

Symmetric dimethylarginine (SDMA) serum levels in rheumatoid arthritis: correlations with insulin resistance and disease activity scores

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Abstract Vascular abnormalities predisposing to atherosclerosis are present in patients with rheumatoid arthritis (RA) and associate with excess cardiovascular risk. Symmetric dimethylarginine (SDMA), an endogenous inhibitor of nitric oxide (NO) synthase activity, has been recognised as novel risk factor for endothelial dysfunction and cardiovascular disease. We aimed to compare SDMA levels in RA patients and controls and to investigate whether they are influenced by demographic, inflammatory or metabolic factors. Serum SDMA levels were measured in 197 RA individuals [median age: 67 years (quartiles: 59–3), 153 (78 %) females] and 82 controls [median age: 44 years [quartiles: 33–55, 50 (61 %) females]. Routine biochemistry tests, lipid profile, glycemic profile [glucose, insulin, homeostasis model assessment (HOMA-IR), quantitative insulin sensitivity check index (QUICKI)], as well as inflammatory markers were measured in all

patients. Paired analysis was employed for the comparison of SDMA in two groups and multivariable regression models were performed to identify predictors of SDMA in the RA cohort. SDMA was significantly lower in RA than control patients in both unpaired and paired analyses ($P < 0.001$ and $P = 0.005$, respectively), with the magnitude of the difference being similar in both models. QUICKI ($P = 0.005$) and disease activity score-28 ($P = 0.007$) were positively related to SDMA in the RA cohort, whilst a negative correlation with renal function (eGFR) was detected ($P = 0.005$). The molecular explanation of lower serum SDMA is unclear, but the established relationships with indices of disease activity and insulin resistance, may underline the pathogenetic role of the L-arginine/NO pathway dysregulation in the development of atherosclerosis in RA. The biological and clinical importance of SDMA in RA remains to be evaluated in clinical and experimental studies.

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Abbreviations

ADMA	Asymmetric dimethylarginine
AGXT2	Alanine-glyoxylate aminotransferase2
CRP	C-reactive protein
CV	Cardiovascular
DAS 28	Disease activity score 28
DDAH	Dimethylamine dimethylaminohydrolase
eGFR	Estimated glomerular filtration rate
ESR	Estimated sedimentation rate
HAQ	Health assessment questionnaire
HOMA-IR	Homeostasis model assessment of insulin resistance
NO	Nitric oxide

QUICKI	Quantitative insulin sensitivity check index
RA	Rheumatoid arthritis
SDMA	Symmetric dimethylarginine

Introduction

Accelerated atherosclerosis leading to excess cardiovascular (CV) risk has been recognised as a major cause of premature morbidity and mortality in patients with rheumatoid arthritis (RA) (Gonzalez-Gay et al. 2005). In addition to higher frequency of CV disease risk factors in RA compared to the general population (Chung et al. 2012), autoimmunity and systemic inflammation have a pivotal role in the development of vascular disease, as rheumatoid synovial and atherosclerotic plaques share a common inflammatory cellular and molecular signalling *milieu* (Abou-Raya A and Abou-Raya S 2006).

Endothelial dysfunction is a central event in the development of atherosclerosis and contributes to all stages of coronary artery disease, from the early formation to the rupture of atherosclerotic plaque. Balanced production of endothelium-derived vasoactive substances maintains the integrity of vascular endothelium, providing an atheroprotective environment within the vessel. Nitric oxide (NO) is considered the most important of these substances and factors affecting its bioavailability are involved in the pathophysiology of vascular damage and injury (Sandoo et al. 2010). In chronic high-grade inflammatory conditions such as RA, increased systemic expression of inflammatory mediators exerts detrimental effects on vascular haemostasis, mainly by affecting NO metabolism, resulting in functional and structural changes, such as dysregulation of vascular tone and disorganization of the vascular architecture that promote thrombosis and atherosclerosis (Desideri and Ferri 2005).

Dimethylarginines are endogenous guanidine-substituted analogues of L-arginine, the precursor of NO, which are naturally liberated in biological fluids following proteolysis. During endogenous protein turnover asymmetrically and symmetrically methylated L-arginine residues are released as asymmetric (ADMA) and symmetric (SDMA) dimethylarginine, respectively. Several lines of evidence support the adverse effect of these molecules on vascular health, as they hold a key homeostatic role in NO production by inhibiting NO synthase (Caplin and Leiper 2012). ADMA, the most studied dimethylarginine today, has been linked to vascular outcomes and overall mortality in different CV disease settings as well as in the general population (Böger et al. 2009; Leong et al. 2008). ADMA has also been considered as a potential CV disease risk factor in patients with rheumatic diseases, with reports demonstrating higher levels in patients with RA compared to controls

(Sandoo et al. 2012a), as well as associations with morphological markers of premature atherosclerosis (Surdacki et al. 2007) and RA-related inflammatory burden (Sandoo et al. 2014).

Symmetric dimethylarginine (SDMA), the structural counterpart of ADMA, was initially considered biologically inert. However, SDMA is a weak inhibitor of neuronal NO synthase (Tsikas et al. 2000). Recent insights suggest that SDMA influences NO synthesis indirectly, by enhancing formation of reactive oxygen species and/or by inhibiting cellular transport of L-arginine and other amino acids (Schepers et al. 2009; Strobel et al. 2012). SDMA has emerged as an independent CV risk factor in the general population and some high-risk populations (Siegerink et al. 2013; Schulze et al. 2010; Schwedhelm et al. 2014). Coronary artery disease is the leading cause of death in patients with RA (Levy et al. 2008) and there is increasing interest in biomarkers that might better identify patients at higher risk for CV disease who may benefit from early preventive strategies and targeted therapeutic interventions for CV risk factors. Little attention has been paid in the role of SDMA and other arginine analogues such as homoarginine (Kayacelebi et al. 2014) in vascular pathology characterising RA.

Therefore, we aimed to compare serum SDMA levels in patients with RA and healthy controls and to explore the associations between SDMA and indices of disease activity and insulin resistance.

Materials and methods

Study population

One hundred and ninety-seven consecutive Caucasian RA patients were recruited from the rheumatology outpatient clinics of the Dudley Group NHS Foundation Trust, UK, between March 2011 and March 2013. All patients met the retrospective application of the 1987 revised RA criteria of the American College of Rheumatology (Arnett et al. 1988). Patients with previous confirmed acute coronary syndrome, evidence of chronic kidney disease or serious psychiatric disorders according to their medical records were excluded. Eighty-two healthy participants were recruited from hospital staff and their friends. The exclusion criteria were the same as for the RA patients. The study received approval from the Black Country Research Ethics Committee and all patients gave their written informed consent according to the Declaration of Helsinki.

Demographic data were recorded and all participants underwent a thorough assessment including a detailed review of their medical history, hospital records and a physical examination. Disease activity was assessed by

the 28-joint disease activity score—ESR (DAS28-ESR) (Prevoo et al. 1995) and physical function using the anglicised version of the Health Assessment Questionnaire (HAQ) (Kirwan and Reeback 1986). All current medications including biologic and non-biologic disease-modifying drugs were recorded.

Insulin resistance was evaluated from fasting glucose and insulin using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) (Matthews et al. 1985). The formulas used for the calculation were as follows (Wallace et al. 2004; Chen et al. 2003):

$$\text{HOMA-IR} = (\text{Fasting Plasma Insulin} \times \text{Fasting Plasma Glucose}) / 22.5$$

$$\text{QUICKI} = 1 / (\log_{10} [\text{Fasting Plasma Insulin}] + \log_{10} [\text{Fasting Plasma Glucose}])$$

Blood tests for biochemical and serological markers were carried out in the Research Laboratory at Russells Hall Hospital. Total cholesterol, high-density lipoprotein cholesterol, triglycerides and C-reactive protein (CRP) were measured using the Vitros 5.1chemistry system (www.orthoclinical.com). Estimated glomerular filtration rate (eGFR) was calculated using modification of diet in renal disease study equation (Levey et al. 1999). Levels of erythrocyte sedimentation rate (ESR) were determined using the Westergren method via a Starrsed Compact (Mechatronics BV, Netherlands). This is an immunoturbidimetric method.

Measurement of SDMA

SDMA was measured in the serum of the study participants. The SDMA assay is based on the method of competitive enzyme using a commercial ELISA kit (Immunodiagnostik, Bensheim, Germany). The sample preparation includes the addition of a derivatization reagent for SDMA coupling. During the incubation period, the target SDMA in the sample competes with the SDMA derivative (tracer) immobilised on the wall of the microtiter plates for the binding of the polyclonal antibodies. The SDMA in the sample displaces the antibodies out of the binding to the tracer. Therefore, the concentration of the tracer-bound antibody is inversely proportional to the SDMA concentration in the sample. The absorbance is measured at 450 nm and patient samples are read from a standard curve. The intra-assay relative standard deviation was 7.5 % and inter-assay was 6 %. The lowest concentration detected was 0.05 μM .

Statistical analysis

Initially, SDMA levels, and other relevant factors, were compared between patients and controls using Mann–Whitney and Fisher’s exact tests. To account for the effects of potentially confounding factors such as age, renal function, inflammation, individuals within the groups were then matched, using a propensity score, and the resulting subset of paired individuals were compared using Wilcoxon’s signed rank and McNemar’s tests.

Within RA patients, associations between a range of variables and SDMA were then assessed using univariable regression models. Factors that were found to be significantly associated with the outcomes were then included in multivariable regression models, to account for potentially confounding factors. Prior to the analysis, the distributions of all of the variables were assessed by plotting histograms. Where skew was detected, \log_2 -transformations were applied, after adding a constant, where applicable, to remove zero values (Bland and Altman 1996). All analyses were performed using IBM SPSS Statistics 22 (IBM Corp. Armonk, NY, USA), with $P < 0.05$ deemed to be indicative of statistical significance.

Results

Baseline characteristics

SDMA data were available for 197 RA and 82 healthy control patients. The demographic characteristics and basic biochemical evaluation of the study participants are summarized in Table 1. RA patients were significantly older than controls, with significantly greater predominance of females, as expected. The RA group was found to have significantly higher triglycerides and CRP levels and lower eGFR.

Comparison of SDMA between RA and controls

Initial comparisons between groups (Table 1) demonstrated that serum SDMA levels were significantly lower in the RA patients compared to controls with medians of 0.47 μM (quartiles: 0.41–0.53) and 0.52 (0.49–0.57) μM , respectively ($P < 0.001$).

In an attempt to remove the potential confounding effects of different distributions of age and biochemical variables between patients and controls, a propensity score was produced. This was based on a multivariable binary logistic regression model, with the patient cohort being the dependent variable (Supplementary Table S1). eGFR was not included in the propensity model, due to issues with

Table 1 Demographic, biochemical and RA-related characteristics and results of unpaired analysis between patients and controls

	RA patients (<i>N</i> = 197)	Healthy controls (<i>N</i> = 82)	<i>P</i> value
General characteristics			
Age (years)	67 (59–73)	44 (33–55)	<0.001*
Gender			
Male	44 (22 %)	32 (39 %)	
Female	153 (78 %)	50 (61 %)	0.005*
Biochemical parameters			
Creatinine (μM)	69 (60–79)	71 (64–81)	0.141
eGFR (ml/min/1.73 m ²)	79 (69–>90)	86 (77–>90)	<0.001*
HDL cholesterol (mM)	1.4 (1.1–1.7)	1.5 (1.2–1.6)	0.575
TG (mM)	1.2 (0.8–1.5)	0.9 (0.7–1.2)	<0.001*
SDMA (μM)	0.47 (0.41–0.53)	0.52 (0.49–0.57)	<0.001*
Disease characteristics			
Disease duration (years)			
	16 (11–25)		
Rheumatoid factor positive <i>N</i> (%)	148 (74)		
Anti-CCP positive <i>N</i> (%)	123 (61)		
DAS28	3.2 (2.5–4.0)		
C-reactive protein (mg/l)	3.0 (<3–8.0)	<3 (<3–<3)	<0.001*
ESR (mm/h)	12 (5–24)		
HAQ	1.8 (1.0–2.1)		
RA medications			
Methotrexate, <i>N</i> (%)	120 (61)		
Hydroxychloroquine, <i>N</i> (%)	47 (24)		
Prednisolone, <i>N</i> (%)	49 (25)		
NSAIDS, <i>N</i> (%)	25 (13)		
Anti-TNFα therapy, <i>N</i> (%)	55 (28)		
Tocilizumab, <i>N</i> (%)	3 (2)		
Cardiovascular medications			
Antihypertensive, <i>N</i> (%)	72 (37)		
Antihypercholesterolemic, <i>N</i> (%)	72 (37)		
Beta-blocker, <i>N</i> (%)	22 (11)		
Calcium channel blocker, <i>N</i> (%)	27 (13)		

Continuous data reported as medians and quartiles, with *P* values from Mann–Whitney tests. Categorical data reported as *N* (%), with *P* values from Fisher's exact test

Standard values ESR, 1–20; CRP, <3; DAS 28, 0–2.6 low disease activity; 2.6–5.1 moderate disease activity, >5.1 high disease activity

HDL high-density lipoprotein, *TG* triglycerides, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *DAS28* disease activity score, *HAQ* health assessment questionnaire, *NSAIDS* non-steroid anti-inflammatory drugs, *anti-CCP* anti-citrullinated protein antibodies, *TNFα* tumour necrosis factor-alpha, *eGFR* estimated glomerular filtration rate, *SDMA* symmetric dimethylarginine

* Significant at *P* < 0.05

multicollinearity, as it was highly correlated with both age and creatinine. CRP was converted into a categorical variable, since a large proportion of individuals had values <3 mg/l, which was a cutoff for truncation at the point of data collection.

Individuals from the two cohorts were then paired on the resulting propensity score. A nearest neighbour approach was used, using a match tolerance of 0.05, and with matches selected at random in the case of ties. Matches

could be found for a total of 44 individuals from each group, which are compared in Table 2. No significant differences between the groups were detected for any of the variables from the propensity score on which the patients were matched. However, the difference in SDMA levels between the groups remained significant (*P* = 0.005), and of a similar magnitude to the unpaired analysis, with medians of 0.46 μM (quartiles: 0.40–0.54) in the RA group, and 0.53 μM (0.49–0.56) in controls.

Table 2 Paired Analysis of propensity matched individuals

	RA (N = 44)	Control (N = 44)	P value
SDMA (μM)	0.46 (0.40–0.54)	0.53 (0.49–0.56)	0.005*
Age (years)	54 (46–63)	54 (45–60)	0.542
Gender			0.774 ^a
Male	11 (25 %)	9 (20 %)	
Female	33 (75 %)	35 (80 %)	
eGFR (ml/min/1.73 m ²)	83 (76–90)	82 (74–89)	0.807
HDL (mM)	1.5 (1.2–1.8)	1.5 (1.2–1.7)	0.964
TG (mM)	1.0 (0.7–1.5)	1.0 (0.7–1.4)	0.764
Creatinine (μM)	66 (60–75)	70 (63–78)	0.525
CRP (<3) (mg/l) ^b	34 (77 %)	34 (77 %)	0.637

Data reported as medians and quartiles, or N (%), as applicable, with P values from Wilcoxon's test, unless stated otherwise

SDMA symmetric dimethylarginine, HDL high-density lipoprotein, TG triglyceride, CRP C-reactive protein, eGFR estimated glomerular filtration rate

* Significant at $P < 0.05$

^a P value from McNemar's test

^b The majority of patients had CRP levels <3, which was the lower limit of truncation during data collection, hence the data are reported as a rate

Associations between SDMA with disease-related and metabolic factors

A second analysis was performed within the RA cohort. Initial review of the distributions of serum SDMA concentrations showed that they were highly skewed, and so log₂-transformations were applied. The resulting variables closely followed a normal distribution, with the exception of 11 (5.6 %) patients with serum SDMA levels >1 μM , who did not fit into the distribution of the remainder (Supplementary Figure S1). These outliers (Supplementary Table S2) were excluded from subsequent analysis, meaning that the remaining data met the normality assumptions, allowing parametric analyses to be used. This left 186 patients, with a median age of 66 years (range 32–89), of whom 144 (77.4 %) were females.

Univariable regression models identified three factors that were significant predictors of SDMA (Table 3). Both DAS28 ($P = 0.010$) and QUICKI ($P = 0.032$) showed significant positive associations, with SDMA, whilst eGFR was significantly negatively associated ($P = 0.024$) with SDMA. In multivariable analysis, the three factors associated with SDMA remained significant (Table 4). A one-unit increase of DAS28 was found to be associated with 3.7 % higher SDMA serum levels (95 % CI 1.1 %, 6.3 %; $P = 0.005$). An increase of 0.1 unit in QUICKI was associated with a 10.8 % higher SDMA levels (95 % CI 2.8 %, 19.5 %; $P = 0.007$). For eGFR, a one-unit increase was

Table 3 Predictors of SDMA ratio in univariable regression models

Factor	SDMA	
	Coefficient (95 % CI) (%)	P value
Age (per decade)	2.0 (–0.8, 4.8)	0.164
BMI (kg/m ²)	–0.5 (–1.1, 0.0)	0.063
Log ₂ CRP [#]	–1.1 (–3.4, 1.3)	0.375
Log ₂ ESR [#]	1.2 (–0.8, 3.3)	0.244
DAS28	3.4 (0.8, 6.1)	0.010*
Log ₂ Insulin [#]	–3.1 (–6.4, 0.4)	0.081
Log ₂ HOMA-IR ^a	–3.1 (–6.2, 0.0)	0.052
Log ₂ Homocysteine ^a	5.6 (0.0, 11.5)	0.052
QUICKI (per 0.1 Units)	8.7 (0.7, 17.3)	0.032*
eGFR (ml/min/1.73 m ²)	–0.3 (–0.5, 0.0)	0.024*
Gender (male)	4.5 (–2.8, 12.2)	0.234
RF (positive)	3.4 (–3.5, 10.8)	0.344
Anti-CCP (positive)	6.1 (–0.2, 12.8)	0.060
TNF-alpha inhibitors (yes)	1.8 (–4.8, 8.8)	0.599
Steroids (yes)	4.3 (–2.8, 11.8)	0.239
Methotrexate (yes)	0.7 (–5.4, 7.1)	0.837
Sulfasalazine (yes)	–0.8 (–7.7, 6.5)	0.816
Hydroxychloroquine (yes)	–0.8 (–7.7, 6.5)	0.816
Leflunomide (yes)	1.3 (–11.4, 15.8)	0.853
DMARDS (yes)	1.9 (–5.2, 9.6)	0.601
NSAID (yes)	–3.1 (–11.8, 6.4)	0.503
Antihypercholesterolemic (yes)	1.0 (–5.2, 7.5)	0.765
Antihypertensive (yes)	1.0 (–5.2, 7.5)	0.765

The original coefficients from the model were anti-logged, and converted into percentage changes in the outcome per unit increase of the factor

ADMA asymmetric dimethylarginine, SDMA symmetric dimethylarginine, BMI body mass index, CRP C-reactive protein, eGFR estimated glomerular filtration rate, ESR erythrocytes sedimentation rate, DAS28 disease activity score, HOMA-IR Homeostasis Model Assessment of Insulin Resistance, QUICKI Quantitative Insulin Sensitivity Check Index, RF rheumatoid factor, anti-CCP Anti-citrullinated protein antibodies, TNF-alpha tumour necrosis factor-alpha, DMARDS disease-modifying drugs, NSAIDS non-steroid anti-inflammatory drugs

* Significant at $P < 0.05$

^a For the factors that were log-transformed, the coefficient represents the increase in the outcome for a twofold increase in the untransformed factor

associated with 0.3 % lower SDMA (95 % CI 0.1 %, 0.6 %; $P = 0.005$).

A sensitivity analysis was then performed, to test whether removing the outliers had introduced bias into the analysis. SDMA values were correlated with the three factors found to be significant, using Spearman's rho on all 197 patients, and Pearson's r on the 186 with outliers excluded. Coefficients for DAS28 ($\rho = 0.218$, $r = 0.157$), eGFR ($\rho = -0.206$, $r = -0.194$) and QUICKI ($\rho = 0.108$,

Table 4 Multivariable Regression models for SDMA

Factor	Coefficient (95 % CI) (%)	P value
DAS28	3.7 (1.1, 6.3)	0.005*
QUICKI (per 0.1 units)	10.8 (2.8, 19.5)	0.007*
eGFR (ml/min/1.73 m ²)	−0.3 (−0.6, −0.1)	0.005*

The original coefficients from the model were anti-logged, and converted into percentage changes in the outcome per unit increase of the factor

SDMA symmetric dimethylarginine, eGFR estimated glomerular filtration rate, DAS28 disease activity score, QUICKI quantitative insulin sensitivity check index

* Significant at $P < 0.05$

$r = 0.153$) were similar in both analyses, hence it was concluded that there was no evidence to suggest that excluding the outliers had adversely affected the conclusions of the analysis.

Discussion

The main finding of our study is that serum SDMA levels are lower in patients with RA compared to healthy controls, and are associated with the QUICKI index, a commonly used measure of insulin sensitivity. We also found positive correlations between SDMA and disease activity score.

Unlike ADMA, SDMA may affect vascular haemostasis through other mechanisms, such as the stimulation of reactive oxygen species by modulating calcium channels in monocytic leukocytes (Schepers et al. 2009). Such proinflammatory effects have not been described with ADMA which, on the other hand, upregulates endothelial adhesion molecules and contributes to oxidative stress by inhibiting NO isoenzymes in various tissues via different mechanisms (Karbach et al. 2014; Kielstein et al. 2007). These observations suggest that these NO synthase inhibitors may promote endothelial insult via the involvement of different cell types and mechanisms (Lüneburg et al. 2012). Until recently, the role of SDMA in clinical studies assessing the relationship between dimethylarginines and CV disease was underestimated but, over the last few years, numerous reports suggest that SDMA may be a predictor of CV mortality and morbidity in a wide range of cardio- and cerebro-vascular disease (Meinitzer et al. 2011; Riccioni et al. 2014; Siegerink et al. 2013; Schulze et al. 2010). It is worth noting that ADMA and SDMA had similar predictive values for cardiovascular risk in a population-based cohort of older individuals (Kiechl et al. 2009) and higher SDMA levels have been linked with increased overall mortality in the general population (Gore et al. 2013; Schwedhelm et al. 2014).

To the best of our knowledge, this is the first study reporting on SDMA concentrations in RA patients and controls as well as on associations between SDMA insulin sensitivity and inflammation. A similar trend of decreased SDMA in RA was found in a small study including 29 RA patients and 26 controls, but the results did not reach statistical significance (Klimek et al. 2014). Given that RA is associated with enhanced CV risk, and SDMA has been proposed as a potential marker of atherosclerosis and vascular dysfunction, the results of our study look at first glance fundamentally counterintuitive. However, reduced SDMA levels have been reported in insulin-resistant patients (Zsuga et al. 2007) and a strong negative relationship between SDMA and insulin resistance has been described in Caucasians (Schutte et al. 2010). In concordance with these data, we demonstrated a positive relationship between SDMA and insulin sensitivity assessed by QUICKI in individuals with RA. A similar insignificant tendency to a negative correlation between SDMA and HOMA-IR has been reported to 80 non-diabetic men with stable angina in whom ADMA levels predicted deterioration of glucose tolerance (Surdacki et al. 2013).

RA and insulin resistance share several atherogenic processes, including vascular injury, acute phase response and aberrant lipid metabolism which might play a role in the increased CV risk observed in these patients (Dessein et al. 2003). Insulin resistance is the central mechanism in the development of metabolic syndrome, which is highly prevalent in RA, and is considered an additional aggravating factor for heightened CV morbidity and mortality in this population (da Cunha et al. 2012). In individuals with RA, insulin resistance maintains a chronic low grade inflammatory activity irrespective of effective—or not—control of joint disease, whereas systemic high-grade RA-related inflammation has adverse effects on vascular pathology precipitating endothelial dysfunction caused by components of the metabolic syndrome (Ferraccioli and Gremese. 2011). This reciprocal relationship is reflected in studies clearly demonstrating that the magnitude of CV risk in RA is virtually identical to that of diabetes mellitus, the prototypic disease carrying high CV risk burden (Lindhardsen et al. 2011; Stamatelopoulos et al. 2009). The lower SDMA concentrations in RA and insulin-resistant patients, as well as previous reports of associations between ADMA and insulin resistance in RA (Dimitroulas et al. 2013) suggest that common pathogenic mechanisms driven by abnormal regulation of L-arginine/NO pathway are involved in the development of accelerated atherosclerosis in both conditions.

The mechanisms related to low SDMA are incompletely understood; however decreased insulin sensitivity results in hyperinsulinemia that directly increases overexpression of γ + transporters, the main route of intracellular migration

of endogenous dimethylarginines (Simmons et al. 1996; González et al. 2004). Taking into account that SDMA competitively inhibits ADMA for entry into the cells via y^+ transporters, it is tempting to speculate that increased SDMA cellular uptake contributes to raised serum ADMA levels, the subsequent inhibition of NO synthesis and the evolution of vascular abnormalities leading to atherosclerosis. Recent reports have shed more light in the mechanisms governing SDMA clearance and degradation, indicating alanine-glyoxylate aminotransferase2 (AGXT2) as an alternative enzymatic cleavage route in healthy controls (Sepälä et al. 2014) and diabetic patients (Anderssohn et al. 2014). AGXT2 strongest mRNA expression was identified in renal and liver tissue (Lüneburg et al. 2014) suggesting that, apart from renal excretion, other organs may also contribute to SDMA clearance. Whether metabolic activation of hepatocytes triggered by proinflammatory cytokines in RA upregulates hepatic metabolic pathways of SDMA remains to be determined. It is worth noting that dimethylarginine dimethylaminohydrolase (DDAH) enzymatic action is not involved in SDMA degradation, despite its leading role in ADMA clearance (Caplin and Leiper 2012). High DDAH activity has been reported in RA and spondyloarthropathies (Chobanyan-Jürgens et al. 2011) but we did not find any relationship between DDAH gene variants and ADMA levels in our RA population (Dimitroulas et al. 2014).

The multivariable analysis confirmed previous studies in other populations, showing the close relationship between SDMA and eGFR which have led to the view that SDMA is a marker for renal disease (Kielstein et al. 2006). As a result, it has been suggested that SDMA may constitute a more reliable indicator of CV disease in patients with renal insufficiency, while ADMA may be more important in the presence of normal renal function (Schulze et al. 2010). Given that renal dysfunction is common in patients with RA, and is associated with classic CV risk factors (Daoussis et al. 2010), the potential of SDMA as a surrogate marker of CV disease in this population is promising but warrants further in-depth studies.

Systemic inflammatory burden has been considered one of the main drivers of vascular damage and derangement of endothelial haemostasis in RA. In the present study, and despite the lack of association between SDMA and acute phase response, the positive relationship with clinical markers of disease activity reinforces the hypothesis that ADMA and SDMA may mediate endothelial dysfunction not only by directly diminishing NO bioavailability, but also by exhibiting proinflammatory effects on the vascular wall. In that respect, ADMA and SDMA may affect vascular haemostasis in RA by other NO-independent mechanisms in keeping with observations in a German population (Willers and Hahn 2012). The decreased serum SDMA levels found

in our study 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 (Anderssohn et al. 2010). It has been reported that interstitial ADMA seems to play a minor role in NO-dependent regulation of adipose tissue blood flow and metabolism in obese and type 2 diabetic patients (May et al. 2014), suggesting that other NO-independent mechanisms may be involved in endothelial dysfunction in insulin-resistant patients. The complexity of CV disease in RA with multilevel interrelations between traditional, novel and disease-related risk factors pose significant difficulties in the establishment of biochemical markers able to encompass such a variety of pathophysiologic processes. Dimethylarginines can capture different elements of this puzzle and improve diagnostic capabilities in CV risk stratification, but translating these assumptions into clinical practice requires replication in multiple settings, ideally with large prospective studies to prove the predictive value of ADMA and SDMA in the RA population.

The findings of our study should be considered against the background of its strengths and limitations. We acknowledge that the RA and control groups were not matched for age, gender and renal function but we took particular care to attenuate the impact of these differences on the outcomes with the propensity matched paired analysis. The lack of variation in the magnitude of SDMA difference between the two groups in unpaired and paired analysis is reassuring regarding the validity of our results. In the regression analyses, we excluded SDMA outliers from the data, to achieve a normal distribution, to maximise the fit and reliability of the final models. However, a sensitivity analysis found that conclusions would have been similar if these patients were included. Patients in our cohort had, on average, moderate to low disease activity which may have contributed to the lower SDMA levels observed in these RA individuals; therefore our findings should not be extrapolated to all RA populations, particularly to patients with active uncontrolled disease, without confirmation in further studies. Despite the cross-sectional design, our study is the first to assess SDMA in RA patients and controls. In fact very few studies assessing SDMA in other conditions characterised by increased CV risk have utilised a control group, either in a cross-sectional or prospective manner (Zsuga et al. 2007). Another strength of the present work is the inclusion of a large cohort of real-life RA patients on a variety of disease-modifying drugs and steroids. We took particular care to consider the influence of antirheumatic medications on SDMA levels in our analysis, none of which were found to be significantly predictive of SDMA in univariate analysis. In addition, biologic and non-biologic disease-modifying drugs do not appear to affect ADMA levels in previous cohorts of RA patients

(Turiel et al. 2010; Sandoo et al. 2012b). Last but not least our RA population was almost exclusively Caucasian, diminishing the effect of ethnic differences previously reported for dimethylarginines (Schutte et al. 2010).

Conclusion

We showed that serum SDMA is lower in RA patients and that it is inversely correlated with insulin resistance. Preliminary data indicate SDMA as a useful tool for determining CV risk, but there is still inadequate understanding of its role in promoting endothelial dysfunction by altering NO metabolism. The link between dimethylarginines and parameters of disease activity and insulin sensitivity supports the notion that ADMA and SDMA induce vascular damage in chronic high-grade inflammatory conditions, such as RA, but further longitudinal studies are warranted to elucidate the importance of L-arginine/NO pathway in the development of atherosclerosis and CV risk in patients with RA.

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants The study received approval from the Black Country Research Ethics Committee. All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study according to the Declaration of Helsinki.

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