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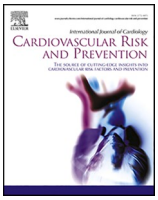

Inflammatory cytokines differ between patients with high versus low CHA2DS2-VASc scores in sinus rhythm-a possible mechanism for adverse cardiovascular events☆

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ABSTRACT

Background: The CHA_2DS_2-VASc score was shown to predict systemic thromboembolism and mortality in certain groups of patients in sinus rhythm (SR). Previous data showed that patients in SR with high CHA_2DS_2-VASc score have higher plasma levels of inflammatory markers such as sP-selectin and *C*-reactive protein. We further investigated this group.

Methods: Blood samples were collected from consecutive patients in SR. Plasma was extracted and stored at −80 °C. Concentrations of a panel of soluble markers IL-1β, IL-6, IL-8, IL-10, TNF-α and VEGF were measured by Magnetic Luminex Performance Assay. The PLF4 cytokine blood level was measured by ELISA.

Results: 66 patients were enrolled (age 53 ± 18 years, 60% women). Patients with high CHA_2DS_2-VASc scores (n = 23) had significantly higher median IQR concentrations of TNF-α [10.34 (8.55,14.92) vs. 7.69 (6.06, 9.85) pg/ml, p = 0.009] and a trend towards higher levels of IL-1β [0.59 (0.4,0.8) vs. 0.44 (0.31, 0.62) pg/ml, p = 0.07] and IL-8 [5.92 (4.5,9.4) vs. 5.04 (3.63, 6.04) pg/ml, p = 0.07], compared to the group with low scores (n = 43). Median IQR concentrations of VEGF, IL-6, IL-10 and PF4 did not significantly differ between the CHA_2DS_2-VASc score groups.

Conclusion: Patients in SR with high versus low CHA_2DS_2-VASc scores have high plasma concentrations of systemic inflammation cytokines. The already proven high levels of sP-selectin, that promotes release of inflammatory cytokines from leukocytes, is in line with these results. This pro-inflammatory state in patients with high CHA_2DS_2-VASc scores, may explain the higher rate of adverse cardiovascular events associated with elevated CHA_2DS_2-VASc score even without atrial fibrillation.

1. Introduction

The CHA_2DS_2-VASc score, based on clinical risk factors (congestive heart failure, hypertension, age, female gender, diabetes mellitus, history of stroke and vascular disease), was first recommended by the European Society of Cardiology (ESC) Practice Guide in 2010 [1]. This score is currently used in daily clinical practice to assess the risk of stroke and systemic thromboembolism in patients with atrial fibrillation (AF) and guide the use of anticoagulation treatment [2]. However, trials failed to demonstrate a clear temporal association between AF and stroke occurrence, and only around 30–40% of subclinical AF events are temporally related to stroke events [3–5]. Thus, the association between AF and stroke is complex [6], and stroke in patients with AF probably involves mechanisms additive to cardiac embolism, such as: myocardial fibrosis or hypertrophy, myocyte dysfunction, left atrial appendage dysfunction, and also a pro-inflammatory state.

Pre-existing inflammation can activate AF. AF further produces an inflammatory reaction that promotes atrial remodeling as well as AF persistency and progression [7,8]. In addition, elevated levels of interferon gamma (IFN-γ) were shown to be an independent risk factor of stroke and all-cause mortality in patients with new-onset AF, and IFN-γ levels had an incremental prognostic value supplementary to that obtained from the CHA_2DS_2-VASc score [9].

Sex type is included in the CHA_2DS_2-VASc score. In women with paroxysmal AF, inflammatory biomarkers were higher, as opposed to men with paroxysmal AF, where biomarkers for vascular remodeling

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were higher [10]. Thus, pathophysiological mechanisms in women and man in AF may be different and may underlie the higher risk for stroke in women with AF and other co-morbidities included in the CHA_2DS_2-VASc score [11].

Similar to AF patients, the CHA_2DS_2-VASc score was recently demonstrated to have a predictive role for systemic thromboembolism [12] and mortality in patients with sinus rhythm (SR) and no history of AF [13–15]. This was specifically demonstrated in patients with heart failure [16,17], sick sinus syndrome (SSS) [18], elderly patients [12], and in middle-aged individuals without AF [15]. An increased CHA_2DS_2-VASc score was associated with increased risk of developing new AF in patients with [19] and without prior ischemic stroke [15,20]. Our recent work demonstrated that patients in SR with high CHA2DS2-VASc score have higher plasma levels of *C*-reactive protein (CRP), neutrophil/lymphocyte ratio (NLR) and soluble *P*-selectin (sP-selectin) [21].

In the current study we further investigated the underlying mechanism of higher risk of stroke in patients at SR and high CHA_2DS_2-VASc scores, compared to patients with low scores, by a systematic comparison of serum cytokines between the two groups.

2. Methods

2.1. Study design and participants

Patients in SR without acute coronary syndrome (ACS)/ST elevation myocardial infarction and without a history of AF were considered for inclusion in the study. The patients were enrolled from cardiology or internal medicine departments and from cardiology outpatient clinic at our medical center between March 2018 and December 2020. Excluded were patients with anemia (hemoglobin <10 g/dL), thrombocytopenia (absolute platelet count $<120 \times 10^3/\mu l$), chronic hemato – oncologic diseases (leukemia, lymphoma etc.), blood products transfusion in the prior 30 days and pregnancy. Any patient with ACS, an infectious or acute inflammatory disease, or history of AF was not enrolled as well. Clinical and demographic data were obtained from medical charts. All echocardiographic exams were analyzed by a specialized cardiologist in our center.

Blood samples (5 ml) were collected from all the patients that were included. After centrifugation, plasma was extracted and stored at −80 °C. Human High Sensitivity Cytokine A pre-mixed Magnetic Luminex Performance Assay (7 Plex, R&D systems) was used to measure the concentrations of a panel soluble markers Interleukin (IL)-1 b, IL-6, IL-8, IL-10, Tumor Necrosis Factor-alfa (TNF-α) and Vascular Endothelial Grown Factor (VEGF) in one sample at the same time. The Platelet Factor 4 (PLF4) cytokine blood level was measured by ELISA (Quantikine ELISA DPF40, R&D systems).

The patients were divided into two groups: those with low CHA2DS2-VASc score (male with score 1 or 0 and female with score ≤2) and those with high CHA2DS2-VASc score (male with score≥2 and female with score ≥3). The definition of low and high CHA2DS2-VASc score was selected according to the current ESC AF guidelines, which differ across genders regarding class I indication for anticoagulation treatment in AF patients [2,11]. The association between CHA_2DS_2-VASc score and each biomarker (IL-1β, IL-6, IL-8, IL-10, TNF-α, PLF4 and VEGF) was evaluated. The study was approved by the local investigational review board (ethics committee) of our hospital, and all subjects provided written informed consent.

2.2. Statistical analysis

The results are presented as the mean ± standard deviation (SD) for continuous variables with normal distribution, as median with interquartile range-IQR (25th; 75th percentiles) for continuous variables with abnormal distribution, and as number and percentage of total patients for categorical data. T test was used for comparison of continuous variables. When the distribution was abnormal, the Mann-Whitney test was applied accordingly. Chi-square test and Fisher's exact test were used for categorical data. A two-sided p-value < 0.05 was considered as statistically significant. The statistical analysis was performed with SPSS version 21 statistical software (IBM Inc. Chicago, Illinois).

Stepwise multivariate logistic regression analysis was performed with TNF-α levels as the dependent variable and the predictors were: age, sex, diabetes mellitus, hypertension, congestive heart failure (CHF), dyslipidemia, smoking, ischemic heart disease (IHD), history of cerebrovascular accident (CVA), and chronic renal failure (CRF). The Stepwise criteria were as follow: Probability-of-F-to-enter≤0.050, Probability-of-F-to-remove≥0.100.

3. Results

3.1. Baseline characteristics

A total of 66 consecutive patients in sinus rhythm with no history of AF were included in the study. Mean age was 53 ± 18 years, 40 (60%) were women, and 10 (15%) had diabetes. Of them, 23 patients (35%) had high CHA_2DS_2-VASc score, and 43 (65%) had low CHA_2DS_2-VASc score. Baseline characteristics of the patients based on the CHA_2DS_2-VASc score are presented in Table 1.

Patients with low CHA_2DS_2-VASc score were younger (mean age 42.4 ± 9.7 vs. 72.7 ± 11.6 y, $p < 0.001$), had less comorbidities and smaller left atrium (LA) area than those with high scores [15 (IQR 12.2,21) vs. 22.6 (19,28.3) cm^2, $p = 0.002$]. Left ventricular ejection fraction (LVEF) was not significantly different between the groups. Patients with low CHA_2DS_2-VASc score were less often treated with statins, beta-blockers, angiotensin converting enzyme inhibitors, anti-platelet medications and anticoagulants, compared to patients with high scores (Table 1).

3.2. Cytokines levels

Table 2 describes biomarker's levels according to CHA_2DS_2-VASc score. Patients with high scores had significantly higher median IQR concentrations of TNF-α [10.3 (8.6,14.9) vs. 7.7 (6.1, 9.9) pg/ml, $p = 0.009$]. This group of patients with high scores also had a trend toward higher levels of IL-1β [0.59 (0.4,0.8) vs 0.44 (0.31, 0.62) pg/ml, $p = 0.073$] and IL-8 [5.9 (4.5,9.4) versus 5 (3.6, 6) pg/ml, $p = 0.073$], compared to the group with low scores. No statistically significant differences were observed between the groups regarding the median IQR concentrations of VEGF, IL-6, IL-10 and PF4 (Table 2).

The distribution of TNF-α levels stratified by CHA_2DS_2-VASc score is presented graphically in Fig. 1. TNF-α concentrations were significantly different between CHA_2DS_2-VASc score = 3 and CHA_2DS_2-VASc score = 4 [median 9.61 (6.66, 9.76) vs. 13.05 (9.70, 14.92) pg/ml respectively, $p = 0.038$] and between CHA_2DS_2-VASc score = 3 and CHA_2DS_2-VASc score = 7 [median 9.61 (6.66, 9.76) vs. 18.41 (15.62, 19.22) pg/ml, respectively, $p = 0.015$].

3.3. Conditioning of TNF-α concentrations by different factors

Despite the small study group, TNF-α was shown to be conditioned by some components of the CHA2DS2-VASc score and by some other factors (Table 3): Age difference had significant impact on TNF-α concentrations; Hypertension, CHF, dyslipidemia and history of IHD disease also had significant influence on the levels of TNF-α. However, diabetes mellitus, sex, smoking, history of stroke and CRF did not significantly affect these levels.

Multivariate logistic regression analysis was performed to investigate whether the differences in TNF-α levels between the groups were independent from patient's associated factors. Multivariate regression analysis (stepwise) was performed with TNF-α levels as the dependent variable and the predictor variables were: age, sex, diabetes mellitus,

Table 1
Comparison between baseline clinical characteristics of patients with high versus low CHA_2DS_2-VASc scores.

Clinical Characteristics	All Patients (n = 66)	High CHA_2DS_2-VASc (n = 23)	Low CHA_2DS_2-VASc (n = 43)	*P*-Value
Age (years), mean ± SD	53.0 ± 17.9	72.7 ± 11.6	42.4 ± 9.7	<0.001
Men, n (%)	26 (39.4)	11 (47.8)	15 (34.9)	0.31
Diabetes Mellitus, n (%)[b]	10 (15.2)	8 (38.4)	2 (4.7)	0.002
Dyslipidemia, n (%)	31 (47.0)	22 (95.7)	9 (20.9)	<0.001
Hypertension, n (%)	27 (40.9)	21 (91.3)	6 (14.0)	<0.001
Smoking, n (%)[b]	9 (13.6)	6 (21.6)	3 (7.0)	0.04
Ischemic Heart Disease, n (%)[b]	13 (19.7)	13 (56.6)	0 (0)	<0.001
Stroke, n (%)[b]	6 (9.1)	6 (26.1)	0 (0)	0.001
Peripheral Artery Disease, n (%)[b]	1 (1.5)	1 (4.3)	0 (0)	0.35
Chronic Heart Failure, n (%)[b]	7 (10.6)	7 (30.4)	0 (0)	<0.001
Chronic Renal Failure, n (%)[b]	4 (6.1)	4 (17.4)	0 (0)	0.01
Permanent Pacemaker, n (%)[b]	5 (7.8)	4 (18.2)	1 (2.4)	0.04
Sick sinus syndrome, n (%)[b]	3 (4.6)	2 (3.1)	1 (2.4)	0.28
Laboratory tests				
Hemoglobin (g/dL), mean ± SD	13.6 ± 1.5	13.4 ± 1.5	13.8 ± 1.5	0.40
Platelets (10^3/dL), median (IQR)[a]	239 (201,287)	230 (196,279)	243 (210,306)	0.36
Creatinine (mg/dL), median (IQR)[a]	0.9 (0.7,1.0)	0.9 (0.9,1.3)	0.9 (0.7,1.0)	0.40
Echocardiographic parameters				
LVEF, %, median (IQR)[a]	60.0 (50.0,60.0)	60 (43.8,60.0)	60 (60.0,60.0)	0.14
LA diameter, mm, median (IQR)[a]	3.9 (3.2,4.4)	4.3 (3.8,4.5)	3.2 (3.0,3.9)	0.002
LA Area, cm2, median (IQR)[a]	20.6 (16.0,24.0)	22.6 (19.0, 28.3)	15.0 (12.2,21.0)	0.002
SPAP, median (IQR)[a]	30.0 (23.5,39.0)	26.2 (20.3,31.9)	26.0 (21.5,27.0)	0.01
Treatment				
Statins, n (%)	27 (41.5)	21 (91.3)	6 (14.3)	<0.001
Beta blockers, n (%)	16 (25.0)	15 (65.2)	1 (2.4)	<0.001
ACE inhibitors or Angiotensin receptor blockers, n (%)	15 (23.4)	13 (56.6)	2 (4.9)	<0.001
Antiarrhythmics, n (%)[b]	1 (1.6)	1 (4.3)	0 (0)	0.359
Aspirin, n (%)	16 (24.6)	15 (65.2)	1 (2.4)	<0.001
Antiaggregant, n (%)[b]	7 (10.8)	7 (30.4)	0 (0)	<0.001
Anticoagulants, n (%)[b]	3 (4.7)	3 (13.0)	0 (0)	0.043

LVEF- left ventricular ejection fraction, LA-left atrium, SPAP- systolic pulmonary artery pressure, ACE-angiotensin converting enzyme, SD−standard deviation, IQR- Interquartile range (25th; 75th percentiles).
[a] Mann-Whitney *U* test.
[b] Fisher Exact test.

hypertension, CRF, dyslipidemia, smoking, IHD, CRF and CVA. The differences in TNF-α between the groups were dependent on age and CHF (p value < 0.05) and were not dependent on sex, diabetes mellitus, hypertension, dyslipidemia, smoking, IHD and history of CVA or CRF.

4. Discussion

In the current study we found that plasma concentrations of TNF-α were significantly higher in patients with SR and high CHA_2DS_2-VASc scores versus those with low scores. TNF-α levels were mainly dependent on age and history of CHF. This is in line with previous reports on the significance of these factors in predicting long-term cardiac and thrombo-embolic adverse outcomes [12,15–17]. The group of patients with high scores also had a trend toward higher levels of cytokines IL-1β and IL-8, compared to the group with low scores. No statistically significant differences were observed between the groups in the plasma concentrations of VEGF, IL-6, IL-10 and PF4.

Table 2
Cytokines' plasma levels stratified by CHA_2DS_2-VASc score.

Cytokines	High CHA_2DS_2-VASC (n = 23)	Low CHA_2DS_2-VASC (n = 43)	*P* value
TNF alpha, median (IQR), pg/ml	10.34 (8.55–14.92)	7.69 (6.06–9.85)	0.009
VEGF, median (IQR), pg/ml	11.8 (6.98–15.23)	8.15 (4.37–16.01)	0.240
IL-1b, median (IQR), pg/ml	0.59 (0.40–0.80)	0.44 (0.31–0.62)	0.073
IL-6, median (IQR), pg/ml	2.58 (2.12–3.79)	1.61 (1.13–2.69)	0.691
IL-8, median (IQR), pg/ml	5.92 (4.5–9.4)	5.04 (3.63–6.04)	0.073
IL-10, median (IQR), pg/ml	1.07 (0.66–1.44)	0.80 (0.54–1.32)	0.317
PF4, median (IQR), pg/ml	946.22 (624.47–1060.92)	843.92 (341.22–1011.64)	0.268

Similar to AF patients, the CHA_2DS_2-VASc score was recently demonstrated to have a predictive role for systemic thromboembolism and mortality in patients with SR and no history of AF [12–15]. A high CHA_2DS_2-VASc score was also associated with increased risk of developing new AF, even in patients without prior ischemic stroke [15,20].

We have recently shown that patients with high CHA_2DS_2-VASc score in SR have higher plasma levels of inflammatory markers, such as CRP, NLR and sP-selectin [21], compared to patients with low scores. Taken together with our current findings, we suggest that ongoing inflammation may contribute to the development of AF, thrombotic events, and higher mortality in patients with high CHA_2DS_2-VASc scores.

TNF-α is a major inflammatory cytokine regulator that is upregulated in many inflammatory diseases. It is produced by activated macrophages/monocytes during acute inflammation, but is expressed also in endothelial and epithelial cells, smooth muscle cells and cardiac myocytes. It is responsible for a diverse range of signaling events within cells, including activation of genes participating in inflammatory and immunoregulatory responses, proliferation, growth inhibition, and cell death [22–25]. It has been shown that activation of TNF-α signaling can promote atrial electrical, structural, and contractile remodeling [26]. In addition, elevated TNF-α in mice may promote atrial fibrosis, slow conduction and alteration of extra-cellular matrix [23–26]. TNF-α represents a ligand for two types of TNF-α receptors. As demonstrated in animal models, the deleterious effects of TNF-α are mainly mediated by the prolonged or excessive activation of TNFR1, which is involved in disease progression, worse heart function and increased mortality, while TNFR2-dependent pathways mainly activate cardioprotective processes [23–25].

IL-1β is a member of the interleukin-1 family of cytokines. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). IL-1β production is critically regulated by cytosolic molecular complexes, termed inflammasomes. The mature form is secreted and serve as an inflammatory signaling-amplifier to perpetuate and spread the inflammatory response. This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [22,27]. Emerging evidence points to an important role of NOD-like receptor 3 (NLRP3) inflammasome in a number of cardiovascular diseases,

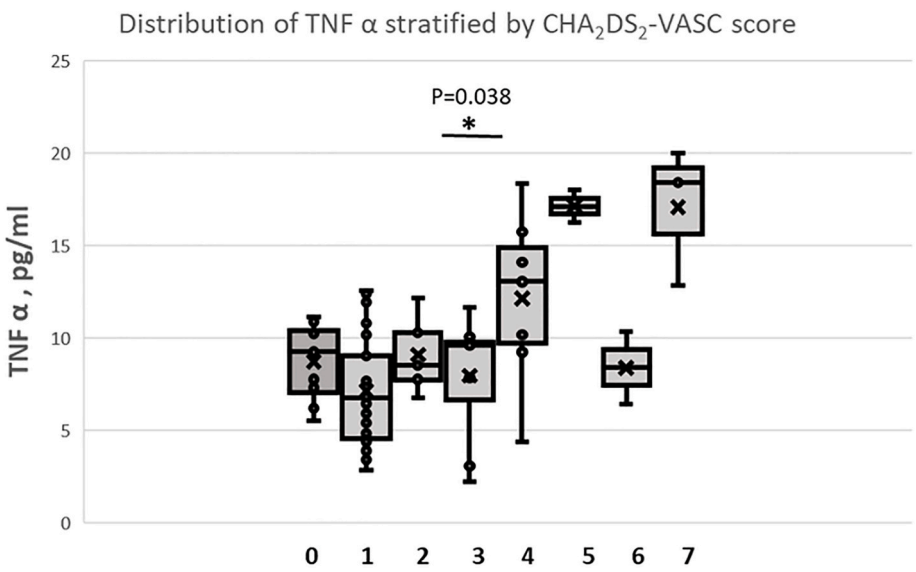

Fig. 1. Distribution of TNF α stratified by CHA_2DS_2-VASC score.

Table 3
The conditioning of TNF-alfa concentrations by different factors.

		analysis	P value	with	without
1	**Age**	Pearson correlation = 0.575	**P < 0.01**		
2	DM	T test, equal variances not assumed, 2-tailed	p = 0.104	9	53
3	**HTN**	T test, equal variances not assumed, 2-tailed	**P < 0.01**	25	37
4	**CHF**	T test, equal variances not assumed, 2-tailed	**P = 0.003**	7	55
5	Sex	T test, equal variances not assumed, 2-tailed	P = 0.117	36	26
6	**Dyslipidemia**	T test, equal variances not assumed, 2-tailed	**P = 0.02**	29	33
7	Smoking	T test, equal variances not assumed, 2-tailed	P = 0.117	9	53
8	**IHD**	T test, equal variances not assumed, 2-tailed	**P = 0.008**	13	49
9	Stroke	T test, equal variances not assumed, 2-tailed	P = 0.813	5	57
10	CRF	T test, equal variances not assumed, 2-tailed	P = 0.058	4	58

DM = diabetes mellitus, HTN = hypertension, CHF = congestive heart failure, IHD = ischemic heart disease, CRF = chronic renal failure.

including ischemic cardiomyopathy, atherosclerosis, which is likely attributed to its dual functions promoting both abnormal cytokine release (and structural remodeling) and perhaps cell death [28].

IL-8 is an important protein related to inflammation, where it plays a key role in the recruitment of neutrophils and other immune cells to the site of infection [8]. In addition to macrophages, *IL-8* is also released by epithelial cells, airway smooth muscle cells, and endothelial cells. IL-8 expression is induced by TNF-α through the JNK pathway, mediated by AP-1.

These three cytokines were found to be elevated in the group of patients with SR and high CHA_2DS_2-VASc score in the current study. TNF-α, IL-1β and IL-8 are all secreted from macrophages following induction by sP-selectin [29], a factor that was found to be increased in this group of patients in our previous work [21]. *P-selectin* is an adhesion molecule implicated in the initial interactions between leukocytes and platelets or endothelium that lead to inflammatory responses. It plays a crucial role in the recruitment of leukocytes to inflammatory and hemorrhagic sites, and it also affects cytokine production by leukocytes [29,30]. It was proved that *P*-selectin-mediated-cell-adhesion induces and potentiates monocytes to secrete TNF-α, but also IL-1β and IL-8. The secretion of IL-8 in a later phase is caused, at least in part, by stimulation with TNF-α secreted in the early phase [29]. These cytokines (along with others) are involved in cascade reactions of inflammation processes.

Inflammation plays an important role in the progression of many types of cardiovascular disease. Systemic inflammatory conditions enhance atherogenesis, leading to coronary artery disease, but can also promote diastolic and systolic heart failure [25], vascular dysfunction (by disruption of endothelial barrier, effects on NO formation, stimulation of vascular peroxidase production, apoptosis of endothelial cells), insulin resistance, and adverse cardiac remodeling after myocardial infarction [23]. In patients with AF, inflammatory markers, such as Interferon-γ, were found to predict AF ablation outcome, as well as stroke and mortality [8,9]. Inflammation is associated with electrophysiological remodeling through ion channel regulation and calcium homeostasis. It is also involved in structural remodeling, myocardial fibrosis, and prothrombotic states in AF [5,6,22]. There is accumulating evidence that inflammation plays an important role in the etiology of stroke and other cardiovascular diseases in addition to traditional risk factors such as arterial hypertension and hyperlipidemia, even in patients with no AF [31,32]. Moreover, previous studies had demonstrated that low grade inflammation is an independent risk factor for total mortality in an apparently healthy adult general population [33]. The presence of low-level inflammation may therefore explain the association of adverse cardiovascular events in patients with SR and elevated CHA_2DS_2-VASc score.

The significance of this finding is the potential anti-inflammatory treatment strategy that can target this underlying mechanism and hopefully improve prognosis in subgroups of patients with elevated CHA_2DS_2-VASc score. For example, TNF-α inhibition with etanercept, TNF-α gene ablation, or p38 inhibition – all prevented atrial structural remodeling and reduced AF inducibility in an exercise-induced AF model [34]; There is growing interest in selective blockade of soluble TNF-α or of TNFR1 while leaving TNFR2 signaling fully functional [23,25,27]; Inhibition of NLRP3 reduced the susceptibility to pacing-induced AF in mice [35]; and colchicine (an anti-inflammatory drug) and low-dose methotrexate (an anticancer drug with anti-inflammatory properties) also inhibit the NLRP3 inflammasome [36,37]; The clinical study CANTOS showed that suppression of IL-1β by the neutralizing antibody canakinumab, can significantly reduce cardiac events in patients at risk [38]; In addition, some clinical studies suggest

that inhibition of coagulation factors may also have anti-inflammatory effects [39]. Future large prospective clinical studies are needed to provide the effectiveness of this anti-inflammatory treatment approach.

5. Limitations

The main limitation of this study is the small study group; however, despite its small size, we showed significant differences in inflammatory markers between the two groups. Due to the small size of the study, we could not look into subgroups of patients (such as heart failure or SSS). In addition, the trend in high levels of IL-1β and IL-8 may have turned into a statistically significant value if the study group was bigger. Yet, the difference in the level of inflammatory markers between the groups directs to the underlying mechanism of the worse prognosis in patients with high CHA_2DS_2-VASc. The clinical implications of our findings remain to be determined, and larger studies are warranted.

6. Conclusions

Patients in SR with high versus low CHA_2DS_2-VASc scores have high plasma concentrations of TNF-α and a trend toward elevated IL-8 and IL-1β, which are systemic inflammation cytokines released from macrophages. The previously proven higher plasma levels of sP-selectin (that activate macrophages to produce these cytokines ex-vivo) are in line with current results. These findings of a pro-inflammatory state in patients with high CHA_2DS_2-VASc scores (even without AF), may explain the higher rate of adverse cardiovascular events associated with elevated CHA_2DS_2-VASc score even without history of AF.

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Author contribution

Avishag Laish-Farkash, Ziv Sevilya and Eli Lev performed the conception and design, the laboratory work and the analysis and interpretation of data, as well as drafting the manuscript. Lior Fortis collected the blood samples from the patients after signing an informed consent and revised the article critically. Olga Perelshtein Brezinov performed the statistical workup and revised the article critically for important intellectual content.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of abbreviations

ACS	acute coronary syndrome
AF	atrial fibrillation
CRP	*C*- reactive protein
ESC	European Society of Cardiology
LA	left atrium
NLR	neutrophils to lymphocytes ratio
PLF4	Platelet Factor 4
SR	sinus rhythm
TNF-α	Tumor Necrosis Factor-alfa
VEGF	Vascular Endothelial Grown Factor

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