Review Article

Indian J Med Res 120, July 2004, pp 13-23

Thrombophilia in coronary artery disease: A double jeopardy

D. Mohanty, K. Ghosh, A. Khare & B. Kulkarni

Institute of Immunohaematology (ICMR), Mumbai, India

Received August 11, 2003

Thrombophilia can be defined as an increased risk of thrombosis. The central event to the pathogenesis of any thrombotic episode is the perturbation of haemostasis, the cause of which may be genetic or environmental. The clinical manifestations of the chronic development of coronary artery atheroma are angina and acute myocardial infarction. In recent years literature is emerging on the role of different factors of blood coagulation in arterial thrombosis. Different coagulation factors, natural anticoagulants, platelet antigens and other factors such as homocysteine, lipoprotein (a), have been studied as risk factors for coronary artery disease (CAD). The results of many of these studies are contradictory. In India, there is an alarming rise in the number of young patients with myocardial infarction (MI) and an interesting feature is that a large majority of these patients lack the conventional risk factors. There have been scattered studies on the thrombophilia status among Indians. The management of thrombophilia can be done by a regimen of different drugs which has been evaluated in different clinical trials. Since the cost of thrombophilia investigations is quite phenomenal for a developing country like India, the selection of these investigations assumes an utmost importance.

Key words Coronary artery disease - factor V Leiden - fibrinogen polymorphisms - homocysteine - platelet alloantigens - protein C pathway

Thrombophilia can be defined as an increased risk of thrombosis-hereditary or acquired. The hereditary causes of thrombophilia are deficiency of protein C, protein S and antithrombin III, factor V Leiden, hyperhomocysteinaemia, prothrombin G20210A polymorphism and elevated levels of factor VIII, von-Willebrand factor (vWF) and fibrinogen levels. The acquired causes are antiphospholipid antibodies, cancer and hyperhomocysteinaemia due to mild nutritional folic acid, vitamin B_{12} or B_6 deficiency or associated hyperlipidaemia. Acquired thrombophilia can also occur in persistently inflammatory conditions and myeloproliferative disorders[1].

The clinical manifestations of thrombophilia are venous thromboembolism at a young age, thrombosis at different sites (cerebral sinuses, portal), recurrent foetal loss, pre-eclampsia, premature atherosclerosis, myocardial infarction at a young age without any conventional risk factors and family history of venous thromboembolism[1]. The main event in the pathogenesis of any thrombotic event is the perturbation of haemostasis[1]. This perturbation may be genetically determined or environmental. But, since the onset of thrombosis in majority of cases is at a later stage, it is unlikely that genetic causes may be the sole determinant. This points towords a gene-environment interaction in thrombosis. There are certain distinctions between arterial and venous thrombosis. The thrombus is platelet rich in arterial thrombosis and fibrin rich in venous thrombosis. Additionally in arterial thrombosis, there is presence of atheroma in arterial thrombosis which represents vascular wall damage[2].

Atherosclerotic disease is a generalized disorder of the vascular tree characterized by long-term atheromatous plaque formation and culminating in atherothrombotic obstructive lesions that lead to tissue

damage[2]. Angina and acute myocardial infarction (MI) are the clinical manifestations of the chronic development of coronary artery atheroma. The underlying processes that lead to atheroma formation and coronary thrombosis are complex involving various systems that regulate vasoactivity, adhesion molecules, inflammation, lipid metabolism, coagulation and the fibrinolytic pathways[2].

The pathogenesis of arterial thrombotic disease involves multiple genetic and environmental factors related to atherosclerosis and thrombosis. The generally well accepted risk factors include smoking, hypertension, hyperlipidaemia, obesity, diabetes and a positive family history[2,59].

Although environmental influences may account for a rise in MI in recent years, genetic background can also be expected to play important role in influencing the disease. In recent years there is a rapidly growing literature on the relationship between the haemostatic system, the environment and arterial thrombosis. This literature mainly comprises the variation in genes for blood coagulation factors, inhibitors, fibrinolytic factors and platelet membrane receptors.

Thus, thrombophilia does play an important role in the pathogenesis of coronary artery disease (CAD).

Thrombophilia in CAD

Here the different thrombophilia markers studied in patients of arterial thrombosis, are summarized in brief.

Fibrinogen: Elevated levels of fibrinogen have been known to be most consistently associated with occlusive vascular disorders. Studies such as the Northwick Park Heart study[3] have prospectively related fibrinogen to MI and stroke[3]. However, relationship between fibrinogen polymorphisms and disease is less clear though there is compelling evidence of associations between fibrinogen level and arterial disease, and between fibrinogen level and certain polymorphisms[4-6].

Factor V Leiden: It was observed that activated protein C (APC) addition to plasma from certain patients with inherited thrombophilia did not show a prolongation in clotting time[7]. This APC resistance was found to be associated with a point mutation in the factor V gene at the cleavage site for APC. This is known as factor V Leiden and this mutation is a G-A substitution at position 1691 as a result of which Arg_{506} is replaced by Gln_{506}[8]. This results in a loss of cleavage site for APC due to which factor V becomes resistant to inactivation by APC. The role of factor V Leiden as a risk factor for thrombophilia in young patients with MI is unclear. The reports have been contradictory with some studies finding an association[9,10] and others finding no association[11,12].

Homocysteine: Homocysteine is an intermediary metabolite of essential dietary amino acid methionine. The adverse effects of hyperhomocysteinaemia are endothelial injury, smooth muscle proliferation, increased production of platelet aggregating substances like thromboxane A_2, inhibition of natural anticoagulation pathway via protein C, thrombin- thrombomodulin system[13,14]. Many studies have shown that mild hyperhomocysteinaemia was associated with an increased risk of CAD[15-17]. Hyperhomocysteinaemia is known to be associated with a variety of acquired and genetic causes which include age, sex, medications, diseases, such as renal failure, diabetes mellitus and hypothyroidism defects of enzymes involved in homocysteine metabolism and nutritional deficiencies (B_6, B_{12} and folic acid)[13,14].

One of the genetic causes for mild hyperhomocysteinaemia is associated with a thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) that is due to a C to T substitution at nucleotide 677[13]. As a result of this variant, the enzyme methylenetetrahydrofolate reductase has a decreased specific activity at 37ºC. This enzyme plays a role in the remethylation pathway of homocysteine to methionine and is essential for the conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate.

Prospective studies examining the risk of arterial thrombosis with hyperhomocysteinaemia have yielded mixed results with most studies showing weak or no association[18,19]. This raises the point whether elevated homocysteine levels are a cause or a consequence of CAD.

MTHFR C677 T has been studied as a risk factor for CAD with some studies finding an association[20] and

others failing to find any association[21]. A second genetic polymorphism in MTHFR has also been described. This involves an A to C change at position 1298[22]. There has been one study which found that A1298C mutation was associated with early onset of CAD[23].

Platelet antigens: Of the different platelet antigens, the most extensively studied one in relation to MI is the human platelet alloantigen-1 (HPA-1) polymorphism. It is located on the platelet glycoprotein IIb-IIIa. There have been contradictory reports with studies finding an association[24,25] and others failing to find such an association[26,27]. Altered platelet reactivity in HPA-1b individuals has also been studied with contradictory results[28,29]. The other platelet antigens studied are GPIIb Ile843Ser, GPIa C807T and Kozak polymorphism with conflicting results and warranting additional studies to confirm these observations[30-35].

Prothrombin gene polymorphism: It has been found that a nucleotide change (G to A transition) at a position 20210 in the 3'untranslated region of prothrombin gene is associated with elevated prothrombin levels[36]. Studies on association of this polymorphism with arterial disease have yielded contradictory results[37,38].

Lipoprotein (a) [Lp (a)]: Lp (a) is a complex serum lipoprotein composed of an low density lipoprotein (LDL) particle linked to apolipoprotein B-100. Lp (a) has a close structural homology to plasminogen. Due to its LDL-like properties, it accumulates in the arterial walls leading to atherosclerosis. Lp (a) has also been shown to stimulate the secretion of plasminogen activator inhibitor and interfere with fibrinolysis[39]. This has been studied most commonly in Indian patients with CAD[39-41].

Fibrinolytic system: The fibrinolytic system consists of plasminogen which is converted to plasmin by tissue plasminogen activator (t-PA) and urokinase[42]. Plasminogen activator inhibitor-1 (PAI-1) is a mjaor inhibitor of the fibrinolytic system and is synthesized by a variety of cells which include platelets, endothelial cells and vascular smooth muscle cells[42]. Plasma concentration of t-PA antigen correlates well with PAI-1 activity rather than with t-PA activity. Elevated levels of t-PA antigen have been found in patients with MI and therefore it may be a predictive risk factor for recurrent or future MI[42-44]. The reasons for increased t-PA antigen levels are unclear. It may be due to the prevalent endothelial dysfunction or may represent a net activation of endogenous fibrinolysis in response to the underlying atherosclerosis. The gene for t-PA is present on chromosome 8. An Alu repeat insertion-deletion polymorphism has been found in the t-PA gene which influences the release rate of t-PA. The role of this polymorphism in arterial thrombosis is unclear with conflicting reports[45-47].

Increased PAI-1 levels have also been found to be related to risk of arterial thrombosis[48]. A common insertion/deletion polymorphism 4G/5G of the promoter region of the PAI-1 has been described. The 4G allele has been associated with inability to bind a transcriptional repressor protein, resulting in an increased PAI-1 mRNA expression and increased PAI-1 levels. This polymorphism has been studied as a risk factor for CAD and MI with contradictory results[49,50]. To summarize, the role of the fibrinolytic system in cardiovascular disease in unclear. Further studies are warranted to understand better the role of this system.

Protein C, protein S, and antithrombin III: The role of PC, PS, ATIII deficiencies in arterial thrombosis is unclear with isolated case reports[51].

Factor VIII and von-Willebrand factor (vWF:Ag): Increased levels of factor VIII and vWF: Ag are known to be associated with fatal and non-fatal arterial thrombotic events[52,53]. In prospective studies involving patients with underlying atherosclerosis, vWF levels were found to be independently associated with risk of acute thrombotic event[53].

Other factors : Factor VII is a vitamin-K dependent coagulation protein which on activation binds to tissue factor present in damaged vascular subendothelium and initiates coagulation. The interest in this protein was aroused by the findings of the Northwick Park Heart study that elevated levels of factor were prospectively linked to fatal but not to non-fatal MI[3]. In addition, several intragenic factor VII polymorphisms which influence plasma factor VII levels have been described. Most of the attention has been focused on Arg353-Gln substitution which is located in the catalytic domain of factor VII. The evidence to date does not suggest the association of factor VII levels or factor VII polymorphisms as risk factors for arterial disease[54-56].

Factor XIII is a transglutaminase that forms covalent bonds between fibrin monomers and stabilizes a fibrin clot. Factor XIII circulates as a zymogen composed of two catalytic A subunits and two carrier B subunits. Thrombin cleavage of a 37 amino acid N-terminal peptide results in activation of the catalytic subunit. A Val34Leu polymorphism of the catalytic subunit is located 3 amino acid residues from the thrombin cleavage site. This polymorphism is associated with increased factor XIII activity. Studies have found conflicting results regarding the protective effect of the Leu34 allele against MI[57,58].

CAD in India

MI is one of the commonest causes of death in the developing and developed world. From being a disease of the old age, MI in Indian people is claiming a large number of lives even before the patients are reaching their fortieth birthday. Stress, strain, change in food habits and environmental pollution probably contribute to the recent epidemic of MI which is being observed in India today[59,60]. The alarming fact is that the incidence, prevalence, hospitalization and mortality due to CAD in Asian Indians is three to four times greater than in American and European counterparts[59].

Epidemiologcal studies have been carried out in India to determine the prevalence of CAD in both the rural and urban populations in the country since late 1950s and early in 1960s in Agra and Delhi. The prevalence of CAD has increased from 1.05 per cent in 1960 to 9.67 per cent in 1995 which in other words means a nine-fold increase over a period of 35 yr[57]. The rural areas have not been spared either. The increase in rural areas is two-fold in a span of twenty years[60]. Another serious revelation is the increased prevalence of CAD in both men and women of the younger age groups *i.e.*, men in age groups of 20-39 yr and women in age groups of 20-49 yr. In the Chennai urban population study, the prevalence rate of CAD in urban south Indians was 11 per cent, which was ten times higher than that reported forty years ago[61]. Studies show that cardiovascular disease has reached alarming proportions in India and it cannot be neglected[62,63].

Thus, the prevalence of CAD in India has increased considerably over the past few years and could become the number one killer if interventions are not done. The most striking feature of premature CAD in Indians is the low prevalence of traditional risk factors. Hence, there is an urgent need to look into the other factors that can lead to an increased predisposition for CAD. This warrants the investigations for thrombophilia in MI patients, in India.

Thrombophilia markers in MI in India

The traditional risk factors have been studied in Indians in relation to CAD. However, this does not totally explain the high incidence of CAD among Indians. To date, there have been scattered studies which have investigated some of the thrombophilia markers unlike the western countries.

The most frequently studied of these markers is Lp(a). Several case-control studies have shown that patients with CAD have significantly higher Lp(a) levels[39-41]. A recent study from Bangalore which recruited 300 CAD patients, did not find any correlation of Lp(a) with CAD[64]. Thus, Lp(a) may be an important risk factor for Indians which should be confirmed by larger studies. Our limited studies have not shown it to be an important risk factor (unpublished data). Of the natural anticoagulants, protein C levels were found to be raised in patients with previous MI in a study from Chennai[65]. Among the coagulation factors, elevated levels of fibrinogen have been found to be a risk factor for CAD in Indians[66,67]. Fibrinogen, tPA and PAI-1 have also been found to be associated with CAD in south Indian males[68].

Studies on homocysteine in Indians have yielded conflicting results[67,69-74]. A study in UK[69] found elevated homocysteine levels in patients with coronary heart disease as compared to controls and higher levels in UK Indians as compared to Europeans. However, four studies from India have failed to find any association between homocysteine and CAD[67,70-72]. Another recent study from Mumbai found that the thermolabile MTHFR variant was associated with hyperhomocysteinaemia in patients with coronary heart disease[73]. However, no significant difference was found in homocysteine levels between the patients and controls. Recently one study reported homocysteine as an independent risk factor for CAD in young Indians[74]

with mean homocysteine levels twice in patients as compared to controls.

In our previous study, prothrombin gene polymorphism was not found in patients with venous thrombosis and in normal control[75]. This observation has been confirmed in an other study on Indians in Punjab[76], suggesting very low prevalence or absence of this polymorphism in India. In our preliminary study, factor V Leiden was found to be a risk factor for MI in the young Indian patients[77]. The role of MTHFR C677T has been studied in cases of venous thrombosis[75] but its role in CAD on Indian patients is unknown. The remaining markers have not been studied in our patients, and need to be studied to get a clear picture of thrombophilia status in our patients.

Management of thrombophilia

Thrombophilia management comprises drugs, exercise and a strict diet regimen.

Antithrombotic agents: These agents prevent the formation of thrombus associated with MI and inhibit platelet function by blocking the cyclooxygenase enzyme and preventing subsequent aggregation. Examples are aspirin (inhibits cyclooxygenase), heparin (augments the activity of antithrombin III and prevents the conversion of fibrinogen to fibrin), warfarin (inhibits the effect of vitamin K on several clotting factors).

Thrombolytic agents: These agents prevent recurrent thrombus formation, lysing the thrombus and aid in rapid restoration of haemodynamic disturbances. Thrombolytic treatment should be started within 30 min of arrival of patients. Maximum benefit occurs when administered within 1-3 h of onset of symptoms. Examples include alteplase (fibrin specific agent with a half-life of 5 min and adjunctive therapy with iv heparin if necessary), t-PA (forms tertiary complex with fibrin and plasminogen), reteplase (recombinant plasminogen activator that forms plasmin after facilitating cleavage of endogenous plasminogen), streptokinase (acts with plasminogen to convert plasminogen to plasmin which degrades fibrin clots and fibrinogen) and tenecteplase (modified version of alteplase).

Platelet aggregation inhibitors: These inhibit platelet aggregation and reduce mortality. ADP receptor inhibitors like clopidogrel and ticlopidine are the main agents. The latter one leads to agranulocytosis; hence monitoring blood investigations is necessary. Clopidogrel was found to be more beneficial over aspirin in the CAPRI trial[78]. Others include GP IIb-IIIa inhibitors like eptifibatide, tirofiban (antagonists of GpIIb-IIIa receptor), abciximab (human-murine monoclonal antibody used in urgent/elective percutaneous coronary intervention). The beneficial effect of abciximab in combination with alteplase in thrombolysis was observed in TIMI 14 trial[79].

Low molecular weight heparin (LMWH): LMW heparins are produced from unfractionated heparin using a combination of column chromatography, chemical cleavage or by microbial heparinase treatment[80]. There are innumerable products in the market and these are not strictly bioequivalent. Low molecular weight and synthetic heparionoids have multiple modes of action. These inhibit factor X, improve fibrinolysis and also have substantial antithrombin activity. LMWH produce less osteoporosis and less incidence of heparin induced thrombocytopaenia[80]. It normally does not require laboratory monitoring unless given in very high dosage. Dalteparin was found to be a better drug in the FRISC trial[81].

Other thrombin inhibitors: (*i*) Hirudin or its analogues-Hirudin is isolated from the salivary glands of medicinal leech *Hirudo medicinalis* and is very costly. The analogues of hirudin are bivalirudin and lepirudin. Hirudin binds irreversibly to thrombin and its effect lasts for a long time. Hence, its dosage should be chosen very carefully. The advantages are high bioavailability after subcutaneous injection, weak immunogenecity and easy monitoring with thrombin time (TT) or activated partial thromboplastin time (APTT). Hirudin was found to be as effective as heparin in the TIMI 9B trial[82], and (*ii*) synthetic thrombin inhibitors-Argatobran and efegatran, are synthetic thrombin inhibitors. These compounds will play an important role in post-acute MI management when laboratory investigations show persistent thrombin generation.

Results of the laboratory investigations of thrombophilia will have a direct bearing on the management of the patient, because the drug of choice will depend on that aspect of haemostasis which is abnormal or deficient in the patient.

Laboratory diagnosis of thrombophilia

The major problem for investigating the thrombophilia markers is the prohibitive cost of each of these investigations. In a developing country like India, a very small proportion of patients will be able to afford the cost of these investigations. Hence, it is necessary to chalk out a plan by which only certain important thrombophilia markers can be investigated which may aid in the future management of the patient, and at the same time would not lead to a financial burden on the patient. For this, the investigations can be divided in three phases with the most urgent ones being done in the initial phase and the other important ones done later.

Phase 1: ABO blood grouping, screening coagulation tests, sensitive C-reactive protein level, fibrinogen, VWF:Ag, factor VII, activated protein C resistance (clotting test), homocysteine, folic acid, B12, D-dimer, Lp(a), anti-cardiolipin antibody, lupus anticoagulant.

These tests could help in the primary treatment of the patient. For example, in case of hyperhomocysteinaemia, the patient can be immediately put on folic acid. Abnormally high fibrinogen levels could mean inadequate fibrinolysis and can be a possible signal for re-infarction.

Phase 2: Protein C, protein S, antithrombin III, factor V Leiden, and Euglobulin clot lysis time.

These investigations can aid in a better management of the patient. In case of deficiency of any of the natural anticoagulants, the patient can be treated by oral anticoagulants and/or various low molecular weight heparin or heparinoids.

Phase 3: Spontaneous platelet aggregation, platelet antigen genotyping, detailed fibrinolytic studies.

These can indicate whether the patient is on adequate medications. This is particularly true in case of anti-platelet agents. If the patient's platelets show the tendency of platelet aggregation, this shows the inadequate medication and additional measures may be taken. Fibrinolytic potential of the blood may be improved by reducing hyperlipidaemia, exercise and using certain drugs such as metformin.

Which tests should be done in which phase will also be influenced by the prevalence of the particular thrombophilia marker. For example, in the middle east countries, factor V Leiden has a high prevalence[83] so it would be a part of phase 1 investigations and not phase 2 as mentioned above.

Unresolved issues

There have been a large number of epidemiological studies investigating the different haemostatic factors in relation to CAD. But to date, the problem is not resolved because a large number of studies including small number of patients have been done and published their findings which have often been disproved subsequently by studies involving a larger patient group. Elevated level of plasma fibrinogen is the only haemostatic marker which has been clearly identified as a risk factor for arterial thrombosis. The decision to do a screening for a haemostatic factor will depend mainly on whether screening offers a cost-effective identification of individuals who could be benefited from the treatment.

CAD affects Indians in the prime of their lives and careers and has significant socio-economic consequences. Of all ethnic groups, people of Indian origin have one of the highest incidences of CAD and it occurs frequently in Indians at a younger age. The prevalence of CAD in Indians is 3-4 times greater than in the western world[60].

The most striking feature of premature CAD in Indians is the low prevalence of conventional risk factors[60]. Hence, there is an urgent need to look into the other factors that can lead to an increased predisposition for CAD. Very little is known about the pathogenesis of atherosclerosis and CAD in Indians. Elevated fibrinogen levels and Lp (a) levels in plasma are the only haemostatic markers which have been found as a risk for CAD in Indians. Though four studies[67,70-72] have failed to find an association between homocysteine and CAD, it should be confirmed on a nationwide basis by recruiting a large number of patients from different parts of the country. The reason for this is three-fold : (*i*) Folic acid deficiency is not uncommon is our country; (*ii*) the traditional methods of cooking lead to the loss of folate and B12 from the food; and (*iii*) predominance of vegetarianism.

With the knowledge on human genome and the rapid technological advances in the field of biotechnology and molecular biology, new candidate genes will emerge for thrombophilia which would help to understand the pathogenesis of athreosclerosis in a better manner. Also, the human gene sequence would provide a better correlation between genotype and phenotype and may also help to identify individuals at an increased risk of CAD.

Thus, it may be worthwhile to investigate the different thrombophilia markers which have not been studied in our patients. This could be done initially in specific types of patients *e.g.*, young age group where a majority of them lack conventional risk factors. Depending on the results, these investigations could be extended to other patients as well. The outcome of all these studies will enable us to decide whether investigations for thrombophilia should form a part of the routine investigations done in patients with CAD.

Conclusion

The question is what a busy practicing cardiologist should do to investigate thrombophilia in patients with atherosclerotic heart disease? Should he investigate all such patients irrespective of their age and existing risk factors and what should he investigate for? Moreover, thrombophilia work-up is costly, and if the patients are not properly selected for these tests, they will unnecessarily incur heavy expenditure without being benefitted.

Plasma fibrinogen level, factor VIII/vWF levels and C-reactive protein (CRP), and lupus anticoagulant screening and confirmation should be done in all patients with significant atherothrombotic disease. These tests are relatively cheap and detect predispositions to future incidents like acute MI. High vWF level in patients' blood suggests continuing endothelial damage and repeat studies after nitrates and vitamins (folate, B12, pyridoxine) therapy will tell whether continuing endothelial damage has been arrested.

An improved ELISA assay for homocysteine is now available[84,85] and as most of the hospitals even at the district level have access to an ELISA reader, this test can be easily adopted. Presently measuring plasma homocysteine level costs around Rs.1000/- per test. If the patient has associated hyperlipidaemia, administration of some of the statins also leads to reduction of plasma fibrinogen levels as well as CRP levels by anti-inflammatory action through blocking the mevalonate pathway and isoprenoid synthesis. If the patient is young (<40 yr), has strong family history (~10% of patients with acute MI), and is a woman in her reproductive years, then apart from the above investigations rest of the thrombophilia markers like anticardiolipin antibody, PC, PS, ATIII levels, APC resistance test and factor V Leiden should also be evaluated.

In a small proportion of cases, more than one thrombophilia marker may be positive and these patients probably should be on some kind of oral anticoagulants apart form aspirin therapy, because risk of future events in these patients is high, though this intervention has not been studied by a formal randomized trial.

If all the investigations of thrombophilia are negative in a young patient with atherothrombosis, the formal fibrinolytic studies should be undertaken. This kind of study should involve a global test for fibrinolysis *i.e.*, euglobulin lysis time (ECLT) pre-and post-venous occlusion. Though ECLT is an extremely variable test and is influenced by a lot of variables, it is the only test which can be done without incurring much expenditure and being a global test for fibrinolytic and anti-fibrinolytic activity, it provides us an idea of the balance between these two systems. The result following post-venous occlusion tells about fibrinolytic reserve.

Ideally these young patients need more exploration of their fibrinolytic system by measuring PAI-1 and t-PA levels.

Platelet alloantigen HPA-1b/1b as a predisposition to acute MI and CAD has not yet been proved conclusively but laboratory results do suggest decreased threshold of these patients to ADP-induced platelet aggregation. Our preliminary study showed that homozygosity for HPA-1b was significantly greater in MI cases as compared to controls (unpublished data). Hence, human platelet alloantigen testing may also be added in future to the list of thrombophilia work-up as these patients may possibly be helped by more aggressive anti-platelet therapy.

References

1. Middeldrop S, Buller HR, Prins MH, Hirsh J. *Approach to the thrombophilia patient. Hemostasis and Thrombosis. Basic Principles and Clinical Research*, 4th ed. PA, USA : Lippincott Williams and Wilkins; 1085-1100.
2. Ross R. Atherosclerosis - an inflammatory disease. *N Engl J Med* 1999; *340* : 115-26.
3. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WR, *et al*. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986; *ii* : 533-7.
4. Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med* 1984; *311* : 501-5.
5. Tybjaerg-Hansen A, Agerholm-Larsen B, Humpries SE, Abildgaard S, Schnohr P, Nordestgaard BG. A common mutation (G455-A) in the β-fibrinogen promoter is an independent predictor of plasma fibrinogen but not ischaemic heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. *J Clin Invest* 1997; *99* : 3034-9.
6. Curran JM, Evans A, Arveiler D, Luc G, Ruidavets JB, Humphries SE, *et al*. The α-fibrinogen T/A 312 polymorphism in the ECTIM study. *Thromb Hemost* 1998; *79* : 1057-8.
7. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci USA* 1993; *90* : 1004-8.
8. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, *et al*. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; *369* : 64-7.
9. Rosendaal FR, Siscovick DS, Schwartz SM, Beverly RK, Psaty BM, Longstreth WT, *et al*. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. *Blood* 1997; *89* : 2817-21.
10. Doggen CJ, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation* 1998; *97* : 1037-41.
11. Cushman M, Rosendaal FR, Psaty BM, Cook EF, Valliere J, Kuller LH, *et al*. Factor V Leiden is not a risk factor for arterial vascular disease in the elderly: results from the Cardiovascular Health Study. *Thromb Haemost* 1998; *79* : 912-5.
12. Gardemann A, Arsic T, Katz N, Tilmanns H, Hehrlein FW, Haberbosch W. The factor II G20210A and factor V G1691A gene transitions and coronary heart disease. *Thromb Haemost* 1999; *81* : 208-13.
13. Townend J, O'Sullivan J, Wilde JT. Hyperhomocysteinaemia and vascular disease. *Blood Rev* 1998; *12* : 23-34.
14. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999; *354* : 407-13.
15. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA* 1995; *274* : 1049-57.
16. Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Association between total homocyst(e)ine and the likelihood for a history of acute myocardial infarction by race and ethnicity: results form the Third National Health and Nutrition Examination Survey. *Am Heart J* 2000; *139* : 446-53.
17. Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB. Plasma homocysteine in the acute and convalescent phases after stroke. *Stroke* 1995; *26* : 795-800.
18. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. *Thromb Haemost* 1999; *81* : 165-76.
19. Christen WG, Ajani UA, Glynn RJ, Hennekens CH. Blood levels of homocysteine and increased risks of cardiovascular disease: causal or casual? *Arch Intern Med* 2000; *160* : 422-34.
20. Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, *et al*. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996; *58* : 35-41.
21. Anderson JL, King GJ, Thomson MJ, Todd M, Bair TL, Muhlestein JB, *et al*. A mutation in the methylenetetrahydrofolate reductase gene is not associated with an increased risk for coronary artery disease or myocardial infarction. *J Am Coll Cardiol* 1997; *30* : 1206-11.
22. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 1998; *64* : 169-72.
23. Szczeklik A, Sanak M, Jankowski M, Dropinski J, Czachor R, Musial J, *et al*. Mutation A1298C of methylenetetrahydrofolate reductase: risk for early coronary disease not associated with hyperhomocysteinemia. *Am J Med Genet* 2001; *101* : 36-9.
24. Zotz RB, Winkelmann BR, Nauck M, Giers G, Maruhn-Debowski B, Marz W, *et al*. Polymorphism of platelet membrane glycoprotein IIIa: human splatelet antigen 1b (HPA-1b/P1A2) is an inherited risk factor for premature myocardial infarction in coronary artery disease. *Thromb Haemost* 1998; *79* : 731-5.

25. Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagaliabue L, *et al*. Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood* 1999; *94* : 46-51.

26. Herrmann SM, Poirier O, Marques-Vidal P, Evans A, Arveiler D, Luc G, *et al*. The Leu33/Pro polymorphism (P1A1/P1A2) of the glycoprotein IIIa (GPIIIa) receptor is not related to myocardial infarction in the ECTIM Study. *Thromb Haemost* 1997; *77* : 1179-81.

27. Laule M, Cascorbi I, Stangl V, Bielecke C, Wernecke KD, Mrozikiewiez PM, *et al*. A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study. *Lancet* 1999; *353* : 708-12.

28. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, *et al.* Platelet GPIIIa P1 (A) polymorphisms display different sensitivities to agonists. *Circulation* 2000; *101* : 1013-8.

29. Meiklejohn DJ, Urbaniak SJ, Greaves M. Platelet glycoprotein GPIIIa polymorphism HPA 1b ($P1^{A2}$): no association with platelet fibrinogen binding. *Br J Haematol* 1999; *105* : 664-6.

30. Hato T, Minamoto Y, Fukuyama T, Fujita S. Polymorphisms of HPA-1 through 6 on platelet membrane glycoprotein receptors are not a genetic risk factor for myocardial infarction in the Japanese population. *Am J Cardiol* 1997; *80* : 1222-4.

31. Bottiger C, Kastrati A, Koch W, Mehilli J, Seidl H, Schomig K, *et al*. HPA-1 and HPA-3 polymorphisms of the platelet fibrinogen receptor and coronary artery disease and myocardial infarction. *Thromb Haemost* 2000; *83* : 559-62.

32. Moshfegh K, Wuillemin WA, Redendo M, Lammle B, Beer JH, Liechti-Gallati S, *et al*. Association of two silent polymorphisms of platelet glycoprotein Ia/IIa receptor with the risk of myocardial infarction: a case-control study. *Lancet* 1999; *353* : 351-4.

33. Croft SA, Hampton KK, Sorrell JA, Steeds RP, Channer KS, Samani NJ, *et al*. The GPIa C807T dimorphism associated with platelet collagen receptor density is not a risk factor for myocardial infarction. *Br J Haematol* 1999; *106* : 771-6.

34. Frank MB, Reiner AP, Schwartz SM, Kumar PN, Pearce RM, Argogast PG, *et al*. The Kozak sequence polymorphism of platelet glycoprotein Ibα and risk of nonfatal myocardial infarction and nonfatal stroke in young women. *Blood* 2001; *97* : 875-9.

35. Douglas H, Michaelides K, Gorog DA, Durante-Mangoni E, Ahmed N, Davies GJ, *et al*. Platelet membrane glycoprotein Ibα gene -5T/C Kozak sequence polymorphism as an independent risk factor for the occurrence of coronary thrombosis. *Heart* 2002; *87* : 70-4.

36. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; *88* : 3698-703.

37. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood* 1997; *90* : 1747-50.

38. Eikelboom JW, Baker RI, Parsons R, Taylor RR, van Bockxmeer FM. No association between the 20210 G/A prothrombin gene mutation and premature coronary artery disease. *Thromb Haemost* 1998; *80* : 878-80.

39. Gambhir JK, Kaur H, Gambhir DS, Prabhu KM. Lipoprotein (a) as an independent risk factor for CAD in patients below 40 years of age. *Indian Heart J* 2000; *52* : 411-5.

40. Gupta R, Vashisht S, Bahl VK, Wasir HS. Correlation of lipoprotein (a) to angiographically defined coronary artery disease in Indians. *Int J Cardiol* 1996; *57* : 265-70.

41. Isser HS, Puri VK, Narain VS, Saran RK, Dwivedi SK, Singh S. Lipoprotein (a) and lipid levels in young patients with myocardial infarction and their first degree relatives. *Indian Heart J* 2001; *53* : 463-6.

42. Ridker PM, Vaughan DE, Stampfer MJ, Manson J, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. *Lancet* 1993; *341* : 1165-8.

43. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari G. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardia infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. *Circulation* 1998; *98* : 2241-7.

44. Wiman B, Andersson T, Hallqvist J, Reuterwall C, Ahlbom A, deFaire U. Plasma levels of tissue plasminogen activator/ plasminogen activator inhibitor-1 complex and von-willebrand factor are significant risk factors for recurrent myocardial infarction in the Stockholm Heart Epidemiology Program (SHEEP) study. *Arterioscler Thromb Vasc Biol* 2000; *20* : 2019-23.

45. van der Bom JG, de Knijff P, Haverkate F, Bots ML, Meijer P, de Jong PT, *et al*. Tissue plasminogen activator and the risk of myocardial infarction. The Rotterdam study. *Circulation* 1997; *95* : 2623-7.

46. Ridker PM, Baker MT, Hennekens CH, Stampfer MJ, Vanghan DE. Alu-repeat polymorphism in the gene coding for tissue type plasminogen activator (t-PA) and risks of myocardial infarction among middle-aged men. *Arterioscler Thromb Vasc Biol* 1997; *17* : 1687-90.

47. Steeds R, Adams M, Smith P, Channer K, Samani NJ. Distribution of tissue plasminogen activator insertion/deletion polymorphism in myocardial infarction and control subjects. *Thromb Haemost* 1998; *79* : 980-4.

48. Margaglione M, Di Minno G, Grandone E, Veechione G, Glentano E, Cappucci G, *et al*. Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke. *Arterioscler Thromb* 1994; *14* : 1741-5.

49. Eriksson P, Kallin B, van't Hoft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen activator inhibitor-1 gene is associated with myocardial infarction. *Proc Natl Acad Sci USA* 1995; *92* : 1851-5.

50. Anderson JL, Muhlestein JB, Habashi J, Carlquist JF, Bair TL, Elmer SP, *et al*. Lack of association of a common polymorphism of the plasminogen-activator inhibitor 1 gene with coronary artery disease and myocardial infarction. *J Am Coll Cardiol* 1999; *34* : 1778-83.

51. Coller BS, Owen J, Jesty J, Horowitz D, Reitman MJ, Spear J, *et al*. Deficiency of plasma protein S, protein C or antithrombin III and arterial thrombosis. *Arteriosclerosis* 1987; *7* : 456-62.

52. Smith FB, Lee AJ, Fowkes FG, Price JF, Rumley A, Lowe GD. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol* 1997; *17* : 3321-5.

53. Rumley A, Lowe GD, Sweetnam PM, Yarnell JW, Ford RP. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. *Br J Haematol* 1999; *105* : 110-6.

54. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and the incidence of coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 1997; *96* : 1102-8.

55. Iacoviello L, Di Castelnuovo A, De Knijiff P, D'Orazio A, Amore C, Arboretti R, *et al*. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. *N Engl J Med* 1998; *338* : 79-85.

56. Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagliabue L, *et al*. Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood* 1999; *94* : 46-51.

57. Corral J, Gonzalez-Conejero R, Iniesta JA, Rivera J, Martinez C, Vicente V. The F XIII Val34Leu polymorphism in venous and arterial thromboembolism. *Haematologica* 2000; *85* : 293-7.

58. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost* 1998; *79* : 8-13.

59. Enas EA. Dhawan J, Petkar S. Coronary artery disease in Asian Indians: lessons learnt and the role of lipoprotein (a). *Indian Heart J* 1997; *49* : 25-34.

60. Gupta R, Gupta VP. Meta-analysis of coronary heart disease prevalence in India. *Indian Heart J* 1996; *48* : 241-5.

61. Mohan V, Deepa R, Rani SS, Premalatha G. Prevalence of coronary artery disease and its relationship to lipids in a selected population in South India. The Chennai Urban Population Study (CUPS No.5). *J Am Coll Cardiol* 2001; *38* : 682-7.

62. Reddy KS. Neglecting cardiovascular disease is unaffordable. *Bull World Health Organ* 2001; *79* : 984-5.

63. Reddy KS. Cardiovascular diseases in the developing countries: dimensions, determinants, dynamics and directions for public health action. *Public Health Nutr* 2002; *51* : 231-7.

64. Govindaraju V, Neelam, Manjunath CN, Sundar KK. Lipoprotein (a) in coronary artery disease in Indian population. *J Indian Med Assoc* 2003; *101* : 458-60.

65. Ganapathyraman L, Shanti P, Baba Krishnan K, Madhavan M, Lakshminathan C. Protein C levels in ischaemic heart disease. *Indian Heart J* 1996; *48* : 125-7.

66. Jose J, Selvakumar D, Selvakumar R, Kanagasabapathy AS, Jeyaseelan L. Plasma fibrinogen- an independent risk factor for ischaemic heart disease. *Indian Heart J* 1998; *50* : 45-8.

67. Gheye S, Lakshmi AV, Krishna TP, Krishnaswamy K. Fibrinogen and homocysteine levels in coronary artery disease. *Indian Heart J* 1999; *51* : 499-502.

68. Deepa R, Velmurugan K, Saravanan G, Dwarakanath V, Agarwal S, Mohan V. Relationship of tissue plasminogen activator, plasminogen activator inhibitor-1 and fibrinogen with coronary artery disease in south Indian male subjects. *J Assoc Physicians India* 2002; *50* : 901-6.

69. Chambers JC, Obeid OA, Refsum H, Ueland P, Hackett D, Hooper J, *et al*. Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. *Lancet* 2000; *355* : 523-7.

70. Chacko KA. Plasma homocysteine levels in patients with coronary heart disease. *Indian Heart J* 1998; *50* : 295-9.

71. Deepa R, Velmurugan K, Saravanan G, Karkuzhali K, Dwarkanath V, Mohan V. Absence of association between serum homocysteine levels and coronary artery disease in south Indian males. *Indian Heart J* 2001; *53* : 44-7.

72. Sastry BK, Indira N, Anand B, Kedarnath, Prabha BS, Raju BS. A case-control study of plasma homocysteine levels in south Indians with and without coronary artery disease. *Indian Heart J* 2001; *53* : 749-53.

73. Nair KG, Nair SR, Ashavaid TF, Dalal JJ, Eghlim FF. Methylenetetrahydrofolate reductase gene mutation and hyperhomocysteinemia as a risk factor for coronary heart disease in the Indian population. *J Assoc Physicians India* 2002; *50* (Suppl) : 9-15.

74. Puri A, Gupta OK, Dwivedi RN, Bharadwaj RPS, Narain VS, Singh S. Homocysteine and lipid levels in young patients with coronary artery disease. *J Assoc Physicians India* 2003; *51* : 681-5.

75. Ghosh K, Shetty S, Madkaikar M, Pawar A, Nair S, Khare A, *et al*. Venous thromboembolism in young patients from western India: a study. *Clin Appl Thromb Hemost* 2001; *7* : 158-65.

76. Herrmann FH, Salazar-Sanchez L, Schroder W, Grimm R, Schuster G, Jimenez-Arce G, *et al*. Prevalence of molecular risk factors FV Leiden, FVHR2, FII 20210 G>A and MTHFR 677CT in different populations and ethnic groups of Germany, Costa Rica and India. *Int J Hum Genet* 2001; *1* : 33-9.

77. Mohanty D, Shetty S, Anil CR, Pathare A, Ghosh K, Yeolekar ME. Factor V Leiden gene mutation in young Indian patients with myocardial infarction. *Curr Sci* 1998; *74* : 1078-80.

78. CAPRIE Steering Committee. A randomized, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996; *348* : 1329-39.

79. Antman EM, Giugliano RP, Gibson CM, McCabe CH, Coussement P, Kleiman NS, *et al*. Abciximab facilitates the rate and extent of thrombolysis: results of the thrombolysis in myocardial infarction (TIMI) 14 trial. The TIMI 14 investigators. *Circulation* 1999; *99* : 2720-32.

80. Linhardt RJ, Gunay NS. Production and chemical processing of low molecular weight heparins. *Semin Thromb Hemost* 1999; *25* (Suppl 3) : 5-16.

81. Low molecular weight heparin during instability in coronary artery disease, Fragmin during Instability in Coronary Artery Disease, (FRISC) Study Group. *Lancet* 1996; *347* : 561-8.

82. Antman EM. Hirudin in acute myocardial infarction thrombolysis and thrombin inhibition in myocardial infarction (TIMI) 9B trial. *Circulation* 1996; *94* : 911-21.

83. Awidi A, Shannak M, Bseiso A, Kailani MA, Kailani MA, Omar N, *et al*. High prevalence of factor V Leiden in healthy Jordanian Arabs. *Thromb Haemost* 1999; *81* : 582-4.

84. Frantzen F, Faaren AL, Alfheim I, Nordhei AK. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clin Chem* 1998; *44* : 311-6.

85. Yu HH, Jourbran R, Asmi M, Law T, Spencer A, Jouma M, *et al*. Agreement among four homocysteine assays and results in patients with coronary atherosclerosis and controls. *Clin Chem* 2000; *46* : 258-64.

Reprint requests: Dr D. Mohanty, Emeritus Medical Scientist, Institute of Immunohaematology (ICMR), 13th Floor
New M.S. Building, KEM Hospital, Parel, Mumbai 400012, India
e-mail : mohanty@bom5.vsnl.net.in