Comprehensive arginine metabolomics and peripheral vasodilatory capacity in rheumatoid arthritis: a monocentric cross-sectional study.

Gian Luca Erre (e.gianluca@libero.it)
Azienda Ospedaliero-Universitaria di Sassari https://orcid.org/0000-0001-6818-7666
Arduino Aleksander Mangoni
The University of Adelaide Discipline of Clinical Pharamcology
Giuseppe Passiu
Universita degli Studi di Sassari Facolta di Medicina e Chirurgia
Stefania Bassu
Universita degli Studi di Sassari Facolta di Medicina e Chirurgia
Floriana Castagna
Universita degli Studi di Sassari Facolta di Medicina e Chirurgia
Ciriaco Carru
Universita degli Studi di Sassari
Matteo Piga
Universita degli Studi Di Cagliari Cittadella Universitaria
Angelo Zinellu
Universita degli Studi di Sassari
Salvatore Sotgia
Universita degli Studi di Sassari

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Comprehensive arginine metabolomics and peripheral vasodilatory capacity in rheumatoid arthritis: a monocentric cross-sectional study.

Gian Luca Erre[1,2], Arduino Aleksander Mangoni[3], Giuseppe Passiu[1,2], Stefania Bassu[2], Floriana Castagna[2], Ciriaco Carru[4], Matteo Piga[5], Angelo Zinellu[4], Salvatore Sotgia[4].

[1]Department of Clinical and Experimental Research, University of Sassari, Sassari, Italy

[2]UOC di Reumatologia, Dipartimento di Specialità Mediche, Azienda Ospedaliero-Universitaria di Sassari, Sassari, Italy

[3]Discipline of Clinical Pharmacology, College of Medicine and Public Health, Flinders University and Flinders Medical Centre, Adelaide, Australia

[4]Departiment of Biomedical Sciences, University of Sassari, Sassari. Italy.

[5]UOC di Reumatologia, Policlinico Universitario e Università degli Studi di Cagliari, Italy

Corresponding Author

Gian Luca Erre, MD PhD

Department of Clinical and Experimental Research, University of Sassari, Sassari, Italy

Dipartimento di Specialità Mediche, UOC Reumatologia, Azienda Ospedaliero-Universitaria di Sassari, Sassari, Italy

Viale San Pietro, 8

07100 Sassari, Sassari, Italy

Phone: +39079228317

Fax: +39079216282

email: gianluca.erre@aousassari.it

e.gianluca@libero.it

ABSTRACT

Background: the relationship between plasma arginine metabolites influencing vascular homeostasis and peripheral vasodilatory capacity in rheumatoid arthritis (RA) patients is not known. **Methods:** L-arginine (Arg), monomethyl-L-arginine (MMA), L-homoarginine (hArg), asymmetric dimethyl-L-arginine (ADMA), symmetric dimethyl-L-arginine, and L-citrulline (Cit) were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) in 164 RA patients and 100 age- and sex-matched healthy controls without previous cardiovascular events. Log-transformed reactive hyperemia index (Ln-RHI) evaluated by flow-mediated pulse amplitude tonometry (PAT, EndoPAT2000 device) was assessed as surrogate measure of peripheral vasodilatory capacity in RA patients. Ln-RHI values <0.51 indicated peripheral endothelial dysfunction (ED). The relationship between plasma arginine metabolite concentrations, RA descriptors and peripheral vasodilatory capacity was evaluated by bivariate correlation and regression analyses. **Results:** Plasma ADMA concentrations were significantly higher, and plasma hArg concentrations significantly lower, in RA patients than in controls (0.53 ± 0.09 *vs* 0.465 ± 0.07 μmol/L and 1.50 ± 0.60 *vs* 1.924 ± 0.78 μmol/L, respectively; p<0.001 for both comparisons). Bivariate correlation analysis demonstrated no significant correlation between arginine metabolites and disease descriptors. In regression analysis in RA patients, higher plasma ADMA concentrations were independently associated with presence of ED [OR(95%CI) = 77.3(1.478 - 4050.005), *p*=0.031] and lower Ln-RHI [B coefficient(95%CI) =-0.57(-1.09 to -0.05), *p*=0.032]. **Conclusions**: ADMA was significantly, albeit weakly, associated with impaired microcirculatory vasodilatory capacity and peripheral endothelial dysfunction in RA. This suggests an important pathophysiological role of this metabolite in the vascular alterations observed in this patient group.

KEYWORDS

Rheumatoid arthritis; L-homoarginine; ADMA; SDMA; endothelial dysfunction; flow-mediated pulse amplitude tonometry.

BACKGROUND

Rheumatoid arthritis (RA), is an autoimmune disease characterized by chronic articular and systemic inflammation, bone damage and excess of atherosclerotic cardiovascular mortality[1]. The increased prevalence of atherosclerotic disease in RA patients compared to the general population has been attributed to premature endothelial dysfunction (ED) and arterial stiffening[2–4].

ED, the earliest step of the pathological process leading to arterial wall atherosclerosis and plaque formation, has been documented both in early and long-standing RA and linked to chronic inflammation[5–7]. Recently, we demonstrated, using flow-mediated pulse amplitude tonometry (PAT), a relatively high prevalence (about 30%) of peripheral microvascular ED in a large cohort of RA patients. However, ED was weakly associated with conventional cardiovascular risk factors, suggesting that other pathways are involved[8]. Reactive hyperaemia of small digital artery measured by PAT, a surrogate measure of peripheral microcirculatory vasodilatory capacity, is significantly correlated with coronary vasodilatory capacity and predicts coronary heart disease and cardiovascular events[9–11].

Several clinical and experimental evidences have convincingly shown that alterations of arginine metabolism are involved in the development of ED[12]. The methylarginines N^G-monomethylarginine (MMA), N^G,N^G-dimethyl-L-arginine (ADMA), and N^G,$N^{G'}$-dimethyl-L-arginine (SDMA), are generated by proteolysis of post-translationally methylated tissue proteins. MMA and ADMA are endogenous inhibitors of nitric oxide synthases and, therefore, negatively modulate nitric oxide availability. In particular, the accumulation of ADMA has been linked to impaired vascular homeostasis in different patient groups and is widely considered as a strong and independent

predictor of cardiovascular and all-cause mortality[13]. Increased ADMA concentrations have been demonstrated in RA[14], and have been linked to ED and impaired endothelial repair[15]. However, little is known about the relationship between methylarginines, and other arginine metabolites potentially influencing vascular homeostasis such as arginine, L-homoarginine (hArg), and L-citrulline (Cit), and peripheral vasodilatory capacity in RA.

Therefore, we sought to address this issue by investigating the significance and the strength of the association between arginine metabolomics and peripheral microcirculatory ED in RA patients free from previous cardiovascular events. Moreover, plasma levels of arginine metabolites in RA patients were also compared to those of heathy controls to assess the role of RA itself in arginine metabolism.

The identification of specific arginine metabolites associated with ED in RA might have important biological and clinical implications because of the lack of clear associations between ED and conventional cardiovascular risk factors previously reported in this group.

METHODS

Patients and controls

Consecutive RA patients aged 45–85 years, free from previous cardiovascular events, prospectively enrolled in the Bio-RA study (Evaluation of new BIO-markers of atherosclerosis in Rheumatoid Arthritis) between October 2015 and July 2017 were evaluated. The Bio-RA study is a sub-study of the Endothelial Dysfunction Evaluation for Coronary Heart Disease Risk Estimation in Rheumatoid Arthritis study (EDRA study. ClinicalTrials.gov: NCT02341066).

Inclusion criteria of the Bio-RA study were: a) Men and women aged >45 and <85 years; and b) RA defined by ACR/EULAR 2010 RA classification criteria[16].

Exclusion criteria of the Bio-RA study were: a) Previous cardiovascular or cerebrovascular events (acute coronary syndrome, stable angina, stroke, interventional procedures, carotid endarterectomy,

symptomatic peripheral artery ischemia); b) Serious infections in the previous 6 months; c) Concomitant severe illness (overt hepatic and/or renal disease, glomerular filtration rate <30 mL/min, calculated by the Cockcroft-Gault formula); d) Recent diagnosis of cancer; and e) Pregnancy.

Healthy controls, matched for age, gender and cardiovascular risk factors, were enrolled from the blood donor bank of the Azienda Ospedaliero-Universitaria of Sassari (Italy).

Clinical and laboratory variables

The following clinical and laboratory characteristics were assessed on the same day of PAT assessment: hypertension (blood pressure≥140/90mmHg or treatment with antihypertensive medications), diabetes (patient history and/or treatment with insulin or oral hypoglycaemic agents), dyslipidemia (lipid profile data collected within 3 months prior to the study as routine clinical practice, patient history and/or treatment with hypolipidemic drugs) and current smoking habit. In RA patients, the following disease specific scores, disease descriptors, and treatment data were collected on the same day of the PAT evaluation: current treatment with non-steroidal anti-inflammatory drugs (NSAIDs), steroids, synthetic or biological disease-modifying anti-rheumatic drugs (DMARDS); number of swollen joints; number of tender joints; C-reactive protein (CRP) concentrations; erythrocyte sedimentation rate (ESR); Disease Activity Score-28 (DAS-28); Clinical Disease Activity Index (CDAI); Health Assessment Questionnaire (HAQ); positivity for Rheumatoid Factor (RF) and anti-citrullinated cyclic peptide antibodies (ACPA).

Flow-mediated pulse amplitude tonometry (PAT)

Eligible RA patients were studied in a fasting state. Briefly, finger probes consisting of thimble a-shaped sensor cap, containing an inflatable chamber which continuously registers pulsatile volume changes, were placed on the middle finger of both hands. Digital volume changes were sensed by pressure transducers, registered over time and recorded as pulse amplitude by the EndoPAT 2000

device (Itamar Medical Inc., Caesarea, Israel).

After a 5-min baseline data acquisition period, arterial flow in the brachial artery was interrupted by inflating a blood pressure cuff to suprasystolic pressures (200 mmHg or 60 mmHg above baseline systolic blood pressure) for 5 min. Then, following cuff release, the digital pulse amplitude was recorded for a further 5 min. The ratio of the post-ischemic pulse amplitude signal compared with baseline was calculated, normalized for the baseline signal, indexed to the contralateral one and reported in standardized arbitrary units. The log-transformed ratio, expressed as Ln-RHI, reflects the small artery reactive hyperemia. We used a Ln-RHI cutoă value<0.51 to define the presence of significant ED as reported by Bonetti[17].

Arginine metabolomics by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Arginine metabolites were measured according to the method developed by Sotgia et al[18]. Briefly, 200-µL of plasma were spiked with 1 µL of a solution containing L-homoarginine-d4 dihydrochloride (d4-hArg), N^G,N^G-dimethyl-L-arginine-d6 dihydrochloride (d6-ADMA), and N^G,$N^{G'}$-dimethyl-L-arginine-d6 (d6-SDMA) used as internal standards. After vigorous vortex-mixing, tubes were placed in a block heater for 5 min at 100 °C then cooled to room temperature. A 400-µL volume of ultrapure water was added and, to displace the clot from the bottom of the vial, tubes were vortexed vigorously for 10 s. Tubes with dislodged clots were then heat-treated again for 5 min at 100 °C, cooled down to room temperature, and centrifuged at 17,000×g for 5 min. Clear supernatant (200 µL) was recovered and mixed with 20 µL of potassium phosphate monobasic buffer (100 mmol/L, pH 7.0) and 40 µL of diethylpyrocarbonate (33 mmol/L). After vortex-mixing, tubes were left at room temperature for 1 min and then analyzed by an Agilent LC-MS/MS system (Agilent Italia, Milan, Italy) using a 100 mm × 4.6 mm Agilent Zorbax Eclipse Plus C18 3.5 µm column and a mixture of an aqueous solution of 0.4% v/v formic acid and acetonitrile (95:5) as a mobile phase, delivered isocratically at a flow-rate of 0.8 mL/min. Mass detection was accomplished in positive ion mode by MRM of the precursor-product ion transitions *m/z*

247.14→142, 248.17→142, 261.28→70, 261.28→84, 275.33→46, and 275.33→70 for Arg, Cit, MMA, hArg, ADMA, and SDMA, respectively, as well as *m/z* 265.28→88, 281.3→52, and 281.3→70 for d4-hArg, d6-ADMA, and d6-SDMA, respectively.

Statistical analyses

Data were assessed for normality using the Kolmogorov-Smirnov test. Continuous variables are presented as mean ± standard deviation (SD) and median and interquartile range, while categorical variables are presented as frequencies (n) and percentages (%). Statistical differences between groups were assessed using unpaired Student's t-test or Mann–Whitney rank sum test, as appropriate. Differences between categorical variables were evaluated by chi-squared test or Fisher exact test as appropriate. Correlations coefficients between variables were assessed by Pearson's correlation or Spearman's correlation as appropriate and presented as a heat map. Simple and multiple linear regression analyses were performed to evaluate the association between arginine metabolites and Ln-RHI. Variables associated with ED either statistically ($p<0.1$) or biologically were entered into multiple logistic regression models in which the presence of ED was the dependent variable. Results are expressed as odds ratio (OR) and 95% confidence interval (95% CI). Analyses were performed using SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Heat map was created using GraphPad Prism 8.0 (GraphPad Software 7825, Fay Avenue, Suite 230, La Jolla, CA 92037 USA). A $p \leq 0.05$ was considered statistically significant.

RESULTS

Patients and controls

A total of 146 RA and 100 healthy controls were studied. Age, gender distribution, and prevalence of cardiovascular risk factors were similar across groups as per matching protocol (see Table I). As

expected, the female gender was prevalent. Mean concentrations of triglycerides were significantly higher in RA patients than in controls (see Table I). RA patients generally had moderate, long-standing, disease treated with DMARDs and other biologic drugs (see Table II).

Arginine metabolomics in RA and controls

ADMA was significantly higher in RA than in controls (0.53 ± 0.09 *vs* 0.46 ± 0.07 μmol/L, p<0.001) (see Table III). The inter-individual variability of ADMA in controls was 15%, similar to the accepted value of 12% suggested by other authors[19,20].On the contrary, hArg was significantly lower in RA than controls (1.50 ± 0.60 *vs* 1.92 ± 0.78 μmol/L, respectively; p<0.001) (see Table III). There were no significant differences in plasma Arg, Cit, MMA and SDMA concentrations between RA and controls (see Table III).

Correlation between arginine metabolites and peripheral vasodilatory capacity

The median(IQR) of Ln-RHI for the overall RA population was 0.68(0.44). In bivariate correlation analysis, higher plasma ADMA concentrations and current smoking habit status were inversely correlated, albeit weakly, with Ln-RHI (see Table IV). In multiple regression analysis, plasma ADMA concentrations remained independently associated with lower Ln-RHI [B coefficient(95%CI) =-0.57(-1.09 to -0.05), *p*=0.032] even after adjustment for smoking habit (see Table IV and Figure 1). About a quarter (24.8%) of RA patients exhibited ED (Ln-RHI<0.51). In binary logistic regression analysis, higher plasma ADMA concentrations and lower systolic blood pressure were associated with presence of ED (see Table V). In multiple logistic regression analysis, higher plasma ADMA concentrations were significantly, albeit weakly, associated with presence of ED [OR(95%CI) = 77.3(1.478 - 4050.005), *p*=0.031] even after adjustment for systolic blood pressure (see Table V).

Correlation between arginine metabolites and RA features

Bivariate correlation analysis showed no significant correlations between arginine metabolites and RA serology, disease activity and treatment (see Figure 2).

DISCUSSION

The aims of this study were a) to evaluate whether patients with RA exhibit alterations in arginine metabolites and b) to assess the relationship between arginine metabolites, RA descriptors and peripheral endothelial dysfunction.

To this purpose, we carried out a comprehensive targeted arginine metabolomics assessment by LC-MS/MS. The latter is a robust approach for the analysis of plasma concentrations of arginine and some of its chemically related metabolites and analogs allowing reproducible quantitative determination from large samples[18].

Consistent with previous reports[14,15], RA patients had higher plasma ADMA concentrations than controls. Different mechanisms have been reported to explain the increase of plasma ADMA concentrations in RA [21], including an inhibitor effect of inflammatory cytokines on ADMA catabolism by dimethylarginine dimethylaminohydrolase[22]. However, conflicting results have been reported regarding the association between systemic inflammatory markers and ADMA concentrations in RA[23–27]. Similarly, evidences about the effect of immunosuppressive drugs on ADMA concentrations are inconsistent[28–32]. Accordingly, in our series of RA patients, we found no significant correlations between plasma ADMA concentrations, inflammatory markers, and type of immunosuppressive drugs used.

In our study, the mean increase of plasma ADMA concentrations in RA patients without overt cardiovascular disease, when compared to healthy subjects with similar cardiovascular risk profile, was 0.8 μmol/L. This difference in plasma ADMA concentrations is clinically relevant as, in a meta-analysis of ~20,000 participants from 22 cohort studies and long-term follow-up, even slight

elevations of ADMA were significantly associated with increased risk of non-fatal and fatal cardiovascular events[33]. Zoccali et al.[34] reported an increase of 21% of major cardiovascular events and of 28% of all-cause mortality for each 1 μmol/L increase in plasma ADMA concentrations in patients with end-stage renal disease. Valkonen et al.[35] reported a 27-fold increase in relative risk of acute coronary events for each 0.1 μmol/L increase in serum ADMA concentrations.

Higher ADMA concentrations have been reported to be significantly associated to different surrogate markers of ED[21]. The primary novel finding of this study is the presence of a significant and inverse correlation between plasma ADMA concentrations and peripheral vasodilatory capacity measured by PAT, independent of conventional cardiovascular risk factors barring smoking. In fact, both smoking status and high plasma ADMA concentrations were inversely related to digital reactive hyperaemia, while lower blood systolic blood pressure and plasma ADMA concentrations were positively associated with presence of ED. Although vascular resistance in digital arteries is primarily modulated by sympathetic activity, nitric oxide plays a role in regulating digital blood flow[36]. It has been reported, indeed, that pharmacological inhibition of nitric oxide synthase blunts approximately one-half of the digital hyperaemic response in healthy subjects[37]. Therefore, we could speculate that increased concentrations of ADMA, a potent endogenous inhibitor of nitric oxide synthase, may impair digital reactive hyperaemia in RA patients by reducing nitric oxide availability. This observation has significant clinical and therapeutic implications as therapeutic strategies specifically activating ADMA catabolic pathways might restore endothelial function, curbing atherosclerotic cardiovascular risk in RA as well as other disease states characterized by ADMA accumulation[38].

We also documented significantly lower plasma concentrations of hArg, an analogue of L-arginine, in RA patients than in healthy controls. Thus far, hArg has been investigated in RA in two small studies[39,40]. In contrast to our results, Kayacelebi et al.[39] did not report any significant differences in plasma hArg concentrations between 100 RA patients and healthy controls. Similarly, a relatively

small study by Radhakutty et al.[40] did not report significant differences in plasma hArg concentrations between 18 RA patients and 20 healthy controls. It is conceivable that differences in sample sizes and heterogeneity in analytic methodologies used to measure the analyte may account for the lack of concordance of these results.

hArg may exert a protective role for endothelium health by enhancing nitric oxide availability. Although this has been thought to be secondary to hArg-mediated inhibition of the enzyme arginase, with consequent increased availability of the substrate L-arginine for nitric oxide synthase, the current evidence supporting this hypothesis is controversial [41,42]. This issue notwithstanding, low hArg concentrations were associated with 3.6-fold higher cardiovascular mortality and 2.7-fold higher all-cause mortality, after adjustment for potential confounders, in a prospective study of 3,305 subjects referred for coronary angiography (LUdwigshafen RIsk and Cardiovascular Health Study)[43]. Accordingly, a recently published meta-analysis of 13 studies for a total of 11,964 participants demonstrated an inverse association between circulating hArg concentrations and all-cause mortality[44].

However, in our study plasma hArg concentrations were not significantly associated with ED measured by flow-mediated pulse amplitude tonometry. Therefore, the biological effects of hArg on vascular homeostasis warrant additional experimental and human studies.

Our study has some potential limitations. First, the vast majority of RA patients were under treatment with immunosuppressive and anti-inflammatory drugs at the time of enrolment; However, as previously acknowledged, the impact of immunosuppressive drugs on arginines metabolites is not currently established[28–32]. In addition, in our series of RA patients the treatment regimen was not significantly associated with either arginine metabolite concentrations or peripheral endothelial function. Second, the observational cross-sectional design of this study did not allow establishing a cause-effect relationship between ADMA concentrations and microvascular function. Finally, the lack of PAT data in the controls prevented the assessment of correlations between arginine

metabolites and peripheral endothelial function in this group.

CONCLUSIONS

RA patients had higher plasma ADMA concentrations, and lower hArg concentrations, when compared to the general population. Higher plasma ADMA concentrations in RA patients were significantly and independently associated with lower peripheral vasodilatory capacity and presence of ED.

ABBREVIATIONS

RA: rheumatoid arthritis; Arg: L-arginine; MMA: monomethyl-L-arginine; hArg: L-homoarginine; ADMA: asymmetric dimethyl-L-arginine; SMA: symmetric dimethyl-L-arginine; CIT: L-citrulline; LC-MS/MS: liquid chromatography tandem mass spectrometry; Ln-RHI: Log-transformed reactive hyperemia index; PAT: flow mediated pulse amplitude tonometry; ED: endothelial dysfunction; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; NSAIDs: non-steroidal anti-inflammatory drugs; DMARDs: disease-modifying anti-rheumatic drugs; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: Disease Activity Score-28; CDAI: Clinical Disease Activity Index; HAQ: Health Assessment Questionnaire; RF: positivity for Rheumatoid Factor; ACPA: anti-citrullinated cyclic peptide antibodies. OR: Odds ratio; CI: confidence interval. Bio-RA study: BIO-markers of atherosclerosis in Rheumatoid Arthritis study; EDRA study: Endothelial Dysfunction Evaluation for Coronary Heart Disease Risk Estimation in Rheumatoid Arthritis study.

DECLARATIONS

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AUTHORS'CONTRIBUTIONS

GLE is the chief investigator responsible for the study, conceived the original idea, led the study team, supervised the conduct of the study, analysis and reporting and drafted the manuscript. GLE, MP, SS, CC, AZ and AAM jointly conceived the original idea and led on the study design. GLE led on the statistical analysis. SS performed the analytical analysis. GLE, SS, FC, SB, AZ and CC, along with all other authors, contributed to methods of data collection, patient materials and data management. GLE and SS managed the study with AZ, AAM, CC, FC, GP including data collection, data entry and validation. GLE, FC, and GP led recruitment. All authors reviewed, discussed and helped interpret the findings, commented on and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The dataset used and analysed during the current study is available from the corresponding author on reasonable request, after approval by the Italian Ministry of Health.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Bio-RA and the EDRA studies were approved by the Ethics Committee of Azienda ASL 1 of

Sassari (Italy) (2126/CE-2015 and 2219/CE-2015) and conducted in accordance with the Declaration of Helsinki. All patients gave their signed informed consent to participate to this study.

CONSENT FOR PUBLICATION

No individual person's data are present in this manuscript. All data are completely anonymized.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table I

Demographic features and cardiovascular risk factors of RA patients and controls

	Controls n=100	RA n=164	*p* value
Age	55.0 ± 5.6	55.0 ± 6.8	0.993
Female, n(%)	51(51.0)	102(62.2)	0.074
Hypertension, n(%)	26(26.0)	47(28.7)	0.639
Dyslipidemia, n(%)	21(21.0)	36(22.0)	0.855
Diabetes, n(%)	6(6.0)	9(5.4)	0.684
Current Smoker, n(%)	19(19.0)	46(28.0)	0.098
Total cholesterol, mg/dL	212.1 ± 36	207.0 ± 36	0.294
Triglycerides, mg/dL	82.3 ± 34	95.3 ± 45	0.013
Creatinine, mg/dL	0.84 ± 0.1	0.82 ± 0.2	0.567

Table II

Rheumatoid arthritis specific features

Variable	Value
DAS-28 ESR	3.70 ± 1.1
CDAI	11.22 ± 8.5
HAQ	0.59 ± 0.5
ACPA positivity, %	67.5
RF positivity, %	86.8
CRP, mg/dL	0.61 ± 0.7
ESR, mm/h	27.7 ± 21
Disease duration, months	114.9 ± 99
Steroid use, %	38.9
NSAIDs use, %	20.4
DMARDs use, %	70.4
TNFi use, %	24.7
Abatacept, %	4.9
Tocilizumab use, %	6.8
Rituximab use, %	1.2

Values are mean ± SD. ACPA, Anti citrullinated cyclic peptide antibodies; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS-28, Disease Activity Score-28; DMARDs, disease modifying anti-rheumatic drugs; ESR, Erythrocyte Sedimentation Rate; HAQ, Health Assessment Questionnaire; NSAIDs,

Table III

Plasma L-arginine and methylarginines in rheumatoid arthritis vs controls

	Rheumatoid arthritis n=164	Controls n=100	*p* value
Arg, μmol/L	68.25 ± 18.7	68.94 ± 19.2	0.774
Cit, μmol/L	35.51 ± 11.2	34.47 ± 10.45	0.455
hArg, μmol/L	1.50 ± 0.60	1.92 ± 0.78	<0.001
ADMA, μmol/L	0.53 ± 0.09	0.46 ± 0.07	<0.001
SDMA, μmol/L	0.45 ± 0.08	0.44 ± 0.08	0.465
MMA, nmol/L	80.2 ± 28.0	75.4 ± 29	0.196

Arg, L-arginine; Cit, L-citrulline; hArg, L-homoarginine; ADMA, asymmetric dimethyl-L-arginine; SDMA, symmetric dimethyl-L-arginine; MMA, NG-monomethyl-L-arginine.

Table IV

Independent determinants of Ln-RHI in rheumatoid arthritis population

Independent variable	Simple linear regression B coefficient (95%IC), *p*	Multiple linear regression B coefficient (95%IC), *p*
Smoke habit	-0.13(-0.24 to -0.02), 0.014	-0.13(-0.23 to -0.02), 0.016
ADMA	-0.63(-1.15 to -0.12), 0.016	-0.57(-1.09 to -0.05), 0.032

A linear regression for multiple variables (ENTER method) was performed including into the models variables showing significant association ($p<0.05$) with the dependent variable Ln-RHI at the univariate regression analysis.

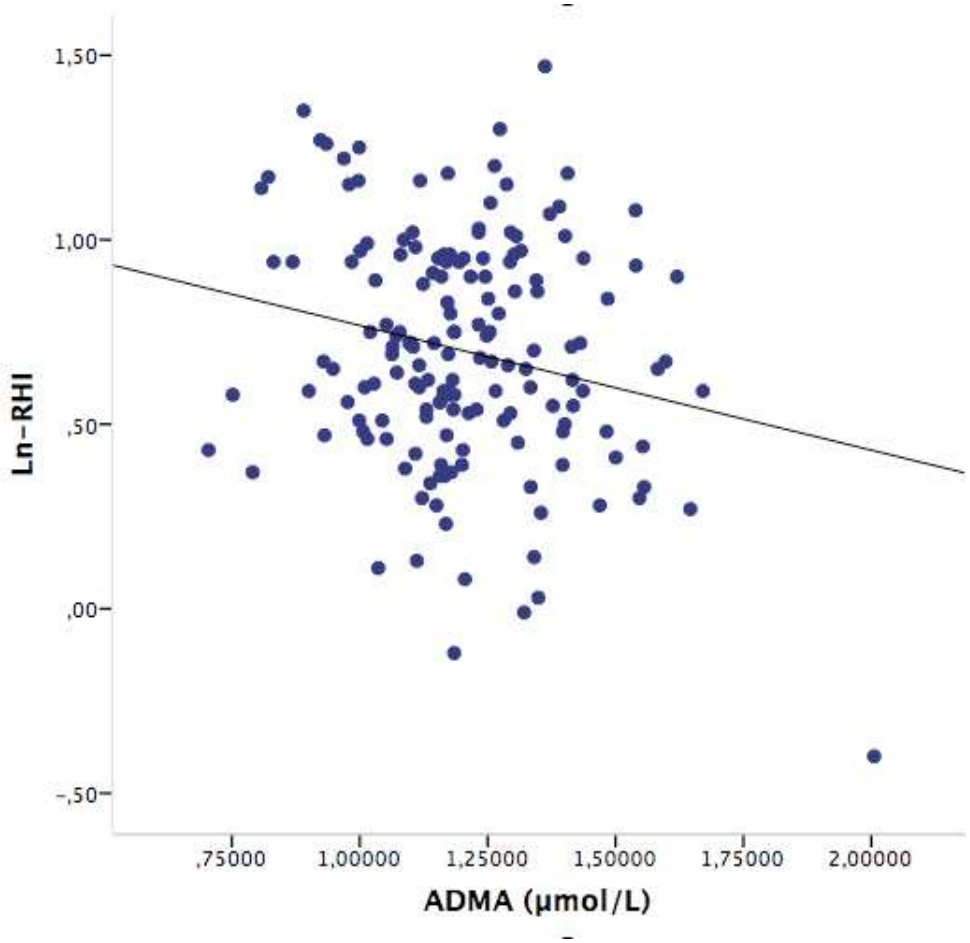
Table V

Independent determinants of peripheral ED in rheumatoid arthritis population

Independent variable	Binary logistic analysis	Multiple logistic analysis
	OR(95%CI), *p*	OR(95%CI), *p*
Systolic blood pressure, mmHg	0.978(0.957-1.000), 0.050	0.975(0.954 - 0.998), 0.031
ADMA, μmol/L	46.4(0.969-2.230.915), 0.052	77.3(1.478 - 4050.005), 0.031

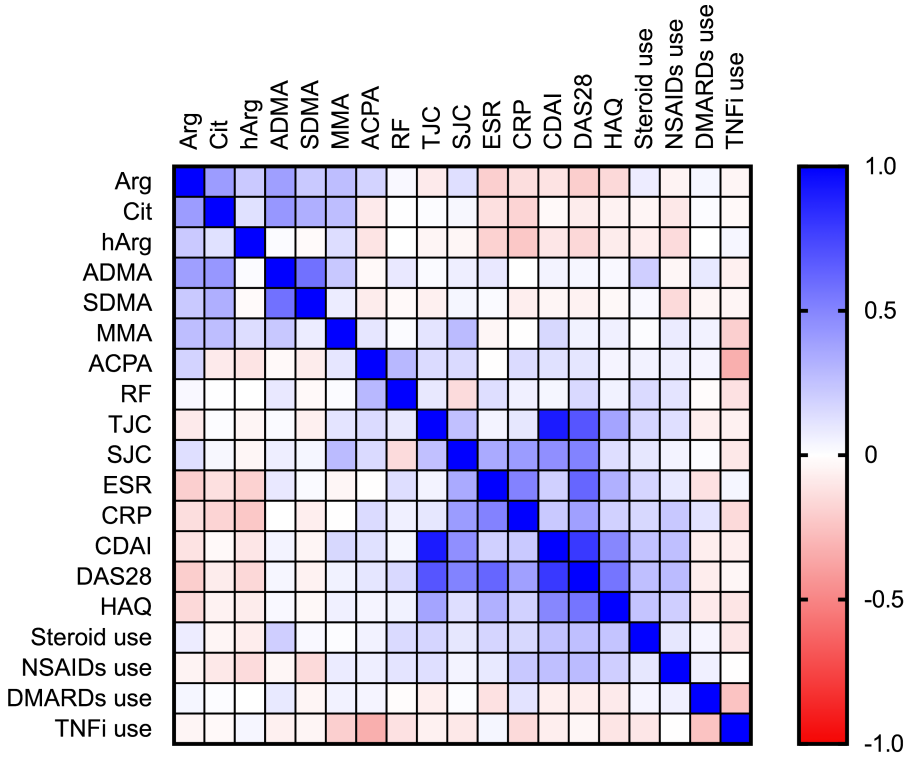
Odds ratio (OR) is based on the risk of the dependent variable (peripheral endothelial dysfunction, ED), given the presence of the independent variable. 95% CI 95% confidence interval. Multivariate logistic analysis (ENTER method) has been performed including in the model variables showing significant association with the dependent variable (ED) at the binary logistic analysis.

Figure 1



Scatter plot depicting relationship between plasma ADMA concentration and log-transformed reactive hyperaemia index (Ln-RHI) in rheumatoid arthritis population

Figure 2



A heat map representation of the Spearman correlation matrix of arginine and metabolites and RA descriptors.