S100A12 and the S100/Calgranulins
Emerging Biomarkers for Atherosclerosis and Possibly Therapeutic Targets
Adam Oesterle, Marion A. Hofmann Bowman

Abstract—Atherosclerosis is mediated by local and systemic inflammation. The multiligand receptor for advanced glycation end products (RAGE) has been studied in animals and humans and is an important mediator of inflammation and atherosclerosis. This review focuses on S100/calgranulin proteins (S100A8, S100A9, and S100A12) and their receptor RAGE in mediating vascular inflammation. Mice lack the gene for S100A12, which in humans is located on chromosome 3 between S100A8 and S100A9. Transgenic mice with smooth muscle cell–targeted expression of S100A12 demonstrate increased coronary and aortic calcification, as well as increased plaque vulnerability. Serum S100A12 has recently been shown to predict future cardiovascular events in a longitudinal population study, underscoring a role for S100A12 as a potential biomarker for coronary artery disease. Genetic ablation of S100A9 or RAGE in atherosclerosis-susceptible apolipoprotein E null mice results in reduced atherosclerosis. Importantly, S100A12 and the RAGE axis can be modified pharmacologically. For example, soluble RAGE reduces murine atherosclerosis and vascular inflammation. Additionally, a class of compounds currently in phase III clinical trials for multiple sclerosis and rheumatologic conditions, the quinoline-3-carboxamides, reduce atherosclerotic plaque burden and complexity in transgenic S100A12 apolipoprotein E null mice, but have not been tested with regards to human atherosclerosis. The RAGE axis is an important mediator for inflammation-induced atherosclerosis, and S100A12 has emerged as biomarker for human atherosclerosis. Decreasing inflammation by inhibiting S100/calgranulin-mediated activation of RAGE attenuates murine atherosclerosis, and future studies in patients with coronary artery disease are warranted to confirm S100/RAGE as therapeutic target for atherosclerosis.

Key Words: apolipoprotein E ● atherosclerosis ● coronary artery disease ● inflammation ● S100/calgranulins ● S100A12

With an estimated 17.5 million deaths yearly, cardiovascular diseases remain the leading cause of death in the world.1 Atherosclerosis is a chronic inflammatory process of the vascular wall mediated by activated macrophages, T lymphocytes, B lymphocytes, and smooth muscle cells (SMC).2,3 Accordingly, multiple inflammatory serum markers have been investigated for their association with atherosclerosis.4–7 Although there is evidence that statins reduce high-sensitivity C-reactive protein (hs-CRP), there have been only few and mostly small randomized controlled trials of immune modulating drugs with respect to atherosclerosis.8,9 Therefore, the results of the 2 large ongoing trials, the Cardiovascular Inflammation Reduction Trial (CIRT), testing low-dose methotrexate in patients with prior myocardial infarction (MI) and either diabetes mellitus (DM) or metabolic syndrome, and the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial, testing the monoclonal antibody Canakinumab,10,11 are eagerly awaited.

The multiligand receptor for advanced glycation end products (RAGE) is a strong candidate for a common pathway mediating arterial inflammation.12 RAGE transmits signals released or upregulated in the inflammatory response. The ligands of RAGE include the S100/calgranulin family, advanced glycation end products, Mac-1, high-mobility group box-1, amyloid-β peptide, β-sheet fibrils, and lysophosphatidic acid.12–14 S100A12 is a member of the S100 family of proteins. Serum concentration of S100A12 has been associated with more extensive coronary atherosclerosis in patients with coronary artery disease (CAD), DM, and chronic kidney disease (CKD).15–20 Furthermore, serum S100A12 has also been associated with disease activity in rheumatoid arthritis and psoriatic arthritis and with cardiovascular complications in lupus. Importantly, the anti-inflammatory effects of methotrexate in patients with inflammatory arthritis were associated with reduction in serum S100A12, raising the hypothesis that S100A12 itself could be a therapeutic target.21,22 Although S100A12 is expressed locally in various cells within the inflamed tissue, including epithelial cells, SMCs, and myeloid cells, the exact mechanisms leading to increased serum concentrations...
Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Apo</td>
<td>apolipoprotein</td>
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<tr>
<td>CAD</td>
<td>coronary artery disease</td>
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<tr>
<td>CD</td>
<td>cluster of differentiation</td>
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<td>CKD</td>
<td>chronic kidney disease</td>
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<td>DM</td>
<td>diabetes mellitus</td>
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<td>hs-CRP</td>
<td>high-sensitivity C-reactive protein</td>
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<td>IL</td>
<td>interleukin</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>Q-compounds</td>
<td>quinoline-3-carboxamides</td>
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<td>RAGE</td>
<td>receptor for advanced glycation end products</td>
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<td>SMC</td>
<td>smooth muscle cell</td>
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<td>TLR-4</td>
<td>toll-like receptor 4</td>
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<td>WT</td>
<td>wild-type</td>
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S100 Proteins and Inflammation

There are at least 24 different members of the low molecular weight (10–14 kDa) S100 protein family, all of which bind calcium and have a conserved calcium-binding motif called the elongation factor hand. Despite the structural and functional differences among the different S100 proteins, the shared characteristic of being soluble in 100% saturated ammonium sulfate at neutral pH lead to the name of S100 proteins. Several names are in use for the S100/calgranulins. S100A8 is also known as calgranulin A or myeloid-related protein 8, S100A9 is also known as calgranulin B or myeloid-related protein 14, and the heterodimer is termed S100A8/A9, myeloid-related protein 8/14, or calprotectin. Other names for S100A12 include calgranulin C, or extracellular newly identified RAGE-binding protein, and calcium-binding protein in amniotic fluid-1. S100 proteins are found both within cells and extracellularly. They act on a variety of target proteins and receptors and are involved in diverse functions, including cellular proliferation, differentiation, apoptosis, calcium homeostasis, energy metabolism, regulation of the cell cytoskeleton, and inflammation. When calcium binds to the elongation factor hand, the protein undergoes changes in the spatial conformation, leading to interaction with various target proteins. Some of the S100 protein-mediated events, such as signaling in the extracellular space, where levels of calcium are already high, are unlikely to be calcium-regulated, and the biological activity is therefore more likely regulated by zinc and copper. S100A8, S100A9, and S100A12 share structural and functional homologies and are often named S100/calgranulins.24–25

The S100/calgranulins are expressed in myeloid cells, including neutrophils, monocytes, and dendritic cells. S100A8/9 comprise 40% of the cytosolic protein in neutrophils, and S100A12 comprises 5%.26,27 At the site of inflammation, myeloid cells release S100/calgranulins from the cytoplasm on cell activation, and for example, S100A8/9 are abundantly enriched in neutrophil extracellular traps, and found to be critically important for the clearance of fungal or bacterial infections.28,29 Other effects of S100/calgranulin include cytokine-like effects with activation of cell surface receptors, including RAGE and toll-like receptor-4 (TLR-4).30–32 Therefore, the S100/calgranulins are part of the pro-inflammatory molecules referred to as damage-associated molecular pattern proteins that activate innate immune pathways. Although the S100/calgranulins are considered proinflammatory, there are significant differences between S100A8, S100A9, and S100A12. For example, oxidation of methionines 63 and 83 on S100A9 and of cysteine 42 on S100A8 inhibits both the chemotactic and repellent effects of these proteins on neutrophils, whereas S100A12 is more resistant to oxidation and remains biological active in conditions of increased oxidative stress.33 Interestingly, S100A8 and S100A9 are more sensitive to oxidation compared with low-density lipoproteins and albumin, and it is proposed that the high amounts of S100A8 and 9 present in atherosclerotic plaque might contribute to oxidant scavenging and protect other tissue components from oxidative damage during inflammation.34

S100/calgranulins bind to other receptors in addition to RAGE. For example, S100A12 has been shown to activate mast cells by releasing histamine and other cytokines, including interleukin (IL)-6 and monocyte chemoattractant protein-1, even in the absence of RAGE expression on mast cells.35 Furthermore, S100A12 has chemotactic activity for mast cells and monocytes, which seems to be mediated by a G-protein–coupled receptor and independent of RAGE.36 S100A8 binds TLR-4 and induces MyD88 translocation and IRAK-1 phosphorylation.32 These studies collectively demonstrate that S100/calgranulin are potent stimulator for acute inflammatory responses even in the absence of RAGE. Other studies, including from our laboratory, showed enhanced transcription of genes critically involved in the regulation of inflammation in white blood cells in S100/calgranulin transgenic mice, and this transcription profile was abolished in mice with global RAGE deficiency,37 demonstrating a critical role of RAGE in mediating activation of white blood cells.

RAGE activates multiple signaling pathways, and its activation is important in endothelial dysfunction and inflammation as demonstrated by attenuated atherosclerosis in apolipoprotein (Apo) E null mice lacking RAGE.37 RAGE was identified in 1992 from bovine lung as a protein that bound advanced glycation end products.38 Advanced glycation end products are formed through nonenzymatic glycation or glycooxidations of proteins, lipids, and nucleic acids, and this process is accelerated during oxidative stress and inflammation, although advanced glycation end product also accumulate in tissues during normal aging.

RAGE contains a V-type domain, 2 C-type immunoglobulin-like domains (C1 and C2), a transmembrane spanning helix, and a cytosolic domain for signaling. S100A12 binds to the V-C1 domain, whereas the S100A8- and S100A9-binding sites...
are not known. It has been demonstrated that increasing concentrations of heparin sulfate minimally impaired the binding of S100A12 to RAGE, but competes with binding of S100A9 or S100A8/9 to RAGE.39 Also, glycan-enrichment of RAGE led to a 30-fold higher binding capacity of S100A12 to RAGE, which was specific for S100A12.40 This suggests that S100A12 may have the strongest potential to activate RAGE. When S100A12 binds to RAGE, a proinflammatory response has been demonstrated in vivo and in vitro with the activation of nuclear factor-κ-light-chain-enhancer of activated B cells and increased secretion of IL-6, IL-1β, and tumor necrosis factor-α. (Figure 1).3,30 Blocking RAGE using anti-RAGE IgG or soluble RAGE, a decoy form of the receptor that limits access of ligands to RAGE, reduced inflammation and atherosclerosis.41–43

**S100A12/RAGE in Animal Models of Vascular Disease**

Both RAGE and TLR-4 play important roles in mediating acute and chronic inflammation and are relevant for atherosclerosis, as demonstrated by decreased atherosclerosis in ApoE null mice lacking either RAGE or TLR-4 signaling.37,44 However, RAGE may play a lesser role in normal physiological development and in healthy homeostasis because mice lacking RAGE develop normally and have normal phenotypes, except mildly increased lung fibrosis in mice aged >15 months. In contrast, lack of TLR-4 signaling contributes to the growth of tumors in various organs.45 This potentially makes RAGE or RAGE-mediated signaling an ideal therapeutic target.

Our laboratory took advantage of the fact that S100A12 is not present in mice and developed transgenic mice expressing human S100A12 in SMCs driven by the SM22α promoter. After backcrossing into hyperlipidemic ApoE-deficient mice, we demonstrated that S100A12 transgenic mice had a 1.4-fold increase in atherosclerotic plaque size and a large increase in necrotic core, calcified plaque area, and reduced extracellular matrix compared with ApoE−/− wild-type (WT) mice (Figure 2A–F).46 Importantly, these are histological criteria characteristic for vulnerable plaques and are often associated with plaque rupture and MI in patients with CAD.47

Mechanisms underlying the accelerated pathological vascular

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**Figure 1.** Ligand-mediated activation of the receptor for advanced glycation end products (RAGE) mediates inflammation. A, Ligands of RAGE, including advanced glycation end products (AGEs), high-mobility group protein box 1 (HMGB1), S100/calgranulins, and lysophosphatidic acid (LPA) are released during cell stress and bind to RAGE on monocytes and smooth muscle cells (B) and endothelial cells (C). This activates pathways, leading to the translocation of transcription factor kappa B (NFκB) and increased reactive oxygen species (ROS), which results in a proinflammatory cell phenotype. Endogenous and pharmacological compounds with the ability to bind RAGE ligands are suggested as possible therapeutic interventions to block activation of RAGE and other targets. IL indicates interleukin; and TNF, tumor necrosis factor. Reprinted from Hofman Bowman and Schmidt3 with permission of the publisher. Copyright ©2013, Springer Science+Business Media.
remodeling observed in the transgenic mice with expression of human S100A12 in SMCs are mediated, at least in part, by enhanced oxidative stress. Oxidative stress can activate Runx-2 in SMCs and thereby regulate the expression of many osteoblastic genes promoting vascular calcification.48 Indeed, aortic tissue or cultured primary aortic SMCs from young S100A12 transgenic mice demonstrated increased ROS and upregulation of many bone-regulating genes even before onset of overt vascular calcification.46 Other potential mechanisms of S100/calgranulins promoting vascular calcification might be mediated by binding of S100 proteins to annexins, a dominant group of proteins in matrix vesicles.49 Matrix vesicles are released from SMCs, macrophages, bone, and other cells and provide a nidus for mineral formation once they accumulate in the extracellular matrix.50–52

Transgenic S100A12 mice with normal lipid profiles (ApoE+/+, C57BL6J) develop scant vascular calcification with aging (Figure 2G and H),46 and other risk factors, aside from hyperlipidemia, accelerate medial aortic calcification as demonstrated in S100A12 transgenic mice with surgically induced CKD.53 In contrast, WT mice lacking S100A12 did not demonstrate medial aortic calcification when subjected to the same model of surgically induced CKD. Calcification of the tunica media usually occurs in the absence of macrophages and hyperlipidemia and is thought to involve SMCs that undergo a phenotypic switch to osteoblast-like cells with

Figure 2. Transgenic expression of human S100A12 targeted to smooth muscle accelerates atherosclerotic remodeling with calcification, necrotic core, and elastic fiber degradation in Apolipoprotein (Apo)E null mice (A–C) compared with wild-type (WT/ApoE) littermate mice (D–F) and promotes medial calcification in normolipidemic C57BL6/J mice (G and H). Alizarin Red stain for calcium (A, D, and G), hematoxylin and eosin stain (B and E) and Verhoeff van Giessen stain for elastic fibers (C and F). L indicates lumen. Insert in B magnifies osteoblast-like cell. Reprinted from Hofman Bowman et al.46 Copyright ©2011, Wolters Kluwer Health.
a gain of a procalcific phenotype. Indeed, our data demonstrate that S100A12 increases the susceptibility of SMCs to gain a transcriptional profile typical of osteoblast-like cells. Mechanistically, pretreatment of cultured aortic SMCs with soluble RAGE, a binding domain of RAGE that limits the access of S100A12 and other ligands to RAGE, as well as knockdown of Nox1, a critical component of NADPH oxidase in SMCs, both attenuated calcification of aortic SMCs. These data indicate that S100A12-mediated increase in reactive oxygen species in vascular smooth muscle activates RAGE, Nox1, or a RAGE-Nox1 signaling complex, which initiates an osteogenic phenotype in an appropriate cellular environment. We speculate that S100A12-mediated arterial calcification as seen in mice with hyperlipidemia, or in chronic uremia induced by ureter ligation, is a possible mechanistic explanation for the strong positive association between elevated serum S100A12 in patients with DM or CKD with accelerated vascular calcification and increased adverse cardiovascular events.

Furthermore, transgenic expression of S100A12 in SMCs mediates other pathological vascular features even without CKD or hyperlipidemia. S100A12 modulates the phenotype of SMCs and promotes a switch from a contractile state to a synthetic state with increased oxidant stress and increased production of matrix metalloproteinases 2/9, IL-6, and Smad2, which leads over time to the formation of thoracic aortic aneurysms in S100A12 transgenic mice. The aortic aneurysms in S100A12 mice have similar histological features of aortic remodeling as seen in mice with a fibrillin-1 mutation, suggesting that different signaling pathways converging in aneurysmal disease share similar histopathological features with loss of SMCs, disruption of elastic fibers, and increased extracellular matrix. Although S100A12 is not expressed in healthy human vascular SMCs, there is growing experimental evidence that S100A12 and other S100 proteins are upregulated in SMCs in response to injury, including lipopolysaccharide or endothelial wire injury, and importantly, S100A12 is highly expressed in SMCs in ruptured coronary artery plaques associated with sudden death and in SMCs in dissecting human aortic aneurysms. Although acute MI and aortic dissections are clinically distinctly different vascular diseases, increased apoptosis or other forms of cell death leading to an acute loss of SMCs may possibly be a shared mechanism underlying both vascular diseases characterized by a sudden instability of the vasculature. Indeed, treatment of various cell lines with recombinant S100A8/9 protein was previously shown to induce apoptosis, and our laboratory showed a significant reduction of apoptosis and inflammation in primary aortic SMCs harvested from patients with thoracic aortic aneurysms on silencing of S100A12. This suggests that upregulation of S100A12 in SMCs in response to cellular injury may initiate pathways of cell death and, thereby, potentially renders a previously stable vascular lesion unstable. This view is supported by recent findings of increased incidence of acute type A thoracic aortic dissection to coincidence with influenza activity and by the association of S100A12 with acute MI.

There is growing evidence that implicates enhanced myelopoiesis as a potential mechanism for vascular inflammation relevant to atherosclerosis and MI, with some of the effects mediated by the RAGE axis. Diabetic mice with either chemically (streptozotocin) or genetically (C57BL/6-Lns2+/−) induced islet cell destruction had increased number of circulating Ly6-C+ proinflammatory monocytes and neutrophils, and this leukocytosis was because of enhanced bone marrow myelopoiesis with increased production of granulocyte macrophage progenitors and common myeloid progenitors. The myelopoiesis induced by hyperglycemia was driven by S100A8/9 and RAGE as demonstrated by increased proliferation of WT and myd88−/− bone marrow in response to S100A8/9, whereas RAGE−/− cells did not proliferate in excess. Mechanistically, S100A8/9 dramatically elevated macrophage colony-stimulating factor, granulocyte-macrophage colony stimulating factor, and granulocyte colony stimulating factor in bone marrow cells via nuclear factor-κ-light-chain-enhancer of activated B cells, and this was abolished in cells lacking RAGE. Interestingly, anti-diabetic therapy achieving normoglycemia corrected enhanced myelopoiesis, reduced circulating Ly6-C+ cells and their entry into atherosclerotic lesions and thereby regressed atherosclerotic lesions in this diabetic LDLR−/− mouse model of atherosclerosis. Collectively, these findings suggest that hyperglycemia per se promotes monocyte production as a contributing factor for atherosclerosis and provides a rational to develop strategies that aim at therapeutic targets in the signaling pathways linking hyperglycemia to S100A8/9/RAGE-mediated myelopoiesis and atherosclerosis. Dutta et al demonstrated enhanced myelopoiesis after experimental MI as a causative factor for further progression of atherosclerosis in ApoE null mice and suggests this pathway as a potential therapeutic target because inhibition of extramedullary hematopoiesis by either splenectomy or b3-adrenoreceptor blockade attenuated progression of atherosclerosis after MI.

As a result of their association with chronic inflammation, the S100/calgranulins have been looked at as potential therapeutic targets. Quinoline-3-carboxamides (Q-compounds) have been around for over 30 years and decrease autoimmune and inflammatory disease in multiple animal models, with relative preservation of innate immunity. Until recently, their exact mechanism of action was unknown. It was demonstrated that 6 different Q-compounds, including ABR-215757 (Paquinimod), currently in development for the treatment of lupus, bind S100A9 in peripheral blood mononuclear cells with high affinity and, thereby, decrease the binding of S100A9 to TLR-4 and RAGE. Mice treated with ABR-215757 and injected with lipopolysaccharide demonstrated the same degree of inhibition of tumor necrosis factor-α production as it was seen when mice were treated with monoclonal antibodies to S100A9, and this identified S100A9 as one molecular target of the Q-compounds.

We demonstrated that ABR-215757 also binds S100A12 in vitro with a slightly different kinetic profile than S100A9. Moreover, when S100A12 transgenic ApoE−/− mice with established atherosclerotic disease were treated with ABR-215757,
Figure 3. Treatment with ABR-21757 improves features of atherosclerotic plaque morphology (A–J) and reduces vascular inflammation (K–O). Innominate artery lesions from control S100A12/apoE−/− (A–D) and in mice receiving treatment with ABR-215757 (E–H) stained with Masson trichrome (A and E), Alizarin Red S (calcium phosphate in red; B and F), Verhoeff–Van Gieson (elastic fibers in black; C and G), and Hematoxin & Eosin (D and H). Original magnification, 10×, scale bare, 10 μm. Quantification of lesion characteristics for necrotic area (I) and Alizarin Red–stained plaque area (J). K–M, Protein level for S100/calgranulin and RAGE in aortic tissue lysates and mRNA (N–O) in aortic tissue in WT/ApoE−/− and S100A12/ApoE−/− mice after 5 weeks of ABR-215757 or vehicle treatment. Reprinted from Yan et al39 with permission of the publisher. Copyright ©2013, Elsevier.
we found a 20% reduction in atherosclerotic lesion size in the innominate artery and aortic root compared with placebo-treated mice. Importantly, they also had smaller necrotic core size, decreased intima and media calcification, minimal elastic fiber disruption, and more plaque area covered with SMCs (Figure 3). Additionally, the expression of leukocyte markers cluster of differentiation (CD) 68, CD4, and CD11c was reduced by 55% to 60% in the S100A12 mice treated with ABR-215757, indicating a reduction in inflammation. ApoE−/− WT mice (without S100A12) exhibited less of an effect from ABR-215757, showing no effect on atherosclerosis in the aortic root, but a reduction in atherosclerotic lesion size and cellular complexity in the innominate artery, likely related to ABR-215757 targeting S100A9 in WT mice. There was also a significant reduction in T cell accumulation in the atherosclerotic lesions, but no change in macrophage accumulation, indicating that the effect of ABR-215757 may be primarily related to T cell lymphocytes. These findings suggest that ABR-215757 treatment may attenuate or even halt S100/calgranulin-mediated acceleration of atherosclerosis.

**Association of S100/Calgranulins With Human Vascular Disease**

Elevated serum concentrations of the S100/calgranulins have been correlated with disease activity in chronic inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, asthma, and Kawasaki vasculitis, and collectively, levels of S100/calgranulins are considered biomarkers of inflammation. The S100/calgranulins and sRAGE have also been associated with traditional risk factors for vascular disease, including hyperglycemia and insulin resistance, and with the presence of vascular disease itself. A study of S100A12 levels and soluble RAGE levels in subjects with and without DM demonstrated that S100A12 levels were increased in diabetics and inversely related to soluble RAGE levels. High S100A12 levels and low soluble RAGE levels were also associated with increased risk of cardiovascular disease as determined by the Framingham score, and other cross-sectional studies confirmed serum S100A12 as an independent predictor of increased glycosylated hemoglobin levels. Serum S100A12 levels have been found elevated in patients with CAD in many studies as listed in Table. Importantly, intervention with a moderate-intensity exercise program in diabetics showed a significant improvement of glucose control and lipid profiles and increases in soluble RAGE levels compared with baseline. Similarly, S100A12 mRNA in peripheral blood cells was significantly reduced (among 142 other genes that were differentially expressed from baseline) after 52 weeks of a rigorous cardiovascular disease risk reduction program with comprehensive lifestyle changes in 63 participants with CAD or ≥2 CAD risk factors. This suggests that S100/calgranulins play an important role within the inflammatory networks, acting as inflammation-responsive proteins but yet amplifying inflammatory signals, ultimately contributing to a positive feedback cycle conducive to unchecked inflammation that subsequently may promote development and progression of atherosclerosis.

The S100/calgranulins are a biomarker of obesity, and S100A8/9 serum level are associated with body mass index even after adjustment for hs-CRP, tumor necrosis factor-α, and inversely related to soluble RAGE levels. High S100A12 and soluble RAGE levels in subjects with and without DM have been found elevated in patients with CAD in many studies as listed in Table. Importantly, intervention with a moderate-intensity exercise program in diabetics showed a significant improvement of glucose control and lipid profiles and increases in soluble RAGE levels compared with baseline. Similarly, S100A12 mRNA in peripheral blood cells was significantly reduced (among 142 other genes that were differentially expressed from baseline) after 52 weeks of a rigorous cardiovascular disease risk reduction program with comprehensive lifestyle changes in 63 participants with CAD or ≥2 CAD risk factors. This suggests that S100/calgranulins play an important role within the inflammatory networks, acting as inflammation-responsive proteins but yet amplifying inflammatory signals, ultimately contributing to a positive feedback cycle conducive to unchecked inflammation that subsequently may promote development and progression of atherosclerosis.

**Table. Studies Linking S100A12 to Human Cardiovascular Disease**

<table>
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<tr>
<th>Year</th>
<th>Study Type</th>
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<th>Patient Population</th>
<th>Outcome</th>
<th>Statistical Significance</th>
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<tr>
<td>2004&lt;sup&gt;46&lt;/sup&gt;</td>
<td>Autopsy</td>
<td>112</td>
<td>Diabetic and non-diabetic with sudden cardiac death</td>
<td>Serum S100A12 mRNA expression in SMCs and macrophages</td>
<td>Not quantified</td>
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<td>2008&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>41.251, 86&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Angiographically significant CAD</td>
<td>Plasma S100A12, carotid intimal medial thickness</td>
<td>S100A12 gene expression in monocytes</td>
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<td>2009&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>72</td>
<td>Patients on HD</td>
<td>S100A12 expression in SMCs and macrophages</td>
<td>P=0.014</td>
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<tr>
<td>2009&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Cross-sectional</td>
<td>67</td>
<td>Symptomatic angiographically significant CAD</td>
<td>Serum S100A12 in patients with and without CAD</td>
<td>775±87.6 ng/mL vs 282.1±22.9 ng/mL, P&lt;0.0002</td>
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<td>2010&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Retrospective</td>
<td>184</td>
<td>Patients on HD</td>
<td>Serum S100A12, cardiovascular mortality</td>
<td>HR 3.23, CI 1.48–7.01, P=0.003</td>
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<td>2010&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>526</td>
<td>Non-diabetics referred for angiography</td>
<td>Panel of genes (including S100A12), obstructive CAD</td>
<td>ROC AUC 0.70±0.02, P&lt;0.001 for the entire panel</td>
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<td>2011&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Cross-sectional</td>
<td>550</td>
<td>Patients on HD</td>
<td>Plasma S100A12, presence of cardiovascular disease</td>
<td>OR 1.28, CI 1.13–1.44, P&lt;0.001</td>
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<td>2012&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>652</td>
<td>PCI for stable CAD</td>
<td>Plasma S100A12, MACE</td>
<td>HR 1.64, CI 1.06–2.53, P=0.025</td>
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<td>2013&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>188</td>
<td>Diabetics referred for angiography</td>
<td>Plasma S100A12, CAD</td>
<td>OR 1.018, CI 1.009–1.016, P&lt;0.001</td>
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<td>2013&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>237</td>
<td>Patients with inactive lupus</td>
<td>Serum S100A12, history of cardiovascular disease</td>
<td>305 ng/mL vs 177 ng/mL, P=0.03</td>
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<td>2014&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>240</td>
<td>Patients with CAD referred for angiography</td>
<td>Serum S100A12, lesion complexity on angiography</td>
<td>OR 1.02, CI 1.01–1.04, P&lt;0.01</td>
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<td>2015&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Prospective</td>
<td>839</td>
<td>Healthy people without CAD</td>
<td>Serum S100A12 level, incident CAD</td>
<td>HR 1.30, CI 1.06–1.59</td>
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AUC indicates area under the curve; CAD, coronary artery disease; CI, 95% confidence interval; HD, hemodialysis; HR, hazard ratio; MACE, major adverse cardiac event; mL, millilitre; ng, nanogram; OR, odds ratio; PCI, percutaneous intervention; ROC, receiver operator curve; SCD, sudden cardiac death; and SMC, smooth muscle cell.

*The association was studied in multiple cohorts in this study.
and neutrophil granulocyte counts in nondiabetic obese subjects. Serum S100A8/9 levels decrease to normal levels in obese subjects who lose significant weight. There is genetic evidence that supports a possible role for S100/calgranulins as mediator of obesity beyond serving as a biomarker. Changes in fat-free body mass after a 20-week endurance exercise program were strongly linked to a polymorphism in the S100 gene cluster on chromosome 1 in the Heritage family study of 364 sibling pairs from 99 white families. A cardiovascular genome study including 1252 men identified that obese subjects homozygous for the S/S allele in the RAGE G82S polymorphisms have significantly increased hs-CRP compared with obese subjects with the common G/G or G/S variant of the RAGE G82S. Our laboratory was the first to identify the G82S variant as a variant within the ligand-binding domain of RAGE that promotes enhanced binding of S100A12 with subsequently increased nuclear factor κ-light-chain-enhancer of activated B cells, IL-6, and tumor necrosis factor-α signaling in peripheral blood mononuclear cells in response to S100A12. Taken together, this supports a role for S100/RAGE activation as a highly relevant pathway to promote inflammation and obesity in human subjects. This view is further supported by experimental data demonstrating reduced weight gain on feeding of a high-fat diet in mice with global deficiency in RAGE.

Regarding cardiovascular disease, a nested case study among patients enrolled in the TIMI 22 study identified the serum concentration of S100A8/9 and hs-CRP obtained 30 days after acute coronary syndrome as predictors of recurrent cardiovascular events over the ensuing 18 to 36 months. A similar predictive power was shown for serum S100A12 concentration in 2 cross-sectional studies of patients undergoing chronic hemodialysis where serum S100A12 levels predicted both the presence of cardiovascular disease and cardiovascular mortality. In those studies, the level of S100A12 remained a predictor of adverse cardiovascular outcomes even after stratification for other inflammatory markers, like hs-CRP and IL-6 levels. An autopsy study of victims of sudden cardiac death found enhanced expression of S100A12 and RAGE in macrophages and SMCs in the ruptured coronary artery plaque, suggesting a potential role of locally expressed S100A12 within the atherosclerotic lesion for increased plaque instability. Additional support linking S100/calgranulin to acute MI stems from a gene expression study on platelet-derived mRNA, which demonstrated increased gene expression of only 2 genes, CD69 and S100A9, in patients with ST-elevation MI, compared with patients with stable CAD, because the mRNA in platelets is a remnant from the bone marrow, the gene expression levels found in platelets are likely reflective of conditions before the onset of ST elevation MI, suggesting that increased levels of CD69 and S100A9 in platelets could be causal for acute MI. Other studies found that gene expression of S100A12 in peripheral blood mononuclear cells was an independent predictor of angiographically confirmed obstructive CAD in the PREDICT trial, a multicenter trial of 526 nondiabetic patients. Another study looked at 652 patients with stable CAD who underwent percutaneous intervention and found that plasma S100A12 was a significant independent predictor for major adverse cardiac events, where as hs-CRP was not. A recent Chinese study demonstrated that S100A12 was increased in patients with acute coronary syndrome and lesion complexity angiographically, demonstrating that S100A12 may be a marker for plaque vulnerability in humans. Moreover, in patients with end-stage renal disease, S100A12 plasma levels were associated with increased carotid intimal medial thickness and with peripheral arterial disease.

Although these mostly small case–control studies mentioned above and shown in Table demonstrate an association between S100A12 and CAD in specifically selected populations, it has only recently been studied longitudinally. The Rotterdam study, a population-based cohort study of 839 participants without coronary heart disease followed for 10.6 years, identified S100A12 as the only biomarker among 16 other measured biomarkers of inflammation that significantly associates with CAD when adjusted for age and sex. Participants in the highest tertile of S100A12 levels had a 2.6-fold higher risk of developing CAD compared with participants in the lowest tertile (hazard ratio, 2.59; 95% confidence interval, 1.52–4.40). (Figure 4). Further adjustments for other inflammatory markers and traditional risk factors (DM, hypertension, CKD, smoking, body mass index, hyperlipidemia) did not change the association. Given that S100A12 was associated with CAD even after correction for hs-CRP, CD40, IL-8, IL-18, and other inflammatory markers, this suggests that S100A12 represents a distinct inflammatory pathway. There was also a stronger association for S100A12 with future MI and CAD mortality compared with revascularization, which suggests that, as confirmed in animal studies and suggested in cross-sectional data, S100A12 may
be a determinant of plaque instability. These results suggest that S100A12 predicts future cardiovascular events and may have a role in primary prevention and intensive risk factor modifications.

**Therapeutic Potential**

Genetic ablation of S100A9\(^\text{65}\) or RAGE\(^\text{37}\) in atherosclerosis-susceptible ApoE null mice results in reduced atherosclerosis. Importantly, S100A12 and the RAGE axis can also be modified pharmacologically. Q-compound ABR-215757 binds with S100A12 in vitro and reduces atherosclerosis in hyperlipidemic mice with transgenic S100A12 expression, demonstrating that S100A12 can serve as a pharmacological target.\(^\text{39}\)

The current Q-compounds undergoing clinical study include ABR-215757 (Paquinimod), Laquinimod, and Tasquinimod. It should be noted that a previous-generation Q-compound, Linomide, was in phase III clinical trials for multiple sclerosis, but those trials were terminated because of increased cardiovascular events—primarily MI.\(^\text{96}\) The molecular mechanisms of Linomide leading to MI remains unclear; however, there were preclinical data on Linomide demonstrating proinflammatory arteritis in medium-sized arteries, hemorrhage, necrosis, and fibrosis of the myocardium in beagle dogs that were not seen in mice studies.\(^\text{87}\) The newer Q-compounds are derivatives of the original compound Linomide, and the propensity for proinflammatory response of different derivative Q-compounds were studied in beagle dogs.\(^\text{88}\) Based on these data, it was suggested that the proinflammatory response could be minimized by replacing a methyl group with an ethyl group in the N-carboxamide moiety of the compound.\(^\text{88}\) Laquinimod and Paquinimod both share this substitution and thus far have not demonstrated the unexpected proinflammatory response in dogs that was seen with Linomide.\(^\text{88}\) Paquinimod is currently approved in the United States as an orphan drug for systemic sclerosis and has been demonstrated to be safe in patients with lupus.\(^\text{90}\) In phase III trials, Laquinimod has slowed the progression of disability and reduced the rate of relapse in patients with multiple sclerosis.\(^\text{92}\) Tasquinimod was tested for metastatic castrate-resistance prostate cancer in a phase clinical III trial.\(^\text{93}\)

Besides the Q compounds, 3 distinct anti-allergic drugs, amlexanox, cromolyn, and tranilast, bind S100A12, although their effect on atherosclerosis has not been examined in murine or human disease.\(^\text{94}\)

One anti-inflammatory drug that is currently tested in a prospective randomized clinical trial to examine whether it may reduce cardiovascular disease burden and mortality is methotrexate. Anti-inflammatory effects of methotrexate are multifactorial, it improves clinical end points of CAD is methotrexate (target dose of 20 mg/ week) in the ongoing CIRT study. Although the anti-inflammatory effects of methotrexate are multifactorial, it is possible that reducing circulating levels of S100A12 as was previously demonstrated in patients with systemic arthritis\(^\text{21}\) might be a potential underlying mechanism of any potential beneficial effects of methotrexate on vascular inflammation. Therefore, it will be of interest to examine the S100/calgranulins as potential biomarker to predict response to methotrexate therapy in CIRT.

In summary, S100/calgranulins and the RAGE axis are exciting emerging pathways linking inflammation to atherosclerosis and to vascular calcification. S100A12 may have a new role as a marker of future cardiovascular disease and may be able to be incorporated into primary prevention and risk factor modification. However, there is no definitive evidence that S100A12 is a therapeutic target in human atherosclerosis because it is associated with other mechanisms of inflammation and has not been specifically targeted in prospective clinical trials. Further prospective studies are required. With this caveat, S100/calgranulins have emerged as possible therapeutic targets of the Q-compounds, and with further drug development, modification of the RAGE axis may reduce cardiovascular disease burden and mortality.

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**Disclosures**

None.

**References**

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S100/Calgranulins and Atherosclerosis


Although significant advances have been made in the treatment of atherosclerosis, it remains the leading cause of death worldwide, and further treatment avenues are needed. The proinflammatory S100/calgranulins, in particular S100A8, S100A9, and S100A12, and their interaction with receptor for advanced glycation end products are important mediators of inflammation and atherosclerosis as it has been demonstrated in both mouse and human models. S100A12 is a biomarker for coronary artery disease in a recent longitudinal population-based study. S100A9 and S100A12 are therapeutic targets for a new class of immune modulators, the quinoline-3-carboxamides, and one of them is undergoing a phase III clinical trial for multiple sclerosis. It is possible that these compounds have potential to improve outcomes in atherosclerosis, and further studies may yield important therapeutic potential.
S100A12 and the S100/Calgranulins: Emerging Biomarkers for Atherosclerosis and Possibly Therapeutic Targets
Adam Oesterle and Marion A. Hofmann Bowman

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