Pleiotropic effects of anti-thrombotic therapies: have direct oral anticoagulants any anti-inflammatory effect?

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ABSTRACT

Direct oral anticoagulants (DOACs) are currently recommended by European guidelines as the first line therapy for both stroke prevention in patients with atrial fibrillation (AF) and the prevention and the treatment of venous thromboembolism (VTE). Recently, it has been speculated that DOACs have anti-inflammatory capabilities in reducing the abnormal release of pro-inflammatory factors in addition to inhibiting the activation of factor X or factor II of the coagulation cascade. However, this hypothesis is based on limited pathophysiological data with small sample size, often on *in vitro* studies. Real-world, *in vivo*, and large clinical data are scarce. The aim of the present study was the evaluation of the possible anti-inflammatory and anti-proliferative effects of DOACs treatment in a cohort of patients affected by AF or VTE, by analyzing an extensive panel of cytokines and molecules involved in the process of vascular and tissue remodeling. Our data evidenced that DOACs treatment is associated with variations in systemic inflammation markers and in metalloproteinases. Further studies with larger number of patients are required to confirm these data.

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INTRODUCTION

Direct oral anticoagulants (DOACs) represent a valid substitute for vitamin K antagonists in the prevention and the treatment of thromboembolic events due to their characteristics that make them more manageable and safer. The European guidelines recommend DOACs as a first line therapy both for stroke prevention in patients with atrial fibrillation (AF) and for the prevention and the treatment of venous thromboembolism (VTE).[1,2]

Their clinical efficacy and safety have been reported in various clinical trials,[3-5] but few studies have evaluated their possible pleiotropic effects, particularly anti-inflammatory and anti-proliferative effects.

The study of the modulation of inflammation by oral anticoagulants represents a relevant subject both from a pathophysiological and a clinical point of view, supported by the literature on inflammation as a common substrate for AF and VTE. Inflammation is linked to a series of pathological processes, including oxidative stress, apoptosis and fibrosis, that are capable of creating a pathological substrate for AF, through their contribution to atrial remodelling.[6] In the biomarker study of the ARISTOTLE trial, which evaluated more than 14,000 AF patients on oral anticoagulation (warfarin or apixaban), the inflammatory markers [C Reactive Protein (CRP) and interleukin-6] were significantly associated with an increased risk of mortality.[7] Moreover, inflammation is also associated with the occurrence of thromboembolic event, due to the capability of inflammatory markers to induce endothelial dysfunction, platelets and coagulation activation, thus contributing to thrombus formation. Recently, evidence has emerged on the role of inflammation also in

VTE. Pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) promote a pro-coagulant state, inducing the occurrence and the progression of VTE and their complication.[8,9] The association between anti-inflammatory effect and cardiovascular disease has been supported by the CANTOS study, in which the use of the anti-IL-1β antibody canakinumab was associated with the reduction of cardiovascular mortality.[10] Other cardiovascular drugs such as statins, heparins and anti-PCSK-9 antibodies are endowed with anti-inflammatory effects. The reduction of the cardiovascular mortality observed with statin treatment was ascribed not only to the LDL-lowering effect but also to the anti-inflammatory effect of statins, which contributes to plaque stability and decrease endothelial dysfunction.[11,12] *In vitro* and *ex-vivo* studies showed that heparins, in addition to their anticoagulant activity, provided anti-inflammatory effect.[13,14] Recently, it has been suggested that also the novel lipid lowering therapy anti-PCSK-9 antibodies had anti-inflammatory properties.[15]

The modulation of DOACs on inflammation, extracellular matrix, cellular proliferation and angiogenesis was studied by *in vitro* and animal models. In *in vitro* studies, the anti-inflammatory activities of DOACs may be ascribed to their capability to inhibit the activation of the protease-activated receptor (PAR)-1 or PAR-2 signaling pathway,[16-19] or to down-regulate platelet activation, by a mechanism involving Glycoprotein VI (GPVI) shedding reduction.[20] Animal models showed that rivaroxaban and edoxaban attenuated atrial fibrosis induced by transverse aortic constriction in mice and reduced vulnerability to AF, suggesting that FXa inhibition might exert a cardioprotective effect against atrial remodelling.[21-23] Furthermore, rivaroxaban significantly attenuated neointima formation in rat injured arteries, and reduced expression of cytokines (as IL-1β e TNF-β) both in injured arteries and in mouse peritoneal macrophages.[24]

In humans, few and contrasting studies about the effect of DOACs treatment on inflammation and atrial fibrosis are available. In patients with AF dabigatran, rivaroxaban and apixaban administration was associated with significant decrease of inflammatory markers.[25,26] In the same clinical setting, it has been also observed that the anti-Xa drugs significantly reduce the urinary excretion of TxB2, and soluble GPVI, a peptide derived from GPVI shedding, suggesting that they possess an antiplatelet effect.[27] In acute ischemic stroke patients both apixaban and dabigatran treatment reduced CRP and IL-6 levels.[28] At variance, other studies failed to reveal any significant effect of DOACs treatment on inflammation.[29]

The aim of the present study is to evaluate the anti-inflammatory and anti-proliferative effects of DOACs treatment through evaluation of cytokines and molecules involved in the process of vascular and tissue remodeling in patients on DOACs (apixaban, dabigatran and rivaroxaban) treatment.

MATERIALS AND METHODS

This is a prospective, observational cohort study with the aim of evaluating pleiotropic effects of DOACs.

Three hundred and fifty-eight patients were enrolled in the period between 10th October 2016 and 19th October 2017 (dabigatran n=90, rivaroxaban n=143 e apixaban n=125) at Careggi University Hospital. We present here data on the first 54 patients (dabigatran n=16, rivaroxaban n=20 e apixaban n=18) without acute or chronic inflammatory disease from whom two venous samples were available: one collected at one month after the beginning of treatment (T1) and the second one after 6 months (T2). Inclusion criteria were the diagnosis of AF or of VTE, therapy with DOACs (dabigatran, rivaroxaban or apixaban), written informed consent for venous sample. Exclusion criteria were the presence of acute or chronic inflammatory disease.

The study design consisted of a clinical evaluation at the time of the first contact, with venous blood sample taken at one month and at six months after the beginning of the treatment.

Demographic data, clinical characteristics, risk factors and treatment modalities were collected at the time of first contact.

Blood samples

Whole venous blood was collected in tubes without anticoagulant and with 3.2% trisodium citrate (9:1 v/v, blood/anticoagulant). Blood was centrifuged within 1 h from collection at 2000 × g for 15 min. Aliquots of serum or plasma were quickly frozen and stored at −80°C until testing. Blood samples were collected 1 and 6 months after treatment with DOACs at C-trough level, obtained at 12 h from the last dose intake for dabigatran and apixaban, and at 24 h from the last dose intake for rivaroxaban. Levels of different inflammatory markers [IL-1RA, IL-6, IL-8, IL-10, TNF-alpha, vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)] were determined using BioPlex suspension array system and Biorad Kits (Bio-Rad Laboratories Inc., Hercules, CA, USA). Metalloproteinases (MMP1, MMP2, MMP3, MMP7, MMP8, MMP9 and MMP10) were assessed in the same patients using Bio-Plex suspension array system (Bio-Rad Laboratories Inc., Hercules, CA, USA) and R&D Kits (R&D System, Milan Italy) following manufacturer's instructions. The coefficient of variation of inflammatory markers, and MMPs assays was <6%.

Apixaban, rivaroxaban and dabigatran plasma levels,

expressed as drug concentration (ng/mL), were assessed by commercial kits (Werfen Milan, Italy), according to the manufacturer's instructions.

Diluted thrombin, calibrated for dabigatran, and specific anti-FXa assays, calibrated for apixaban and rivaroxaban, were used to measure DOACs plasma levels.

Statistics

Discrete data are expressed as frequencies, and continuous data as mean±SD or median and interquartile range, as appropriate. The χ^2 test was used to compare categorical variables, and the unpaired two-tailed Student's t-test or Mann-Whitney rank-sum test was used to test differences between continuous variables.

In order to evaluate the degree of inflammation, patients treated with DOACs were divided into three categories on the basis of the grade of inflammation (low, moderate and elevated). For this purpose the levels of cytokines IL-1Ra, IL-6, IL-8, IL-10 and TNFα were divided into tertiles and were used to calculate a global grading of inflammation. Patients with high levels of pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) not balanced with level of the anti-inflammatory ones (IL-1Ra, IL-10) were assigned as patients with an elevated grade of inflammation; patients with moderate-high levels of pro-inflammatory cytokines partially balanced with levels of anti-inflammatory ones were assigned as patients with a moderate grade of inflammation, whereas patients with balanced levels of pro- and anti-inflammatory cytokines were assigned as patients with a low level of inflammation.

As for DOACs plasma concentrations, they were divided in two groups in relation to patients' median level. In particular, patients on apixaban therapy were divided in two groups depending on if apixaban plasma levels were above or under 94 ng/mL after one month of treatment and 90 ng/mL after 6 months of treatment. Similarly, patients on rivaroxaban were divided in two groups depending if rivaroxaban plasma levels were above or under 28 ng/mL after 1 month of treatment and 24 ng/mL after six months of treatment, and patients in treatment with dabigatran were divided in two groups on the basis of dabigatran plasma levels (above or under 111 ng/mL after 1 month of treatment, and 90 ng/ml after 6 month of treatment).

RESULTS

Demographic and clinical characteristics of enrolled patients are shown in Table 1. As shown in Table 2, no significant difference in terms of cytokines and metalloproteinases levels was found among the different groups of DOACs , both one and six months after treatment. No significant difference in plasma levels of inflammatory marker were found between patient with AF and TEV

Table 1. Demographic and clinical characteristics.

Demographic characteristics and risk factor	All patients (n=54)
Age (years), mean and SD	73 (15)
Men, n (%)	32 (59.3%)
BMI (Kg/m^2), mean and SD	26.4 (4.0)
Creatinine (mg/dL), mean and SD	0.9 (0.2)
Creatinine Clearance (mL/min), median and IQR	74 (53.3-93.5)
Hb (g/dL), mean and SD	13.5 (1.8)
RBC (10^6/μL), median and IQR	4.7 (4.4-5.4)
Platelets (10^3/μL), mean and SD	223.07 (64.1)
ALT (U/L), median and IQR	23 (17-32)
AST (U/L), median and IQR	20 (17-24.5)
Hypertension, n (%)	38 (70.4)
Smoking habit, n (%)	4 (7.4)
Diabetes, n (%)	9 (16.7)
Family history for CVD, n (%)	15 (27.8)
Medical history, n (%)	
Non valvular atrial fibrillation (NVAF)	33 (61.1)
Cronic NVAF	15 (27.8)
Parossistic NVAF	18 (33.3)
Venous thromboembolism	21 (38.9)
DOACs, n (%)	
Dabigatran	16 (29.6)
Rivaroxaban	20 (37.0)
Apixaban	18 (33.3)
CHADSVASc score of NVAF patient (n=33), n (%)	
1	2 (6.1)
2	5 (15.2)
3	14 (42.4)
4	6 (18.2)
5	5 (15.2)
6	1 (3.0)
Concomitant therapy, n (%)	
Aspirin	2 (3.7)
Ticagrelor	0
Clopidogrel	1 (1.9)
Prasugrel	0
Antiarrhythmic drugs	9 (16.7)
Antidiabetic drugs	7 (13.0)
Lipid lowering drugs	24 (44.4)
Antihypertensive drugs	35 (64.8)
Ace inhibitors	14 (25.9)
Beta blockers	20 (37)
Calcium channel blockers	10 (18.5)
Diuretics	13 (24.1)
Sartans	14 (25.9)
Nitrate	2 (3.7)
Digoxin	4 (7.4)
Gastroprotectors	28 (51.9)
Antiviral	0
Immunosuppressive drugs	0
Steroids	2 (3.7)
Drugs for thyroid disease	8 (14.8)
Antipsychotic drugs	7 (13)
Antiepileptic	2 (3.7)
Anxiolytics	8 (14.8)
Painkillers	3 (5.6)

SD, standard deviation; BMI, body mass index; IQR, interquartile range; Hb, hemoglobin; RBC, red blood cell counts; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVD, cardiovascular disease; DOACs, direct oral anticoagulants.

(data not shown), therefore we could analyze globally patients' data as a single population.

Inflammatory markers and DOACs treatment

In Table 3 inflammatory markers' levels at one and six months after treatment are shown. Treatment with DOACs for six months is associated with significantly higher circulating levels of anti-inflammatory cytokine IL-10, pro-inflammatory cytokine TNF-α and VEGF-α. Furthermore, circulating levels of the adhesion molecule VCAM-1 are significantly higher at T2 with respect T1 (Table 3).

In Table 4 the ratios between pro-inflammatory and anti-inflammatory cytokines at T1 and at T2 are shown. The ratios between IL-6/IL-10 and IL-8/IL-10 were significantly lower at time T2 compared to the one at time T1, whereas the ratio between TNF-α/IL-1RA was significantly higher at T2 compared to the one at T1 (Table 4).

By using a global inflammatory score, we identify at T1 30 patients (55.5%) in which the global inflammation

Table 2. Cytokines and metalloproteases levels according to DOACs treatment.

	Dabigatran (n=16)	Rivaroxaban (n=20)	Apixaban (n=18)	P
IL-6 (pg/mL)	1.70 (0.76-3.40)	1.60 (0.52-2.36)	1.35 (0.99-2.35)	0.793
IL-8 (pg/mL)	3.16 (1.94-4.43)	3.85 (1.92-6.15)	3.63 (2.30-6.28)	0.760
TNF-α (pg/mL)	0.11 (0.030-2.04)	0.73 (0.030-3.61)	1.99 (0.83-2.69)	0.165
IL-1RA (pg/mL)	276.54 (215.95-526.00)	362.19 (230.055-688.83)	389.79 (279.18-550.72)	0.579
IL-10 (pg/mL)	0.30 (0.20-7.65)	1.07 (0.22-10.47)	0.25 (0.20-11.90)	0.463
VEGF-α (pg/mL)	7.68 (0.030-21.80)	6.37 (3.73-18.66)	9.69 (5.26-16.23)	0.823
ICAM-1 (ng/mL)	184.54 (136.49-216.44)	132.61 (70.52-190.89)	117.72 (142.84-232.88)	0.095
VCAM-1 (ng/mL)	655.42 (462.18-844.55)	738.75 (419.43-922.61)	688.47 (561.07-1002.82)	0.813
MMP-1 (ng/mL)	0.287 (0.207-0.384)	0.380 (0.265-1.070)	0.429 (0.194-1.130)	0.298
MMP-2 (ng/mL)	211.38 (164.59-619.19)	339.89 (156.13-583.01)	483.30 (123.05-744.96)	0.783
MMP-3 (ng/mL)	30.74 (19.61-56.20)	33.41 (18.37-61.74)	49.98 (24.99-101.57)	0.319
MMP-7 (ng/mL)	1.762 (0.770-2.998)	1.378 (1.050-1.678)	1.906 (1.043-3.114)	0.423
MMP-8 (ng/mL)	0.677 (0.332-1.128)	0.722 (0.400-1.013)	0.689 (0.371-1.464)	0.914
MMP-10 (ng/mL)	0.387 (0.210-0.748)	0.543 (0.351-0.748)	0.367 (0.234-0.557)	0.208

IL, interleukin; TNF, tumor necrosis factor; IL-1RA, interleukin -1 receptor antagonist; VEGF, vascular-endothelial growth factor; ICAM, intercellular adhesion molecule; VCAM, Vascular cell adhesion protein; MMP, matrix metalloproteinases. Values are reported as median and interquartile ranges (in parenthesis)

Table 3. Inflammatory markers levels after 1 and 6 months of DOACs treatment.

	T1	T2	P
IL-6 (pg/mL)	1.58 (0.89-2.43)	1.55 (0.80-2.60)	0.652
IL-8 (pg/mL)	3.63 (2.10-5.79)	3.23 (2.04- 5.67)	0.959
TNF-α (pg/mL)	*1.05* (0.03-2.46)	*1.62* (0.65-3.23)	*0.033*
IL-1RA (pg/mL)	354.24 (248.92-572.93)	364.22 (269.55-544.60)	0.997
IL-10 (pg/mL)	*0.30* (0.20-10.21)	*5.04* (0.3-11.71)	*0.005*
VEGF-α (pg/mL)	*7.68* (3.34-18.89)	*16.19* (10.45-21.19)	*<0.001*
ICAM-1 (ng/mL)	143.51 (105.61-212.84)	149.93 (108.96-232.29)	0.997
VCAM-1 (ng/mL)	*686.95* (487.44-9.21.43)	*769.35* (651.96-1002.89)	*0.034*

IL, interleukin; TNF, tumor necrosis factor; IL-1RA, interleukin -1 receptor antagonist; VEGF, vascular-endothelial growth factor; ICAM, intercellular adhesion molecule; VCAM, Vascular cell adhesion protein. Values are reported as medians and interquartile ranges (in parenthesis); values statistically significative are in italics.

Table 4. Pro- and anti-inflammatory cytokines ratios after 1 and 6 months of DOACs.

	T1	T2	P
IL-6/IL-10	*3.46 (*0.20-8.77)	*0.36* (0.12-3.47)	*0.012*
TNF-α/IL-10	0.42 (0.10 -5.15)	0.45 (0.13-2.16)	0.188
IL-8/IL-10	*8.7 (*0.55-18.30)	*0.90* (0.35-2.96)	*<0.001*
IL-6/IL-1RA	0.0044 (0.0023-0.0077)	0.0042 (0.0022-0.0067)	0.887
TNF-α/ IL-1RA	*0.0028 (*0.0001-0.0071)	*0.0049* (0.0020-0.0106)	*0.001*
IL-8/ IL-1RA	0.0094 (0.0059-0.0154)	0.0113 (0.0052-0.0177)	0.608

IL, interleukin; TNF, tumor necrosis factor; IL-1RA, interleukin -1 receptor antagonist. Values are reported as medians and interquartile ranges (in parenthesis); values statistically significative are in italics.

level was low, 10 patients (18.5%) with moderate grade of inflammation and 14 (30%) with high level of inflammation. Treatment for six months with DOACs is associated with a significant reduction of patients classified in the group with high level of inflammation. In fact, 8 out of 14 (57.1%) patients with high level of inflammation at T1 switched to the low level of inflammation group and 1 (7.1%) into the moderate group. Similarly, 5 of 10 patients classified as moderate level of inflammation at T1 after 6 months switched into the low-level category (50%), and in only one patient the grade of inflammation became higher. Among 30 (73.3%) patients with low grade of inflammation at T1 only 8 patients (26.7%) switched to a higher inflammation group. Overall, only 4 patients out of 54 (7.4%) switched from a lower-moderate level of inflammation to a higher one, instead 14 out of 54 patients (25.9%) with high grade of inflammation switched to a lower grade.

Metalloproteinases and DOACs treatment

Treatment with DOACs (1 and 6 months) was associated with significant increase in circulating levels of MMP-7 and MMP-10 (Table 5).

Correlations between inflammatory markers, metalloproteinases and DOACs plasma concentrations

We analyzed the correlation between DOACs plasma levels and circulating inflammatory markers after 1 and 6 months of treatment. After 1 month, apixaban and rivaroxaban plasma levels were positively and significantly correlated with anti-inflammatory cytokines IL-1RA and IL-10 levels. Furthermore, dabigatran plasma levels were positively related with circulating levels of TNF-α (Table 6).

After six months of treatment apixaban levels were inversely correlated with anti-inflammatory cytokines IL-10 and positively related with adhesive molecule ICAM-1, whereas dabigatran levels were positively correlated with circulating levels of IL-1RA.

As MMPs levels are concerned, at T1 dabigatran plasma levels were inversely related with circulating levels of MMP-8 (rho=-0.615; p=0.011) whereas at T2 circulating levels of dabigratan were related with increasing levels of MMP-7 (rho=0.600; p=0.014).

By dividing DOACs plasma concentrations in relation to patients' median levels of drugs, patients with DOACs concentrations at T1 above the median values had signif-

Table 5. Circulating levels of metalloproteinases after 1 and 6 months of DOACs treatment.

	T1	T2	P
MMP-1 (ng/mL)	*0.368* (0.207-1.003)	*0.697* (0.258-1.458)	*0.065*
MMP-2 (ng/mL)	313.88 (158.32-651.4)	245.7 (165.3-714.5)	0.689
MMP-3 (ng/mL)	37.93 (20.33-62.58)	47.60 (26.14-70.38)	0.272
MMP-7 (ng/mL)	*1.59* (1.01-2.58)	*2.16* (1.34-2.82)	*0.003*
MMP-8 (ng/mL)	0.693 (0.367-1.145)	0.806 (0.426-1.471)	0.183
MMP-9 (ng/mL)	28.48 (21.05-43.71)	29.41 (22.065-47.88)	0.499
MMP-10 (ng/mL)	*0.464* (0.308-0.648)	*0.602* (0.463-0.780)	*0.015*

MMP, matrix metalloproteinases. Values are reported as medians and interquartile ranges (in parenthesis); values statistically significative are in italics.

Table 6. Correlations between circulating levels of cytokines and DOACs levels after 1 and 6 months of treatment.

	Apixaban (ng/mL) (N=18)		Rivaroxaban (ng/mL) (N=20)		Dabigatran (ng/mL) (N=16)	
	T1	T2	T1	T2	T1	T2
IL-8 (pg/mL)	-0.013	-0.140	0.023	0.351	-0.026	-0.021
	0.958	0.578	0.922	-0.129	0.922	0.940
TNF-α (pg/mL)	-0.240	-0.044	-0.138	-0.005	*0.575*	0.375
	0.338	0.861	0.562	0.985	*0.020*	0.152
IL-6 (pg/mL)	-0.191	-0.160	0.213	-0.221	0.313	0.279
	0.447	0.526	0.367	0.348	0.237	0.295
IL-10 (pg/mL)	-0.079	*-0.537*	*0.542*	0.013	-0.205	0.186
	0.757	*0.022*	*0.014*	0.957	0.447	0.490
IL-1RA (pg/mL)	*0.690*	0.298	-0.050	0.096	0.235	*0.556*
	0.002	0.229	0.835	0.686	0.380	*0.025*
VEGF-α (pg/mL)	-0.131	0.084	0.226	0.151	-0.032	0.397
	0.605	0.741	0.339	0.526	0.905	0.128
ICAM-1 (ng/mL)	*0.522*	*0.571*	0.123	-0.110	-0.009	-0.135
	0.026	*0.013*	0.605	0.645	0.974	0.617

IL, interleukin; TNF, tumor necrosis factor; IL-1RA, interleukin -1 receptor antagonist; VEGF, vascular-endothelial growth factor; ICAM, intercellular adhesion molecule. T1 and T2, levels after 1 and 6 months of treatment, respectively. Values statistically significative are in italics.

icantly higher levels of IL-1RA compared to the levels observed in patients DOACs concentration under median value (Table 7). Similarly, after 6 months of treatment, patients with DOACs concentration had higher levels of the anti-inflammatory cytokine IL-1RA than the ones with lower concentrations of the drugs (Table 7).

DISCUSSION

Treatment with DOACs for six months was associated with significantly higher levels of IL-10, TNF-α, VEGF-α, and VCAM-1. Furthermore, by analyzing the balance between pro-inflammatory and anti-inflammatory cytokines, we found that after 6 months of DOACs treatment IL-6/IL-10 and IL-8/IL-10 ratios were significantly lower compared to ratios observed at one month. On the contrary, the ratio between TNF-α/IL1RA were significantly higher at six months compared to ratio at one month.

Therefore, our results suggest that prolonged DOACs treatment is able to influence the subtle inflammation, which characterized both venous thromboembolism and atrial fibrillation. Interestingly, our data reveal that DOACs treatment increased the anti-inflammatory cytokine IL-10, whose levels might effectively counterbalance the inflammatory status present in the above-mentioned diseases, suggesting that DOACs treatment reduces the adverse outcomes occurrence not only through their capability of reducing thrombin formation, but also through their action on the inflammation.

In fact, IL-10, produced by B and T lymphocytes and NK cells, is able to inhibit pro-inflammatory cytokines synthesis, therefore reducing leukocyte maturation and recruitment during inflammation. It also has a role in suppressing the capacity to display the antigen to antigen-presenting cells and induces the production of antibodies and lymphocytes B survival.

In our study, DOACs treatment causes a significant increase in TNF-α circulating levels. This datum seems to be inconsistent with the supposed anti-inflammatory effect of DOACs. However, it has to be considered that, beyond the prevalent effect on a single molecule, cytokines are pleiotropic and synergic and some of them can behave as either antagonist or agonist of the other cytokines. The alteration of the expression of one cytokine can significantly modify the profile of many other cytokines. Therefore, it is necessary to evaluate the whole cytokines network and consider the global pattern of pro- and anti-inflammatory cytokines. In actual fact, it is the displacement of pro- and anti-inflammatory ratios that determines a pro-inflammatory phenotype, rather than the increase of a single pro-inflammatory cytokine.

Using a global score of inflammation (low, moderate, and elevated grade of inflammation according to the tertiles of pro- and anti-inflammatory cytokines), almost 60% of patients, after six months of therapy, showed a reduction of the grade of inflammation compared to the first month of therapy. Considering the balance between pro- and anti-inflammatory cytokines, this result indicates a net anti-inflammatory effect of DOACs, mainly driven by the increase of IL-10 levels at six months.

In addition to the anti-inflammatory effect of DOACs treatment, our data show a time-dependent modification of MMP-7 and MMP-10 levels, suggesting that this treatment can modulate, mirroring the effect of DOACs on cytokines patterns, the MMP plasma levels and consequently the remodeling process. MMPs are a family of zinc-dependent proteases, that have a specific role in pathophysiological proteolysis of extracellular matrix and in tissue remodeling. Moreover, MMPs are able to modulate growth factors and cytokines activities. The balance between MMPs and their inhibitors control the extracellular matrix turnover. MMP-7 acts not only on extracellular matrix components but also on pro-inflammatory molecules such as pro-α defensin, Fas-Ligand, TNF-α and

Table 7. Circulating levels of cytokines according to DOACs concentrations above or below the median levels after 1 and 6 months of treatment (N=18).

	T1		P	T2		P
	DOACs above median value n. %	DOACs below median valuen n. %		DOACs above median value n. %	DOACs below median value n. %	
IL-8 (pg/mL)	3.2 (1.9-5.8)	3.7 (2.6-6.3)	0.331	3.5 (2.1-6.0)	3.2 (2.0-5.0)	0.551
TNF-α (pg/mL)	0.6 (0.03-2.2)	1.6 (0.03-2.7)	0.224	1.4 (0.6-2.6)	2.0 (0.7-5.1)	0.337
IL-6 (pg/mL)	1.2 (0.6-2.19	1.8 (0.9-3.5)	0.161	1.6 (1.0-2.5)	1.5 (0.8-3.3)	0.883
IL-10 (pg/mL)	0.3 (0.2-10.6)	0.3 (0.2-10.1)	0.929	5.0 (2.9-14.2)	4.8 (0.3-11.2)	0.386
IL-1RA (pg/mL)	*307.5 (224-520)*	*491.4 (275-699)*	*0.046*	*305 (245-464)*	*399 (334-594)*	*0.049*
VEGF-α (pg/mL)	7.1 (2.4-15.4)	7.9 (3.7-22.3)	0.411	13.4 (7.3-18.3)	14.9 (12.0-22.2)	0.057
ICAM-1 (ng/mL)	140 (92-191)	157 (124-215)	0.346	149 (87-225)	151 (113-306)	0.574

IL, interleukin; TNF, tumor necrosis factor; IL-1RA, interleukin -1 receptor antagonist; VEGF, vascular-endothelial growth factor; ICAM, intercellular adhesion molecule. Values statistically significative are in italics.

E-cadherin. MMP-10 can directly lyse extracellular matrix and act on several proMMPs, activating other MMPs.

Our data are not consistent with those obtained in an experimental animal study, in which rivaroxaban administration was able to inhibit MMP-9 expression on a model of catheter thrombosis in mice external jugular vein.[30] However, some differences between the two studies must be taken into account. First, the animal model of catheter thrombosis is rather different from human venous thrombosis. Second, rivaroxaban dosage is quite different as animals were administered 5mg/kg of rivaroxaban, a dose higher than the one used in humans (0.25 mg/kg). Therefore, we cannot compare the two studies, as a higher dose of rivaroxaban (5mg/kg) cannot be administered to humans.

Until now, only one study about the effect of DOACs on metalloproteinases in human is available.[31] In that study, Japanese AF patients were randomized to receive either rivaroxaban (n=93) or warfarin (n=94) for 24 weeks. Rivaroxaban administration was associated with a slight but significant reduction of MMP-9 levels. However, that study enrolled only Japanese patients, so it is likely that different genetic backgrounds might determine different effect on MMPs. In addition, in the Japanese study the authors evaluated the difference between the pre-treatment sample and the 24-week sample, whereas in our study we evaluated variation of MMP plasma levels between samples obtained after one and six months of treatment.

Moreover, we can't exclude that the small number of patients treated with rivaroxaban in our study could have biased the results.

The correlation between atrial fibrosis and AF is well known.[32,33] MMPs play a central role in AF remodeling process and thus in the development of atrial fibrosis, suggesting a possible pathogenetic role in the occurrence and the progression of AF.[34,35] The possible pathogenetic role of MMP-7 in AF is suggested by an animal model and a clinical study.[36-38] Consistent with these studies, we found that MMP-7 increased according to the duration of anticoagulant therapy and of AF. This increase could be linked to the disease natural history and might not be influenced by oral anticoagulation.

Regarding venous thromboembolism, MMPs are involved in numerous vascular diseases such as deep vein thrombosis (DVT) and post thrombotic syndrome (PTS). Increased levels of MMP, strictly correlated to the inflammation grade, was observed into vein walls after an episode of DVT.[39,40] Some reviews reported that MMP are increased both in the acute phase of DVT and in patients with PTS.[41,42] In a murine model of DVT, MMP-7 and MMP-10 expression in femoral vein was significantly higher than in the murine control group;[43] MMPs may affect the process of DVT through transcription regulation of the fibrinolysis and anti-fibrinolytic system during the course of thrombosis and thrombus resolution.

Data on DOACs plasma concentrations indicate that there was no selection bias in patients' population, since DOACs plasma concentrations observed in our patients were similar to those reported in literature. Furthermore, levels of anticoagulation were stable between one month and six months of treatment.

Interestingly, our results showed, after one month of treatment, a positive and significant correlation between IL-10 and rivaroxaban plasma levels and between IL-1RA and apixaban plasma levels. After six months of treatment only dabigatran plasma levels showed a positive and significant correlation with anti-inflammatory IL-1RA, suggesting that dabigatran could exert a slower anti-inflammatory effect as compared to apixaban and rivaroxaban. However, these preliminary data need to be evaluated in a larger number of patients in order to find possible confounding factors that could have influenced our results.

Surprisingly, our data showed an inverse relationship between apixaban plasma levels and IL-10 after six months of treatment, implying the possibility that, after an initial beneficial effect, there was an inversion in the trend in the following months. Nevertheless, several factors have to be taken into account. First of all, in our study pre-treatment blood samples were not available, so we were unable to evaluate, after several months of treatment, the real degree of inflammation inhibition. Secondly, the small number of patients in treatment with apixaban could have biased the results. Thirdly, the possible noncompliance of patients with treatment procedures has to be taken into account. In addition, it is likely that after an initial decrease in IL-10 levels associated with apixaban intake, a steady state is achieved in which IL-10 is no longer inhibited.

Dividing our population in two groups, patients with DOACs plasma concentration above or below median value, the former patients had significantly higher levels of IL-1RA, both after one and six months of treatment.

Our study presents several limits, such as the lack of a control group (without anticoagulant therapy or in therapy with VKA anticoagulants) and the lack of pre-treatment blood samples, collected before the beginning of the therapy. Most importantly, the low number of investigated patients does not allow us to extrapolate the statistical analysis results to the overall population.

CONCLUSIONS

In conclusion, treatment with DOACs is associated with variations in systemic inflammation markers, and in metalloproteinases, suggesting that, beyond the well-known anticoagulant activities, DOACs may also modulate the inflammatory and remodeling processes and that

thrombin and factor Xa appear to be involved in the pathophysiological mechanisms of non-valvular atrial fibrillation and venous thromboembolism. The modulation of anti-inflammatory cytokines IL-10 and IL-1RA seems at least partially related to DOACs plasma concentration in a dose and time dependent way. Further studies with larger number of patients are required.

REFERENCES

1. Hindricks G, Potpara T, Dagres N, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J 2021;42:373-498.
2. Konstantinides SV, Meyer G, Becattini C, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). Eur Heart J 2020;41:543-603.
3. Nakamura M, Yamada N, Ito M. Novel Anticoagulant Therapy of Venous Thromboembolism: Current Status and Future Directions. Ann Vasc Dis 2017;10:92-8.
4. Zirlik A, Bode C. Vitamin K antagonists: relative strengths and weaknesses vs. direct oral anticoagulants for stroke prevention in patients with atrial fibrillation. J Thromb Thrombolysis 2017;43:365-79.
5. Steffel J, Collins R, Antz M, et al. 2021 European Heart Rhythm Association Practical Guide on the Use of Non-Vitamin K Antagonist Oral Anticoagulants in Patients with Atrial Fibrillation. Europace 2021;23:1612-76.
6. Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. Nat Rev Cardiol 2015;12:230-43.
7. Hijazi Z, Aulin J, Andersson U, et al. Biomarkers of inflammation and risk of cardiovascular events in anticoagulated patients with atrial fibrillation. Heart 2016;102:508-17.
8. Shbaklo H, Holcroft CA, Kahn SR. Levels of inflammatory markers and the development of the post-thrombotic syndrome. Thromb Haemost 2009;101:505-12.
9. Bittar LF, Silva LQD, Orsi FLA, et al. Increased inflammation and endothelial markers in patients with late severe post-thrombotic syndrome. PLoS One 2020;15:e0227150.
10. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med 2017;377:1119-31.
11. Oesterle A, Laufs U, Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. Circ Res 2017;120:229-43.
12. Ren Y, Zhu H, Fan Z, et al. Comparison of the effect of rosuvastatin versus rosuvastatin/ezetimibe on markers of inflammation in patients with acute myocardial infarction. Exp Ther Med 2017;14:4942-50.
13. Mousavi S, Moradi M, Khorshidahmad T, Motamedi M. Anti-Inflammatory Effects of Heparin and Its Derivatives: A Systematic Review. Adv Pharmacol Sci 2015;2015:507151.
14. Litov L, Petkov P, Rangelov M, et al. Molecular Mechanism of the Anti-Inflammatory Action of Heparin. Int J Mol Sci 2021;22:10730.
15. Basiak M, Kosowski M, Cyrnek M, et al. Pleiotropic Effects of PCSK-9 Inhibitors. Int J Mol Sci 2021;22:3144.
16. Spronk HM, de Jong AM, Crijns HJ, et al. Pleiotropic effects of factor Xa and thrombin: what to expect from novel anticoagulants. Cardiovasc Res 2014;101:344-51.
17. Bukowska A, Zacharias I, Weinert S, et al. Coagulation factor Xa induces an inflammatory signalling by activation of protease-activated receptors in human atrial tissue. Eur J Pharmacol 2013;718:114-23.
18. Ruf W. Roles of factor Xa beyond coagulation. J Thromb Thrombolysis 2021;52:391-6.
19. Ten Cate H, Guzik TJ, Eikelboom J, Spronk HMH. Pleiotropic actions of factor Xa inhibition in cardiovascular prevention: mechanistic insights and implications for antithrombotic treatment. Cardiovasc Res 2021;117:2030-44.
20. Al-Tamimi M, Grigoriadis G, Tran H, et al. Coagulation-induced shedding of platelet glycoprotein VI mediated by factor Xa. Blood 2011;117:3912-20.
21. Kondo H, Abe I, Fukui A, et al. Possible role of rivaroxaban in attenuating pressure-overload-induced atrial fibrosis and fibrillation. J Cardiol 2018;71:310-9.
22. Matsuura T, Soeki T, Fukuda D, et al. Activated Factor X Signaling Pathway via ProteaseActivated Receptor 2 Is a Novel Therapeutic Target for Preventing Atrial Fibrillation. Circ J 2021;85:1383-91.
23. Tsujino Y, Sakamoto T, Kinoshita K, et al. Edoxaban suppresses the progression of atrial fibrosis and atrial fibrillation in a canine congestive heart failure model. Heart Vessels 2019;34:1381-8.
24. Hara T, Fukuda D, Tanaka K, et al. Inhibition of activated factor X by rivaroxaban attenuates neointima formation after wire-mediated vascular injury. Eur J Pharmacol 2018;820:222-8.
25. Amini S, Gholami K, Bakhshandeh H, Fariborz Farsad B. Effect of Oral Anticoagulant Therapy on Coagulation Activity and Inflammatory Markers in Patients with Atrial Fibrillation Undergoing Ablation: A Randomized Comparison between Dabigatran and Warfarin. Iran J Pharm Res 2013;12:945-53.
26. Katoh H, Nozue T, Michishita I. Anti-inflammatory effect of factor-Xa inhibitors in Japanese patients with atrial fibrillation. Heart Vessels 2017;32:1130-6.
27. Pignatelli P, Pastori D, Bartimoccia S, et al. Anti Xa oral anticoagulants inhibit in vivo platelet activation by modulating glycoprotein VI shedding. Pharmacol Res 2016;116:484-9.
28. Nakase T, Moroi J, Ishikawa T. Anti-inflammatory and antiplatelet effects of non-vitamin K antagonist oral anticoagulants in acute phase of ischemic stroke patients. Clin Transl Med 2018;7:2.
29. Zemer-Wassercug N, Haim M, Leshem-Lev D, et al. The effect of dabigatran and rivaroxaban on platelet reactivity and inflammatory markers. J Thromb Thrombolysis 2015;40:340-6.
30. Terry CM, He Y, Cheung AK. Rivaroxaban improves patency and decreases inflammation in a mouse model of catheter thrombosis. Thromb Res 2016;144:106-12.
31. Chan MY, Lin M, Lucas J, et al. Plasma proteomics of pa-

tients with non-valvular atrial fibrillation on chronic anticoagulation with warfarin or a direct factor Xa inhibitor. Thromb Haemost 2012;108:118091.
32. Li CY, Zhang JR, Hu WN, Li SN. Atrial fibrosis underlying atrial fibrillation (Review). Int J Mol Med 2021;47:9.
33. Xintarakou A, Tzeis S, Psarras S, et al. Atrial fibrosis as a dominant factor for the development of atrial fibrillation: facts and gaps. Europace 2020;22:342-51.
34. Liu Y, Xu B, Wu N, et al. Association of MMPs and TIMPs With the Occurrence of Atrial Fibrillation: A Systematic Review and Metaanalysis. Can J Cardiol 2016;32:803-13.
35. Zhan G, Wenhua G, Jie H, et al. Potential roles of circulating matrix metalloproteinase-28 (MMP-28) in patients with atrial fibrillation. Life Sci 2018;204:15-9.
36. Mukherjee R, Akar JG, Wharton JM, et al. Plasma profiles of matrix metalloproteinases and tissue inhibitors of the metalloproteinases predict recurrence of atrial fibrillation following cardioversion. J Cardiovasc Transl Res 2013;6: 528-35.
37. Wang W, Zhang HT, Yang XL. Effect of matrix metalloproteinase and their inhibitors on atrial myocardial structural remodeling. J Cardiovasc Med (Hagerstown) 2013;14:265-9.
38. Jia M, Li ZB, Li L, et al. Role of matrix metalloproteinase-7 and apoptosis-associated gene expression levels in the pathogenesis of atrial fibrosis in a Beagle dog model. Mol Med Rep 2017;16:6967-73.
39. de Franciscis S, Gallelli L, Amato B, et al. Plasma MMP and TIMP evaluation in patients with deep venous thrombosis: could they have a predictive role in the development of post-thrombotic syndrome? Int Wound J 2016;13:1237-45.
40. Zhang T, Li Q, Wang L, Li G. Expression variations and clinical significance of MMP-1, MMP-2 and inflammatory factors in serum of patients with deep venous thrombosis of lower extremity. Exp Ther Med 2019;17:181-6.
41. Mosevoll KA, Johansen S, Wendelbo Ø, et al. Cytokines, Adhesion Molecules, and Matrix Metalloproteases as Predisposing, Diagnostic, and Prognostic Factors in Venous Thrombosis. Front Med (Lausanne) 2018;5:147.
42. Saghazadeh A, Hafizi S, Rezaei N. Inflammation in venous thromboembolism: Cause or consequence? Int Immunopharmacol 2015;28:655-65.
43. Zhang YB, Li W, Yao LQ, et al. Expression changes and roles of matrix metalloproteinases in a rat model of traumatic deep vein thrombosis. Chin J Traumatol 2010;13:188-92.