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Prevalence and Significance of Thrombophilia in Peripheral Arterial Disease

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Introduction

In addition to well-established risk factors such as smoking, diabetes, hypercholesterolaemia and hypertension, an increasing number of novel humoral and endothelial factors have recently been implicated in the aetiology and progression of vascular disease. Thrombophilia may be defined as a propensity to thrombosis secondary to abnormalities in haemostasis.[1] Thrombophilia has long been recognised as contributing to venous thrombosis, but is increasingly associated with arterial disease. It is important because screening may identify patients at high risk of thrombosis who may then be offered prophylaxis. This review will focus on the prevalence and significance of thrombophilic states associated with peripheral arterial occlusive disease (PAOD) and discuss possible strategies for screening and treatment.

Prevalence of Thrombophilia

General coagulation activation

If thrombophilia is important in PAOD then there should be evidence of activation of coagulation in affected patients. Thrombin and fibrinogen, and products of their metabolism, including thrombin-antithrombin (TAT) complexes, prothrombin fragments (PF) 1 + 2 and fibrin degradation products (FDPs) can be used to measure coagulation activation. Cross-sectional[2–5] and longitudinal[6] epidemiological studies have demonstrated an association between activation of coagulation and PAOD. Furthermore, in 1988, Boneu showed that PAOD was associated with inhibition of fibrinolysis.[7] In young patients (<51 years old) undergoing lower limb revascularisation, as many as 76% may have a hypercoagulable state (increased platelet aggregation or coagulation abnormality).[8]

Homocysteine

A mild elevation of homocysteine levels (hyperhomocysteinaemia) affects 5% or more of the population and is increasingly recognised as an independent risk factor for atherosclerosis and thrombosis.[9] Hyperhomocysteinaemia can cause increased Factor V activity, possibly via a decrease in thrombomodulin cell surface activity and a corresponding decrease in activated protein C (Fig. 1).[10–13] The prevalence of hyperhomocysteinaemia in PAOD may be between 50 and 60%[14–16] and many cross-sectional studies have demonstrated a clear association between plasma homocysteine levels and PAOD.[17]

Antithrombin III

Anti-thrombin III (AT III) is an endogenous anti-coagulant, produced by the liver, which inactivates thrombin and factor Xa. Deficiency of AT III is inherited in an autosomal dominant fashion. In a population-based study of 7983 subjects over 55 years old 3.1% had deficiency of AT III, defined as <75% activity.[18]

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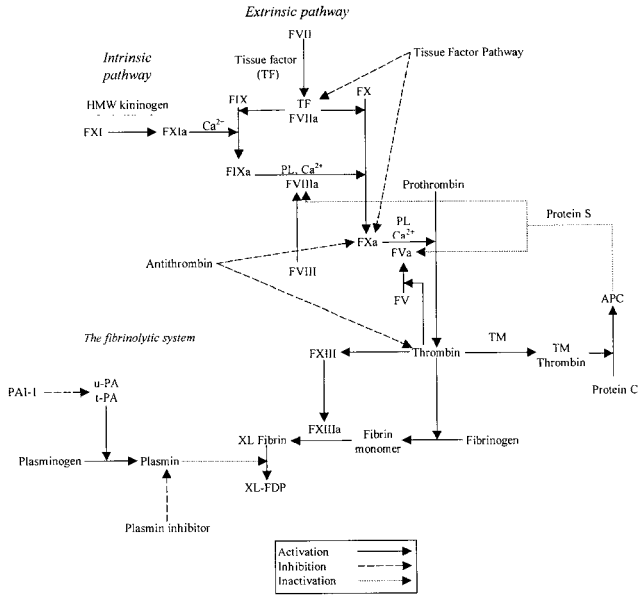

Fig. 1. Overview of coagulation system.

Fibrinogen

Fibrinogen is the substrate on which the end-product of the coagulation cascade, thrombin, acts to produce fibrin, and ultimately, a blood clot. Its effects are diverse and include increases in blood viscosity, red cell aggregation, platelet aggregation and activation.[19] Fibrinogen deposited in the arterial intima may also lead to smooth muscle cell proliferation and leukocyte migration.[20–23] Hyperfibrinogenaemia has long been associated with cardiovascular disease and is present in more than 50% of patients with PAOD.[24–26]

Antiphospholipid antibodies

Antiphospholipid antibodies (aPL) are a group of auto antibodies originally thought to be targeted towards negatively charged phospholipid, although recent work suggests that they are directed against β_2-glycoprotein I.[27] aPLs are of two types, detected by different laboratory methods: the anticardiolipin antibody (aCL) enzyme linked immunoassay and the lupus anticoagulant (LAC) coagulation assay. Although the lupus assay relies on the *in-vitro* effect of aPL to prolong coagulation assays, the *in-vivo* effect is procoagulant, the mechanism for which is uncertain. Antiphospholipid antibodies may inhibit protein C and protein S, and have prothrombotic effects via enhanced platelet reactivity or endothelial cell surface molecules such as heparan sulphate and tissue factor.[27]

Cross-sectional studies have shown that the prevalence of aPL amongst patients with PAOD requiring intervention varies between 26% and 45%.[28–30] This mostly comprises patients with aCL who constitute 84–94% the total. A small proportion of patients with aPL have both LAC and aCL (2–3%).[28,30,31] No studies have yet compared the prevalence of aPL in PAOD with the prevalence in the general population, and no large cross-sectional studies have been performed to give a population prevalence of aPL.

Protein C deficiency

Protein C is a vitamin K dependent protein which, when activated by the thrombin-thrombomodulin complex, inactivates factors Va and VIIIa (Fig. 1). Protein C deficiency is established as a risk factor for venous thrombosis, but its role in arterial pathology is less clear. Few studies have investigated the prevalence of protein C deficiency in PAOD. In a recent study of 116 claudicants, deficiency of protein C was found in 2 (1.7%).[31] Other studies have shown the prevalence of protein C deficiency to be between 2.5 and 15% in PAOD patients, but no comparisons were made with control groups.[8,32–34]

Activated protein C resistance/factor V Leiden

Activated protein C (APC) resistance is the most common inherited risk factor for thrombosis. The prevalence varies in different ethnic populations; in U.K. it is 3.5–4.9%, Africans 0% and in Cyprus 13%.[35,36] The most common cause of APC resistance is a mutation in the factor V gene leading to the replacement of Arginine 506 with Glutamine, (factor V Leiden, fVL) which renders it more resistant to degradation by protein C. This is responsible for 90–95% of APC resistance, the remainder of which is made up of acquired conditions such as aPL, pregnancy and the oral contraceptive pill.[37–40] APC resistance is measured using a plasma assay and exogenous activated protein C, and is indicated by a lowering of the APC ratio (normal range 2.2 to 2.6). This will identify the majority, but not all of patients with fVL. fVL may also be identified directly using genomic analysis, but not all mutations lead to lowering of the APC ratio. fVL is thought to underlie 18–30% of venous thromboses, but its importance in arterial disease is less well defined.

Both APC resistance and fVL have been demonstrated to be more common in patients with PAOD

compared with the general population. Sampram found the prevalence of fVL and APC resistance (defined as ratio <2.6) to be higher (26.4%) in 359 patients with PAOD than in 278 controls (12.2%).[41] A smaller study by Foley in patients who had undergone lower limb arterial bypass surgery reported a 17.8% prevalence of fVL, compared with a local population prevalence of 3.5%.[35] Evans only reported one positive APC resistant patient in 116 claudicants.[31] Variations in these reported figures may be explained by the preferential use of DNA analysis or APC ratio to define fVL; variations in the lower end of the normal range for defining the normal APC ratio and the severity of the presenting PAOD.

Protein S deficiency

Protein S is a vitamin K dependent plasma protein and an essential co-factor for the anticoagulant and profibrinolytic effect of activated protein C.[42] Protein S deficiency has been identified as a cause of venous thrombosis, and more recently has been proposed as a factor in arterial disease. The prevalence of protein S deficiency in the general population is thought to be around 0.7%.[43] There are only a few small studies investigating the prevalence of protein S deficiency in PAOD. Allart in 1990 showed protein S deficiency to be present in 3 out of 45 patients (8%) less than 45 years old who required surgical treatment for PAOD.[42] A study of 33 patients undergoing arterial surgery, and 10 controls found a prevalence of protein S deficiency in PAOD patients of 15%. Although no statistical difference was shown between patients and healthy controls, all five subjects with protein S levels less than normal were PAOD patients.[44]

Prothrombin 20210A

A G to A transition at position 20210A of the prothrombin gene is associated with an increased risk of venous thrombosis, although the underlying mechanism is not clear. The prevalence of this mutation is 1.2% to 4.3% in patients with venous thrombosis, 5.7% in patients with PAOD, and 0.7% in controls.[45,46] However, no specific studies have been performed investigating the association between prothrombin 20210A and arterial disease.

Despite studies screening for different states, using a variety of methods, in patients with a range of disease severity, it is clear that there is an increased prevalence of thrombophilic states in PAOD, perhaps as high as 60%. Although common, the clinical relevance of thrombophilia in PAOD is a more important issue, which will now be discussed.

Significance of Thrombophilia

Studies of general coagulation activation

There is a correlation between the level of coagulation activation and the severity of PAOD as determined by walking distance,[4] ankle–brachial pressure index (ABPI),[47,48] duplex ultrasonography, angiography[49] and clinical symptoms.[25] For example, Ray reported the prevalence of thrombophilia (protein C deficiency, protein S deficiency, antithrombin III deficiency, lupus anticoagulant) to be 11% in controls, 27% in claudicants and 40% in patients who had received a revascularisation.[34]

The importance of a hypercoagulable state in PAOD has also been revealed through the association between coagulation abnormalities and the progression of PAOD. In the Edinburgh Artery Study, whole blood viscosity, plasma viscosity and fibrinogen levels were predictive of the requirement for vascular intervention,[50] or fall in ABPI,[6] over a six-year follow-up period. Furthermore, whole blood viscosity, and fibrinogen levels have been shown to be predictive for the progression of PAOD as determined by walking distance.[51]

Thrombophilic states may also be important causes of failure of arterial interventions. In 1994, Ray studied 124 patients undergoing arterial reconstruction and reported 75 graft occlusions after a mean follow up of 44 months.[34] Almost half (49%) of these were subsequently identified as having a thrombophilia, compared with 27% of patent reconstructions. Abnormalities identified in the graft occlusion group were: protein C deficiency (21% of occlusions), protein S deficiency (17%), lupus anticoagulant (25%) and multiple abnormalities (12%). A subsequent prospective study, investigated the presence of a thrombophilia prior to arterial reconstruction in 60 patients with one-year follow-up.[52] A pre-operative thrombophilia was identified in 65% of patients whose graft subsequently occluded within one year, compared with 20% of those with a patent graft ($p<0.05$). The presence of thrombophilia was particularly significant in early graft failures, where 11 of the 12 occlusions within one month had a pre-operative hypercoagulable abnormality. A prospective study of 137 patients undergoing a mixture of arterial reconstructions identified 14 patients (10%) with a hypercoagulable state.[33] Three of these patients (27%)

suffered a graft thrombosis within 30 days, compared with two of 123 patients with a normal thrombophilia screen (1.6%). Eldrup Jorgensen studied 20 young (<51 years old) patients undergoing aorto-iliac (7) or infra-inguinal (13) vascular surgery.[8] Four patients suffered an early (<30 days) post-operative thrombosis, all of who had thrombophilia identified pre-operatively. Patients with multiple coagulation abnormalities appear to be at special risk. Thus, of 124 patients undergoing revascularisation studied by Ray, 11 had multiple thrombophilias, all of whom had had a previous revascularisation. Nine of these patients had a further occlusion during the follow-up period.[34]

Homocysteine

Hyperhomocysteinaemic patients have an increased rate of vein graft stenosis and increased failure of bypass grafts and angioplasty.[16,53] Patients undergoing peripheral arterial bypass surgery with elevated homocysteine have evidence of pre-existing intimal hyperplasia in saphenous vein biopsies.[15] A prospective study investigating hyperhomocysteinaemia and progression of PAOD, with mean follow-up of 37 months found a trend towards an association, but this was not statistically significant.[54] This may, however, represent a type II error as only a relatively small number of patients (22) were judged to have progression of PAOD during follow-up.

Fibrinogen

Fibrinogen levels correlate with the severity of PAOD, higher levels being associated with more severe disease, as determined by claudication distance,[25] angiography,[55,56] and ABPI.[47,49,57] Hyperfibrinogenaemia has been shown to be predictive for the progression of PAOD, as measured by change in claudication distance,[51] or the requirement for intervention.[50]

Given that hyperfibrinogenaemia is associated with the development and progression of PAOD, it is unsurprising that high levels of fibrinogen are predictive of failure of interventions for PAOD.[58] Prospective studies have shown that hyperfibrinogenaemia is associated with failure of vein and prosthetic femoral popliteal bypass grafts.[59–62] In addition, associations have been demonstrated between raised fibrinogen levels and graft stenosis, implying that it is not simply an increased thrombotic tendency underlying the failure of such interventions.[58,63] Data regarding fibrinogen and patency following percutaneous angioplasty are conflicting. Two prospective studies have shown that hyperfibrinogenaemia is associated with poorer patency rates, while another prospective study showed that high fibrinogen levels measured prior to angioplasty were associated with improved patency rates.[64–66]

At present there are no selective treatments available to lower fibrinogen and consequently no reported trials confirming benefit in treating hyperfibrinogenaemia. While there is a great deal of evidence associating fibrinogen levels and PAOD, it is difficult to conclude that this relationship is causal until such trials are available. Fibrinogen is an acute phase protein, and its increased levels in arterial disease may merely be representative of an underlying low-grade inflammatory process.

Antiphospholipid antibodies

No studies to date have demonstrated an association between the prevalence of aPL and the progression of PAOD. However, aPL and the antiphospholipid syndrome are associated with an increased risk of thrombotic complications of vascular surgery, although the majority of these studies are retrospective.[28,67–69] Ahn retrospectively identified seven patients with aPL who underwent a total of 18 vascular procedures.[70] Three of these patients, none of whom were anticoagulated developed multiple post-operative thrombotic complications and all eventually required amputation. The remaining four vascular patients in this study were taking steroids, anticoagulants, or vitamin K at the time of the initial operation. A similar study found that 16 of 19 patients with aPL undergoing a vascular procedure suffered a thrombosis, 12 of who died.[71] In a retrospective report of 234 patients undergoing vascular surgery, aPLs were associated with a shorter bypass patency period (17 vs 58 weeks) and a risk of occlusion that was 5.6 times greater than patients without aPLs.[28]

The only prospective study to investigate the association between aPL and the outcome of vascular intervention, showed a trend towards the presence of aPL and failure of arterial bypass surgery, but this did not reach statistical significance.[30] This result is unfortunately confounded by the fact that, significantly more of the aPL group were anticoagulated post-operatively thus diminishing any likely difference between the groups.

Activated protein C resistance/factor V Leiden

Ouriel prospectively monitored 76 patients who underwent lower limb revascularisation for a mean of 47 months. 60% of those with APC resistance (defined as APC ratio <2.0) had an occlusion of their graft, while only 24% of those without APC resistance suffered a graft failure ($p<0.02$).[72] A similar finding was seen in Sampram's study in which 32% of those with fVL and 49% of those with APC resistance suffered a graft occlusion (both $p<0.001$).[41] A study from Foley *et al.* reported no association between fVL and graft occlusion, but excluded patients whose graft occluded within six weeks of surgery, a time that others have reported as important in graft occlusion associated with thrombophilia.[35]

Protein S deficiency

Although the prevalence of protein S deficiency is higher in patients with PAOD, its significance is unknown. Allart investigated the families of young (<45 years old) PAOD patients who were found to be heterozygotes for protein S deficiency, but no association was found between likelihood of protein S deficiency, and arterial thrombosis.[42] This finding corroborated a previous study, which showed that relatives of protein S deficient individuals did not have an increased incidence of arterial thrombosis.[73]

Deficiency of protein S was identified in 4 of 20 patients (20%) whose arterial reconstruction failed compared with 6 of 40 (15%) of those with a successful reconstruction at 30 days post surgery, although this difference did not reach statistical significance.[42]

Antithrombin III deficiency

In Van der Bom's population study, examination of 7983 subjects revealed a complex relationship between level of AT III and ABPI.[18] In men, mild PAOD was associated with a high level of ATIII, while severe PAOD was associated with lower levels of ATIII. Whilst in women, there was in an inverse relationship between ABPI and ATIII level. The authors suggest that levels of ATIII rise in the presence of cardiovascular disease as a protective mechanism, but as vascular disease progresses, ATIII becomes consumed, leading to lower levels. The reason for the difference between the sexes is not clear.

The poor results of intervention in patients with thrombophilias, in terms of intervention failure and mortality, reinforce the clinical importance of these states in patients with PAOD. The presence of two or more co-existent thrombophilias, seems to have an additive effect, and be particularly dangerous clinically. However, many thrombophilic states may be asymptomatic for many years and the "two-hit" hypothesis suggests that thrombophilic states only become apparent when a subject is exposed to some other thrombogenic trigger such as surgery, oestrogen-containing medication, dehydration or systemic upset.

Table 1. Patients to be investigated for thrombophilia.

1	Venous thromboembolism before the age of 40–45 years
2	Recurrent venous thrombosis or thrombophlebitis
3	Thrombosis in an unusual site, e.g. mesenteric vein, cerebral vein etc.
4	Unexplained neonatal thrombosis
5	Skin necrosis, particularly if on coumarins
6	Arterial thrombosis before the age 30 years
7	Relatives of patients with thrombophilic abnormality
8	Patients with clear family history of venous thrombosis
9	Unexplained prolonged activated partial thromboplastin time
10	Patients with recurrent foetal loss, idiopathic thrombocytopaenia or SLE

Clinical implications

Screening

The British Committee for Standards in Haematology (BCSH) identified 10 groups of patients who should be screened for thrombophilia (Table 1).[1] The treatment of thrombophilic abnormalities is complex, and the decision for treatment, which may be lifelong anticoagulation, should only be made after careful consideration of the patient, the individual thrombophilia and any triggering factors that may have precipitated a previous thrombosis. It is our recommendation that such patients are referred to a haematologist.

In patients with PAOD who do not fall into one of the groups in Table 1, thrombophilia screening is still likely to reveal an abnormality in approximately 30–60% of patients. In those who are not undergoing a vascular intervention, there is no evidence to suggest that treatment of the thrombophilia will alter the progression of arterial disease. There is evidence however, that patients with a thrombophilia undergoing a vascular intervention have a poor prognosis, with increased risk of graft occlusion, limb loss and death, and this can be partially offset by treatment. It is therefore recommended that all patients undergoing a vascular intervention should be screened for a thrombophilic tendency.

Testing for thrombophilia should depend on the individual abnormality. Antiphospholipid antibodies, activated protein C resistance, and hyperhomocysteinaemia are the commonest abnormalities, and should form the basis of a thrombophilia screen. Screening for protein C and S deficiency, prothrombin 20210A, and antithrombin III deficiency may be useful, but likely to yield less positive results, although no less significant.

Assays for homocysteine have previously been difficult to perform, due to the requirement for immediate cooling of the sample and separation within 1 h. New techniques are being developed to improve the stability of blood samples for homocysteine analysis, increasing the ease by which homocysteine assays can be performed.[74,75]

The cost of thrombophilia screening is used as an argument against its use. However, if screening were targeted to high-risk groups, such as those in Table 1, or those undergoing intervention, the cost of screening may be offset against the reduced risk of failure of vascular intervention. The treatment of intervention failure may include prolonged hospital stay, repeated intervention, or amputation, all with significant costs. A more detailed cost-benefit analysis is beyond the scope of this article, and would be difficult to perform given that the lack of trials in this area means the true benefit of screening and/or treatment cannot be quantified.

Treatment

Although numerous different treatments are available for thrombophilias, they have not been formally studied in patients with PAOD to determine whether improved outcomes can be attained.

Hyperhomocysteinaemia

Patients with hyperhomocysteinaemia, who are undergoing a vascular intervention, should be treated with homocysteine lowering therapy prior to surgery if there is sufficient time. If the surgery is urgent, consideration should be given to formally anticoagulating these patients until the level of homocysteine can be reduced. Hyperhomocysteinaemia may be corrected simply with folic acid, and vitamins B_{12} and/or B_6, although it has yet to be demonstrated whether such treatment will lead to a reduction in cardiovascular risk or improvement in patency rates. Trials are presently being undertaken to determine whether lowering homocysteine levels is beneficial in terms of outcome for vascular patients both in PAOD and in cardiac and cerebrovascular disease. It seems sensible in the absence of current evidence however, to lower homocysteine levels in PAOD patients undergoing intervention.

Anti-coagulation

Anticoagulation in non-thrombophilic patients is of benefit in femoro-popliteal bypass grafts when compared with no treatment, but when compared to aspirin, the data are conflicting.[76] The largest study was a multicentre, randomised controlled trial investigating the effectiveness of oral anticoagulation (to maintain an INR 4.0–4.5) against aspirin (80 mg daily) in 2690 patients undergoing infrainguinal bypass surgery,[77] which showed no overall benefit of either treatment in preventing graft occlusion. Patients with antiphospholipid antibodies who are anticoagulated (with heparin and subsequently warfarin) when they underwent vascular surgery were noticed to suffer fewer complications.[70] No studies to date have prospectively investigated the use of anticoagulation in PAOD patients with a thrombophilia. However, Khamashita *et al*. retrospectively studied the effectiveness of anticoagulation in patients with antiphospholipid syndrome.[78] They showed that anticoagulation with warfarin to an international normalised ratio (INR) of >3 was significantly more effective in preventing recurrent thrombosis than anticoagulating to an INR <3, or aspirin. This study was not confined to patients with PAOD, but is significant in demonstrating a benefit of aggressive anticoagulation in thrombophilia.

Steroids

Whilst the thrombophilias discussed previously are not thought to be associated with a vasculitis, patients with the lupus anticoagulant who are taking steroids seem to have a reduced thrombotic risk.[70] The protective effect of steroids in conjunction with aspirin has been demonstrated previously in obstetric patients, and leads to a decrease in lupus anticoagulant levels.[79] There are no data on the use of steroids for PAOD patients with thrombophilia.

Anti-platelet agents

Aspirin is beneficial in obstetric patients with the lupus anticoagulant.[80] The use of aspirin has not been investigated in PAOD with a thrombophilia, but it is suggested that it be used in patients with aPL, with no history of thrombosis. Patients with aPL undergoing surgery, or with a history of thrombosis should be formally anticoagulated, as these patients are at high risk of thrombosis.

Factor replacement

An alternative treatment for patients with protein C or S deficiency undergoing surgery is the use of perioperative fresh frozen plasma or protein C concentrate. In the case of peripheral vascular surgery, patients will usually require formal anticoagulation to ensure the patency of the graft.

Nucleic acid therapy

Recently, oligonucleotides have been shown to have *in-vitro* anticoagulant effects through specific protein binding.[81] It remains to be seen whether this will translate into improved outcomes in thrombophilias.

Conclusion

The evidence to date supports an association between certain thrombophilias and peripheral vascular disease. Hyperhomocysteinaemia, hyperfibrinogenaemia, APCR and aPL syndrome are more common in PAOD, but there is no clear evidence for the other thrombophilias.

Thrombophilic states in general are associated with an increased failure rate of vascular reconstruction. This is particularly marked when considering patients with multiple thrombophilias, and early intervention failures. No conclusive evidence yet exists to show that treatment of these thrombophilic states can lead to an improvement in the course of PAOD, or the results of intervention. While it may be appropriate to anticoagulate patients identified with a thrombophilia who are undergoing a vascular intervention, it cannot yet be justified to recommend screening of all patients with PAOD for thrombophilia. There is a pressing need for well-designed trials of therapeutic intervention in patients with thrombophilia to determine whether outcomes are genuinely improved.

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