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Original Article

Mean corpuscular volume and red cell distribution width as predictors of left atrial stasis in patients with non-valvular atrial fibrillation

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Abstract: Background: The role of erythrocyte indexes for the prediction of left atrial stasis, assessed by transesophageal echocardiography in patients with non-valvular atrial fibrillation, has not been previously clarified. Methods: Single center cross-sectional study comprising 247 consecutive patients admitted to the emergency department due to symptomatic atrial fibrillation and undergoing transesophageal echocardiogram evaluation for exclusion of left atrial appendage thrombus (LAAT) before cardioversion. All patients had a complete blood count performed up to 12 hours prior to the transesophageal echocardiogram. Markers of left atrial stasis were sought: LAAT, dense spontaneous echocardiographic contrast (DSEC) and low flow velocities (LFV) in the left atrial appendage. Erythrocyte indexes' accuracy for detecting transesophageal echocardiogram changes was evaluated through receiver operating curve analysis. Binary logistic multivariate analysis, using solely erythrocyte indexes and in combination with other variables (i.e. $CHADS_2$, CHA_2DS_2VASc classifications and left ventricle ejection fraction), was used for transesophageal echocardiogram endpoints prediction. Results: LAAT was found in 8.5%, DSEC in 26.1% and LFV in 12.1%. Mean corpuscular volume and red cell distribution width were independent predictors of LAAT and DSEC. Despite adding incremental predictive value to each other, when clinical risk factors from $CHADS_2$ and CHA_2DS_2VASc classifications and left ventricle ejection fraction were added to the models, only mean corpuscular volume remained an independent predictor of LAAT and DSEC. Conclusions: These findings suggest that mean corpuscular volume and red cell distribution width may be linked to left atrial stasis markers.

Keywords: Atrial fibrillation, stroke, left atrial appendage thrombus, mean corpuscular volume, red cell distribution width, spontaneous echocardiographic contrast

Background

Thromboembolism is among the most feared complications of non-valvular atrial fibrillation (AF) [1]. However, the mechanisms and pathways underlying thrombus formation and the presence of prothrombotic milieu in the left atrium have not yet been fully clarified.

The presence of left atrial thrombus is associated with thromboembolism in patients submitted to cardioversion [2] or catheter ablation of AF [3], and that is why these procedures are contraindicated in the case of intracavitary thrombus [4]. Although, transesophageal echocardiogram is the gold-standard for the exclusion of thrombus, this procedure is not devoid of risks, is invasive in nature and may be not tolerable for some patients [5]. Furthermore, thrombi are found in only a minority of patients with non-valvular AF if under anticoagulation treatment (1.6%) [3] and 12% in patients without anticoagulation [6].

Patients with AF and left atrial thrombus [7], dense spontaneous echocardiographic contrast (DSEC) [8] and low flow velocities (LFV) in the left atrial appendage [9] are known to have a higher risk of thromboembolism or adverse prognosis.

A better understanding of the thrombogenic mechanisms and pathways in non-valvular AF could have two main benefits: first, providing

help in foreseeing which patients have a very low risk of having a left atrial appendage thrombus (LAAT) and, therefore, could be spared transesophageal echocardiogram assessment before cardioversion or catheter ablation of AF; second, a more accurate detection of subjects at a high risk of thromboembolism that would derive benefit from anticoagulation therapies.

The assessment of red blood cell indexes is a low cost and very commonly performed laboratory technique that has been previously shown to provide information concerning prothrombotic status or adverse outcome in other spectra of cardiovascular disease [10, 11]. However, the role of these erythrocyte measures as predictors of left atrial stasis in patients with AF remains to be assessed.

Methods

Study population

A single center cross-sectional study was conducted including patients undergoing echocardiographic assessment (comprising transesophageal and transthoracic echocardiogram) due to symptomatic AF, which lead to a hospital admission in a 25-month time period. Among a total of 353 subjects, 302 performed a complete blood count 12 hours prior to transesophageal echocardiogram and were selected for the purpose of our investigation. Among these, 31 subjects with valvular AF (defined as presence of a previous valve repair, a prosthetic valve, rheumatic heart disease, and moderate or severe valve stenosis and/or regurgitation) and 24 with concomitant infection were excluded from analysis. Our study population included the remaining 247 patients. All subjects provided their informed consent to undergo the necessary investigations and to allow the usage of their data for research purposes, preserving their anonymity.

Baseline overall group characterization with demographic, anthropometric, clinical, laboratory and echocardiographic data, alongside with information on medication was obtained for all patients. Data was retrospectively retrieved from clinical records (outpatient clinic evaluations, emergency department and hospital ward admissions). This study was conducted with the approval of our institution's Cardiology Department Supervisor and Ethics Committee.

Echocardiographic data

Transesophageal and transthoracic echocardiogram were performed using a GE Vivid 7 echocardiograph alongside with M4S (1.5-4.0 MHz) and 6T phased array multiplane transesophageal (2.9-7.0 MHz) probes. All examinations were performed by two cardiologists with accreditation in transesophageal and transthoracic echocardiography by the European Society of Cardiology. Transesophageal echocardiography was performed without anesthesia or sedation in more than 98% of patients. Images were later reanalysed using the GE Health Care EchoPac Dimension software, PC version 108.1.4. Left atrium volume was measured using the single-plane area length method. On transesophageal echocardiogram, the left atrium and left atrial appendage were imaged in different tomographic planes to detect the presence of LAAT and DSEC. Spontaneous echo contrast was classified according to the classification (1 to 4+) proposed by *Fatkin et al.* [12]. Grade 3+ or 4+ was defined as DSEC. Left atrial appendage flow velocities were assessed with a pulsed Doppler sample placed 1 cm from the entry of the left atrial appendage into the body of the left atrium. Emptying and filling velocities were estimated from an average of five well-defined emptying and filling waves. Patients with emptying and filling velocity ≤20 cm/s were classified as having LFV.

The cardiologists performing the transesophageal and transthoracic echocardiogram were blinded for the laboratory results and clinical information of the patients, other than the fact that they were in AF and there was need for excluding transesophageal echocardiogram changes that could contraindicate cardioversion.

Laboratory data

After venous blood was drawn, it was immediately transferred into our hospital's laboratory using an automatic internal tube transference system directly connected from different parts of the hospital into the laboratory. On average laboratory measures were performed within 15 minutes of venous blood sampling.

Erythrocyte index assessment was performed using the Cell-Dyn Sapphire Hematology

Analyzer from Abbot Diagnostics. Reference range values according to local calibration from our hospital's laboratory were: red blood cell counting -4.5 to 5.5 x 10^6/uL; hemoglobin -13.0 to 17.5 g/dL; hematocrit -40 to 50%; mean corpuscular volume 80 to 100 fL; mean corpuscular hemoglobin 27 to 32 pg; mean corpuscular hemoglobin concentration 32 to 35 g/dL; red cell distribution width 11.6 to 14%.

C-reactive protein was measured using the CRP VITROS Chemistry Products assay. The lower limit of sensitivity was <0.5 mg/L and the reference interval for normal values was <1.0 mg/L. A rise in C-reactive protein was defined as the observed value over the lower limit of sensitivity (eg. 0.4 mg/L was the observed rise in C-reactive protein in a patient with a value of 0.9 mg/L, assuming the 0.5 mg/L lower limit of sensitivity).

Reference range for activated partial thromboplastin time (aPTT) was 25 to 30 seconds.

Erythrocyte indexes and prediction of transesophageal endpoints

PASW Statistics version 18.0 was used for descriptive and inferential statistical analysis. Comparisons were performed according to the presence/absence of markers of left atrial stasis. Chi-square was used for nominal variables and Student's t-test was used for comparison of continuous variables, where appropriate; the Levene test was used in order to check the homogeneity of variance; equivalent non-parametric tests were used when Kolmogorov-Smirnov was in favor of absence of normal distribution. Results with P<0.05 were regarded as significant.

The discriminative capability of the red blood cell indexes was tested using receiver operating characteristic (ROC) curves and the resulting area under the curve (AUC) summary statistic (c statistic) for the prediction of LAAT. In parameters with an AUC of at least 0.650 we were able to define the optimal cutoff point (Youden index) using the coordinates from the ROC curves. Univariate analysis was then performed using the chi-square test.

Binary logistic multivariate analysis using erythrocyte indexes alone and combined with variables from the $CHADS_2$ and CHA_2DS_2VASc classifications was used for obtaining models for the prediction of transesophageal echocardiogram endpoints. Erythrocyte indexes that were predictors of changes on transesophageal echocardiogram on univariate analysis were used either alone or with the isolated clinical parameters from $CHADS_2$ and CHA_2DS_2VASc and left ventricle ejection fraction for obtaining logistic regression models (using the backward stepwise method trough likelihood ratio; probability for stepwise=0.1) that could predict transesophageal echocardiogram endpoints: LAAT, DSEC and LFV. Continuous variables such as left ventricle ejection fraction were converted into ordinal variables and then used in the logistic regression analysis. Established cutoff points were: ≥55% vs. <55% for left ventricle ejection fraction. The Hosmer-Lemeshow summary statistic was used to assess the goodness-of-fit of the models.

Results

The patients' baseline clinical, echocardiographic and laboratory characterization is shown on **Table 1**. In 41.7% (n=103) of subjects, there was no previously known history of AF. The following markers of left atrial stasis were found on transesophageal echocardiogram: LAAT in 8.5%, DSEC in 27.1% and LFV in 12.3%.

Table 2 illustrates the capability of red blood cell indexes to discriminate LAAT, DSEC and LFV, with the respective area under the curve (AUC) values. Only mean corpuscular volume and red cell distribution width had a moderate accuracy for detecting LAAT (AUC=0.668 and AUC=0.657, respectively). Concerning DSEC and LFV, the discriminative capability was low and non-significant. Youden index values for the variables that performed better on **Table 2** were the following: mean corpuscular volume <91.5 fL (62% sensitivity and 64% specificity for LAAT) and red cell distribution width (57% sensitivity and 70% specificity for LAAT).

Comparisons of patients with mean corpuscular volume < vs. ≥91.5 fL and red cell distribution width < vs. ≥15.0% are shown on **Table 1**. A higher prevalence of females (p=0.024) and diabetes mellitus (p=0.037) was observed in patients with mean corpuscular volume <91.5 fL. Furthermore, a lower prevalence of subjects medicated with statins (p=0.028), alongside

Table 1. Population baseline characteristics and sub-analysis according to mean corpuscular volume and red blood cell distribution width

	Overall (n=247)	MCV <91.5 fL (n=90)	MCV ≥91.5 fL (n=157)	P	RDW <15% (n=166)	RDW ≥15% (n=81)	P
			Demographics				
Age (years)	68.0±10.5	67.0±10.0	68.6±10.7	0.213	67.4±11.3	69.3±8.4	0.383
Female gender	36.4% (90)	45.6% (41)	31.2% (49)	0.024	64.5% (107)	61.7% (50)	0.676
Body Mass Index (Kg/m^2)	29.0±5.0	29.8±5.8	28.6±4.5	0.275	28.8±4.6	29.5±5.9	0.704
			Clinical Data				
Congestive heart failure	49.8% (124)	48.9% (44)	51.0% (80)	0.755	45.2% (75)	60.5% (49)	0.024
Hypertension	83.8% (207)	82.2% (74)	84.7% (133)	0.609	82.5% (137)	86.4% (70)	0.436
Diabetes mellitus	22.7% (56)	30.0% (27)	18.5% (29)	0.037	20.5% (34)	27.2% (22)	0.239
Stroke or TIA	15.4% (38)	14.4% (13)	15.9% (25)	0.757	10.2% (17)	25.9% (21)	0.001
Vascular disease[a]	52.2% (129)	54.4% (49)	51.0% (80)	0.597	52.4% (87)	51.9% (42)	0.934
AF episode duration >1 week	67.6% (167)	65.6% (59)	68.8% (108)	0.601	66.9% (111)	69.1% (56)	0.721
$CHADS_2$ score	2.2±1.3	2.1±1.3	2.2±1.3	0.761	2.0±1.2	2.5±1.4	0.003
CHA_2DS_2-VASc score	3.7±1.8	3.8±1.8	3.7±1.8	0.804	3.5±1.7	4.2±1.9	0.019
			Medication				
Oral anticoagulants	23.1% (57)	25.6% (23)	21.7% (34)	0.484	21.7% (36)	25.9% (21)	0.458
Enoxaparin	44.1% (109)	40.0% (36)	46.5% (73)	0.322	42.8% (71)	46.9% (38)	0.538
Antiplatelet agents	53.8% (133)	48.9% (44)	56.7% (89)	0.237	55.4% (92)	50.6% (41)	0.477
ACE-i or ARB-II	71.3% (176)	67.8% (61)	73.2% (115)	0.361	72.3% (120)	69.1% (56)	0.607
Statin	41.3% (102)	32.2% (29)	46.5% (73)	0.028	41.6% (69)	40.7% (33)	0.902
			Laboratory Assessment				
RBC (10^6/uL)	4.51±0.65	4.48±0.66	4.40±0.62	0.001	4.52±0.61	4.48±0.72	0.658
Haemoglobin (g/dL)	13.8±1.8	13.4±1.8	14.0±1.8	0.029	14.0±1.7	13.2±1.9	0.001
Hematocrit (%)	41.6±5.7	40.7±5.6	42.2±5.6	0.040	42.3±5.2	40.2±6.2	0.005
MCV (fL)	92.7±5.7				93.8±4.4	90.3±7.0	0.001
MCV <91.5 fL	36.4% (90)				28.9% (48)	51.9% (42)	<0.001
MCH (pg)	30.7±2.2	28.8±2.1	31.7±1.4	<0.001	31.1±1.7	29.7±2.7	<0.001
MCHC (g/dL)	33.0±1.3	33.0±1.2	33.1±1.3	0.697	33.1±1.3	32.9±1.2	0.068
RDW (fL)	14.1±1.4	14.6±1.4	13.7±1.3	<0.001			
RDW ≥15	32.8% (81)	46.7% (42)	24.8% (39)	0.001			
INR	1.2±0.5	1.2±0.6	1.2±0.5	0.199	1.2±0.4	1.3±0.7	0.054
INR ≥2.0	7.7% (19)	5.6% (5)	8.9% (14)	0.340	5.4% (9)	12.3% (10)	0.055
aPTT time (s)	33.1±5.5	33.2±5.4	33.0±5.6	0.899	33.2±5.8	32.8±5.2	0.876

Rise in CRP (mg/L)	0.9±2.5	1.0±2.5	0.9±2.5	0.698	0.8±2.0	1.1±3.3	0.158
GFR assessed with MDRD (ml/min)	71.4±28.2	73.6±29.7	70.2±27.2	0.362	75.0±27.2	63.9±28.9	0.004
Transthoracic echocardiogram data							
Indexed left atrial volume (ml/m^2)	62.0±25.5	65.0±27.7	60.4±24.4	0.302	57.5±19.9	73.1±33.8	0.007
Indexed LV diastolic diameter (mm/m^2)	29.7±5.6	30.8±4.5	29.2±5.9	0.011	29.2±5.6	30.9±5.3	0.026
LV ejection fraction <55%	27.9% (69)	21.1% (19)	31.8% (50)	0.070	22.3% (37)	39.5% (32)	0.005

MCV – mean corpuscular volume; MPV – mean platelet volume; TIA – transient ischemic attack; AF – atrial fibrillation; ACE-i – angiotensin converting enzyme inhibitor; ARB-II – angiotensin II receptor blocker; RBC – red blood cells; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; INR – international normalized ratio; CRP – C reactive protein; GFR – glomerular filtration rate; MDRD – modified diet in renal disease formula; LV – left ventricle; aPTT – activated partial thromboplastin time. [a]vascular disease is defined as having at least one of the following: myocardial infarction, peripheral artery disease and complex aortic plaque.

Table 2. Discrimination of left atrial stasis by erythrocyte indexes

Variable	LAAT		DSEC		LFV	
	AUC $CI_{95\%}$	P	AUC $CI_{95\%}$	P	AUC $CI_{95\%}$	P
RBC (10^6/uL)	0.507 0.381-0.634	0.910	0.505 0.429-0.582	0.902	0.533 0.418-0.649	0.597
Hgb (g/dL)	0.430 0.304-0.555	0.285	0.503 0.424-0.581	0.946	0.536 0.425-0.646	0.570
MCV (fL)[a]	0.668 0.558-0.777	0.011	0.528 0.442-0.613	0.505	0.432 0.308-0.556	0.278
Hematocrit (%)	0.434 0.309-0.560	0.320	0.515 0.436-0.594	0.715	0.563 0.450-0.675	0.322
MCH (pg)[a]	0.609 0.484-0.735	0.098	0.507 0.425-0.590	0.865	0.490 0.365-0.615	0.874
MCHC (g/dL)	0.489 0.365-0.614	0.873	0.468 0.386-0.550	0.439	0.435 0.315-0.555	0.302
RDW (%)	0.657 0.544-0.771	0.017	0.570 0.489-0.652	0.089	0.514 0.395-0.634	0.820

LAAT – left atrial appendage thrombi; DSEC – dense spontaneous echo contrast; LFV – low flow velocities in the left atrial appendage; AUC – area under the curve; CI – confidence interval; RBC – red blood cells; Hgb – hemoglobin; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; [a]A lower value was defined as a more positive test for MCV and MCH.

with lower hemoglobin (p=0.029) values was found. Red cell distribution width was higher (p<0.001) in patients with mean corpuscular volume <91.5 fL. Despite the lack of differences concerning depressed left ventricle ejection fraction, left ventricles of patients with mean corpuscular volume <91.5 fL were slightly more dilated.

Concerning patients with red cell distribution width ≥15.0%, they had more frequently previous episodes of congestive heart failure and stroke or transient ischemic attack (TIA) (p=0.024 and p=0.001, respectively), which translated into higher $CHADS_2$ and CHA_2DS_2-VASc scores (p=0.003 and p=0.019, respectively). Lower mean corpuscular volume and estimated glomerular filtration rate (p<0.001 and p=0.004, respectively), alongside with a compromised left ventricle ejection fraction (p=0.005) was also observed among patients with red cell distribution width ≥15.0%.

On univariate analysis (**Table 3**) both mean corpuscular volume and red cell distribution width were predictors of LAAT and DSEC (all p<0.05). However, these variables were not predictors of the presence LFV in the left atrial appendage.

When combined (**Table 4**), mean corpuscular volume and red cell distribution width were both included in backward likelihood ratio logistic regression models for the prediction of LAAT and DSEC. However, when clinical risk factors from $CHADS_2$ and CHA_2DS_2-VASc and left ventricle ejection fraction were added to the models, only mean corpuscular volume remained an independent predictor of LAAT and DSEC.

Discussion

We have identified two parameters (mean corpuscular volume and red cell distribution width) that were linked to an increased prevalence of LAAT and DSEC, which are transesophageal echocardiogram markers that have been previously associated with an increased risk of stroke and adverse outcome in patients with AF [7, 8]. However, despite adding incremental predictive value to each other, when combined with clinical risk factors present within $CHADS_2$ and CHA_2DS_2-VASc classifications and left ventricle ejection fraction, only mean corpuscular volume remained an independent predictor, which may be due to the small size sample.

Thromboembolic risk stratification of patients with AF is currently based on clinical schemes [14, 15]. The $CHADS_2$ [15, 16] and CHA_2DS_2-VASc [17, 18] scores have been shown to possess a moderate ability for the prediction of thromboembolic transesophageal echocardiogram risk factors. Their predictive capability has shown potential for improvement in this setting with the addition of biomarkers like C-reactive protein [19], troponin I [20], von Willebrand factor [21] or D-dimers [22].

To the best of our knowledge, red blood cell indexes had not yet been shown to improve the prediction of LAAT or DSEC provided by clinical risk factors.

Decreased mean corpuscular volume may be a marker of different situations: iron deficiency (caused by inadequate dietary intake or blood loss), chronic disease (e.g. inflammation, chronic kidney disease, hematologic or lymphoproliferative disorders) or thalassemia. Patients with ongoing infection were excluded from our study and univariate analysis showed that no difference was observed concerning C-reactive protein and estimated glomerular filtration rate, according to the mean corpuscular volume cut-off level used. Furthermore, no patients with low mean corpuscular volume were identified as having thalassemia.

A high red cell distribution width (signaling the presence of red blood cells of variable sizes) was more frequent in the subset of patients of low mean corpuscular volume and is known to be compatible with iron deficiency in this situation. A high red cell distribution width has previously been associated with cardiovascular events and adverse outcomes [23] and in some investigations these findings were seen independently of the presence of anaemia [24].

Despite our patients were not anemic, those with a mean corpuscular volume <91.5 fL were below the average of our population (see **Table 1**). Therefore, and despite not having assessed their body's iron stores, we wonder if this microcytic trend may lead to a more prothrombotic behavior.

Takahashi et al. have previously described an increase in mean corpuscular volume in patients with AF [25]. If this holds true for our population, this increase in mean corpuscular

Table 3. Presence of left atrial stasis on transesophageal echocardiogram and sub-analysis according to mean corpuscular volume and red cell distribution width

	Atrial stasis transesophageal echocardiogram markers								
	Overall (n=247)	MCV <91.5 fL	MCV ≥91.5 fL	OR $CI_{95\%}$ P	P	RDW <15%	RDW ≥15%	OR $CI_{95\%}$ P	P
LAAT	8.5% (21)	14.4% (13)	5.1% (8)	3.14 1.25-7.91	0.011	5.4% (9)	14.8% (12)	3.03 1.22-7.53	0.013
DSEC	27.1% (67)	35.6% (32)	22.3% (35)	1.92 1.09-3.41	0.024	22.3% (37)	37.0% (26)	2.05 1.15-3.67	0.014
LFV	12.3% (24)[a]	9.9% (7/71)	13.7% (17/124)	0.69 0.27-1.75	0.431	11.9% (16/135)	13.3% (8/60)	1.14 0.46-2.84	0.771

MPV – mean platelet volume; RDW – red cell distribution width; LAAT – left atrial appendage thrombi; DSEC – dense spontaneous echo contrast; LFV – low flow velocities in the left atrial appendage. [a]only 195 subjects had assessment of left atrial appendage flow velocities due to technical or procedural reasons (see discussion).

Table 4. Binary logistic regression multivariate analysis models for predicting the presence of markers of left atrial stasis

			Erythrocyte indexes alone			
Endpoint	Variable	Wald	B	Exp β $CI_{95\%}$	P	Hosmer and Lemeshow test
LAAT	MCV <91.5 fL	3.864	0.952	2.591 1.003-6.695	0.049	χ^2=2.071 df=2 p=0.355
	RDW ≥15%	3.568	0.903	2.467 0.967-6.296	0.059	
	Constant	15.098	1.369	3.933	<0.001	
DSEC	MCV <91.5 fL	3.078	0.528	1.695 0.940-3.057	0.079	χ^2=3.306 df=2 p=0.191
	RDW ≥15%	3.930	0.604	1.829 1.007-3.323	0.047	
	Constant	1.136	0.286	1.331	0.287	
	Erythrocyte indexes plus clinical risk factors from $CHADS_2$ and CHA_2DS_2-VASc and left ventricle ejection fraction					
Endpoint	Variable	Wald	B	Exp β $CI_{95\%}$	P	Hosmer and Lemeshow test
LAAT	Stroke/TIA	8.275	1.480	4.394 1.603-12.046	0.004	χ^2=1.065 df=3 p=0.786
	MCV <91.5 fL	7.321	1.339	3.816 1.446-10.068	0.007	
	LVEF <55%	3.443	0.921	2.521 0.949-6.648	0.064	
	Constant	0.007	-0.051	0.950	0.932	

DSEC	MCV <91.5 fL	9.872	1.050	2.858 1.484-5.501	0.002	χ^2=6.128 df=6 p=0.409
	Age ≥65	3.307	0.642	1.901 0.951-3.799	0.069	
	LVEF <55%	28.341	1.811	6.118 3.140-11.917	<0.001	
	Stroke	3.188	0.740	2.097 0.930-4.726	0.074	
	Constant	9.732	-1.640	0.194	0.002	
LFV	Stroke/TIA	4.051	1.092	2.980 1.029-8.631	0.044	χ^2=0.629 df=1 p=0.428
	LVEF <55%	3.193	0.841	2.320 0.922-5.838	0.074	
	Constant	0.608	0.469	1.598	0.436	

LAAT – left atrial appendage thrombi; DSEC – dense spontaneous echo contrast; LFV – low flow velocities in the left atrial appendage; LA ABN – left atrial abnormality; CI – confidence interval; TIA – transient ischemic attack; MCV – mean corpuscular volume; LVEF – left ventricle ejection fraction; MPV – mean platelet volume.

volume caused by AF may contribute to partly masking some of the microcytic trend in subjects in the mean corpuscular volume <91.5 fL group.

Patients with heart failure frequently have iron deficiency (most of them have no relevant decrease on serum hemoglobin) and improve their outcomes after intravenous iron replacement [26]. Iron deficiency can have deleterious effects, independently of anemia. A possible explanation is the possibility of altered cardiac function illustrated by mitochondrial swelling, abnormal sarcomere function [27] and impaired mitochondrial electron transport [28]. As far as endothelial dysfunction and procoagulant states are concerned, an association with iron deficiency remains to be proved.

Maguire and colleagues suggested that a relation between iron-deficiency anemia and stroke in young children might be caused the hyperviscosity that may occur in this setting [29]. Other reports have focused the same association in adult and elderly cohorts [30]. As far as markers of left atrial stasis are concerned, and according to the Virchow triad, this hyperviscosity hypothesis fits perfectly as an explanation for the observed association.

Another example of an association between iron deficiency, hyperviscosity and thrombosis may occur in patients with *Eisenmenger* syndrome, where repeated phlebotomies may lead to the formation of microcytic erythrocytes, which are thought to have higher viscosity than their normocytic counterparts and thus predispose to embolic events [31].

In our population, patients with a lower mean corpuscular volume had a more dilated left ventricle. This has been previously described to be an effect of iron deficiency [27]. Moreover, women displayed more frequently lower levels of mean corpuscular volume. We hypothesize that besides hormonal factors, the association of female gender with increased thromboembolic risk in AF may be also explained by iron deficiency.

Based on different hypothesis concerning the composition of spontaneous echocardiographic contrast (aggregates of red blood cells interacting with plasma proteins [32, 33]) we are lead into thinking that the observed association of mean corpuscular volume and red cell distribution width with LAAT and DSEC is not caused by pure chance.

A high bleeding and thrombotic risk frequently coexists in patients with AF, as classifications for bleeding risk (e.g. HAS-BLED [34]) share several common risk factors present within $CHADS_2$ and CHA_2DS_2-VASc scores [13, 14]. Furthermore, some of the recently assessed biomarkers in AF (e.g. troponin I [35], D-dimer [36]) signal both the thromboembolism and bleeding predisposition. The association of anemia or bleeding with adverse outcomes has also been observed in other cardiovascular conditions: in patients with acute myocardial infarction, anemia and bleeding enclose worse outcomes [37] and the same applies to patients with heart failure and concurrent anemia [38].

Study limitations

Due to the small size of our single-centre study sample only a limited number of predictors was included in the logistic regression models. However, we think that our data is already indicative that red blood cell indexes (data mostly supportive of mean corpuscular volume) may play a role in predicting transesophageal echocardiogram changes like LAAT and DSEC.

A low prevalence of patients undergoing oral anticoagulation was found in our sample. Still, we reinforce that 103 patients (41.7%) had no previous diagnosis of AF. If we consider only patients with previously known AF the prevalence of patients undergoing oral anticoagulation rises steeply to 39.6% (57 out of 144).

Fifty-two patients (21.1%) had no evaluation of left atrial appendage flow velocities. This was due to technical reasons (echocardiographic data being classified as unsuitable for the accurate assessment of flow velocities) or lack of probe tolerance by the patient. In these subjects, transesophageal echocardiogram was performed without measurement of left atrial appendage flow velocities if the presence of LAAT and DSEC could be excluded right promptly.

Conclusions

Our findings, using left atrial stasis markers, suggest that mean corpuscular volume and red

cell distribution width may be associated with the presence of left atrial appendage thrombus and dense spontaneous echocardiographic contrast in non-valvular atrial fibrillation. However, only mean corpuscular volume seemed to add incremental predictive power to the clinical risk factors present within $CHADS_2$ and CHA_2DS_2-VASc classifications.

Further validation of these findings using transesophageal endpoints in patients undergoing cardioversion or catheter ablation of atrial fibrillation may be useful in identifying low risk groups that can be spared of this imaging procedure. Furthermore, assessment of mean corpuscular volume and red cell distribution width as predictors of thromboembolism in patients with atrial fibrillation may also be a reasonable approach.

Abbreviations

AF, atrial fibrillation; TEE, transesophageal echocardiogram; DSEC, dense spontaneous echocardiographic contrast; LAAT, left atrial appendage thrombus; LFV, low flow velocities; MCV, mean corpuscular volume; RDW, red cell distribution width; ROC, receiver operating characteristic; AUC, area under the curve.

Conflicts of interest

None to be declared.

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