

NOVEL CARDIOVASCULAR RISK FACTORS

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Introduction

Cardiovascular disease (CVD) is the major cause of death worldwide, claiming 10.7 million lives in 1990.¹ Of these, approximately two-thirds are coronary deaths and one-third are cerebrovascular deaths. The traditional pathophysiological paradigm for CVD includes atherosclerosis, the lipid hypothesis and the influence of conventional CVD risk factors. The concept of risk factor was introduced in the 1960s, along with the results of the Framingham Heart Study.² Epidemiologically, a risk factor is a trait or characteristic which, when present early in life, confers future risk for development of a specific disease. The currently accepted CVD risk factors are dyslipidaemias, hypertension, smoking, age, family history of myocardial infarction (MI) at an early age, diabetes mellitus, physical inactivity, obesity and menopause. Identification and control of risk factors is essential for clinical practice and for the development of primary and secondary prevention strategies in public health. In fact, at least part of the decrease in CVD mortality observed in the last decade may be due to better identification and treatment of high risk populations.

Nevertheless, over half of those who develop CVD do so in the absence of conventional risk factors.^{3,4} In these individuals, alternative factors associated with haemostasis, inflammation and thrombogenesis seem likely to be implicated.⁵ In addition, conventional risk factors appear not to bear the same weight in different ethnic groups. For example, in the African-Caribbean population, there is a low prevalence of CVD despite high rates of diabetes mellitus⁶ and in China the widespread use of tobacco does not increase the incidence of CVD.⁷ As a result of such disparities, alternative risk factors for CVD have been sought and identified over the last 15 years. These novel risk factors can be divided into factors of haemostasis and thrombosis, such as homocysteine, fibrinogen, lipoprotein (a) (Lp [a]), aspirin resistance and inflammatory markers such as selectins P and E, cellular adhesion molecules (CAMs), tumour necrosis factor alpha (TNF- α), interleukin 6 (IL-6) and C reactive protein (CRP).

This review proposes to analyse each of these new risk factors in light of their putative pathophysiological role in the genesis of atherosclerosis, strength of the evidence of their associate risk and their relevance in and implications for routine clinical practice.

Factors of thrombosis and haemostasis

Homocysteine

Homocysteine is an intermediate amino acid enzymatically formed during the metabolism of methionine. Normal levels of fasting homocysteine in Western populations vary between 5 and 15 μ mol/l and can be easily measured by standard laboratory techniques. Increased levels of serum homocysteine have been associated with ageing, menopause, chronic renal insufficiency, heart transplantation and low plasma levels of vitamin cofactors (B₆, B₁₂, folate).

The relationship between serum homocysteine levels and CVD has been well established as patients with homocystinuria, homozygous for β -synthase deficiency, are known to develop severe and premature CVD. The putative mechanisms through which homocysteine promotes CVD include endothelial dysfunction with reduced vascular relaxation,^{8,10} mitotic effect on vascular smooth muscle cells,¹¹ factor V activation,¹² activation of tissue plasminogen activator (tPA)¹³ and endovascular inflammation.¹⁴ In the Physicians' Health Study,¹⁵ where approximately 15,000 men with no prior history of CVD were followed for five years, 7% of MI were attributable to elevated homocysteine levels and the relative risk for MI associated with serum homocysteine in the higher percentiles was 3.4 (95% confidence interval [CI] 1.3-8.8; $p=0.01$). Another study of 802 subjects with angiographically documented coronary disease showed that homocysteine levels correlated linearly with the risk of future cardiovascular events and death.¹⁶ Lastly, a meta-analysis of 27 studies found a positive correlation between serum homocysteine levels >15 mmol/l and increased risk of coronary events, cerebrovascular accidents (CVA), peripheral vascular disease (PVD) and venous thromboembolism.¹⁷

Results of prospective, randomised, clinical trials demonstrating a reduction of CVD risk with folate supplementation in patients with hyperhomocystinaemia are not yet available. However, there is indirect evidence that folic acid improves endothelial function in patients with coronary artery disease and hyperhomocystinaemia¹⁸ and that patients who were supplemented with multivitamins containing folate and B vitamins had a lower incidence of CVD.^{19,20}

As it is well established that hyperhomocystinaemia is

associated with increased CVD risk and that folic acid effectively decreases serum homocysteine levels, routine folic acid supplementation for patients with hyperhomocystinaemia seems warranted. It is also recommended to screen specific populations for increased homocysteine levels.

Fibrinogen

Fibrinogen is a molecule synthesised by the liver consisting of a glycoprotein with three polypeptide chains (α , β and γ) whose expression is regulated by three genes in chromosome 4. By binding to GP1b and GP2b/3a receptors on the surface of platelets, fibrinogen promotes platelet adhesion and aggregation. It is also essential for the formation of the fibrin thrombus and increases in plasma viscosity. In addition to these thrombogenic properties, fibrinogen is a potent acute phase reactant which closely follows rises in sedimentation rates and CRP levels during inflammatory states. It is likely that the pathophysiological role played by fibrinogen in CVD involves both its thrombogenic and inflammatory properties.

Fibrinogen has been identified as an independent CVD risk factor^{21,22} whose interaction with traditional risk factors has been well established.²³ It has been postulated that at least part of the deleterious effects of smoking, LDL cholesterol and diabetes mellitus is mediated by their induction of higher fibrinogen serum levels and consequent platelet hyperactivity.²³ In addition, patients with chronic stable angina and elevated serum fibrinogen levels have a threefold increase in risk for coronary events when compared to those with normal fibrinogen levels.²⁴

Genetic and environmental factors determine serum fibrinogen levels. Male gender, African descent, older age, menopause, tobacco use, diabetes mellitus, increases in LDL cholesterol, Lp (a) and body mass are all associated with increased serum fibrinogen levels.^{21,25} In women, the relationship between increased fibrinogen levels and MI is particularly significant.²⁶

Indiscriminate routine fibrinogen screening is not appropriate. However, in those presenting with CVD in the absence of traditional risk factors, fibrinogen screening may be helpful. In addition, in patients with certain risk factors, such as menopausal women, people with diabetes, smokers and patients with angina, fibrinogen levels may help to further stratify those at the highest risk. In such patients, more aggressive strategies directed at weight loss, smoking cessation, diabetes control and perhaps revascularisation may be warranted. Although at this time there are no medical therapies effective at reducing serum fibrinogen levels, these levels can be reduced by exercise, smoking cessation, moderate alcohol ingestion and oestrogen use.²⁶

Aspirin resistance

Due to the fact that platelets play a central role in the pathogenesis of CVD, a large part of the pharmacological arsenal used to treat and prevent CVD is directed at platelet function inhibition. Aspirin (acetylsalicylic acid) is the most

widely used anti-platelet agent in the prevention of primary and secondary cardiovascular events. It impairs platelet aggregation by the acetylation of platelet cyclooxygenase, resulting in an irreversible inhibition of platelet-dependent thromboxane. However, platelet function and its inhibition also depend on intrinsic platelet characteristics, such as number, size, function and spontaneous aggregation capacity.

Whereas aspirin is an effective agent for prevention of CVD complications,²⁷ offering a 19% risk reduction for cardiovascular events in patients with CVD, it fails to prevent 81% of events in high risk patients. Therefore, one in every eight high risk patients suffers a recurrent cardiovascular event despite continuous use of therapeutic doses of aspirin.

It is well recognised that the anti-platelet effect of aspirin is highly dependent on individual patient variations. In fact, it has been estimated that 4-45% of the population do not obtain adequate anti-platelet effect with aspirin use.^{28,29} Optical platelet aggregation measurements in 326 patients with stable CVD showed that 1 in 20 had laboratory evidence of aspirin resistance.²⁸ The possible mechanisms for aspirin ineffectiveness may include patient non-compliance, insufficient doses, different pathways for platelet activation not affected by aspirin, biosynthesis of aspirin-insensitive thromboxane, glycoprotein receptor polymorphism and states of accelerated platelet turnover.

In a cohort of stable CVD patients followed for two years, optical platelet aggregation identified 5% as being aspirin-resistant.³⁰ The hazards ratio for the composite endpoint of death, MI or CVA was 3.1 (95% CI 1.8-8.2; $p=0.03$) for those who were aspirin-resistant as opposed to those who were aspirin-sensitive. In addition, the Thrombolysis in Myocardial Infarction (TIMI) 11B investigators³¹ identified previous aspirin use as an independent risk factor for poor outcomes in patients with unstable angina and non-ST elevation MI (NSTEMI). The postulated reason for poor prognosis was thought to be aspirin-resistance and, therefore, a prothrombotic state.

In clinical practice, a patient with high risk acute coronary syndrome (ACS) with a history of ongoing aspirin use should be considered for therapy with GP2b/3a inhibitors and stratified for early invasive strategy.³¹⁻³³ Although the implications of aspirin failure in patients with ACS are recognised, there are no data available for patients with stable CVD.

However, given the known risks associated with aspirin resistance, laboratory access to optical platelet aggregation techniques and the availability of potent substitute anti-platelet drugs, screening of well-selected patients may be warranted. Candidates for aspirin resistance screening are patients with multiple recurrent events, high risk profile and angina despite use of aspirin. Unfortunately, there remain some impediments for the widespread screening for aspirin resistance in all patients with CVD. First, there is no standard laboratory definition of aspirin resistance; second, there is no specific therapy for aspirin resistance; and lastly, there is no evidence that substitute anti-platelet therapy in this group of patients improves survival.

Nevertheless, for patients in whom aspirin resistance is strongly suspected, strategies to increase the probability of adequate platelet inhibition should be considered. Such strategies should be directed at those who have recurrent CVD events while on adequate aspirin doses (81-325mg/day). Clopidogrel is a thiopyridine that impairs platelet aggregation through a different mechanism to that of aspirin and should be considered as a substitute or additional therapy. Clopidogrel's superiority to aspirin has been demonstrated when used in lieu of, or in addition to, aspirin in different categories of high risk patients.^{36,37} Other drugs that may be considered are dipyridamole, coumadin and ticlopidine.

Lp (a)

Lp (a) belongs to a family of lipoproteic particles similar to cholesterol LDL both in their lipid composition and in the presence of apolipoprotein (apo) B-100 on their surface. The main difference is the glycoprotein apo (a) moiety attached to apo B-100. Serum levels of Lp (a) vary in different populations and seem to be hereditarily determined, as they remain relatively stable from a young age.³⁸ Interestingly, Lp (a) levels do not correlate with LDL, HDL or total cholesterol levels and are not affected by measures to alter serum levels of these lipoproteins.³⁹ The regulatory mechanisms for Lp (a) are unknown, but it is well known that chronic renal insufficiency, nephrotic syndrome, renal transplantation and dialysis tend to be associated with higher Lp (a) levels.

Metabolic insults such as diabetes mellitus, oestrogen therapy and pregnancy cause fluctuations in Lp (a). Serum Lp (a) levels are higher in black people and post-menopausal women.^{38,40} Lp (a) is strongly associated with premature CVD,^{38,39} although the exact mechanisms behind this association are still poorly understood. As levels of Lp (a) are determined in infancy and tend to remain stable throughout life, this may help explain its association with premature atherosclerosis. In fact, the atherogenic effects of Lp (a) seem to be similar to those of LDL cholesterol,⁴¹ and similarly its oxidation confers greater atherogenic potential to the particle.⁴² Lp (a) also exerts a potent prothrombotic effect by impairing fibrinolysis⁴³ through the competitive binding of its apo (a) moiety to fibrin.

Due to the fact that it is similar in structure to plasminogen, despite lacking fibrinolytic properties, Lp (a) competitively binds to fibrin, displaces plasminogen and thus impairs fibrinolysis.⁴³ Other properties of Lp (a) that may be related to its atherogenic effects include stimulating smooth muscle cell growth, enhancing endothelial expression of intercellular adhesion molecule 1 (ICAM-1), inhibiting plasminogen activation by tPA, increasing expression of plasminogen activation inhibitor 1 (PAI-1) and inactivating the tissue factor pathway inhibitor (TFPI).^{44,46}

As an independent risk factor for premature CVD in men, an elevated serum level of Lp (a) is equivalent to a total cholesterol of ≥ 240 mg/dl or a HDL cholesterol of

< 35 mg/dl.⁴⁷ Plasma concentrations of 20mg/dl or greater have been associated with increased risk of MI, CVA and PVD in men⁴⁷ and women.⁴⁸ Compared to other CVD risk factors, Lp (a) ranked fifth in order of importance as a predictor of cardiovascular events, after high LDL, family history for MI, serum fibrinogen and low HDL.⁴⁹ The association between accelerated progression of coronary lesions on angiography⁵⁰ and high rates of post-angioplasty stenosis⁵¹ may be linked to its prothrombotic effect.

Lp (a) screening should be considered in patients with premature CVD or with a family history of premature CVD, with CVD in the absence of traditional risk factors, and in those with rapid and aggressive progression of CVD. The finding of Lp (a) level > 20 mg/dl should prompt aggressive treatment and screening of family members. Unfortunately, pharmacological treatment of elevated Lp (a) has met with modest success. Even though statins are ineffective in lowering Lp (a),⁵² aggressive treatment of LDL levels is important as it reduces pathogenicity of Lp (a) by reducing angiographic progression of coronary lesions and the incidence of new CVD events.⁵³ Interventions that have been shown to modestly reduce Lp (a) are nicotinic acid,⁵⁴ gemfibrozil,⁵⁵ omega 3 fatty acids⁵⁶ and plasmapheresis.⁵⁷ However, there are no clinical studies to prove that the reduction of Lp (a) levels leads to a decrease in cardiovascular morbidity and mortality.

Serum inflammatory markers

Increased inflammatory activity is believed to be one of the major culprits in the development and progression of atherosclerosis and endothelial dysfunction in CVD.⁵⁸ The initial events in the development of an atherosclerotic lesion involve monocyte chemotaxis, adhesion and transmigration to the vascular endothelium. Subsequent transformation of monocytes into leucocytes with progressive intracellular lipid accumulation results in sponge cells and fatty streaks, the first visible manifestation of atherosclerosis on the vascular lumen. Continuous inflammatory cell recruitment and smooth muscle cell proliferation eventually produce a mature atherosclerotic plaque, with its fibrous capsule. Progressive thinning of the capsule leads to plaque rupture with exposure of prothrombotic material into the vascular lumen and subsequent clot formation. Every step of this process is mediated and accelerated by inflammation. Recently discovered molecules measured in human sera have been recognised as inflammatory markers and have been evaluated as potential novel CVD risk factors.

Selectins P and E

Selectins P and E are molecules involved in the initial stages of vascular inflammation that appear to play a major role in atherogenesis. It is thought that selectins mediate adhesion of monocytes to the vascular wall and their transmigration to the vascular endothelium. In experimental animal studies, a steep increase in P selectin levels was found after a

cholesterol-rich diet,⁵⁹ as well as a decrease in plaque formation and leucocyte migration after infusion of antibodies against P selectin in mice.^{60,61}

The value of P selectin as a potential marker for future CVD events was tested in the Women's Health Study. In this cohort of 28,263 healthy women, initially elevated levels of P selectin were associated with a two-fold increased risk for future cardiovascular events.⁶² At present, however, the clinical utility of selectin P is poorly understood and serum assays are not yet routinely available. Nevertheless, development of therapies aimed at blocking selectin P may be yet another potential strategy to prevent and treat atherosclerosis.

CAMs

The ICAM-1 and the vascular adhesion molecule (VCAM-1) are the members of this class of molecules that seem to be most strongly implicated in the genesis of the atherosclerotic plaque. Along with selectins, ICAM-1 and VCAM-1 regulate macrophage adhesion and transmigration to the vascular endothelium.⁶³ ICAM-1 is expressed by macrophages and endothelial cells following activation by inflammatory cytokines such as interleukin 1 (IL-1), TNF- α and gamma interferon (IFN- γ). Once activated, endothelial cells perpetuate the inflammatory cascade by producing IL-6, monocyte chemoattractant protein 1 (MCP-1), amongst others, which continue to foment plaque growth.

Given its pathogenic properties, the clinical impact of ICAM-1 on the risk of cardiovascular events was prospectively evaluated in a cohort of 15,000 healthy physicians. In the Physicians' Health Study, elevated initial serum levels of ICAM-1 were found to be an independent risk factor for future MI.⁶⁴ In addition, the risk was found to increase with time of follow up, underscoring the role of ICAM-1 in the pathogenesis of the atherosclerotic plaque. Although the value as a marker of cardiovascular risk has been repeatedly confirmed, a meta-analysis compiling 27 studies suggests that its routine use in clinical practice offers little predictive information incremental to that provided by conventional risk factors.⁶⁵

Statins have been found to inhibit ICAM-1 expression in macrophages, a property believed to contribute to their anti-inflammatory effects. Tobacco, on the other hand, is associated with increased ICAM-1 levels and smoking cessation leads to a decrease in its levels. As of yet, there is no evidence to support routine screening of ICAM-1, as its clinical relevance is still under investigation.

TNF- α and IL-6

TNF- α is a pleiotropic cytokine expressed by a great variety of cells including macrophages, endothelial cells and smooth muscle cells. Along with IL-1 and IFN- γ , TNF- α stimulates smooth muscle cell expression of IL-6, which is the major inducer of hepatic CRP synthesis. TNF- α is also implicated in increased oxidised LDL in the atherosclerotic plaque. It has been linked to plaque instability because it facilitates degradation of the collagenous matrix by inducing expression

of matrix metalloproteinases (MMPs).^{66,67}

The adverse predictive value of increases in both TNF- α and IL-6 was demonstrated in acute coronary syndromes and amongst healthy individuals. In patients with unstable angina, high serum IL-6 levels are associated with increased in-hospital morbidity and mortality;⁶⁸ while in stable post-MI patients, high levels of TNF- α have been shown to correlate with increased risk of recurrent events.⁶⁹ A sub-analysis of the Physicians' Health Study found an independent risk association between serum IL-6 levels and future coronary events.⁷⁰ In this analysis, even though levels of IL-6 increased in a parallel fashion to levels of CRP, IL-6 remained an independent risk factor even after correction for CRP levels. This finding suggests the existence of additional mechanisms, other than those mediated by CRP synthesis, through which IL-6 exerts its atherothrombotic effects.

IL-6 and TNF- α expression are inhibited by statins and, more specifically, simvastatin reduces monocyte TNF- α expression. The putative mechanism of this inhibition is thought to occur through the ability of statins to activate nuclear factor κ B, a transcription factor involved in the induction of inflammatory cytokines.

CRP

CRP is an acute phase reactant clinically established as a marker of systemic inflammation. It is synthesised by the liver and arterial tissue and released after direct stimulus by IL-6. Although CRP has been traditionally believed to be a byproduct of systemic inflammation, recent evidence suggests that it may in fact have a pathogenic effect in vascular inflammation. CRP induces expression and synthesis of several pro-inflammatory markers such as IL-1, IL-6, ICAM-1, VCAM-1 and TNF- α .⁷¹ CRP has been found within atherosclerotic plaques,⁷² from where it regulates macrophage phagocytosis of LDL molecules.⁷³ It has also been implicated as a complement activator within atherosclerotic plaques.⁷⁴

CRP has been the most widely studied inflammatory marker and the one found to have the most relevance to clinical practice. Initial CRP values are a strong and independent predictor of risk for MI, CVA, PVD and cardiovascular death in healthy individuals.⁷⁵⁻⁷⁹ Furthermore, CRP is a marker of increased risk of death for patients with stable and unstable angina,^{80,82} MI⁸³ and in patients submitted to myocardial revascularisation.^{84,85}

In patients with ACS, CRP >3mg/l indicates greater likelihood of death, MI or revascularisation.⁸⁰ In patients with MI due to vasospasm, CRP tends to remain normal despite ST segment elevation, suggesting that increases in CRP occur before and not after myocardial necrosis.⁸⁶ CRP may be a greater marker of risk in women than in men⁷⁸ and elevated levels of CRP are predictive of higher risk even in those with normal LDL cholesterol.^{87,88} In patients with normal LDL but elevated CRP, treatment with lovastatin resulted in a relative risk reduction similar to that obtained by treating patients with overt hyperlipidaemia.⁸⁹ These data suggest that including CRP in the routine work-up of CVD patients may

offer additional information pertaining to future risk of cardiovascular events. Patients with normal LDL but elevated CRP may benefit from more aggressive therapeutic strategies to modify conventional risk factors, including pharmacological treatment with statins. Routine screening for CRP levels for assessment of cardiovascular risk should be undertaken in selected patients. Furthermore, elevated CRP levels in the absence of overt infection should lead the clinician towards a more aggressive stance in controlling modifiable cardiovascular risk factors, as well as consideration of use of statins to normalise CRP.

Conclusion

Novel cardiovascular risk factors emerge as important stratifiers of cardiovascular risk. Most can be measured as serum biomarkers using diagnostic assays that are relatively simple and routinely available. It is important that selected

groups of patients be submitted to these tests in order to better stratify their cardiovascular risk. Those more likely to benefit from screening for novel risk markers are those with CVD in the absence of conventional risk factors, premature CVD and aggressively progressive disease. In certain patients, the presence of such factors as CRP, fibrinogen, homocysteine and aspirin resistance should lead to consideration of additional hygienic and pharmacological measures.

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