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Primed to understand fibrinogen in cardiovascular disease

Alisa S. Wolberg, Ph.D.

Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599-7525

Fibrinogen is a 340 kDa glycoprotein that circulates in healthy humans at 2–4 mg/mL; however, fibrinogen is an acute phase protein synthesized in the liver, and its circulating levels can exceed 7 mg/mL during acute inflammation. Elevated fibrinogen levels are associated with increased risk of incident cardiovascular disease (CVD).[1,2] Healthy mice infused with unfractionated human fibrinogen and subjected to $FeCl_3$-mediated carotid artery injury have a shortened time to vessel occlusion and increased resistance of thrombi to acute thrombolysis, suggesting elevated fibrinogen independently contributes to thrombosis.[3,4]

Fibrinogen is composed of two sets of three polypeptide chains: Aα, Bβ, and γ. Alternative splicing of the γA chain leads to synthesis of a γ′ chain containing a unique 20-amino acid sequence at the C-terminus. Between 8–15% of circulating fibrinogen in healthy individuals contains a γ′ chain (γA/γ′). Cross-sectional and retrospective studies have associated elevated circulating levels of the γA/γ′ isoform with increased incidence of coronary artery disease[5], myocardial infarction[6], and ischemic stroke[7–9]. The observation that some patients have an increased γ′-to-total fibrinogen ratio[7–11] suggests γA/γ′ fibrinogen is not simply a biomarker for increased total fibrinogen. Together with data from *in vitro* studies demonstrating clots formed from purified γA/γ′ fibrinogen are composed of abnormally-structured fibers and are highly resistant to fibrinolysis[12–15], these observations have led to the notion that γA/γ′ fibrinogen is an etiologic risk factor for CVD.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Appiah and colleagues report a large prospective study examining the association of plasma γ′ fibrinogen levels with incident CVD endpoints.[16] Their unadjusted analysis shows a positive association of γ′ fibrinogen with incident coronary heart disease, ischemic stroke, peripheral artery disease, heart failure, and CVD deaths. However, adjustment for established CVD risk factors and levels of plasma fibrinogen and C-reactive protein (CRP) as a biomarker for inflammation abolished the associations with coronary heart disease and ischemic stroke, and sharply attenuated the significance of the association with heart failure and peripheral artery disease. In contrast to previous studies, Appiah *et al*. conclude that γ′ fibrinogen levels reflect an inflammatory process that accompanies, and may promote, CVD, but that γ′ fibrinogen does not independently contribute to CVD.[16] Strengths of their analysis include the large number of subjects and its prospective design which are directly responsive to prior calls for this

Address correspondence to: Alisa S. Wolberg, Ph.D., Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, 819 Brinkhous-Bullitt Building, CB #7525, Chapel Hill, NC 27599-7525, Phone: (919) 962-8943; Fax: (919) 966-6718, alisa_wolberg@med.unc.edu.

type of study.[17,18] Limitations include drift in measurements of γ′ fibrinogen over time and the fact that γ′ fibrinogen and CRP measurements were made from samples collected at separate visits.

Given these conclusions, what is the role of γ′ fibrinogen *in vivo*? In addition to its prothrombotic characteristics, fibrinogen has critical anticoagulant functions by adsorbing thrombin during clotting (known as "antithrombin I" activity).[17] Afibrinogenemic patients have elevated markers of coagulation activation and experience acute thrombosis[19,20]. Notably, repletion of afibrinogenemic plasma with γA/γ′ fibrinogen is more effective than γA/γA fibrinogen at reducing thrombin generation.[21] This effect has been attributed to the ability of γ′ fibrinogen to support high affinity non-substrate binding of thrombin.[22–24] Although fibrin-bound thrombin resists heparin-catalyzed inactivation by antithrombin III[24], its activity towards its endogenous substrates is also reduced. Accordingly, *in vitro* studies show that presence of γA/γ′ fibrinogen reduces thrombin-mediated activation of cofactors VIII[25] and V[26], and increases plasma sensitivity to activated protein C[27]. Consequently, the net contribution of γ′ fibrinogen to coagulation *in vivo* – either pro- or antithrombotic – is difficult to predict.

Muthard *et al*.[28] recently found that γ′ fibrin(ogen) reduces thrombin-mediated clot growth at venous, but not arterial shear rates, suggesting the contributions of γ′ fibrinogen are mediated by the vascular bed. Observations from animal models of venous and arterial thrombosis are consistent with this premise. Data from venous thrombosis models demonstrate a net antithrombotic effect of γ′ fibrinogen: 1) transgenic expression of the human γ′ chain reduces venous thrombus volume in mice that are heterozygous for the factor V Leiden mutation[29], and 2) infusion of an 18-amino acid peptide mimicking the γ′ chain C-terminus (γ′ 410–427) reduces fibrin formation in an arteriovenous shunt in baboons[25]. In contrast, in an arterial thrombosis model, mice infused with γA/γA fibrinogen have a shorter time to artery occlusion than control mice, but mice infused with γA/γ′ fibrinogen do not.[4] This finding is notable since mice infused with γA/γ′ fibrinogen have lower circulating levels of thrombin-antithrombin complexes than either control mice or γA/γA fibrinogen-infused mice.[4] Thus, it appears that in this model, the antithrombin I activity of γA/γ′ fibrinogen mitigates, but does not overcome, any procoagulant effects of this molecule. Together with findings from Appiah *et al*.[16], these data suggest the net effect of γ′ fibrinogen during arterial thrombosis is neutral (Figure).

If γA/γ′ fibrinogen does not influence CVD outcomes, why are its levels increased in CVD patients? CVD is a proinflammatory pathology associated with elevated levels of fibrinogen, and the proinflammatory cytokine interleukin-6 preferentially up-regulates hepatocyte production of γA/γ′ fibrinogen *versus* γA/γA fibrinogen.[30] Accordingly, γA/γ′ fibrinogen may be increased to down-regulate inflammation-induced prothrombotic activity in certain situations. Notably, reduced γA/γ′ fibrinogen levels and γ′-to-total fibrinogen ratio are associated with increased risk of venous thromboembolism[31] and thrombotic microangiopathy[32], related pathologies that are also associated with vascular inflammation. These findings are consistent with observations from the animal studies.[25,29] Thus, increased γA/γ′ levels associated with CVD may simply reflect its common etiology with venous disease, in which this molecule has a protective, antithrombotic role. To this end, it

is curious that elevated γ′ fibrinogen appears differentially associated with the different CVD outcomes studied and remains significantly associated with the broad category of CVD deaths, even after adjustment for CVD risk factors, fibrinogen, and CRP. A greater understanding of the common and unique pathophysiologic mechanisms associated with these pathologies may shed light on this issue.

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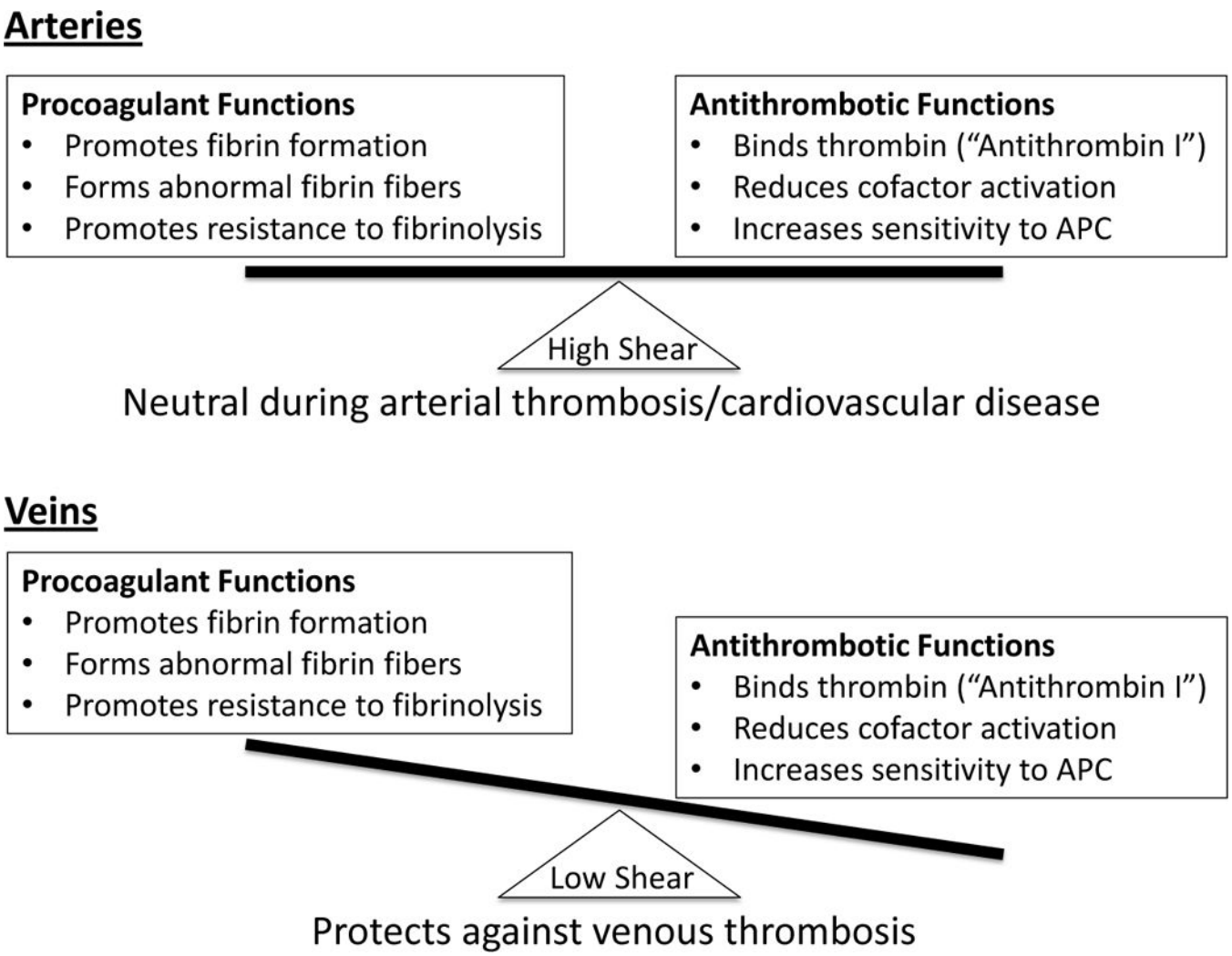

Figure. Model illustrating the procoagulant and antithrombotic functions of γ′ fibrinogen

During thrombosis in high arterial shear (top), the antithrombotic activity of γ′ fibrinogen is not sufficient to overcome the prothrombotic properties of this molecule, resulting in a net neutral effect. In low shear in venous circulation (bottom), the thrombin-binding ability of γA/γ′ fibrinogen reduce cofactor activation and increase sensitivity to APC, outweighing its procoagulant contributions and reducing venous thrombosis risk.