The pre-thrombotic state

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Introduction

Virchow initiated the concept that disordered function of the blood contributes to thrombogenesis [1]. This is evidently true to the extent that thrombi are composed of cells and proteins derived from blood. However, it is not known whether disturbance of haemostatic function is important in the development of most cases of venous and arterial thrombosis. There are a few specific conditions in which abnormality of the blood leads to thrombosis but their general applicability is uncertain. The concept of a pre-thrombotic, or hypercoagulable state, supposes that thrombosis is preceded by loss of the normal haemostatic equilibrium, instability of the system leading to an accelerated tendency to fibrin formation and platelet deposition. Unfortunately the hypothesis is difficult to test in patients. The rate of venous and arterial thrombosis in healthy individuals is low, so that study of a pre-morbid population is a daunting proposition. In addition, it is rarely feasible to time the onset of thrombosis accurately, because available diagnostic techniques cannot exclude the presence of small, asymptomatic thrombi. Accordingly, the pre-thrombotic state remains somewhat hypothetical: it can be demonstrated experimentally and in a few uncommon conditions but has not been established as a prelude to most thrombotic events.

Haemostatic function in a pre-thrombotic state

Blood clots after exposure to non-endothelial surfaces, or because it has been contaminated with tissue factor, a phospholipoprotein. A complicated series of protein interactions ensues, which was first described as an amplification cascade [2] but more accurately functions in a series of inter-linked positive and negative feedback loops [3]. The end product of the coagulation pathway is thrombin, a proteolytic enzyme which subtly degrades the plasma protein fibrinogen to form soluble fibrin monomer. Soluble fibrin spontaneously polymerizes and cross-links form, catalysed by the action of factor XIII, between first the γ- and then the α-chains of fibrin molecules. Above a critical concentration insoluble fibrin is precipitated to form a stable meshwork of fibrin strands, in which the cellular elements of the blood are trapped. Multiple interactions occur between coagulation proteins and blood platelets. Platelets can initiate thrombosis by adhering to damaged vascular surfaces with formation of a microaggregate. In addition, platelets provide phospholipid surfaces on which several coagulation reactions take place, and bind forming fibrin leading to clot retraction, which shrinks and hardens the coagulum. Hypercoagulability could occur when the overall reaction rate of the coagulation system is accelerated, or when sub-critical amounts of soluble fibrin are being formed, due to traces of tissue thromboplastin in the circulation or to low-level contact activation.

Measurement of the overall reaction rate of the coagulation sequence is difficult, although there is a great deal of information about rates of reactions between individual proteins obtained from studies in purified systems [3]. The most precise test available for clinical studies is probably measurement of the fibrinopeptide A (FPA) generation time [4]. FPA, the peptide released from fibrinogen by thrombin, can be assayed at timed intervals in shed blood exposed to plastic and gives a rate for fibrin generation which is consistent in normal individuals [5]. The FPA generation time is shortened in patients with significant thrombosis, but due to thrombin activity in the circulation, the initial concentration of FPA in the sample is also increased. This makes it difficult to be certain whether the overall reaction time of the coagulation sequence is truly shortened. Experience in our laboratory indicates that the activated partial thromboplastin time (APTT), which is the time taken for blood to clot after addition of a contact activator, phospholipid and calcium, is simultaneously shortened, consistent with an accelerated reaction rate in the coagulation cascade.

There are other tests available to detect thrombin activity in blood. It can be argued that
detection of cross-linked X oligomers, which are derived specifically from fibrin [6], or of thrombin-antithrombin complexes [7], in the absence of overt thrombosis, indicates an enhanced tendency for intravascular thrombus deposition. The difficulty lies in deciding whether the results of such tests are predictive of future thrombosis or the consequence of active thrombosis already present.

There is a tendency to assume that high plasma concentrations of coagulation proteins are prothrombotic. There is circumstantial evidence to support this; for example, the APTT is shortened by increasing concentrations of coagulation factors VIII, IX and X in the sample [8]. However, there is no direct evidence that substrate availability is a limiting factor in the clotting process [3] and it is hard to understand why coagulation should be spontaneously accelerated in the presence of higher than normal concentrations of zymogens. In addition, several coagulation proteins take part in the acute-phase response and it is doubtful whether acute-phase changes affect haemostatic equilibrium [9].

Analysis of data from clinical studies is further complicated by the generally held view that the coagulation mechanism is continuously active at a low level. Small amounts of FPA are present in normal plasma and can be partially suppressed by heparin infusion [5]; plasma fibrinogen is probably normally degraded to a small extent by fibrinolytic enzymes [10]; and there is evidence for continuous activity of the fibrinolytic mechanism [11]. Accordingly, the finding of thrombin activity in the circulation is not necessarily inappropriate, so that in characterizing hypercoagulability investigators must decide whether physiological or pathological activation of haemostasis has occurred.

Tests for detecting hyperactive platelets can be equally hard to interpret. Raised concentrations in plasma of platelet release proteins might indicate that platelets have been involved in thrombosis [9] but the tests are susceptible to technical artefact. The threshold of sensitivity to agonists which induce platelet aggregation is also lowered in some patients but the significance of this is uncertain.

Clinical and experimental hypercoagulability

Certain forms of clinical and experimental thrombosis are probably preceded by a pre-thrombotic state.

Eighty years ago it was appreciated that clotting was initiated when blood came in contact with non-endothelial surfaces. This observation stimulated experiments in which contact activation through the intrinsic clotting pathway was used as a model for thrombosis. In animals, venous thrombi can be produced by ligating or clamping the vessel to induce stasis, if the animal has been previously rendered hypercoagulable by infusion of contact activators [12] or serum containing trace amounts of activated clotting factors [13]. The period of stasis required to induce thrombosis is shorter the more potent the procoagulant stimulus. Attempts to provoke arterial thrombosis by similar techniques using hypercholesterolaemia were unsuccessful [14] and some form of injury to the arterial wall seems necessary for initiation of experimental arterial thrombosis. Results of animal studies are consistent with a view that hypercoagulability contributes to venous thrombosis but that vessel wall damage is the significant precipitating factor in arterial thrombosis.

Unsurprisingly, there are few diseases in which a similarly precise chronological order of events can be established. There are points of resemblance between animal experiments and some forms of thrombosis in man. For example, in poisoning by snake bite the venom of Echis carinatus activates prothrombin causing intravascular coagulation [15]; infusion of clotting factor concentrates in treatment of bleeding can cause thrombo-embolic complications, probably due to the presence of traces of activated clotting factors produced during manufacture [16].

Thrombo-embolism resulting from increased plasma concentrations of oestrogen probably provides the best example of a pre-thrombotic state in man. Although the risk of venous thrombosis occurs largely in the puerperium, the haemostatic mechanism is over-active during pregnancy [17] and there are increased levels of soluble fibrin in blood [18], suggesting enhanced thrombin generation. The incidence of venous thrombosis was increased in patients receiving oestrogen for secondary prevention of myocardial infarction [19], during treatment of disseminated cancer of the prostate [20], and for contraception [21]. Oestrogen has a number of metabolic effects and the mechanism by which thrombosis is induced has not been established. However, high plasma oestrogen levels affect haemostasis in ways that are likely to promote coagulation: plasma concentrations of factors II, VII, IX and X and fibrinogen rise [22], combined with a fall in levels of antithrombin III and plasma activator activity [17].

Congenital abnormalities predisposing to thrombosis

Much of our knowledge about the haemostatic mechanism has derived from the study of indi-
individuals with defects of single plasma proteins leading to haemorrhage. Comparable cases in which abnormal proteins predispose to thrombosis are rare and understanding of the role of blood in thrombogenesis has lagged as a result.

The best characterized of these congenital prothrombotic conditions is familial antithrombin III deficiency, first described in 1965 [23]. Antithrombin III is a plasma protein which inactivates the active forms of factors XII, XI, X, IX and II. Heterozygotes, with less than 65% of normal antithrombin III concentration, suffer repeated episodes of venous thrombosis starting in their teens and more rarely episodes of arterial occlusion [24]. More recently, a similar pattern of precocious thrombosis has been discovered in families with protein C deficiency [25]. Protein C inactivates the active forms of factors V and VIII and in addition contributes to fibrinolysis. Homozygotes probably die as neonates [26], and heterozygotes have a severe pro-thrombotic tendency. Deficiency of protein S, which regulates the activity of protein C, probably has similar consequences, but has only recently been described [27].

There are rare congenital aberrant forms of fibrinogen, which are more susceptible to the action of thrombin, resulting in a thrombotic tendency [28]. Functional abnormalities of plasminogen can also be genetically transmitted and lead to a thrombotic tendency [29], presumably from inadequate fibrinolysis.

Patients with homocystinuria have an increased risk both of arterial and venous thrombo-embolism [30]. Experiments in baboons suggested that homocystine in excess damaged endothelium and caused platelet activation, but platelet survival in patients has been shown to be normal [31]. Homocystine causes contact activation in vitro but this mechanism seems inadequate to account for the thrombotic risk.

**Hypercoagulability in patients with an acquired thrombotic tendency**

There are many conditions which can be complicated by thrombosis, often through obscure mechanisms. The relationship between malignancy and thrombosis has been one of the more closely studied. Patients with cancer are more likely to have venous thrombosis than others, occasionally demonstrate striking migratory superficial thrombosis, sometimes develop disseminated intravascular coagulation and can suffer from non-bacterial thrombotic endocarditis. Investigation of cancer patients without overt thrombosis indicates a tendency to raised levels of coagulation factors V, VIII, IX and X and fibrinogen, reduced antithrombin III concentration and partial thromboplastin times, with increased turnover of fibrinogen and blood platelets [32]. Whether these changes are initiators of thrombosis or the response to fibrin deposition provoked by other mechanisms is not clear.

Experimentally, tumour materials can be shown to have procoagulant activity: mucin from adenocarcinomas activates factor X [33]; proteases from colonic, breast and vaginal tumours can similarly induce factor X activation [34]; and a thromboplastin is released from promyeloblasts in promyelocytic leukaemia in which there is a high incidence of disseminated intravascular coagulation (DIC) [35]. A plausible thesis can be advanced that such patients are in a hypercoagulable state owing to low-level release of tumour thromboplastins, which interact with other factors such as venous stasis after bed rest to promote symptomatic thrombosis.

Patients with myocardial infarction provide a similar, though less well characterized, example of the thrombo-embolic risk from a combination of immobility and tissue thromboplastin release. Some patients will have hyperlipidaemia in addition, which is associated with an unfavourable haemostatic profile [36] and enhanced platelet activity [36a]. Around 15% of patients develop small, radioisotopically detectable, calf vein thrombi, though the incidence of deep venous thrombosis has dropped with the reduction in the length of bed rest imposed during treatment [37]. In addition about a third of patients with anterior myocardial infarction develop intraventricular thrombi [38]. Evidence for a mild hypercoagulable state is circumstantial, but there is a rise in FPA concentrations in patients in whom suspected infarction is confirmed [39], probably due to activation of coagulation after release of tissue thromboplastin from damaged myocardium. There are abnormalities of platelet function which are generally consistent with a state of platelet activation in patients with ischaemic heart disease [39a] but they are likely to be a secondary response to the underlying vascular damage.

Thrombo-occlusive vascular disease is a major complication of diabetes mellitus, though the risk of venous thrombosis is probably not enhanced. There have been many (and often conflicting) findings from studies of coagulation [40] and platelet function [41] in diabetic patients. They illustrate well the difficulty of establishing a clear chronological sequence of cause and effect in the study of haemostasis and vascular disease. On balance, it seems unlikely that hypercoagulability
plays much part in the pathogenesis of the vascular complications.

Hypercoagulability is likely to be a powerful element in the combination of risk factors which promotes thrombo-embolism and sometimes DIC in conditions such as sepsis, burns and trauma. In most instances the blood changes are probably a natural response to injury, the result, for example, of endotoxin-mediated vascular damage [42], thromboplastin release [43] or contact activation by immune complexes [44]. A full discussion of mechanisms in DIC is outside the scope of this article (for review see [45]).

Patients with myeloproliferative disorders have a tendency to arterial thrombosis, particularly if the platelet count is very high. The mechanism is not understood and its potential complexities are suggested by the fact that a risk of bleeding and thrombosis can co-exist. The high platelet count is frequently accompanied by defective platelet function.

Prospective studies

General applicability of the concept of a pre-thrombotic state to the common forms of venous and arterial thrombosis can only be established by prospective studies in healthy individuals or patients at risk before a thrombotic episode. Such studies make great demands on the resolution of patients and investigators. Postoperative venous thrombosis has been the most frequently studied model because the event rate is high (about 30% after general abdominal surgery) and the onset of thrombosis can be detected by 125I-labelled fibrinogen leg scanning. The APTT (which is sensitive to changes in components of the intrinsic coagulation system) was found to be shorter pre-operatively and on the first postoperative day in patients who developed calf-vein thrombi [46]. We have used stepwise, logistic discriminant function analysis in studies in Leeds to detect factors measured pre-operatively which predict thrombosis (though, of course, prediction does not imply causation). Four items of clinical information and results of laboratory measurement of fibrin-degradation products and plasminogen activator activity (an indirect measure of fibrinolytic potential) were used to identify correctly 95% of gynaecological patients who subsequently developed positive postoperative leg scans [47]. The method was validated elsewhere and in Leeds with a fresh cohort of patients [48]. We have similarly found that low plasminogen activator activity pre-operatively is an indicator of risk for deep venous thrombosis in general surgical patients [49].

Patients undergoing hip surgery, particularly after fracture of the femur, have a very high risk of developing venous thrombosis. Perhaps not surprisingly, their haemostatic function is disturbed in ways likely to contribute to fibrin formation: plasma antithrombin III concentrations are depressed pre-operatively [50] and anti-factor Xa activity is impaired [51]; plasma fibrinogen is higher than in controls [50]; and post-operative measurements of fibrinopeptide A, β-thromboglobulin and fibrinopeptide Bβ 1–42 have shown respectively that there is persisting thrombin generation, activation of blood platelets and a reduced fibrinolytic response [52]. In a few patients there is evidence of tissue thromboplastin in blood [53].

The results of pre- and post-operative measurements of haemostatic function are consistent with the hypothesis that during surgery there is activation of the haemostatic mechanism. We have found that plasma fibrinopeptide A concentrations rise 5–10-fold during colonic surgery, returning almost to normal levels by the first postoperative day [53a]. This indicates that thrombin generation occurred during an uneventful surgical procedure and supports the other evidence that surgery renders blood hypercoagulable. In patients with other risk factors (for example, sepsis or malignancy, prolonged immobility, or older age) this could well be the trigger which precipitates deep venous thrombosis.

The part which hypercoagulability might play in arterial thrombo-atherosclerotic occlusion is more conjectural. The Northwick Park Heart Study has provided the best (and almost only) evidence for a relationship between an undesirable profile of haemostatic measurements and the development of symptomatic arterial disease. Measurements made in about 3500 London factory workers have been related to the subsequent diagnosis of coronary events [54]. High concentrations of plasma fibrinogen and reduced blood fibrinolytic activity were found to share a common distribution with the major recognized risk factors for coronary heart disease [55]. In 27 patients who had died by 1979 from coronary heart disease, levels of fibrinogen and coagulation factors VII and VIII were significantly higher than in survivors [56]. In an equally large population study from Sweden, high plasma fibrinogen concentration was similarly found to carry an increased risk of death from coronary artery disease and more strongly from stroke [57].

Epidemiological surveys incorporating measurements of platelet function are difficult to carry out, because of the technical problems raised by getting viable platelets to a laboratory for testing.
Populations at higher risk of ischaemic heart disease might have a less favourable pattern of platelet function than those with low risk [58].

Conclusion

Many investigators have tried to show a link between pre-morbid changes in haemostatic function and symptomatic thrombosis. Such studies are of considerable importance for defining mechanisms in thrombogenesis, and improving the sensitivity and specificity of laboratory tests of coagulation and platelet function. There is still a need for investigation of healthy populations to determine whether a pre-thrombotic state contributes to most forms of thrombosis. At a more detailed level, improvements in imaging techniques should help to establish the relationship between abnormal blood tests and fibrin deposition in conditions such as DIC and trauma. For clinicians, laboratory tests of haemostatic function can be ignored in diagnosis of thrombosis, unless DIC is suspected. Present methodology can identify populations at risk but cannot yet detect the individual in whom thrombosis will occur.

References

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