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ORIGINAL ARTICLE

Inflammation and Atrial Electrical Remodelling in Patients With Embolic Strokes of Undetermined Source

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Background About one third of ischaemic strokes are classified as embolic strokes of undetermined source (ESUS). A silent atrial fibrillation (AF) may play a pathogenic role in these strokes and P wave dispersion (PWD), representing an electrocardiographic (ECG) predictor for paroxysmal AF, thereby a potential marker of covert cardioembolism, was found to be increased in cryptogenic stroke. Furthermore, current evidence links AF to inflammation: inflammatory markers, such as high-sensitive C-reactive protein (hsCRP), have been related to the development and persistence of AF, possibly by promoting atrial remodelling. The aim of this study was to evaluate whether a relationship between PWD and hsCRP in patients with ESUS exists, in order to highlight a possible role for inflammation in the atrial electric remodelling, that predisposes to AF.

Methods We enrolled 174 patients (91 males, 83 females; mean age 69 ± 13 years) with ESUS. All patients underwent neuroimaging examination, arterial ultrasound examination, echocardiography and ECG. P wave dispersion and hsCRP were measured in all subjects.

Results A significant positive correlation was found between hsCRP and PWD (Spearman r: 0.35, $p < 0.0001$). In patients with high PWD (>40 msec; n = 102), hsCRP was three-fold higher than in patients with normal PWD (≤40 msec; n = 72)(1.57 ± 2.9 vs 0.42 ± 0.4 mg/dl, p = 0.0005).

Conclusions Our results show increased hsCRP levels in cryptogenic stroke patients with high PWD. These findings provide support for the hypothesis that systemic inflammation plays a role in a fraction of patients with ESUS, by increasing AF risk via atrial electric remodelling.

Keywords Ischaemic stroke • Atrial fibrillation • P wave dispersion • Inflammation • ESUS • ECG

Background

About one third of ischaemic strokes are classified as embolic strokes of undetermined source (ESUS) [1]. Many ESUS are suspected to arise from occult cardiac embolism; in this view, subclinical atrial fibrillation (AF) may play a pathogenic role in these strokes [2]. Recently, an updated model of thromboembolic stroke suggested the importance of systemic and atrial substrate in explaining the relationship between AF and stroke [3].

P wave dispersion (PWD), a well-known electrocardiographic parameter, predictor of AF, may be particularly

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relevant for ESUS: high PWD values reflect the inhomogeneous propagation of sinus impulses and the prolongation of atrial conduction time, a recognised atrial electrophysiological substrate in patients with paroxysmal AF [4].

Furthermore, current evidence links AF to inflammation: systemic inflammatory markers, such as high-sensitive C-reactive protein (hsCRP), have been related to the development and persistence of AF, by contributing to the atrial remodelling [5].

Systemic insults, such as subclinical inflammation, may lead to atrial remodelling, resulting in slowed atrial conduction with inhomogeneous recovery, and reflecting prolonged, inhomogeneous and anisotropic distribution of connections between atrial myocardial fibres [6]. In this view, the aim of this study was to evaluate the relationship between PWD and hsCRP in patients with ESUS to highlight a possible role of inflammation in the atrial electrophysiological remodelling, that predisposes to AF.

Methods

We enrolled 174 patients (91 males, 83 females; mean age 69 ± 13 years), admitted consecutively to the Stroke Unit Department of Siena University Hospital for ESUS.

All patients underwent neuroimaging examination (brain computerised tomography with angio-CT scan and/or brain magnetic resonance imaging), extracranial and transcranial arterial ultrasound examination, transthoracic echocardiography, 12-lead resting electrocardiogram (ECG) and 24-hour ECG monitoring. In patients with age <60 years (n = 42) transoesophageal echocardiography and screening for hypercoagulable state were performed. Strokes were classified as ESUS according to criteria proposed by the Cryptogenic Stroke/ESUS International Working Group [1]. In all patients, hsCRP, potassium and sodium plasma levels were measured. HsCRP was measured within 24 hours of stroke onset and was assayed by a particle-enhanced turbidimetric method using the module C501 of a COBAS-6000 platform (Roche Diagnostics GmbH; Mannheim, Germany). Values were expressed in mg/dl (normal values <0.5).

The study was approved by the Ethics Committee of the University Hospital of Siena, Italy.

P Wave Duration and P Wave Dispersion

Simultaneous 12-lead ECG (25 mm/s and 10 mV/cm) was recorded by means of commercially available imaging system (Cardioline ECT WS 2000, Remco Italia, Vignate-Milano, Italy) in all subjects in supine position (during spontaneous breathing) in the morning hours (between 08.00 and 12.00) of the first 24 hours of admission. Paper-printed ECGs were scanned and digitised in order to achieve greater precision in detecting and measuring P waves. P wave duration was measured by two observers (A.L.M. and M.A.) from the beginning of the P wave deflection from the isoelectric line to the end of the deflection returning to isoelectric line in all simultaneous 12 leads. The following indices were derived from measurements of each ECG: the maximum P wave duration (P max), the minimum P wave duration (P min) and the P wave dispersion (PWD), defined as the difference between P maximum and P minimum. Normal PWD values were lower than 40 ms [7–9].

Statistical Analysis

Statistical analysis was performed with GraphPad InStat (version 3.06 for Windows, GraphPad Software Inc., La Jolla, CA, USA) computer software. All results are presented as means ± SD. Normal distribution of quantitative variables was preliminary tested using the Kolmogorov–Smirnov test to select parametric or non-parametric inferential statistical methods. Pearson's coefficient was used to analyse the intra-observer variability on the first 20 PWD measurements, while Kappa agreement was used to analyse the inter-observer variability. Correlations between P wave indexes and hsCRP were estimated with Spearman's correlation coefficients. Statistical analysis included comparisons of age and hsCRP level in patients with high and normal PWD, using the nonparametric Mann-Whitney test for unpaired data. The two-sided Fisher's exact test was performed to compare risk factors (previous ischaemic stroke, coronary artery disease, arterial hypertension, hypercholesterolaemia, diabetes mellitus, smoking) and minor sources of embolism (mitral valve disease, aortic valve disease, non-atrial fibrillation atrial dysrhythmias, hypokinetic/akinetic left ventricle, moderate-to-severely dilated left atrium, atrial septal aneurysm, patent foramen ovale, aortic arch atherosclerotic plaques) in the two groups of patients. A p value below 0.05 was considered statistically significant.

Results

Descriptive statistics for the data are presented in Table 1. A significant positive correlation was found between CRP and PWD (Spearman r = 0.35, p < 0.0001) (Figure 1, A); furthermore, there was also a significant negative correlation between CRP and P min (Spearman r = −0.15, p = 0.04) (Figure 1, C). No correlation was found between CRP and P max (Spearman r = 0.13, p = 0.07) (Figure 1, B).

Out of 174 patients, PWD was high (>40 ms) in 102 subjects (58%); demographic characteristics of patients with normal and high PWD are depicted in Table 2.

In patients with high PWD, hsCRP was significantly higher than in patients with normal PWD (1.57 ± 2.9 vs 0.42 ± 0.4 mg/dl, p = 0.0005; Mann-Whitney test). In particular, the proportion of patients with high CRP levels (CRP≥ 0.5 mg/dl) was significantly higher in the high-PWD group, than in normal-PWD group (51% vs 26%, p = 0.001 Fisher's exact test). No differences were found regarding the other laboratory parameters evaluated in both groups (sodium, potassium) (Table 2).

Furthermore, there were no differences between patients with high and normal PWD in regard to age, sex,

Table 1 Demographic characteristics of study patients.

	Patients with ESUS (n: 174)
Age (years)	69.2 ± 13.6
Women/men	83:91
Cardiovascular Risk Factors	
Hypertension, n (%)	104 (59%)
Diabetes mellitus, n (%)	37 (21%)
Hypercholesterolaemia, n (%)	70 (40%)
Previous coronary artery disease, n (%)	14 (8%)
Previous stroke, n (%)	8 (4%)
Smoking, n (%)	45(25%)
Minor – risk potential embolic sources, n (%)	
Mitral valve disease	56 (32%)
Aortic valve disease	73 (42%)
Non-atrial fibrillation atrial dysrhythmias	5 (3%)
Hypokinetic/akinetic left ventricle	7 (4%)
Moderate-to-severely dilated left atrium	53 (30%)
Atrial septal aneurysm	26 (15%)
Patent foramen ovale	19 (10%)
Aortic arch atherosclerotic plaques	1 (0.5%)
Carotid artery non-stenotic plaques	114 (65%)
Laboratory findings	
Sodium (mmol/L)	139 ± 3.4
Potassium (mmol/L)	4 ± 0.36
High sensitivity C reactive protein (mg/dl)	1.1 ± 2.3
P wave indexes	
P wave max (ms)	126 ± 16
P wave min (ms)	79 ± 18
P wave dispersion (ms)	47 ± 14

cardiovascular risk factors and minor-risk potential embolic sources, except for mitral valve disease (39% of patients in high-PWD group vs 22% in normal-PWD group; $p = 0.02$) (Table 2). P wave dispersion measurements repeated by the same investigator showed a very good correlation ($r = 0.98$, $p < 0.001$, for both observers; Pearson's test), with a mean intraobserver error of 2.7% and 4.0%, respectively for each observer. The interobserver variability in the P wave measurements was performed by the Kappa test, with values of 0.87 for PWD.

Discussion

The novel findings of our study are the following: PWD positively correlates with hsCRP levels and P min negatively correlates with hsCRP levels in patients with ESUS. In particular, in patients with high PWD (>40 ms), hsCRP levels are significantly higher than in patients with normal PWD.

In our previous study [7] we observed higher PWD values in strokes of unknown cause suggesting the hypothesis that PWD can be a marker of silent atrial fibrillation episodes occurrence [8], that may determine a possible cardioembolic mechanism in these strokes. Indeed, previous studies showed paroxysmal AF occurrence in approximately one third of ESUS patients during the follow-up period [10] and a silent AF can be the cause of ESUS, even if recently some authors suggested the hypothesis that AF episodes, detected remotely during follow-up, may represent an innocent bystander rather than a causative mechanism [11]. Atrial fibrillation could be associated with different factors that cause stroke and, in this context, systemic vascular risk factors may be responsible for atrial cardiopathy, that can result in AF and thromboembolism [3].

Our findings suggest the hypothesis that a systemic low-grade inflammation may determine a more complex pathogenic mechanism in these strokes.

First, inflammation may play a role in determining atrial cardiopathy in these strokes; in fact, it may alter the conduction properties of atrial cells. A huge amount of data demonstrated that inflammatory cytokines play a key pathophysiological role in AF [6], by promoting both structural and electrical atrial remodelling through several mechanisms, including atrial fibroblast activation, gap junction impairment via changes in connexins, and intracellular calcium-handling abnormalities [12]. These phenomena, by both increasing ectopic activity and slowing atrial conduction, impair the homogeneity of impulse propagation throughout the atrium and promote re-entry, a key electrophysiological alteration for AF development [12].

The modulation of these electrophysiological and structural properties results in slowed and heterogeneous atrial conduction with inhomogeneous propagation of sinus impulses and the prolongation of atrial conduction time, detected by PWD [13] and recent evidence suggests that statins could exert protective effects [14], by means of their anti-inflammatory pleiotropic effects. Second, inflammation may play a further pathogenic role by increasing systemic and local thrombogenicity [3]. Many well-known risk factors such as coronary artery disease and hypertension are associated with inflammation and it is possible that these risk factors contribute to ESUS pathogenesis by different mechanisms originating by the effects of proinflammatory cytokines.

Finally, our results show also that in high-PWD group mitral valve disease was more frequent in comparison with normal-PWD group. A previous study demonstrated higher hsCRP values in patients with mitral valve disease, possibly suggesting the contribution of inflammation to valve calcification [15] and, furthermore, it is possible that mitral annulus calcification may contribute to increase P wave dispersion values [16]. These data seems to confirm the hypothesis that inflammation may play a role in ESUS associated to atrial cardiopathy.

This study has some limitations. Firstly, this study was conducted in a single centre. Secondly, all patients underwent 24-hour ECG monitoring, but there are no data about prolonged ECG monitoring of this cohort in order to determine the impact of high PWD and elevated CRP on the risk of incident AF. Further studies will be necessary to evaluate the effects of inflammation and high PWD values on the occurrence of AF.

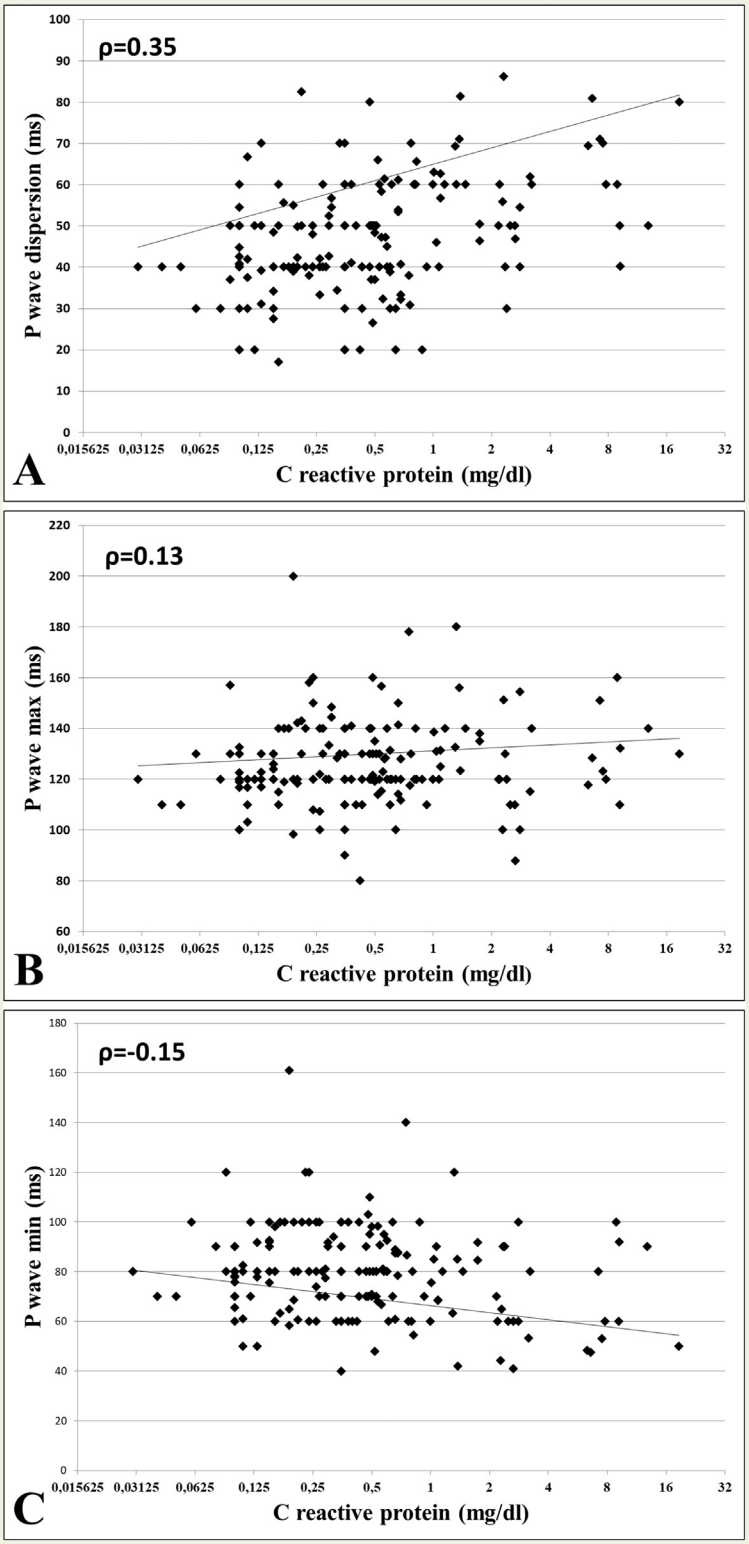

Figure 1 Correlation between C reactive protein and P wave indexes.
A: positive correlation between CRP and PWD (Spearman r = 0.35, p < 0.0001). B: no correlation between CRP and P max (Spearman r = 0.13, p = 0.07). C: negative correlation between CRP and P min (Spearman r = −0.15, p = 0.04).
Abbreviations: CRP, C reactive protein; PWD, P wave dispersion.

Table 2 Characteristics of ESUS patients with high and normal P wave dispersion.

	Patients with PWD > 40 ms (n = 102)	Patients with PWD ≤ 40 ms (n = 72)	P Value
Age (years)	68.5 ± 14.4	70.3 ± 12.4	0.38
Women/men	51:51	32:40	0.53
Cardiovascular Risk Factors			
Hypertension, n (%)	64 (63%)	40 (55%)	0.35
Diabetes mellitus, n (%)	25 (24%)	12 (16%)	0.26
Hypercholesterolaemia, n (%)	42 (41%)	28 (38%)	0.87
Previous CAD, n (%)	9 (8%)	5 (7%)	0.78
Previous stroke, n (%)	6 (5%)	2 (3%)	0.47
Smoking, n (%)	26 (25%)	19 (26%)	1
Minor – risk potential embolic sources, n (%)			
Mitral valve disease	40 (39%)	16 (22%)	**0.02**
Aortic valve disease	45 (44%)	28 (39%)	0.5
Non-atrial fibrillation atrial dysrhythmias	4 (3.9%)	1 (1.3%)	0.40
Hypokinetic/akinetic left ventricle	5 (4.9%)	2 (2.7%)	0.70
Moderate-to-severely dilated left atrium	36 (35%)	17 (24%)	0.13
Atrial septal aneurysm	16 (15%)	10 (14%)	0.83
Patent foramen ovale	11 (10%)	8 (11%)	0.1
Aortic arch atherosclerotic plaques	0 (0%)	1 (1.3%)	0.41
Carotid artery non-stenotic plaques	65 (63%)	49 (68%)	0.62
Laboratory findings			
Sodium (mmol/L)	139 ± 3.5	138 ± 3.2	0.06
Potassium (mmol/L)	4 ± 0.37	4.01 ± 0.33	0.6
C reactive protein (mg/dl)	1.57 ± 2.9	0.42 ± 0.4	**0.0005**
Pts.with high CRP (≥0.5 mg/dl), n (%)	52 (51%)	19 (26%)	**0.001**

Data are expressed as mean ± SD.
Abbreviations: CAD, coronary artery disease; CRP, C reactive protein.
Bold values are statistically significant ($p < 0.05$).

Conclusions

Our results show a positive correlation between CRP and PWD in ESUS, suggesting that the relationship between AF and stroke could be more complex than a simple cause and effect mechanism. Systemic inflammation could play a pathogenic role in atrial cardiopathy observed in this type of stroke, determining an impaired electrical atrial conduction with subsequent increase of PWD and possible increased risk of AF occurrence.

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