Review

Measuring Plasma Fibrinogen to Predict Stroke and Myocardial Infarction

Giovanni Di Minno and Mario Mancini

Epidemiological observations indicate that high plasma fibrinogen levels are strongly correlated with the frequency of two major thrombotic complications of atherosclerosis, stroke and myocardial infarction. Thrombosis is increasingly recognized as a central mechanism in stroke and myocardial infarction, and fibrinogen is involved in events thought to play a major role in thrombosis. Therefore, elucidation of the relationship between fibrinogen and thrombosis may strengthen the predictive value of this protein and suggest new treatment to prevent stroke and myocardial infarction. The current data relating fibrinogen to thrombosis are not easy to reconcile with the available epidemiological observations. In addition, advances in understanding the atherogenic potential of several risk factors for coronary heart disease have used information on the measurement of the risk factors in population-based studies. Thus, measuring plasma fibrinogen to predict stroke and myocardial infarction may be important in gaining insight into the thrombogenic potential of this protein and in inspiring new strategies against the thrombotic complications of atherosclerosis. (Arteriosclerosis 10:1-7, January/February 1990)

In 1980, Meade et al.\(^1\) first reported an association between hemostatic parameters and cardiovascular death. In their prospective study, they found that persons who died from coronary heart disease (CHD) had higher plasma levels of fibrinogen at recruitment than did survivors or persons dying from other causes. In addition, Meade et al. showed that within 5 years after the start of the study, the association of cardiovascular mortality with fibrinogen levels was independent of established CHD risk factors and stronger than the association with serum cholesterol. Lowe et al.\(^2\) reported in the same year that levels of fibrinogen were higher in patients with two or three stenosed coronary arteries than in those with a single stenosed artery or no stenosis. Four years later, Wilhelmsen et al.\(^3\) reported on the synergistic effect of fibrinogen levels and blood pressure on stroke and suggested that high plasma fibrinogen is a risk factor for stroke and myocardial infarction. More recently, other prospective studies\(^4-8\) have given new impetus to these observations and strengthened their clinical relevance. These epidemiological observations did not occur in isolation. Over the last 15 years, the theory that hemostatic components are involved in atherosclerosis and its thrombotic complications has been confirmed and extended.\(9,10,11\) It is now clear that some risk factors for CHD, such as cigarette smoking and high plasma lipid levels, affect the hemostatic process;\(\) that human gelatinous and fibrous plaques are rich in fibrinogen, fibrin, and fibrinogen degradation products;\(\) that fibrinogen and fibrin are involved in tissue proliferation;\(\) that patients with some dysfibrinogenemias (qualitative abnormalities of fibrinogen) are prone to thrombosis;\(\) that thrombosis is a major determinant of myocardial ischemia;\(\) and that drugs that affect hemostatic parameters reduce the recurrence of two major ischemic complications of atherosclerosis, stroke and myocardial infarction.\(\)

Advances in our understanding of the basic mechanism(s) through which some abnormalities are involved in the pathogenesis of atherosclerosis have confirmed a strong relationship between an abnormality and a clinical event, have inspired appropriate strategies of intervention, and have been crucial in identifying risk factors for CHD.\(9,10,11,16,17,19,20,38,39\) Fibrinogen and its naturally occurring derivative, fibrin, are involved in mechanisms such as platelet aggregation, blood rheology, and endothelial cell injury,\(12,19,20,38,39\) which are thought to play a key role in thrombosis and atherosclerosis. Therefore, it is important to evaluate whether the experimental and clinical data on fibrinogen allow for conclusions comparable to those reached for CHD risk factors.

Fibrinogen and Platelets

It is known\(40-43\) that patients with a congenital fibrinogen deficiency have a life-long tendency to bleed and that...
their platelets either do not aggregate or aggregate poorly in vitro in response to a variety of agents. In vitro, a defect comparable to that of these patients' platelets is seen in normal subjects' platelets that have been washed free of fibrinogen.44-48 Since fibrinogen corrects the bleeding tendency and the in vitro defective response of platelets in these two instances, it has been concluded that fibrinogen is important for effective platelet function in vitro and in vivo.

This theory has also been suggested by studies on patients with Glanzmann's thrombasthenia. Like patients lacking fibrinogen, patients with Glanzmann's thrombasthenia have a tendency to bleed,39 and their platelets do not aggregate in vitro in response to various agents.49,50,51 However, in Glanzmann's thrombasthenia, in contrast with afibrinogenemia, fibrinogen does not correct the bleeding tendency or the abnormal in vitro response of platelets.39 In the 1970s, it was shown52-60 that platelets from thrombathrombocytopenic patients lack a glycoprotein complex, the Gpllb-IIIa complex, on their membranes. Ten years later, it was demonstrated61-64 that platelets possess specific receptors for fibrinogen and that these receptors are not found on the surface of platelets from persons with thrombathrombocytopenia. It was then suggested that the Gpllb-IIIa complex is the locus for the binding of fibrinogen to platelets, and that this binding is important for effective hemostasis as well as for normal in vitro platelet aggregation.

Studies with murine62-65 and human66-68 monoclonal antibodies to the platelet receptor for fibrinogen have further supported this concept and suggested a mechanism for the thrombogenic potential of fibrinogen. In vivo aggregation of platelets has been demonstrated in patients with unstable angina or sudden cardiac death.32,33,34 In vitro platelet hyperreactivity to aggregating agents has been reported in some patients who are prone to thrombosis and during episodes of myocardial or peripheral ischemia.69-74 Effective inhibition of fibrinogen binding to platelets in some of these patients corrected their hyperaggregable state.70,71,72 Although suggestive, these data should be taken with caution. In humans, if sensitivity of platelets to adenosine diphosphate (ADP) is correlated to plasma levels of fibrinogen,73 the maximal aggregation of washed human platelets is achieved in response to concentrations of fibrinogen that are much lower than the concentrations that are predictive for stroke or myocardial infarction.38 Furthermore, only a limited number of the patients who are prone to thrombosis show platelet hyperreactivity. In our experience, only 7% to 10% of diabetics and 30% to 35% of hypercholesterolemic patients have platelets with an increased in vitro sensitivity to aggregating agents. Finally, the presence of an association between in vitro platelet hyperreactivity and the ischemic complications of atherosclerosis does not satisfy the minimum criteria for establishing a causal relationship between thrombotic episodes and blood test results.75

**Fibrinogen and Blood Rheology**

Studies on the physiology of Newtonian fluids (i.e., noncomplex fluids such as water or plasma) show that the amount of flow through a straight tube depends upon the force applied and on the resistance opposed to the flow. The latter is related to the length and the radius of the tube and also to some inherent properties of the fluid such as elasticity and viscosity.36,78 The elasticity of blood is likely to be related to the reversible deformation of erythrocyte aggregates,79,80 and the pathophysiological significance of this elasticity remains to be defined.

In contrast, the relevance of viscosity is well established. Viscosity measures the internal resistance of blood to flow. When a fluid flows through a tube, it forms concentric layers that slide over each other. This laminar flow is defined as shearing, and shear stress is the force per unit of area that causes shearing motion.36,78 The constant of proportionality between shear stress and the rate of shear (shear rate) is the viscosity of the fluid. For Newtonian fluids, the relationship between shear rate and shear stress is linear and depends upon the specific properties of the fluid. In fact, the viscosity of plasma is 1.6-fold higher than that of water because plasma contains large proteins such as fibrinogen.79,80 At shear rates below 200 sec⁻¹, blood is a nonNewtonian fluid, and its shear rate increases nonlinearly with shear stress. Experimental data81,82 indicate that this nonlinear increase is correlated to the volume of packed red blood cells and that the increase involves the ability of erythrocytes to aggregate, a phenomenon largely dependent on plasma fibrinogen.81,82

Several lines of evidence suggest a role for blood viscosity in thrombosis. It has been shown83-86 that blood viscosity is involved in platelet adhesion to the vessel wall; in the presence of fibrinogen, changes in the viscosity of the platelet-suspending medium greatly enhance the sensitivity of platelets to subthreshold concentrations of aggregating agents such as ADP.87-90 The regulation of the availability of a platelet procoagulant activator, PF3, has been shown to involve blood viscosity.91 Finally, patients with hyperviscous syndromes are prone to thrombosis and to abnormalities of the cerebral and retinal circulation that can be corrected by correcting the hyperviscous state.97-103 However, only a limited number of thrombosis-prone patients have a hyperviscous syndrome.

**Fibrinogen and the Vessel Wall**

Studies in rodents104,105,106 demonstrate that the thrombi formed after a limited endothelial injury (similar to injuries thought to initiate atherosclerosis in humans) are rich in fibrin, platelets, and leukocytes. A few days after formation, these thrombi become indistinguishable from fibrous atherosclerotic plaques. In vitro experiments with cultured endothelial cells show that fibrin and some fibrinogen degradation products trigger a variety of mechanisms thought to play a major role in atherogenesis: endothelial injury, disorganization of the normal architecture of the endothelial monolayer, increased DNA synthesis and pinocytotic activity (a mechanism probably regulating the normally high water content of early proliferative lesions), cell growth, proliferation and migration of fibroblasts and smooth muscle cells, and release of von Willebrand factor.107-114 Furthermore, several fibrinogen degradation products that are formed when
fibrogen(ogen) is exposed to plasmin increase chemotaxis, vascular permeability, smooth muscle cell contractility, synthesis of collagen, and angiogenesis.\(^{115-122}\)

The evidence indicates a relationship between the interaction of fibrinogen with the vessel wall and thrombosis, as well as atherosclerosis. Erythrocytes from normal subjects do not adhere to endothelial cells, while red blood cells from thrombosis-prone patients (such as those with diabetes mellitus) do adhere, and fibrinogen is a major modulator of this adhesion.\(^{123}\) Perturbed cultured endothelial cells or fibroblasts bind fibrinogen,\(^{124,125}\) and this interaction is important for the retraction of fibrin clots.\(^{126,127}\) A phenomenon that may be involved in the consolidation of a physiological plug or a pathological thrombus. However, the amounts of fibrinogen required to achieve these effects are much lower than the ones that are correlated to the frequency of stroke and myocardial infarction.

### Thrombogenic Potential of Fibrinogen: Other Directions

In recent years, several aspects of the physiology of fibrinogen have been clarified (Table 1), and some directions to be followed to elucidate the thrombogenic potential of this multifaceted protein have emerged.

In addition to platelets and endothelial cells, fibrinogen also interacts with monocytes/macrophages.\(^{126-134}\) These cells are thought to play a major role in atherosclerosis,\(^{107}\) and the binding of fibrinogen to monocytes/macrophages is associated with the triggering of procoagulant and fibrinolytic activities. However, the maximal binding of fibrinogen to perturbed monocytes/macrophages is achieved in response to concentrations of fibrinogen comparable to those required for platelets.

The estrogen, 17-\(\beta\)-estradiol, has been shown to decrease the synthesis of fibrinogen.\(^{135}\) Likewise, a variety of personal and environmental factors (Table 2) have been shown to affect plasma levels of fibrinogen.\(^{136,137,138}\)

Furthermore, recent observations suggest a reduction of plasma levels of fibrinogen during treatments with ticlopidine, pentoxifylline, stanozolol, cod liver oil, or some fibrates. Although suggestive, these data are only circumstantial and are not likely to elucidate the thrombogenic potential of fibrinogen. Most of these conditions also affect other mechanisms thought to play a role in thrombosis.

Human fibrinogen is a covalently dimerer formed in the liver consisting of three pairs of disulfide-bonded polypeptide chains designated as \(A\alpha, B\beta,\) and \(\gamma.\)\(^{136}\) In the hepatocytes, there are intracellular pools of \(\alpha\alpha-\) and \(\gamma\)-chains; the synthesis of the \(B\beta\)-chain is the limiting step in the assembly of the protein.\(^{136,140}\) Recent data\(^{141}\) have shown that a polymorphism of the \(B\beta\)-chain is associated with plasma concentrations of fibrinogen comparable to the ones that are correlated to an increased risk of ischemic heart disease. However, variations of the fibrinogen \(\beta\)-gene locus account for only a limited number of the total phenotypic, individual variations in fibrinogen levels.\(^{138,141}\)

Some molecular variations of fibrinogen are associated with an abnormally high tendency to thrombosis.\(^{24,136,142}\) The \(\gamma\)-chain of human fibrinogen exists in two variants produced by alternative mRNA processing,\(^{143}\) and the pathophysiological significance of these variants remains to be determined. However, only about half of dysfibrinogenemias are symptomatic, and only a limited number are associated with arterial thrombosis.\(^{24}\)

### Perspectives

Epidemiological data indicate that measurements of plasma levels of fibrinogen should be included in the cardiovascular risk factor profile.\(^7\) On the other hand, the results of most experimental and clinical studies that relate fibrinogen to the thrombotic complications of atherosclerosis are suggestive but only circumstantial and are not easily reconciled with epidemiological data. Furthermore, advances in the understanding of the thrombogenic potential of established CHD risk factors have often been based on information on these risk factors from population-based studies.\(^36,37\) Thus, the implications emerging from the screening studies on fibrinogen should be pursued to gain insights into the role of this protein in...
thrombosis, to define new strategies for preventing the thrombotic complications of atherosclerosis, and to establish fibrinogen as a risk factor for stroke and myocardial infarction. Some examples will help clarify the concept.

A "hematological stress syndrome" in atherosclerosis has been described, and a variety of stress conditions (Table 2) have been shown to enhance plasma fibrinogen levels. This raises the possibility that high plasma levels of fibrinogen may be a response of an acute-phase reactant to the severity of the atherosclerotic vascular damage taking place. The results of studies evaluating the contribution of genetic heritability to the regulation of plasma levels of fibrinogen suggest that high plasma fibrinogen levels are not just an epiphenomenon of unknown events in thrombosis and atherosclerosis. This conclusion is also suggested by the independence of high plasma fibrinogen levels from known risk factors for stroke and myocardial infarction. However, confirmation of a causal relationship between fibrinogen and stroke and myocardial infarction will depend on the results of ad hoc studies aimed at addressing the issue of whether high fibrinogen levels identify a group of patients with an abnormally high tendency to the thrombotic complications of atherosclerosis.

In view of the fact that CHD risk factors act synergistically, the notion of fibrinogen as an independent variable for stroke and myocardial infarction implies that high fibrinogen levels may greatly enhance the injurious effects of other risk factors. Thus, the issues addressed should be 1) whether high fibrinogen levels identify the persons for whom strong interventions on established risk factors for stroke and myocardial infarction are of the highest priority (or for whom strong interventions on established risk factors for stroke and myocardial infarction are needed) and 2) whether these interventions should take into account personal and environmental factors involved in the regulation of plasma levels of fibrinogen.

In addition to its obvious pathophysiological significance, this information will help organize the large and diverse body of data on plasma fibrinogen and will suggest new strategies against the thrombotic complications of the commonest cardiovascular disease.

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FIBRINOGEN AND ARTERIAL THROMBOSIS  Di Minno and Mancini


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