

THE COLLECTIVE RISK HYPOTHESIS: FIBRIN NETWORK ARCHITECTURE AND CARDIOVASCULAR DISEASE

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ABSTRACT

Fibrinogen is an important risk factor for atherosclerosis, stroke and cardiovascular disease (CVD). This risk is increased when associated with high serum cholesterol, increased blood pressure, obesity and smoking. However, it is also believed that not only the fibrinogen itself, but also the quality of resultant fibrin networks may be a predisposing risk factor for the development of CVD. Changes in the fibrin network architecture are caused by changes in the fibrin polymerisation conditions in the blood. Kinetic and modulating factors determine polymerisation conditions. The kinetic factors are thrombin and fibrinogen concentrations. The modulating factors affect the fibrin structures independent of the kinetic factors. Such factors include all biochemical and physical properties of the direct surrounding within which the circulating fibrinogen molecule finds itself. Current literature describes only the concentration of the circulating plasma fibrinogen to have predictive value for CVD. The main aim of this article is to present a hypothesis that provides a novel way of looking at fibrinogen related CVD risk. We will refer to this hypothesis as the Collective Risk Hypothesis, in which it is postulated that the fibrin network architecture provides a better estimate of CVD risk than just plasma fibrinogen concentration. The fibrin network architecture depends not only on the fibrinogen concentration alone, but also on the collective effect of all other chemical and physical interactions within its direct environment (the modulating factors), which also includes that of the other more established independent CVD risk factors, such as LDL-C, glucose and smoking. This model provides the potential for additional laboratory analysis to establish CVD risk by means of fibrin network architecture analyses, in addition to providing a valuable tool for prevention, diagnosis and monitoring of patients with CVD.

Keywords: Plasma fibrinogen, fibrin network architecture, cardiovascular disease

1. INTRODUCTION

The pathology of cardiovascular disease is well investigated and understood. Yet, in the developed world cardiovascular disease (CVD) remains a burden. Also, in developing countries, such as South Africa the prevalence of CVD is expected to increase¹. Many clinical and epidemiological studies have undertaken the responsibility to establish those factors that contribute towards the development and/or prevention of CVD. The degrees of risk to which different factors contribute towards the clinical manifestation of CVD varies and have caused many an ordeal between scientists, as well as the clinical profession. The importance of some of these risk factors was accepted without any a doubt, such is the role of serum-Total Cholesterol and its different sub-fractions. In addition to epidemiological evidence, hypotheses such as these were soon to be found biologically plausible. Other risk factors, such as plasma fibrinogen, were met with much caution and even so, resistance. Especially the processes involved in fibrinogen related CVD risk are not yet well understood. The scientific community is burdened with finding appropriate lifestyle factors that can decrease fibrinogen related CVD risk. Lifestyle intervention, such as diet and exercise, showed promising results as a means of decreasing cholesterol related CVD risk. This however should not allow one to disregard the multifactorial nature of CVD and it is yet it is this characteristic that remains disregarded. Some investigators recognise this aspect of CVD and have developed a "risk calculator", which base its calculation of CVD risk on a compilation of established lifestyle and biochemical risk markers (total serum-cholesterol, low-density lipoprotein cholesterol, blood pressure, age, smoking and the presence of diabetes). It is available from <http://hin.nhlbi.nih.gov/atp/iii/calculator.asp>. CVD is preventable and early detection of individuals prone to its manifestation is important. In this article we present a novel theory of establishing CVD risk, using fibrin network architecture as a possible indicator of collective CVD risk in human subjects.

2. PLASMA FIBRINOGEN

Prospective epidemiological studies from 1980 to 1989 accumulated evidence of a possible relationship between cardiovascular disease (CVD) and plasma fibrinogen concentration². These studies include the Gothenborg Study³, the Framingham Study⁴, the Northwick Park Heart Study⁵, the Leigh Study⁶ as well as the Caerphilly/Speedwell Study⁷. All of these studies predict a strong correlation between plasma fibrinogen concentration and CVD risk. Plasma fibrinogen was therefore also strongly associated with other known cardiovascular risk factors, including blood pressure, serum total cholesterol, obesity, glucose intolerance, etc. However, it soon became evident that raised circulating plasma fibrinogen levels, causing hypercoagulable states, involve complex and multifactorial processes that are difficult to predict. According to Tarallo *et al.* (1992) the presence of known risk factors explains nearly 15% of the total variance in the circulating fibrinogen concentration⁸. Some of these factors have been examined extensively:

hypercholesterolaemia⁹;
hypertriglyceridaemia¹⁰;
diabetes mellitus⁴;
age⁴;
hypertension^{4, 11};

obesity¹²;
smoking³;
oral contraceptives¹²;
psychological stress and social class¹³;
physical inactivity¹⁴.

Results from the Northwick Park Heart Study indicated an 85% increase in the predictability of the prevalence of cardiovascular disease in the following 5 years with a fibrinogen level of only 0.6 g/L above the average of 2.9 g/L¹⁵. Consequently it is important to realise that hypercoagulability is associated with other risk factors of cardiovascular disease. This elevates the relevance of studying the haemostatic variables together with these risk factors².

3. FIBRIN NETWORK ARCHITECTURE

The study of fibrin network architecture is not new. Yet, it seems as if little interest in this science was stimulated in the pathology laboratory by those that managed to develop and show its promising applications. But, it is a field of study with much potential for application in the diagnosis and treatment monitoring of cardiovascular disease, and those abnormal pathologies related to it. The methods of fibrin network architecture analyses have widely been used widely for the successful investigation of genetic abnormalities of the haemostatic system. As a method of screening it provides a biochemical summary of the cardiovascular risk status of a patient blood sample, taking into consideration the collective effect of the separately established independent risk factors. Knowledge of the structure of fibrinogen and of the properties of the fibrin polymers that are formed on activation of fibrinogen by thrombin or other enzymes has expanded considerably during the past two decades. Evaluation of fibrin network properties has become easy and accessible in almost any standard haematology or clinical chemistry laboratory.

3.1 Fibrin network architecture regulation

The physical and biochemical structure of the fibrin network depends upon the polymerisation conditions¹⁶. It is known that any given network comprises of a major network of thicker fibres and a minor network of thinner fibres¹⁷. According to Blombäck *et al.* (1992) these gel structures are determined by kinetic factors and modulating factors¹⁸.

Kinetic factors: The kinetic factors are thrombin and fibrinogen concentrations. Increasing the kinetic factors will result in tighter, less porous networks with thinner fibres and a higher density of nodes. These structures are dense, rigid and flow of a liquid through it is impaired. Conversely, low concentrations of kinetic factors result in porous networks with thick fibres and fewer nodes. These structures are less tightly packed, deformable and plastic and consequently fluid easily escapes through the structure. It is evident that the initial fibrin polymers create nuclei for the growth of linear polymers in different spatial directions. A network structure is thus formed. The faster the activation the larger the density of polymeric nuclei and the tighter the network structure¹⁸. The rate of activation of fibrinogen by thrombin will increase significantly with increasing fibrinogen concentration and this leads to a drastic change in the fibrin gel structure¹⁹.

Modulating factors: The modulating factors include proteins and ions in direct contact with the fibrinogen molecule¹⁷. Modulating factors change the

architecture of the network by either interacting with the surrounding fluid during fibrin formation or by binding to either fibrinogen or to the fibrin strands in the established network.

Modulating factors include:

- fibrinogen composition, non-enzymatic glycosylation, carbohydrate content, and any factors causing a change in primary and/or secondary structure of the molecule^{20; 21};
- albumin^{22; 23};
- cations, pH and temperature^{24; 25; 26; 17};
- blood platelets²⁷;
- plasma antithrombin-III²⁸;
- homo poly (L-amino acids)²⁹;
- dextran³⁰;
- glucose and antidiabetic drugs^{31 ; 32};
- gamma-globulin³³;
- fibronectin³²;
- insulin, growth hormone and estrogen³⁴;
- acetylsalicylic acid³⁵; and
- a variety of long- and short-chain fatty acids³⁶.

3.2 Fibrin network architecture and fibrinolysis

Fibrin network structure contributes to the regulation of the fibrinolytic rate³⁷. As the fibrin network size is decreased, the fibrinolytic rate also decreases. Thin fibrin fibres have a decreased rate of conversion of plasminogen to plasmin by tissue plasminogen activators (tPA) and thin fibres are lysed more slowly than thick fibres³⁷. The number of tPA binding sites and fibrinolytic rate are dependent on fibrin structure³⁸. Since structural features of fibrin at the end of fibrin assembly may determine the potential number of binding sites for the fibrinolytic enzymes, it is possible that the thin fibres possess fewer plasmin binding sites, possibly a function of the smaller surface area of the thin fibres³⁷. This may be due to (i) a decrease in the number of binding sites resulting from the smaller surface area of thin fibres, or (ii) steric factors caused by the curvature of the thin fibres which prohibit stable interaction between tPA and its binding site³⁷. Fibrin, with its ordered structure appears to exert its rate-enhancing effect by presenting its binding-sites for interaction with tPA and plasminogen, thus concentrating and correctly orienting these two reactants on its surface and inducing conformational changes which lead to higher catalytic activities³⁹. Alternative explanations include a decreased collision rate between plasmin and fibrin based on the fibre size³⁷. This description is only true for the early part of fibrinolysis. As the fibrin structure undergoes lysis, the fibre diameter decreases, while the porosity and subsequently the specific permeability increases¹⁶. However,

thicker fibres are known to undergo substantial inner fibre lysis before break-up²⁶.

3.3 Fibrin network architecture and cardiovascular disease

It is suspected that not only fibrinogen concentration, but also the quality of fibrin networks may directly be related to CHD risk¹⁸. Patients with an elevated plasma fibrinogen concentration have a considerably lower fibrin gel permeability compared with normofibrinogaemic patients. The network fibres appear long and thin. Also, circulating LDL-C is correlated inversely with permeability and the mass-to-length ratio of the fibres¹⁸. Significant inverse relations, which are independent of plasma fibrinogen or lipoprotein concentrations, are detected between the permeability of plasma generated fibrin networks and mass-to-length ratio within the fibrin fibres and the severity of coronary artery stenosis as determined by angiography³⁵. Also, Blombäck *et al.* (1992) indicated that a grossly abnormal gel structure rather than simply the tightness and rigidity of the architecture is associated with progression of the atherosclerotic process¹⁸. Since these patients often have elevated plasma fibrinogen levels, an association can be found between tight and rigid fibrin networks *in vitro* and the process *in vivo*. Further research is required to elaborate on these associations.

4. THE COLLECTIVE RISK HYPOTHESIS

Current literature describes only the concentration of the circulating plasma fibrinogen to have predictive value for CVD. However, extensive studies in our laboratory show that changes in plasma fibrinogen concentration need not be present to induce alterations in fibrin network architecture^{40; 41}. In these studies a CVD risk reducing diet promoted the formation of "healthier" fibrin networks, believed to be less atherogenic, without inducing a fibrinogen lowering effect. These alterations were complemented by significant changes in serum-Total Cholesterol. The implication of this is far reaching. This confirms that fibrinogen related cardiovascular risk can be reduced without alterations in the concentration of the protein itself. In the past, a variety of studies investigated a variety of lifestyle means to lower the concentration of plasma fibrinogen in human subjects (i.e. dietary intervention and exercise). None of these studies were successful. Yet, the scientific community is not convinced that fibrinogen can not be regulated by means of lifestyle changes. This leaves us with two considerations, of which (i) question the specific method of fibrinogen concentration determination used by respective research laboratories, and (ii) that the value of plasma fibrinogen concentration as CVD risk factor is misunderstood. Both these considerations provide adequate understanding as to why fibrinogen related research declined dramatically over the past decade.

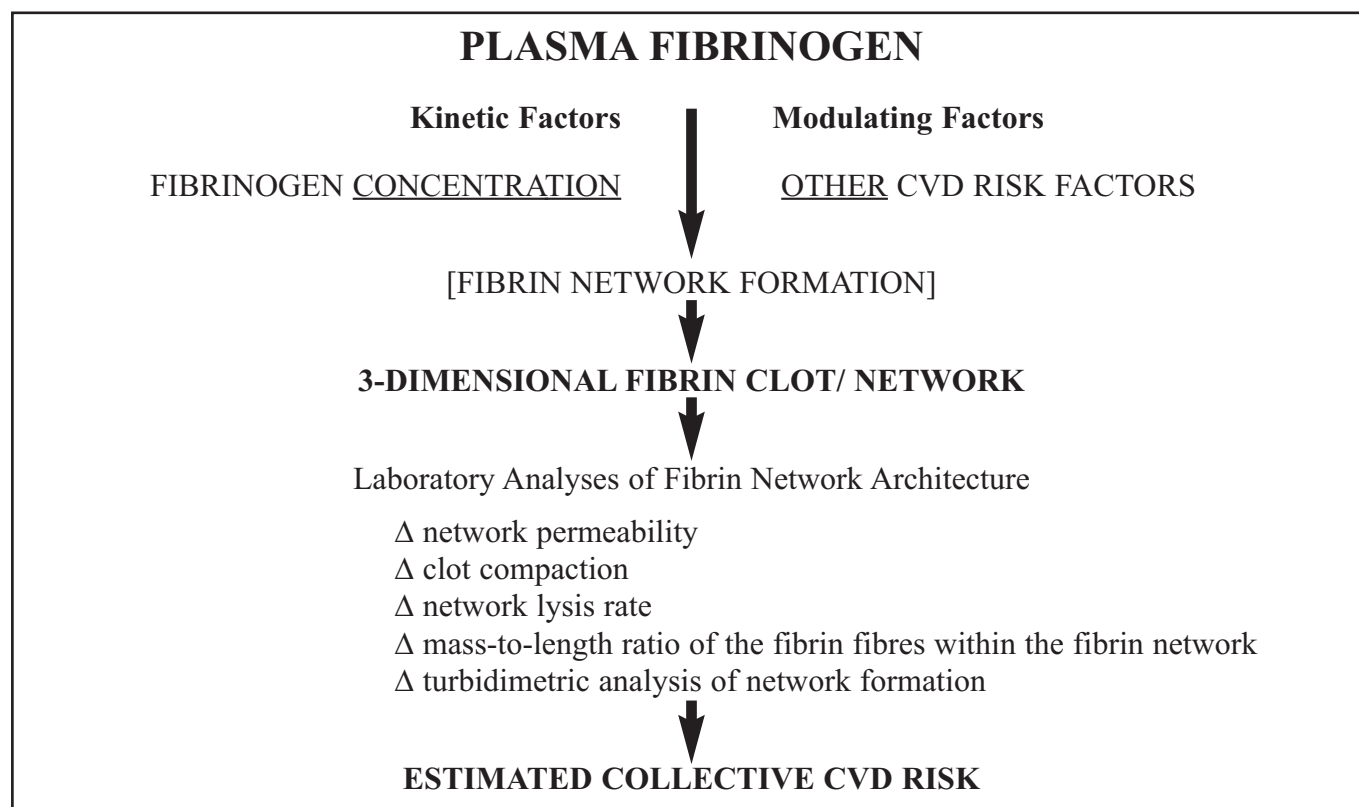


Figure 1 The Collective Risk Hypothesis, explaining that fibrin network architecture determines the degree to which plasma fibrinogen can be used to predict the occurrence of CVD, and in turn, that the fibrin network architecture depends on the association of fibrinogen with its concentration, as well as with other CVD risk factors in its direct environment.

Available methodology for determination of the circulating fibrinogen concentration in plasma was not well understood and/or defined until the turn of the century. Studies were performed using different principles of measurement, including enzymatic, colorimetric, precipitation, chromatographic and immunological methods. Today it is known that the immunological determination of plasma fibrinogen provides an estimate of both active and inactive portions of the circulating fibrinogen molecule, whereas enzymatic determination, which today is the most widely used, provides an estimate of only the active portion of the circulating protein molecule. These differences create confusion during interpretation of results, especially when comparing results obtained from different research groups.

Consideration (ii) suggests that the current exclusive focus of attention on the concentration of the circulating fibrinogen protein does not provide an adequate estimate of CVD risk. It is the intention of the author to present a novel hypothesis that states that fibrin network architecture, which also includes the concentration of the circulating fibrinogen protein as a determinant, provides a stronger estimate of CVD risk, due to its dependence on the collective effect of all kinetic and modulating factors within its direct environment. It therefore provides an immediate evaluation of collective CVD risk that includes possible factors that contemporary science is not yet aware of, as well as those factors, whether dependent or independent, already recognised, based on the assumption that our hypothesis is correct. This is what defines the model as proposed within the Collective Risk Hypothesis (see figure 1).

5. CONCLUSION

Fibrin network architecture analyses are well described elsewhere^{32; 33}. The methods are cheap, easy to standardise, require limited apparatus and are reliable. Due to its limited use the methods are not yet automated and therefore labour intensive. Also, for use in a routine pathology laboratory would require that quality control samples are developed, especially for inter-laboratory standardisation purposes. However, once these measures are taken care of, fibrin network analyses should provide valuable information as a tool to define individual CVD risk, to the benefit of prevention, diagnosis and treatment thereof. Patients on therapeutic agents for blood clotting abnormalities could be monitored for their response, others screened for and/or diagnosed with early/pre-clinical symptoms of CVD. However, in this article it is reasoned that without grasping the basic tenets of the Collective Risk Hypothesis, that the benefit of fibrin network architecture analyses will not be clearly understood.

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