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Letter to the Editor

Arginine in older patients with persistent atrial fibrillation: A link to endothelial dysfunction?☆

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Dear Editor, atrial fibrillation (AF) is the most frequent sustained arrhythmia observed in clinical practice. Still now, thromboembolic stroke is the most relevant acute clinical complication of AF. Indeed, the incidence of such an event significantly grows with age, reaching 8.9% at five-years in those older than 85 years. Moreover, AF can be observed in 24% of patients presenting an embolic stroke of undetermined source (ESUS) when using a monitoring technique with an appropriate recording length [1]. Current evidence supports the hypothesis that in many cases AF is directly responsible of a thromboembolic event. However, a temporal disconnection between the presence of the arrhythmia and stroke development can often be noticed. Thus, present findings suggest that AF is at the same time a cause and a "bystander" of such a dramatic complication, with the arrhythmia representing a marker of underling thrombogenic atrial conditions, ultimately responsible of a thrombo-embolic event [2]. On this basis, the concept of atrial myopathy was introduced in clinical practice. The condition appears to be characterized by atrial enlargement, alterations of coagulation, electrical remodeling, myocyte dysfunction, fibrosis and hemodynamic disturbances [3]. Endothelial dysfunction has been also advocated as another plausible component of atrial myopathy [3], and changes in the Virchow's triad - alterations in vascular endothelium, blood flow, and hypercoagulability - could contribute to explain an enhanced thrombogenesis [2,4]. Accordingly, aim of this study was to evaluate the existence of endothelial dysfunction in older patients with persistent forms of AF, particularly exposed to the most severe complications of the arrhythmia [4]. Hence, we conducted this pilot study analyzing the concentration of an amino acid - marker of endothelial function - arginine, from which nitric oxide (NO) is produced through the activity of NO synthase [5].

For this purpose, we enrolled 50 consecutive older patients with persistent AF admitted to the Day-Hospital of our institution. They underwent a standard clinical evaluation, including echocardiogram, coupled with multidimensional geriatric assessment. In particular, neuro-cognitive status, depressive symptoms and physical performance were explored, respectively, with the Mini-Mental State Examination (MMSE; abnormal score: $<$24/30), the 15-item Geriatric Depression Scale (GDS; abnormal score: $>$5/15), and the Short-Physical Performance Battery (SPPB; abnormal score: $\leq$6/12). We measured also the ankle-brachial index (ABI) and arterial stiffness, using the Cardio-Ankle Vascular Index (CAVI) [6]. A venous blood sample was drawn in iced tubes at 8 a.m. with patients in fasting conditions. The sample was centrifuged at 2500 rpm (4 °C) for 15 min [6]. Then, plasma was stored at −80 °C until inflammatory mediators' concentrations were measured using commercially available ELISA kits, and acylcarnitines and amino acids levels determined with liquid chromatography–tandem mass spectrometry. A blood sample was also drawn from 22 subjects without persistent arrhythmias, heart failure, acute or chronic coronary syndrome, cancer, severe chronic renal or hepatic conditions, and acute infections. This control population (healthy subjects) was further divided into a younger ($<$65 years) and an older ($\geq$65 years) group. Statistical analysis was performed using SPSS for Mac ver. 28 (64 bit-edition). Continuous variables are expressed as mean ± s.d. or as median value with 25th – 75th percentile. Categorical variables are presented in raw numbers and percentages. Differences of continuous variables between two or more categories of subjects were explored using Student's t-test or analysis of variance, respectively. In the case of a non-normal distribution, the Mann-Whitney and the Kruskal-Wallis tests were chosen. Univariate linear regression analysis models measured the associations between continuous variables. Differences in distribution of categorical variables were evaluated with the chi-square test. All variables significantly correlated with arginine levels, our endpoint measure, were entered into a multivariate linear regression analysis model with, at each step, the backward deletion of the variable showing the lowest statistical association. A 2-tailed p-value $<$0.05 was considered to indicate the statistical significance.

Heart rate, systolic and diastolic arterial pressure of AF patients

Abbreviations: AF, atrial fibrillation; ESUS, embolic stroke of undetermined source; GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination; NO, nitric oxide; SPPB, Short-Physical Performance Battery.

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were, respectively, 76±15 bpm, 131±18 mmHg and 80±11 mmHg; the CHA_2DS_2-VASc score was: 3.8 ± 1.6. Overall, when compared to control subjects, the prevalence of women in AF patients was lower (32.0 vs. 63.6%, $p = 0.019$), but no difference in age was observed (patients: 76 ±6 vs. healthy subjects: 70±14 years, $p = 0.083$). More in detail, mean age was, respectively, 56±4 and 79±14 years in healthy young ($N = 8$) and old ($N = 14$) subjects. Arginine concentration was determined in all but one patient due to technical reasons. The amino acid levels were significantly lower when AF was present (56±17 vs. 71±23 μmol/L; $p = 0.003$); a post-hoc analysis showed a statistically significant difference between healthy young subjects and patients; no differences were observed between healthy young and old individuals, and between healthy old individuals and patients (Fig. 1). A multivariate linear regression analysis model ($R = 0.547$, $p<0.001$) found an inverse association linking arginine concentration to age ($\beta = -0.93\pm0.22$; $p<0.001$) and to the presence of AF ($\beta = -9.71\pm4.58$; $p = 0.038$), whereas sex was deleted from the model ($p = 0.666$).

When limiting the analysis to patients with the arrhythmia, we found that hypertension and heart failure were the most represented comorbidities. DOACs were prescribed in 46.9% of cases ($N = 23$). Antagonists of the renin-angiotensin system and β-blockers were used in the majority of patients (83.7 and 77.6%, respectively), while anti-arrhythmic therapy with amiodarone was adopted in 23 subjects (46.9%) (Table 1).

Once again, in AF patients, we observed an inverse relation linking arginine to age. No association of the amino acid concentration was found with sex, body size, heart rate, hypertension, diabetes, dyslipidemia, chronic renal failure, left ventricular ejection fraction, history of coronary artery, cerebrovascular and peripheral artery disease, and other comorbidities (Table 1). Accordingly, also the CHA_2DS_2-VASC score was unrelated to arginine concentration, as well as the measures of neurocognitive profile, depressive symptoms and physical performance. The amino acid levels were associated with white blood cells count, and with iron and glutamic-pyruvic transaminase concentration (Table 1). Interestingly, interleukin-6 did not show any correlation with arginine, as observed for arterial stiffness, and the most important cardiovascular drugs (Table 1). Citrulline ($\beta = 0.85\pm0.16$; $R = 0.619$; $p<0.001$) levels were directly related to arginine concentration. A multivariate linear regression analysis model ($R = 0.626$, $p<0.001$) confirmed the age-related arginine decrease in patients with AF ($\beta = -0.91\pm0.37$; $p = 0.019$). Also, it showed the inverse association between arginine and iron levels ($\beta = -0.19\pm0.07$; $p = 0.009$) and the direct correlation between the amino acid and the glutamic-pyruvic transaminase concentration ($\beta = 0.21\pm0.07$; $p = 0.037$), whereas white blood cells count was deleted from the model ($p = 0.318$).

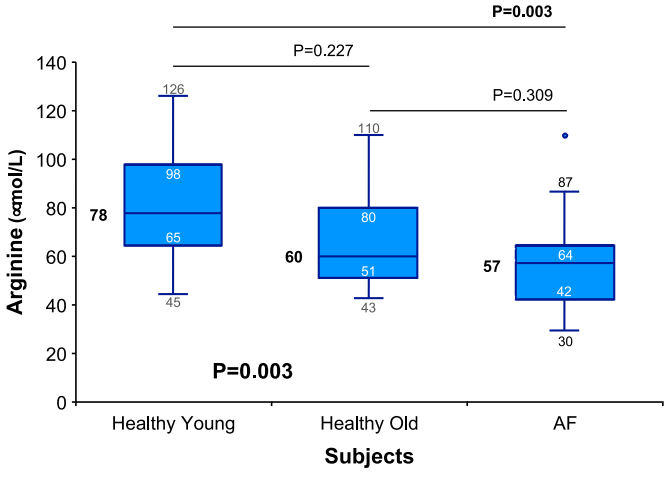

Fig. 1. Arginine concentration in healthy subjects and in atrial fibrillation (AF) patients. The median, the 25th and the 75th percentile values are reported for each category.

Table 1
Clinical characteristics of the enrolled population and their association (categorical variables - upper panel; continuous variables - lower panel) with arginine concentration (μmol/L).

Categorical Variables	All patients (N,%)	Condition Present	Absent	p-value
Woman	15 (30.6)	59±18	56±17	0.582
Lives alone	11 (22.4)	49±13	58±18	0.132
Current smoker	4 (8.2)	55±9	57±18	0.822
CAD	13 (26.5)	62±24	54±14	0.299
CKD	10 (20.4)	58±24	56±16	0.743
COPD	9 (18.4)	51±14	58±18	0.284
CVD	6 (12.2)	50±19	57±17	0.309
Diabetes	8 (16.3)	57±16	56±18	0.918
Dyslipidemia	22 (44.9)	60±21	54±14	0.217
Heart failure	27 (55.1)	57±18	56±17	0.971
Hypertension	42 (85.7)	57±18	51±14	0.346
Hyperuricemia	15 (30.6)	56±21	57±16	0.898
PAD	10 (20.4)	48±13	59±18	0.104
Thyroid disease	10 (20.4)	55±17	57±18	0.839
DOACs	23 (46.9)	56±17	57±19	0.973
Antiplatelets	6 (12.2)	56±11	57±18	0.898
β-blockers	38 (77.5)	58±18	50±13	0.156
Amiodarone	23 (46.9)	53±18	59±16	0.227
Digoxin	18 (36.7)	56±14	57±19	0.782
α-blockers	12 (24.5)	52±12	58±19	0.356
ACE-I / ARBs	41 (83.6)	57±18	51±11	0.354
Dihydropyridine Ca-antagonists	9 (18.4)	56±23	56±16	0.982
Diuretics	31 (63.2)	55±17	59±19	0.465
Oral Antidiabetics	5 (10.2)	58±16	56±18	0.852
Insulin	3 (6.1)	55±20	57±17	0.881
Statins	22 (44.9)	58±21	55±14	0.523
Anti-uric agents	10 (20.4)	49±16	58±18	0.152
Continuous Variables	**All patients (mean ±sd)**	**β±es**	**R**	**p-value**
Age (years)	76±6	−1.14 ±0.36	0.417	0.003
Height (cm)	171±9	/	0.077	0.599
Weight (Kg)	78±12	/	0.139	0.341
HR (bpm)	76±15	/	0.074	0.615
SAP (mmHg)	131±18	/	0.042	0.775
DAP (mmHg)	80±11	/	0.212	0.143
MMSE (score)	28±2	/	0.003	0.986
GDS (score)	2.8 ± 2.3	/	0.202	0.217
SPPB (score)	9.4 ± 2.1	/	0.072	0.621
CHA_2DS_2-VASc (score)	3.8 ± 1.6	/	0.190	0.191
LAD (mm)	55±6	/	0.077	0.605
EDD (mm)	52±8	/	0.153	0.310
ESD (mm)	34±10	/	0.004	0.977
LVEF (%)	58±12	/	0.060	0.684
CAVI	10.1 ± 1.9	/	0.118	0.426
ABI	1.1 ± 0.2	/	0.080	0.593
Creatinine (mg/dL)	1.0 ± 0.3	/	0.034	0.826
Glycemia (mg/dL)	99±23	/	0.231	0.137
GPT (U/L)	33±24	0.33±0.10	0.446	0.002
Cholesterol (mg/dL)	165±35	/	0.075	0.740
Triglycerides (mg/dL)	112±54	/	0.113	0.646
Fibrinogen (mg/dL)	414±71	/	0.181	0.240
Ferritin (ng/mL)	111±108	−0.06 ±0.03	0.338	0.035
Iron (μg/dL)	77±32	−0.19 ±0.08	0.339	0.026
NT-proBNP (pg/mL)	2120 ±2153	/	0.009	0.956
Uric acid (mg/dL)	6.0 ± 1.6	/	0.246	0.100
Hemoglobin (g/dL)	13.5 ± 1.6	/	0.042	0.784
WBC (n.10^{-3}/mm^3)	6.6 ± 2.2	2.58±1.13	0.318	0.028
Platelets (n.10^{-3}/mm^3)	195±73	/	0.218	0.136
IL-6 (pg/mL)	3.9 ± 3.1	/	0.103	0.487
OPG (pmol/L)	4.7 ± 2.5	/	0.075	0.614

CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; CVD: cerebrovascular disease; PAD: peripheral

artery disease; DOACs: direct oral anticoagulants; HR: heart rate; SAP/DAP: systolic/diastolic arterial pressure; MMSE: Mini-Mental State Examination; GDS: 15-item Geriatric Depression Scale; SPPB: Short-Physical Performance Battery; LAD: left atrium diameter; EDD/ESD: end-diastolic/end-systolic diameter of the left ventricle; LVEF: left ventricular ejection fraction; CAVI: Cardio-Ankle Vascular Index; ABI: Ankle-Brachial Index; GPT: glutamic-pyruvic transaminase; WBC: white blood cells count; IL-6: interleukin-6; OPG: osteoprotegerin; β: change of the dependent variable for a unitary variation of the independent variable.

To the best of our knowledge, this pilot experience is one of the first showing that arginine concentration, directly involved in NO synthesis, and for this reason, expression of endothelial function, is significantly lower in patients with AF than in a control group without the arrhythmia. A previous study demonstrated that NO synthase expression and NO production were, respectively, 46 and 73% lower in atrial endocardium isolated from pigs with pace-induced AF when compared to controls. Interestingly, these changes corresponded to an increased activity of the prothrombotic protein plasminogen activator inhibitor 1 (PAI-1) [7]. Also, subjects with ESUS showed higher values of L-arginine and a reduced carotid intima-media thickness than patients with stroke and the arrhythmia [8]. Importantly, in our study, the relation between arginine and AF is independent and additive to that found for age. Indeed, endothelial dysfunction is highly prevalent in older subjects, and it might contribute to the development or the worsening of important age-related conditions, such as dementia, loss of physical function, hypertension, heart and renal failure [5]. Interestingly, despite the existence of conflicting evidence, it was shown, in older individuals, that arginine concentration could be increased through oral supplementation, with this change correlated to the improvement of endothelial function [5]. In our patients, we found also an inverse relation between iron levels and arginine concentration. Indeed, the association between arginine and iron is complex. NO - derived from the amino acid - exerts its action oxidizing, nitrating and nitrosylating regulatory proteins and enzymes. Among these, the nitrosilation of the heme iron brings to the activation of the soluble guanylyl cyclase, which catalyzes GTP conversion to cGMP, a key step in vasodilation. Historical data support the hypothesis that NO generation could be associated with changes in iron homeostasis, due to an enhanced iron release from intracellular ferritin stores. Also, it was shown that macrophages activated by interferon-gamma synthetize a flavoprotein promoting the conversion of arginine to NO, with this last mediator responsible of the efflux of the metal from neoplastic and infected cells. Importantly, these macrophage target cells could significantly reduce their iron uptake from plasma if the concentration of arginine is low [9]. These last findings support the existence of an inverse relationship between the concentration of iron and that of arginine or its derivative, NO. The direct association between the amino acid concentration and the glutamic-pyruvic transaminase levels could be justified by the involvement of the enzyme in the complex catabolic cascade of arginine itself and by the key role played by the liver in its synthesis, one of the main steps of the urea cycle [10].

Limitations of this pilot experience are represented by the small number of AF patients and controls. Given the nature of the study, we were not able to collect instrumental data of healthy subjects. Also, a possible home supplementation of nutraceuticals containing arginine could have been missed. However, such a support of the amino acid should have reduced, and not increased, the magnitude of the findings we observed. Furthermore, we could not differentiate L- from D-arginine and we could not measure the concentration of asymmetric dimethylarginine (ADMA), an inhibitor of NO synthase. However, the levels of citrulline, which derives from arginine after NO synthesis, had a significant correlation with arginine concentration.

In conclusion, this pilot analysis seems to support the hypothesis that AF is independently associated with endothelial dysfunction, as expressed by reduced arginine plasma levels. This condition worsening atrial cardiomyopathy and left atrium remodeling could augment the incidence of thromboembolic complications. Importantly, age, synergistically interacting with AF, could further increase the risk profile of patients. Specific studies with an adequate sample size should address these issues to prevent arrhythmia-related morbidity, disability and mortality.

Informed consent. All enrolled patients gave their informed consent to participate to the study.

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Declaration of Competing Interest

The authors declare they have no conflict of interest.

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