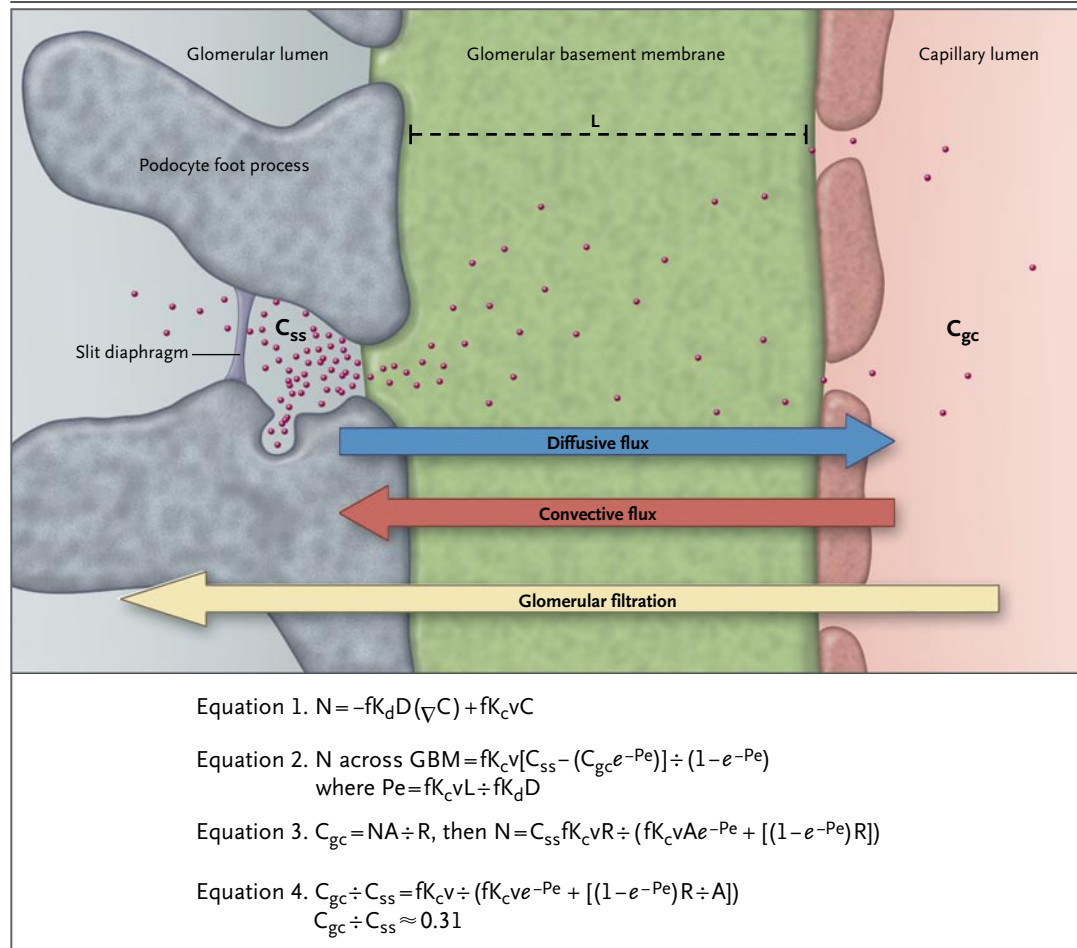


## VEGF Inhibition and Renal Thrombotic Microangiopathy

**TO THE EDITOR:** The article by Eremina et al. (March 13 issue)<sup>1</sup> suggests that the survival of glomerular endothelial cells depends on vascular endothelial growth factor (VEGF) from podocytes. The authors propose that VEGF might be transported across the glomerular basement membrane



**Figure 1. A Model for VEGF Transport against Glomerular Filtration Flow.**

This model assumes that the glomerular basement membrane (GBM) is the only barrier of VEGF transport from podocyte foot processes into the glomerular capillary lumen and that there is a negligible concentration of VEGF (C) in the systemic circulation. Because of size selectivity of the slit diaphragm, VEGF secreted by podocyte foot processes accumulates in the subslit space, generating a concentration gradient across the GBM, despite continuous glomerular filtration flow. VEGF transport across the GBM is influenced by diffusive flux in and convective flux out (equations 1 and 2). If there is a negligible concentration of VEGF in the systemic circulation, the VEGF concentration in the glomerular capillary will be determined by the ratio of the solute delivery from the subslit space to renal plasma flow. As a result, net solute flux (N) could be expressed as a function of the VEGF concentration in the subslit space (equation 3). The ratio of the VEGF concentration in the glomerular capillary to that in the subslit space ( $C_{gc}:C_{ss}$ ) could then be calculated by means of equation 4 and the following estimates<sup>2-4</sup>: the base of the natural logarithm ( $e$ )  $\approx 2.7182818$ ; total glomerular surface area (A) = 6000 cm<sup>2</sup>; solute diffusivity in water (D) = 0.00000094 cm<sup>2</sup> per second, calculated from a molecular-weight–based relationship for globular protein with the use of a VEGF molecular mass of 45 kD; the product of the partition coefficient and the hindrance factor for convection ( $fK_c$ ) = 0.154, and the product of the partition coefficient and the hindrance factor for diffusion ( $fK_d$ ) = 0.034, calculated from Deen et al.<sup>4</sup> with the use of a VEGF radius of 26 Å; the thickness of the GBM<sup>4</sup> (L) = 0.00004 cm; renal plasma flow (R) = 10 ml per second; and velocity vector (v) = -0.00033 cm per second, calculated from a glomerular filtration rate of 2 ml per second divided by A (6000 cm<sup>2</sup>) (the negative value indicates the reverse direction of convective flow). Pe denotes Peclet number.

simply by diffusion. However, their proposal is contrary to the general concept that it is unlikely for any molecules to move against the flow of glomerular filtration. Therefore, we used a simple model to calculate whether VEGF could ultimately reach the capillary lumen (Fig. 1).

To our surprise, we calculated that VEGF secreted from podocyte foot processes would reach the capillary lumen and accumulate there at a magnitude of up to nearly one third of the concentration generated in the subslit space. Apart from a decreased secretory capacity of the podocytes, this model and the derived equation (equation 4) suggest that the endocapillary VEGF concentration would also be adversely affected by decreased size selectivity of the slit diaphragm (proteinuria), increased thickness of the glomerular basement membrane (diabetes), and glomerular hyperperfusion and hyperfiltration (chronic kidney disease). Until more data are available, we suggest that these conditions might be regarded as potential risk factors for renal thrombotic microangiopathy in patients receiving VEGF inhibitors.

Pisut Katavetin, M.D.

Paravee Katavetin, M.D.

Chulalongkorn University  
Bangkok 10330, Thailand  
pkatavetin@yahoo.com

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**TO THE EDITOR:** Eremina et al.<sup>1,2</sup> have reported that VEGF is important in glomerular disease, elucidating mechanisms of glomerular lesions of thrombotic microangiopathy in the mutant mouse and, now, in humans, when VEGF synthesis by podocytes is inhibited. Thrombotic microangiopathy in mice resulted from direct targeting to delete VEGF from podocytes and, in humans, resulted from exposure to an agent that inhibits VEGF.

We previously reported thrombotic microangiopathy in a patient receiving anti-VEGF treatment for renal-cell carcinoma.<sup>3</sup> The glomeruli showed

numerous capillary-loop double contours, fibrin thrombi, endotheliosis, and mesangiolytic.<sup>3</sup> The thrombotic microangiopathy was dose-dependent, disappearing clinically on cessation of anti-VEGF therapy, then relapsing when therapy was resumed. Podocytes that were identified with two specific markers, anti-VEGF and podocalyxin, showed that podocytes adjacent to mesangiolytic were diminished or absent. Anti-VEGF staining revealed the presence of isolated pedicels on the glomerular wall, although the bodies of the podocytes had disappeared. In humans, anti-VEGF thrombotic microangiopathy is similar to preeclampsia, both morphologically and clinically, in that each can resolve when the placenta is delivered<sup>4</sup> or anti-VEGF is stopped.<sup>3</sup>

Dominique Nochy, M.D.

Georges Pompidou European Hospital  
75015 Paris, France  
dominique.nochy@egp.aphp.fr

Carmen Lefaucheur, M.D.

Saint Louis Hospital  
75010 Paris, France

Gary Hill, M.D.

Georges Pompidou European Hospital  
75015 Paris, France

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**THE AUTHORS AND A COLLEAGUE REPLY:** We are grateful to Katavetin and Katavetin for presenting a kinetic model of VEGF synthesis that supports our suggestion that VEGF diffuses from the podocytes to the endothelial cells. The Peclet number (Pe) reveals the importance of convection relative to diffusion<sup>1</sup> in the following way:

$$Pe = \frac{(\Phi K_c) \times v \delta}{(\Phi K_d) \times D_\infty}$$

where  $v$  denotes filtration velocity,  $\delta$  membrane thickness,  $\Phi$  partition coefficient,  $D$  diffusion con-

stant for the solute, and  $K_c$  and  $K_d$  hindrance factors for convection and diffusion, respectively.

Measurements on isolated glomerular basement membranes<sup>2</sup> suggest that  $\Phi K_c = 0.2$  and  $\Phi K_d = 0.02$ . The latter value shows that diffusion is markedly impaired in the glomerular basement membrane as compared with diffusion in water (2% of D), but convective transport is also restricted. The key point is whether diffusion is more limited than convection; the Pe reflects this. A Pe above unity indicates that transport occurs mainly by convection, and a Pe of less than 1 reflects diffusion-dominated transport.

The Pe in glomerular basement membrane for VEGF can be estimated by using a value of  $v$  close to  $4 \times 10^{-4}$  cm per second and  $\delta$  close to  $2 \times 10^{-5}$  cm. VEGF has a Stokes–Einstein radius ( $r_s$ ) of 2.6 nm, which implies that  $D_{2.6\text{nm}}$  is  $1.26 \times 10^{-6}$  cm<sup>2</sup> per second. Inserting these values into equation 1 results in a Pe of 0.063. It is safe to conclude that VEGF is transported by diffusion, and diffusion alone, across the glomerular basement membrane.

The model presented by Katavetin and Katavetin has the virtue of simplicity but would appear to be an oversimplification. For example, one needs to incorporate the effects of serial barriers<sup>1</sup> to allow for better predictions of concentrations at the endothelium.

Also, as stated by Katavetin and Katavetin, the general perception is that transport across the glomerular barrier is completely dominated by convection. In fact, the opposite is true.<sup>2</sup> Thus, the flow conditions in the glomerular basement membrane resemble not a waterfall but, rather, a great lake with slow flow velocity. Consequently, diffusion dominates the transport of most solutes in the glomerular basement membrane.<sup>2</sup>

We thank Nochy et al. for pointing out an additional published case report by Frangié et al.<sup>3</sup> The issue of reversibility of thrombotic microangiopathy raised by the authors is important.<sup>4</sup> Although renal function improved in the six cases we reported, we were unable to determine whether it returned to baseline. Frangié et al. found that although hypertension and hemolysis improved, the proteinuria persisted between cycles; this may reflect podocyte loss and segmental sclerosis, as shown in their report. We are aware of a patient at the University of Toronto in whom irreversible chronic kidney injury apparently developed and progressed to end-stage renal failure, despite discontinuation of the VEGF inhibitor.

Borje Haraldsson, M.D., Ph.D.

Sahlgrenska University Hospital  
SE-413 45 Gothenburg, Sweden

Laura Barisoni, M.D.

New York University School of Medicine  
New York, NY 10016

Susan E. Quaggin, M.D.

University of Toronto  
Toronto, ON M5S 1A8, Canada  
quaggin@mshri.on.ca

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## Case 11-2008: Mental-Status Changes after Liver Transplantation

**TO THE EDITOR:** We would like to make two points about the case of disseminated cryptococcosis in a liver-transplant recipient, discussed in the Case Records by Fishman et al. (April 10 issue).<sup>1</sup> The first point concerns delayed diagnosis of this infection in patients with negative tests for human immunodeficiency virus infection. We believe that in the case presented, a correct diagno-

sis could have been suspected from day 13 onward on the basis of the patient's recurrent headaches. In immunocompromised patients, severe and persistent headache should prompt the performance of lumbar puncture.<sup>2</sup>

The second point is the low awareness among physicians of the diagnostic utility of cryptococcal antigen determination in serum.<sup>3</sup> As shown