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Stroke risk factor analysis in patients with patent foramen ovale

ABSTRACT OF THE PhD THESIS

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Abstract of the PhD Thesis

Introduction

This thesis details the findings from four studies conducted during my doctoral research, under the guidance of Prof. Dr. Bogdan Ovidiu Popescu.

The primary objective of this research was to enhance our understanding of the patent foramen ovale's (PFO) role in ischemic cerebrovascular disorders, whether it is manifested by ischemic strokes, transient ischemic attacks (TIAs), or even silent cerebral ischemic lesions. Additionally, the research aimed to identify certain risk factors, including modifiable ones, that, in the setting of an existing PFO, increase the risk of ischemic cerebrovascular incidents.

Given the scarcity of literature on identifying PFO patients at risk of experiencing their first ischemic cerebrovascular event and the lack of primary preventive interventions, our findings offer a significant preliminary step toward a personalized management strategy for asymptomatic PFO patients and the development of primary preventive treatments for PFO-related ischemic strokes.

1. Current level of knowledge

1.1. PFO associated stroke

The foramen ovale is a normal anatomical structure in intrauterine life, necessary for blood circulation through the fetal atrial septum. It serves a pivotal role in shunting the pulmonary circulation, which is non-functional during fetal development (1). The failure of fusion between the two interatrial septa (septum primum and secundum), results in the formation of a patent foramen ovale (PFO). This feature is a remnant of normal fetal cardiac anatomy and is present in over 25% of the general population (2).

The term 'PFO-associated stroke' was first proposed in 2020 by the international task force responsible for the study of ischemic stroke in patients with PFO (3). The diagnosis of PFO associated stroke is a diagnosis of exclusion, and requires the existence of a non-lacunar stroke (with a diameter >1.5 cm), or retinal ischemia in patients who present a PFO with anatomical characteristics that can turn it into a risk factor for ischemic stroke, and in whom any other cause of ischemic stroke was excluded after an extensive work-up (3).

Although not formally named until 2020, PFO has been recognized in most ischemic stroke classification systems as a potential etiology for cryptogenic strokes or embolic strokes of unknown source (ESUS). Systems such as the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) (4) and SSS-TOAST (Stop-Stroke Study TOAST) (5) categorize PFO as a low- or intermediate-risk cardioembolic source of stroke. Conversely, the ASCO/ASCOD classification system (6) assesses the likelihood of stroke secondary to PFO based on the clinical context in which it occurs (7).

Currently, the pathophysiological mechanisms underlying ischemic strokes associated with PFO remain only partially understood. The literature published up to this point identifies three primary mechanisms believed to contribute to ischemic cerebrovascular events in patients with PFO: paradoxical embolism (8), 'in-situ' thrombi formation (9), and a possible susceptibility for atrial arrhythmias in patients with PFO (10). An aspect that should not be ignored when analyzing the physiology of PFO, is its role in disrupting the systemic biochemical homeostasis, due to the persistence in arterial blood of specific chemical compounds that would normally be filtered in the pulmonary circulation (11), and this retention of vasoactive mediators could constitute a plausible pathophysiological mechanism underlying ischemic strokes associated with PFO.

Risk factors associated with PFO-associated stroke

Current knowledge concerning the risk factors that predispose patients with PFO to ischemic strokes is primarily derived from studies analyzing the risk of recurrent ischemic

cerebrovascular events and from case-control studies. To date, prospective studies assessing the risk factors for a first PFO-related ischemic stroke event in younger patients are lacking. The main risk factors suggested until now to be involved in PFO-associated strokes are linked to the anatomical characteristics of PFO, while the roles of genetic predisposition or biological mechanisms precipitating ischemic events among PFO patients have been addressed to a more limited extent.

PFO morphological features most frequently associated with an increased risk of ischemic stroke include an interatrial septal aneurysm (ASA) (12), a prominent Eustachian valve, the presence of a Chiari network (13), excessive mobility of the interatrial septum (14), large PFOs, high-grade interatrial shunt, and a less than 10 degrees angle between the inferior vena cava and the PFO valve (15).

Some coagulation disorders are reported with greater frequency in patients with ischemic strokes associated with PFO compared to the general population (16). However, conclusive evidence linking procoagulant states with an increased risk of ischemic stroke in PFO patients remains elusive. This uncertainty is reflected in current good clinical practice guidelines, which recommend that the management of PFO patients with associated prothrombotic states should be the same as that of patients without procoagulant conditions. Furthermore, these guidelines suggest that additional testing is not required for patients who have experienced an ischemic stroke associated with PFO (17).

Establishing a causal relationship between a PFO and an ischemic stroke presents considerable challenges. However, the specific circumstances of the stroke event, combined with the presence of the morphological risk factors for PFO discussed earlier, can significantly aid in explaining this relationship. Several risk scales have been developed in order to aid in a more objective assessment of PFO's role in ischemic stroke occurrences. The first such scale is the Risk of Paradoxical Embolism (RoPE) score, with a maximum of 10 points, and decreasing with the presence of traditional cardiovascular and cerebrovascular risk factors. Despite the notable advancement the development of the RoPE score represented, its sensitivity remains relatively low (18). In response, the PASCAL score (‘PFO-Associated Stroke Causal Likelihood’) has been more recently introduced (19). This new scoring system enhances the RoPE score by incorporating additional clinical and anatomical risk factors associated with PFO; consequently, the PASCAL score offers an improved framework for more reliably diagnosing ischemic strokes attributable to PFO.

However, special attention must be paid to the way in which the RoPE score was developed. It is important to understand that it was derived from a cohort comprising patients who had experienced ischemic strokes associated with PFO, but the control group was selected from patients with cryptogenic stroke without a PFO. Consequently, it can be misinterpreted that the RoPE has the ability to assess the risk of ischemic stroke in patients with PFO, but such a conclusion cannot be drawn as the study on which its development was based did not include patients with asymptomatic PFO (20). Thus, although the RoPE score indicates that patients with ischemic PFO associated stroke are generally younger, more likely to smoke, and have lower incidences of hypertension and diabetes compared to those with cryptogenic strokes without PFO, these observations should not lead to the conclusion that such risk factors are irrelevant for stroke occurrence in PFO patients, and emphasizes the need for further research to clarify their impact and contribution to stroke risk in this population.

2. Special part

2.1. Research concept and methodology

Ischemic stroke associated with PFO was first distinctly recognized in the specialized literature in 2020 (3). Despite its formal recognition, substantial gaps persist in our understanding of the pathophysiological mechanisms underlying PFO-associated strokes, as well as the reasons why some individuals with PFO experience ischemic events while others remain asymptomatic throughout their lives. There is also a lack of recommendations concerning effective strategies for preventing initial ischemic cerebrovascular events in younger patients with PFO.

Additionally, the fact that PFO-associated ischemic stroke has quite a low incidence in the general population, estimated at about 4% of the total number of ischemic strokes, is probably a factor contributing to the lesser attention it has been afforded compared to atherothrombotic or cardioembolic strokes (21). However, it occurs particularly in the young (22), leading to notable social and economic repercussions (23, 24). Apart from its role in ischemic stroke, PFO has also been associated with other pathologies, such as migraine (25) and, rarely, silent cerebrovascular disease (26). However, data on the relationship between these entities are also mostly speculative and based on epidemiological studies.

Despite the efforts of the scientific community to clarify the role of PFO in neurological disorders, the reality is that the only substantial evidence, currently applicable in medical practice and derived from methodologically rigorous studies, pertains to the secondary prevention of ischemic strokes in young patients with PFO. The pathophysiology of ischemic stroke associated with PFO, predisposing factors for initial strokes, the potential role of migraines in these events, and the presence of silent ischemic cerebrovascular disease in PFO patients remain largely unexplored.

This doctoral thesis seeks to address these critical gaps by identifying the various factors that may convert a typically 'benign' PFO into one capable of precipitating ischemic strokes or inducing a silent cerebrovascular disease. For this purpose, 4 studies were conducted, whose study populations overlap. The first one ('Analysis of risk factors for ischemic stroke in patients with PFO') is an observational, cross-sectional, case-control study, which consecutively enrolled patients aged 18-65 years, evaluated in the Neurology Department of the University Emergency Hospital of Bucharest, between January 2018 and December 2023. The first working hypothesis proposed in this study explores the idea that certain anatomical, biological or demographic factors increase the susceptibility of patients with PFO to suffer an ischemic stroke. Consequently, the main objective of this research was to identify those risk factors associated with an increased risk for ischemic stroke in patients with a PFO. A second primary objective was to develop a risk score to assess ischemic cerebrovascular risk among patients with PFO. The purpose of this score is to facilitate the identification of PFO patients who may benefit from personalized preventive interventions based on their individual risk.

The second study ('Prospective analysis of the risk of ischemic cerebrovascular events in people with PFO'), is a prospective study in which the occurrence of a new ischemic cerebrovascular event in patients with PFO was followed longitudinally. The study represents a subgroup analysis of patients enrolled in the first study between January 2018 and January 2023. The main objective of the study was to estimate the incidence of new or recurrent ischemic cerebrovascular events associated with PFO, as well as to identify risk factors contributing to their occurrence. Secondary objectives of this study were to evaluate the performance of the score developed in the first study in identifying patients at risk for a new ischemic stroke, to determine the frequency of residual shunt among patients undergoing PFO closure, and whether the persistence of postprocedural shunt increases the risk of recurrence of ischemic cerebrovascular events.

The third study ('Silent ischemic cerebrovascular disease in patients with PFO') is a retrospective, case-control study whose population was selected from the population enrolled in the first study. Only patients who had brain magnetic resonance imaging (MRI) examinations compatible with the Quantib^{TM} analysis system were selected for this study. The main objective of this study was to investigate the hypothesis of the existence of a silent ischemic cerebrovascular disease in patients with PFO. The secondary objectives of this study were to identify potential risk factors for the development of silent cerebrovascular disease in patients with PFO and to evaluate the impact of silent cerebral white matter lesions on the cognitive performance of patients with PFO.

The fourth and final study of this doctoral thesis, titled 'The relationship between headache and cerebrovascular disease in patients with patent foramen ovale', is a retrospective, observational study. The participant pool, like in the previous studies, was selected from the cohort enrolled in the first study. The research hypothesis is based on the existing literature, suggesting that patients with a cryptogenic ischemic stroke who also experience migraine with aura exhibit a significantly high prevalence of ischemic stroke (27). Therefore, through this study, I aimed to investigate the prevalence of headache among the young patients having suffered an ischemic stroke, categorizing headache types according to the 3rd edition of the International Classification of Headache Disorders (ICHD-3) (28). Additionally, the study sought to determine whether patients with specific types of headaches have an increased risk of ischemic cerebrovascular events in patients with PFO.

Patient selection and data recorded for statistical analysis

The main inclusion criterion across all four studies was a diagnosis of ischemic stroke or TIA associated with PFO. For establishing the diagnosis the presence of a non-lacunar ischemic stroke (with measured dimensions > 1.5 cm) was required, confirmed by brain MRI or CT scans, or TIA in which any other plausible etiology for the occurrence of ischemic stroke was excluded (3). This was done after an extensive clinical, biological and imaging evaluation, which included: ultrasonographic evaluation of the cervico-cerebral vessels and/or contrast-enhanced CT examination of the supra-aortic and intracerebral vessels (in cases where ultrasonography could not adequately explore the intracerebral vasculature). Additionally, cardiac rhythm was monitored using Holter monitoring for a minimum of 24 hours. Comprehensive cardiac evaluation was conducted using both trans-thoracic and transesophageal echocardiography to exclude any other cardiac pathologies with embolic potential.

The exclusion criteria for the studies included the presence of any pathology that could independently cause an ischemic stroke. These included atrial fibrillation, other cardiac arrhythmias with known cardioembolic potential such as atrial flutter, and significant atheromatosis of the cervico-cerebral vessels, defined specifically as a stenosis of the vascular lumen exceeding 50% (29), the presence of unstable atheromatous plaques as identified by ultrasonographic examination (30), arterial dissection, congestive heart failure with left ventricular ejection fraction under 50%, endocarditis, intracardiac thrombosis, multiple sclerosis, or autoimmune diseases with possible central nervous system (CNS) involvement.

The control group was composed of asymptomatic volunteers, including employees of the University Emergency Hospital of Bucharest, as well as patients who were evaluated in the neurology department during the same time period for symptoms such as vertigo or headache. To ensure the validity of comparisons, these control participants were selected based on the absence of any history of ischemic stroke, immune-mediated disorders, or systemic diseases with potential central nervous system involvement. To definitively exclude TIA in patients presenting with vertigo, it was required that the symptoms persist for more than 24 hours. Additionally, cerebral MRI was employed to rule out any acute ischemic lesions or other central nervous system pathologies (31). Patients who met the inclusion criteria were invited to be evaluated for the presence of a PFO by contrast-enhanced transcranial doppler (c-TCD), and if positive, further by transesophageal echocardiography (TEE) to identify and characterize the PFO. All people included in the control group were evaluated by ultrasonography of cervical and cerebral vessels, trans-thoracic cardiac ultrasound, and some of them also by TEE.

For all participants enrolled in the study, demographic and clinical data including age, sex, and medical history of hypertension (HT), diabetes mellitus (DM), excessive alcohol consumption, and tobacco use were collected for statistical analysis. For individuals who smoked, a smoking index (SI) was calculated using the formula: SI = (number of cigarette packs smoked per day) × (number of years of smoking). Additionally, the study retained results from specific biological tests, which included serum concentrations of triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine, fibrinogen, hemoglobin, as well as leukocytes and platelets counts (expressed per cubic millimeter – mm^3). While the absolute neutrophil and lymphocyte counts were recorded, these values were not directly included in the statistical analysis. Instead, the neutrophil-to-lymphocyte

ratio (NLR) was calculated and used as an inflammatory marker. Furthermore, detailed anatomical features of the patent foramen ovale (PFO) were meticulously documented through transthoracic and transesophageal echocardiography. This included measurements of PFO length, defined as the longest overlapping distance between the septum primum and septum secundum, and PFO height, the maximum distance between these structures during a Valsalva maneuver. Other noted features included the presence of an ASA, defined by a displacement of the interatrial wall greater than 10 mm, the Chiari network, the prominence of the Eustachian valve, and the severity of the shunt. Shunt severity was assessed using c-TCD and quantified on the Spencer logarithmic scale: grade 0 indicated no microembolic signals (MES); grade 1, 1–10 MES; grade 2, 11–30 MES; grade 3, 31–100 MES; grade 4, 101–300 MES; and grade 5, indicative of a 'curtain pattern' with more than 300 MES (32).

Subsequent to the initial assessment, participants of the second study underwent biannual follow-up examinations until the conclusion of the study period in December 2023. To ensure a minimum of two clinical evaluations per participant, enrollment was limited to individuals who commenced participation no later than January 2023. These follow-up assessments, conducted either in-person or via telephone interviews, included a comprehensive review of each patient's clinical status. Data recorded for statistical analysis included the incidence of any new acute cerebrovascular events, specifying the type of event when applicable. Additionally, information was gathered regarding antithrombotic therapies administered during the preceding six months and whether the patients had undergone PFO closure, specifying whether the closure was achieved through surgical or interventional radiological methods.

In cases where patients had undergone PFO closure, c-TCD ultrasonography was conducted every six months during the first year post-closure to monitor for any residual shunts. As in the first study, shunt severity was assessed by using c-TCD, quantified on the Spencer logarithmic scale (grade 0–5) and recorded for analysis. Participants from the initial study who declined to continue the monitoring process for at least 12 months, or who could not be reached by telephone for follow-up assessments, were excluded from the secondary data analysis.

The third study followed the general inclusion and exclusion criteria previously established. It further specified additional criteria for inclusion based on the quality of brain imaging assessments. Participants were required to have brain MRI scans of a quality sufficient for analysis with the semi-automated Quantib^{TM} system (Quantib BV, Rotterdam,

The Netherlands). For the statistical analysis, both the volumes of cerebral white matter lesions resulting after an ischemic stroke (where applicable) and silent cerebral white matter lesions, measured using the Quantib^{TM} system, were retained, alongside results from neuropsychological tests designed to evaluate cognitive status. These included the Mini-Mental State Examination (MMSE) (13) and the clock drawing test (14).

The fourth study was a retrospective study, whose patients were also selected from those included in the first study, the inclusion and exclusion criteria being identical to those previously presented. In addition to the data collected for the first study, data concerning the presence and type of headache in patients with PFO were recorded for statistical analysis. These data were extracted from both physical and electronic medical records of the participants. When information in the medical reports was deemed incomplete or inaccurate, a telephone interview was conducted to gather comprehensive data. To accurately classify the type of headache, a standardized interview was employed, which was developed in accordance with the third edition of the International Classification of Headache Disorders (ICHD-3) (33), thus allowing for the standardization of the diagnosis and the comparison of the results with those of other international studies. Patients lacking complete data, those who could not be contacted by telephone despite multiple attempts, or who declined participation were excluded from the analysis.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 29.0 (Armonk, NY: IBM Corp).

The study was approved by the Ethics Committee of the Bucharest University Emergency Hospital, approval number 38721, and complied with international ethical norms and the basic principles of bioethics. Subjects' participation was based on informed consent obtained in accordance with the Helsinki guidelines.

2.2. Study 1: Analysis of risk factors for ischemic stroke in patients with PFO

The first study aimed to identify factors associated with an increased risk for the occurrence of ischemic cerebrovascular events in patients with PFO and to model a score capable of dynamically estimating ischemic cerebrovascular risk associated with PFO.

The study included a cohort of 182 individuals with PFO, who had an average age of 41.92 ± 10.83 years and a balanced gender distribution (55.49% female and 44.51% male).

Of these participants, 122 (67.03%) were diagnosed with PFO-associated ischemic stroke, while the remaining 60 (32.97%) represented the asymptomatic control group.

The average age of patients with PFO-associated ischemic stroke (40.53 ± 10.74) was not significantly different from those with asymptomatic PFO (42.60 ± 10.85), t(180)=-1.21, p=.228. However, gender distribution analysis showed a male predominance among patients with ischemic PFO-associated stroke, with women showing a significantly lower risk of ischemic stroke compared to men (OR = 0.28, CI 95% [0.14, 0.56]).

In terms of risk factor analysis, hypertension and excessive alcohol consumption emerged as significant risk factors for cerebrovascular ischemic events in PFO patients (OR = 5.64, 95% CI [2.25, 14.14] and (OR = 5.35, 95% CI [1.20-23.79]). Additionally, although diabetes and chronic smoking were observed more frequently among patients with a history of ischemic stroke than in those with asymptomatic PFO, these differences did not reach statistical significance.

Statistical analysis of laboratory tests indicated that patients with a history of PFO associated stroke had significantly higher concentrations of serum triglycerides (99.50 mg/dL, IQR 71.50-142.00) and lower concentrations of serum HDL (45.00 mg/dL, IQR 37.00-56.00), compared to patients with asymptomatic PFO (58.00 mg/dL, IQR 56.00-109.00 and 51.30 mg/dL, IQR 48.00-58.00, respectively).

Analysis of the NLR revealed significant differences between the two groups. Patients with PFO-associated stroke/TIA had significantly higher median NLR values (2.56 [1.84-3.47]) compared to those with asymptomatic PFO, (2.00 [1.62-2.66]), (U = 3452.50, p = .007), with a standardized test value of Z=2.71. Further stratification of NLR data indicated that the odds of having an NLR greater than 3 were significantly higher among stroke patients compared to those without a stroke history (Odds Ratio = 2.83, 95% CI [1.22, 6.54], p = .017).

No significant statistical differences were observed in the analysis of various thrombophilia tests, with similar frequency distributions across both groups.

Concerning the anatomical features of PFOs, analysis showed that PFO height was significantly greater in patients with a history of ischemic stroke (3.50 mm, IQR 2.00-4.50) compared to those with asymptomatic PFO (2.00 mm, IQR 2.00-3.00). However, other

characteristics, including shunt severity assessed by c-TCD, did not exhibit significant differences between the groups.

Factors that showed significant statistical differences in univariate analyses were subsequently incorporated into a binomial logistic regression model to refine the prediction of ischemic stroke occurrence in PFO patients. In alignment with the literature data, and to enhance the model's predictive accuracy, the presence of an ASA was also included in the regression analysis (34).

Thus, the binomial logistic regression model was conducted to assess the effects of the presence of hypertension, excessive alcohol consumption, serum HDL concentrations, NLR, and PFO-specific anatomical characteristics such as the presence of ASA aneurysm and PFO height, on the likelihood of ischemic stroke in PFO patients. The resulting model was statistically significant $\chi2(7) = 36.052$, $p < .001$, explaining 27.8% of the variance in the diagnosis of ischemic PFO-associated stroke as indicated by the Nagelkerke R2. It correctly classified 72.1% of cases, with a sensitivity of 85.2% and a specificity of 42.0%. The model's ability to discriminate between patients with and without ischemic stroke was medium to high, as reflected by an Area Under the Curve (AUC) of 0.778 (95% CI 0.706-0.850).

Detailed analysis of the model revealed that hypertension was the most significant predictor (B = 1.43, SE = 0.50, p = .005, OR = 4.16, 95% CI [1.51, 11.17]). Gender analysis further indicated that women had a statistically significantly lower risk of developing ischemic stroke associated with PFO compared to men (B = -1.01, SE = 0.43, p = .018, OR = 0.37, 95% CI [0.16, 0.84]), echoing the findings from the univariate analysis.

Contrary to the results of the univariate analysis, where ethanol consumption emerged as a significant differentiator between groups with and without a history of stroke/TIA, this factor did not retain statistical significance in the multivariate analysis. Nonetheless, the inclusion of ethanol consumption as a predictor enhanced the overall predictive capability of the model. A similar observation was made regarding serum HDL concentrations; while independently these did not show a statistically significant effect on the risk of PFO-associated ischemic stroke in the multivariate context, their inclusion in the model notably improved its discriminatory capacity. This improvement is evidenced by the receiver operating characteristic (ROC) curve, which yielded an AUC of 0.778, alongside increases in both sensitivity and specificity of the model. These findings underscore that while ethanol consumption may not be a significant independent predictor, its role as a

contributing factor in the pathogenesis of ischemic stroke associated with PFO warrants further investigation.

The NLR value was also identified as a significant risk factor in differentiating the two groups, an increase of 1 unit in this ratio being associated with an increase in the probability of the diagnosis of ischemic PFO associated stroke of up to 45%.

Although in univariate analysis the presence of ASA aneurysm did not reach statistical significance in differentiating patients with asymptomatic PFO from those with ischemic stroke/TIA, it was entered into the logistic regression model based on literature data suggesting that it plays an important role in the pathogenesis of cerebrovascular disease. Thus, in the multivariate analysis, the presence of the ASA aneurysm was shown to have a significant role in explaining the diagnosis of ischemic stroke in patients with PFO. Conversely, while there was an observed trend that suggested an increased probability of ischemic stroke diagnosis with greater PFO height, this relationship did not achieve statistical significance ($p = .185$).

Based on the results of the logistic regression test, a score was developed to calculate the cerebrovascular risk in patients with PFO.

The resulting risk score formula was = (1.43 × hypertension) + (-1.01 × Sex) + (1.13 × ASA) + (-0.02 × HDL) + (0.37 × NLR) + (0.17 × PFO Height) + (0.49 × Excessive ethanol consumption) + 0.83. The presence of an ASA, female gender, and alcohol consumption are scored as binary variables, contributing one point each if present. HDL, NLR, and PFO height values are inputted directly into the formula.

To convert the risk score into a percentage probability of cerebrovascular risk in PFO patients, the score is entered into the following formula:$= \frac{e^{\log odds}}{1+ e^{\log odds}}$, where e is the base of the natural logarithm, approximately equal to 2.72, and 'log odds' represents the calculated score. The probability intervals for categorizing ischemic cerebrovascular risk into low, medium, or high were determined based on sensitivity and specificity metrics derived from the ROC curve and the Youden index: 1) low ischemic cerebrovascular (CVI) risk for scores <60% (sensitivity 80%, specificity 60%), 2) medium CVI risk for scores between 60-75% (sensitivity 67%, specificity 76%), and 3) high CVI risk for scores ≥76% (sensitivity 44%, specificity 90%). To simplify further discussion of the developed score, we propose the name CVI-PFO score.

In conclusion, this study has successfully identified several key demographic, biological, and anatomical risk factors that are more prevalent among patients with ischemic stroke compared to those with asymptomatic PFO. Notably, hypertension, male sex, chronic ethanol consumption, elevated PFO height as measured by TEE, altered serum HDL levels, and an abnormal NLR were all significantly associated with the diagnosis of ischemic stroke/TIA associated with PFO. Using these findings, a binary logistic regression model was constructed that included the presence of an ASA as an additional predictor. This model was able to accurately predict ischemic cerebrovascular disease in approximately 70% of patients presenting with PFO.

To enhance the robustness of the predictive model, it was validated using advanced statistical methods, including neural network analysis, which can examine non-linear relationships among variables. Based on the logistic regression results, we developed a cerebrovascular disease risk score, the CVI-PFO score, capable of stratifying patients into three categories of cerebrovascular disease risk: low, medium, and high. This tool offers a nuanced approach to risk assessment, aiding in the clinical decision-making process for patients with PFO, potentially guiding targeted interventions based on individual risk profiles.

2.3. Study 2. Prospective analysis of the risk of cerebrovascular events in patients with PFO

Long-term follow-up data were available for 167 patients who were enrolled in the study, with a mean follow-up duration of 19.67 ± 10.84 months. The cohort had an average age of 42.07 ± 10.86 years, and comprised a higher proportion of females (95, 56.89%) compared to males (72, 43.11%). Of these participants, 113 (67.66%) had experienced a previous ischemic stroke, while 54 (32.34%) were categorized as having asymptomatic PFO. In terms of treatment, 58 patients (34.73%) underwent PFO closure, with 4 receiving surgical closure and 54 undergoing interventional PFO device-closure.

A new ischemic cerebrovascular event occurred in 4 (2.40%) of the 167 monitored subjects, of which 2 ischemic strokes and 2 TIAs. All acute ischemic cerebrovascular events occurring during the follow-up period occurred in patients with a history of ischemic stroke/TIA associated with PFO, while none of the patients with asymptomatic PFO experienced an ischemic cerebrovascular event during the follow-up period. Three of them had previous ischemic stroke and one had a TIA. Thus, we can estimate the risk of recurrence

of ischemic cerebrovascular events in the studied population at 2.83%. Further analysis was performed to calculate the stroke recurrence rate per 100 patient-years. In the group of patients with PFO and a history of ischemic stroke/TIA, who were followed for a total of 273.08 patient-years, the recurrence rate of ischemic events was determined to be 2.16 per 100 patient-years (CI 95%, 0.04-4.28).

An assessment of the recurrence risk differentiated by treatment modality revealed that patients who underwent PFO closure exhibited a substantially lower risk of recurrence, calculated at 1.05 per 100 patient-years. This contrasts with a higher recurrence rate of 3.13 per 100 patient-years observed in patients who managed their condition with pharmacological treatment alone, following a history of ischemic PFO-associated stroke.

Due to the limited number of patients who experienced an acute ischemic stroke/TIA during the follow-up period, the possibility to perform advanced statistical tests was limited. Nonetheless, an analysis of the anatomical characteristics observed in TEE among patients who suffered a new ischemic event revealed notable differences. Specifically, these patients exhibited significantly greater medians for PFO height and length compared to those who did not experience a cerebral ischemic recurrence (height: 7.00 mm [6.00 - 8.00] vs. 3.00 mm [2.00 - 4.50]; length: 8.00 mm [3.00 - 11.00]). Moreover, three out of the four patients with recurrent ischemic events were found to have an ASA. Intriguingly, despite these anatomical predispositions, all four patients demonstrated only low to moderate interatrial shunt severity on the Spencer logarithmic scale, as assessed via c-TCD. Notably, the one patient who had undergone PFO closure displayed a residual shunt of grade 1 on the Spencer scale during c-TCD assessment.

With regard to the CVI-PFO score, developed in the first study of this doctoral thesis, it was observed that the average score for patients who experienced a recurrent stroke or TIA was significantly higher at 93.65% (±3.90%) compared to 82.25% (±15.64%) in patients with a history of ischemic stroke but without recurrence, and 65.73% (±15.18%) in patients with asymptomatic PFO. Furthermore, according to the classification described in the first study, all 4 patients who had recurrent ischemic strokes were in the high-risk group for ischemic cerebrovascular disease and constituted 3.85% of the total number of patients categorized as high risk in the study.

One of the secondary objectives of this study was to assess the frequency of residual shunts in patients who underwent PFO closure, through either surgical or interventional

methods, and to explore the potential role of these shunts in recurrent cerebrovascular events. The incidence of residual shunt post-PFO closure aligned with existing literature, estimated at approximately 25% one year following the procedure. Notably, the frequency and severity of residual shunts observed at the 12-month follow-up were reduced compared to the 6-month assessments, a change likely attributable to progressive endothelialization of the closure device.

Although the sole patient with a closed PFO who experienced a recurrent ischemic event during follow-up exhibited a residual shunt, the limited number of such cases limits any definitive assessment of the shunt's role in precipitating ischemic strokes. Consequently, while suggestive, the link between residual shunts and recurrent cerebrovascular events could not be conclusively determined through advanced statistical analysis given the available data.

2.4. Study 3 - Silent cerebrovascular disease in patients with PFO

The third study, following additional inclusion and exclusion criteria, encompassed 67 patients. Within this cohort, 41 individuals (61.19%) were diagnosed with ischemic PFO-associated stroke, while the remaining 26 (38.81%) were categorized as having asymptomatic PFO. The mean age of participants was 40.00 ± 10.62 years, with females constituting a slight majority (38 persons, 56.72%) over males (29 persons, 43.28%).

A comparative analysis between the two groups revealed no significant age differences. However, the gender distribution displayed a statistically significant disparity; the group with a history of ischemic stroke/TIA was predominantly male (23 individuals, 56.10%), in contrast to the asymptomatic PFO group, where males only comprised 23.07% (6 individuals). This difference was statistically significant, $\chi 2 = 7.06$, $p = 0.011$.

Volumetric analysis of cerebral white matter lesions in patients with PFO was conducted to assess differences between total lesion volumes, which included both ischemic stroke-related lesions and clinically silent lesions. This comparison revealed no statistically significant differences between patients with a history of ischemic PFO-associated stroke and those with asymptomatic PFO regarding the overall lesion volume. However, in the strict comparative analysis of the volume of silent cerebral white matter lesions, excluding those

related to overt ischemic strokes, it was observed that patients with asymptomatic PFO exhibited a significantly higher volume of silent lesions (median 0.27 cm^3, [interquartile range 0.03 - 0.60]) compared to those who had experienced a previous ischemic stroke (median 0.08 cm^3, [IQR 0.02 - 0.18]), with a Mann-Whitney U value of 351.50 and a p-value of .019. This disparity remained statistically significant even after adjusting for classic cardiovascular and cerebrovascular risk factors such as age, sex, hypertension, glomerular filtration rate (GFR), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides, ($F=7.27$, $p=0.010$). These findings suggest that the burden of silent cerebral lesions may be greater in patients with asymptomatic PFO, underscoring the potential for subclinical vascular changes in this population.

In the study's assessment of classic cardiovascular and demographic risk factors, as well as the anatomical characteristics of patent foramen ovale (PFO), no statistically significant associations were found with the volume of silent cerebral white matter lesions. However, there was a notable trend indicating a positive relationship between patient age and the volume of silent cerebral lesions, suggesting an increase in lesion burden correlating with advancing age.

Further exploratory analysis on the relationship between PFO dimensions and the volume of silent cerebral lesions revealed nuanced findings. Specifically, when considered in the context of a patient's history of ischemic stroke or TIA, a greater PFO height was associated with greater volumes of silent cerebral white matter lesions only among patients with a history of ischemic events. Conversely, in patients with asymptomatic PFO, the largest volumes of silent cerebral lesions were observed in those with lower PFO heights. This observation suggests the potential existence of two distinct pathophysiological mechanisms contributing to the development of cerebral white matter lesions.

The impact of silent cerebral white matter lesions on cognition has been documented in patients with other comorbidities such as hypertension, diabetes, or atrial fibrillation (35, 36). However, the specific role of these lesions in contributing to neurocognitive disorders in patients with PFO remains unexplored. In this study, a notable observation was that patients with larger volumes of silent cerebral white matter lesions exhibited a poorer cognitive performance, as evidenced by lower scores on the clock drawing test and the MMSE. Although these findings did not achieve statistical significance, it is my opinion that it reflects the reality of clinical practice. Nevertheless, future research should aim to include a larger cohort of patients and perhaps integrate more sensitive cognitive assessment tools

to thoroughly investigate the cognitive consequences of silent cerebral lesions in individuals with PFO. This approach would not only validate the current findings but also enhance our understanding of the broader implications of PFO-associated cerebral pathology.

2.5. Study 4: Relationship between headache and cerebrovascular disease in patients with patent foramen ovale

The fourth study included 177 participants with an average age of 42.29 ± 10.76 years, of whom 97 (54.80%) were female. The analysis identified four types of primary headaches according to the ICHD-3 criteria within the PFO population. Tension-type headache was the most prevalent, affecting 27 patients (15.25%), followed by migraine without aura in 20 patients (11.30%), and migraine with aura in 15 patients (8.47%). Additionally, a solitary case of autonomic trigeminal headache, specifically short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing (SUNCT), was diagnosed in one patient (0.56%).

An intriguing finding from this study pertains to the differences in headache types between patients with PFO who have a history of ischemic stroke and those with asymptomatic PFO. Notably, patients with asymptomatic PFO experienced migraines more frequently than those with a history of ischemic stroke associated with PFO, who displayed an equal distribution of tension headaches and migraines. Contrary to existing literature (37), our data reveal that migraine without aura was more commonly reported among patients with a history of ischemic stroke, whereas migraine with aura was predominantly observed in the asymptomatic PFO group.

The analysis of the anatomical features of patent foramen ovale (PFO) revealed no significant role in the occurrence of headaches among PFO patients. Interestingly, however, the data indicated that the intracardiac shunt was more severe in patients who did not experience headaches. Further comparison across different headache types showed variations in shunt severity: consistent with findings by Moaref et al. (38), patients with migraine with aura exhibited the highest degree of shunt severity. Conversely, those with tension-type headaches presented with a lower severity of the right-to-left intracardiac shunt.

To our knowledge, this is the first study to systematically explore the types of headaches encountered in patients with PFO and to investigate the association between these headaches and the occurrence of PFO associated stroke. While providing valuable insights,

the study is not without limitations. Being retrospective and observational in nature, it constrains the ability to establish causal relationships between the types of headaches, the presence of PFO, and the occurrence of ischemic cerebrovascular events.

3. Conclusions and personal contributions

This doctoral thesis contributes to our understanding of the spectrum of cerebrovascular diseases associated with PFO and offers new perspectives on the evaluation of patients with PFO who have not yet experienced an ischemic cerebrovascular event. The four studies conducted as part of this research demonstrate that the risk of ischemic stroke in patients with PFO is a dynamic process, influenced not only by anatomical characteristics of the PFO, but also by situational factors that could precipitate the ischemic events. Thus, our findings suggest that ischemic stroke associated with PFO is not solely a result of singular etiological factors. Rather, it likely occurs in individuals with specific anatomical predispositions, such as an ASA or elevated PFO height, who are also exposed to particular conditions that promote thrombogenesis, whether due to metabolic imbalances, or systemic inflammation.

An important contribution of this study is that it allowed a deeper understanding of the mechanisms underlying ischemic cerebrovascular events in patients with PFO, but also identified various risk factors for ischemic stroke associated with PFO, including modifiable ones. While the results of this research are promising, they require validation in larger patient cohorts through prospective studies to confirm their generalizability and reliability. Nonetheless, these findings mark a pivotal step toward the development of personalized treatment strategies aimed at the primary prevention of ischemic stroke in patients with PFO, shifting from a one-size-fits-all method of managing PFO patients, to one tailored to individual risk profiles.

Another significant finding from this research is the finding that PFO contributes not only to clinically manifest ischemic strokes, but also to silent ischemic cerebrovascular disease. While these silent events do not produce acute neurological symptoms, they could be clinically significant, potentially impacting the long-term cognitive performance of affected individuals.

All four studies achieved their proposed objectives, the main conclusions being the following:

1. Classic cardio- and cerebrovascular risk factors, such as hypertension and excessive alcohol consumption, contribute significantly to the risk of PFO-related ischemic stroke.

2. Lower serum concentrations of HDL and higher neutrophil/lymphocyte ratios are associated with an increased likelihood of acute ischemic cerebrovascular events in PFO patients.

3. The presence of cardiovascular risk factors, particularly hypertension and excessive alcohol consumption, in conjunction with metabolic disorders, notably in patients with a tall PFO and ASA, markedly increases the risk of cerebral ischemia.

4. Based on the above conclusions, a novel risk assessment tool, the CVI-PFO score, was developed to dynamically evaluate the cerebrovascular ischemic risk in patients with PFO. This score successfully classified 72.1% of the cases into accurate risk categories during validation, and it allows the stratification of PFO-patients into low, medium, or high risk of cerebrovascular events, based on their individual clinical and biochemical profiles.

5. The presence of prothrombotic states does not influence the risk of cerebral ischemia in patients with PFO.

6. Patients who have experienced an initial PFO-related ischemic stroke exhibit a significantly higher risk of subsequent ischemic stroke or TIA compared to those with asymptomatic PFO.

7. The risk of ischemic stroke recurrence in patients with PFO is estimated at 2.16 per 100 patient-years (95% CI 0.04-4.28).

8. Patients with PFO-related ischemic stroke who benefit from PFO closure have a lower risk of recurrent ischemic cerebrovascular events (1.05 per 100 patient-years) than those pharmacologically treated (3.13 per 100 patient-years).

9. Approximately 30% of patients undergoing PFO closure exhibit a residual shunt at six months after the procedure. However, both the frequency and severity of these shunts progressively decrease, likely due to the endothelialization of the implanted device. The impact of residual shunts on the occurrence of ischemic stroke remains unclear, indicating the need for further study involving a larger cohort and extended monitoring to elucidate their clinical significance.

10. PFO can lead to silent ischemic cerebrovascular disease, characterized by the presence of a large volume of cerebral white matter lesions, with a potential role on cognitive performance. Intriguingly, the aforementioned risk factors associated with

PFO-related stroke do not appear to influence the incidence of this silent cerebrovascular pathology.

11. Patients with PFO exhibit a frequency of headache comparable to that of the general population. Furthermore, the presence and type of headache in these patients do not significantly influence the occurrence of ischemic cerebrovascular events.

This study emphasizes the significant role of modifiable metabolic factors in the occurrence of ischemic stroke among patients with PFO. However, it is likely that these factors do not account for all mechanisms contributing to PFO-related strokes. Consequently, it is imperative to broaden research initiatives to encompass the examination of more subtle biomarkers that identify PFO patients with an elevated risk for ischemic cerebrovascular incidents. Furthermore, longitudinal studies are required to determine whether targeted modifications of these metabolic factors can reliably and sustainably reduce the risk of ischemic strokes associated with PFO. Additionally, the present research reveals a novel, probably underrecognized pathological entity associated with PFO, namely silent ischemic cerebrovascular disease. Nevertheless, further studies are needed to investigate the impact of an increased load of cerebral white matter lesions on cognitive function and quality of life in PFO patients, as well as to delineate risk factors contributing to this pathology. Enlarging the patient cohort in future studies will be essential to validate these findings and extend their generalizability.

3.1. Personal contributions

- Identified risk factors for PFO associated stroke, some non-modifiable, such as the height of the PFO and the presence of interatrial septal aneurysm, but also some modifiable risk factors, such as hypertension, excessive alcohol consumption and low serum HDL values (Chapter 5.3.2 - Risk factors for ischemic stroke or TIA associated with PFO).

- Developed the CVI-PFO score, a dynamic assessment tool for estimating the cerebral ischemic risk in patients with PFO (Chapter 5.3.4. Modeling of a cerebrovascular risk score in patients with PFO).

- Analyzed the recurrence of ischemic strokes in patients with PFO, comparing the effectiveness of PFO closure versus pharmacological treatment in preventing new ischemic cerebrovascular events (chapter 6.3.3. Incidence of new ischemic cerebrovascular events in patients with PFO).

- Established the frequency of residual shunts following PFO closure (chapter 6.3.2. Residual shunt).

- Conducted the first objective and quantitative evaluation of cerebral lesion volume in patients with PFO, identifying silent ischemic cerebrovascular disease as a new pathological condition associated with PFO (chapter 7.3.2. Volume of cerebral white matter lesions in patients with PFO).

- Described the frequency and types of headaches in patients with PFO according to the ICHD-3 classification, investigating their influence on the incidence of ischemic strokes associated with PFO (chapter 8.3.2. Types of headaches according to the ICHD-3 classification in patients with PFO).

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1. **Badea, R. Ş.**, Ribigan, A. C., Grecu, N., Terecoasă, E., Antochi, F. A., Bâldea Mihăilă, S., Tiu, C., & Popescu, B. O. (2023). Differences in clinical and biological factors between patients with PFO-related stroke and patients with PFO and no cerebral vascular events. Frontiers in neurology, Volumul 14, 1104674. **IF 3.393 –** capitolul 5

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