

Galectin-3 as a Predictor for Thromboembolic risk in Patients with Non-valvular Atrial Fibrillation Assessed by CHA₂DS₂-VASc Scoring

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- thromboembolic complication

Abstract

Background: Circulating remodeling markers such as Galectin-3 (Gal-3) are significantly higher in atrial fibrillation (AF) patients. Nevertheless, there is still scarcity in the published literature regarding Gal-3 levels and risk of stroke in AF patients. The aim of this study was to evaluate the correlation of Gal-3 blood levels with the predicted thromboembolic risk factors according to the current standard scoring system (CHA₂DS₂-VASc) as well as some other echocardiographic and laboratory parameters, among patients with paroxysmal or persistent non-valvular AF. **Methods:** Three groups were enrolled in this cross-sectional study: low (70 patients), moderate (50 patients) and high risk (40 patients) categorized according to CHA₂DS₂-VASc score. Serum concentrations of Gal-3 were detected by an enzyme-linked immunosorbent assay (ELISA). In addition, activated partial thromboplastin time (aPTT) was estimated in all patients. Echocardiography was carried out to evaluate valvular functions and left ventricular ejection fraction and left atrial volume index (LAVI). **Results:** Patients with high risk had significantly higher Gal-3 level, lower aPTT duration and higher LAVI values than moderate and low risk. There were statistically significant positive correlations between CHA₂DS₂-VASc score and serum Gal-3 level and LAVI. Similarly, there was statistically significant negative correlation between CHA₂DS₂-VASc score and aPTT. Female patients at all age groups had significantly higher Gal-3 levels than male patients. **Conclusion:** Serum Gal-3 level might be a predictor for thromboembolic complication in AF patients with potential clinical benefit.

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INTRODUCTION

The most frequent persistent arrhythmia encountered in clinical practice is atrial fibrillation (AF), which is considered as a significant contributor to cardiovascular morbidity and mortality. When compared to those without AF, the probability of stroke is four to five times higher, and this risk is further reduced by the frequent usage of oral anticoagulation [1]. In 2010, the CHA₂DS₂-VASc score for thromboembolism risk assessment among non-valvular AF patients was developed [2]. In 2016, the European Society of Cardiology guidelines recommend using the CHA₂DS₂-VASc score to predict stroke in AF patients and to use oral anticoagulants to male patients who scored ≥ 2 and females who scored ≥ 3 .

Galectin-3 (Gal-3) is a multifunctional galectin found in the heart, kidney, blood vessels, and macrophages, where it plays a role in tissue fibrosis, immunology, and the inflammatory response [3]. Gal-3 had involved in the cardiovascular remodeling pathogenesis as well as inflammatory processes [4]. As a result, higher serum Gal-3 levels have been found in all forms of cardiovascular illness, and their predictive usefulness for various clinical outcomes in patients, including AF, has been widely investigated [5]. Gal-3 promoted fibrosis and cardiac remodeling and was reported as a well-established cause of arrhythmias [6]. Significantly higher levels of circulating remodeling markers such as Gal-3 and matrix metallo-proteinase-9 (MMP-9) have been reported in AF patients.

Nonetheless, there is a paucity of information in the literature about the relationship between the level of Gal-3 and the risk of stroke in AF patients. Therefore, the current cross-sectional

study was carried out to clarify Gal-3 levels in non-valvular AF patients either paroxysmal or persistent divided into 3 groups according to the risk of stroke that assessed by CHA₂DS₂-VASc score. Also, to check the correlation between Gal-3 blood level and CHA₂DS₂-VASc score, aPTT and LAVI among such patients.

Patients and methods

This study is a cross-sectional study (registered at clinical trial.gov ID: NCT03413072) that recruited patients diagnosed as Non-Valvular AF. It included 160 patients diagnosed as non-valvular AF either paroxysmal or persistent according to CHA₂DS₂-VASc score, divided as follows:

- Low risk group: which included 70 patients with score zero. The median age of the patients was 37.5 years (33-45), and all the patients were males.
- Moderate risk group: which included 50 patients with score one. The median age of the patients was 60 years (50-67), and most of the patients were male (66%).
- High risk group: which included 40 patients with score two or more. The median age of the patients was 65.5 years (55-75), and about three-quarters of patients were female (72.5%).

The Inclusion criteria were adults (age > 18 years), of both genders (male and female), patients who diagnosed as non-valvular AF either paroxysmal (self-terminating, usually within 48 hours; although, some AF paroxysms can extend up to one week), or persistent (AF that lasts more than a week, including cardioversion-terminated episodes). The Exclusion criteria to avoid confounder causes of elevated Gal-3 level as much as we can. Exclusion criteria included those with heart disease either valvular, congenital, or ischemic, systemic inflammatory diseases, thyroid

disease, cancer, and pregnancy.

EPI info 2000 statistical package was used for sample size calculation. The following parameters were applied: expected frequencies from previous studies using 95% confidence interval, 80% power of the study and worst acceptable results 5% and accordingly the minimum required sample was 140 patients.

Clinical assessment

All patients were being subjected to clinical evaluation comprising detailed medical history included CV risk factors which were known to be hypertension, diabetes and dyslipidemia, medical examination (general and cardiac) and ECG. Also, echocardiography examination was performed by a single physician who is not aware of any clinical or biochemical data to evaluate echo finding and interpret the abnormalities.

Biochemical analysis

Peripheral venous blood sample was drawn from all patients after written informed consent at the time of admission. Drawn samples were centrifuged at 4000 rpm for 20 min and serum was stored at -20°C till assayed. Gal-3 serum levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Sino Gene Clon Biotech Co., Ltd). Catalog No: SG-11634. Activated partial thromboplastin time (aPTT) levels were estimated by photometric method using laser beam to detect clot formation.

Statistical Analysis

Data analysis was carried out using SPSS software (Statistical Package for the Social Sciences, version 20, SPSS Inc, Chicago, IL, USA). Shapiro-Wilk Test was used to examine the normality of the continuous variables. Quantative data were expressed as mean \pm standard deviation (SD)/median and interquartile range (IQR) as appropriate. Qualitative data were expressed as frequency and percentages. Mann-Whitney test was used to compare the medians of non-

parametric quantitative variables. Chi-square test was used to analyze difference in frequency in categorical variables. Spearman rank correlation was applied to investigate the correlation between $\text{CHA}_2\text{DS}_2\text{-VASc}$ score and other variables. Significance was set at a p-value of < 0.05 .

Results

Demographic data of the studied groups

Table 1 showed that age, female gender, hypertension, diabetes, and vascular disease, $\text{CHA}_2\text{DS}_2\text{-VASc}$ score, AF duration, systolic and diastolic blood pressure were significantly different among the studied groups ($p < 0.001$).

Serum Gal-3 level and aPTT of the studied groups

As shown in fig. 1, high risk group had significantly higher Gal-3 level than moderate and low risk ($p < 0.001$). Also, aPTT duration was significantly shorter in patients with high risk than the other groups ($p < 0.001$).

Gal-3 levels according to sex

Sex difference was recorded between groups as shown in table 2. Female patients either below, equal, or above 65 years old had significantly higher Gal-3 levels than males ($p < 0.001$). On the other hand, no significant difference was detected in the serum levels of Gal-3 between female patients below, equal, or above 65 years old for pairwise comparisons (Table 3).

Predictive value of Gal-3

The data in table 4 showed the receiver operator characteristic (ROC) results of Gal-3 i.e., it was found to be a considerable predictor of stroke in AF female patients. At a cutoff value of ≥ 5.4 ng/ml, Gal-3 yielded a sensitivity of 95% and specificity of 86% for high-risk stroke prediction in AF patients. At a cutoff value of ≥ 4.5 ng/ml, Gal-3 yielded a sensitivity of 95% and specificity of 70% for moderate-risk stroke prediction in AF patients.

LAVI and EF of the studied groups

Moderate and high-risk group patients had higher LAVI values than low risk group (p <0.001). Moreover, those in the high-risk group had significant lower EF% than low risk group (p <0.05) (Fig. 2).

Correlation between CHA₂DS₂-VASc score and stroke determinants

Table 5 demonstrated significant positive correlations between CHA₂DS₂-VASc score, and serum Gal-3 level (r =0.93, p <0.001) and LAVI (r =0.87, p <0.001). Similarly, a significant negative

correlation was found between CHA₂DS₂-VASc score and aPTT (r =-0.84, p <0.001).

Correlation between serum Gal-3 level and stroke determinants

Fig. 3 revealed significant positive correlations between serum Gal-3 level and patients' age (r =0.69, p <0.001), systolic blood pressure (r =0.63, p <0.001) and LAVI (r =0.85, p <0.001). Likely, a significant negative correlation between serum Gal-3 level and aPTT was reported (r =- 0.79, p <0.001).

Table 1 Demographic data of the studied groups

Variables	Low risk (N =70)	Moderate risk (N =50)	High risk (N =40)	P-value
Age (y) Median (Range)	37.50 (32.75-45.00)	60.00 (50.00-66.75)**	65.50 (55.00-75.25)**	<0.001
Female gender (n, %)	0 (0%)	17 (34%)**	29 (72.5%)**,**	<0.001
HTN (n, %)	0 (0%)	23 (46%)**	31 (77.5%)**	<0.001
DM (n, %)	0 (0%)	20(40%)**	24(60%)**	<0.001
Vascular D and Stroke (n, %)	0 (0%)	0 (0%)	10(25%)**,**	<0.001
CHA ₂ DS ₂ -VASc score (n, %)	0----70 (100%)	1-----30 (60%) 2-----20 (40%)	3-----19 (47.5%) 4-----15 (37.5%) 5-----6 (15%)	<0.001
Type of AF: (n, %)				
-Paroxysmal	44 (62.9%)	33 (66%)	24 (60%)	NS
-Persistent	26 (37.1%)	17 (34%)	16 (40%)	
AF duration (months) Median (Range)	8 (6-12)	17 (12-20)**	29 (27-35)**,**	<0.001
SBP (mmHg) Median (range)	110 (110-120)	130 (120-140)**	140 (130-150)**	<0.001
DBP (mmHg) Median (range)	70 (70-80)	80 (80-86.25)**	90 (85-90)**	<0.001

N= number of patients per group; NS: non-significant; HTN: hypertension; DM: diabetes mellitus; Vascular D: vascular disease; AF: atrial fibrillation; CHA₂DS₂-VASc (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke, vascular disease, age 65-74 years, and female sex); SBP: systolic blood pressure; DBP: diastolic blood pressure; Data presented as median (Range), or number (%); P value represent significance between the three studied groups by using Kruskal Wallis Test followed by Mann-Whitney tests to determine significance between groups; Chi-square test was used to analyze categorical variables; *: significance at p value <0.05 versus low risk group and moderate risk groups; ** significance at p value <0.001 versus low risk group and moderate risk groups.

Table 2 Gal-3 levels according to sex

Variables	male	female	P-value
Serum Gal-3 (ng/ml) Median (range) < 65 years	3.452 (1.29 - 6.09)	5.42 (4.57 - 9.04)	<0.001*
Serum Gal-3 (ng/ml) Median (range) ≥ 65 years	4.49 (4.22 – 6.22)	6.155 (4.55 – 7.89)	<0.001*

*: significance at p value <0.05

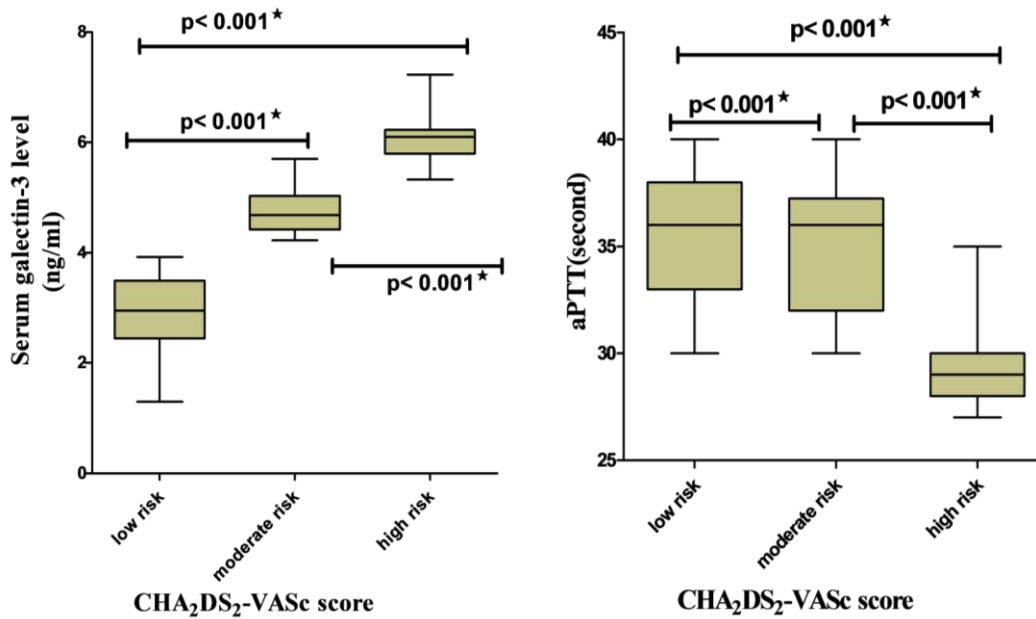


Fig. 1 Serum Gal-3 level and aPTT of the studied groups. CHA₂DS₂-VASc (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke, vascular disease, age 65-74 years, and female sex); aPTT: activated partial thromboplastic time; Data presented as median (Range), or number (%); P value represent significance between the three studied groups by using Kruskal Wallis Test followed by Mann-Whitney tests to determine significance between groups; *: significance at p value <0.05

Table 3 Gal-3 levels according to age in female patients

Variable	Female < 65 years	Female ≥ 65 years	P-value
Serum Gal-3 (ng/ml)	5.42 (4.57 - 9.04)	6.155 (4.55 – 7.89)	NS
Median (range)			

NS: non-significant

Table 4 The area under the curve (AUC) and the predictive value of Gal-3

Variable	AUC, 95%CI	P-value	Cut-off points	Sensitivity	Specificity
High risk versus low risk	0.89 (0.84-0.93)	0.000	≥5.4	95%	86%
moderate risk versus low risk	0.67 (0,6-0.75)	0.01	≥4.5	95%	70%

AUC: area under curve; CI: Confidence interval. A p-value < 0.05 is considered statistically significant

Table 5. Correlation analysis between CHA₂DS₂-VASc score and stroke risk factors

Variables	CHA ₂ DS ₂ -VASc score	
	Correlation Coefficient (r)	P- Value
Serum Gal-3 (ng/ml)	0.93	<0.001
LAVI (ml/m ²)	0.87	<0.001
aPTT (seconds)	-0.84	<0.001

BP: blood pressure; LAVI: left atrial volume index; aptt: activated partial thromboplastic time; CHA₂DS₂-VASc (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke, vascular disease, age 65-74 years, and female sex); Spearman correlation analysis was used to investigate the correlation between CHA₂DS₂-VASc score and other variables. A p-value < 0.05 is considered statistically significant; Data was presented as correlation coefficient (r) and 95% confidence interval.

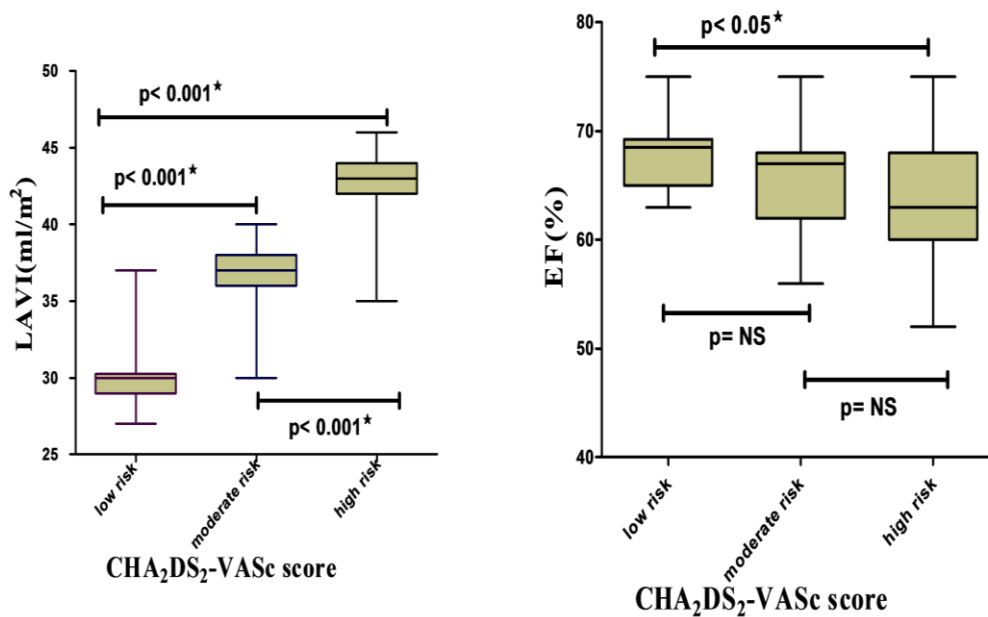


Fig. 2 LAVI and EF of the studied groups

LAVI: left atrial volume index; CHA₂DS₂-VASc (congestive heart failure, hypertension, age ≥75 years, diabetes mellitus, stroke, vascular disease, age 65-74 years, and female sex); Ns: non significance; EF: ejection fraction; Data presented as median (Range), or number (%); P value represent significance between the three studied groups by using Kruskal Wallis Test followed by Mann-Whitney tests to determine significance between groups; *: significance at p value <0.05

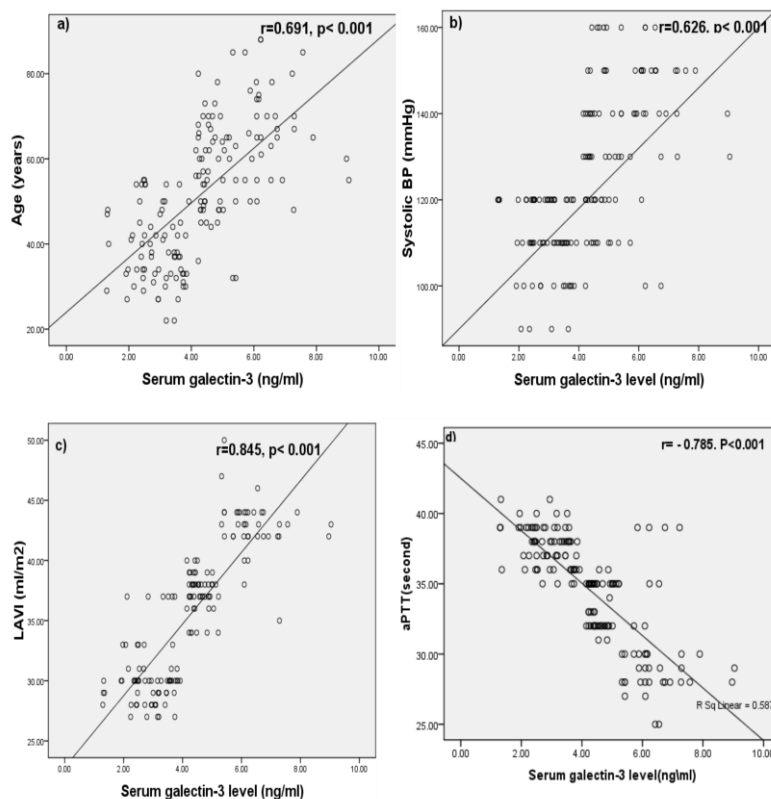


Fig. 3 Correlation coefficient between serum Gal-3 level and stroke risk factors

BP: blood pressure; LAVI: left atrial volume index; aPTT: activated partial thromboplastic time; Spearman correlation analysis was used to investigate the correlation between serum Gal-3 level and other variables. A p-value <0.05 is considered statistically significant; Data was presented as correlation coefficient (r) and 95% confidence interval

Discussion

AF is closely linked to several cardiovascular complications including mortality, stroke, and HF [7]. Increased Gal-3 levels were

linked to an increased chance of developing AF. Circulating remodeling markers such as Gal-3 and MMP-9 levels were significantly higher in AF patients [8].

In this study, patients with high risk for thromboembolism according to CHA₂DS₂-VASc score had significantly higher Gal-3 level and lower aPTT duration than moderate and low risk groups. This finding was in line with a previous study showed that elevation in serum Gal-3 was linked to an increased risk of major disability or death within two weeks after an ischemic stroke [9]. Another study showed that shortened aPTT was associated with acute ischemic strokes [10]. Potential mechanisms which might explain the increased Gal-3 levels in high-risk patients are possible multifactorial, as observed in previous reports. First, galectins, particularly Gal-3 played a key part in the chain of events that led to the inflammatory response and fibrogenesis induced by oxidative stress [11]. Second, a study by Akoum et al. [12] found that the degree of LA fibrosis found in paroxysmal AF patients with intact LV function was linked with serum Gal3 levels. Gal-3 was extensively expressed in fibrotic tissues and upregulated in chronic inflammatory and fibrotic circumstances in humans, hence it increased in cardiac fibrosis [13]. Third, Gal 1, 3, and 8, either in a soluble or immobilized form, have been found to be capable of eliciting a wide spectrum of platelet response, which explains why serum Gal-3 was enhanced and aPTT was lowered [14], including adhesion, aggregation, release of granule content and P-selectin expression.

Additionally, this study found that Gal-3 levels were positively correlated with CHA₂DS₂-VASc score in patients with AF. So, high Gal-3 level put AF patients at a higher risk of embolic ischemic strokes or indicated this higher risk. Gal-3 has been linked to several modifiable stroke risk factors, including age, hypertension, atherosclerosis, and diabetes. Many of these risk

factors also had a part in the pathogenesis of atherosclerosis, which was heavily influenced by inflammatory states that played a role in its development [15].

Our result demonstrated that there were statistically significant differences between male and female patients. Female patients either below, equal, or above 65 years old had significantly higher Gal-3 levels than male patients. This finding was in accordance with previous studies demonstrated that Gal-3 level was higher in women than men [16-17]. Another study found that serum Gal-3 levels differed by gender, with women having greater levels than men and the link between Gal-3 and cardiovascular risk factors being stronger in women than in men [18]. Furthermore, De Boer et al. [19] reported that Gal-3 levels were higher among elderly and women, sex difference was explained by hormonal regulation, while age effect could be caused by AF-related co-morbidities and other factors [20]. This discrepancy was due to several sex variations in macrophage function, including activation levels, phagocytic capacity, and cytokine production. Numerous cytokines released by macrophages were modulated by estradiol, progesterone [21]. Also, immune responses were stronger in adult females than in males, and these responses were partially influenced by sex hormones [22]. However, in this work there was insignificant difference of the level of Gal.3 in female patients of different age groups which is not matching with CHA₂DS₂-VASc score assumption that females at age above 65 are more prone than males for thromboembolism and so a female above 65 years age is given additional point in the score unlike males of the same age and unlike females of younger age. Maybe we

need to have larger numbers of patients in different age groups to confirm this.

Gal-3 could be a major predictor of stroke risk in AF patients, according to the current findings. This finding was in line with a prior study concluded that the Gal-3 serum level might be used to diagnose stroke. The specificity for acute ischemic stroke prediction was 89.3% and the sensitivity was 75.8% at a cut-off value of 53.5pg/ml [23]. In our study, at a cutoff value of ≥ 5.4 ng/ml, Gal-3 was yielded a sensitivity of 95% and specificity of 86% for prediction of high risk of stroke in AF patients. These values may represent a potential discriminating test that may help decision making regarding anticoagulation in moderate risk non-valvular AF patients which are still left optional for anticoagulation or not in the latest updated guidelines of AF management.

The current study revealed that those with high-risk had higher LAVI values than low/moderate risk groups. Several studies showed an association between left atrial enlargement (LAE) and embolic events [24, 25]. Furthermore, as LAVI increased, patients were more likely to develop AF or higher cardiac thromboembolic risk irrespective of AF [26]. In this research, Gal-3 serum level had a strong positive connection with LAVI. A similar relationship existed between serum Gal-3 and LAVI as reported by Gurses et al. [27] who found that serum Gal-3 and LAVI levels were considerably higher in patients with AF, suggesting that Gal-3 levels in AF patients may be linked to AF-induced atrial remodeling [28].

In the present study, there was a positive correlation between serum Gal-3 level and age. This was in agreement with other report that showed Gal3 levels may rise early in the course of

inflammation, before those processes lead to the formation of clinically visible stroke risk factors as HTN and diabetes [29]. With advancing age, these clinically evident risk factors become more prominent. [30]. In our study, there was a positive correlation between serum Gal-3 level and systolic blood pressure. Gal-3 likely represented as a biomarker of fibrosis and correlation had been observed between elevated serum Gal-3 and negative outcome of HTN such as adverse cardiac remodeling [31]. Gal-3 could be a valuable biomarker in cardiovascular disorders under the recommended criteria [32].

Moreover, negative correlation between serum Gal-3 levels and aPTT was reported. Higher Gal-3 levels were linked to increased thrombogenicity in AF patients, as seen by decreased LA appendage flow velocity, appendage remodeling, and thrombus formation on transesophageal echocardiography as reported in several studies [33, 34]. These results proposed that baseline Gal-3 levels could be one of the risk factors of stroke in patients with AF [35].

Conclusion: Serum level of Gal-3 were found to be higher in patients with paroxysmal or persistent AF with higher risk for stroke. Also, elevated Gal-3 levels were found to be positively correlated with CHA₂DS₂-VASc score in AF patients. According to the finding of this study, serum Gal-3 level could be used as a predictor for thromboembolic complication in AF patients with potential clinical benefit.

Recommendation

To evaluate thromboembolism risk in AF patients, serum Gal-3 levels could be used in conjunction with the existing stroke risk stratification measures and help clinicians in the decision of treatment (for example, if a cutoff

value of ≥ 5.4 ng/ml in clinically borderline or moderate CHA₂DS₂-VASc risk (score1)). More research is needed to confirm the current findings and the accurate serum levels to be used as a reference.

limitations

This study has encountered some limitations; a bigger study population and a longer period of clinical follow-up are needed to demonstrate the predictive relevance of serum Gal-3 levels in determining thromboembolic risk in patients with AF.

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