The growing significance of myeloperoxidase as an inflammatory marker in coronary artery disease and other non-infectious diseases

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The Growing Significance of Myeloperoxidase as an Inflammatory Marker in Coronary Artery Disease and Other Non-Infectious Diseases

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July 22, 2004
Dedications

I would like to dedicate this project to some very special people in my life who have always been there for support whenever I needed it. Thank you; to my roommate, Kristi Klein, to my boyfriend, Nick Felkey, and to all of my immediate family members, Mom, Dad, Shawn, and Randy.
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Introduction

Numerous studies have evaluated several inflammatory markers to determine their clinical significance in predicting the risk of developing coronary artery disease (CAD) and many other non-infectious diseases. Some of the inflammatory markers that have been recently studied include C-reactive protein (CRP), antineutrophil cytoplasmic antibody (ANCA), cytokines, such as, tumor necrosis factor (TNF) and interleukin 6 (IL-6), homocysteine, 15-lipoxygenase, and myeloperoxidase. C-reactive protein is the inflammatory marker that has been most extensively studied. Therefore, the most conclusive evidence exists about CRP and its significance in the inflammatory process. Myeloperoxidase, MPO, is the most recently studied inflammatory marker. Many studies conclude that MPO is not only a significant inflammatory marker for CAD, but also for systemic vasculitis, leukemia, lung cancer, brain infarctions, multiple sclerosis and Alzheimer’s disease.

Although several inflammatory markers have been studied and are proven to be linked to the development of disease, none have met the criteria to be acceptable for use in the clinical realm. First, there must be an abundant amount of data from several large-scale studies that link the inflammatory marker to the prediction of risk. Today, it is unclear whether one inflammatory marker will prove superior to another. It is even harder to determine whether that same inflammatory marker can be used to predict risk in both the acute and chronic phase of disease (Vorhheimer and Fuster, 2001). Secondly, levels of the marker must decrease with an assessed improvement of risk. For example, are specific therapies available to reduce the serum level of the marker of inflammation? Thirdly, there must be a standardized assay available for the marker. In other words, a test for the specific inflammatory marker should be available to order
through the laboratory. Finally, screening for the marker should potentially direct therapeutic intervention (Blake and Ridker, 2002). Do therapies that lower serum levels of inflammatory markers reduce risk to the disease? CRP seems to meet the most of the criteria listed due to it having been studied the most. The significance of MPO as an inflammatory marker is growing each day as more and more studies demonstrate its link it to risk of developing specific diseases.

In one article, MPO is defined as being an abundant enzyme secreted from activated neutrophils, monocytes, and certain tissue macrophages (Zhang et al., 2001). In another article, MPO is defined as functioning as a glycoprotein released by activated polymorphonuclear neutrophils, which take part in defense of the organism through production of hypochlorous acid (HOCl), a potent oxidant (Carr, McCall, and Frei, 2000). Because MPO is an enzyme, it causes changes to occur that are measurable through laboratory tests that can predict the risk of developing CAD and many non-infectious diseases.

In this literature review several markers and their significance in the inflammatory process will be compared and contrasted. A detailed discussion of the atherosclerotic process is presented to provide information of where specific inflammatory markers take action or cause damage. The action of each marker will be discussed. It compares evidence supporting the conclusions of different studies done on the same marker. Nonetheless, the main focus will be the extensively studied CRP and the most recently studied MPO. This article presents evidence that supports the action of MPO becoming an inflammatory marker used in clinical practice. The association between myeloperoxidase levels and the risk of developing CAD and other non-infectious diseases is unknown.
Atherosclerosis/Arteriosclerosis

Atherosclerosis is the term used for the three patterns of vascular disease, all of which cause thickening, inelasticity and damage to the arteries of the human body. The first type of arteriosclerosis is a medial calcific sclerosis. This process occurs in the media of muscular arteries where calcifications take form of medial plates or transverse rings (Kumar, Cotran, and Robbins, 2003). Because these lesions do not encroach on the vessel lumen, this type of arteriosclerosis is not of much importance in studies or in the cause of death. The second type is the disease of small arteries and arterioles. This process of disease causes thickening of the vessel walls with luminal narrowing which may become so severe is causes ischemic injury to tissues or organs (Kumar et al., 2003). The third type is the most severe and complicated type of arteriosclerosis. This dominant pattern is known as atherosclerosis. This disease process is responsible for the most deaths and has stimulated more research than any other disease in the United States.

Atherosclerosis is a step-wise process that occurs in both men and women. Some risk factors predispose a person to the disease more than others. For example, overall, males are more prone to the disease than females. Females are at equal risk of developing the disease if they have diabetes mellitus, hyperlipidemia, or severe hypertension. Otherwise, they do not meet equal risk with males until after menopause. Age is also a dominant factor. The more advanced a person’s age, the greater the incidence of disease or death from myocardial infarction. There is a well-defined familial predisposition as well. Atherosclerosis may be related to a family history of hypertension, diabetes mellitus, or excessively high blood lipid levels, known as hyperlipidemia. Hypercholesterolemia and derangements in the metabolism of
some lipoproteins are a few genetically inherited traits that predispose a person to developing atherosclerosis. There are also major acquired factors that are in some part amendable to control. Hyperlipidemia, hypertension, cigarette smoking, and diabetes mellitus are among these risk factors. Smoking is the dominant preventable risk factor. Smoking is thought to account for the recent increase in incidence of atherosclerosis among women (Kumar et al., 2003). If hypertension, hyperlipidemia and diabetes mellitus are not acquired due to family history, then these risk factors are amendable through specific diet indications and therapies. Other secondary factors that have been studied and may have an affect on atherosclerosis are insufficient, sedentary physical activity, obesity, stressful lifestyle, the use of oral contraceptives, low HDL (high density lipoprotein), and hyperhomocysteinemia, which will be discussed later in this review.

Atherosclerosis begins in early childhood and progresses slowly over the decades. The disease targets mainly the coronary arteries and the cerebral arteries causing either a myocardial infarction or a stroke, respectively. The atherosclerotic process is stimulated by chronic endothelial injury. Endothelial injury can be caused by any of the risk factors mentioned earlier. The endothelial injury is responded to by cell stimulation, increased endothelial permeability and increased leukocyte adhesivity (Kumar et al., 2003). Leukocytes normally do not bind to undamaged endothelium, but when injury occurs cell adhesion molecules are expressed by the endothelium, which binds circulating leukocytes (Blake and Ridker, 2002). There are many types of cell adhesion molecules that aid in this step of atherosclerosis. After this step, monocytes begin to collect near the injury and migrate subendothelialy where they become macrophages and engulf lipoproteins and oxidized LDL (low density lipoproteins) (Kumar et al., 2003). The macrophage becomes known as a foam cell when it cannot engulf any more of its
surroundings. Smooth muscle cells (SMC) migrate from the media to the intima where they act similar to the macrophages, by engulfing lipids. A fatty streak is then formed, which is an accumulation of foam cells. The continuous migration of SMC from the media forms the fatty streak into a fibrofatty atheroma. SMC have the ability to synthesize collagen, elastin, and glycoproteins or proteoglycans (Kumar et al., 2003). These connective tissues are prominent on the intimal aspect of the endothelium and form a fibrous cap (Kumar et al., 2003). After the fibrous cap is formed on top of the atheroma, it becomes known as a fibrous plaque. These fibrous plaques are continuously forming throughout the disease process of atherosclerosis.

The atherosclerotic process takes time to complete and has deleterious affects. The affects of the atherosclerotic process on the human body have triggered much advancement toward treatment and many studies on inflammatory markers to prevent the disease. Myeloperoxidase (MPO) is among the many inflammatory markers that have been studied. In the disease process that is described above, MPO has the potential to be a significant inflammatory marker due to its effects on LDL. MPO is an enzyme that promotes LDL oxidation, which in turn leads to more uptake by macrophages and forming more foam cells and larger plaques (Blake and Ridker, 2002). From this evidence several investigators have concluded that MPO plays a direct role in causing atherosclerosis and may serve as an early inflammatory marker.
**Myeloperoxidase**

One of the most interesting facts about MPO is that it has the ability to act in host defense and at the same time, it can cause damage to the host. *(Table 1).* MPO is stored within granules of neutrophils and monocytes and is not activated until leukocyte activation or degranulation of cells occurs (Zhang et al., 2001). Degranulation is a release of chemical mediators from the pre-formed storage depots in cells. Leukocyte activation can occur by infection of the host or by endothelial injury as discussed earlier. MPO responds in the exact same manner no matter what source causes the leukocyte activation. MPO forms free radicals and oxidants, which have an antimicrobial affect and can cause host tissue damage at the sites of inflammation. MPO has also been found to be an enzyme that promotes oxidation of LDL, which has an additive, damaging affect in atherosclerosis (Zhang et al., 2001). MPO can cause endothelial injury by more than just one pathway. MPO has the ability to oxidize chloride, tyrosine, or nitrogen into reactive nitrogen species that readily oxidize LDL. All of these processes require H$_2$O$_2$ to be present and are independent of free metal ions. Hydrogen peroxide is readily formed by the dismutation of O$_2$ and halides, such as Cl- Br- or SCN-to form hypohalous acids (Hoy, Leininger-Muller, Kutter, Siest, Visvikis, 2002). The reaction occurs most often with Cl- because of its high concentration in serum compared with the other halides.
Klebanoff SJ believes that the main function of MPO lies in the defense of the host organism through production of hypochlorous acid, a powerful oxidant (Hoy et al., 2002). The reaction products derived from the MPO-H$_2$O$_2$-Cl system have a potent activity against a wide range of viruses, bacteria and fungi (Hoy et al., 2002). It is understood that the MPO-H$_2$O$_2$-Cl system is stimulated by the ingestion of bacteria by PMNs (polymorphonuclear lymphocytes) in host cell defense. MPO, which was released due to leukocyte activation, responds by forming hypochlorous acid (HOCl) to fight off the bacteria. At the same time, the powerful oxidants released by this system are affecting vessel endothelium and MPO is continuously oxidizing LDL for uptake in atherosclerotic plaques. Therefore, the question has been raised as to whether an infection of such magnitude, such as Chlamydia pneumoniae, may have a role in the pathogenesis of atherosclerosis.

Other studies show that this MPO-H$_2$O$_2$-Cl system may also affect the HDL available in the human body. HDL is the “good” lipoprotein in the body which functions by reversing transport of cholesterol/lipid to cells and bringing them to the liver for extraction in bile rather than the lipids getting deposited in atherosclerotic plaques (Kumar et al., 2003). Evidence from a study performed by Hazen et al. illustrates that HOCl, when in contact with human HDL, increases uptake of lipid and cholesterol particles by the macrophages, therefore increasing the availability of potential plaque content, such as foam cells, and decreasing the removal of cellular cholesterol (Hoy et al., 2002). This is the only information found indicating that MPO has a bad impact on HDL.
One study shows how the MPO-H$_2$O$_2$-Cl system is linked to Alzheimer’s disease (Hoy et al., 2002). The authors propose that MPO storage in the brain could be dangerous to surrounding neurons when released inadvertently. This MPO-HOCl system has been shown to oxidize Apolipoprotein E in the brain. Apo E is responsible for the maintenance of cerebral lipid homeostasis and Apo E polymorphisms are strongly linked to Alzheimer’s Disease.

**MPO-Nitric Oxide System (MPO-H$_2$O$_2$-NO)**

MPO may also contribute to atherosclerotic disease by promoting endothelial dysfunction. Recent studies show that nitric oxide (NO) is dependent upon MPO for oxidation to form the harmful nitric oxide (NO$_2^-$) species (Podrez, Abu-Soud, Hazen, 2000). Nitric oxide is present in human serum as a short-acting, soluble free radical gas. When nitric oxide comes in contact with oxygen and superoxide molecules, in the presence of MPO, there is a reaction that occurs to form reactive nitrogen species (RNS) (Carr et al., 2000). These RNS contribute to lipid peroxidation, providing another link between MPO dependent biochemistry and atherosclerosis.

This harmful oxidative system not only oxidizes lipids in LDL, it also oxidizes proteins that are present in the LDL (Nauseef, 2001). The exact mechanism of how the proteins in LDL get oxidized is not known, but it appears to change LDL to a more atherogenic form.

Recent studies show that the reactive species formed through this system is one of the primary mechanisms for promoting LDL peroxidation and LDL protein nitration. The interesting biochemical step of this pathway is that once the nitrogen species is oxidized by MPO, in the presence of H$_2$O$_2$, it is converted to a form (NO$_2$-LDL) that is avidly taken up and degraded by macrophages (Podrez et al., 2000). This specific form of nitric oxide and LDL is
easily recognized by macrophages that have a CD36 receptor. Because these reactive nitrogen species are the only oxidized species that convert to recognizable form, they get engulfed faster and easily provide for the development of an atherosclerotic plaque.

Nitrogen also has an additive affect on the blood vessels by way of its primary reason of being available within human endothelium. Nitric Oxide is primarily responsible for regulating blood flow. When NO is functioning properly it is a species used in vasodilatation. NO is released by endothelial cells and activates guanylate cyclase in vascular smooth muscle cells resulting in increased cyclic guanine monophosphate (cGMP) and, ultimately, leads to smooth muscle relaxation or vasodilation (Kumar et al., 2003). This is where nitric oxide’s secondary affect on atherosclerosis is understood. If much of the available NO has been oxidized by MPO into reactive nitrogen species that is taken up by macrohages, then there is a decrease in the amount of available NO to vasodilate when it is stimulated by endothelial cells. Therefore, the vessels continue to get constricted by the atherosclerotic plaque build up unless medication is added to control homeostasis and hemodynamics. Also, MPO is then considered a catalytic sink for NO, limiting its bioavailalibility and function, most importantly. This is the explanation of how nitric oxide can cause endothelial dysfunction.
Tyrosine is a phenolic amino acid present in human plasma. The tyrosine system is similar to the HOCl and NO systems. In the presence of MPO and H$_2$O$_2$, a relatively unreactive oxidant, tyrosine is converted to tyrosyl radicals (Heinecke, 1998). These tyrosyl radicals then cross-link tyrosine residues on proteins, forming $o,o'$-dityrosine. In this reaction, free tyrosine is considered the rate-limiting reagent. MPO requires free tyrosine to form tyrosyl radicals then protein $o,o'$-dityrosine cross-links (Heinecke, 2002).

One study presented data, which indicated that MPO can oxidize free tyrosine into a diffusible intermediate that promotes lipid peroxidation (Heinecke, 2002). The diffusible, reactive intermediate is the tyrosyl radical (Heinecke, 1998). These radicals have the potential to initiate peroxidation of LDL. It has also been demonstrated that $o,o'$-dityrosine, cross-links in proteins of tyrosyl radicals, initiate LDL lipid peroxidation in vivo (Heinecke, 2002).

$o,o'$-dityrosine was assayed in human vascular tissue. The study compared normal aortic tissue to atherosclerotic aortic tissue. Dityrosine was present by more than 11-fold in the earliest lesion of atherosclerosis, the fatty streak. Protein bound dityrosine was elevated 6-fold in advanced atherosclerotic lesions, the atheromatous plaque (Heinecke, 2002). This accumulation of dityrosine in atherosclerotic aortic tissue is consistent with the hypothesis that tyrosyl radicals oxidize artery wall proteins. Tyrosyl has two reactive species; the tyrosyl radical that is formed by tyrosine oxidation and the dityrosine protein cross-link that is formed by two or more tyrosyl radicals. Neither of these reactive species can form without free tyrosines being present in human plasma.
C-Reactive Protein (CRP)

Of all of the inflammatory markers that have been studied, C-reactive protein (CRP) is the most widely studied, and there is now an abundance of evidence supporting CRP as an inflammatory marker. (Table 2) Due to all of the evidence, CRP has been known as a strong independent predictor of future cardiovascular risk (Blake and Ridker, 2003).

It has recently been reported that an inflammatory response is related to the development of atherosclerosis (Jeong, 2003). This inflammatory response may have an effect on the stability of the atherosclerotic plaques. It may accelerate the rupture of atheromatous plaques and cause coronary artery thrombosis. CRP is one of the inflammatory markers that, when detected in patients with coronary artery disease, increase the risk of future coronary events. Factors that stimulate the rise in CRP are not well known, but a few suggested chronic infectious pathogens include; Helicobacter pylori (H. pylori), Cytomegalovirus, and Chlamydia pneumoniae (C. pneumoniae) (Jeong, 2003). A study was performed among 272 patients who were diagnosed with having coronary artery disease and were positive for antibody titers of H. pylori. The study concluded that the antibody titers of H. pylori were higher in patients with coronary artery disease, and the levels of CRP and ESR (erythrocyte sedimentation rate) were significantly higher as well (Jeong, 2003).
What is CRP?

CRP is an inflammatory marker that has recently become a clinical lab test that is used to predict the risk of myocardial infarction and is used as a screening tool to evaluate the treatment of revascularization. Table 3 shows examples of how CRP is associated with cardiovascular disease. CRP reaches a peak 2-4 days after the initial event of the myocardial infarction and often falls thereafter (Gomes, 2002). C-reactive protein (CRP) is a protein that is produced in the liver in response to interleukin-6 (IL-6). Interleukin is considered an acute phase reactant that stimulates the activation of CRP (Blake and Ridker, 2003). CRP is also linked to many other inflammatory markers that will be discussed later in this review. It was once thought that CRP was just a bystander marker of vascular inflammation, in other words, it was considered an inactive marker. But recent studies suggest that CRP plays an active role in the pathogenesis of atherosclerosis.

Several studies concluded that LDL-cholesterol opsinized by CRP is readily engulfed by macrophages, therefore, CRP appears to contribute to the formation of foam cells (Gomes, 2002). It has been measured in many stages of the coronary artery disease process and has proved a highly reliable predictor of cardiovascular risk. An article published in the American Magazine Fortune on June 24, 2004 established that most myocardial infarctions are caused by inflammation in the arteries rather than coagulation problems.

CRP levels are frequently higher in such individuals with established vascular disease, those who present with acute coronary syndrome, those who are considered obese by clinical standards, those who have uncontrolled diabetes mellitus or metabolic syndrome, and those who smoke. But yet there is no specific treatment for an elevated CRP. According to Ridker, who
aggressively studies CRP, CRP should be measured routinely with a lipid profile in the primary prevention setting. Ridker and his laboratory group have established cut-off points for CRP levels. A CRP level of less than 1mg/L is normal (Abrams, 2003). A CRP level of 1-3 mg/L is intermediate and a CRP level of greater than 3 mg/L is abnormally high (Abrams, 2003). Ridker also determined that a baseline CRP level of greater than 10 mg/L suggests a non-cardiovascular source of inflammation, such as infection or a chronic inflammatory state.

Three major questions must be answered before routine clinical application of inflammatory markers is advocated. First, does the marker independently predict a risk? Second, are specific therapies available to reduce levels of the inflammatory marker, and third, do therapies that lower plasma levels of the inflammatory markers also reduce cardiovascular risk? Of the questions above, CRP currently meets most, if not all of the criteria. It has already been discussed how CRP is an independent inflammatory marker for cardiovascular disease risk. And it is already used in the clinical realm. Recent clinical studies have shown that statin therapy can lower CRP levels (Blake and Ridker, 2003). There is currently no data that states lowering CRP levels decreases cardiovascular events or improves survival (Blake and Ridker, 2003).

Although CRP seems like a well-defined inflammatory marker, it actually is a relatively non-specific marker of inflammation (Pepys and Hirschfield, 2003). CRP response is triggered by many disorders unrelated to cardiovascular disease (Table 2). When the levels of CRP are elevated it is hard to associate one disease process to the elevation without other clinical information, such as, history and physical. In conclusion, CRP is a good inflammatory marker, but it is not as specific for cardiovascular disease as MPO is understood to be.
Anti-Neutrophil Cytoplasmic Antibody (ANCA)

Anti-Neutrophil Cytoplasmic Antibody (ANCA) is related to MPO. The target molecule of MPO-ANCA is a lysosomal enzyme, MPO, that usually acts to kill bacteria, viruses, and fungi and that causes damage to the tissue due to the toxicity of its product, hypochlorite. This matter was discussed earlier in this review. MPO is more known as an activated neutrophil, a neutrophil that gets activated in host defense or during an inflammatory process, while ANCA is known as an antibody against neutrophils. There are two profiles of ANCA. They are perinuclear ANCA (P-ANCA) and cytoplasmic ANCA (C-ANCA) (Suzuki, 2001). The target molecules for P-ANCA and C-ANCA are myeloperoxidase and proteinase-3 (PR3), respectively (Suzuki, 2001). ANCA is known to play an important role in pathogenesis in the vessel wall by activating neutrophils and increasing adhesivity between neutrophils and endothelial cells via adhesion molecules (Suzuki, 2001).

The PR3-ANCA complex is associated with lung disease, such as Wegener’s granulomatosis, while the MPO-ANCA complex is associated with renal disease or vasculitides, such as glomerulonephritis or microscopic polyangiitis (Bartunkova, Tesar, Sediva, 2003). Wegener’s granulomatosis is described as a granulomatous inflammation involving the respiratory tract and necrotizing vasculitis involving small vessels (Stegeman, 2002). Microscopic polyangiitis is described as necrotizing vasculitis with few or no immune deposits mostly affecting small vessels, but some medium sized vessels may be involved (Stegeman, 2002). Both of these conditions are closely related and share the vasculitide involvement of small sized vessels. The most important distinction between the two diseases is the severity of the respiratory tract infection and the presence of granulomatous tissue formation (Franssen,
Wegener’s granulomatosis is more severe in the upper respiratory tract than microscopic polyangiitis. Microscopic polyangiitis is usually mild and limited to rhinitis and/or sinusitis (Franssen, 2000).

MPO-ANCA is not only associated with renal disease. Elevation in the levels of MPO-ANCA have been observed in patients with Kawasaki disease, systemic lupus erythematos (SLE), vasculitis, and rheumatic disease (Teo, 1998). This complex has an affect on blood vessels causing damage and the infiltration of inflammatory cells. Because of its association to so few diseases, ANCA is now a test performed routinely in diagnostic immunology laboratories.
15-Lipoxygenase is another inflammatory marker that has not been studied as much as CRP. Its pathophysiology in plaque formation is known and some experimental studies show that its mRNA and proteins have been detected in human atherosclerotic plaques.

15-Lipoxygenase is an intracellular enzyme that peroxidizes polyunsaturated fatty acids into oxygenated lipids with potent biological effects (Heinecke, 1998). As mentioned in the beginning of this review, oxygenated lipids get engulfed by macrophages, which is the beginning of the formation of the atherosclerotic plaque. Lipoxygenase may promote atherosclerosis by several different mechanisms. The enzyme might directly oxidize LDL without having to be triggered by some other component first (Heinecke, 1998). Or the reverse, 15-lipoxygenase may indirectly oxidize LDL by releasing or triggering secondary components that promote the oxidation of LDL. Finally, oxygenated products produced by lipoxygenase may have a powerful biological effect on the artery wall without the direct involvement of LDL oxidation (Heinecke, 1998).

In conclusion, 15-lipoxygenase is not a good inflammatory marker to follow. It has not been widely explored, therefore, not much information is available. Its deleterious effects on the vessel wall is known, but its process of oxidizing LDL is yet to be determined.
**Interleukin-6 (IL-6)**

The induction and regulation of immune responses involve the interactions of multiple cell types. These cell types include lymphocytes, monocytes and other inflammatory cells that affect the endothelium. Many interactions require direct cell-cell contact while others are mediated by short-acting soluble mediators called cytokines. Cytokines are low molecular weight polypeptides that are secreted by lymphocytes and effector cells (Kumar, 2003).

Cytokines induce their effects in three ways: 1) they act on the same cell that produces them (autocrine cells), 2) they affect other cells in their vicinity (paracrine cells), or 3) they affect cells systematically (endocrine cells) (Kumar, 2003). Interleukin-6 is a multifunctional cytokine with endocrine and paracrine effects (Bennet et al., 2003). It mediates several functions in host defense and promotes atherogenesis, dyslipidemia, hypertension, and insulin resistance through activated macrophages and lymphocytes. It is also an important stimulant of C-reactive protein (CRP). There are several different types of cytokines. The cytokines are organized into five categories based on their general properties. Interleukin-6 falls under the category of cytokines that mediate innate immunity (Kumar, 2003). It is one of the three inflammatory markers that exist under this category. The other two include tumor necrosis factor (TNF) and interleukin-1 (IL-1). These cytokines initiate nonspecific proinflammatory responses, such as the activation of endothelium and mononuclear inflammatory cells and induction of acute-phase reactant synthesis by the liver (Kumar, 2003). Some of their specific effects on the body that promote atherosclerosis are the increase in leukocyte adherence and an increase in procoagulant activity (Rosenfeld, 1994). There is also an increase in acute phase proteins, an increase in fibroblast proliferation and an increase in collagen synthesis (Rosenfeld, 1994).
A study was performed to determine the association between elevated IL-6 and CRP to the risk of developing myocardial infarction. The studies results support the role of inflammatory markers in coronary artery disease, but it still remains unclear whether IL-6 has a causative function or is just a marker for a previous myocardial infarction (Bennet et al., 2003). High IL-6 readings could just be due to increased IL-6 synthesis in response to inflammation related to the myocardial infarction. IL-6 is equally, likely rise due to exposure to infections or other immune stimuli in conjunction with blood sampling (Bennet et al., 2003).

In conclusion, circulating IL-6 levels are likely to have important systemic effects that increase the hazardous effects of common risk factors for coronary artery disease. Since the exact reason for the elevation of IL-6 is still unclear, and it is a non-specific inflammatory marker for coronary artery disease, IL-6 is not a reliable inflammatory marker for coronary artery disease.
Homocysteine

Homocysteine is another inflammatory marker associated with atherosclerosis. Currently, estimates indicate that as many as 40% of patients with coronary, cerebrovascular, or peripheral vascular disease have elevated concentrations of homocysteine (Temple, Luzier, Kazierad, 2000). Epidemiological data have demonstrated that elevated plasma homocysteine concentrations are an independent risk factor for atherosclerosis and thrombosis. Elevated homocysteine concentrations have been associated with other disease processes, such as, psoriasis, pernicious anemia, diabetes mellitus, and chronic renal failure (Temple et al., 2003).

Homocysteine is a nonessential, sulfur-containing amino acid produced from the demethylation of methionine, an essential amino acid from dietary protein (Temple et al., 2003). Elevated homocysteine concentrations have many damaging effects on human blood vessels, which causes it to be an independent risk factor for atherosclerosis. Hyperhomocysteinemia may injure endothelial cells, alter platelet activity, inhibit vasodilatation of vessels, or cause thrombogenesis (Temple et al, 2003). Homocysteine causes endothelial injury when it fails to become fully metabolized. The only way that homocysteine does not get fully metabolized is if there is an abnormality that causes it to be oxidized (Robinson, Mayer, Jacobsen, 1994). This abnormal oxidation occurs when the levels of homocysteine are too high in human plasma. Auto-oxidation of homocysteine creates superoxide ions and hydrogen peroxide, which in turn, oxidize LDL (Robinson et al., 1994). Oxidized LDL is easily engulfed by macrophages to from foam cells, which are the beginning products of the atherosclerotic process, as discussed earlier. Even the complete metabolism of homocysteine has an effect on the development of an atherosclerotic plaque. The by-products of metabolized homocysteine, such as homocysteine thiolactone, reacts
with LDL to form aggregates that are readily taken up by macrophages (Robinson et al., 1994). Homocysteine thiolactone is also known to increase platelet aggregation by stimulating the release of thromboxane and prostaglandin-platelet aggregates.

Recent studies demonstrate that homocysteine can react with nitric oxide and cause vascular dysfunction. One of two things can happen. First, homocysteine can combine with nitric oxide to form S-nitrohomocystiene, a molecule that has vasodilatory and antiplatelet effects and may be one way to detoxify excess homocysteine in the blood (Lee and Frenkel, 2003). This reaction does not cause endothelial dysfunction. Second, high levels of homocysteine can indirectly impair the synthesis of nitric oxide through the inhibition of nitric oxide synthase (Lee and Frenkel, 2003). This reaction impairs endothelial dependent vasodilatation. Studies have shown an increase in mortality in patients with elevated homocysteine concentrations compared to healthy individuals, and a strong correlation between myocardial infarctions and hyperhomocysteinemia in premenopausal women (Temple et al., 2000). In addition, another study showed a positive correlation between high homocysteine concentrations and the prevalence of type II diabetes mellitus and end stage renal disease. A study performed by the Cleveland Clinic in December of 1994 showed that fasting homocysteine levels were higher in patients with coronary artery disease than the controls with normal angiographic findings (Robinson et al., 1994). Levels were also found to be higher in patients with cerebrovascular disease and peripheral vascular disease compared to controls.
There is a genetic disorder called hyperhomocysteinemia, that is a rare autosomal recessive disorder that results from defective activity of an enzyme known as, cystathionine-3-β-synthase (Mehrabi et al., 2002). Patients have severe hyperhomocysteinemia and many abnormalities, which include, early death from myocardial infarction, stroke, or vascular thromboembolism. Levels have been set to separate normal, mild, moderate, and severe homocysteinemia. Normal is set at 5-15 µmol/L. Mild ranges from 16-30 µmol/L, moderate ranges from 31-100 µmol/L, and anything >100 µmol/L is considered severe homocysteinemia (Lee and Frenkel, 2003). Because so many factors such as, age, gender, diet, smoking, and genetic abnormalities have impacts on homocysteine levels, these levels have not been set as standard laboratory values.

Are there any treatment regimens to lower the levels of homocysteine in the blood? A study done by Jonathan Abrams and Andrew Chai from the Department of Internal Medicine of the University of New Mexico concluded that homocysteine concentrations are reduced with combinations of folic acid, Vitamin B₆, or Vitamin B₁₂ (Chai and Abrams, 2001). Homocysteine is metabolized by one of two ways: transsulfuration or remethylation. If it is fully metabolized, it causes less damage to the endothelium of blood vessels than when it is not metabolized and eventually oxidized. If it is remethylated, it is converted back to methionine by one of two ways. One pathway is referred to as the folate cycle. A methyl group is simply transferred to homocysteine from 5-methyltetrahydrofolate (Kircher and Sinzinger, 2000). This reaction is catalyzed by methionine synthase, which is dependent on Vitamin B₁₂ and folate. The other pathway is not Vitamin dependent. In the transsulfuration pathway, homocysteine is broken down and its metabolites are excreted in the urine. This pathway requires cystathionine β
synthase and cystathionase, both of which require Vitamin B$_6$ as a cofactor (Chai and Abrams, 2001). Therefore, deficiencies in Vitamin B$_6$, B$_{12}$, and folate could have a significant effect on plasma homocysteine levels.

In conclusion, lowering LDL, smoking cessation and controlling elevated blood pressure are all ways to reduce risk of developing coronary artery disease. Avoiding these risk factors is the most effective means of preventing atherosclerotic disease (Chai and Abrams, 2001).
Discussion

The focus of this review was to compare and contrast several different inflammatory markers to determine which one is superior as an independent risk factor of atherosclerosis, or more specifically, coronary artery disease. Of these inflammatory markers, CRP, ANCA, and homocysteine are available to order as laboratory studies in the clinical realm. Although none of these inflammatory markers have met the criteria to be a laboratory study, all three are acceptable for use. First, there must be an abundant amount of data from several large-scale studies that link the inflammatory marker to the prediction of risk. Second, levels of the marker must decrease with an assessed improvement of risk. Third, there must be a standardized assay available for the marker. Finally, screening for the marker should, potentially direct therapeutic intervention.

CRP seems to meet the majority of these criteria. It is the most widely studied inflammatory marker. It was once thought that CRP was just an inactive marker of vascular inflammation, but recent studies show that it plays an active role in the pathogenesis of atherosclerosis (Gomes, 2002). Several studies have showed that the levels of CRP are frequently higher in such individuals with established vascular disease, acute coronary syndrome, obesity, uncontrolled diabetes mellitus, metabolic syndrome or those who smoke (Jeong et al., 2003). But there is still no treatment for elevated CRP. It is still unclear what exactly causes the elevation of CRP.

Myeloperoxidase is among the many inflammatory markers that have been studied. MPO is an interesting inflammatory marker because its normal action in the human body is to protect it from infection whether that infection is caused by a fungus, a bacteria, or a virus. While MPO is fighting infection from its host, it can also cause damage to the host. It does this
by forming free radicals and oxidants that can cause host tissue damage at sites of inflammation. So far, MPO is understood to have the ability to react with hypochlorous acid, tyrosine, or nitrogen. The product of these reactions, then oxidize LDL for easy uptake into atherosclerotic lesions. MPO also affects vasodilatation by oxidizing all the available nitric oxide. Although MPO has not been widely studied, its affects on the atherosclerotic process and coronary artery disease is clearly understood.

Antineutrophil cytoplasmic antibody (ANCA) is an inflammatory marker that is not a good predictor of risk of coronary artery disease. It is more associated with kidney and lung inflammation. It is a good predictor of glomerulonephritis and microscopic polyangiitis in the kidneys and wegener’s disease in the lungs.

Several studies show that 15-lipoxygenases mRNA and proteins have been found in atherosclerotic plaques, but since it has not been widely studied, its presence in the plaque is still not understood (Heinecke, 1998). Interleukin-6 activates the endothelium and acute phase reactant synthesis in the liver when triggered by inflammation. But like 15-lipoxygenase, it has not been widely studied, so its association to coronary artery disease and atherosclerosis is not well known.

Homocysteine is tightly linked to the development of atherosclerosis. It has been shown to injure endothelial cells, alter platelet activity, inhibit vasodilatation of vessels, and cause thrombosis (Temple et al., 2003). It has also been estimated that as many as 40% of patients with vascular have elevated homocysteine levels (Temple et al., 2003). The pathogenesis of how homocysteine affects the endothelium of blood vessels is well known. Elevated levels of homocysteine cause it to be auto-oxidized, which in turn, cause damage to blood vessels. It becomes elevated in the blood when it is no longer being metabolized. The metabolism requires
the presence of specific vitamins; vitamin B₆, vitamin B₁₂, and folic acid to function properly. Homocysteine is a good inflammatory marker to follow when watching the risk of developing coronary artery disease. It is an independent marker that only affects the vasculature of the body, therefore it is relatively specific for vascular disease.
In summary, the use of MPO, CRP and other inflammatory markers to predict the risk of developing coronary artery disease is a novel idea. CRP is still the marker most widely used to determine a patient’s risk of developing CAD. It is used the most because it has been studied the most and there is an abundance of information that shows that CRP gets released during an inflammatory process, such as the atherosclerotic process. But, information also shows that it is not a specific marker for CAD (Table 2). Myeloperoxidase is a better inflammatory marker to use. MPO has been shown to be an excellent marker for determining prediction of CAD (Brennan et al., 2003). It is released by leukocytes, one of the acute phase reactants to an inflammatory process. The first phase of the atherosclerotic process occurs when the endothelium of blood vessels gets injured and surrounding leukocytes get triggered. The activation of these leukocytes causes MPO to form free radicals and oxidize LDL. This makes MPO one of the first active reactants in the atherosclerotic process. Therefore, if laboratory studies could measure levels of MPO then, atherosclerotic disease and coronary artery disease could potentially be prevented. MPO is elevated and catalytically active in vulnerable plaques. It has been linked to factors affecting the development and stability of a plaque. MPO has been found in all stages of the atherosclerotic process (Figure 1). Its focus is mainly contributing to the atherosclerotic plaque, although it also acts in the host’s defense (Table 1).

A study was done among patients who were negative for troponin, a cardiac enzyme that is quick to rise in the event of a myocardial infarction, at their presentation. Results showed that elevated MPO at presentation was predictive of subsequent major adverse cardiovascular events, although the patient had negative troponin levels. CRP levels predicted the risk of MI at
presentation of the cohort, but did not predict major adverse cardiac events in the group that was
negative for troponin (Brennen et al., 2003). This indicates that MPO may elevate faster and be
more specific for CAD than both CRP and troponin. A separate study had results supporting the
role of CRP as an inflammatory marker for CAD. The study showed that the levels of CRP did
not change after balloon angioplasty in patients with stable or unstable angina who had normal
pre-procedural levels of CRP, but CRP did increase after angioplasty in unstable patients with an
elevated CRP at baseline (Blake and Ridker, 2003). Moreover, even diagnostic angiography
without intervention caused an increase in CRP levels among patients with elevated levels at
baseline. This suggests that elevated levels of CRP may be a marker of the body’s
responsiveness of the inflammatory process to even small stimuli, such as balloon angioplasty or
an angiogram. Therefore, this raises the question as to which raised the levels of CRP, the small
stimuli or the atherosclerotic disease process. CRP may be a more specific predictor of plaque
vulnerability and hence future cardiovascular events, rather than the extent of atherosclerosis
(Blake and Ridker, 2002). Therefore, patients already have atherosclerotic plaques present and
prevention is out of the question. Although MPO has not been extensively studied, it has been
determined that MPO is a specific inflammatory marker that independently predicts the risk of
developing coronary artery disease (Brennan et al., 2003).
References


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Table 1- Biological Activities of MPO

- Bactericidal through enzymatic production of hypochlorous acid
- Functions as a peroxidase to produce free radicals causing lipid peroxidation of LDL
- Produces oxidants that activate cell-signaling pathways
- Produces advanced glycation end products at sites of inflammation
- Internalized by endothelial cells causing increased free radical production
- Tyrosine nitration of vascular ECM proteins

“Table 1. Biological activities of MPO”

Note. From “Understanding the pathogenesis of ANCA: Where are we today?,” by Gloria A. Preston, Jin Jia Yang, Hong Xiao and Ronald J. Falk, Cleveland Clinic Journal of Medicine, 69, p. SII-52. Copyright- name not provided
**Table 2- Routine clinical uses of CRP measurements**

Screening test for organic disease

Assessment of disease activity in inflammatory conditions
- Juvenile chronic (rheumatoid) arthritis
- Rheumatoid arthritis
- Ankylosing spondylitis
- Reiter disease
- Psoriatic arthropathy
- Vasculitides
  - Behçet syndrome
  - Wegener granulomatosis
  - Polyarteritis nodosa
  - Polymyalgia rheumatica
- Crohn disease
- Rheumatic factor
- Familial fevers including familial Mediterranean fever
- Acute pancreatitis

Diagnosis and management of infection
- Bacterial endocarditis
- Neonatal septicemia and meningitis
- Intercurrent infection in systemic lupus erythematosus
- Intercurrent infection in leukemia and its treatment
- Postoperative complications including infection and thromboembolism

Differential diagnosis/classification of inflammatory disease
- Systemic lupus erythematosus vs. rheumatoid arthritis
- Crohn disease vs. ulcerative colitis

“Table 2. Routine clinical uses of CRP measurement”

Table 3- Possible specific associations of CRP with cardiovascular disease

- CRP binds selectively to LDL, especially “damaged” LDL
- CRP is deposited in most atherosclerotic plaques
- Aggregated and/or ligand-complexed CRP activates complement and can be proinflammatory
- CRP is co-deposited with activated complement in all acute myocardial infarction lesions
- Human CRP and complement increase final myocardial infarction size in experimental models

CRP may therefore be a therapeutic marker

“Table 3. Associations of CRP to cardiovascular disease”

“Figure 1. Presence of specific inflammatory markers at each stage of atherosclerosis”

References


Preston, Gloria A., Yang Jia Jin, Xiao, Hong, Falk, Ronald J. Understanding the Pathogenesis of ANCA: where are we today? *Cleveland Clinic Journal of Medicine*, 69(suppl II), S51-54.


