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ΑΝΑΣΚΟΠΗΣΗ
REVIEW

Η συμμετρική και ασύμμετρη διμεθυλαργινίνη ως βιοχημικοί δείκτες της ενδοθηλιακής δυσλειτουργίας και της αθηροσκλήρωσης στη Ρευματοειδή Αρθρίτιδα.

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ΠΕΡΙΛΗΨΗ

Η Ρευματοειδής αρθρίτιδα (ΡΑ) χαρακτηρίζεται από αυξημένη καρδιαγγειακή νοσηρότητα και θνητότητα που αποτελεί την κύρια αιτία του μειωμένου προσδόκιμου επιβίωσης που παρατηρείται στους ασθενείς που πάσχουν από τη νόσο. Καρδιαγγειακά συμβάματα όπως εγκεφαλικά επεισόδια και εμφράγματα του μυοκαρδίου είναι συχνά και έχουν χειρότερη έκβαση στους ασθενείς με ΡΑ σε σχέση με το γενικό πληθυσμό γεγονός που οφείλεται σε πρώιμη υποκλινική αθηρωματική νόσο των εγκεφαλικών και στεφανιαίων αγγείων. Η δυσλειτουργία του ενδοθηλιακού κυττάρου αποτελεί το πρόδρομο στάδιο της δημιουργία αθηρωματικής πλάκας και σήμερα γνωρίζουμε ότι αγγειακές βλάβες στους ασθενείς με ΡΑ εμφανίζονται πριν την κλινική εκδήλωση των συμπτωμάτων. Το ενδοθήλιο αποτελεί το σημαντικότερο όργανο-στόχο των παθολογικών καταστάσεων που αποτελούν παράγοντες κινδύνου για την εμφάνιση καρδιαγγειακών νοσημάτων (υπερχοληστεριναιμία, σακχαρώδης διαβήτης, κάπνισμα κτλ.), ενώ οι ασθενείς με ΡΑ εμφανίζουν διαταραχή της ενδοθηλιακής λειτουργίας σε σχέση με μάρτυρες της ίδιας ηλικίας και φύλου.

Το οξείδιο του αζώτου (NO) αποτελεί μία από τις βασικές αγγειοδραστικές ουσίες και διαδραματίζει πρωταρχικό ρόλο στον έλεγχο της αγγειακής λειτουργίας. Διαταραχή της απρόσκοπτης παραγωγής και ελευθέρωσης του NO από τα ενδοθηλιακά κύτταρα είναι πιθανόν να αποτελεί βασικό παθοφυσιολογικό μηχανισμό της ενδοθηλιακής βλάβης, στην οποία φαίνεται να εμπλέκονται οι διμεθυλαργινίνες που είναι ενδογενείς αναστολείς της συνθετάσης του NO – του ένζυμου που είναι υπεύθυνο για την παραγωγή του NO. Η ασύμμετρη (ADMA) και η συμμετρική (SDMA) διμεθυλαργινίνη έχουν πλέον καθιερωθεί ως βιοχημικοί δείκτες της ενδοθηλιακής δυσλειτουργίας αλλά και ως νεότεροι παράγοντες αυξημένου καρδιαγγειακού κινδύνου γενικότερα, καθώς αυξημένα επίπεδα ADMA και SDMA έχουν συσχετισθεί με πλήθος νοσολογικών οντοτήτων όπως σακχαρώδης διαβήτης, υπέρταση, νεφρική ανεπάρκεια κτλ., που οδηγούν σε αθηροσκλήρυνση και στεφανιαία νόσο.

Στη ΡΑ η αυξημένη επίπτωση καρδιαγγειακών νοσημάτων έχει οδηγήσει στην ανάγκη εύρεσης απλών, μη επεμβατικών βιοδεικτών που θα συμβάλλουν στην κατανόηση των πολύπλοκων παθοφυσιολογικών μηχανισμών της αγγειακής βλάβης και την αναγνώριση των ασθενών που βρίσκονται σε μεγαλύτερο κίνδυνο για την ανάπτυξη αθηρωματικής νόσου. Μελέτες έχουν

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δείξει αυξημένα επίπεδα ADMA σε ασθενείς με ΡΑ και συσχέτιση με άλλες παραμέτρους της ενδοθηλιακής δυσλειτουργίας όπως με το πάχος του τοιχώματος των καρωτίδων. Αν και μεγάλες αναδρομικές μελέτες δεν έχουν πραγματοποιηθεί σε πληθυσμούς ΡΑ πιστεύεται ότι οι διμεθυλαργινίνες μπορούν να αποτελέσουν μία εναλλακτική οδό προσέγγισης και διερεύνησης των αγγειακών διαταραχών στους ασθενείς αυτούς. Η παρούσα ανασκόπηση συνοψίζει τα δεδομένα για τη χρησιμότητα της ADMA και της SDMA στην εκτίμηση της ενδοθηλιακής δυσλειτουργίας και του καρδιαγγειακού κινδύνου στους ασθενείς με ΡΑ.

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Λέξεις-Κλειδιά: ασύμμετρη διμεθυλαργινίνη, συμμετρική διμεθυλαργινίνη, Ρευματοειδής Αρθρίτιδα, αθηροσκλήρωση

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Symmetric and asymmetric dimethylarginines as biochemical markers of endothelial dysfunction and atherosclerosis in Rheumatoid Arthritis.

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ABSTRACT

Rheumatoid arthritis (RA) is associated with reduced life expectancy due to excess cardiovascular (CV) disease. Endothelial dysfunction is common in patients with RA, and accelerated coronary and cerebrovascular atherosclerosis are the major contributors to higher rates of CV events in RA compared to the general population. Nitric oxide (NO) produced by L-arginine is an important vasoactive agent for the maintenance of vascular health. The derangement of the NO/L-arginine pathway leads to vascular changes, predisposing to atherosclerosis. Nitric oxide metabolism is disrupted in RA, with a growing body of evidence suggesting that circulating inhibitors of NO synthase play a crucial role. Asymmetric (ADMA) and symmetric (SDMA) dimethylarginines have been recognized as emerging novel markers of endothelial dysfunction and CV morbidity and mortality in several CV disease settings; for example, coronary artery disease, stroke, lipids disorders, etc., as well as in the general population. Thus, it not surprising that they have been evaluated as indicators of vascular disease in RA. This paper provides an overview of the potential role and utility of these molecules in the pathophysiology of CV disease and the evaluation of endothelial function with a specific focus on RA patients.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive inflammatory disease affecting the joints and other parts of the body. In addition to physical disability, patients suffering from RA die earlier than the general population with life expectancy being reduced by 10-15 years[1] despite the dramatic advances observed in this field in general population since the 1960s.[2] It is now well recognized that cardiovascular (CV) disease is the biggest contributor to premature mortality, accounting for up to 50% of deaths in RA individuals.[3] Although classical CV disease risk factors such as hypertension,[4] dyslipidaemia,[5] physical inactivity and obesity[6] are more prominent in RA, they are not sufficient on their own to explain the higher rates and worse outcomes of CV events amongst RA individuals compared to the general population.[7] It has been suggested that disease-specific factors, including systemic inflammation and autoimmune activation, trigger pathways of atherosclerosis and/or thrombosis, and, combined with traditional risk factors, lead to increased CV morbidity and mortality.[8,9]

Increased CV burden in RA is mainly associated with accelerated atherosclerosis, resulting in coronary artery disease. High intensity of systemic inflammation underlies structural and morphological changes in vasculature which can exacerbate or accelerate atherosclerosis.[10] Indeed, the inflammatory process in rheumatoid synovium and atherosclerotic vascular wall share several similarities in terms of inflammatory cell migration and activation, the production of pro-inflammatory cytokines and the expression of adhesion molecules. Endothelial dysfunction – the initial step to atherogenesis – has been reported in several studies, assessing vascular morphology in RA,[11] and it appears that the pathophysiological process may actually precede the clinical appearance of the disease.[12] Thus, the prompt recognition of early, pre-atherogenic changes may contribute to the introduction of protection measures and more effective CV risk management in RA individuals.

In that respect, a European League Against Rheumatism (EULAR) task force made a commendable effort in producing recommendations for CV risk management in patients with inflammatory arthritis;[13] however, the performance of risk factor assessment algorithms for predicting CV risk in RA is suboptimal, as they seem to underestimate CV risk in this population.[14] Consequently, other approaches including validated biomarkers routinely used in the management of RA such as C-reactive protein (CRP) have been suggested in the assessment of CV risk.[15] While CRP indicates systemic inflammatory load and in parallel is associated with accelerated atherosclerosis[16] and CV events,[17] the search for novel, sensitive, surrogate markers of endothelial dysfunction continues.

Dimethylarginines are formed during endogenous protein turnover as guanidino-substituted analogues of L-arginine and interfere with numerous metabolic and signaling pathways. Asymmetric dimethylarginine (ADMA) is the most potent endogenous nitric oxide (NO) synthase inhibitor whilst its structural isomer symmetric dimethylarginine (SDMA) reduces NO synthesis indirectly by enhancing formation of reactive oxygen species[18] and/or by inhibiting cellular transport of L-arginine and other amino acid.[19] Both molecules have emerged as potential new markers of endothelial dysfunction with independent roles in CV disease[20] and predictions of CV events.[21] In fact, ADMA correlates with traditional and non-traditional CV disease risk factors[22] and it is associated with CV mortality and morbidity in various conditions such as coronary artery disease,[23] peripheral vascular disease,[24] chronic renal failure[25] as well as in general population.[26] Although SDMA has not been studied to a similar extent, recent data demonstrates that elevated circulating SDMA levels are associated with worse prognosis in patients suffering from stroke[27] and increased risk of cardiac death in individuals referred for coronary angiography[28] and diagnosed with myocardial infraction.[29] It is worth noting that ADMA and SDMA have been found to have the same prognostic significance for CV risk in the general population.[30] Finally, recent insights indicate ADMA as a biomarker and mechanistic bridge between renal cyclooxygenase-2 inhibition and systemic vascular dysfunction, suggesting that endogenous NO synthase inhibitors have an important role in the adverse CV events and outcomes reported with nonsteroidal anti-inflammatory drug usage.[31]

As CV disease is one of the major and most important co-morbidities in RA, dimethylarginines have attracted interest as potential sensitive laboratory markers of endothelial impairment and atherosclerosis in this population. Specifically, the role of ADMA has been investigated not only in RA, but also in other cardiovascular complications of immunologic abnormalities.[32] The aim of the current review is to summarize the available amount of data and evidence regarding the utility of ADMA and SDMA in assessing CV risk in RA.

NO as a regulator of vascular function

The vascular endothelium forms the innermost lining of the vasculature and represents one of the largest endocrine organs in the human body. The endothelium performs vital tasks crucial for several physiological functions, including tissue fluid balance, angiogenesis, vessel tone, and host defense.[33] Amongst others,

endothelial cells control vascular function and structure by secreting various hormones and vasoactive substances which are involved in a broad variety of regulatory mechanisms of CV system by affecting vasomotion, coagulation, vessel wall growth and inflammation.[34] Nitric oxide (NO) is an endothelium-derived vasodilator of particular importance with inhibitory effects on vascular muscle cell proliferation and constriction, and cell-to-cell interactions in blood vessels, such as platelet aggregation and leucocyte adhesion. Nitric oxide regulates blood flow by maintaining basal vasodilator tone and protects vasculature from multiple atherothrombotic biological processes, representing a significant regulator of vascular function and health.

The production of NO is catalyzed by the enzyme NO synthase which converts L-arginine to NO. Three isoforms of NO synthase have been identified; classified by the cells they were originally found in: neuronal isoform, which functions as a retrograde neurotransmitter; inducible or macrophage isoform, which is upregulated in an oxidative environment after activation of macrophages; and endothelial isoform, which is responsible for the constitutional production and release of NO in the vasculature.[35] The later physiological function of endothelial isoform requires dimerization of the enzyme, the presence of the substrate l-arginine, and the essential cofactor (6*R*)-5,6,7,8-tetrahydro-l-biopterin (BH_4); one of the most potent naturally occurring reducing agents.[36] Physiologically, NO diffuses from endothelial cells to subendothelial layers of the vascular wall or to the cells of flowing blood to induce vascular dilatation and other atheroprotective properties, mainly by increasing production of the second messenger cyclic guanosine monophosphate via activation of soluble guanylate.

As L-arginine/NO pathway is thought to be the major effector of endothelial control of vascular homeostasis, alterations in endothelial biology due to impaired NO synthesis and particularly the imbalance between the secretion of vasodilators and endothelium-derived contracting factors result in endothelial dysfunction, which represents a key early event in the long road towards atherosclerotic vascular disease.[37] Reduction of endothelial NO synthase activity and the consequent reduction in NO bioavailability lead to abnormal vasoreactivity and endothelial cell activation accompanied by derangement of fibrinolysis and prothrombotic status; abnormalities which characterize atherosclerotic plaque formation, progression and rupture.[38]

Dimethylarginines, endothelial dysfunction and CV disease

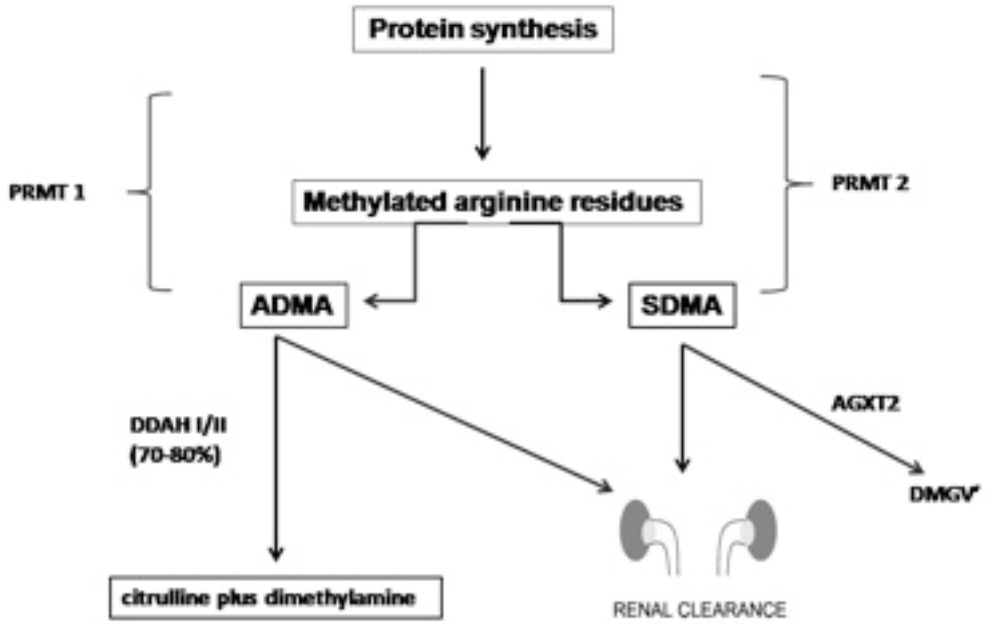
Synthesis and Metabolism

Dimethylarginines are naturally occurring components of human plasma derived from the proteolysis of proteins with methylated arginine residues. The methylation is catalyzed by arginine methyltransferases and gives rise to three types of compounds: ADMA, SDMA and L-NG-monomethylarginine (L-NMMA).[39] According to their specific catalytic activity, protein arginine methyltransferases (PRMT) are classified into two types: Type I catalyzes the formation of ADMA and L-NMMA, while Type II catalyzes the formation of SDMA and L-NMMA.[40] After proteolysis, free dimethylarginines are released by the cells. As the structure of ADMA is similar to L-arginine, it competes with L-arginine for NO synthase binding; thereby inhibiting NO formation whilst SDMA is a weaker indirect inhibitor of NO synthase activity.[41] Despite common synthetic pathways, clearance of ADMA and SDMA occurs through different mechanisms. ADMA is predominantly metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to citrulline and dimethylamine, with other pathways such as liver degradation and renal excretion having a minor contribution.[42] Dimethylarginine dimethylaminohydrolase has two isoforms (DDAH-1 and DDAH-2) which are distributed differently across tissues, with DDAH-1 being primarily an enzyme of epithelial cells, whereas DDAH-2 is present in the vasculature.[43] On the other hand, SDMA seems to be strictly eliminated by renal excretion and it is considered as a very reliable marker of renal function.[44] Recently, it has been suggested that enzymatic activity of alanine–glyoxylate aminotransferase 2 represents an alternative cleavage route for SDMA degradation in different population settings (Figure 1).[45,46]

The role of dimethylarginines in atherosclerosis

Impairment of endothelial function represents the most common process through which several CV risk factors such as hypertension, smoking, obesity, etc., exert adverse effects on vascular architecture and function. There is evidence that a number of vascular conditions are characterized by reduced NO generation, suggesting that endogenous control mechanisms of L-arginine/NO pathway underlie pathogenesis of CV disease.[39] Such observations have provided the rationale for *in vitro* and *in vivo* studies exploring the possible role of dimethylarginines in aberrant regulation of NO metabolism. Particularly, the role of ADMA has been an area of intense research in recent years.

Experimental evidence suggests that even small changes of circulating ADMA levels affect endothelial homeostasis and modulate vascular function. For example, in a mouse model, overexpression



ADMA: Asymmetric dimethylarginie
AGTX2: Alanine:glyoxylate aminotransferase 2
DDAH: Dimethylarginine dimethylaminohydrolase
SDMA: Symmetric dimethylarginine
PRMTs: Protein arginine methyltransferases

Figure 1. Metabolic pathway of dimethylarginines. Arginine residues that lie within appropriate consensus sequences in proteins can be post-translationally methylated by the action of PRMTs. PRMT I catalyzes asymmetrical dimethylation and monomethylation of arginine residues and produces ADMA, whereas 32 type II catalyzes symmetrical dimethylation and monomrthylasion and forms symmetric dimethylarginine - the biologically inactive stereoisomer of ADMA. The former is degraded via DDAH action to citrulline plus dimethylarginine and the latter is predominantly eliminated through kidneys. Although there is data in mice for contribution of AGXT2 to ADMA degradation, these findings have not been confirmed in humans.

of the DDAH-1 gene improved blood pressure and vascular resistance through enhanced NO production.[47] Furthermore, overexpression of DDAH-1 in apolipoprotein E-deficient mice reduced plaque formation in the aorta and improved endothelial function as assessed by endothelium-dependent vasodilatation.[48] In contrast, exogenous ADMA treatment in cell culture models of human umbilical vein endothelial cells blocks endothelial cells differentiation and senescence, induces monocytoid cells adhesion, smooth muscle cell migration and foam cell formation; processes involved in the initial steps of atherosclerosis.[49,50] In addition, ADMA inversely interferes with differentiation and mobilization of endothelial progenitor cells in patients with coronary artery disease.[51]

In the context of clinical data in humans, Miyazaki *et al*. demonstrated that ADMA levels were correlated with age, mean blood pressure, glucose tolerance and carotid artery intima media thickness in 116 healthy individuals without apparent CV disease;[52] suggesting that ADMA may have a prognostic significance in the development of vascular disease before it manifests clinically. Plasma ADMA levels were also significantly correlated with carotid artery intima thickness in patients with subclinical carotid atherosclerosis, even in those who were not taking any medication.[53] Elevated ADMA levels have been reported in several conditions associated with vascular pathology and excess CV risk, such as hypertension,[54] hypercholesterolemia,[55] hyperhomocysteinemia,[56] peripheral vascular disease,[57] renal failure,[58] and heart failure of various etiologies.[59,60] Thus, it is not surprising that ADMA has been evaluated as a novel surrogate marker of endothelial dysfunction and CV risk in different disease settings and general population, with outcomes indicating its prognostic value for both occurrence and outcomes of CV events, as well as overall CV morbidity and mortality.[61]

The appreciation that the - originally considered biologically inert - SDMA affects vascular homeostasis

through NO-dependent but also through NO-independent mechanisms[19,20] has led to studies assessing its clinical significance in populations with high CV risk. Although the amount of data is limited, the current level of evidence suggests that elevated SDMA levels are linked with increased CV risk in conditions such as coronary and peripheral artery disease[62] with the prognostic impact of SDMA in some populations – stroke,[63] for example - being reported superior to that of ADMA. The interrelationship between the two molecules has not been fully understood today; however, they modulate NO metabolism, and potentially hold an important role in the development and progression of CV disease.

Atherosclerosis and CV risk in RA

Rheumatoid arthritis patients are more susceptible to developing CV disease, which accounts for the excess morbidity and mortality. Although cardiac disease in RA is heterogeneous including a wide spectrum of clinical manifestations such as systolic and diastolic heart failure, (myo)pericarditis, valvular disease and pulmonary hypertension, it appears that heightened CV risk and reduced life expectancy in this population are specifically attributable to accelerated coronary artery and cerebrovascular atherosclerosis.[64] Rheumatoid arthritis patients are at higher risk of myocardial infractions and cerebrovascular events compared to the general population[65] that are characterized by higher rates of re-occurrence and worse long-term outcomes.[66] Rheumatoid arthritis is now considered a novel risk factor for CV disease[67] and a coronary artery disease equivalent.[68]

Appreciation has increased that upregulation of proinflammatory cytokines such as tumor necrosis factor-alpha and IL-6 which are abundantly present in the inflamed synovium and the systemic circulation leads to endothelial dysfunction and contributes to the development of atherosclerosis in RA.[69] Systemic inflammation can cause vascular damage through several mechanisms, including promotion of oxidative stress via uncoupling of endothelial NO synthase and reduced NO bioavailability, increased extent of coronary artery calcification, induction of secondary dyslipidemia, abnormal fibrinolytic activity and enhancement of prothrombotic propensity.[70] In addition, the intensity of vascular inflammation in RA not only drives the formation and progression of atherosclerotic plaque, but more importantly, contributes to greater plaque instability and subsequently clot formation that causes acute events.[71] Last but not least, chronic high-grade systemic inflammation precipitates the adverse effects of traditional CV risk factors on vascular wall underlying the complexity of the links between RA-related factors and impairment of endothelial function.[72]

The central role of inflammation in CV risk is further supported by clinical observations suggesting that high sensitive C-reactive protein (CRP) has been described as a predictor of future coronary heart disease in general population.[73] In RA higher CRP levels are associated with subclinical atherosclerosis assessed by carotid-artery intima thickness – a validated morphological marker of CV disease.[74] High erythrocyte sedimentation rate (ESR) values and severe, active RA defined by erosive bone disease, extraarticular involvement and serological abnormalities are significantly associated with increased CV morbidity and mortality.[75,76] The observations that rheumatoid factor and antinuclear antibodies positive individuals are in higher risk of CV events and death[77] and anti-cyclic citrullinated peptide (anti-CCP) antibodies in patients with RA are independently associated with the development of ischemic heart disease[78] indicate that the systemic autoimmune process may play a role to the genesis of endothelial damage and atherosclerosis in RA patients.

Dimethylarginines, endothelial dysfunction and inflammation in RA

Growing amount of evidence demonstrates that ADMA levels are raised in RA individuals, even in the absence of atherosclerotic disease or conventional CV risk factors (Table 1).[79-82] Although several mechanisms have been proposed to explain elevated ADMA such as inflammation-induced upregulation of PRMTs and increased endothelial cell turnover in the inflamed synovium with liberation of ADMA during cell catabolism,[83] it appears that the most important parameter is DDAH dysfunction.[84] Inflammatory mediators, oxidative stress and high levels of NO production following overexpression of inducible NO synthase inhibit DDAH activity leading to ADMA accumulation. In this way, ADMA acts in a multiplicative manner in promoting endothelial dysfunction, not only by reducing NO synthesis, but also by evoking endothelial NO synthase uncoupling; switching it to a superoxide synthase.[85] Thus, ADMA may represent a novel link between endothelial dysfunction and vascular inflammation: this relationship may be of high importance in chronic, systemic inflammatory conditions such as RA. To lend more support to the former, higher ADMA levels in RA patients do not seem to have a genetic background, as they were not associated with DDAH gene variants in a recent study.[86] However, the association between ADMA and systemic inflammation remains controversial. Although Surdacki *et al*. described positive associations between ADMA and RA-specific autoantibodies,[87] preliminary reports failed to establish a direct relationship between inflammatory markers and ADMA[79,80,88] whilst others[89] demonstrated positive correlations between oxidative

Table 1. Overview of the studies assessing ADMA and SDMA in RA patients.

Authors	Patients	Parameters assessed	Assessment tools	ADMA	SDMA	Associations
Surdacki et al.[79]	30 RA/20 controls	Atherosclerosis	Carotid U/S	↑RA	Not assessed	IMT, endothelial progenitor cells count
Sandoo et al.[80]	67 RA/29 controls	Microvascular / macrovascular function Arterial stiffness	LDI with iontophoresis of ACh and SNP FMD with high-resolution U/S of the brachial artery Augmentation index	↑RA	Not assessed	No associations
Klimek et al.[81]	29 RA/29 controls	Disease activity	DAS28	↑RA with high DAS28	No difference	
Surdacki et al.[87]	20 RA	Autoimmunity	APCA		Not assessed	APCA
Korkosz et al.[88]	29RA/23 controls	Disease activity		↑RA	No difference	
Kwaśny-Krochin et al.[89]	46 RA/50 controls	Disease activity	Inflammatory markers Disease activity and disability scores	↑RA	↑RA	CRP, DAS28, HAQ
Sandoo et al.[91]	201 RA	Cumulative inflammation	Quarterly measurement of CRP and ESR / year		Not assessed	Cumulative inflammatory load
Dimitroulas et al.[92]	197 RA	Cumulative inflammation	Quarterly measurement of CRP and ESR / year	Not assessed		No associations
Turiel et al.[93]	25 RA/25 controls	Coronary circulation	Dipyridamole trans-thoracic stress U/S	↑RA	Not assessed	Coronary flow reserve
Sandoo et al.[96]	35 RA	Response to biologics		(-) after treatment	Not assessed	
Turiel et al.[97]	10 RA					
Di Franco et al.[82]	20 RA/20 controls	Response to DMARDS		↓ ADMA after treatment	Not assessed	
Dimitroulas et al.[103]	67 RA	Classic CVD risk factors	HOMA, QUICKI, BMI, Reynolds score		Not assessed	HOMA
Dimitroulas et al.[105]	197 RA/ 82 controls	Disease activity Classic CVD risk factors	CRP, ESR,HOMA, QUICKI, BMI, Reynolds score, DAS28	Not assessed	↓ RA	QUICKI, DAS28
Dimitroulas et al.[108]	201 RA	Coronary microvascular perfusion	Subendocardial viability ratio		Not assessed	No associations

ACh: acetylcholine, ADMA: asymmetric dimethylarginine, APCA: anti-citrullinated protein antibodies, BMI: body mass index, CFR: coronary flow reserve, CRP: C-reactive protein, CVD: cardiovascular disease, DAS28: disease activity score, ED: endothelial dysfunction, ESR: erythrocyte sedimentation rate, HOMA: haemostatic model assessment, FMD: flow mediated dilatation, HAQ: health assessment questionnaire, IMT: intima-media thickness, LDI: laser Doppler imaging, QUICKI: quantitative insulin sensitivity check index, RA: rheumatoid arthritis, SDMA: symmetric dimethylarginine, U/S: ultrasound

stress and CRP in 46 RA patients with active disease. These cross-sectional studies included only one single measurement of ESR and/or CRP and subsequently did not accurately reflect cumulative inflammatory load over a longer period of time, which is considered to have a larger impact on endothelial injury and the development of atherosclerosis in chronic conditions such as RA.[90] A recent publication shed more light on this unresolved issue as ADMA was found to be associated with cumulative inflammatory load in a

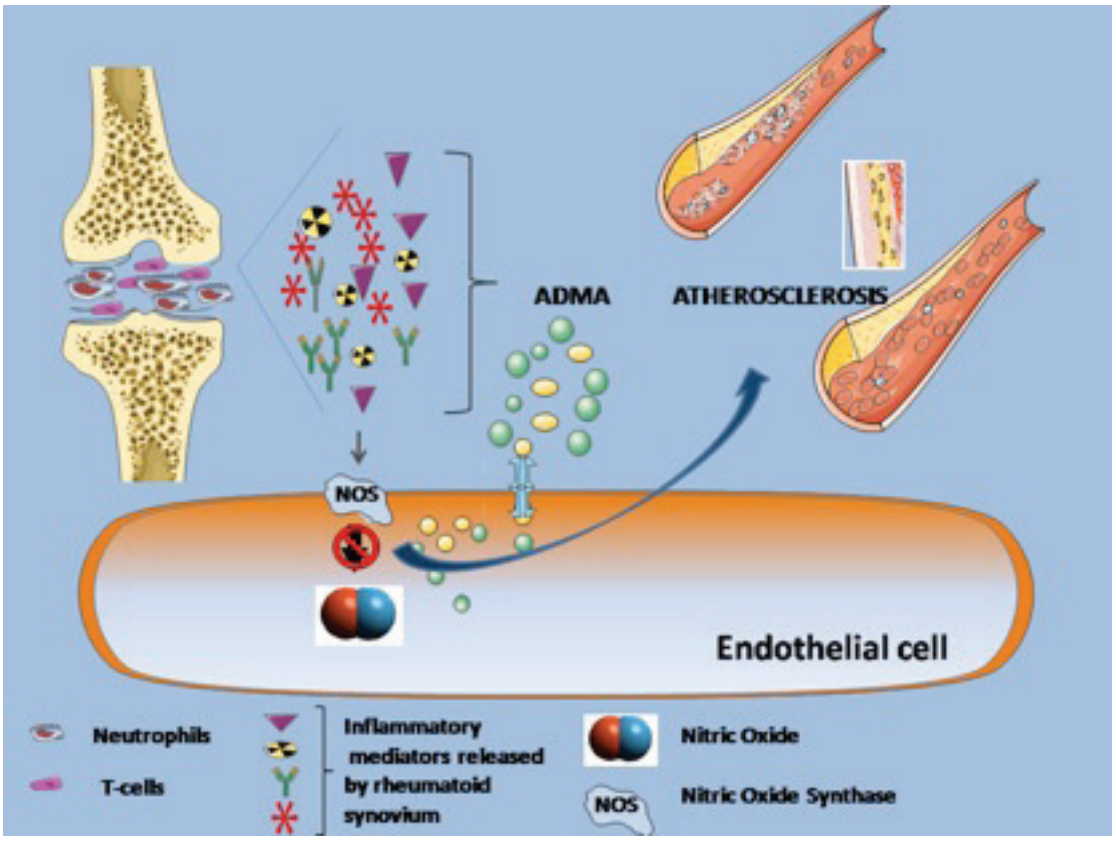


Figure 2. Schematic overview of NO synthase inhibition in RA. Increased production and release of various pro-inflammatory cytokines and mediators by activated immune cells in rheumatoid synovium has a dual role in mediating vascular injury: a potential direct inhibitory effect on NO synthase activity, and a significant input on ADMA synthesis upregulation through various mechanisms. ADMA inhibits NO synthase and the ensuing disruption in endothelial hemostasis results in vascular damage and promotion of various stages of atherosclerosis.

well-characterized cohort of RA patients in a 6-year follow-up study; suggesting a further mechanistic link between chronic inflammation and vascular injury, and an additional pathway for excessive CV morbidity in RA.[91] It is worth noting that a similar study investigating the relationship between SDMA and cumulative inflammation in the same RA population yielded negative results,[92] indicating that SDMA may have less proinflammatory properties compared to ADMA, and that NO synthase inhibitors may promote endothelial insult via different pathways[20].

With regards to other surrogate markers of atherosclerosis and vascular abnormalities, ADMA has been associated with morphological and functional parameters of endothelial dysfunction in some, but not all, studies. Significant correlations have been established between ADMA and carotid artery intima-media thickness[79] as well as coronary flow reserve in patients with early RA.[93] In contrast, no significant relationship between ADMA and assessments of *in vivo* endothelium-dependent and -independent microvascular and macrovascular function was established in 67 RA patients with moderate disease activity.[80] These inconsistent reports emphasize the complexity of vascular pathology in RA, indicating that assessments of vascular function and morphology cannot be used interchangeably to assess vascular health, as previous studies have found that microvascular and macrovascular endothelial function are independent of each other in this population.[94] Asymmetric dimethylarginines - a biochemical marker with no known specificity for the microvasculature or microvasculature - can be a useful, additional tool in the global evaluation of endothelial function and vascular health complementing information providing by clinical examination and other assessments of vascular function.

Whether clinical remission following treatment with anti-inflammatory drugs improves CV risk in RA remains to be determined as observational studies and data from registries did not always demonstrate a decrease in CV events.[95]

As ADMA is high in RA patients, the effects of treatment with conventional and biologic disease-modifying drugs on ADMA have been investigated in longitudinal studies. Most of the studies in patients with longstanding disease have not demonstrated any statistically significant difference in ADMA levels prior and after treatment with tumor necrosis-alpha inhibitors and/or synthetic anti-rheumatic drugs;[96-98] suggesting that mechanisms of ADMA accumulation in RA may be independent of tumor necrosis factor-alpha mediated pathways. On the other hand, a small study including 20 disease modifying drugs-naïve patients with early RA showed reduction of ADMA levels after 12 months of treatment with conventional and biologic agents.[81] This finding may imply that early intervention may be helpful in restoring endothelial function in RA but further studies are required.

The role of dimethylarginines in insulin resistance

Rheumatoid arthritis shares numerous pathophysiological processes with metabolic syndrome, such as acute phase response and vascular injury along with potential prognostic implications.[99] Insulin resistance is one of the main components of metabolic syndrome, and it is an important contributor to the CV risk associated with it. The chronic state of low-grade inflammation present in the metabolic syndrome and the multiple pleiotropic effects of adipokines on the vasculature have been implicated in the pathogenesis of vascular injury in RA, further augmented by high levels of systemic inflammation and immune activation.[100] It is now well-recognized and accepted that the magnitude of CV risk in RA is comparable to that of diabetes mellitus; the prototypic disease carrying increasing risk for atherosclerotic CV events.[101,102]

Nitric oxide production and vascular homeostasis are disrupted in RA and insulin resistance and dimethylarginines have been proposed as significant regulators of endothelial dysfunction in both conditions. For example, Homeostasis Model Assessment, a strong indicator of insulin resistance, has been established as an independent predictor of high ADMA levels in RA individuals.[103] Other studies have confirmed a strong relationship between insulin resistance and ADMA[104] reinforcing the hypothesis that a bidirectional influence between ADMA and insulin resistance might exist, and it is possible that the coexistence of high ADMA and abnormal insulin metabolism in RA act together in inducing and promoting inflammatory vascular reaction leading to atherosclerosis.

Similarities between RA and insulin resistance have been also described with other dimethylarginines. In contrast to ADMA, SDMA values are lower in RA patients and are correlated with insulin sensitivity[105]in line with observations in insulin resistant patients[106]. These findings may reflect a mechanism associated specifically with RA; similarly to what occurs in diabetes mellitus, where intriguing interactions between ADMA and CV events have been described.[107]

Conclusions and clinical implications

There is well-established evidence for considerable overlap between pathogenetic processes characterizing RA and atherosclerosis, resulting in high CV mortality and morbidity in these individuals. Cardiovascular risk management is an integral part of the care of RA patients, requiring – besides tight control of systemic inflammatory disease - modification of traditional CV risk factors. Complex interactions between environmental and genetic indices affect the immune system and vasculature, contributing to the disruption of endothelial function, and the initiation, development and destabilization of atherosclerotic lesions. Abnormal NO metabolism is considered to be the most important pathway through which systemic inflammation exerts deleterious effects on vascular wall, although NO-independent mechanisms have also been suggested to participate in accelerated atherosclerosis in RA. Dimethylarginines which have been recognized as regulators of NO/L-arginine pathway as well as mediators of inflammatory vascular disease encompass different aspects of pathogenesis of vascular disease in RA. The current level of evidence indicates that these molecules may be useful tools in the assessment of endothelial dysfunction in RA but large prospective studies are lacking. The role of ADMA and SDMA as biomarkers for CV stratification and earlier therapeutic intervention as part of a multilevel risk assessment tool needs to be examined in long term trials with specific hard endpoints.

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