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- Abstract -

**CARDIOVASCULAR RISK FACTORS AND *MTHFR* POLYMORPHISMS
INVOLVED IN CARDIOEMBOLIC STROKE DUE TO NON-VALVULAR
ATRIAL FIBRILLATION**

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TABLE OF CONTENTS

GENERAL PART

Chapter 1. Risk factors involved in non-valvular atrial fibrillation and cardioembolic stroke	1
1.1. Non-valvular atrial fibrillation	1
1.1.1. Definition, prevalence and classification of NVAf	1
1.1.2. Pathophysiology of atrial fibrillation	2
1.1.3. AF-related thrombogenesis	4
1.1.4. Clinical evaluation of non-valvular AF. Echocardiographic parameters	5
1.1.5. Risk factors and risk scores in AF	7
1.2. Current scientific background regarding cardioembolic stroke	11
1.2.1. Definition and epidemiology of cardioembolic stroke	11
1.2.2. Classification of ischemic stroke subtypes	12
1.2.3. Evaluation of clinical features in CES	15
1.2.3.1. Clinical presentation of an acute cardioembolic stroke	15
1.2.3.2. Neuroimaging studies in cardioembolic stroke	16
1.2.4. Stroke severity evaluation by clinical scores	19
1.2.4.1. NIHSS scale (National Institute of Health Stroke Scale)	19
1.2.4.2. Modified Rankin Scale (mRS)	20
1.2.5. Aspects regarding mortality, risk of early embolic recurrences and clinical outcome in CES	20
1.3. MTHFR polymorphisms as cardiovascular genetic risk factors	21
1.3.1. MTHFR gene and the folate metabolism	21
1.3.2. MTHFR gene polymorphisms and their clinical involvement in different cardiovascular diseases	23
1.3.2.1. Hypertension	23
1.3.2.2. Coronary artery disease	24
1.3.2.3. Type II Diabetes Mellitus	24
1.3.2.4. Carotid atheromatosis	24
1.3.2.5. Dyslipidemia	25
1.3.3. MTHFR polymorphisms and atrial fibrillation	25
1.3.4. MTHFR polymorphisms and stroke	26

SPECIAL PART

Chapter 2 RESEARCH MOTIVATION	28
2.1. Research design for relationship between cardiovascular risk factors-MTHFR polymorphisms-non-valvular atrial fibrillation-cardioembolic stroke	29
2.2. Materials and Methods	33
2.2.1. Patients selection	33
2.2.2. Data collection	25
2.2.3. Statistical analysis	36
Chapter 3 RESULTS	37
3.1. Personal contribution: Study of relationship between echocardiographic parameters and cardiovascular risk factors for stroke incidence in non-valvular AF	37
3.1.1. Current scientific background	37
3.1.2. Objectives of research	38
3.1.3. Materials and methods	39
3.1.3.1. Study protocol	40
3.1.3.1.1. Clinical evaluation	41
3.1.3.1.2. Biochemical evaluation	43

3.1.3.1.3. Echocardiographic evaluation	44
3.1.3.1.4. Doppler carotid echography	45
3.1.3.1.5. Stroke risk evaluation	45
3.1.4. Results	47
3.1.5. Discussion	56
3.1.6. Conclusions	58
3.2. Personal contribution: Study of MTHFR gene polymorphisms prevalence and cardiovascular risk factors involved in cardioembolic stroke type and severity	59
3.2.1. Current scientific background	59
3.2.2. Study objectives	61
3.2.3. Materials and methods	62
3.2.3.1. Patient population	62
3.2.3.2. Clinical and biochemical evaluation	63
3.2.3.2.1. Clinical evaluation	63
3.2.3.2.2. Electrocardiogram	63
3.2.3.2.3. Biochemical evaluation	63
3.2.3.2.4. Assessment of cardiovascular comorbidities	64
3.2.3.2.5. Inflammatory state detection	64
3.2.3.2.6. Thromboembolic and bleeding risk scores	65
3.2.3.2.7. INR determination	65
3.2.3.3. Evaluation of stroke severity	65
3.2.3.4. Imagistic evaluation	66
3.2.3.5. Genetic testing of MTHFR (C677T and A1298C) polymorphisms	66
3.2.3.6. Ultrasound evaluation of the heart and carotid arteries	68
3.2.4. Results	70
3.2.5. Discussions	82
3.2.6. Conclusions	88
3.3. Personal contribution: A case report of thrombophilia and cardioembolic stroke in a young patient	89
3.3.1. Current scientific background	89
3.3.2. Case presentation	89
3.3.3. Discussions	94
3.3.4. Conclusions	95
Chapter 4 GENERAL CONCLUSIONS	96
BIBLIOGRAPHY	98
APPENDIX	I

Keywords: non-valvular atrial fibrillation, cardioembolic stroke, cardiovascular risk factors, MTHFR polymorphisms A1298C and C677T, thrombophilia

General part

Atrial fibrillation (AF) is known as the most frequent cardiac arrhythmia associated with a high morbidity and mortality rate (1). As AF becomes more frequent in civilized countries associated with the current lifestyle of the population, outcomes of AF are becoming a social and economic burden for most states. Romania is still a country associated with a very high cardiovascular mortality rate, mostly due to the predominance of rural sites with population that has difficulties in accessing medical care.

Structural and functional atrial substrate changes together with stasis and a prothrombotic state are the main pathogenic mechanisms that lead to thrombogenesis in AF. While LA size is considered an independent stroke risk factor (2), LV dysfunction is also regarded as a thrombogenic risk factor in AF by LV remodeling and increased LV diastolic filling pressures. An increase in D-dimers favors thromboembolic events by generating thrombus formation in the LV (3). The role of inflammation in thrombogenesis is still on debate, however certain inflammatory biomarkers have been linked to both AF initiation and perpetuation such as hs-CRP (high sensitive C-reactive protein) and IL-6 (interleukin 6). Moreover, increased hs-CRP could be related to stroke risk and prognosis (4).

Several cardiovascular risk factors have been studied in relation to non-valvular AF (NVAf) and some of them are specific stroke risk factors. However, the general profile of NVAf patients is still incomplete, as some cases can have multiple stroke causes with high thrombotic states that can go undetected by the stroke risk scores in actual practice. Numerous comorbidities such as heart failure, hypertension, obesity, coronary artery disease, diabetes mellitus and valvular heart diseases that come along with the ageing of today's population and are correlated to the lifestyle of the present are increasing the prevalence of AF. Naser et al studied the impact of risk factors and comorbidities on AF incidence and revealed that habits like smoking or alcohol abuse can trigger atrial fibrillation (5). Heart failure followed by stroke are the main consequences of AF (6), while sudden cardiac death is the leading cause of cardiovascular death for this disease, which can be predicted by the presence of previous myocardial infarction, LV hypertrophy, digitalis treatment and the absence of beta-blocker use, heart failure, low EF, high cardiac frequency and the male gender (7).

Cardioembolic stroke, known as the most severe subtype of ischemic stroke, is associated with a high disability and mortality rate (8). Also as cardioembolic stroke is associated with a higher stroke recurrence rate, the overall hospitalization and post stroke care costs are significantly higher than in other stroke subtypes (9). The prevalence of CE stroke is

14-30% from all stroke types (10,11). Arboix et al. also studied the incidence of CES and their findings indicate a higher incidence in the population aged 85 years and older with 36%, mainly due to the higher prevalence of AF at this age. A frequent complication of cardioembolic stroke and also a clinical suggestive feature is the hemorrhagic conversion (or transformation) of an initially ischemic infarct (12).

Clinical evaluation of an ischemic stroke patient requires a complex association of clinical data regarding neurological status, neuroimaging studies and treatment response. The most comprehensive and used scale in neurological practice is the NIHSS scale. The complexity of the analyzed neurological items makes this scale the principal tool in clinical assessment of clinical evolution for recanalization techniques, such as thrombolysis and mechanical thrombectomy, but also in clinical research studies. The modified Rankin scale is a good predictor of global clinical outcome and disability and therefore frequently used in follow-up. However, the utility of this score in acute stroke is the measurement of the primary outcome in treatment response (13,14).

Genetic risk factors as predictors for cardioembolic stroke in NVAF could determine a step forward in the new generation of treatments, including perhaps future targeted gene therapies. The methylenetetrahydrofolate reductase (*MTHFR*) gene, which has an important role in the folate metabolism, could also play an essential role in the phenotype of various cardiovascular diseases. The folate metabolism plays a crucial role in the amino acid synthesis, respectively in the one-carbon metabolism process, and in the DNA methylation pathways. (15) The main circulating form of folate, 5-methyltetrahydrofolate, is also involved in the process of homocysteine (Hcy) transformation into methionine. Moreover, methionine is the main precursor for S-adenosylmethionine, the principal methyl donor found in various reactions throughout the cellular metabolism. Dietary folic acid intake from aliments or supplements is of great importance, as the human body is unable to self-synthesize folates and thus prevents some of the clinical conditions associated with folate deficiency (16). 5-methylenetetrahydrofolate reductase (*MTHFR*) is the main enzyme that catalyzes the reduction of 5,10-methylenetetrahydrofolate into 5-methyl-tetrahydrofolate. This particular enzyme is an essential element involved in the Hcy metabolism, therefore *MTHFR* deficiencies lead to numerous diseases associated with accumulation of increased plasma levels of homocysteine.

The *MTHFR* gene location is at the 1p36.3 position on chromosome 1. The main genetic variants of *MTHFR* are *C677T* and *A1298C* with an overall prevalence of 10% for homozygous and heterozygous form for both variants, while the rest of the population (60-70%) inherits at least one genetic variant. (17) Single nucleotide polymorphisms (SNP) in the *MTHFR* gene,

respectively *A1298C* and *C677T*, have proven to be important indicators of cardiovascular risk in various diseases (18). The clinical implications of these SNPs result from a mild impairment of the *MTHFR* enzymatic activity leading to moderate elevation of Hcy levels.

So far study results are indicating that AF could have multiple potential genetic risk factors (19) although information about *MTHFR* polymorphisms involvement in AF determination is scarce. Only a few studies have researched this connection and some their results revealed an important association between AF and the role of elevated tHcy as genetic risk factor in AF (20). Clinical data regarding the role of *MTHFR* polymorphisms in cardioembolic stroke is very limited. However, studies were conducted on different polymorphisms regarding their role in ischemic stroke, as genetic risk factors. More studies need to be conducted on larger cohorts to establish the role of these inherited polymorphisms in NVAf and cardioembolic stroke, as information about the role of these SNPs in the *MTHFR* gene are insufficient.

This current research aims to find a general profile of NVAf patients, which includes echocardiographic parameters as predictors for cardioembolic stroke. Another study aim is to find if other cardiovascular comorbidities like coronary artery disease, hypertension, heart failure, diabetes mellitus type II and dyslipidemia are associated with the outcome of stroke in NVAf. Regarding the genetic risk, this research investigates if *A1298C* and *C677T* polymorphisms in the *MTHFR* gene are linked with the outcome of cardioembolic stroke in NVAf. This is based on the premise that if proven to be connected, these genes could be used in future clinical practice as genetic predictors for stroke outcome in NVAf patients. By looking for further connections of these SNPs in the *MTHFR* gene with cardioembolic stroke, we searched for their role in the determination of stroke type, severity and localization.

Special Part

While new clinical approaches in diagnostic and treatment options for non-valvular AF and cardioembolic stroke are currently available in literature and the role of *MTHFR* polymorphisms has been studied in numerous cardiovascular diseases, there still is lack of data regarding involvement of *MTHFR* genetic variants in cardioembolic stroke due to NVAf.

This study aims to find answers to the following issues in clinical practice:

- Are certain echocardiographic parameters such as LAV, LVEDV and LVEF linked to the outcome of stroke in NVAf?

- Are other cardiovascular comorbidities like coronary artery disease, hypertension, heart failure, diabetes mellitus type II and dyslipidemia associated with the outcome of stroke in NVAF?
- Can *MTHFR* A1298C and C677T polymorphisms be associated with the outcome of cardioembolic stroke in NVAF patients? Could these genetic variants be used in future clinical practice as genetic predictors for NVAF patients for stroke outcome?
- Are *MTHFR* polymorphisms associated with a more severe outcome in cardioembolic stroke and with a certain lesion localization? Also is there a connection between the presence of these mutations and hemorrhagic conversion of CES in NVAF patients

Study background: The current research was made starting from the following premises:

- While NVAF is the most common cause of cardioembolic stroke and NVAF incidence is continually growing each year causing a rise in overall mortality of cardiovascular diseases, the present study tries to find an association between certain clinical and echocardiographic parameters that could predict the outcome of stroke for patients with NVAF.
- Several studies have been conducted on genetic risk factors involved in ischemic stroke, but there is a lack of information in literature regarding *MTHFR* polymorphisms and cardioembolic stroke due to NVAF. *MTHFR* polymorphisms have been studied in numerous diseases (18) and studies have revealed an important connection with cardiovascular diseases, but there are no studies regarding the role of these genetic variants and cardioembolic stroke and NVAF.

Type of study: retrospective (for ambulatory care patients) and prospective (for hospital care patients), cross- sectional, analytical and interdisciplinary

Studied populations:

- NVAF patients with or without stroke in ambulatory care, selected according to ESC Guidelines for the diagnosis and treatment of atrial fibrillation (21). NVAF patients with cardioembolic stroke in hospital care, selected according to TOAST criteria for ischemic stroke (22) and ECASS criteria for hemorrhagic conversion of cardioembolic stroke (23).
- From the group of patients in hospital care for stroke, a patient with thrombophilia, paroxysmal AF and cardioembolic stroke was selected for presentation as a case report.

Overall objectives: The purpose of this current study was to identify a connection between:

- Echocardiographic parameters, cardiovascular risk factors and the outcome of stroke in NVAf
- *MTHFR* gene polymorphisms prevalence and cardioembolic stroke, as a consequence of NVAf

Specific objectives:

- Correlations between echocardiographic parameters and cardiovascular risk factors, the types of NVAf and the presence of chronic stroke lesions
- Associations between *MTHFR* polymorphisms *A1298C* and *C677T* and cardioembolic stroke for NVAf patients, prevalence of these *MTHFR* genetic variants and cardioembolic stroke lesion type and localization
- Prevalence of *MTHFR* polymorphisms *A1298C* and *C677T* and cardiovascular risk factors in cardioembolic stroke due to NVAf

Studied parameters:

1. **Clinical evaluation:** age, gender, body mass index (BMI), personal history of hypertension (HTN), coronary artery disease (CAD), peripheral arterial disease (PAD), diabetes mellitus type II (T2DM), heart failure (HF)
2. **Hemodynamic risk parameters:** systolic blood pressure (SBP), diastolic blood pressure (DBP)
3. **Echocardiographic parameters:** left atrium volume (LAV), left ventricle end-diastolic volume (LVEDV) and left ventricle ejection fraction (LVEF)
4. **Carotid atheromatosis indicators:** intima media thickness (IMT) and the presence of carotid plaques on carotid Doppler echography findings
5. **Stroke risk factors:** CHA₂DS₂-VASC, HASBLED scores
6. **Stroke severity indicators:** National Institute of Health Stroke Scale (NIHSS) and modified Rankin scale (mRS)
7. **Serum biomarkers for cardiovascular risk:** lipid profile values (total cholesterol-TC, triglycerides-TGL, low-density lipoprotein cholesterol- LDLc, high-density lipoprotein cholesterol- HDLc) and glycosylated hemoglobin (HbA1c)
8. **Inflammatory biomarker:** high-sensitive C reactive protein (hsCRP)

9. Genetic risk indicators: MTHFR polymorphisms A1298C and C677T

The first part of research: “Personal contribution: **Study of relationship between echocardiographic parameters and cardiovascular risk factors for stroke incidence in non-valvular AF**” aimed to determine a general profile of NVAf ambulatory patients regarding incident stroke by analyzing certain echocardiographic parameters and applying the CHA₂DS₂-VASC and HASBLED scores, evaluation of lipid profile and HbA1c and presence of carotid and/or coronary artery disease. Types of NVAf (paroxysmal, persistent and permanent AF) and the presence of chronic stroke lesions (personal history of stroke) were analyzed in association with cardioembolic risk factors and echocardiographic parameters. Our results indicated that parameters such as LAV, LVEDV and LVEF% are associated with cardioembolic risk in NVAf (especially LVEDV), but are also useful in detecting patients at higher risk to develop ischemic heart failure (LAV in particular). Also modified lipid profile values and the presence of comorbidities such as coronary artery disease and T2DM are important indicators for the possible outcome of stroke in NVAf.

The second part of research: “Personal contribution: **Study of MTHFR Gene Polymorphisms Prevalence and Cardiovascular Risk Factors Involved in Cardioembolic Stroke Type and Severity**” is an original study with the purpose to determine the prevalence of both single nucleotide polymorphisms (SNPs) in the *MTHFR* gene: C677T and A1298C in cardioembolic stroke due to non-valvular atrial fibrillation and their correlations with cardiovascular risk factors, localization and severity of stroke. Our results revealed the prevalence of *MTHFR* gene polymorphisms in a population with cardioembolic stroke and NVAf. The C677T mutation was significantly associated with stroke lesion type and localization, but also with recurrent stroke lesions and stroke severity. Also, this mutation in the *MTHFR* gene is associated with a higher incidence of cardiovascular comorbidities (HTN, HF, dyslipidemia, T2DM with high HbA1c and increased inflammatory state), thromboembolic and bleeding risk compared to patients without this genetic variant. The A1298C mutation was associated with a higher previous lacunar stroke incidence and stroke recurrence rate, while only dyslipidemia was the main cardiovascular comorbidity. Although larger cohort studies are needed to confirm these findings, these results highlight the importance of this study in the aim to evaluate the genetic risk for patients with cardioembolic stroke and NVAf as a background for possible future gene therapies.

Case presentation: “Personal contribution: **A case report of thrombophilia and cardioembolic stroke in a young patient**” is a case presentation of a relatively young patient with multiple comorbidities such as paroxysmal AF, multiple infarct ischemic stroke with right ataxic hemiparesis and dysarthria, thrombophilia with homozygous PAI-1 4G/4G, heterozygous *MTHFR A1298C* and Factor II G20210A heterozygous mutation and chronic hypertension.

The case particularity for this patient is the co-existence of multiple stroke causes at a relatively young age. The decision to present this case was motivated by the lack of clinical data regarding the interference of these comorbidities that lead to high thrombotic states.

Patient selection

The present study divided the selected patients into two distinct categories: non-valvular AF patients in ambulatory care, who were selected from a Cardiology Ambulatory Praxis in Arad (Rubio Medical Center in Arad) and patients with NVAF in hospital care, selected from the database of the Arad County Clinical Emergency Hospital. These two studies were conducted from October 2018 until March 2020.

The first part of this research is a retrospective, cross-sectional study and patients were selected according to the established inclusion/ exclusion criteria to meet study objectives. The second part of the research is a prospective, cross-sectional study and eligible patients were selected consecutively based on the predefined inclusion/ exclusion criteria. Patient selection for this research is illustrated in Fig.1

This study was approved by the Scientific Research Ethics Committee of the University of Medicine and Pharmacy “Victor Babes” Timisoara and the Ethics Committee of Arad County Clinical Emergency Hospital and conformed to the Declaration of Helsinki. All patients that were included in the study provided written informed consent.

Study definitions:

- NVAF with or without stroke in ambulatory care, according to ESC Guidelines for the diagnosis and treatment of atrial fibrillation (21)
- NVAF with CES in hospital care, according to TOAST criteria for ischemic stroke (22) and ECASS criteria for hemorrhagic conversion of CES (23)
- Case report of a patient with thrombophilia, paroxysmal NVAF and CES

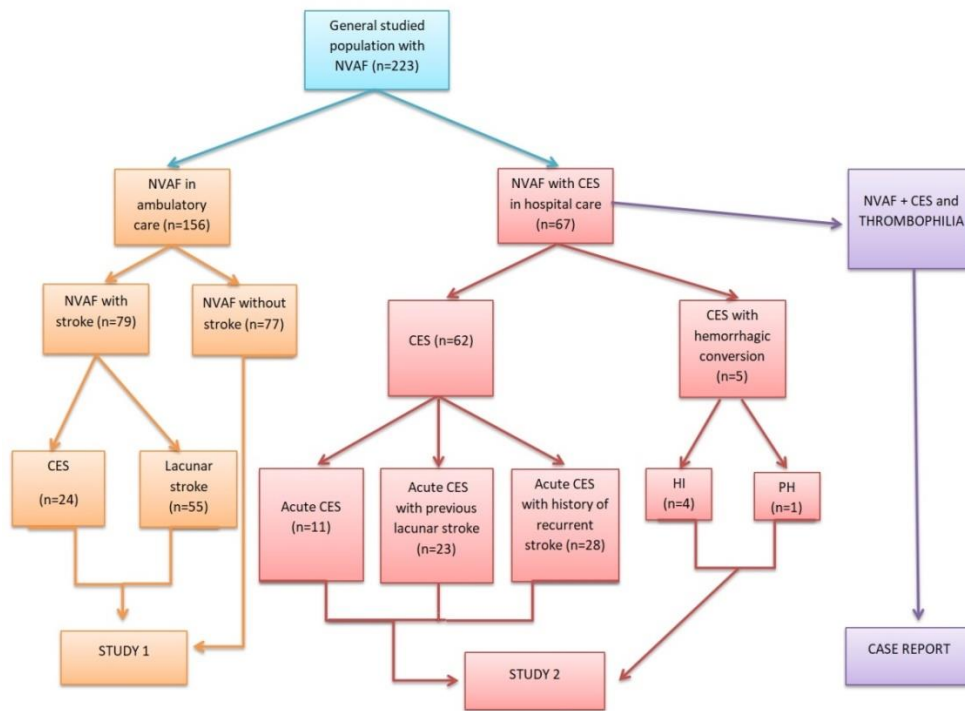


Fig. 1 Selection of study population

Results

Study 1

LAV values and CHA₂DS₂_VASC scores were significantly increased in patients with permanent NVAF vs. paroxysmal NVAF ($p=0.016$ in the case of LAV, $p=0.017$ for CHA₂DS₂_VASC, Mann-Whitney U non-parametric test). Cardioembolic stroke group vs. non-stroke had significantly decreased values of HDLc ($p=0.011$) and increased LAV values ($p=0.043$)(Mann-Whitney non-parametric test). Significantly increased values of LDLc were found for patients with lacunar stroke vs. non-stroke ($p=0.039$, Mann-Whitney non-parametric test).

A multivariate regression data analysis was made using stroke as an independent variable related to LAV, LVEDV, LVEF%, IMT and HbA1c showed a statistically significant direct correlation with LAV ($\rho=0.274$, $p=0.015$). HbA1c and IMT were significantly and directly correlated with CHA₂DS₂_VASC scores ($\rho = 0.246$, $p = 0.001$ for HbA1c, and $\rho = 0.196$, $p = 0.007$ for IMT). In both cases the correlations between CHA₂DS₂_VASC scores and LVEDV,

respectively LVEF%, were negative ($\rho = -0.134$ with $p = 0.038$ in the case of LVEDV and $p < 0.001$, $\rho = -0.266$ in the case of LVEF %).

By using Chi2 tests in the comparison of stroke vs. non-stroke patients, high values of TC ($p=0.014$; OR=2.51 with confidence interval of 95% 1.19;5.27), LDLc ($p=0.026$, OR=4.22 with confidence interval of 95% 1.13;15.78), TGL ($p=0.020$; OR=3.45 with 95% confidence interval 1.28; 9.29) and low values of HDLc ($p=0.022$; OR=2.18 with 95% confidence interval 1.12; 4.28) were found to be important risk factors for stroke incidence in NVAf.

Regarding echocardiographic parameters, patients with increased LVEDV had a higher stroke incidence (Chi2 test, $p=0.031$; OR=2.05 with confidence interval of 95% 1.06; 3.94). Another important risk factor for developing stroke in patients with NVAf was HbA1c with values over 6,5% (Chi2 test, $p=0.019$; OR=2.2 with confidence interval of 95% 1.13;4.27). Stroke incidence was significantly higher for patients with coronary artery disease (Chi2 test, $p=0.004$).

Study 2

Significantly increased values for DBP ($p = 0.007$), TC ($p = 0.003$), LDLc ($p = 0.003$), HbA1c ($p = 0.004$), TGL ($p < 0.001$), LVEF ($p = 0.047$) and hsCRP ($p = 0.015$) were found in the C677T mutation group vs the non-mutation group. In addition, values for NIHSS ($p = 0.001$), mRS ($p = 0.003$), CHA₂DS₂VASC ($p = 0.029$) and HASBLED ($p = 0.025$) scores were significantly increased in this group of patients.

Chi² tests revealed that the proportion of ischemic CES in the sylvian artery territory was significantly higher in the non-mutant group ($p = 0.002$), while the proportion of vertebrobasilar CES ($p = 0.002$), hemorrhagic conversion of CES, respectively the hemorrhagic infarction subtype ($p = 0.020$) and previous lacunar stroke ($p = 0.003$) was significantly higher in patients with C677T mutation. Also recurrent stroke was significantly higher in the C677T mutation group ($p < 0.001$).

In patients with *MTHFR* A1298C mutation, stroke severity was significantly increased with higher NIHSS ($p = 0.006$) and mRS ($p = 0.020$) scores. Also significant differences were found in patients with A1298C mutation regarding the proportion of previous lacunar stroke lesions ($p = 0.019$) and the presence of recurrent stroke ($p = 0.024$). The values for the lipid profile of patients with A1298C mutation were significantly higher vs. patients without this mutation: TC ($p = 0.001$), LDLc ($p < 0.001$) and TGL ($p < 0.001$) and significantly lower HDLc ($p = 0.001$).

Logistic regression (Forward Stepwise (Wald) method) was performed considering the C677T mutation as a dependent variable and variables such as DBP, HbA1c and TGL were

significantly associated with the *MTHFR* C677T mutation in both homozygotes and heterozygotes.

Patients with C677T mutation had a higher risk for increased HbA1c values (OR = 1.982, 95%CI= (1.171, 7.799)) and hypertriglyceridemia (OR = 1.392, 95%CI = (1.192, 3.994)). Mann–Whitney U test also revealed that patients with C677T mutation were associated with significantly higher HbA1c ($p = 0.004$) and TGL ($p < 0.001$) values compared to the group without the mutation.

Logistic regression performed using the *MTHFR* A1298C mutation as a dependent variable (Forward Stepwise (Wald) method) revealed that patients with the A1298C mutation had a risk for developing high TGL values (OR = 2.983, 95%CI = (1.972, 7.994)). Significantly higher TGL values were found in patients with A1298C mutation compared to the non-mutant group (Mann–Whitney U Test, $p < 0.001$).

Multiple comparison analysis showed a significant association between previous lacunar stroke ($p = 0.001$, Chi² Test), respectively, recurrent stroke and the presence of *MTHFR* polymorphisms (Chi² Test, $p < 0.001$). NIHSS and mRS scores were significantly lower for patients without *MTHFR* polymorphisms when compared to patients with one or both genetic variants (Kruskal–Wallis test, $p < 0.001$).

Case presentation

The case particularity is having multiple stroke causes at a relatively young age with relatively low CHA₂DS₂-VASC score on admission, despite having such a high thrombotic state (with homozygous PAI-1 4G/4G, heterozygous *MTHFR* A1298C and Factor II G20210A heterozygous mutation). Also, a moderate clinical stroke severity on admission with very good in-hospital and at-home recovery is another case particularity.

Discussions

The volume of the left atrium is of superior value when it comes to predicting the outcomes in AF and echocardiographic parameters related to stroke risk are increased diastolic and systolic diameters of LV, increased LA size, increased E/A ratio and reduced LVEF% (24). The ENGAGE AF-TIMI trial 48 highlighted that paroxysmal AF was associated with fewer thromboembolic events than permanent AF and that high CHA₂DS₂-VASC scores were found in NVAf patients with important impairment of the diastolic functions of the left heart (25). In our study patients with permanent AF were more likely to develop increased left atrium volumes and have higher CHA₂DS₂-VASC scores. Also increased LA volumes were correlated with increased LVEDV and lower LVEF% values and significantly higher CHA₂DS₂-VASC scores.

Parameters such as LAV, LVEDV and LVEF% are associated with cardioembolic risk in NVAf (especially LVEDV), but are also useful in detecting patients more prone to develop ischemic heart failure (LAV in particular), as confirmed by the present study.

Studies have shown associations between a modified lipid profile and different types of stroke, for instance large artery atherosclerotic stroke is associated with dyslipidemia, while lacunar and embolic stroke seem to present low or almost no association (26). Both stroke groups showed a modified lipid profile, which can be explained by a low compliance of our study population to lipid lowering therapy. Coronary artery disease can be very common in patients with AF (17% to 46,5%) and studies have predicted that future stenting could be required to 5%-15% of patients with AF (27). The study results from Study 1 showed that a modified lipid profile, the presence of coronary artery disease and diabetes are important indicators for the possible outcome of stroke in NVAf.

Study 2 demonstrated the prevalence of *MTHFR* gene polymorphisms in a cardioembolic stroke population with NVAf. This study highlighted the relationship between *C677T* mutation and stroke lesion type and localization (especially vertebrobasilar CES, hemorrhagic conversion of CES and previous lacunar stroke), but also with recurrent stroke lesions and stroke severity (high NIHSS and mRS scores). While a study demonstrated that the *C677T* mutation was associated with a high risk for hemorrhagic stroke, especially the T allele (28), other authors demonstrated that the two genetic *MTHFR* variants are independent genetic risk factors for stroke, both ischemic and hemorrhagic (29).

The *C677T* mutation in patients with NVAf was associated with a higher incidence of cardiovascular comorbidities (HTN, HF, dyslipidemia, T2DM with high HbA1c and increased inflammatory state), thromboembolic and bleeding risk compared to patients without this genetic variant. Another study also demonstrated the link between *C677T* polymorphism and the risk for ischemic stroke in patients with T2DM. (30). Stroke severity in patients with the *C677T* mutation was also higher in patients with increased NIHSS ($p = 0.001$) and mRS ($p = 0.003$) scores in our study.

A meta-analysis performed by Kang S. et al. discovered a link between the high ischemic stroke risk and the presence of the *A1298C* polymorphism (31). The *A1298C* mutation was also associated with a higher previous lacunar stroke incidence and stroke recurrence rate, while only dyslipidemia was the main cardiovascular comorbidity.

Cardiovascular risk factors such as DBP, hypertriglyceridemia and elevated HbA1c were important risk factors associated with the *C677T* mutation, while only hypertriglyceridemia was associated with the *A1298C* mutation.

General Conclusions

- Non-valvular atrial fibrillation is a clinical entity that must be carefully evaluated in order to predict severe outcomes that are associated with a high mortality, such as cardioembolic stroke and sudden cardiac death. Some echocardiographic parameters have proven to be useful predictors for cardioembolic risk: LAV, LVEDV and LVEF%.
- LVEDV has a stronger association with cardioembolic risk, while LAV is more useful in the detection of patients more prone to ischemic heart failure.
- Cardiovascular comorbidities such as coronary artery disease, diabetes mellitus type II and a modified lipid profile are important stroke predictors in NVAf patients.
- Genetic risk in cardioembolic stroke due to NVAf should be seriously taken into consideration, as our study indicates *MTHFR* polymorphisms *A1298C* and *C677T* are prevalent in patients with these two intricate pathologies.
- The *C677T* polymorphism in the *MTHFR* gene was significantly associated with stroke lesion type (hemorrhagic conversion of CES and previous lacunar stroke) and localization (the vertebrobasilar arterial system)
- *C677T* mutation is also significantly associated with recurrent stroke and with higher NIHSS and mRS scores.
- A higher incidence of cardiovascular comorbidities such as hypertension, heart failure, dyslipidemia and T2DM with high HbA1c was observed in patients with *C677T* mutation in our study.
- *C677T* polymorphism is also associated with an increased inflammatory state (higher hsCRP values).
- A considerably higher thromboembolic and bleeding risk with higher CHA₂DS₂-VASC and HASBLED scores were found in patients with *C677T* mutation.
- The *A1298C* mutation was associated with a higher previous lacunar stroke incidence and stroke recurrence rate.

- DBP, hypertriglyceridemia and elevated HbA1c were important risk factors associated with the *C677T* mutation, while only hypertriglyceridemia was associated with the *A1298C* mutation.
- Patients with thrombophilia and *MTHFR* gene polymorphisms can have multiple stroke causes and therefore clinical investigations should be done extensively. Cardiac rhythm disturbances can be associated in these patients and thus be additional stroke risk factors.

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