Novel Risk Factors for Atherosclerosis

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Abstract: Epidemiologic studies demonstrated that the classical cardiovascular risk factors explain only a part of the increased cardiovascular morbidity and mortality. Large scale studies have shown that novel cardiovascular risk factors, including increased plasma homocysteine, fibrinogen, C-reactive protein, uric acid levels, and increased white blood cells count as well as low adiponectin levels, might have a key role in the pathogenesis of the cardiovascular disease. This review examines recent literature data on the effect of novel risk factors on cardiovascular morbidity and mortality in healthy subjects as well as in subjects at high cardiovascular risk. In addition, the pathogenetic mechanisms linking the effects of the novel risk factors with atherosclerosis are discussed.

Keywords: Cardiovascular disease, risk factors, novel, white blood cells, fibrinogen, uric acid, homocysteine, adiponectin, C-reactive protein.

INTRODUCTION

Despite of the great contribution of the established risk factors to the cardiovascular disease (CVD), they fail to predict all cardiovascular events [1, 2]. A recent analysis of more than 120,000 patients with coronary artery disease (CAD), showed that about 20% of the patients had no evidence of hyperlipidemia, hypertension, diabetes, or smoking, and more than 50% had only one of the above risk factors [1]. Furthermore, another large analysis, with a follow-up for 30 years, showed that 85% to 95% of the subjects with CAD had at least one established risk factor, but so did the subjects without CAD [2].

The above observations have focused the clinical and experimental research over the past decade to the identification and evaluation of novel risk factors, including the number of white blood cell count (WBC), plasma concentrations of fibrinogen, uric acid (UA), homocysteine (Hcy), adiponectin and C-reactive protein (hsCRP). Therefore, the aim of the present review is to provide literature data on the potential relationship between the novel cardiovascular risk factor and CVD and the results of the various studies are summarized in Table **1**. In addition, the pathogenetic mechanisms linking the effects of the novel risk factors with atherosclerosis are discussed.

WHITE BLOOD CELL COUNT

The WBC count has been associated with CVD since the 1920s [3]. The first study to report a clear relationship between WBC count and cardiovascular morbidity was published in 1974 [4]. This study showed that the WBC count was a strong predictor of myocardial infarction (MI) similar to total serum cholesterol and blood pressure [4]. Later studies reported that subjects with increased WBC count (>9,000 vs. <6,000 mm^3 and >10,000 vs. <4,000 mm^3) had excess risk of MI [5, 6] and thrombotic strokes [7] independently of gender, smoking habits, blood pressure and cholesterol levels. The multiple risk factor intervention trial (MRFIT) demonstrated also a strong relationship between total WBC count and the risk of CVD [8].

A subanalysis in a cohort of dyslipidemic men from the Helsinki Heart Study of coronary atherosclerosis primary prevention, found that WBC count at baseline was higher in subjects who suffered from acute coronary syndromes than in controls [9]. The above relationship was more profound in smokers with elevated WBC count [9]. In the Hiroshima and Nagasaki Adult Health Study, the WBC count correlated positively with the incidence of CVD in a large population of individuals free of disease at baseline [7]. Recently, a large, retrospective 5-year study showed that high WBC count at baseline correlated with the development of acute coronary syndromes, especially in participants who were free of CVD at baseline (9,209 vs. 6,205/ mm^3) [10].

Other studies have shown that the relationship between WBC count and CVD exists not only in subjects free of CVD but also in patients with overt CVD even after adjustment for other cardiovascular risk factors. The PARIS-1 study showed that the baseline WBC count was strongly associated with coronary event recurrence and with total mortality 2 to 60 months after MI, even after adjustment for other variables including smoking [11]. Furthermore, it was found that the number of WBC count on admission in patients with acute MI was an independent predictor of early ventricular fibrillation [12]. In a study of patients who have had acute MI within the previous six months, a high WBC count (52,000 vs. 10,600/mm^3) was associated with increased risk of re-infarction or death [13]. In the TACTIS-TIMI-18 trial, which included patients with acute coronary syndromes, elevated WBC count at baseline was associated with poorer prognosis, more severe CAD and increased mortality at 6 months [14]. The TIMI-10A and -10B trials found that a relatively high WBC count was associated not only

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with new-onset congestive heart failure but also with higher mortality [15].

The role of total WBC count and WBC subtypes has also been evaluated in a high-risk population in the CAPRIE trial [16]. This study showed that an increased neutrophil count was strongly contributed to the increased cardiovascular risk, while the impact of monocytes was smaller [16]. In another large study of patients with or at high risk for CVD, the neutrophil/lymphocyte ratio was the strongest predictor of death or MI [17]. In agreement with the previous findings are the results of a recently published meta-analysis from seven long-term prospective studies in 30,374 subjects, which showed that the neutrophil count was the strongest predictor of CVD [18].

In addition, other studies reported relationships between CVD and different WBC subtypes. The Hiroshima and Nagasaki Adult Health Study showed a positive association between moderately elevated eosinophil count and CVD [7]. The Paris Prospective Study II [19] showed that the risk of CVD increased by 1.15 times for an increase of 100 cells/mm^3 in monocyte count. In two studies from the United Kingdom [20], a positive association was found between neutrophil and eosinophil count and the incidence of CAD. One retrospective study in patients with CVD [21] showed that the 5-year survival was significantly better for patients who had normal in comparison with those who had low lymphocyte count.

Several possible pathogenetic mechanisms have been proposed in order to explain the relationship between elevated WBC and CVD. WBC may influence the development of CVD through their ability to induce proteolytic and oxidative damage to coronary arteries [22], to promote the release of inflammatory mediators [23] and to affect blood flow through the cardiac microvasculature, because WBC are larger and have stiffer membrane than the red blood cells and the platelets [24]. Another mechanism that WBC may influence the development of CVD is by inducing a hypercoagulation state in response to acute MI, since they correlate positively with plasma fibrinogen and factors VII and VIII levels [25]. In addition, higher WBC count after acute MI has been associated with poorer myocardial reperfusion, thromboresistance, and greater thrombus burden [26]. Finally, a study showed that a high WBC count predicted ventricular fibrillation in patients with acute MI by affecting the electrical activity of the heart [27].

FIBRINOGEN

Fibrinogen is both a coagulation factor and an acute phase reactant and thus increased plasma fibrinogen levels may also be a marker of the inflammation associated with the atherosclerotic process [28]. The ECAT Angina Pectoris Study showed that higher plasma fibrinogen levels (> 300 mg/dL) resulted in hypercoagulation and thromboembolic events [29]. Plasma fibrinogen levels are associated with other risk factors for CVD, such as male sex, obesity [30], diabetes [31], hypertension [32], high LDL cholesterol and triglycerides concentrations [33] as well as nephropathy [34]. Smoking is the strongest known determinant of fibrinogen levels in healthy persons [35]. This relationship is dose-dependent and reversible after smoking cessation [35].

The first study to suggest the role of increased plasma levels of fibrinogen in CVD was published in the 1980s [36]. It was followed by the results of the Framingham study which showed that elevated plasma fibrinogen levels were associated with the classical CVD risk factors [37]. Two meta-analyses have summarized the results of 18 and 22 prospective studies and have shown a significant and independent association between elevated fibrinogen levels and cardiovascular morbidity and mortality [38, 39].

In patients with CAD, plasma fibrinogen levels are associated with the severity of the disease [40] and with higher risk of re-stenosis after angioplasty [41]. The Northwick Park Heart Study [42] and the PRIME study [43] demonstrated that increased baseline levels of fibrinogen were associated with future coronary events compared to normal fibrinogen levels, even after adjustment for other cardiovascular risk factors. Two prospective studies [44, 45] in subjects with CAD showed a significant relationship between plasma fibrinogen levels and a second ischemic event as well as cardiovascular mortality.

Previous data have shown that fibrinogen levels peak after an acute stroke [46]. Furthermore, elevated plasma fibrinogen levels at the time of the stroke are strongly associated with the recurrence of cardiovascular events within the next 2 years [47] and with cerebrovascular mortality [48]. The above findings have been confirmed by large studies which demonstrated the role of fibrinogen in cerebrovascular disease. The Gothenburg Study [36], the Framingham Study [37], and the Scottish Heart Study [49], showed a significant association between fibrinogen levels and cerebrovascular disease.

Longitudinal data from patients with peripheral arterial occlusive disease showed that high fibrinogen predicted reocclusion of the femoropopliteal vein grafts [50]. Finally, the Edinburgh Artery Study demonstrated a significant association between plasma fibrinogen levels and clinical and subclinical arterial disease [51].

The mechanisms by which fibrinogen may promote atherosclerosis and thrombosis are still not fully understood. Fibrinogen promotes atherogenesis by increasing vascular permeability and collagen synthesis [52], by promoting endothelial injury [53], and by promoting smooth muscle cell proliferation and migration [53]. Furthermore, it is known that fibrinogen binds to receptors on the platelet membrane enhancing their aggregation *in vivo* [54]. Fibrinogen is also integrated directly into arteriosclerotic vascular lesions, where it is converted to fibrinogen degradation products and binds to low-density lipoproteins [55]. Both fibrinogen and fibrinogen degradation products stimulate smooth muscle cell proliferation and migration [55]. These effects suggest that fibrinogen is involved in the earliest stages of plaque formation.

URIC ACID

In humans, UA is the end product of purine metabolism catalysed by the enzyme xanthine oxidoreductase [56]. UA is degraded in most mammals by urate oxidase (uricase) to allantoin which is freely excreted in the urine. However, during the Miocene period, two parallel mutations occurred in early hominoids that rendered the uricase gene nonfunctional [57]. As a result, humans have higher uric acid levels

(>2 mg/dL) compared with most mammals. A simple explanation for the uricase mutation is that the antioxidant action of UA may have provided an evolutionary advantage and that this may account for the greater longevity of humans compared with most other primates [58]. Therefore, the increase in serum UA in subjects with CVD might reflect a mechanism to counter the oxidative stress that occurs in these conditions [59].

UA levels are higher in postmenopausal women because estrogens have uricosuric effects and in men [60]. Hyperuricemia is common in subjects with obesity, insulin resistance and dyslipidemia because insulin stimulates sodium and urate reabsorption in the proximal tubule [60]. Hyperuricemia predicts the development of CVD in the general population, in subjects with hypertension, and in subjects with preexisting CVD [61]. Hyperuricemia also predicts stroke in diabetic and nondiabetic subjects [62] and predicts the development of hypertension [63] and renal disease in the general population [64].

Existing data regarding the potential role of serum UA to CVD are conflicting. The Framingham study [65] failed to demonstrate any association between serum UA and CVD, while the Chicago Heart Association Detection Project and the NHANES I study found an independent relationship, but this association occurred in women but not in men [66, 67]. In the MONICA Augsberg and Gubbio cohorts, the relationship of UA to MI was suggestive and not significant [68, 69].

On the other hand, the Honolulu Heart study and the Hypertension Detection Follow-up Program study demonstrated a consistent independent relationship between serum UA levels and CVD [70,71]. Another study showed a significant association between serum UA and CAD mortality, which was more profound in women [72]. Furthermore, in low cardiovascular risk populations within the NHANES I follow-up study, UA was an independent predictor of cardiovascular mortality [73].

Serum UA levels are increased in subjects with metabolic syndrome (MS) [74]. Moreover, as the components of MS cluster, there is a parallel rise in plasma UA concentrations [75]. Serum UA levels may also be a reliable predictor of MS in obese youths [76]. Several studies revealed that insulin resistance is the pathogenetic link between elevated serum UA levels and MS [74, 77].

High plasma UA levels are common in subjects with arterial hypertension [78]. The increase in serum UA in hypertension may be due to the decrease in renal blood flow which accompanies the hypertensive state since a low renal blood flow stimulates urate reabsorption [78]. In the Olivetti Heart Study, the baseline serum UA level was the strongest independent predictor of new-onset hypertension and a 1 mg/dL increment in serum UA was associated with a 23% increase in the risk of hypertension during a 12-year follow-up period [62]. Similar were the findings from the Kaiser Permanente Multiphasic Health Checkup study [79]. Two recent epidemiological studies in hypertensive subjects reported a strong, independent association between serum UA at baseline and during therapy and cardiac morbidity and mortality [80, 81]. The Syst-Eur trial was the only study in hypertensive patients that did not find a significant association between serum UA levels and hypertension [82].

Several studies demonstrated an effect of serum UA levels on prediction of stroke and stroke outcomes [62, 83, 84]. One study has shown that serum UA levels predicted independently of other factors the stroke in elderly patients [62]. In patients admitted with stroke, serum UA was an independent predictor of future cardiovascular events in the next 2 years [83]. Furthermore, is has been demonstrated that UA predicted worse early outcome after acute stroke [84]. In diabetic subjects elevated serum UA levels increase the risk of future stroke events [85].

In type 2 diabetic patients elevated plasma UA levels were associated with reduced aortic distensibility, which is considered an index of the total arterial stiffness and an important cardiovascular risk factor [86]. In addition, a recent study found an independent relationship between serum UA and peripheral arterial disease in Asian diabetic patients [87]. In patients with chronic heart failure, a strong relationship was found between elevated serum UA levels and all cause mortality [88].

Medications used for the management of hyperlipidemia and hypertension may reduce plasma UA levels and part of their effect on the reduction of cardiovascular risk is attributable to the reduction in serum UA levels [89, 90]. In the GREACE study [88], an average of 0.8 mg/dL lower serum UA concentrations in the aggressively treated group with statin were associated with lower coronary event rate compared with the control group. In the LIFE study [90], UA levels were associated with the incidence of cardiovascular events independently of other risk factors. Furthermore, the same study showed that a proportion of 29% of the benefit associated with treatment with losartan in terms of cardiovascular risk reduction was attributable to the decreased levels of UA in comparison with the treatment with atenolol [90].

Several possible mechanisms have been proposed in order to explain the association between serum UA levels and CVD. First, there is evidence that increased UA levels promote oxidation of low density lipoprotein cholesterol and facilitate lipid perioxidation [91]. In addition, increased UA levels are associated with increased production of oxygen free radicals [92] which contribute to the initiation and progression of atherosclerosis. Moreover, it has been suggested that elevated UA levels are associated with increased platelet adhesiveness, [93] and this effect could potentiate thrombus formation in patients with acute coronary syndromes. UA has been shown to inhibit nitric oxide (NO) bioavailability [94]. It has been reported that UA infusion in healthy humans resulted in impaired acetylcholine-induced vasodilation in the forearm, thereby documenting impaired endothelial NO release [95]. Finally, UA stimulates rat vascular smooth muscle cell proliferation *in vitro* [96].

HOMOCYSTEINE

Hcy is a sulfur-containing amino acid absent in naturally occurring dietary sources. Hcy levels increase with aging, male gender and in the presence of CVD [97]. An elevated plasma level of Hcy was first suspected to be associated with atherogenic and thrombogenic tendencies in patients with classic homocystinuria. This is a rare autosomal recessive

disease caused by cystathionine b-synthase deficiency and results in high plasma Hcy levels and early onset of atherosclerosis [98].

Several studies reported an association between mild hyperhomocysteinemia and CAD, stroke, and peripheral arterial disease [99, 100]. A meta-analysis [99] of 27 retrospective case-control studies demonstrated that a 5-μmol/L rise in Hcy levels was associated with 1.6- to 1.8-fold increase of in the relative risk for CAD, cerebrovascular disease and peripheral vascular disease. A recent meta-analysis of observational studies also suggested that elevated Hcy was a modest independent predictor of ischemic heart disease and stroke in healthy subjects [101].

Previous studies have shown that the risk for MI is higher among subjects with higher plasma Hcy levels than in subjects with normal concentrations of plasma Hcy [102, 103]. In a longitudinal cohort of 1,368 women in Gothenburg, Sweden, after 24-years of follow-up, individuals with high baseline Hcy showed an increased risk for acute MI and mortality from MI compared with individuals with normal Hcy levels [104]. Recent data from the Framingham Heart trial data showed a strong association between Hcy concentrations and the risk for CVD in both men and women with no prior history of CAD [105]. Furthermore, in patients admitted with acute MI, elevated plasma Hcy levels were associated with increased risk of recurrent MI or death [106]. A Norwegian study showed that baseline Hcy levels predicted 10-year mortality in middle aged subjects with MI [107]. Another study including 549 patients with CAD who had at least one vessel disease, plasma Hcy levels predicted nonfatal MI, stent reocclusion and cardiac death after successful coronary angioplasty in the next year [108].

However, other prospective studies have shown conflicting results regarding the relationship between plasma Hcy levels and CVD. The Physicians' Health Study showed that higher plasma Hcy concentrations were not associated with higher risk of CAD, angina pectoris, and stroke [109]. Additionally, the Multiple Risk Factor Intervention Trial cohort [110], the Atherosclerosis Risk in Communities Study cohort [111] and the North Karelia Project [112] failed to show significant associations between elevated Hcy levels and the risk of major coronary events or stroke.

Several potential mechanisms have been proposed on the potential role of Hcy in CVD, including impairment of endothelial function [113], oxidation of low-density lipoproteins [114], increased monocyte adhesion to the arterial wall [115], increased lipid uptake and retention [115], activation of the inflammatory pathway [115], stimulatory effects on smooth-muscle proliferation [115], and thrombotic tendency mediated by activation of coagulation factors [113] and platelet dysfunction [116].

Homocysteine can be lowered by supplementation with folate, vitamin B6, and B12 [117]. In patients with markedly increased homocysteine levels, vitamin treatment was associated with a decrease in CVD risk in a controlled trial [118]. However, the results of 4 large randomized controlled trials in high-risk patients failed to provide conclusive evidence for a benefit of Hcy lowering on major cardiovascular events. The Vitamin Intervention for Stroke Prevention [119] study showed that treatment with a high dose of vitamin B had no effect on recurrent stroke, coronary events or deaths. The Norwegian Vitamin Trial [120] showed that treatment with folic acid decreased Hcy levels by about 27% but had no impact on the composite primary endpoint of MI, stroke and sudden cardiac death. The Heart Outcomes Prevention Evaluation-2 trial [121] showed that reduction of homocysteine had no effect on the primary outcome of the study which was the composite of cardiovascular death, MI and stroke. Finally the Women's Antioxidant and Folic Acid Cardiovascular Study [122] reported no cardiovascular benefits for the combined folic acid and vitamins B6 and B12 supplementation administered for an average of 7.4 years in 5,442 middle-aged women who had preexisting CVD or at least three cardiovascular risk factors, in spite of a reduction in plasma Hcy levels by an average of about 18%.

ADIPONECTIN

Adiponectin is an adipocytokine with important metabolic effects [123]. It is derived only from adipose tissue and is abundantly present in circulating blood [123]. Plasma levels of adiponectin are lower in obese patients [124], in patients with type 2 diabetes [125] and in patients with CAD [126]. A significant negative relationship exists between body mass index and plasma adiponectin levels [127]. Also, plasma adiponectin concentrations are negatively associated with the total body fat, waist-to-hip ratio and intra-abdominal fat [128].

Prospective studies in adults have consistently shown that low serum adiponectin concentrations predict development of type 2 diabetes mellitus [129-131]. Moreover, hypoadiponectinemia is a marker for predisposition to hypertension in men [132]. Patients with hypertension have significantly lower plasma adiponectin levels than the normotensive counterparts [133]. One study found increased plasma adiponectin concentrations in hypertensive men with renal dysfunction but not in women [134]. Another study found [135] that young Japanese men with high-normal blood pressure had lower adiponectin levels.

Previous data have shown lower adiponectin levels in patients with CAD in comparison with matched controls [126]. In addition, high plasma adiponectin levels protect from MI [136]. A recent study reported that low plasma adiponectin levels were independently associated with CAD even after adjustment for several risk factors [relative risk: 0.75 (95% confidence intervals: 0.39-1.42) for adiponectin levels lower than 7.0 μg/mL] [137]. Cross-sectional studies have shown that serum adiponectin levels are lower in patients with cerebral [138] and peripheral arterial disease [139], as well as in men with CAD [140]. In prospective studies, high adiponectin levels protect from CAD both in subjects with and without diabetes [141]. However, an association between plasma adiponectin concentrations and the risk of CAD could not be demonstrated in three studies: the Strong Heart Study [129], the British Women's Heart Health Study [142] and a large study in British men with coronary heart disease [143].

In a nested case-control study, plasma adiponectin levels were not associated with future risk of stroke [144]. However, patients with ischaemic stroke had lower plasma levels of adiponectin than controls [138]. Furthermore, low adiponectin levels are associated with poor outcome after first-

event ischemic stroke independently of other factors [145]. Interestingly, in patients with heart failure, plasma levels of adiponectin increase as a function of the severity of the disease (NYHA class) and reduction in body fatness [146]. Thus, in subjects with heart failure, higher adiponectin levels predict mortality independently of other risk factors [147].

In a large Austrian study of healthy middle-aged men and women, serum adiponectin levels were associated inversely with the carotid artery intima-media thickness (IMT) even after adjustment for other cardiovascular risk factors [148]. The negative relationship between adiponectin and IMT was also seen in obese and non-obese children as well as in adolescents [149] and in a study of Swedish men who had a family history of diabetes, whereas no such association was found in the control group [150].

High sensitivity C-reactive protein (hsCRP) is a risk factor for CAD. A significant inverse association was observed between CRP and adiponectin mRNA levels in subcutaneous adipose tissue in humans with atherosclerosis [151]. Circulating adiponectin concentrations are also related to lipid metabolism as indicated by the negative relationship between plasma adiponectin concentrations and plasma concentrations of triglycerides and LDL-cholesterol, and the positive association between circulating adiponectin and fat oxidation as well as HDL-cholesterol levels [152]. In addition, low adiponectin levels have been associated with endothelial dysfunction in the coronary arteries [153] and with the severity of the CAD [154].

Adiponectin influences various aspects of endothelial function. Thus, adiponection inhibits TNFα-induced expression of VCAM-1, ICAM-1, and E-selectin in human aortic endothelial cells *in vitro* [125]. Moreover, TNFα-stimulated adhesion of monocytes on endothelial cells is inhibited by adiponectin [125]. Importantly, adiponectin directly stimulates NO production in human and bovine aortic endothelial cells [155]. Apoptosis of human endothelial cells is also suppressed by adiponectin [156]. These functions of adiponectin explain its role in the process of atherosclerosis.

C-REACTIVE PROTEIN

C-reactive protein (CRP), an acute-phase reactant, is synthesized in the liver in response to interleukin-6 [157]. CRP is a marker of vascular inflammation and plays an active role in atherogenesis [157]. A number of large, prospective epidemiologic studies demonstrated that hsCRP is a strong independent predictor of future cardiovascular events, including MI, ischemic stroke, peripheral vascular disease, and sudden cardiac death in individuals without CVD [158]. Thus, the American Heart Association has published a statement suggesting that subjects with CRP levels <1 mg/L to be considered as low-risk, 1 to 3 mg/L as average risk, and >3 mg/L as high-risk for CVD [159].

In the Physicians' Health Study, subjects with high baseline levels of hsCRP had a 2-fold increase in the risk of ischemic stroke or peripheral vascular disease and a 3-fold increase in the risk of MI in comparison with subjects with low hsCRP levels [160]. The Honolulu Heart Program showed that the hsCRP levels were associated with coronary events that occurred as many as 15 years later and that the risk of MI increased in parallel with increasing hsCRP levels [161]. In the primary prevention Women's Health Study, women with the highest baseline hsCRP levels had a 5-fold higher risk of suffering from vascular events and a 7-fold higher risk of MI or stroke than women with low hsCRP levels (≥ 7.3 vs. ≤1.5 mg/L) [162]. The Nurses' Health Study and the Health Professionals Follow-up Study confirmed the results of the Women's Health Study and showed that the hsCRP was an independent predictor of CVD (≥ 3.0 vs. ≤1 mg/L) [163]. The Honolulu Heart Program showed a positive association between hsCRP levels and risk of stroke over a follow-up period of 20 years [164]. Analysis of data from the Framingham Heart Study showed also an association between hsCRP levels and stroke [165].

Increased hsCRP concentrations have been shown to be a strong predictor of future cardiovascular risk in patients with established CVD. In the Scandinavian Simvastatin Survival Study, elevated hsCRP levels predicted mortality in patients with stable ischemic heart disease [166]. A meta-analysis of 14 prospective long-term studies on the relationship between hsCRP and the risk of nonfatal MI or CVD death demonstrated an increased relative risk for individuals with increased baseline hsCRP levels in comparison with those with lower hsCRP levels [167].

Other studies have shown variable results. The Multiple Risk Factor Interventional Trial found that increased hsCRP levels predicted increased risk of cardiovascular disease in middle-aged men, but that the relationship was statistically significant only for smokers [168]. Another study found that the association between hsCRP levels and development of future ischemic heart disease was abolished after adjusting for other known risk factors [169].

Obesity is associated with elevated hsCRP [170]. HsCRP levels are also associated with increased blood pressure and are predictive of the development of hypertension [171]. High levels of hsCRP have been shown to be an independent predictor of cardiovascular risk for all degrees of severity of the MS [172]. In a cohort of the Women's Health Study, women who had plasma concentrations in the upper quartile had an increased relative risk of diabetes, even after adjusting for body mass index, family history of diabetes mellitus and smoking, in comparison with the women who had plasma concentrations of hsCRP in the lowest quartile [173]. Similarly, the West of Scotland Coronary Prevention Study indicated that high hsCRP levels are an independent predictor of type 2 diabetes mellitus in healthy middle-aged men [174].

Experimental data have shown that CRP influence vascular vulnerability directly by a variety of mechanisms. Endothelial dysfunction is associated in epidemiological studies with CRP production [175] and CRP exerts direct effects on the endothelial NO synthesis [176]. Expression of adhesion molecules in endothelial cell cultures is also increased by *in vitro* exposure to CRP [177]. The expression and activity of plasminogen activator inhibitor-1 by human aortic endothelial cells is upregulated by CRP [178]. Another mechanism by which CRP contributes to CVD is the complement activation as CRP is able to activate the classical route of complement activation [179]. CRP also appears to be involved in the recruitment of monocytes, the infiltration of monocytes into the vessel wall and the subsequent formation of the foam cells. CRP is deposited in the vessel wall at sites of atherogenesis [180] and has been shown to be chemotactic for freshly isolated human blood monocytes [181].

Table 1.

Risk Factor	Ref.	Sample Size	Population	Setting	Gender	Follow-up (yrs)	Cut-off Values	Clinical end-Points	Hazard Ratio	95% CI
WBC	[5]	7,206	Healthy subjects	France	M	6.5	<6,000 vs. ≥9,000/mm^3	Fatal/nonfatal MI	4.5	2.5-7.8
	[6]	15,909	Healthy subjects	U.S.	M/F	16	<4,000 vs. >10,000/mm^3	Angina, MI, CHD death	1.82	NA
	[9]	420	Healthy subjects	Finland	M/F	5	NA	CHD incidence	1.13 3.07 (non-smokers) 1.86 (smokers)	1.00-1.27 2.23-8.19 0.81-4.28
	[10]	6,021	Healthy subjects	Japan	M/F	NA	9,209 vs. 6,205/ mm^3	ACS incidence	0.038	1.042-4.016
	[13]	1,294	Subjects with acute MI or unstable angina	U.S./Canada	M/F	2.1	52,000 vs. 10,600/mm^3	CHD death	1.18	1.00-1.40
	[15]	975	Subjects with acute MI or unstable angina	U.S.	M/F	NA	NA	Death/CHF/shock	0.24 (death) 0.24 (CHF/shock) 0.19 (death/CHF/shock)	0.07-0.75 0.08-0.74 0.07-0.47
Fibrinogen	[29]	2,700	Subjects with angina	Europe	M/F	2.0	300 vs 328 mg/dL	coronary event	NA	NA
	[36]	792	Healthy Subjects	Sweden	M	13.5	330 vs 360 mg/dL	CVD event	NA	NA
	[37]	554, 761	Healthy subjects	U.S.	M/F	12	291.4 mg/dL	CVD event	NA	NA
	[51]	617	Intermittent claudication	Scotland	M/F	1.0	NA	Coronary death	NA	NA
	[43]	9,489	Healthy subjects	France Ireland	M	5	NA	MI	1.56	1.29-1.95
Uric Acid	[68]	1,044	Healthy subjects	Germany	M/F	8	>0.373 mmol/L	All cause mortality CVD mortality MI	2.8 2.2 1.7	1.6-5.0 1.0-4.8 0.8-3.3
	[73]	5,926	Healthy subjects	U.S.	M/F	16	>0.416/0.333 mmol/L	CV mortality All cause mortality	1.17 1.26	1.06-1.28 1.16-1.36
	[61]	13, 504	Healthy subjects	U.S.	M/F	8	>0.447/0.369 mmol/L	CHD events	M: 1.02 W: 1.18	0.69-1.51 0.62-2.26
	[72]	9,701	Healthy subjects	Belgium	M/F	10	NA	A. CV mortality B. CHD mortality	M: 1.58 1.67 F: 1.56 8.58	1.10-2.28 1.06-2.63 0.92-3.70 1.91-38.6
	[69]	2,469	Healthy subjects	Italy	M/F	6	>0.428 mmol/L	CHD events CVD events	1.15 1.18	0.94-1.40 1.00-1.39
	[62]	3,282	Elderly subjects	Italy	M/F	14	>0.38 mmol/L	Stroke mortality	1.61	1.14-2.10
	[85]	1,017	NIDDM subjects	Finland	M/F	7	>0.295 mmol/L	Stroke events	1.91	1.24-2.94
	[83]	3,731	Subjects with stroke	U.K.	M/F	2.7	>0.38 mmol/L on admission	Subsequent events 90-day placement	1.27 0.78	1.18-1.36 0.67-0.91
Homocys-teine	[103]	791	Past history of CVD	U.S.	F	3	per 5-µmol/L tHcy incre-ment	Fatal and non-fatal CHD	1.74	1.13-2.64

(Table 1). Contd…..

Risk Factor	Ref.	Sample Size	Population	Setting	Gender	Follow-up (yrs)	Cut-off Values	Clinical end-Points	Hazard Ratio	95% CI
	[110]	712	Past history of morbidity (not explained)	U.S.	M	11	per 5-μmol/L tHcy increment	Non-fatal MI, fatal CHD	0.98	0.83-1.15
	[111]	NA	Past history of CHD, stroke, or TIA	U.S.	M/F	3.3	per 5-μmol/L tHcy increment	Fatal and non-fatal CHD	1.15	0.68-1.92
	[112]	7,424	Past history of CVD	Finland	M/F	9	per 5-μmol/L tHcy increment	Fatal and non-fatal MI	1.03	0.66-1.53
	[104]	1,368	Subjects free of previous MI	Sweden	F	32	NA	MI Death due to MI	1.86 5.14	1.06 - 3.26 2.22-11.92
	[105]	2,491	Subjects free of CHF	U.S.	M/F	1979-82 1986-90	NA	CHF	1.84 1.93	1.06-3.17 1.19-3.14
Adi-ponectin	[142]	4,286	Women free of CAD	U.K.	F	4	NA	CAD	0.93	0.78-1.11
	[137]	225	Men underwent coronary angiography	Japan	M	-	<4.0 μg/mL <5.5 μg/mL <7.0 μg/mL	CAD	2.05 1.22 0.75	1.29-4.95 0.68-2.19 0.39-1.42
	[145]	160	Subjects with ischemic stroke	Greece	M/F	5	<4 μg/mL	Death	5.2	2.1-18.4
	[147]	195	CHF patients	Denmark	M/F	2.6	≤11.6 vs >19.8 μg/mL	Death	3.23	NA
	[129]	4,549	Healthy subjects	U.S.	M/F	11	NA	CHF	0.97	0.81 - 1.16
	[136]	18 225	Healthy subjects	U.S.	M	6	NA	nonfatal MI	0.39	0.23-0.64
	[143]	5661	Healthy subjects	U.S.	M/F	4	<8.32 vs ≥ 13.33μg/mL	CHD	0.76	0.59 - 0.98
CRP	[162]	122	Healthy Subjects	U.S.	M/F	3	≥ 7.3 vs. ≤1.5 mg/l	CVD event MI or stroke	4.80 7.30	2.3-10.1 2.7-19.9
	[163]	504	Healthy subjects	U.K.	M/F	6-8	≥ 3.0 vs. ≤1 mg/L	CAD	1.79	1.27-2.51
	[165]	1,462	Healthy subjects	U.S.	M/F	12-14	NA	Ischemic strokes	M: 2.0 W: 2.9	NA
	[166]	-	129 who died / 129 matched participants	Italy	M/F	5	NA	CAD	2.36	1.06-5.26
	[168]	-	98 MI cases /148 CAD deaths / 491 controls	U.S.	M/F	17	> 3.3 vs <1.2 mg/dL	CAD mortality	4.30	1.74-10.8
	[164]	8,006	Healthy subjects	Japan	M	20	NA	Stroke	2.5	1.2 - 5.1
	[174]	5,974	Non diabetics	U.K.	M/F	5	NA	Diabetes	1.30	1.07-1.58
	[173]	27,628	Healthy subjects	U.S.	F	4	≤ 0.14 vs. > 0.48 mg/dL	Diabetes	4.2	1.5 -12.0

M: males, F: females; ACS: acute coronary syndrome; CAD: coronary artery disease; CVD: cardiovascular disease; MI: myocardial infarction; CHF: congestive heart failure; CHD: chronic heart disease; NIDDM: non–insulin-dependent diabetes mellitus; NA: non-available.

In conclusion, there is abundant evidence today from observational, experimental and epidemiological studies that novel risk factors of atherosclerosis exist which exerts their effects on the arteries either in combination with or above and beyond the classical risk factors. In the novel risk factors are included the number of the WBC count, higher plasma concentrations of fibrinogen, UA, homocysteine and CRP and lower plasma levels of adiponectin. However, there are limited data to suggest that interventions aiming at modification of the novel risk factors reduce CVD morbidity and mortality and further prospective studies are needed to address this issue.

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