Disease Markers 26 (2009) 217–225
DOI 10.3233/DMA-2009-0646
IOS Press

Markers of inflammation and risk of coronary heart disease

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Abstract. Cardiovascular disease is the leading cause of global mortality, with coronary heart disease (CHD) its major manifestation. Although inflammation, the body's response to noxious stimuli, is implicated in several stages of CHD development, the relevance of circulating levels of markers of inflammation to CHD risk remains uncertain. This review summarizes available epidemiological evidence for four emerging inflammatory markers implicated in CHD (fibrinogen, C-reactive protein, lipoprotein-associated phospholipase A_2 and interleukin-6); considers their likely utility in cardiovascular risk prediction; and outlines areas of outstanding uncertainty.

Keywords: inflammation, coronary, cardiovascular, prediction

1. Introduction

Cardiovascular disease is the leading cause of global mortality, accounting for almost one in every two adult deaths worldwide, with coronary heart disease (CHD) its major manifestation [1]. CHD results from atherosclerotic narrowing of the coronary arteries and the formation of an occlusive thrombus after plaque rupture [2]. The tendency for CHD to cluster in families (coefficient of familial clustering [λs] estimated to be between 2 and 7) suggests that genetic variation importantly influences CHD risk [3]. On the other hand, studies of migrant populations indicate that CHD risk increases following movement from low-risk to high-risk regions (eg, Japanese in the USA) [4], suggesting that lifestyle and environmental factors also contribute importantly [5].

Inflammation, the body's response to noxious stimuli, is implicated in several stages of CHD development, including atherosclerosis, plaque destabilization, plaque rupture and post-ischaemia damage to the myocardium [6,7]. Much uncertainty remains, however, about whether circulating levels of markers of inflammation are related to CHD risk. In particular, it remains unclear whether such markers are (i) causal in disease risk; (ii) correlates of conventional cardiovascular risk factors; (iii) markers of subclinical or prevalent disease; or some combination of these possibilities.

This review summarizes available epidemiological evidence for four emerging inflammatory markers implicated in CHD development; considers their likely utility in cardiovascular risk prediction; and outlines areas of outstanding uncertainty.

2. Fibrinogen

First isolated from horse plasma in 1876, fibrinogen is the most abundant clotting protein in circulation. A very large (340 KDa) glycoprotein synthesized in the liver, fibrinogen can bind to GpIIB/IIIa surface proteins creating bridges between platelets and is the precursor to fibrin [8]. In addition to being involved in the coagulation cascade, fibrinogen is thought to stimulate smooth-muscle-cell migration, promote platelet aggregation and increase blood viscosity [8,9]. It has been suggested that fibrin may bind to lipoproteins in

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ISSN 0278-0240/09/$17.00

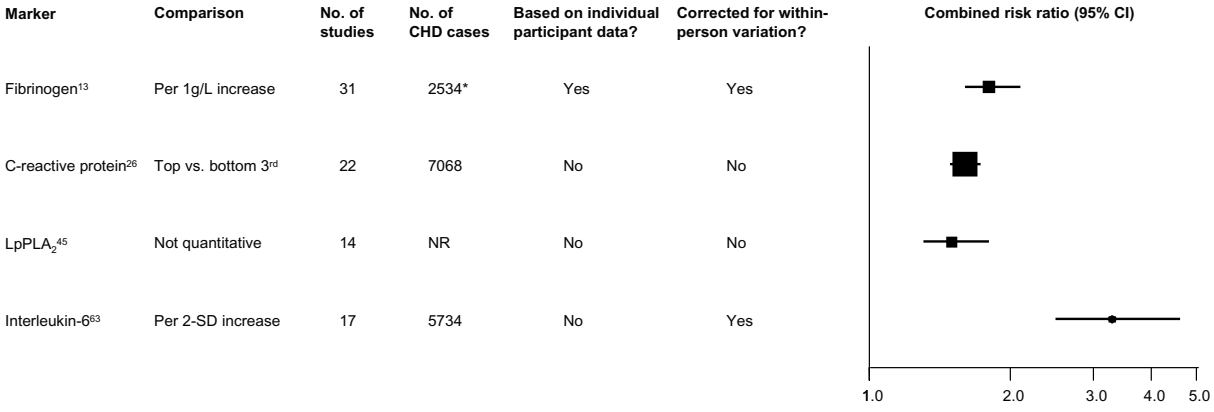
the vascular wall, enhancing lipid accumulation in fibrous plaques and leading to plaque growth [8,9]. Fibrinogen is thought to be a "downstream" marker of the inflammatory process governed by more proximal mediators (such as interleukin-6), reflecting observed spikes in circulating levels of fibrinogen during periods of inflammatory stress [8,9].

Several prospective epidemiological studies have assessed the association of circulating fibrinogen concentration with CHD risk, but have yielded apparently conflicting results. In the absence of individual studies of very large size, appropriate synthesis of the available data by meta-analysis should provide a better indication of the relevance of risk markers to CHD than can individual studies typically involving just a few hundred cases. This is because meta-analyses are less likely to be subject to random error than single studies, which due to their inherent statistical uncertainties may produce false-positive and false-negative results [10]. The Fibrinogen Studies Collaboration (FSC) is an individual participant meta-analysis of data from 154,211 participants in 31 prospective studies, including 6944 first-ever non-fatal myocardial infarctions or stroke events and 13,210 deaths recorded during 1.38 million person-years of follow-up (Table 1) [11]. This collaborative re-analysis of available prospective evidence on fibrinogen and cardiovascular disease risk demonstrated that about 7% of the variation in fibrinogen levels is explained by conventional vascular risk factors (notably, positive associations with smoking and body mass index and an inverse association with HDL-C) and a further 10% is explained by other inflammatory markers [12]. There were approximately log-linear relationships of circulating fibrinogen levels with risk of several vascular and non-vascular diseases including CHD, stroke, other vascular mortality and cancer mortality, with no evidence of a threshold within the range of usual fibrinogen level at any age [13]. The risk ratio for CHD per 1 g/L increase in long-term 'average' fibrinogen concentration was 1.8 (1.6–2.1) after adjustment for several conventional vascular risk factors and correction for measurement error (Fig. 1), and did not vary materially according to sex, smoking status, level of blood pressure or blood lipids, or laboratory and study characteristics [13].

Observational studies are limited in their ability to help judge causality, particularly as they are susceptible to bias by reverse association and by confounding [14] (although such distortion of associations can be minimised, but not eliminated, by prospectively studying initially disease-free individuals and by appropriately adjusting risk estimates for potential known confounders). Comparison of disease rates in groups of individuals between whom the only difference is the exposure of interest, with random distribution of all other factors across the groups, should be free of such residual confounding and provide a more reliable assessment of the causal relevance of the exposure. Several interventions that lower lipid levels also influence levels of inflammatory markers. Of these, the two most studied in relation to both their impact on circulating inflammatory markers and rates of CHD are statin and fibrate medications. Since both these medications are associated with substantial changes in several markers (particularly lipids), they cannot specifically assess the causal relevance of any inflammatory marker to CHD [15]. In the absence of large-scale randomised controlled trials of suitable interventions that show specific and important changes in inflammatory markers, the study of genetic variants can provide an alternative approach to assess the causal relevance of such markers to disease risk [16]. Since the presence of particular genetic variants is effectively allocated randomly at conception, this should render associations of such variants with levels of risk markers or with coronary disease risk unaffected by subsequent development of disease (i.e. avoidance of "reverse association" bias) and, by analogy with randomized controlled trials, minimize the influence of potential confounders [16]. Identification of genetic variants that are associated with important and specific changes in circulating levels of inflammatory markers would, therefore, provide an opportunity to conduct such "Mendelian randomisation" experiments to assess the causal relevance of inflammatory markers to CHD. Several genetic determinants of fibrinogen have been identified [17], including a single nucleotide polymorphism at position −148 in the beta-fibrinogen gene promoter (beta −148C/T). A meta-analysis of 20 studies of beta-fibrinogen genotypes involving a total of 12,220 coronary disease cases and 18,716 controls was reported in 2006 [18]. This investigation demonstrated that for each T allele inherited, carriers of beta −148C/T had 0.14 g/l higher mean fibrinogen concentration, with little evidence of any important change in levels of several conventional vascular risk factors. Using data from the FSC, a 0.14 g/l higher usual plasma fibrinogen concentration would be expected to be associated with a risk ratio for MI of 1.17 (95% CI 1.14–1.19). The observed combined odds ratio for MI per T allele of the beta -148C/T polymorphism, however, was 1.00 (95% CI 0.95–1.04) [18]. The finding that genotypes that produce (presumably) lifelong

Table 1
Large-scale individual participant data meta-analyses of inflammatory markers and coronary heart disease

Collaboration	Focus	Total no. of studies	Total no. of participants	Anticipated reporting of empirical findings
Fibrinogen Studies Collaboration (FSC) [11–13]	Aetiological associations and predictive value of fibrinogen in prospective studies	31	154k	2005–2010
Emerging Risk Factors Collaboration (ERFC) [27]	Aetiological associations and predictive value of CRP and other inflammatory markers in prospective studies	104	1.1M	2009–2010
$LpPLA_2$ Studies Collaboration (LSC) [46]	Aetiological associations of $LpPLA_2$ in prospective studies	32	150k	2009–2010
CRP CHD Genetics Collaboration (CCGC) [32]	Mendelian randomisation assessment of CRP	35	150k	2010–2011



*2534 out of a total of 7118 CHD cases had complete information on all potential confounders adjusted for
NR Number of CHD cases were not reported separately from cases of any cardiovascular disease

Fig. 1. Available meta-analyses of prospective studies of circulating concentration of inflammatory markers and risk of coronary heart disease.

and specific differences in fibrinogen concentration are not materially associated with CHD risk, together with the non-specific associations of fibrinogen levels with risk of several chronic vascular and non-vascular diseases [13], suggest that fibrinogen is unlikely to be causal in coronary disease.

Although many published prospective studies have commented on the potential value of particular markers in risk prediction, they have often reported on measures of strength of association only (e.g., odds ratios, hazard ratios), which do not directly address the accuracy of a marker in risk prediction or stratification. Instead, such accuracy is commonly assessed by two independent criteria, discrimination and calibration. Discrimination is the ability to separate individuals at higher risk from those at lower risk, while calibration is the ability to correctly estimate the risk or probability of a future event [19]. Each of these approaches may impart somewhat different information. To date, prospective studies that have used such methods to assess the potential improvement in vascular risk prediction upon measurement of fibrinogen levels in addition to conventional vascular risk factors have yielded conflicting results [20–23]. Relevant investigations in the FSC should help provide a more robust assessment about whether measurement of circulating fibrinogen concentration can help better identify individuals at increased risk of CHD than measurement of conventional risk factors alone.

3. C-reactive protein (CRP)

CRP, a nonglycosylated 224-residue plasma protein, is probably the most studied circulating marker of inflammation. Produced by hepatocytes, CRP synthesis is closely regulated by upstream pro-inflammatory cytokines (such as interleukin-6) and resultantly massive spikes in circulating CRP levels are observed in response to inflammatory stimuli [24]. Whereas older less sensitive assays were only able to identify such acute phase responses of CRP (during which levels of CRP can rise up to several-thousand fold), more recent "high sensitivity" immunoassay methods have en-

abled measurement of circulating "baseline" levels and assessment of chronic low-grade inflammation [24].

The first population-based prospective study of circulating CRP concentration and incident CHD risk, reported in 1996, was a nested case-control comparison of 246 CHD cases and 491 controls within The Multiple Risk Factor Intervention Trial [25]. Since this study, more than 40 such studies of CRP and CHD risk have been reported. A literature-based meta-analysis of 22 prospective epidemiological studies published by 2004, involving a total of 7068 incident CHD cases, reported a combined risk ratio for CHD of 1.6 (1.5–1.7) in a comparison of individuals in the top third with those in the bottom third of the baseline distribution of CRP concentration in the population [26] (Fig. 1). It remains unclear, however, whether associations of CRP levels with incident CHD risk are "independent" from conventional vascular risk factors and other inflammatory markers, specific to CHD (or vascular disease), or importantly modified under different circumstances (such as by age, sex, smoking status or different levels of conventional vascular risk factors). The Emerging Risk Factors Collaboration (ERFC) is an individual participant meta-analysis of data from 110 prospective cohorts which builds on the FSC [27]. It has established a central database in which individual data records from over 1.1 million participants have been harmonised to a consistent format, including from large subsets of participants with information on inflammatory (including CRP) and other emerging risk markers, lipids and other conventional risk factors and characteristics, as well as major cardiovascular morbidity and cause-specific mortality (Table 1) [27]. Information on repeat measurements on relevant characteristics has been collected in approximately 320,000 participants to enable estimation of and adjustment for within-person variability in measured values. This collaborative initiative should enable more precise and detailed characterisation than has previously been possible of the shape and strength of the age- and sex-specific associations of circulating CRP levels with incident CHD outcomes under a wide range of circumstances [27].

Several genetic variants in the CRP gene that control CRP concentration have been identified [28]. A meta-analysis of studies of the +1444C>T polymorphism in the CRP gene assessed the association of this variant with circulating CRP concentration and CHD in a total of 18,637 participants, including 4610 CHD cases [29]. This meta-analysis reported that the combined geometric mean CRP was 1.14 (1.11–1.18) higher per each T allele inherited, with negligible difference in levels of several conventional vascular risk factors [29]. The association of the same variant with CHD risk was 0.96 (0.90–1.03) per T allele [29]. A separate study assessed associations of 4 different variants in the CRP gene (1081G>A, 223C>T, −390C>T>A, 3678T>G) in a total of over 50,000 participants (including 6545 with CHD) [30]. A combination of these 4 variants was associated with a 64% increase in circulating CRP concentration and, based on the observed association of CRP levels with CHD risk, was predicted to be associated with a 32% increase in CHD risk [30]. The same genetic combination, however, was not significantly associated with CHD [30]. These findings from studies of CRP genotypes do not support a causal association of CRP in CHD. However, as any associations of common genetic variants with disease risk are likely to be modest, genetic studies require information from upward of 15,000 patients with coronary disease to reliably evaluate the likelihood and magnitude of any causal association between CRP and CHD risk [31]. The CRP CHD Genetics Collaboration (CCGC) has established a central database containing individual data on CRP polymorphisms, circulating CRP levels and major coronary outcomes as well as several other relevant characteristics [32] (Table 1). This collaboration comprises a total of over 37,000 CHD outcomes and over 120,000 controls in 35 studies and should help clarify whether CRP is involved in the pathogenesis of CHD [32]. Study of interventions that specifically modify circulating CRP levels may help directly assess whether CRP is likely to be causal in CHD. Although such interventions are in development, these interventions currently focus on whether anti-CRP agents may have a role in minimising tissue damage subsequent to an MI (rather than help prevent development of CHD) [33].

Several prospective studies have reported on the potential utility of CRP measurements for CHD risk prediction. A recent review assessed the predictive performance of CRP in two prospective cohort studies (involving a total of 309 CHD outcomes) and reported a systematic review of relevant data from 31 published prospective studies (involving a total of 11,252 CHD outcomes) [34]. This review concluded that although raised circulating CRP levels are consistently associated with increased CHD risk, measurement of CRP levels provides little improvement in CHD risk prediction when assessed using several metrics of predictive value [34]. Despite such reservations stemming from findings from observational studies, there has been substantial interest in whether measurement of CRP levels should be used for risk stratification and prioritiza-

tion of preventative treatment in individuals who, according to current clinical guidelines, would otherwise be ineligible for such treatment [35]. The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvasatin (JUPITER) trial randomly assigned 17,802 men and women with LDL-C levels $<$ 3.4 mmol/L (ie, below thresholds for treatment according to current clinical guidelines) and high-sensitivity CRP levels $\geqslant$ 2.0 mmol/L to receive 20 mg rosuvastatin or placebo [36]. The trial was stopped after a median follow-up of 1.9 years, with an observed reduction in LDL-C and CRP of 50% and 37%, respectively, in people receiving rosuvastatin. The hazard ratio for MI in this trial was 0.46 (0.30–0.70) in people receiving rosuvastatin compared to people receiving placebo [36]. A separate report from the JUPITER trial reported that compared to participants receiving placebo, the hazard ratio for a combination of any of non-fatal MI, non-fatal stroke, unstable angina, revascularization or cardiovascular death in participants allocated to receive rosuvastatin was: 0.45 (0.34–0.60) in people who achieved LDL-C $<$ 1.8 mmol/L; 0.38 (0.26–0.56) in people who achieved CRP $<$ 2 mg/L; and 0.35 (0.23–0.54) in people who achieved LDL-C $<$ 1.8 mmol/L and CRP $<$ 2 mg/L [37]. As acknowledged in this report, however, such assessments are no longer randomized and, therefore, potentially susceptible to distortion by confounding [37]. Assessment of the relevance of CRP levels in other long-term trials of statin medications are ongoing [38].

4. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2)

Originally named platelet-activating factor acetylhydrolase after its ability to catalyse the hydrolysis of platelet-activating factor *in vitro*, Lp-PLA_2 appears to link arterial retention and oxidative modification of LDL in the coronary artery wall with localised inflammation and subsequent plaque destabilization [39]. Expressed by hematopoietic cells, around 70–80% of Lp-PLA_2 is carried on LDL [40]. By virtue of this association, Lp-PLA_2 is carried into the arterial wall where it can hydrolyze the *sn-2* fatty acids in the phospholipids of LDL as they become truncated by oxidation [41]. For example, Lp-PLA_2 mediates the oxidative modification of phosphatidylcholine (a common phospholipid in LDL) and its subsequent hydrolysis to lysophosphatidylcholine and oxidized free fatty acids. These products elicit several potentially proinflammatory and proatherogenic effects [42]. In turn, inflammatory cells attracted to the arterial wall by the products of Lp-PLA_2 activity express further Lp-PLA_2, potentially creating a positive feedback loop [43]. On the other hand, Lp-PLA_2 has also been proposed to play a protective role against atherosclerosis, based on the observation that its substrates (rather than the products it generates) can show proinflammatory and proatherogenic activities [44].

Despite ongoing debate over the role of Lp-PLA_2 in atherosclerosis, several prospective epidemiological studies have reported generally positive associations between circulating levels of Lp-PLA_2 and subsequent risk of cardiovascular disease. A literature-based meta-analysis of 14 observational studies reported a relative risk of 1.5 (1.3–1.8) for Lp-PLA_2 and cardiovascular disease risk [45] (Fig. 1). The validity of that meta-analysis was limited, however, because it combined information from prospective and retrospective studies (increasing the potential for selection and reverse association biases); considered heterogeneous populations, disease outcomes and Lp-PLA_2 exposures (potentially conflating any divergent associations); and did not standardize reported risk estimates to a consistent comparison [45]. The relevance of Lp-PLA_2 to CHD risk remains uncertain. In particular, the magnitude and shape of any dose-response relationships, the extent of any coronary or vascular specificity, and the degree of independence from conventional cardiovascular risk factors (particularly the lipoproteins on which Lp-PLA_2 is carried) have yet to be characterized in detail. The Lp-PLA_2 Studies Collaboration (LSC) is a consortium of investigators of prospective studies of Lp-PLA_2 and cardiovascular disease [46] (Table 1). The LSC will include data from 32 prospective studies involving a total of approximately 15 000 patients with major cardiovascular disease outcomes and should help to determine more reliably than previously possible the strength and shape of any independent association, the magnitude of associations in different circumstances, and sources of heterogeneity between studies [46].

Family studies have suggested that around half of the variation in Lp-PLA_2 activity may be heritable [47], and several common variants in the Lp-PLA_2 gene (*PLA2G7* on chromosome 6) have now been identified. One such variant, V279F is found almost exclusively in East Asian populations and results in Lp-PLA_2 activity being significantly reduced in heterozygotes and almost undetectable in individuals homozygous for the T allele [48]. In contrast to V279F which has only been reported in East Asian populations, oth-

er common *PLA2G7* variants have been found in all populations [49] and Mendelian randomization experiments (such as those described above) involving study of these variants may provide a framework to help judge whether Lp-PLA_2 is causal in CHD.

Because circulating Lp-PLA_2 levels have generally been positively associated with cardiovascular diseases in published prospective studies, a consensus panel recently recommended the incorporation of Lp-PLA_2 mass measurement into risk assessment guidelines for individuals at 'intermediate' risk of CHD [50]. As noted above, however, assessments of the magnitude and independence of associations with disease outcomes do not directly address the potential utility of a risk marker in classifying or predicting disease risk. The few previous studies to have directly assessed the predictive ability of Lp-PLA_2 in vascular disease have reported generally modest improvements in risk prediction on addition of Lp-PLA_2 to conventional risk factors [51–55]. Interpretation of these findings has been complicated, however, by the fact that these relatively few studies have used different population settings and endpoints; have added Lp-PLA_2 to risk prediction models that include different sets of conventional cardiovascular risk factors; have typically involved fewer than 10 years of follow-up; and have generally used the area under the receiver-operator characteristic curve (AUROC) to assess discrimination, a technique that is only appropriate for binary data and does not consider time to event or allow for censoring. Furthermore, these previous studies did not evaluate the ability of Lp-PLA_2 measurements to reclassify predicted CHD risk; analyses that may be more clinically informative than discrimination *per se*. Future prospective studies with long-term follow-up in large numbers of initially healthy participants are therefore needed to help clarify the value of Lp-PLA_2 measurements to cardiovascular risk prediction.

A number of studies have shown that medications that lower lipid levels and reduce CHD risk also lower Lp-PLA_2. For example, statins lower Lp-PLA_2 activity by around 20–40% [56]. However, because statins do not limit secretion of Lp-PLA_2 from macrophages, the observed reductions in Lp-PLA_2 activity are thought to result from drug-induced enhanced clearance of LDL [57]. By contrast, darapladib (SB480848; GlaxoSmithKline), a reversible substrate-competitive pyrimidone, reduces Lp-PLA_2 activity by around two thirds (with more modest effects on Lp-PLA_2 mass) [58]. In late 2008 the STABILITY (Stabilisation of Atherosclerotic plaque By Initiation of darapLadib TherapY) trial was initiated [59]. This randomised, placebo-controlled trial will assess the impact of long-term treatment with darapladib compared to placebo on the composite endpoint of major cardiovascular events (including cardiovascular death, nonfatal MI and nonfatal stroke) in over 15,000 CHD patients receiving standard care [59], and help elucidate the importance of Lp-PLA_2 inhibition therapy to the secondary prevention of CHD.

5. Interleukin-6 (IL-6)

IL-6, a pleiotropic 184-amino acid monomer, is secreted by T cells, macrophages and endothelial cells and propogates inflammatory cascades in response to inflammatory stimuli [60,61]. IL-6 can either accelerate or inhibit the inflammatory process. Part of the role of IL-6 in the acute phase response is to upregulate several downstream markers, including CRP [60, 61]. IL-6 levels are also elevated in response to muscle contraction, produced by smooth muscle cells in blood vessels and may have a role in lipid catabolism and insulin resistance [60,61]. Excess circulating IL-6 concentration has been linked to several autoimmune disorders, especially rheumatoid arthiritis [62].

Although less studied than its down-stream acute phase reactants such as fibrinogen and CRP, several prospective studies have reported associations of circulating IL-6 concentration with CHD risk. A literature-based meta-analysis of 17 prospective epidemiological studies published by 2008, involving a total of 5730 CHD cases recorded during follow-up, reported a combined risk ratio for CHD of 1.6 (1.4–1.8) per 2-SD increase in baseline IL-6 measurements [63]. However, owing to the short half-life and substantial within-individual fluctuations in circulating IL-6 levels, failure to make allowance for such within-individual variation may substantially underestimate the magnitude of any association of IL-6 concentration with CHD risk. After correction for within-individual variability of IL-6, the combined risk ratio for CHD in this meta-analysis was 3.3 (2.5–4.6) per 2-SD increase in IL-6 levels [63] (Fig. 1); potentially comparable to the strength of observed associations of some conventional vascular risk factors with CHD risk. Further studies of IL-6 concentration and CHD risk are warranted, therefore, to confirm such associations and to assess whether measurement of IL-6 levels can usefully contribute to CHD risk prediction algorithms. Studies of genetic variants in the IL-6 gene have predominantly focused on the $-174G>C$ promoter variant, but this variant does not

appear to be materially associated with circulating IL-6 concentration [64,65] (so is unlikely to provide any causal inference about the relevance of IL-6 levels to CHD). Several IL-6 receptor antagonists are under development and are being trialed for the treatment of rheumatoid arthritis [62,66]. Such trials may help indicate whether anti-IL-6 therapy may also be a potential therapeutic target for CHD prevention.

6. Other markers of inflammation

Several other markers of inflammation have also been assessed in relation to CHD, including albumin [67], leukocyte count [68] (both of which are being assessed in the ERFC), CD40 ligand [69], tumour necrosis factor alpha [70], matrix metalloproteinases [71], IL-1 [72] and IL-18 [73]. Ongoing studies of these and other related markers of inflammation should help progress understanding of the potential relevance of such markers to CHD.

7. Summary

Although it is generally accepted that inflammation is importantly involved in the development of CHD, it remains unclear whether circulating markers of inflammation are causal or whether their measurement can help improve CHD risk prediction. Ongoing large-scale epidemiological and genetic studies should help to resolve such outstanding uncertainties.

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