Controversies in cardiovascular medicine

Clinical implications of inflammation for cardiovascular primary prevention

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While many questions remain unanswered in this challenging arena, the emerging status of biomarkers of inflammation provides an excellent illustration of how application of basic and clinical science of inflammation can lead to advances in clinical care. Yet we need to do better. We need to improve the number needed to treat. We should strive for further refinements in risk prediction to individualize interventions. Imaging and genetic biomarkers will likely find their place in clinical practice alongside traditional risk factors and biomarkers of inflammation in the years to come. We must aim to assure that individuals targeted for statin therapy do not lessen their adherence to a healthy lifestyle, believing that they enjoy pharmacological protection from unhealthy behaviours. At the other extreme, we need to counsel carefully certain patients to avoid creating a cohort of ‘cardiac neurotics’ with above median high-sensitivity C-reactive protein readings. We need to devise measures to optimize lifestyle change at both a medical and societal level. For our individual patients, implementation of sustained lifestyle change has proved very challenging in practice, given the multiplicity of behaviours that require vigilance. The clinical use of biomarkers of inflammation may provide the practitioner with a tool to help gauge residual risk, and chart a course for its optimal management.

Keywords JUPITER study • Inflammation • Biomarkers

Inflammation: a fundamental driver of atherothrombosis

A growing body of evidence points to inflammation as a primordial driver of all stages of atherothrombosis. A combination of in vitro and in vivo laboratory experiments and considerable evidence from human studies support this notion.1,2 In the laboratory, pro-inflammatory cytokines activate cellular functions related to atherothrombosis. For example, cytokine stimulation augments the expression of leucocyte adhesion molecules on the endothelial surface that promote the binding of monocytes to their surface. Recent results indicate that under hypercholesterolaemic conditions, a particularly proinflammatory subset of monocytes accumulate in peripheral blood and may undergo selective recruitment to nascent atheroma.3 Chemokines beckon the bound blood cells to enter the arterial intima. Further protein mediators of inflammation such as macrophage–colony stimulating factor (M–CSF) mediate monocyte maturation into macrophages within the arterial wall, and induce the expression of scavenger receptors for modified lipoproteins that permit lipid accumulation and favour foam cell formation.4 Other inflammatory mediators such as fas ligand expressed by activated T cells within atherosclerotic plaques can promote the local apoptosis of macrophages and hasten development of a lipid core and the generation of thrombogenic microparticles. Oxidative stress, manifested by local production of reactive oxygen species such as hypochlorous acid or superoxide anion, often accompany inflammation, as pro-inflammatory cytokines stimulate the production of these pro-oxidants. Cytokines also change the normally anticoagulant and pro-fibrinolytic properties of endothelium to an activated state that fosters thrombus formation and stalls fibrinolysis. Locally acting cytokines can perturb the haemostatic balance in the ‘solid-state’ of the plaque itself. In addition, circulating cytokines, notably interleukin 6, can signal hepatic overproduction of fibrinogen and plasminogen activator. Heightened levels of these proteins in the ‘fluid phase’ of blood can amplify and sustain thrombosis initiated locally in the plaque. The inflammatory mediator CD 40 ligand can induce vascular cells and mononuclear...
phagocytes alike to produce tissue factor, a potent trigger for thrombus formation.\(^5,6\)

Laboratories around the world have constructed atherosclerosis-susceptible mice with genetically engineered disruption of inflammatory pathways whose analysis provides indisputable laboratory evidence for the in vivo significance of inflammation as a regulator of atherosogenesis and aspects of plaques related to clinical complications.\(^7\) Activated inflammatory cells release proteases that degrade the arterial extracellular matrix and favour plaque rupture, a frequent trigger to fatal coronary thrombosis.\(^8\) When inflammation runs amok in experimental atherosclerotic plaques, due to a prevalence of activating factors over dampening by anti-inflammatory pathways, aneurysm formation, plaque disruption, and thrombosis can result. This extensive experimental database has advanced the notion that inflammation incites atherothrombosis.

While such experimental data point to inflammatory triggers responsible for plaque growth, including dyslipidaemia, smoking, hypertension, and hyperglycaemia, the mechanisms responsible for the sudden transition from coronary stability to instability remain largely unknown, in part because of the lack of experimental preparations which simulate human acute coronary syndromes (Figure 1). In particular, we understand poorly why many patients with severe and extensive atherosclerosis remain stable for years without developing acute coronary syndromes, while others develop acute events as the first manifestation of ischaemic heart disease despite less severe coronary atherosclerosis.\(^9,10\) Many studies have established, however, that patients with acute coronary syndromes have higher levels of systemic soluble biomarkers of inflammation than those with stable disease. Clinical studies have also highlighted the changes in peripheral leucocytes in acute coronary syndromes. First, coronary instability associates with a transient and short lasting activation of neutrophils at the site of the culprit stenosis.\(^11,12\) Second, the activation of inflammatory cells involves not only the local culprit stenosis but also extends throughout the coronary circulation\(^13–14\) and may even involve extracoronary arterial beds.\(^15\) Third, a profound perturbation of circulating T-cell population occurs in acute coronary syndrome patients, notably an accentuation of an unusual subset of T cells that express the CD4+CD28 null phenotype.\(^16,17\) These CD4+CD28 null T cells produce the highly proinflammatory cytokine interferon-gamma (IFN-\(\gamma\)). The release of IFN-\(\gamma\) in unstable angina patients may activate monocytes/macrophages in the circulation as well as in lesions. The finding that CD28 null T cells have cytolytic capability implicates further immune reactions in tissue damage.\(^17,18\) Environmental as well as genetic mechanisms could underlie the perturbation of the T cell repertoire. Since the defect in CD28 cell surface expression may result from chronic exposure to antigen, the expansion of CD4+CD28 null T cells may reflect a persistent immune response to microorganisms or autoantigens contained in atherosclerotic plaques.\(^19,20\)

Human biomarker studies and observations on human plaque specimens argue for the clinical relevance of these in vitro and in vivo laboratory experiments that implicate inflammation in atherothrombosis. Biomarkers of inflammation, notably high-sensitivity C-reactive protein, add to traditional risk factors in providing prognostic information in populations that range from apparently well individuals to those with manifest cardiovascular disease.\(^21,22\) Yet there remains a great gap in closing the loop of causality between inflammation and atherogenesis and its complications in humans\(^23–25\) — hence, the keen interest of the cardiovascular community in clinical trials that address this question. The recent Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) did not address the causality of inflammation in atherosclerosis, but did support the use of inflammatory status to guide event-reducing therapy in apparently well people otherwise considered at low cardiovascular risk, a finding with considerable implications for the primary prevention of cardiovascular events.\(^26\)

**The challenge of primary prevention of cardiovascular diseases**

Primary prevention of cardiovascular diseases represents an increasing challenge worldwide. As communicable diseases wane, the epidemics of obesity and consequent dysmetabolism, together with the ageing of the population, have elevated cardiovascular disease to a leading cause of death and loss of useful life years globally. As we now possess interventions that can reduce cardiovascular events even in apparently well individuals, we must not merely wait until individuals declare themselves as having cardiovascular risk by developing symptoms or enduring a cardiovascular event. Indeed, many cases of sudden cardiac death and many acute myocardial infarctions occur without warning. One approach to global risk reduction would be a ‘one size fits all’ deployment of a ‘poly-pill’.\(^26–28\) The proposed exposure of large swaths of individuals to the potential hazards of therapy without assurance of benefit and the logistic and economic burden of indiscriminate targeting of...
pharmaceutical intervention highlight the contemporary challenge of primary prevention of cardiovascular disease.

Although the traditional risk factors have great utility in predicting cardiovascular risk, a majority of patients with coronary artery disease have only one or none of these risk factors. Equally important, many individuals with multiple risk factors may never experience a cardiovascular event and instead succumb to non-cardiac disease. Fortunately, advances in understanding the clinical and basic biology of atherosclerosis and its complications offer the possibility of using biomarkers to sharpen our assessment of cardiovascular risk to tailor interventions in primary prevention.

The role of soluble biomarkers in cardiovascular prevention

Soluble biomarkers measured in plasma have a venerable place in cardiovascular risk prediction and indeed figure prominently among the traditional risk factors, e.g. low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Certain emerging plasma biomarkers have garnered considerable interest as potential contributors to improving cardiovascular risk stratification. Novel putative biomarkers of cardiovascular risk include those that report on oxidative stress, infection, and inflammation (Figure 2). Of these proposed markers of cardiovascular risk, those that reflect inflammation have come to the fore as clinically translatable fruit of basic research in atherosclerosis. The concept that inflammation participates pivotally in all phases of atherosclerosis, from initiation through progression and including clinical complications, has gained wide currency. The clinical application of this burgeoning basic science has led to investigations of biomarkers of inflammation as additions to the traditional, well-validated risk factor panels in cardiovascular event prediction. Large prospective studies have brought this concept to clinical reality. Biomarkers of inflammation have a number of applications among the traditional risk factors, e.g. low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Certain emerging plasma biomarkers have garnered considerable interest as potential contributors to improving cardiovascular risk stratification. Novel putative biomarkers of cardiovascular risk include those that report on oxidative stress, infection, and inflammation (Figure 2). Of these proposed markers of cardiovascular risk, those that reflect inflammation have come to the fore as clinically translatable fruit of basic research in atherosclerosis. The concept that inflammation participates pivotally in all phases of atherosclerosis, from initiation through progression and including clinical complications, has gained wide currency. The clinical application of this burgeoning basic science has led to investigations of biomarkers of inflammation as additions to the traditional, well-validated risk factor panels in cardiovascular event prediction. Large prospective studies have brought this concept to clinical reality. Biomarkers of inflammation have a number of applications...
in the cardiovascular field: prediction of first-ever cardiovascular events, determination of prognosis in those with established disease, providing a target of therapy (much as we follow blood pressure serially), or serving to guide therapy (much as we use LDL cut points currently).

For this purpose, C-reactive protein measured by a highly sensitive assay (high-sensitivity C-reactive protein) that can reliably discriminate levels of C-reactive protein well below the excursions measured in those with acute inflammatory states holds much promise. An ongoing debate surrounds the degree of clinical utility of the information added by inflammatory biomarkers. Much of this discussion revolves around the statistical tools employed, e.g. the C statistic or a reclassification scheme. C-reactive protein reports on inflammation regardless of the instigator (Figure 2). This very ‘non-specificity’ may underlie much of C-reactive protein’s utility in risk prediction; C-reactive protein integrates overall inflammatory status, capturing aspects otherwise difficult to measure directly. For example, assessment of ‘lifestyle’ variables presents a practical challenge in the clinic. C-reactive protein may provide an overall readout that incorporates such otherwise difficult to quantitate elements associated with risk. Indeed, C-reactive protein may reflect low-grade chronic infections in various sites such as bronchitis and periodontitis or genetically determined hyper-reactivity of inflammatory cells, which can exacerbate atherosclerosis. Furthermore, the INTERHEART study implicated risk factors not included in the Framingham Risk Algorithm, appears to hold great appeal, they may remain too expensive and/or entail too high a radiation exposure to prove cost-effective or cost-beneficial for screening of unselected populations of unknown cardiovascular risk. At least for the midterm, cost-effectiveness and risk benefit analyses will probably favour a tiered approach for the deployment of imaging in cardiovascular risk assessment in apparently well individuals (Figure 3).

Family history of premature coronary artery disease, a variable not included in the Framingham Risk Algorithm, appears to confer clinically useful information regarding cardiovascular risk in both men and women. These observations underscore the contribution of genetic complement to cardiovascular risk. The power of modern genetic technology and the achievement of sequencing the human genome have stimulated great interest in harnessing genetic markers to improve cardiovascular risk assessment. Many early genetic studies often had inappropriately chosen control groups, and/or lacked ethnic diversity. Hence, many initial studies of the utility of genetic markers in cardiovascular risk prediction proved unreproducible. In contrast, the application of genome-wide association screen technology (GWAS) has identified reproducible regions of the human genome where variants track with cardiovascular outcomes in large studies. These observations, e.g. the identification of the chromosome 9p21 risk region, hold immense promise for providing novel insight into pathophysiology.48,49 These GWAS discoveries should stimulate functional genomic research to help

Potential roles of other biomarkers in primary prevention: endothelial function, imaging, and genetics

Endothelial-dependent vasodilatation assessed by a variety of techniques correlates with many risk factors, but not with complete fidelity. Imaging approaches, both anatomical and functional, have generated great interest for cardiovascular risk prediction. Carotid intima-media thickness correlates well with cardiovascular outcomes. Emerging evidence from cohort studies affirms that calcium scores derived from electron beam computed tomography may also add information regarding cardiovascular risk to traditional algorithms.44,45 Computed tomographic angiography shows promise for probing coronary anatomy non-invasively. Molecular imaging aims to interrogate functional aspects of atherosclerotic lesions that go beyond mere anatomical features, including aspects of inflammation directly implicated in plaque stability and thrombogenic potential.46 Although imaging approaches hold great appeal, they may remain too expensive and/or entail too high a radiation exposure to prove cost-effective or cost-beneficial for screening of unselected populations of unknown cardiovascular risk. At least for the midterm, cost-effectiveness and risk benefit analyses will probably favour a tiered approach for the deployment of imaging in cardiovascular risk assessment in apparently well individuals (Figure 3).
unravel the underlying pathogenic pathways. To date, however, none of the genetic markers that have emerged from GWAS have yet proved appropriate for routine clinical use. While the future holds considerable promise and may teach a great deal regarding disease mechanism, the conundrum of the genetics of common diseases may frustrate the harnessing of genetic markers to help us with the challenge of primary prevention. A myriad of genes may make small contributions to genetic predispositions to develop a complex, chronic, and non-Mendelian disease such as atherosclerosis.\textsuperscript{50} The expectation that a small number of genetic variants may prove pivotal in routine clinical risk stratification may prove vain.

**The JUPITER trial in the context of primary prevention**

Many studies have addressed interventions to reduce events in primary prevention. The WOSCOPS,\textsuperscript{51} AFCAPS/TexCAPS,\textsuperscript{52} ASCOT,\textsuperscript{53} and CARDS\textsuperscript{54} studies all enrolled individuals without manifest cardiovascular disease. Of note, in WOSCOPS and in AFCAPS/TexCAPS, lipoprotein levels accounted for much of the cardiovascular risk. The mean LDL-C level was 192 mg/dL in WOSCOPS and 156 mg/dL in AFCAPS/TexCAPS, whose participants had mean HDL levels of 36 mg/dL. In ASCOT and CARDS, hypertension and/or diabetes drove much of the risk, while LDL-C levels were lower (mean levels 132 and 117 mg/dL, respectively). Taken together, these findings indicate that statin treatment improves the outcome regardless of the mechanisms responsible for the risk (Figure 4).

With respect to targeting of therapy, a post-hoc analysis of AFCAPS/TexCAPS generated the hypothesis tested by the recent large JUPITER clinical trial. Dr Paul Ridker collaborated with the AFCAPS/TexCAPS investigators to analyse the outcomes in participants stratified into four groups: those with above and below median LDL or above and below median C-reactive protein.\textsuperscript{55} As expected, the two groups with above median LDL responded to therapy in an effective manner. Those with below median LDL and below median C-reactive protein derived no demonstrable benefit from statin treatment over the term of the study. The provocative cell in this analysis showed that those with below median LDL, but above median CRP, did enjoy benefit from statin therapy to the same extent as those in the high LDL groups. This post-hoc analysis suggests that one might pick individuals from a general population without known cardiovascular disease who might benefit from statin therapy based on their inflammatory status in the absence of overt dyslipidaemia.

JUPITER tested this hypothesis prospectively in over 17,000 individuals in a multi-national and multi-ethnic randomized placebo-controlled blinded clinical trial.\textsuperscript{24} The participants included more than 6000 women, a group often under-represented in cardiovascular clinical trials. On the recommendation of the independent academic data/safety monitoring committee, the investigators stopped the study after a mean treatment period of some two years, two years before the anticipated completion, because of overwhelming benefit. The primary endpoints and all of the pre-specified secondary endpoints, including total mortality, showed a statistically significant benefit of the statin intervention (rosuvastatin 20 mg daily) over placebo in a group whose median baseline LDL was 105 mg/dL, a level considered near ideal by most national guidelines. The population selected because of a C-reactive protein >2.0 mg/L had a higher event rate than predicted by LDL levels alone and similar to that observed in other statin trials in primary prevention (Figures 4 and 5).

A pre-specified analysis of JUPITER indicated that risk reduction associated with both LDL reduction, a key traditional risk factor, and lowering of C-reactive protein (a novel risk marker that reflects inflammation).\textsuperscript{56} JUPITER cannot, however, disclose a mechanism of benefit of the statin intervention, as the agent used lowers both LDL and C-reactive protein. The effect of a more selective anti-inflammatory therapy on atherosclerotic risk will require further study.\textsuperscript{57}

The ensemble of statin trials in primary prevention and in patients with stable ischaemic heart disease suggests that the long-term benefits of these agents accrue from effects on the lipoprotein profile. Indeed, in JUPITER, the observed reduction of cardiovascular events in participants randomized to rosvastatin, compared with those receiving placebo, resembled that predicted by the absolute reduction of LDL-C levels (Figure 5). In contrast, patients who have survived an acute coronary syndrome, associated with a heightened inflammatory state, enjoy a protection from recurrent events within the first weeks after instituting intensive statin treatment. This early benefit probably derives partly from an anti-inflammatory effect apart from LDL lowering. Notably, in observational studies, statin treatment associated with lower levels of CD 28 null T cells, and statin treatment in vitro can mute T-cell function.\textsuperscript{58} Accordingly, in the setting of acute coronary syndromes, the improvement of short-to-medium-term outcome correlates better with the reduction of C-reactive protein than with the reduction of LDL levels.\textsuperscript{59} Modulation by statins of haemostasis can also contribute this early benefit in acute coronary syndrome survivors. Statins may confer protection from thrombotic complications as they activate a transcription factor known as Kruppel-like factor 2 (KLF2).
that modulates thrombomodulin, an endogenous anti-coagulant associated with the endothelial cell surface.60 In this regard, rosuvastatin significantly reduced the occurrence of symptomatic venous thromboembolism, a pre-specified endpoint in JUPITER.61 As cholesterol probably does not cause venous thromboembolism, this reduction likely involved the statin’s antithrombotic action at the level of the vessel wall. Thus, the mechanism of statin benefit appears to vary depending on the clinical state of the population studied.

The example of C-reactive protein indicates how a biomarker of inflammation may help identify individuals without known cardiovascular disease who might benefit from therapy. Of course, in all primary prevention programmes, lifestyle modification including diet, physical activity, maintaining an ideal weight, and smoking cessation should receive the highest priority. The results of JUPITER, however, indicate that statin therapy may prove useful when these measures do not lower C-reactive protein <2.0 mg/L in individuals deemed by traditional risk instruments not otherwise to have substantial cardiovascular risk.

Lessons from JUPITER for risk stratification

We are at an exciting juncture in the primary prevention of cardiovascular diseases. Demographic trends and globalization lend immediacy to the challenge of primary prevention of cardiovascular disease. The tried and true traditional risk factors (Figures 2 and 3) rightly form the foundation for cardiovascular risk assessment. The accumulating evidence supporting the use of biomarkers of inflammation in cardiovascular risk prediction may permit us to improve upon the success of traditional risk algorithms and target individuals for intensive risk reduction in a more clinically effective manner. The application of emerging markers of risk will require large-scale clinical validation. Application of JUPITER suggests that statin treatment can prevent many events in individuals not viewed as eligible for statin therapy by traditional guidelines. Extrapolating JUPITER eligibility criteria using NHANES data estimate that 6.5 million adults ineligible for statin therapy according to current guidelines could nonetheless benefit from this intervention. This strategy could prevent 260,000 events over 5 years in the US alone. The number needed to treat to prevent one event in 5 years in JUPITER is 25, a level that compares favourably with therapies well accepted in primary prevention practice such as treatment of hypertensives with diuretics (see ref. 62).

Anti-inflammatory treatment of atherosclerosis: perspectives

The putative direct anti-inflammatory benefits of statins and the recognition that inflammation contributes to cardiovascular risk independent of LDL levels raises the possibility that other anti-inflammatory treatments might reduce cardiovascular events. Many of our currently available anti-inflammatory strategies have drawbacks in this regard. Non-steroidal anti-inflammatory agents (NSAIDS) effectively combat inflammation, but if anything, seem to augment atherosclerotic complications. This apparent paradox may result from a pro-thrombotic effect due to interference...
with prostacyclin synthesis, particularly with the COX-2 selective agents. Moreover, NSAIDS tend to cause slight elevations in blood pressure that over time can adversely affect cardiovascular outcomes. Glucocorticoids also exert strong anti-inflammatory effects, yet may accelerate atherosclerosis rather than abate its consequences. This class of agents causes many metabolic perturbations that may mitigate their anti-inflammatory actions. For example, glucocorticoids cause dyslipidaemia, hypertension, and insulin insensitivity, each an important risk factor for atherosclerotic events.

Experimental work has implicated a burgeoning number of cytokines and chemokines in atherogenesis. These inflammatory signaling pathways tend to have great redundancy, such that neutralization of any one of these effectors may not suffice to halt the inflammatory process in the plaque. Moreover, some of the specific anti-cytokine agents, such as those that target TNF or IL-6, have adverse effects on the lipid profile that may limit their utility as therapeutics in a chronic disease such as atherosclerosis. Pharmacological inhibitors of certain other effectors of inflammatory pathways, such as the matrix metalloproteinases, have also exhibited undesired actions that render them unsuitable for prevention of atherosclerotic events. A recently proposed clinical trial would test whether low-dose metotrexate, and effective C-reactive protein lowering, anti-inflammatory intervention, can reduce event rates in patients with established coronary artery disease over and above standard therapies, including statins. The continued search for selective anti-inflammatory agents that complement existing agents such as statins remains a high priority for future research in this arena.

Is the glass half empty?

Although the emerging status of biomarkers of inflammation provides an excellent illustration of how application of basic and clinical science of inflammation can advance clinical care, we still need to do better. We must improve the number needed to treat. We should strive for further refinements in risk prediction to individualize interventions. Imaging and genetic biomarkers will likely find their place in clinical practice alongside traditional risk factors and biomarkers of inflammation in the years to come.

We must hasten to assure that individuals targeted for statin therapy do not lessen their adherence to a healthy lifestyle, believing that they enjoy pharmacological protection from unhealthy behaviours. At the other extreme, we need carefully counsel certain patients to avoid creating a cohort of ‘cardiac neurotics’ with above median high-sensitivity C-reactive protein readings. We need to understand more about the mechanisms and clinical significance of unwanted actions of statins, including dysglycaemia. We must remain mindful of the challenge of asking apparently well individuals to adhere to therapy with multiple preventive therapies.

We need to devise measures to optimize lifestyle change at both a medical and societal level. For our individual patients, implementation of sustained lifestyle change has proved very challenging in practice, given the multiplicity of behaviours that require vigilance. The clinical use of biomarkers of inflammation may provide the practitioner with a tool to help gauge residual risk, and chart a course for its optimal management.

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References