOLOGICAL

The search for the effects produced by this intravitreal VEGF inhibition was also examined on a larger scope [12▪▪,13]. Avery *et al*. noted a higher risk of cerebrovascular accident in an early study [22,23,35,36–38]. Later Hanhart reported an increase in post intravitreal VEGFi induced cerebrovascular event mortality, post myocardial infarction mortality, and most concerning an elevation of all-cause mortality [35,36,37]. Dalvin *et al*. and Starr *et al*. were unable to confirm these analyses [39,40]. The differential exposure to total dose of drug, differential absorption, and the relationship of VEGFi tending to produce worse outcomes in patients who had comorbidities are potential explanations for the divergent results seen in many larger case studies [11▪]. Other studies reviewed showed that there was an increased risk of thrombotic events in VEGFi use [38], but no one agent was identified as safer [38,41]. See Table 1.

CLINICAL REPORTS OF GLOMERULONEPHRITIS

There have been a total of 32 biopsy proven or clinically documented events showing thrombotic microangiopathy, de-novo nephrotic range proteinuria, severe hypertension exacerbation, collapsing focal and segmental sclerosis, minimal change disease, worsening eGFR [42], among other phenotypes [10▪▪,11▪,13,14▪,15▪▪,42–50,51▪,52–57]. These cases occurred in a temporal relationship to VEGFi initiation intravitreally. Our group has reported 14 such cases [10▪▪,11▪,13,14▪,15▪▪,45,51▪,58]. These cases were often diagnosed through extremely careful analysis of the clinical course, with biopsy being pursued when the acute renal injury or proteinuria deviated from the expected pattern of slow deterioration seen in diabetic nephropathy coupled with more aggressive intravitreal VEGFi administration [10▪▪,11▪,13,14▪,15▪▪,45,51▪,58]. Attention to timing following VEGFi therapy is critical and guidelines to help diagnose difficult cases have been published elsewhere (Hanna *et al*. Kidney International) [12▪▪]). Figure 2 details the pathological phenotypes seen in our group's reported cases. Table 2 catalogues the known clinically described cases of intravitreal VEGFi nephrotoxicity and systemic toxicity.

DISCUSSION

This concise review describes in detail the progress being made on the newly rediscovered and popularized notion that intravitreal VEGFi can be systemically toxic. Nephrotoxicity in a manner congruent with prior reports of toxicity after VEGFi has been shown [11▪]. A future challenge is to design studies that more predictably capture high-risk patients that are likely to be the most susceptible to these complications [11▪].

The reasons for having reported cases in the literature with positive and negative results is multifactorial [11▪]. First it is reasonable to expect that absorption from the retinal compartment to the vascular compartment is not uniform. This is hinted at by the fact that the systemic levels of absorbed VEGFi after intravitreal injection vary between different conditions (DME and AMD). We have previously postulated that this may be due to different

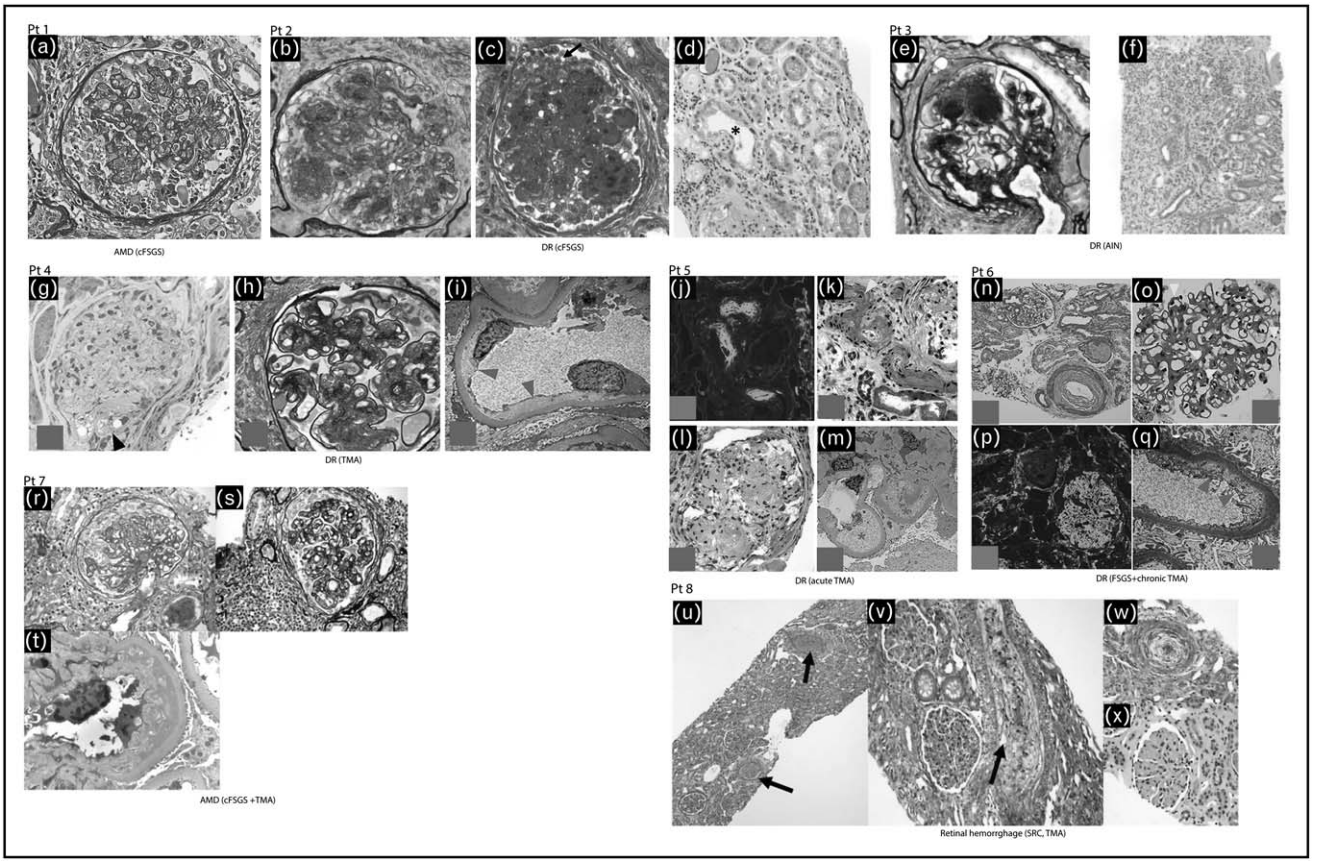

FIGURE 2. Patient biopsies: Adapted from: Nobakht N., Kamgar M., Abdelnour L. *et al.* Development of Collapsing Focal and Segmental Glomerulosclerosis in a Patient Receiving Intravitreal Vascular Endothelial Growth Factor Blockade. Kidney Int-Rep. 2019. (Under open access license with attribution). 1 a-d) Native kidney biopsy findings in 2019 demonstrating collapsing focal and segmental sclerosis (hematoxylin and eosin, original magnification 40 light microscopy). Patient 2 b-d): Shye *et al.* CKJ (Adapted under open access license with attribution) [14▪]. Renal biopsy micrographs for patient showing diabetic nephropathy and focal and segmental sclerosis with collapsing features. Renal biopsy reveals underlying diffuse and nodular diabetic glomerulosclerosis (b, Jones methenamine silver 600×). There were lesions of segmental sclerosis with focal collapsing features (c, Trichrome stain 600×) characterized by capillary luminal obliteration by insudates and segmental tuft deflation, with overlying podocyte hyperplasia and prominent cytoplasmic vacuolization (black arrow). There was also concomitant acute tubular necrosis (d, asterisk, hematoxylin and eosin, 200×), with interstitial edema and a mixed interstitial inflammatory infiltrate (interstitial nephritis). Adapted from: -Shye M, Hanna RM, Patel SS, *et al.* Worsening Proteinuria and Renal Function after Intravitreal Vascular Endothelial Growth Factor Blockade for Diabetic Proliferative Retinopathy. Clin Kidney J. 2020. (Under open access license with attribution). Patient 3 e-f): Shye *et al.* CKJ (Adapted under open access license with attribution) [14▪]. Renal biopsy micrographs for patient showing diabetic nephropathy and drug-induced acute interstitial nephritis. Renal biopsy revealed diffuse and nodular diabetic glomerulosclerosis (e, Jones methenamine silver 600×). There were diffuse interstitial edema and extensive interstitial inflammation (f) with associated tubular inflammation and acute necrosis, consistent with interstitial nephritis. Adapted from: -Shye M, Hanna RM, Patel SS, *et al.* Worsening Proteinuria and Renal Function after Intravitreal Vascular Endothelial Growth Factor Blockade for Diabetic Proliferative Retinopathy. Clin Kidney J. 2020. (Under open access license with attribution). Patient 4 g-i): Hanna *et al.* FIM-Nephrology (Adapted under open access license with attribution) [10▪▪]. Biopsy findings in patient with diabetic retinopathy and nephropathy treated with bevacizumab and subsequent thrombotic microangiopathy. (g) One glomerulus showed segmental luminal obliteration by insudates and lipid, with adherence to Bowman's capsule consistent with segmental glomerulosclerosis (arrowhead, methylene blue stain, 400×). (h) Few glomeruli demonstrated segmental duplication of glomerular basement membranes (arrowhead, Jones methenamine silver stain, 400×). (i) Ultrastructural analysis revealed segmental subendothelial electron lucent widening, with very early duplication of basement membrane material (arrowheads, 20 000×). The light and ultrastructural findings were consistent with chronic thrombotic microangiopathy. Adapted from: Thrombotic Microangiopathy and Acute Kidney Injury Induced After Intravitreal Injection of Vascular Endothelial Growth Factor Inhibitors VEGF Blockade-Related TMA After Intravitreal Use. Frontiers in Medicine-Nephrology. 2020. (Under open access license with attribution). Patient 5 j-m): Hanna

levels of retinal inflammation that alter blood vessel permeability to VEGFi agents [11▪]. Volume of distribution can additionally vary between patients [11▪]. Patients may have different degrees of podocyte pathology due to diabetes or other causes of glomerular dysfunction and the effect of VEGF inhibition on overall glomerular function can therefore vary [11▪,13,14▪,15▪▪]. Ultimately two important steps remain to put into effect. First is to create a large national registry to collect all possible side effects of these agents as noted by physicians and patients as this concept is popularized [11▪,13,14▪,15▪▪]. Application of pharmacoepidemiology should be utilized to narrow the gap between clinical pharmacology and epidemiology and pharmacovigilance is urged to concomitantly monitor currently known and possible undiscovered adverse drug events from systemic and intravitreal VEGFi''

Second, a well powered study to include a large group (~50%) of patients with advanced evidence of proteinuria and glomerular dysfunction is needed [10▪▪,59,60]. Measurements of novel markers of renal injury, renal fibrosis, a thorough documentation of VEGFi dose and blood levels [61], estimation of retinal absorption, pharmacokinetics [62], and an epidemiological survey of outcomes would be essential to definitively address all major remaining questions pertaining to the toxicity induced by intravitreal VEGFi [11▪,13,14▪,15▪▪].

CONCLUSION

Intravitreal VEGF blockade is capable of causing systemic pathology and increasingly deleterious renal outcomes are being observed. Larger scale basic, clinical, translational, and epidemiologic research are needed to provide a clearer scale of

et al. FIM-Nephrology (Adapted under open access license with attribution) [10▪▪]. Biopsy findings in patient with diabetic retinopathy and nephropathy treated with bevacizumab and subsequent thrombotic microangiopathy. (j) Immunofluorescence microscopy revealed scattered arterioles which displayed strong amorphous intraluminal and vessel wall staining for fibrinogen (400×). (k) Examination of hematoxylin–eosin (H&E)-stained sections from the frozen tissue demonstrated that the fibrin staining corresponded with changes of arteriopathy, including mucoid intimal thickening (arrowhead) and considerable luminal narrowing, consistent with acute thrombotic microangiopathy (400×). (l) Glomeruli showed changes of diffuse and nodular diabetic glomerulosclerosis (600×). (m) Ultrastructural analysis revealed glomerular basement membranes which showed prominent subendothelial electron lucent widening with accumulation of flocculent debris (20 000×). Overall, the findings were consistent with acute thrombotic microangiopathy. Adapted from: Thrombotic Microangiopathy and Acute Kidney Injury Induced After Intravitreal Injection of Vascular Endothelial Growth Factor Inhibitors VEGF Blockade-Related TMA After Intravitreal Use. Frontiers in Medicine-Nephrology. 2020. (Under open access license with attribution). Patient 6 n-q): Hanna *et al.* FIM-Nephrology (Adapted under open access license with attribution) [10▪▪]. Biopsy findings showing arteriopathy and chronic thrombotic microangiopathy in patient 3. (a) There is severe arterial sclerosis, associated with focal global glomerulosclerosis, periodic acid-Schiff (PAS) stain, 200×. (b) Nonsclerosed glomeruli reveal irregular thickening and segmental remodeling of the capillary loops, with occasional double contours (yellow arrowheads), PAS stain, 400×. (c) There is dull reactivity for fibrin along the glomerular capillary walls on immunofluorescence microscopy, fluorescein isothiocyanate (FITC) stain, 200×. (d) On electron microscopy, glomerular capillary walls reveal subendothelial widening by electron lucent material (red arrowhead), 10 000×. Adapted from: Thrombotic Microangiopathy and Acute Kidney Injury Induced After Intravitreal Injection of Vascular Endothelial Growth Factor Inhibitors VEGF Blockade-Related TMA After Intravitreal Use. Frontiers in Medicine-Nephrology. 2020. (Under open access license with attribution). Patient 7 r-t): Phakde *et al.* CKJ (Adapted under open access license with attribution) [14▪]. Biopsy data showing cFSGS and TMA after intravitreal VEGF injections. (r) Periodic acid–Schiff stain, 40×, showing features of collapsing FSGS. (s) Silver stain, 40×, showing double contouring of glomerular basement membrane seen in chronic TMA lesions. (t) Electron microscopy showing splitting/double contouring of glomerular basement membrane seen in chronic TMA lesions. Adapted from: -Phadke G, Hanna RM, Ferrey AJ, *et al.* Review of intravitreal vascular endothelial growth factor inhibitor toxicity, and report of collapsing focal and segmental sclerosis with thrombotic microangiopathy in a patient with age-related macular degeneration. Clin Kidney J. 2021. (With attribution under open access license) (Under open access license with attribution). Patient 8 u-x): Hanna *et al.* SAGE Open Access Medicine Case Reports (Adapted under open access license with attribution) [45]. Features of active thrombotic microangiopathy on renal biopsy: (u) arteries with near-complete luminal occlusion by fibro-intimal proliferation (arrows) and adjacent ischemic tubulopathy (periodic acid Schiff stain, 100×); (V) artery with mucoid intimal hyperplasia (arrow) (trichrome stain 200×); (w) artery with 'onion skin' change (trichrome stain 400×); and (x) 'bloodless' glomerulus (H&E stain, 400×). Adapted from: Hanna RM, Abdelnour L, Zuckerman JE, *et al.* Refractory scleroderma renal crisis precipitated after high-dose oral corticosteroids and concurrent intravitreal injection of bevacizumab. SAGE Open Med Case Rep. 2020;8 : 2050313X20952650. (Under open access license with attribution).

Table 2. Reported clinical cases of intravitreal VEGF inhibitor use and systemic toxicity

Reference	N	Agent used	Clinical effect(s), renal pathology
Hanna *et al.* [10▪▪]	3	Bev (Cases 1,2) Aflib (Case 3)	Case 1 and Case 2: Diabetic nephropathy and chronic TMA (biopsy+) Case 3: FSGS with chronic TMA features (biopsy +)
Hanna *et al.* [11▪]	1	Bev→Ran	Worsening HTN and proteinuria, lessened with Ran use versus Bev
Hanna *et al.* [13]	4	Bev & Ran	Case 1 de-novo MCD (biopsy+), Cases 2–4 Increased proteinuria, CKD progression, HTN worsening
Phadke-Hanna *et al.* [14▪]	1	Ran→Aflib	(Biopsy +) CFSGS + Chronic TMA Low serum VEGF level Worsening renal disease and HTN with switch from low potency agent (Ran) to high potency agent (Aflib)
Shye-Hanna *et al.* [15▪▪]	3	Case 1 Bev→Ran Case 2 Bev Case 3 Bev→Ran	All: Increased proteinuria, CKD progression, HD Case 1 Worsening proteinuria, CKD progression, HD (biopsy +) Case 2 DN + FSGS with collapsing features + AIN (biopsy +) Case 3 DN + AIN + low systemic VEGF level
Diabetic retinopathy study *et al.* [42]	3	Bev	decreased eGFR
Cheungpasitporn *et al.* [43]	2	Bev	Case 1, MGN, Case 2 TMA (biopsy+)
Georgalas *et al.* [44]	2	Ran & Bev	Decreased eGFR. HD started
Hanna *et al.* [45]	1	Bev	Case 1: Scleroderma renal crisis and TMA induced after intravitreal VEGFi and oral corticosteroids.
Jamrozy-Witkowska *et al.* [46]	1	NR	Decreased eGFR
Kenworthy *et al.* [47]	1	Bev	Increased proteinuria
Khneizer *et al.* [48]	1	Bev	MGN (biopsy+)
Miwako *et al.* [49]	1	Aflib	Case 1: Hypertensive hemorrhage with undetectable VEGF plasma levels after intravitreal injection (preprint)
Morales *et al.* [50]	1	Ran	DN (biopsy+)
Nobakht *et al.* [51▪]	1	Bev→Ran→Aflib	CFSGS (biopsy+) + low systemic VEGF level
Pelle *et al.* [52]	1	Ran	TMA (biopsy+)
Perez-Valdivia *et al.* [53]	1	Bev	Relapsed MCD (biopsy+)
Sato *et al.* [54]	1	Bev	Relapsed MCD (biopsy+)
Touzani *et al.* [55]	1	Bev	endotheliosis/ possible TMA (biopsy +)
Tran *et al.* [56]	1	Bev	AIN (biopsy+)
Yen *et al.* [57]	1	Bev	TMA (biopsy+)

Aflib, aflibercept; AIN, acute interstitial nephritis; AMD; age related macular degeneration; Bev, bevacizumab; Biopsy+, biopsy obtained; CC, current case; CFSGS, collapsing focal and segmental sclerosis; CKD, chronic kidney disease; DM, diabetes mellitus; DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; FSGS, focal and segmental sclerosis; HD, hemodialysis started; HTN, hypertension; IP, MCD, minimal change disease; MGN, membranous glomerulonephritis; n, number of patients; NR, not recorded; Ran, ranibizumab; TMA, thrombotic microangiopathy; UACR, urine albumin to creatinine ratio; UR, under review. Biopsy only if (Biopsy+) stated. Data from Shye *et al*, Clin Kidney J 2020 and Phadke *et al.*, Clin Kidney J 2021. Adapted under open access license and with proper attribution [14▪,15▪▪].

the adverse events [63]. Caution should be advised in using high potency VEGF inhibitors in patients with pre-existing proteinuria, hypertension, and chronic kidney disease.

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Conflicts of interest

None.

This research work does not contain human subject research material, as it is an individual anonymized case series. The work herein conforms with the Declaration of Istanbul.

Ethical permission / consent for publication: IRB permission was not applied for as it is not required for individual case reports or case series with three patients or less in our institution (University of California Los Angeles). Consent was obtained from the patient and documented, on condition that the no identifiable data be published.

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