Inflammatory Biomarkers and Atherosclerosis

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Summary

Atherosclerosis has been regarded as a form of chronic vascular inflammation. Numerous biomarkers associated with inflammation have been identified as novel targets to monitor atherosclerosis and cardiovascular risk. C-reactive protein (CRP) is one of the most actively studied and established inflammatory biomarkers for cardiovascular events. However, CRP response is triggered by many disorders unrelated to cardiovascular disease, which interferes with the clinical application. This review describes established and traditional inflammatory biomarkers including CRP as well as novel inflammatory biomarkers reflective of local atherosclerotic inflammation. In addition, we focus on the potential usefulness of inflammatory biomarkers in developing anti-atherosclerotic therapeutic approaches. (Int Heart J 2016; 57: 134−139)

Key words: Epicardial adipose tissue (EAT), Inflammation, MicroRNA (miRNA), Pentraxin 3 (PTX3)

Atherosclerosis has been regarded as a form of chronic vascular inflammation since Ross and colleagues presented the “Response to Injury Hypothesis” in 1973, which asserted that atherosclerosis is a series of biological responses triggered by injury to the endothelial tissue. The onset of atherosclerosis is characterized by infiltration of low-density lipoprotein (LDL) into the arterial intima, where it undergoes oxidation. Subsequently, leukocytes (mainly monocytes and lymphocytes), recruited by adhesion molecules expressed by inflamed endothelium, penetrate into the intima and produce a variety of inflammatory cytokines and chemokines. Infiltrating monocytes differentiate into macrophages, ingest oxidized LDL, and slowly turn into large foam cells, which in turn promote the growth of plaques. Macrophages and foam cells secrete matrix metalloproteinases (MMPs), which assist in the degradation of the extracellular matrix, thereby weakening the fibrous cap and destabilizing the atherosclerotic plaque. Eventual rupture of the atherosclerotic plaque causes thrombus formation. If the plaque rupture occludes the coronary artery, acute coronary syndrome will develop. Different types of inflammatory reactions are thus involved in the initiation and progress of atherosclerosis. Consequently, various inflammatory biomarkers have been studied extensively with the goal of improving cardiovascular risk prediction.

Current Status of Inflammatory Biomarkers for Atherosclerosis

Among the biomarkers reported to date, C-reactive protein (CRP) is probably the most promising indicator for vascular inflammation. CRP, one of the acute-phase proteins, is primarily produced in the liver during episodes of acute inflammation or infection. CRP is also detected at local sites of inflammation or injury. However, the levels of CRP produced in response to vascular inflammation are generally very low. High-sensitivity CRP (hs-CRP) assay methods have therefore been developed to detect small changes in CRP concentrations. Hs-CRP is independent of the Framingham risk score that captures classic cardiovascular risk factors. The combination of hs-CRP and Framingham risk score has been shown to achieve a higher predictive accuracy than that derived by either measure alone. High levels of hs-CRP have been associated with a higher incidence of cardiovascular events in patients with multiple complex plaques. However, attention should be paid to the poor specificity of this biological indicator. Hs-CRP levels are known to be significantly influenced by infection and tissue damage, as well as obesity, old age, hypertension, diabetes mellitus, smoking, and other cardiovascular risks. Cytokines are a class of high molecular weight polypeptides that deliver cell signals in the context of immunological responses, inflammatory reactions, hematopoiesis, and other basic biological functions. For example, interleukin (IL)-6 and tumor necrosis factor (TNF)-α, members of the inflammatory cytokine family released from vascular smooth muscle cells, endothelial cells, monocytes, macrophages, and so forth, have been shown to be deeply involved in atherosclerosis. In an observational study by Ridker and colleagues that followed up 14,916 healthy male adults for 6 years, blood IL-6 concentrations were significantly elevated in individuals who developed myocardial infarction as compared with those who did not. In the 7-year prospective Health ABC cohort study that examined 2,225 community residents aged 70 to 79 years old, IL-6 was correlated with the incidences of ischemic cardiac disease, stroke, and heart failure events. Chemokines, such as IL-8, are a group of cytokines that form a chemotactic gradient directing leukocytes towards an injury site. Chemokines are produced in large amounts at inflammatory sites, and recruit leukocytes to the damaged tis-
sue. Some chemokines, such as IL-8, interferon-inducible protein of 10 kD (IP-10), and monocyte chemoattractant protein 1 (MCP-1), are believed to play a significant role in the development of atherosclerosis. However, as is the case with CRP, such chemokines are not specific to the formation or progress of atherosclerosis, and careful attention should be made in interpreting the data because chemokine levels are elevated in response to inflammation associated with heart failure and other disorders.

It is commonly held that atherosclerosis is triggered by arterial infiltration and oxidation of LDL, as described earlier. Oxidized LDL particles are readily taken up by macrophages, and cause transformation of macrophages into foam cells. They are also known to accumulate in atherosclerotic lesions. These findings support the idea that oxidized LDL mediates the development and progression of atherosclerosis, and it is therefore looked upon as an effective biological indicator for the disease. In practice, these autoantibodies can therefore be viewed as an in vivo marker of LDL oxidation. However, oxidized LDL particles consist of macromolecules of varying composition, and antibodies against oxidized LDL recognize only part of the three-dimensional configuration of the molecules. Consequently, the assay sensitivity, selectivity, and specificity for such antibodies require careful evaluation. Oxidation of LDLs results in the formation of reactive aldehyde products. Of these, malondialdehyde-modified (MDA-) LDL is commonly utilized as an indicator for oxidized LDL, because MDA is the most abundant aldehyde arising from lipid peroxidation, and its chemical structure is readily identifiable. A recent clinical study has demonstrated that circulating MDA-LDL levels are associated with the presence of thin-cap fibroatheromas determined by optical coherence tomography in patients with coronary artery disease.

Researchers have examined the clinical significance of lectin-like oxidized LDL receptor-1 (LOX-1), a major receptor for oxidized LDL in many cell types, such as endothelial cells, monocytes/macrophages, platelets, and smooth muscle cells. LOX-1 mediates plaque destabilization through apoptosis of smooth muscle cells and enhancement of MMP secretion by mature foam cells. It also mediates thrombosis after plaque rupture via activation of platelets. LOX-1, expressed on the cell surface of macrophages and smooth muscle cells, can be cleaved by proteases and transformed into soluble forms. The blood levels of soluble LOX-1 have been shown to rise earlier than those of cardiac troponin T during the onset of acute coronary syndrome, and are useful for its diagnosis.

Researchers have shown that migrating monocytes, T cells, and other types of leukocytes attach to the endothelial cell layer, and then gradually enter into the subendothelial space. Such heterotypic adherence is facilitated by the binding of the surface-expressed adhesion molecules of different types of cells acting as ligands and their receptors. Soluble forms of adhesion molecules have been shown to circulate in the blood, and the accumulation of such molecules on the cell surface is of pathological significance. In fact, the serum levels of soluble forms of cell adhesion molecules expressed on the endothelial cell surface, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin, have been shown to increase in association with inflammation in atherosclerotic lesions. In a study conducted by the present authors and colleagues, the plasma concentrations of the glycoprotein platelet-endothelial cell adhesion molecule-1 (PECAM-1) were significantly higher in patients presenting within 3 hours after myocardial infarction and patients with unstable angina pectoris, as compared with patients with exertional angina pectoris and controls. The results of this prior study suggested the clinical usefulness of PECAM-1 for the early diagnosis of acute coronary syndromes.

It is estimated that about 60% of cases of acute coronary syndrome are caused by plaque ruptures, which do not necessarily involve significant stenosis. The characteristics of vulnerable or unstable plaques include cholesterol abundance, thinning of the fibrous cap, cap with a predominance of inflammatory cells (e.g., macrophages, T lymphocytes), presence of a necrotic core, and mild to moderate vasa vasorum neovascularization, possibly leading to intraplaque hemorrhage. In particular, MMPs and other matrix-degrading enzymes produced by macrophages have been found to play an important role in fibrous cap thinning and eventual plaque rupture, and consequently have been investigated as possible inflammatory markers. MMP-1, MMP-3, MMP-8, and MMP-9 are primarily detected in the shoulder regions of vulnerable plaques, indicating their involvement in fibrous cap thinning. Studies revealed that patients with acute coronary syndrome had increased levels of MMP-1, MMP-2, MMP-3, and MMP-9; in addition, a significantly higher cardiovascular mortality rate was reported for patients with high plasma MMP-9 concentrations in an observational study of patients with coronary artery disease with a mean follow-up period of 4.1 years.

Myeloperoxidase (MPO), an important enzyme that catalyzes the formation of potent oxidants by neutrophils and macrophages, has been shown to induce LDL oxidation in blood with high antioxidant status. In fact, an increase in local oxidative stress is observable at the sites of plaque inflammation in connection with neutrophil and macrophage activation in unstable plaques, generation of reactive oxygen species, and extracellular release of MPO. Serum MPO level is an independent risk factor for coronary artery disease and has been shown to predict cardiovascular events in patients presenting to the emergency department with active chest pain. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an inflammatory enzyme that catalyzes the hydrolysis of oxidized phospholipids into lysophospholipids on the surface of lipoproteins. Approximately 80% of serum Lp-PLA2 is found on the surface of LDL particles, migrating across the vascular endothelial cell layer together with LDL. Coronary arterial lesions have a high concentration of Lp-PLA2 in the necrotic core, which suggests the involvement of Lp-PLA2 in plaque rupture. In a recent meta-analysis of 79,036 participants from 32 prospective studies, Lp-PLA2 activity and mass showed roughly linear associations with the risk of coronary heart disease, and the magnitude of the risk was similar to that associated with non-HDL cholesterol and systolic blood pressure.

**Quest for Novel Predictive Markers**

**Pentraxin 3:** As explained above, hs-CRP has been proven to be the most reliable biomarker for atherosclerosis, despite its non-specific correlation with infections and chronic inflammatory diseases. Pentraxin 3 (PTX3), another acute-phase response protein of the pentraxin family that includes CRP, has
Figure 1. Inflammatory markers secreted from various cells in atherosclerotic lesion. Primary proinflammatory markers such as cytokines, pentraxin-3, MMPs, LOX-1, and microRNAs are produced abundantly by various cells in atherosclerotic lesions including macrophages, endothelial cells, and vascular smooth muscle cells in atherosclerotic lesion, while CRP is mainly produced in the liver via the messenger cytokine IL-6. These inflammatory markers are released into the circulation at different stages in atherosclerosis from different mechanisms. CRP indicates C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; and MMP, matrix metalloproteinase.

Figure 2. Plasma pentraxin (PTX) 3 concentrations and coronary plaque vulnerability. There was no significant difference in peripheral plasma PTX3 concentrations between angina pectoris (AP) patients and control (upper left). Plasma PTX3 concentrations in the coronary sinus were higher in patients with AP than in control subjects (lower left). Furthermore, peripheral plasma PTX3 concentrations negatively correlated with CT density (upper right). In contrast, no correlation was seen between CT density and peripheral plasma concentrations of high sensitive-CRP (lower right). (Soeki T, et al. J Cardiol 2011; 58: 151-7. 31)

MicroRNA: MicroRNA molecules (miRNAs) are small (20–25 bases), single-stranded, non-coding RNAs produced endogenously. These non-coding RNAs bind to complementary sequences in the 3'-untranslated regions of the target miRNAs, and modulate the expression of genes by suppressing their translation. 38 In 2008, miRNAs were detected in circulating blood in a remarkably stable form despite the presence of ribonucleases in the blood. 37 It is believed that miRNAs are secreted in exosomes, small vesicles with diameters between 30 and 100 nm. 39

After the discovery of stable serum miRNAs, rapid progress was made in various fields of miRNA research, including cardiovascular diseases. For instance, clinical studies detected significant changes in the blood levels of the following miRNAs in patients with cardiovascular diseases, in particular coronary artery disease (Table): 1) miR-1, miR-133a, miR-208a, miR-208b, and miR-499 in patients with acute myocardial infarction; 2) miR-17, miR-92a, miR-126, miR-133, miR-140, miR-145, miR-155, miR-182, and miR-208a in patients with coronary arterial diseases; and 3) miR-18b, miR-122, miR-126, miR-129-5p, miR-423-5p, miR-499, miR-622, and miR-654-3p in patients with heart failure. 40

Moreover, in a study that compared the concentrations of circulating miRNAs in coronary venous sinus and aortic plasma samples collected simultaneously (transcoronary concentration gradients), muscle-enriched miR-499 and miR-133a were significantly elevated during transcoronary passage in patients with acute coronary syndrome, whereas vascular miR-126 decreased during transcoronary passage. 40 These results
suggested that miR-499 and miR-133a are released into the coronary circulation during myocardial injury, while miR-126 is consumed during transcoronary passage, providing interesting insight into the role of miRNAs in atherosclerotic diseases (notably acute coronary syndrome). The involvement of vascular miR-126 has also been suggested in inflammation control and leukocyte adherence. During the apoptotic process in vascular endothelial cells, miR-126 is released in apoptotic bodies, which are shown to confer protection against atherosclerosis. Furthermore, we recently have found that plasma miR-100 levels are higher in the coronary sinus than in the aorta in patients with angina pectoris. We also have found that transcoronary concentration gradients of circulating miR-100 are significantly correlated with the percentage of lipid volume and fibrous volume determined by integrated backscatter intravascular ultrasound. These findings suggest that miR-100 might be released into the coronary circulation from vulnerable coronary plaques (Figure 1) and might stabilize plaques at least in part by suppression of the mammalian target of rapamycin (mTOR) signaling pathway. Altogether, the accumulated body of evidence indicates the potential usefulness of miRNAs as a novel type of atherosclerotic biomarker, promising rapid progress in the coming years.

**Adipose tissue and inflammatory markers:** Adipose tissue, commonly regarded as a major reservoir of energy, is gradually emerging as an active organ secreting various biologically important substances. In fact, visceral fat produces and releases various cytokines, such as IL-6, TNF-α, and plasminogen activator inhibitor-1 (PAI-1), and as the amount of visceral fat increases, blood levels of the anti-inflammatory agent adiponectin decrease. Of note is the relationship between cardiovascular diseases and adiponectin, extensively examined in clinical studies; the risk of coronary arterial disease was about twice as high in individuals with plasma adiponectin concentrations below 4 μg/mL, and the incidence of myocardial infarction was significantly lower in the population with high blood adiponectin levels than in populations with medium and low blood levels of adiponectin. Scientists used to believe that such adipose tissue-derived inflammatory cytokines activated inflammatory signals in remote tissues in the presence of excess free fatty acids, thereby inducing insulin resistance and chronic inflammatory diseases such as atherosclerosis.

However, recent studies conducted by the authors and colleagues suggested that increased levels of activated M1 macrophages in epicardial adipose tissue (EAT) were involved in the development of atherosclerotic inflammation in a paracrine manner. This was supported by the finding that the levels of inflammatory cytokines such as IL-6 and TNF-α were higher in the EAT of patients whose coronary lesions were treated by coronary bypass grafting than in the EAT of heart valve replacement patients who had no coronary artery disease. In addition, examination of the surface antigens of the macrophages infiltrating into the EAT revealed a higher ratio of M1 to M2 macrophages in patients with coronary artery disease than in patients without. These findings are compatible with those of another recent study showing a significant association between

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**Table.** Circulating MicroRNAs as Biomarkers in Coronary Artery Disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Study design</th>
<th>miRNA biomarkers</th>
<th>Source</th>
<th>Age/sex differences between groups?</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI 33 AMI; 33 non-AMI with chest pain; 30 healthy subjects</td>
<td>miR-1, miR-133a, miR-208a, miR-499</td>
<td>Plasma</td>
<td>No</td>
<td>Wang GK, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 33 AMI; 17 healthy subjects</td>
<td>miR-1, miR-133a, miR-133b, miR-499-5p</td>
<td>Plasma</td>
<td>Controls &gt; 10 years younger</td>
<td>D’Alessandra Y, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 32 AMI; 36 non-AMI with AP</td>
<td>miR-208b, miR-499</td>
<td>Plasma</td>
<td>No</td>
<td>Corsten ME, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 14 AMI; 10 healthy subjects</td>
<td>miR-499</td>
<td>Plasma</td>
<td>Controls &gt; 25 years younger</td>
<td>Adachi T, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 93 AMI; 66 healthy subjects</td>
<td>miR-1</td>
<td>Plasma</td>
<td>No</td>
<td>Ai J, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 29 AMI; 42 nonacute CAD</td>
<td>miR-1, miR-133a</td>
<td>Serum</td>
<td>Sex differences</td>
<td>Kuwabara Y, et al</td>
<td></td>
</tr>
<tr>
<td>AMI 31 AMI; 20 healthy subjects</td>
<td>miR-1</td>
<td>Serum</td>
<td>Age- and sex-matched controls used</td>
<td>Cheng Y, et al</td>
<td></td>
</tr>
<tr>
<td>CAD 67 CAD; 31 healthy subjects</td>
<td>Increased: miR-133, miR-208a Decreased: miR-126, miR-17, miR-92a, miR-155, miR-145</td>
<td>Plasma</td>
<td>Controls &gt; 30 years younger and sex differences</td>
<td>Fichtlscherer, et al</td>
<td></td>
</tr>
<tr>
<td>CAD 12 CAD; 12 healthy subjects</td>
<td>miR-140, miR-182</td>
<td>Whole blood</td>
<td>No</td>
<td>Taurino C, et al</td>
<td></td>
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<tr>
<td>CAD 50 CAD; 20 healthy subjects</td>
<td>Increased: miR-135 Decreased: miR-147</td>
<td>PBMC</td>
<td>Age- and sex-matched controls used</td>
<td>Hoeckstra M, et al</td>
<td></td>
</tr>
<tr>
<td>CAD 32 CAD (AP)</td>
<td>miR-100 Associated with coronary plaque vulnerability</td>
<td>Plasma (Aorta, Coronary sinus)</td>
<td>No</td>
<td>Soeki T, et al</td>
<td></td>
</tr>
</tbody>
</table>

AMI indicates acute myocardial infarction; AP, angina pectoris; and CAD, coronary artery disease. (Modified Creemers EE, et al, Circ Res 2012; 110: 483-95.)
studies with over 200,000 participants, shedding light on this issue were published recently. Inflammation or the result of it. Several large-scale studies specific inflammation markers (eg, IL-6, IL-6R) are the cause of therapies targeting specific inflammation markers will open a new door for the development and application of anti-atherosclerotic treatment modalities.

Conclusions: It is widely accepted that atherosclerosis involves the chronic inflammation of vessel walls. This article reviewed the relationship between atherosclerosis and the dynamics of various inflammatory biomarkers reported or extensively investigated to date, focusing on the development and progression of coronary artery diseases. The initial stages of atherosclerosis are often asymptomatic; however, when an atherosclerosis patient becomes symptomatic, his or her quality of life is significantly impaired. Therefore, early detection, diagnosis, and treatment of atherosclerosis must be sought. For that purpose, potential atherosclerosis patients should be classified based on their risk factors. These circumstances underpin the diagnostic values of inflammatory markers, warranting their routine clinical application. Likewise, further studies should focus on the use of inflammatory biomarkers in developing anti-atherosclerotic therapeutic approaches.

The EAT volume and the severity of coronary artery disease. There are currently no simple, reliable blood assays for detecting such changes; we anticipate significant progress in this area.

Therapies Targeting Specific Inflammation Markers

Despite a convincing body of evidence demonstrating the involvement of inflammatory reactions in the onset and progression of atherosclerosis, it was long unknown whether specific inflammation markers (eg, IL-6, IL-6R) are the cause of inflammation or the result of it. Several large-scale studies shedding light on this issue were published recently.

According to a collaborative meta-analysis of 82 clinical studies with over 200,000 participants, the minor allele frequency of the IL-6R gene Asp358Ala was 39%. There was no significant association between Asp358Ala and classical risk factors for atherosclerosis, such as lipid concentrations and blood pressure. Interestingly, for every copy of Asp358Ala inherited, the risk of coronary heart disease was reduced by 3.4% (Figure 3). In addition, for every copy of Asp358Ala inherited, the mean concentrations of IL-6 and IL-6R increased by 14.6% and 34.3%, respectively, whereas the mean concentrations of CRP and fibrinogen decreased by 7.5% and 1.0%, respectively (Figure 3). These results were similar to the pattern of IL-6R inhibition by tocilizumab (humanized anti-IL-6R antibody) observed in randomized clinical trials of patients with rheumatoid arthritis.

In a separate large-scale study of tocilizumab, the Mendelian randomization principle was applied to analyze single nucleotide polymorphisms (SNPs) in more than 133,000 individuals enrolled in 40 studies. An evaluation of 25,458 coronary heart disease cases and 100,740 controls showed that the IL6R rs7529229 SNP was associated with a decreased odds ratio of coronary heart disease events (per allele odds ratio 0.95). This study suggested that IL-6R inhibitors could be a novel class of drugs for the prevention of coronary artery diseases, supporting the idea that the blockade of inflammatory pathways can be an effective modality for reducing cardiovascular risk. Efforts targeting specific inflammation markers will open a new door for the development and application of anti-atherosclerotic treatment modalities.

References

5. Goldstein JA, Chandra HR, O’Neill WW. Relation of number of complex coronary lesions to serum C-reactive protein levels and major adverse cardiovascular events at one year. Am J Cardiol 2005; 96: 56-60.
10. Virella G, Koskinen S, Krings G, Onorato JM, Thorpe SR, Lopes-Virella M. Immunochemical characterization of purified human...


