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EDITORIAL

Plasma RANTES: a molecular fingerprint of the unstable carotid plaque?

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This editorial refers to 'Relationship between circulating levels of RANTES (regulated on activation, normal T-cell expressed, and secreted) and carotid plaque characteristics: the Atherosclerosis Risk in Communities (ARIC) Carotid MRI Study'†, by S.S. Virani *et al.* on page 459

Rupture of atherosclerotic plaques in epicardial coronary or carotid arteries may lead to myocardial infarction or stroke—the leading causes of morbidity and mortality in Western countries.[1] Thus, timely detection of these unstable plaques before they rupture is a great medical need.[2] Non-invasive plaque visualization using duplex ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) provide the advantage of longitudinal analyses, but limitations exist in terms of moderate spatial resolution and exposure to X-rays when using CT. Hence, their sensitivity and specificity for detecting unstable atherosclerotic plaques is moderate. To optimize the cost–benefit ratio, we need to tailor our tools for identifying patients with increased cardiovascular risk harbouring plaques prone to rupture.

Ideally, high-resolution plaque imaging is combined with one or several emerging biomarkers that provide incremental information about plaque biology and patient prognosis. Adding to the wealth of experimental, clinical, and epidemiological evidence that identified inflammation as an integral element throughout the different stages of atherosclerosis, biomarkers of inflammation have been validated in clinical trials.[1,3] Among them, C-reactive protein stands out as the prototype inflammatory biomarker that adds valuable information in terms of cardiovascular risk stratification.[1] However, in the absence of firm evidence for a causal role of C-reactive protein in atherosclerosis by applying Mendelian randomization for gene variants in the C-reactive protein locus,[4] C-reactive protein does not meet the criteria of a cardiovascular risk factor. Several additional inflammatory mediators involved in experimental atherogenesis have been tested clinically with regard to their prognostic value. However, short half-life, low plasma levels, and susceptibility to both *in vivo* and *ex vivo* confounding factors such as altered plasma levels by heparin or release from platelets during coagulation complicate the quest for THE biomarker of cardiovascular risk. Moreover, given the complex interplay of a multitude of inflammatory mediators in atherogenesis, it appears likely that a set of multiple biomarkers will be needed for complete risk assessment.

The ideal cardiovascular inflammatory biomarker is a stable protein, sufficiently present in a non-invasively accessible compartment for cost-effective and reproducible detection. Reflection of the stage and progression of atherosclerosis would confer both prognostic value and its implementation as a monitoring tool for emerging anti-inflammatory therapies.[3] C-reactive protein does not combine all of these desirable criteria but, to date, portrays the closest approximation. Therefore, the characteristics described for C-reactive protein are what emerging biomarkers such as RANTES need to run up against.

The CC chemokine ligand-5 (CCL5/RANTES, regulated upon activation, normal T cell expressed, and secreted) is an inflammatory cytokine that recruits leucocytes into sites of inflammation. Rapid release from activated platelets leads to deposition of RANTES on activated endothelia, and induces monocyte and T cell arrest, followed by transendothelial diapedesis (*Figure* 1).[5] CXCL4, another platelet-derived chemokine, has been reported to augment the RANTES-mediated chemotaxis and vice versa, potentially through the formation of CXCL4–CCL5 heterodimers.[6]

RANTES is also highly expressed within the atheroma, where it colocalizes with T cells, monocyte-derived macrophages, and smooth muscle cells (*Figure* 1).[7] Animal studies have highlighted the pro-atherogenic effects of both RANTES and its receptor CCR5: targeted disruption of the RANTES receptor CCR5, present on both plaque-resident and circulating leucocytes as well as on endothelial cells, reduced advanced atherosclerotic lesions in mice.[6] In parallel, pharmacological blockade using the antagonizing antibody MET-RANTES diminished atherosclerosis and decreased both mononuclear cell infiltration and the T

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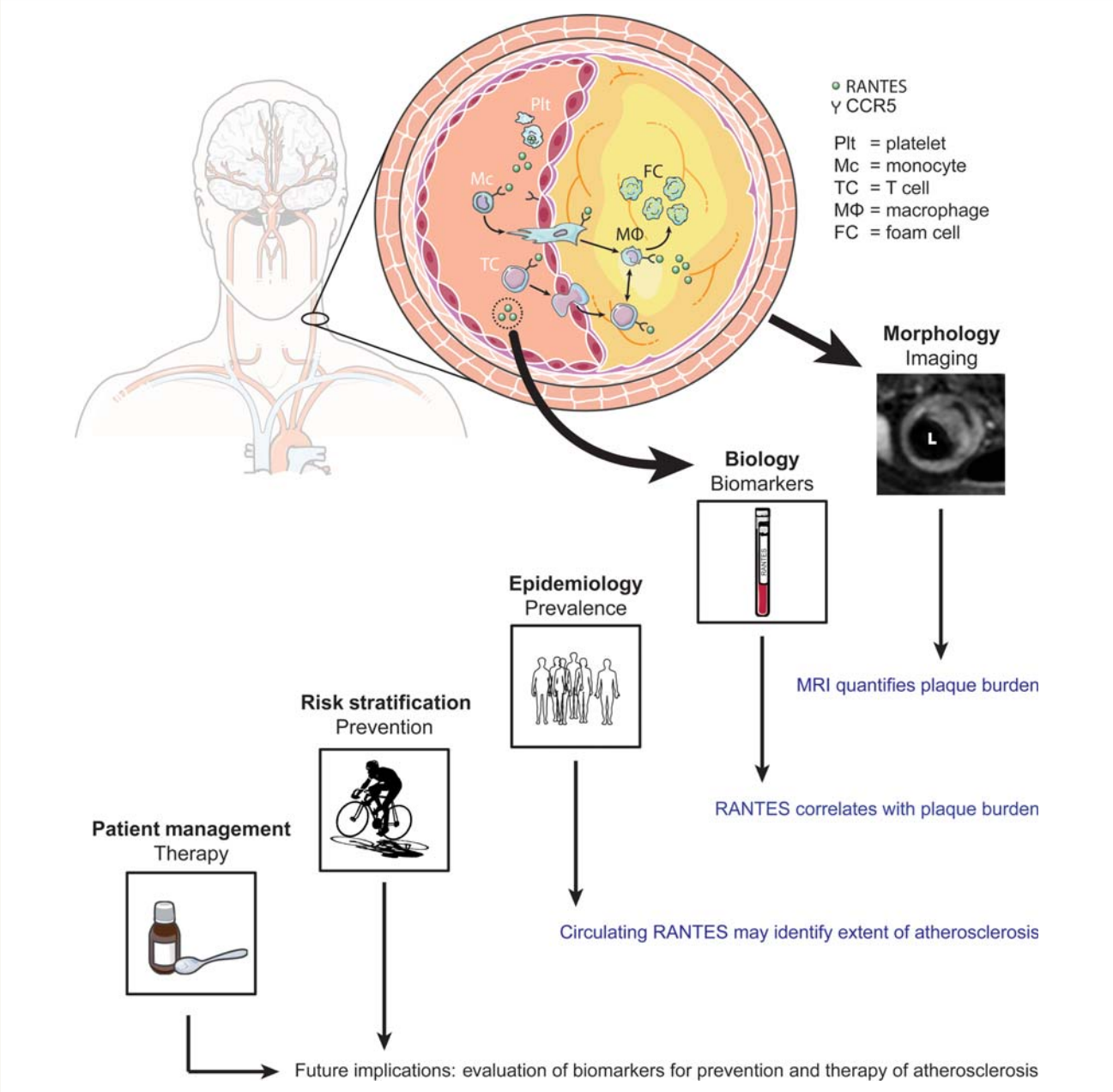

Figure 1 Plasma RANTES (regulated upon activation, normal T cell expressed and secreted/CCL5) and atherosclerosis: secretion of RANTES by activated platelets and expression of its receptor CCR5 on circulating monocytes, T cells and activated endothelial cells enhance leucocyte arrest and transendothelial diapedesis, thereby promoting plaque development. Virani and colleagues investigated plaque morphology using magnetic resonance imaing and plaque biology assessing RANTES plasma levels in a large cohort. Their key findings (blue) indicate the potential of plasma RANTES levels to identify the extent of atherosclerosis as assessed by magnetic resonance imaing. Evaluation of this emerging inflammatory biomarker for risk stratification or potential therapeutic intervention needs to be assessed in future studies.

helper 1 (Th1)-type immune response in atherosclerotic mice.[6] However, in patients with coronary artery disease (CAD), the value of plasma RANTES levels remains controversial. In patients with stable CAD RANTES levels were decreased compared with healthy controls. [8] In contrast, transiently raised levels of RANTES were observed in ischaemic patients with unstable angina compared with patients with stable CAD.[9] Therefore, the value of the RANTES plasma level as a biomarker or prognostic factor in CAD warrants further investigation.

In this issue of the *EHJ* Virani and colleagues[10] report the investigation of the association between plasma levels of RANTES and morphological plaque parameters using MRI in a large cohort of the Atherosclerosis Risk in Communities (ARIC) study. High-risk patients with increased carotid intima-media thickness were

selected by carotid duplex ultrasound and underwent carotid MRI. The authors found that plasma RANTES levels positively correlated with total plaque burden.

Using C-reactive protein as a reference inflammatory biomarker, the authors describe a weak correlation between C-reactive protein and RANTES plasma levels. This association, however, was not reported in patients receiving statins. These findings are intriguing and necessitate future research to prospectively assess the effects of statins on both circulating C-reactive protein and RANTES. Ethnic variations of RANTES levels as described by Virani and coworkers deserve distinct attention since specificity and sensitivity may vary markedly. In addition, low plasma RANTES levels ranging magnitudes below the respective C-reactive protein levels as well as its limited stability may impede routine detection of RANTES as a biomarker in CAD. Moreover, originating to a considerable extent from platelets, *ex vivo* release during coagulation of plasma samples may confound results. On the other hand, the skewed distribution of plasma RANTES and its putative causative role in experimental atherosclerosis might redeem some of the impairments to its use as a biomarker.

Since plasma RANTES levels may not reflect its expression pattern in atherosclerotic lesions, a correlation with the biological activity of RANTES in atherogenesis remains to be determined. Therefore, prospective clinical studies correlating circulating RANTES and C-reactive protein levels with plaque histology and clinical presentation appear critical for the evaluation of the biological significance of plasma RANTES levels.

To evaluate RANTES as a therapeutic target in atherosclerosis, the risk–benefit ratio of RANTES antagonism deserves attention. Importantly, the atheroprotective effects of MET-RANTES[11] in rodents appear to be outweighed by an impaired systemic immune response.[12] Therefore, CCL5–CXCL4 heterodimers may emerge as a better therapeutic alternative mediating reduced monocyte recruitment into the atherosclerotic plaque and attenuating its growth.[13]

To date, clinical evidence for a role of RANTES in atherosclerosis is scarce. A word of caution along this line comes from current large-scale genome-wide association studies. In this regard, genetic evidence substantiating a critical role for RANTES in CAD is lacking, whereas in type 1 diabetes RANTES single nucleotide polymorphisms were found to be associated with RANTES serum concentrations and the development of disease.[14]

Virani and colleagues provide data correlating circulating RANTES with plaque burden and lipid-core volume (in univariate analysis). However, a clear association with further features of unstable plaques was not detected. Unexpectedly, elevated RANTES plasma levels were associated with a thicker fibrous cap, suggestive of a more stable plaque phenotype. The limited spatial and temporal resolution of MRI in pulsating carotid arteries may, at least in part, explain this shortcoming. Moreover, preselecting patients using duplex ultrasound might have introduced a bias.

Plasma RANTES—a molecular fingerprint of the unstable carotid plaque? Circulating RANTES levels may help to identify the extent of atherosclerosis but appear to be of limited value for the identification of unstable lesions. As an inflammatory biomarker, plasma RANTES lags behind C-reactive protein regarding its biochemical properties and stable correlation with inflammation. Reflecting an intricate pathophysiological process in atherosclerosis, however, RANTES may take hold as a biomarker of the plaque burden in atherosclerosis. Since statins appear to blunt the observed correlation, broad clinical applicability in patients on this lipid-lowering medication appears questionable. Moreover, assessment of the prognostic value of circulating RANTES or its significance as a therapeutic target warrants future research.

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