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Closing the gap

Between patent foramen ovale and ischemic stroke

Maikel H.M. Immens

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Closing the gap

Between patent foramen ovale and ischemic stroke

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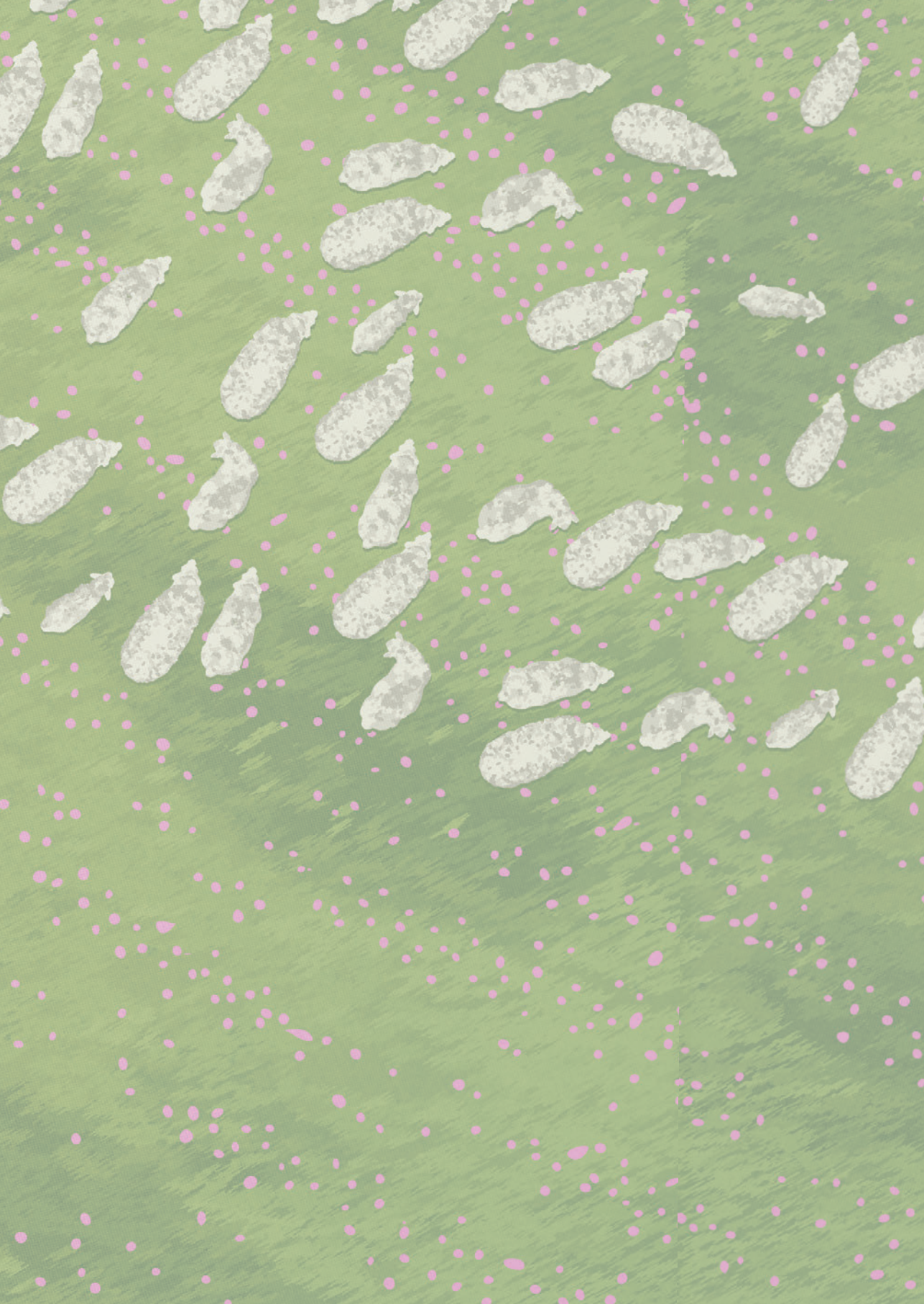
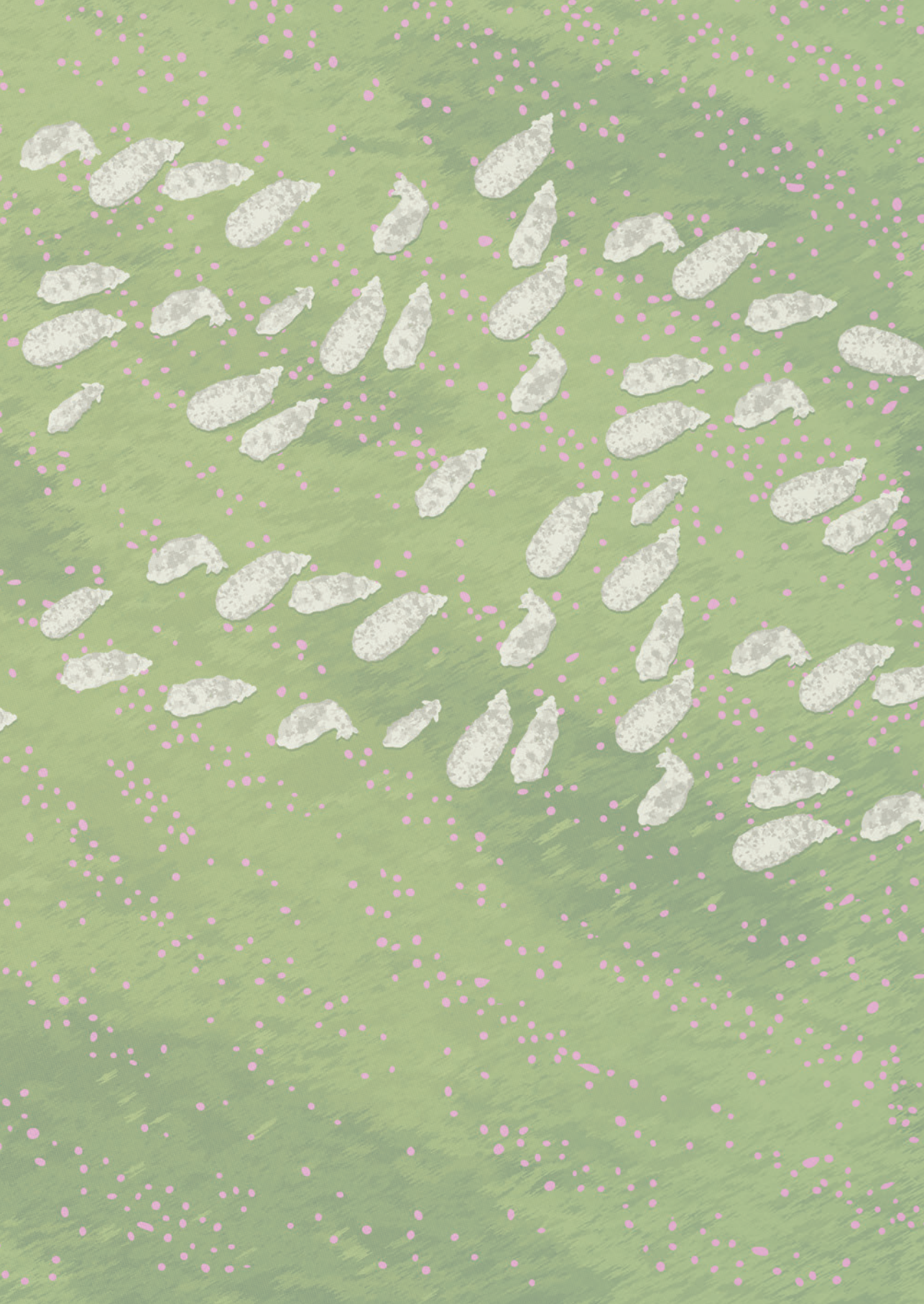


Part I

Introduction



1

General introduction, aims and outline

Ischemic stroke in young patients

Stroke is the leading cause of disability worldwide.[1] Approximately 10-15% of all strokes occur in people aged 18-50 years.[2] Since these young adults are in the prime of their lives—working, building a family and developing new skills—the consequences of stroke can be devastating, leading to significant socio-economic problems. To prevent recurrent strokes, it is crucial to identify the cause as accurately as possible, since different etiologies require different treatments. The cause of stroke is often classified by the so-called TOAST criteria (Trial of Org 10172 in Acute Stroke Treatment), which distinguish five major causes, with "large vessel atherosclerosis" being the most prevalent.[3] In contrast to elderly individuals, strokes in young adults are less likely to be caused by cardiovascular risk factors leading to atherosclerosis or small vessel disease. A relatively common cause of stroke, particularly in young adults, is a patent foramen ovale (PFO).

Patent foramen ovale associated stroke

PFO-associated stroke accounts for approximately 12% of strokes in young adults.[4] PFO is a congenital variant, failed post-partum closure of the atrial septum, which is very common (prevalence ranges from 15-31%) in the general population.[5, 6] PFO can facilitate right-to-left shunting of deoxygenate blood. Intracardiac shunting can be responsible for dyspnea and hypoxemia (e.g. Platypnea-orthodeoxia syndrome) but is also thought to be a cause of stroke. The hypothesized mechanism by which a PFO causes a stroke includes the passage of a thrombus, presumably originating from the venous circulation, then bypassing the lung circulation through the orifice of the PFO, into the arterial circulation (i.e. paradoxical embolism hypothesis). Histological analysis of thrombi from PFO-associated strokes may help determine whether they are of venous origin. An alternative pathogenesis involves the development of thrombi within the tunnel of the PFO itself. A study by Yan et al. (2023) using optical coherence tomography (OCT) demonstrated an exceptionally high frequency of in situ thrombi within the PFO tunnel in patients with stroke, while no thrombi were found in asymptomatic individuals with a PFO. This suggests that in situ thrombus formation contributes to stroke in patients with a PFO.[7] Another potential cause of PFO-associated stroke could be changes in the left atrial function due to alterations in hemodynamics caused by the PFO.[8] PFO facilitates right-to-left shunting and these changes in hemodynamics could cause turbulent blood flow and stasis of blood, therefore mimic an "atrial fibrillation like" pathogenesis for stroke.[9] Nowadays, left atrial function can be assessed using left atrial strain (LAS).

LAS is a measure of atrial wall deformation during cardiac cycle.[10] It is increasingly used to evaluate cardiac function in cardiovascular diseases[11] and can be quantified using transthoracic echocardiography (TTE) and two-dimensional speckle tracking echocardiography (2D-STE).[10] The LAS reflects left atrial function, which may be impaired in patients with PFO-associated stroke. A fundamental step in finding a treatment is understanding the underlying causes and mechanisms of the disease. To date, the true pathogenesis of a PFO-associated stroke remains unresolved. Having an understanding of where the thrombi in PFO-associated stroke originate is an essential first step toward optimizing individualized treatment strategies.

Pathological conversion

Contrary to vascular risk factors and atherosclerosis, a PFO is present from birth. However, it is completely unknown how an anatomical structure that is already present at birth in a large proportion of the population can convert into a PFO that causes stroke in a few. The relatively new concept of trigger factors may elucidate this pathological conversion. A trigger factor is a short-lasting exposure to a trigger (i.e. toxins, caffeine, sexual activity, physical exercise, or infection), that may subsequently create a (short-lasting) condition (e.g. a prothrombotic state or increase in blood pressure) that may predispose to stroke.[12-14] To date, only one case series (n = 4) investigated the relation between a trigger factor (exercise-induced Valsalva) and PFO-associated stroke.[15, 16] In practice, only a small percentage of patients who experience a PFO-associated stroke reportedly performed a Valsalva-like maneuver prior to the event. Besides, more than 50% of patients with a PFO have permanent right-to-left shunting, also without a Valsalva-like maneuver.[17]

Recent studies reported a significant association between trigger factors (cola consumption, vigorous physical exercise, sexual activity, illicit drug use, fever and flu-like disease) and ischemic stroke in young adults (< 50 years).[18, 19] Better understanding of which trigger factors could convert a PFO into a stroke causing one may shine light on the pathophysiology and could help in de decision-making for PFO closure.

Treatment

In recent years, studies have shown that the most effective way to treat a PFO-associated stroke is through percutaneous closure, which involves sealing the orifice using a double-disc occluder device (Figure 1). After several randomized

clinical trials that reported no effect on recurrent ischemic stroke following PFO closure, four newer trials demonstrated that PFO closure was beneficial in preventing ischemic strokes compared to best medical treatment.[20-23] The number needed to treat (NNT) to prevent one stroke during two years for PFO closure compared to best medical therapy remains relatively high - 21 for patients with high-risk anatomical PFO features and 37 overall.[24] Additionally, adverse events (e.g. atrial fibrillation) following closure are a realistic risk (approximately 5%).[25] Given the high prevalence of PFO in the general population, it is important to accurately identify which patients have a vulnerable "stroke-causing" PFO and which patients have an "innocent bystander" PFO. Furthermore, incorrectly treating patients with PFO closure when another cause of stroke is present will result in unnecessary treatment risk and prevent patients from receiving appropriate medical treatment regarding their true cause of stroke.

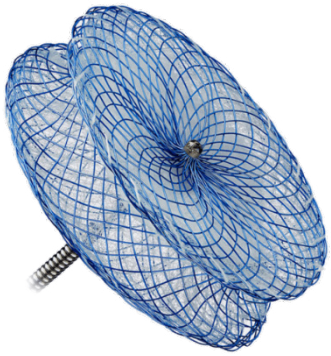


Figure 1. Image of the Amplatzer occluder by Abbott

Selecting for PFO closure

To estimate which patients would benefit most from PFO closure Kent et al. developed two scoring systems: the RoPE (Risk of Paradoxical Embolism) score and PASCAL (PFO-Associated Stroke Causal Likelihood) score. These scores estimate the likelihood that the PFO is pathologically related to the stroke, rather than being an "innocent bystander".[26, 27] While these scoring systems serve as helpful tools, they lack the specificity needed to determine which patients should undergo PFO closure. Due to the large uncertainty in the selection process and the heterogeneity between centers, both the European Association of Percutaneous Cardiovascular Interventions (EAPCI) and The Society for Cardiovascular Angiography and Interventions (SCAI) have published position papers on the management of patients with PFO and stroke.[5, 28] These papers advocate for the installment of

an interdisciplinary Heart-Stroke Team (HST), which should include at least a neurologist and an interventional cardiologist, to assess whether the stroke can be attributed to the PFO. The HST is expected to improve the selection process, by excluding patients who would not benefit from PFO closure. These exclusions could prevent unnecessary surgery and wrongly treated patients. Although, widely accepted, the HST has not yet been evaluated in terms of its added clinical value. In other words, there is no data available on the effect of the HST and on whether its implementation actually prevents unnecessary surgical procedures.

To determine which patients are most likely to benefit from PFO closure, patients undergo a thorough diagnostic work-up, with the outcome evaluated and discussed by a multidisciplinary team. Key factors in the assessment include: patient characteristics (e.g. risk factors and medical history), echocardiographic features of the PFO (e.g. diameter, shunt size, tunnel length, atrial function and presence of an atrial septal aneurysm), and stroke characteristics (imaging confirmation of the stroke/TIA, type of stroke [territorial vs. lacunar stroke], concomitant small vessel disease). Additionally, alternative, more likely causes of stroke (e.g. cervical artery dissection) should be excluded. To better understand the anatomical features of PFOs associated with stroke, it is important to analyze these characteristics in patients who have undergone PFO closure. Such an evaluation could inform how much weight should be given to anatomical features when considering closure. Especially since these characters are increasingly used in the selection process, take for example the PASCAL classification, which focusses on high-risk anatomical features of the PFO.[27]

Aims of this thesis

The aim of this thesis was to assess how a common anatomical variant, the patent foramen ovale, becomes vulnerable. Furthermore, we aimed to investigate how to distinguish vulnerable PFOs in stroke patients from innocent bystander PFOs. Finally, we aimed to gain a deeper understanding of the pathogenesis of PFO-associated stroke.

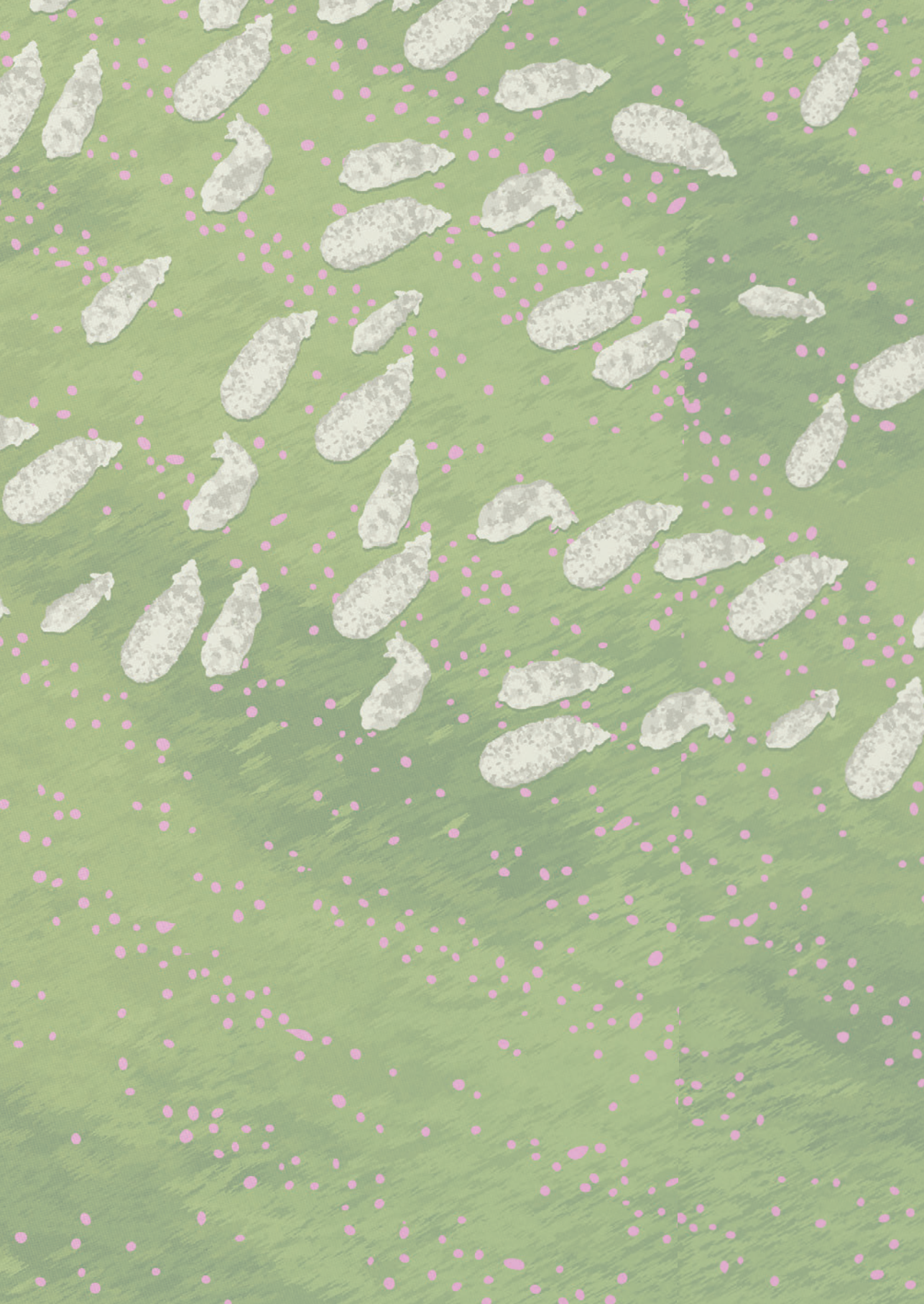
Outline of the thesis

In **chapter 2,** we evaluated the *anatomical* characteristics of the PFO of all patients with a PFO-associated stroke who underwent percutaneous closure at two congenital heart disease institutions in The Netherlands (Radboudumc and Amsterdam UMC) from 2016 to 2022. This retrospective analysis aimed to determine

the role of anatomical features in PFO-associated stroke. **chapter 3** discusses left atrial strain (LAS) as a *physiological* measurement for atrial function in patients with a stroke and PFO. It explores its potential role in the diagnostic work-up of these young patients and investigates whether atrial dysfunction contributes to the pathogenesis of PFO-associated strokes. To gain a better understanding of the pathogenesis of PFO-associated stroke, **chapter 4** compares the *histological* composition of thrombi from patients with PFO-associated stroke to venous thrombi from patients with iliofemoral deep venous thrombosis (DVT). Data on these thrombi were retrieved from the MR CLEAN Registry, a Dutch nationwide, multicenter, prospective registry of patients who underwent endovascular treatment (EVT) for ischemic stroke between March 2014 until June 2016. In **chapter 5**, we investigated *trigger factors* as potential risk contributors to PFO-associated stroke using data from the ODYSSEY cohort. The ODYSSEY study is a Dutch multicenter prospective cohort study on the risk factors and prognosis of 1492 patients aged 18 to 50 years with a first-ever ischemic stroke or intracerebral hemorrhage, in 17 centers in the Netherlands, between May 2013 and February 2021. **chapter 6** focuses on the role of an interdisciplinary HST in *deciding on PFO closure*, describing the team's diagnostic approach and management and the resultant proportion of patients accepted for closure.

The final part of the thesis includes a general discussion (**chapter 7**) and a Dutch summary (**chapter 8**).

Part of the studies reported in this thesis are based on data from the Observational Dutch Young Symptomatic StrokE studY (ODYSSEY)[29] and the Multicenter Randomized Clinical trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN) Registry.[30]



Part II

Anatomy

2

Anatomical features of percutaneously closed patent foramen ovale in patients with cryptogenic stroke

Published as

Immens MHM*; Lars S. Witte*; Abdelhak el Bouziani; Anthonie Duijnhouwer, Berto J. Bouma, Jan G.P. Tijssen, Frank-Erik de Leeuw, Rob J. de Winter and Tim J.F. ten Cate. Anatomical Features of Percutaneously Closed Patent Foramen Ovale In Patients With Cryptogenic Stroke. *Netherlands Heart Journal, 2025 October.*

*These authors contributed equally

Abstract

Background

Patent foramen ovale (PFO) is increasingly recognized as a cause of stroke, with a prevalence of approximately 25% in the general population. Consequently, the likelihood of encountering a 'bystander-PFO' in young patients who have experienced a stroke seems significant. To aid in identifying patients with a PFO related cryptogenic stroke, an interdisciplinary Heart-Stroke Team (HST) has been established. This team evaluates patients who suffered from stroke and were diagnosed with a PFO to assess its potential contribution. Understanding the anatomical features of PFOs associated with stroke is essential for decision-making. This study examines the PFO characteristics of all patients who underwent percutaneous PFO closure for cryptogenic stroke at two congenital heart disease institutions in The Netherlands.

Methods

Data on all patients that underwent PFO closure from 2016 to 2022 were collected. Anatomical characteristics were measured using transesophageal echocardiography and analyzed by two cardiologists.

Results

In total, 223 patients underwent PFO closure. Mean age was 42.8 ± 10.7 years, with 115 (51.6%) being male. Approximately 80% of all patients had at least one risk-enhancing PFO feature (moderate to severe shunt and/or atrial septal aneurysm of >10mm).

Conclusion

Although all patients accepted for percutaneous PFO closure were individually assessed by a dedicated HST, 20% had a PFO without risk-enhancing features but were still accepted for closure due to other reasons. This highlights the importance of careful individual assessment of young stroke patients with a PFO. Future studies are needed to identify the characteristics that contribute to stroke in these patients.

Background

The prevalence of patent foramen ovale (PFO) in the general population is approximately 25%.[6] PFO-induced right-to-left shunting (RLS) is recognized as a potential contributing factor to stroke, particularly in younger patients between 18 and 60 years.[31] A meta-analysis demonstrated that in younger patients (<55 years) with cryptogenic stroke, the prevalence of PFO was six times higher compared to young stroke patients with alternative etiologies.[32] This suggests a possible association between PFO and stroke.[33] Percutaneous closure of PFO has been shown to reduce the risk of recurrent stroke in selected patients.[20-22] Given the high prevalence of PFO in the general population, the likelihood of encountering a 'bystander-PFO' in young patients who experienced a stroke seems realistic.[34] To differentiate between patients with a PFO that is considered stroke-related and those with a bystander PFO, the interdisciplinary Heart-Stroke Team (HST) has been established.[35] This team systematically evaluates all stroke patients diagnosed with a PFO to assess its potential contribution to the cerebrovascular event. The evaluation process involves a comprehensive assessment, including neuroimaging, blood tests to screen for coagulability disorders, and a cardiac evaluation for identify potential cardiac sources of stroke. As part of this assessment, echocardiography is performed to confirm the presence of a PFO.

Several anatomical PFO characteristics have been associated with an increased risk of stroke.[27] These include the size of the right-to-left shunt (RLS) and various other anatomical characteristics,[36-39] which collectively enhance the likelihood that the PFO is causally related to stroke.[27] This study retrospectively evaluates the anatomical characteristics of all patients who underwent percutaneous PFO closure for cryptogenic stroke after acceptance by a dedicated HST at two congenital heart disease institutions in the Netherlands.

Methods

Patients

We retrospectively analyzed all consecutive patients that underwent percutaneous PFO closure with a double disc device in the Radboudumc, Nijmegen and Amsterdam UMC, Amsterdam, The Netherlands between 2016 and 2022. Patient who received PFO closure before the instigation of the HST (before July 2018 for Radboudumc and before January 2016 for Amsterdam UMC) were excluded.

All procedures were performed with either the Amplatzer PFO occluder or the Amplatzer Multifenestrated septal occluder (Abbott, Abbott Park, Illinois, USA). The decision on which device size was most suitable for the patients was left to the discretion of the implanting cardiologist. The decision was based on TEE findings during the procedure.

All patients underwent a standardized work-up to rule out other potential causes of stroke. This included at least 48 hour rhythm monitoring to rule out atrial fibrillation, imaging of the carotid arteries to rule out atherosclerosis, testing for coagulation disorders and saline contrast echocardiography to assess the RLS. Additional diagnostic tests were performed when indicated. Furthermore, classical cardiovascular risk factors were identified. The Risk of Paradoxical Embolism (RoPE) score was calculated for all patients.[26] Following this assessment, the HST reviewed all cases to determine the potential contribution of the PFO to the stroke. When the patients were assessed the PFO-Associated Stroke Causal Likelihood (PASCAL) classification was not yet available.[27] This classification may improve patient selection for PFO closure. To assess this in our series, the PASCAL-classification was retrospectively applied on the available data for all patients.

Echocardiography

All patients underwent standard TTE with saline contrast to determine the presence of a RLS and to rule out other cardiac causes of the stroke. Patients with an intracardiac thrombus, severe calcification or stenosis of the aortic or mitral valve were excluded for PFO closure.

All echocardiograms were analyzed by three independent cardiologists (TtC, AD, BB). Classification of the RLS was done by measuring the number of bubbles of agitated saline present in the left atrium within 5 heart beats after opacification of the right atrium. Patients who did not receive agitated saline as contrast but only color doppler were excluded. The Valsalva maneuver was applied if there was no

shunt at rest or if the referring cardiologist deemed Valsalva necessary to better evaluate the shunt size. Shunt severity was graded in 5 groups: none (no bubbles), small Grade 1 (1-5 bubbles), mild Grade 2 (6-25 bubbles), moderate Grade 3 (>25 bubbles) and severe Grade 4 (opacification of the left ventricle).

The results of TEE imaging were also recorded to further assess the PFO characteristics. Anatomical PFO characteristics were measured with standard TEE imaging (Epiq, Phillips, Best, The Netherlands and Vivid E95, GE Healthcare, Horten, Norway). The PFO size was measured with two-dimensional TEE before PFO intervention as an "unstretched diameter" in 114 patients (Figure 1) or with three-dimensional TEE during PFO intervention, after placement of a guidewire through the PFO resulting in a maximal "stretched diameter" in 109 patients (Figure 2). Atrial septal excursion was measured on an image with the best cross section of the atrial septum between 30 to 60 degrees (Figure 1).

As described in the PASCAL score, a risk-enhancing feature was defined as a Grade 3 shunt or higher and/or an ASA with at least 10mm of excursion from midline (see Figure 3) [10].

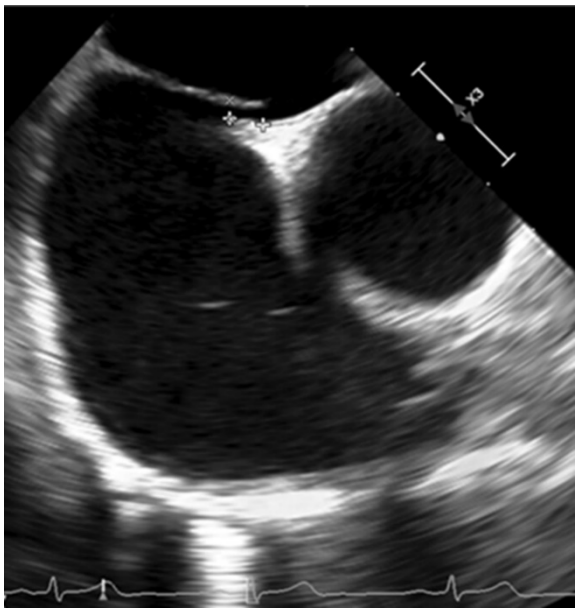


Figure 1. TTE image of an unstretched PFO

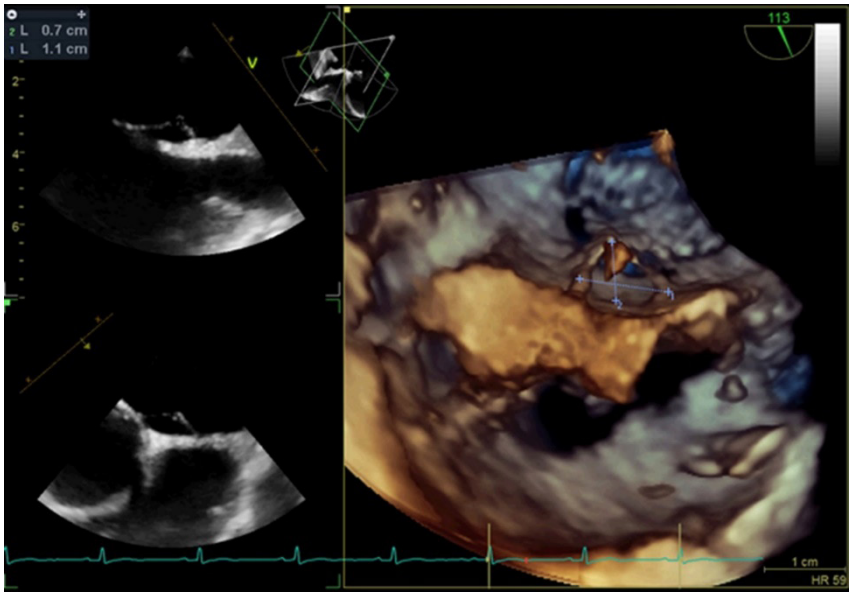


Figure 2. TEE image of a stretched PFO

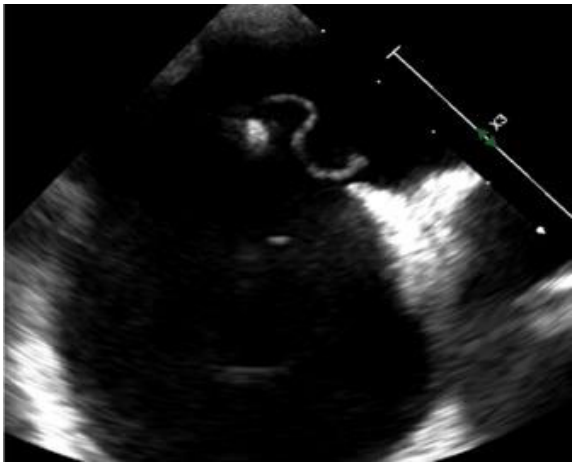


Figure 3. TTE image of a mobile ASA with an excursion of more than 15mm

Statistical analysis

Shapiro-Wilk Test was performed to test for normality. Categorical variables were summarized as the number of subjects with percentages, continuous variables with normal distribution as mean with standard deviation and continuous variables with non-normal distribution as median with interquartile ranges. Statistical analyses were performed with IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, New York).

Results

Between January 2016 and April 2022, 223 consecutive patients who underwent percutaneous PFO closure were included, 114 patients at Radboudumc, Nijmegen and 109 patient at Amsterdam UMC, Amsterdam, The Netherlands. The mean age was 42.8 ± 10.7 years, 115 patients (51.6%) were males, the median risk of paradoxical embolism (RoPE) score was 7 [IQR 6 - 8]. The RLS grade at baseline with contrast bubble study was small in 17 patients (7.6%), mild in 43 patients (19.2%), moderate in 67 patients (30.0%) and severe in 96 patients (43.0%). In 44 out of 223 patients (19.7%) Valsalva maneuver was not performed. In total, 73% had a Grade 3 shunt or higher. Of all patients included, 70 (31.4%) had an atrial septal aneurysm (ASA) (≥ 10mm excursion of the septum).

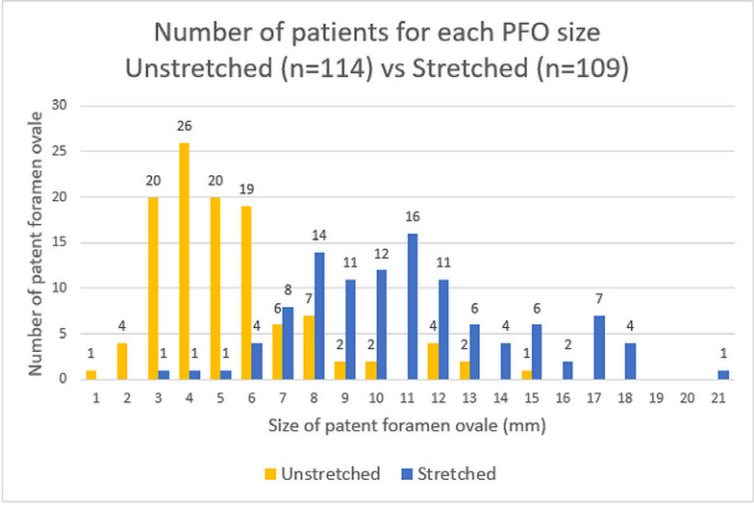


Figure 4. Distribution of the number of patients for each PFO size in mm. In yellow, the unstretched diameter opening, measured using echocardiography. In blue, the stretched diameter opening, measured during the closure procedure using a guidewire.

The mean PFO diameter in patients with cryptogenic stroke was 5.3 ± 2.5mm in the unstretched group and 11.0 ± 3.5mm in the stretched group (Figure 4). The PASCAL classification was retrospectively applied to all patients, the classification was unlikely in 14 patients (6.3%), possible in 109 patients (48.9%) and probable in 100 patients (44.8%) (Table 1).[26, 27] Overall, 80% of patients had at least one risk-enhancing feature, as defined in the PASCAL score.[27]

In our cohort, 82 patients had a RoPE-score <7. Of these, 68 patients were classified as having a possible PFO-associated stroke based on the PASCAL classification. This was due to a large shunt in 37 patients, a combination of a large shunt and an ASA in 26 patients, and the presence of an isolated ASA in 5 patients.

Table 1 Characteristics of patients who underwent PFO closure.

Number of patients	n= 223
Mean age at time of referral, years [SD]	42.8 [±10.7]
Male, N (%)	115 (51.6)
Median RoPE Score, [IQR]	7 [6 - 8]
PASCAL Classification System, N (%)	
Unlikely	14 (6.3%)
Possible	109 (48.9%)
Probable	100 (44.8%)
Shunt grade, N (%)	
Small	17 (7.6)
Mild	43 (19.2)
Moderate	67 (30.0)
Severe	96 (43.0)
≥ 10mm excursion of septum, N (%)	70 (31.4)
≥ 15mm excursion of septum, N (%)	35 (15.7)
Mean unstretched diameter opening, mm [SD]*	5.3 [±2.5]
Mean stretched diameter opening, mm [SD]†	11.0 [±3.5]
Any residual shunt after 6 months, N (%)	64 (28.7)
Any residual shunt after 12 months, N (%)	44 (19.7)

Shunt size is measured using contrast bubble study during TTE. The excursion of the septum and unstretched diameter opening are measured using echocardiography. The stretched diameter opening is measured during the closure procedure using a guidewire. Residual shunt is defined as any crossing of bubbles on TTE with contrast bubble study within 5 heartbeats.

* n= 114 unstretched diameter is measured using TEE, without manipulating the orifice.

† n= 109 stretched diameter is measured after placing a wire through the PFO orifice during the closure procedure, measuring the maximal stretched diameter.

PASCAL, PFO-associated stroke causal likelihood; *PFO,* patent foramen ovale; *RoPE,* risk of paradoxical embolism; *TTE,* transthoracic echocardiography

The 20% of patients without risk-enhancing features (median RoPE-score of 7, IQR 6-8) were accepted for PFO closure for various reasons: a high RoPE-score (21 patients), absence of an alternative cause of stroke (10 patients), multiple stroke events (7 patients), recurrent event despite antithrombotic therapy (2 patients), presence of Factor V Leiden without an alternative cause (2 patients), antiphospholipid syndrome without an alternative cause (1 patient), and occupation-related risk (1 patient; a flight attendant with a possible increased risk of stroke recurrence).

Discussion

This study shows that despite that all PFOs were considered in the HST as contributing factor to the stroke, risk- enhancing anatomical features were not present in 20%. Looking into these risk-enhancing characteristics in more detail, former studies showed that the PFO diameter in patients with a cerebral event was significantly larger (4 ± 2mm) compared to those without (2 ± 1mm, $p<0.001$).[40] Notably, an unstretched PFO diameter greater than 4mm was associated with an increased risk of stroke and TIA.[40] In our study, we observed larger unstretched diameters that can be classified as "high-risk", with a mean of 5.4 ± 2.6mm compared to the previous report's mean of 4.0 ± 2.0mm.[40] This difference may be caused by differences in the methodology used to measure the PFO. Moreover, prior studies demonstrated that the risk of stroke increases with the presence of an ASA. An ASA, defined as an excursion of the inter atrial septum >10mm, was present in 31.4% in our series. This is in concordance with previous reports.[41]

Autopsy studies and cohorts of patients who underwent TEE for reasons other than evaluation of a cardiac origin of stroke reported a prevalence of ASA of about 1-5%.[42, 43] Notably lower than the 32% we observed.

Additionally, the presence of an ASA or a hypermobile septum may facilitate a larger opening of the PFO. Potentially allowing passage of thrombus from the venous circulation through the PFO, formation of thrombus in the tunnel of the PFO or local thrombus formation in the septal aneurysm.[44] Future research is needed to substantiate these hypotheses.

The anatomical characteristics of PFOs associated with stroke are diverse, but a large diameter and the presence of ASA appear to be indicative. Since there is significant heterogeneity in the anatomical features of pathological PFOs, the role these features should play in the decision-making remains unclear. Using the

PASCAL score, one risk-enhancing anatomical feature can shift a PFO-related stroke from 'unlikely' to 'possible'. The interpretation of these terms remains difficult. In our study more patients had a 'possible' outcome rather than a 'probable' one, and 20% did not have any risk-enhancing features despite all patients having a PFO-associated stroke.

Given the heterogenicity of PFOs and the still high number needed to treat to prevent a single stroke a thorough understanding of the individual patient and a multidisciplinary assessment are essential before deciding on PFO closure.[5]

Limitations

Our study has several limitations. We lack information on the PFO characteristics of patients with a PFO who were declined for percutaneous closure. Therefore we are not able to make any comparisons. Additionally, while we demonstrate that PFOs can be stretched, resulting in a larger opening, the relationship between this characteristic and stroke risk remains unclear. The stretched diameter can only be obtained with an invasive measurement and is not a useful parameter for identifying patients eligible for PFO closure. Furthermore, due to the lack of information on patients' baseline characteristics, we are unable to provide detailed assessment of the selection process or identify potential areas of improvement. Lastly, there may be a larger number of patients with a severe shunt size, as 15% of the patients with a shunt grade lower than severe (opacification) did not undergo a Valsalva maneuver.

Conclusion and clinical implications

Although all patients accepted for percutaneous PFO closure were individually assessed by a dedicated HST, 20% had a PFO without risk-enhancing features but were still accepted for closure due to other reasons. This highlights the importance of careful individual assessment of young stroke patients with a PFO. Future studies are needed to identify the characteristics that contribute to stroke in these patients, including follow-up for stroke recurrence in patients who were accepted and patients who were declined for percutaneous closure.

Sources of funding

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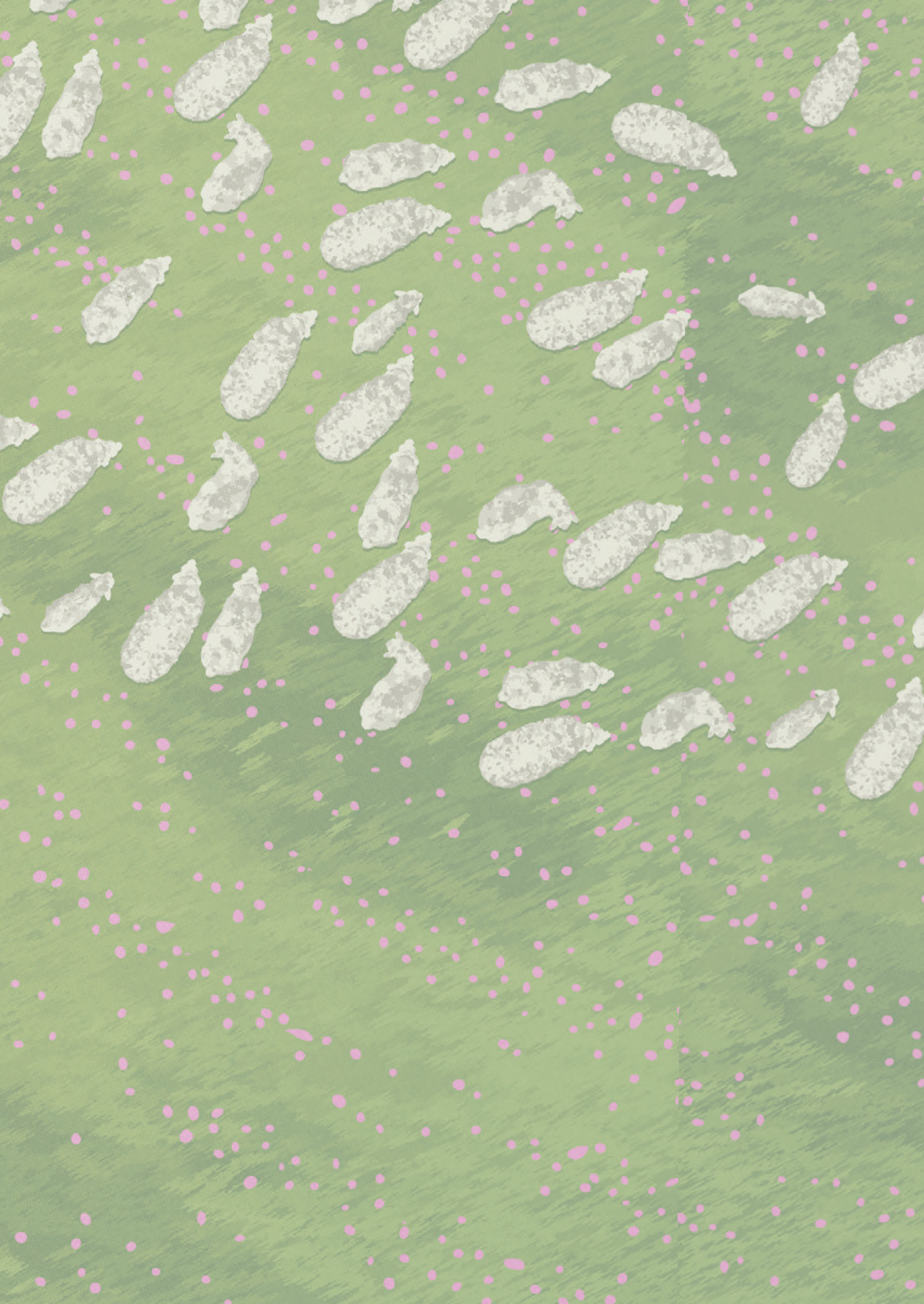
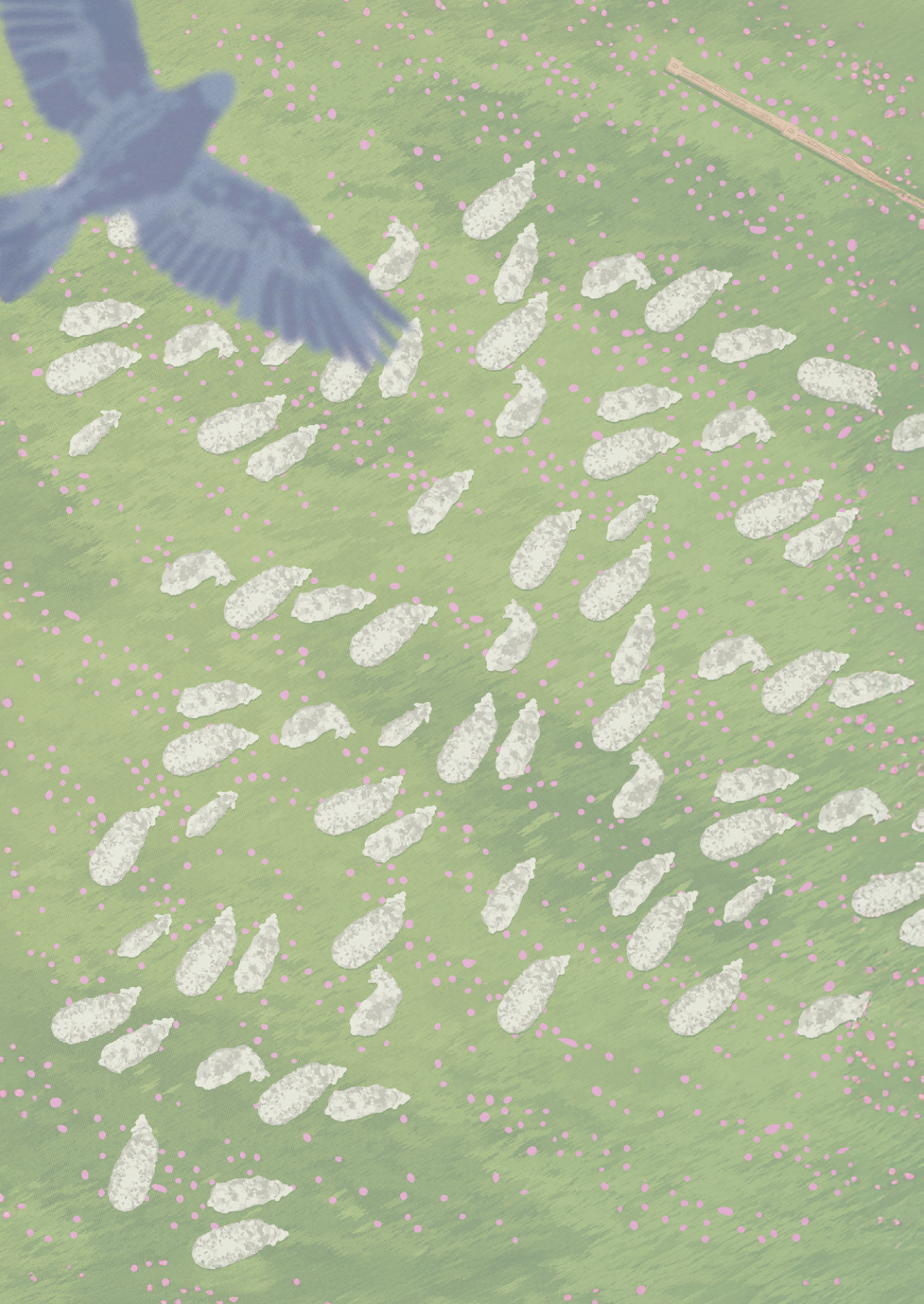
Conflict of interests

There was no role of any sponsor or funder in the design and conduct of the study, in the collection, analysis or interpretation of the data. Neither was there a role in the preparation of the manuscript. There were no conflict of interests. Dr. Tim ten Cate and dr. Berto Bouma are associate editors of Netherlands Heart Journal.



Part III

Physiology



3

Left atrial strain as a diagnostic marker in PFO-associated stroke

Published as
Immens MHM, C de Wildt, A.L. Duijnhouwer, H.F. de Leeuw, T.J.F. ten Cate.
Left atrial strain as a diagnostic marker in PFO-associated stroke. *Submitted.*

Abstract

Introduction

In younger patients with stroke, a patent foramen ovale (PFO) is increasingly recognized as a possible cause of the stroke. The prevalence of PFO is about 25% in the general population. How an innocent PFO becomes vulnerable and causes a stroke remains unclear. Atrial dysfunction assessed by left atrial strain (LAS), may also contribute to converting an asymptomatic PFO into a vulnerable stroke-causing PFO. To determine whether LAS is impaired in PFO-associated stroke, we analyzed the LAS and compared them to patients with an asymptomatic bystander PFO and stroke of other etiology.

Patients and Methods

This is a single-center, retrospective analysis of all patients who were evaluated for PFO closure by a Heart-Stroke Team (HST). To minimize instrumentation bias, we only included patients who underwent transthoracic echocardiography at the Radboud University Medical Center, Nijmegen, Netherlands. TTE images were collected and analyzed using Tomtec/ Agfa EI software. PFO characteristics were obtained during the closure procedure using transesophageal echocardiography.

Results

We analyzed the LAS of 64 patients with PFO-associated stroke and 12 patients with a PFO and stroke of other etiology. LAS in patients with a PFO-associated stroke was (35.6%), compared to patients with stroke of other etiology (33.0%). LAS appeared to be more impaired (i.e., lower) in patients with a PFO-associated stroke who had a large shunt (33.7%), atrial septal excursion ≥ 10mm (29.6%) and particularly in those with both anatomical features (27.5%).

Conclusion

LAS is impaired in the presence of high-risk PFO features. The value mimics that of patients with paroxysmal atrial fibrillation. Whether this causes an innocent bystander PFO to become vulnerable and whether LAS can be used to differentiate between a PFO-associated stroke and stroke of other etiology remains to be determined in future research.

Introduction

In young patients (18-50 years) with an ischemic stroke, a patent foramen ovale (PFO) is increasingly recognized as a possible cause. The exact underlying mechanism of a PFO-associated stroke remains unknown. The most commonly accepted hypothesis is that a thrombus from the venous circulation directly enters the arterial circulation through the PFO, a so called paradoxical embolus.[45]

The relationship between deep vein thrombosis (DVT) and PFO-associated stroke remains unclear.[46] An observation challenges this hypothesis is the prevalence of PFO in the general population, estimated at approximately 25%, whereas only a small minority will ever experience a stroke. In a large prospective registry of young patients with stroke, a PFO was considered the cause in only 11.9% of cases.[4] This stands in contrast to the annual incidence of venous thromboembolism: several tens of thousands of cases of DVT are reported in the Netherlands each year, and according to "Het Longfonds", the leading authority on pulmonary diseases in the Netherlands, the incidence of a pulmonary embolism (PE) is 10,000-12,500 cases annually. If we assume that PFO-associated stroke shares a similar pathophysiological mechanism with PE, we would expect the annual incidence of PFO-associated stroke to be at least several thousand cases. Nevertheless, the estimated incidence in the Netherlands is only 300–1,500 cases per year.[4, 15] Furthermore, PFO-associated stroke is more common in younger patients, whereas DVT is rare in younger individuals (approximately 1 per 10,000 person-years between the ages of 25 and 30) and increases exponentially with age (about 8 per 8,000 person-years in individuals aged 85 years and older).[47] This inverse age relationship makes it less likely that DVT plays a causal role in PFO-associated stroke. These findings imply that other mechanisms are needed to turn an innocent PFO into a stroke-causing, vulnerable PFO.

It has been demonstrated that certain trigger factors, including infection, may convert an innocent PFO into a vulnerable, stroke-causing PFO.[4] An alternative hypothesis is that the clot is formed in the PFO itself. This may be caused by reduced, turbulent blood flow in the foramen ovale and/or left atrium.[7] Turbulent blood flow is related to left atrial dysfunction, which was already been shown in atrial fibrillation.[48] Therefore, further support for this hypothesis may emerge if atrial dysfunction can be demonstrated in patients with a PFO-associated stroke.[8] Left atrial function can be assessed using standard transthoracic echocardiography (TTE), including measurements of left atrial strain (LAS). LAS measures the atrial wall deformation within the cardiac cycle.[10] If atrial dysfunction plays a role in PFO-

associated stroke, it may be a contributing factor in the progression of an innocent PFO into a vulnerable PFO. We therefore analyzed LAS in patients with PFO-associated stroke as assessed by a dedicated Heart-Stroke Team (HST) to determine whether it differs from that in patients with a PFO and stroke of other etiology.[49]

Methods

Study design and patients

All patients referred to the HST of the Radboud University Medical Center (Radboudumc), Nijmegen, The Netherlands, between June 2018 and December 2024 were eligible.[49] To minimize instrumentation bias, we only included patients who had a TTE performed at the Radboudumc. This ensures that all measurements were done using the same standardized echocardiography protocols and equipment. Patients with echocardiography performed outside the Radboudumc and patients with insufficient echocardiography ultrasound windows for strain measurement were excluded. All patients with an ischemic stroke or transient Ischemic attack (TIA) and a PFO were included. All patients with an ischemic stroke had imaging proof of the lesion (preferably an MRI). The work-up process of the HST is explained previously.[49]

Echocardiographic work-up

TTE's were acquired using an Epiq ultrasound scanner (Philips Medical Systems, Netherlands) or Vivid E95 ultrasound scanner (GE Ultrasound, USA). The standard protocol includes agitated saline contrast imaging to demonstrate the presence of a right-to-left shunt. At least 1 sufficient acquisition without Valsalva and 2 with Valsalva maneuvers are obtained. The protocol instructs that right atrial and right ventricular opacification are obtained for a contrast study of sufficient quality. The presence of a PFO was defined as any contrast visible in the left atrium within five heartbeats after entering the right atrium on TTE. Shunt size was classified as (0) None, (1) Small, <5 bubbles, (2) Moderate, 5-30 bubbles, (3) Large, >30 bubbles, (4), Opacification or near opacification of the left ventricle.[50] The anatomical PFO characteristics were obtained using transesophageal echocardiography (TEE) during the PFO closure procedure. Atrial septal aneurysm (ASA) was defined as at least 10mm of excursion from the midline visible on TEE as used by the Systematic Collaborative, PFO closure Evaluation (SCOPE).

2D speckle-tracking echocardiography

LAS measurement using 2D-STE was performed by one experienced operator (MI). Assessment of LAS was done on a standard 2D apical 4 chamber view with at least

one pulmonary vein visible. A minimal frame rate of 50 frames per second (fps) was used. Images acquired for LAS measurement consisted of three consecutive heartbeats. Analysis was done off-line using Tomtec post-processing software. Delineation of the left atrium was done by automated tracking software. The automated tracking was visually verified and manually corrected when necessary (Figure 1). The mean left atrial peak strain is shown as a percentage. A higher value reflects a better function.

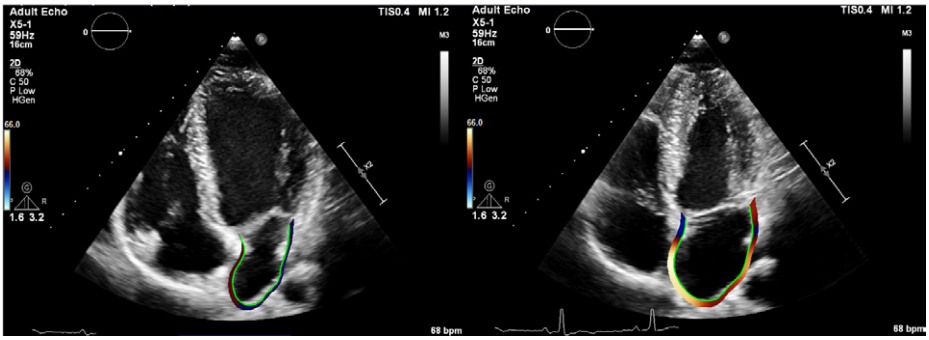


Figure 1. Two-dimensional speckle tracking echocardiography.
Thoracic echocardiogram of a patient with a cryptogenic stroke, this picture shows the measurement of the left atrial strain using 2D-STE at the beginning and the end of the atrial diastolic phase.

Statistical analyses

Statistical analysis was done using IBM SPSS Statistics 26. Data are shown as number (%) for categorical data. Continuous data are expressed as mean ± SD or median and range depending on the Gaussian distribution. No statistical analyses were performed due to an imbalance in the number of patients between groups.

Results

From June 2018 and December 2024, a total 505 patients were referred to the Radboudumc and evaluated by the HST for PFO closure. A total of 301 patients were excluded either because the echocardiogram was performed outside the Radboudumc or due to inadequate ultrasound windows for strain measurement. A PFO-associated stroke was present in 64 patients, mean age was 41 (SD 6.0) years, 33 (51.6%) were females. We identified 12 patients with a stroke of other etiology, mean age 52 (SD 4.0) years, 6 (50.0%) were females (Table 1). The TOAST criteria for stroke etiology[3] in this group was large artery disease in 2, small vessel disease in 6, arterial dissection in 2 and cryptogenic but not related to the PFO in 2.

Table 1. Demographics.

	PFO-associated stroke (n = 64)	Stroke of other etiology (n = 12)
Female, n (%)	33 (51.6)	6 (50.0)
Mean age (SD)	41 (6)	52 (4)
Mean body mass index [IQR]	25 [24-27]	25 [21-30]
Vascular risk factors, n (%)*		
History of smoking	21 (32.8)	7 (58.3)
Hypertension	7 (10.9)	2 (16.7)
Diabetes mellitus	1 (1.6)	2 (16.7)
Hypercholesterolemia	20 (31.3)	8 (66.7)
History of pulmonary embolism, n (%)	5 (7.8)	0 (0.0)
History of deep venous thrombosis, n (%)	3 (4.7)	1 (8.3)
Mean RoPE score [IQR]	7 [6-8]	6 [5-7]

* Information was missing in the following number of patients: 1 in "Mean body mass index", 1 in "History of smoking", 11 patients in "History of pulmonary embolism", 21 patients in "History of deep venous thrombosis".

¤ Reached significance level of 0.05. *p*-Values were calculated two-sided using a chi-squared test to assess the equality of proportion.

Table 2. Echocardiographic findings.

	PFO-associated stroke (n = 64)	Stroke of other etiology (n = 12)
PFO characteristics*		
Median shunt without Valsalva [IQR]	1 [1-2]	1 [1-2]
Median shunt with Valsalva [IQR]	3 [2-4]	2 [1-3]
10mm excursion of atrial septal, n (%)	14 (22.0)	2 (16.7)
Mean tunnel length, mm (SD)	7.6 (4.4)	x
Mean diameter opening, mm (SD)	7.0 (3.4)	x
Mean left atrial strain, percentage (SD)**	35.6 (13.4)	33.0 (13.1)
10mm excursion of atrial septal	29.6 (10.0)	30.2 (1.2)
No atrial septal aneurysm	36.7 (13.6)	32.4 (14.7)
Small shunt	36.2 (11.1)	33.9 (14.3)
Large shunt	33.7 (14.7)	29.0 (0.6)
Large shunt and 10mm excursion	27.5 (10.1)	29.4 (0.0)

* Information was missing in the following number of patients: 5 patients in "Median shunt without Valsalva", 3 patients in "Median shunt without Valsalva", 4 patients in "Median shunt with Valsalva", 15 patients in "Excursion of atrial septal", 25 patients in "Mean tunnel length", 23 patients in "Mean diameter opening".

**In total, in patients with a PFO-associated stroke: 14 patients had ≥ 10mm excursion of atrial septal, 37 patients had no atrial septal aneurysm, 30 patients had a small shunt (type 1 or 2), 31 patients had a large shunt (type 3 or 4), 10 patients had a large shunt and ≥ 10mm excursion of atrial septal. In patients with stroke of other etiology: 2 patients had ≥ 10mm excursion of atrial septal, 8 patients had no atrial septal aneurysm, 10 patients had a small shunt (type 1 or 2), 2 patients had a large shunt (type 3 or 4), 1 patients had a large shunt and ≥ 10mm excursion of atrial septal. No data on atrial excursion or shunt size was considered missing and not included in the calculations.

Patients with a PFO-associated stroke had a large shunt (≥ grade 3) in 31 patients and 14 (22%) had an atrial septal aneurysm (>10mm excursion from the midline). Further echocardiographic findings are presented in Table 2. The median normative value of LAS in the general population, as established in the Copenhagen City Heart Study, is 39.4% (23.0-67.6%). LAS in patients with a PFO-associated stroke was 35.6%, compared to 33.0% in those with stroke of other etiology. LAS appeared to be more impaired (i.e., lower) in patients with a PFO-associated stroke who had a large shunt (33.7%), and when the atrial septum was aneurysmatic (29.6%). When a patient had both echocardiographic characteristics, the LAS was even further impaired (27.5%).

Discussion

PFO is an accepted contributor to cryptogenic stroke especially in younger patients. The pathophysiological mechanism remain unclear. Our findings suggest that left atrial function is impaired in patients with a PFO-associated stroke. LAS was lowest (28%) in the patients with the most high-risk PFO characteristics, i.e. a large shunt and aneurysmatic atrial septum. Patients with a PFO-associated stroke had a lower LAS compared to the presumed median normative value observed in the Copenhagen Heart Study (36% versus 39%).[51] Unfortunately, as LAS is a relative new parameter, there are no definitive data indicating the threshold of percentage decrease that is clinically relevant. However, a reduction in LAS has been shown to be associated with left atrial pathology. For example, studies on patients with paroxysmal atrial fibrillation have reported mean LAS values ranging between 22% and 28%.[52 53]

LAS was also lower in patients with a PFO that was not considered related to the stroke (33%). This may be explained by the high proportion of patients with small vessel disease and large vessel atherosclerosis in this group (67%). It was previously demonstrated that LAS is lower in the presence of cardiovascular risk factors such as hypertension, smoking and obesity. These factors are also strong contributors to the development of both large vessel atherosclerosis and small vessel disease.[54]

Previous studies found that several PFO characteristics increase the risk of a PFO-associated stroke.[38] Both a larger shunt size and more pronounced atrial septal excursion were found to increase this probability. The underlying pathophysiological mechanisms remain unclear. We demonstrate that LAS was lowest when more risk-enhancing PFO features are present. When both a large shunt and an ASA are present, the LAS was 28%. In other words, the probability of a PFO-associated stroke appears to correlate with a reduction of atrial strain. These findings are in line with a

previous reports that showed that LAS was lower in patients with a PFO-associated stroke with ASA compared to patients without ASA.[9] We hypothesize that the reduction in LAS with presumed impaired left atrial function could result in blood flow alterations, causing shear stress and increasing the risk of subsequent local thrombus formation.

Similar to the local thrombus formation in the left atrial appendage in atrial fibrillation, the PFO tunnel may serve as a preferred site for thrombus formation in patients with impaired atrial function. This hypothesis is supported by a recent observation that identified local in situ thrombus formation within the PFO tunnel.[55] Therefore the reduced LAS may be one of the triggers that turn an innocent PFO into a vulnerable PFO. We assume that hemodynamic changes within the left atrium take years before causing atrial impairment, which could explain why PFO-associated strokes are extremely rare in childhood.

We previously described flu-like disease and vigorous exercise as potential trigger factors for PFO-associated stroke.[4] It is possible that, in the presence of such triggers, the combination of risk-enhancing anatomical characteristics and atrial dysfunction could transform an otherwise innocent PFO into a vulnerable one.

This hypothesis, that a PFO-associated stroke involves multiple pathophysiological mechanisms, beyond the traditional theory of paradoxical embolism, may help explain the weak correlation observed between PFO-associated stroke and deep vein thrombosis.[4] Future studies are needed to further clarify the relationship between PFO and stroke and to define criteria, possibly using LAS as a diagnostic tool, to identify which strokes are truly related to PFO. This, could reduce the number of PFO closures needed for effective stroke prevention.[27]

Limitations

The primary limitation of this study is the small sample size of patients, especially with stroke of other etiology. Due to an imbalance in number of patients between groups, no reliable statistical analyses could be performed. Since only echocardiograms performed at Radboudumc were deemed suitable for analysis, patients referred to our center who underwent their cardiac work-up elsewhere were excluded. This may impose a selection bias. Since our cohort primarily consists of Dutch patients of Caucasian ethnicity, treated in an academic hospital, extrapolation of these results to different populations should be approached with caution.

Conclusion and implications

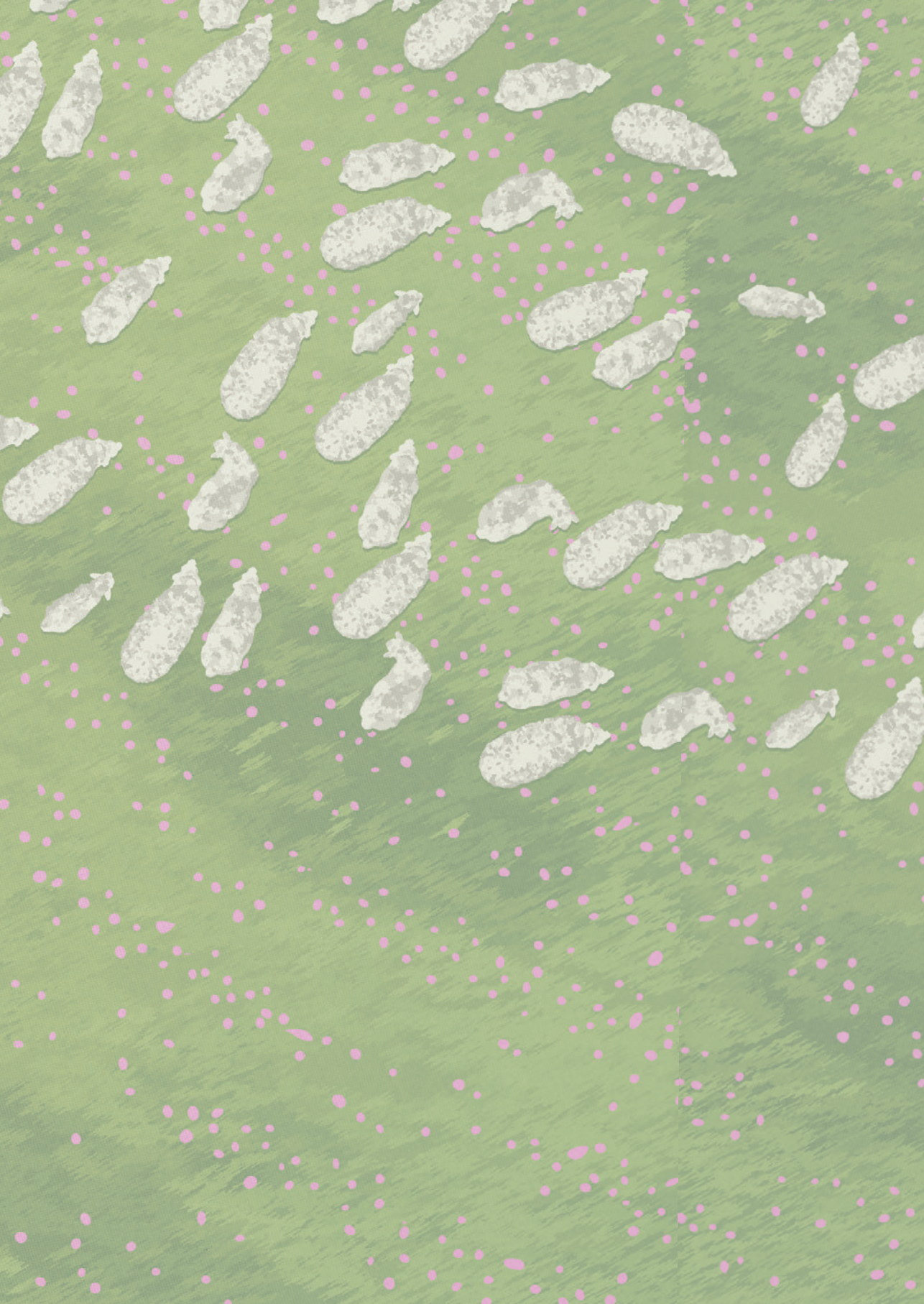
LAS is impaired in the presence of high-risk PFO features. The value mimics that of patients with paroxysmal atrial fibrillation. Whether this causes an innocent bystander PFO to become vulnerable and whether LAS can be used to differentiate between a PFO-associated stroke and stroke of other etiology remains to be determined in future research.

Disclosures

TJF ten Cate and FE de Leeuw institutional research support by Abbott vascular. The other authors describe no conflicts of interest.

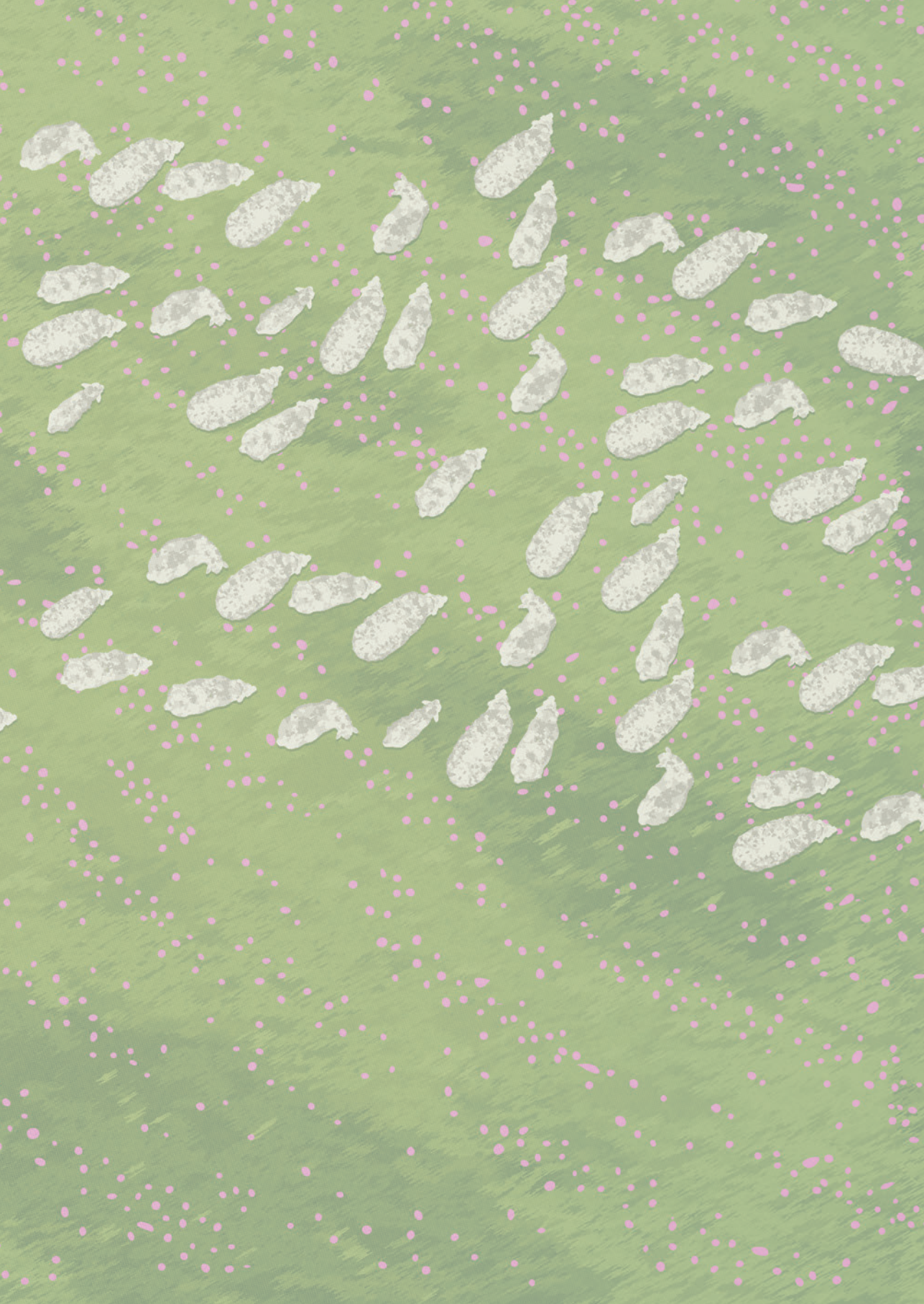
Ethical approval

The Ethics Committee of the Radboudumc, Nijmegen, Netherlands waived the need for ethics approval and the need to obtain consent for the collection, analysis and publication of the retrospectively obtained and anonymized data for this non-interventional study.



Part IV

Histology



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Histology of PFO-associated stroke thrombus compared to iliofemoral deep vein thrombus: an explorative study

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Abstract

Purpose

A patent foramen ovale (PFO) is as a cause of thrombo-embolic stroke. It is thought that the thrombus originates from the venous circulation, although this has never been proven. The histological composition of the thrombus might help to identify its origin. The aim of this exploratory pilot study is to compare the histological composition of thrombi of patients with PFO-associated stroke with venous thrombi from patients with iliofemoral deep venous thrombosis (DVT).

Methods

We retrieved thrombi from the MR CLEAN Registry, a Dutch nationwide, multicenter, prospective registry of patients who underwent endovascular treatment for ischemic stroke. Furthermore DVT thrombi were obtained as fully anonymous waste material and analyzed retrospectively.

Results

Thrombi were available for three patients treated for PFO-associated stroke and four patients treated for DVT. The thrombi of patients with PFO-associated stroke contained less red blood cells (RBC) and more fibrin and platelets (Fib+Plt) than those with DVT (30.2% vs 91.3% RBC and 67.4% vs 8.5% Fib+Plt). The PFO-associated stroke thrombi were most comparable to thrombi from the same cohort classified as cardioembolic (RBC 25.8% and 67.1% Fib+Plt). As this is a descriptive histological analysis, no definitive comparisons between different thrombi can be made.

Conclusion

We observed that the composition of the three thrombi from patients with PFO-associated stroke differs from that of the four DVT thrombi in our cohort. Prospective studies are needed to determine whether thrombi in PFO-associated stroke are all similar in composition and share a similar pathophysiology with venous thrombi.

Data access statement

The raw and anonymized data used in this study can be made available to other researchers on request. Written proposals can be addressed to the MR CLEAN Registry group.

Background

A PFO is present in about 25% of all healthy people, but may in rare instances convert to a cause of stroke.[6, 34] Because of the high prevalence of "innocent bystander PFOs", it is crucial to separate them from stroke-causing PFOs in order to prevent unnecessary treatment of asymptomatic PFOs. The mechanism by which a PFO causes a stroke includes the passage of a thrombus, presumably originating from the venous circulation to the arterial circulation, though this has never been directly demonstrated. An alternative pathological mechanism suggests in situ thrombus formation within the PFO tunnel. [55] The histopathological composition of thrombi might reveal the actual origin of the thrombus as arterial thrombi mainly consist of fibrin and platelets (Fib+Plt), while venous thrombi mostly contain red blood cells (RBC). [56] The aim of this exploratory pilot study is to characterize and compare the histopathology of thrombi of patients with presumed PFO-associated stroke, symptomatic iliofemoral deep venous thrombosis (DVT) and strokes of other etiologies.

Methods - patients

Patients with an ischemic stroke

We used data from the MR CLEAN Registry.[57] In short, the MR Clean registry is a Dutch nationwide, multicenter, prospective registry of patients who underwent endovascular treatment (EVT) for ischemic stroke between March 2014 until June 2016. For the purpose of our particular substudy we only selected patients between 18 and 60 years. After analyzing hundreds of medical discharge letters, we observed that patients diagnosed with PFO-associated stroke during initial admission were relatively young (below the age of 60). We reviewed all medical discharge letters from the MR CLEAN Registry for patients up to 65 years of age and found no applicable candidates between the ages of 60 and 65. Therefore, we did not examine medical release letters for patients older than 65, as we did not expect to identify additional suitable candidates for our study.

Stroke etiology was determined based on the diagnosis at discharge. The presence of a PFO was assessed with transthoracic echocardiography (TTE) and/or transesophageal echocardiography (TEE) following a bubble test with agitated saline. Patients with an atrial septal defect (ASD) were not included. A PFO was considered the cause of stroke if patients had undergone CT angiography of the cervical arteries to rule out atherosclerosis and had at least 24-hour cardiac rhythm

monitoring to rule out atrial fibrillation. In the Netherlands, screening for DVT is not part of the standard diagnostic workup for stroke patients presenting with PFO, unless there is a clinical suspicion of DVT, which was not the case in these patients. [3] Thrombi available for histopathological analysis were analyzed.

Patients with a DVT

Thrombi of patients with a symptomatic DVT were part of a separate cohort. They underwent percutaneous thrombectomy within the iliofemoral vein due to severe symptoms. These thrombi were obtained as fully anonymous waste material and analyzed retrospectively. The thrombi were retrieved and stored at the Erasmus Medical Center, Rotterdam, The Netherlands, for undetermined research purposes. Due to anonymization, no patient data were available.

According to Dutch law, fully anonymous waste material can be used without medical ethics approval.

Patients with a cardioembolic or non-cardioembolic stroke

Furthermore, we looked at the analyzed thrombi from the MR CLEAN Registry, categorized as cardioembolic or non-cardioembolic. The quantification of these thrombi is described in a previous study by Hund et al., who also investigated the association between thrombus composition and stroke etiology in the same cohort.[58]

Processing and histopathological analysis of the thrombi

Thrombi were collected, fixed, photographed and stored in 4% buffered formaldehyde prior to paraffin embedding. DVT samples contained a large number of fragments, sometimes more than 60 fragments per patient. Hence a random set of maximally three blocks with multiple fragments were taken for histological analysis. All thrombus fragments collected during EVT were stored in one block and thus all segments were analyzed.

Samples were stained for hematoxylin-eosin as a routine stain and digitalized using the Hamamatsu scanner.(5) In short, digitized images were histologically analyzed for RBC, Fib+Plt and leukocytes using a machine learning algorithm (Orbit Image Analysis software (Orbit Image Analysis, Idorsia Ltd.). Orbit Image Analysis shows good performance as an image analysis tool compared to manual visual control. [59] The weighted average of the sections was used for data-analysis.(5) In case of missing areas of RBC due to processing artefacts, these areas were manually traced and data was then corrected for these areas. Quantitative analysis of thrombus

composition was performed for all images within the same session using Orbit analysis software; hence, the analysis was by definition blinded to the source. Qualitative analysis was conducted by an experimental pathobiologist who was not blinded to the source.

Statistical analyses

The composition of thrombi from PFO-associated stroke was compared to thrombi obtained from patients with DVT. Statistical analysis was done using GraphPad Prism 8.0.1. Data are shown as mean percentage (%) ± standard deviation. No statistical tests were performed to compare the groups due to the small number of patients. We compared the quantification of the thrombus characteristics from PFO and DVT with the analyzed cardioembolic and non-cardioembolic thrombus from the same cohort as described by Hund et al.[58]

Results

We identified 18 patients with a PFO-associated stroke in the MR CLEAN Registry. Of these, three patients (aged 30-50 years) had thrombi available for analysis (Table 1).

Table 1 Description of patients with PFO-associated stroke.

Patient	Gender	Age	Medical history	Cardiovascular risk factors	Event	Intravenous thrombolysis
PFO-1	Female	50 years	Tetralogy of Fallot, angina pectoris, TIA in 2009 and 2011, since 2009 a known PFO, which was not closed due preference for best medical treatment	Dyslipidemia and smoking	Left M2 occlusion	Yes
PFO-2	Female	30 years	Migraine with aura	None	Right M1 occlusion	No
PFO-3	Female	48 years	Tonsillectomy at young age, usage of oral contraceptive pill	Dyslipidemia	Right M1 occlusion	Yes

Investigations according to national stroke guidelines did not reveal any other cause of stroke than the PFO.

Histological results

Thrombi of PFO-associated stroke showed that they consisted for 30.2±6.1% of RBC, 67.4±7.3% of Fib+Plt and 2.4±2.2% of leukocytes (Figure 1). Thrombi of patients with DVT consisted for 83.4±6.2% of RBC, 15.9±6.2% of Fib+Plt and 0.7±0.5% of leukocytes (Figure 2). The thrombus composition of PFO-associated strokes was different compared to DVT. The percentage of RBC's was lower and the Fib+Plt higher in thrombi of PFO-associated stroke compared to thrombi of patients with DVT (Figure 3, panel 1). In contrast, the PFO-associated stroke thrombi were similar to thrombi from patients with a cardioembolic cause of stroke (RBC 25.8% and 67.1% Fib+Plt) (Figure 3, panel 2). As this is a descriptive histological analysis, no definitive comparisons between different thrombi can be made.

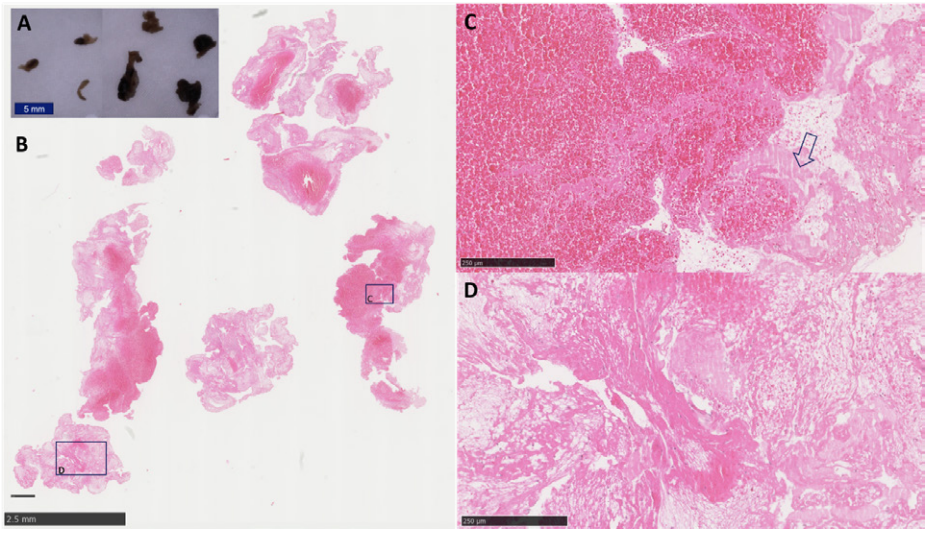


Figure 1. Stroke thrombus histology (PFO-2). Macroscopy (A) and microscopy (B-D) of thrombi retrieved for acute ischemic stroke. The diversity in thickness and length of the fragments is illustrated in the macroscopic image of the fixed thrombi (A). Histology (B-D) illustrates the presence of both erythrocyte rich (C) and fibrin rich areas (D). Zahn-lines observed in panel C (arrow), which can be seen in both venous and arterial thrombi indicate that part of the thrombus was formed under flowing blood conditions. Hematoxylin Eosin stain (B-D). Bars indicate magnification.

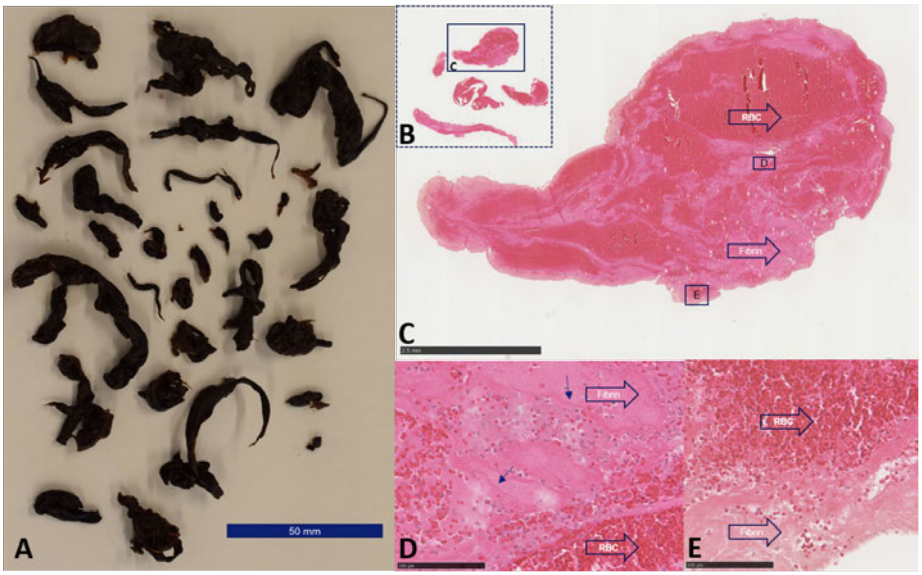


Figure 2. Histology of iliofemoral deep venous thrombus (DVT1). Macroscopy (A) and microscopy (B-D) of thrombi retrieved from patients with an iliofemoral deep venous thrombosis. The diversity in thickness and length of the thrombi is illustrated in the macroscopic image of the fixed thrombi (A). Histology (B-D) illustrates the presence of both erythrocyte rich areas (RBC, arrowheads) and fibrin rich areas (Fibrin, arrowheads) in the center of the thrombus fragments (C) as well as at the edges of the fragments (D). Zahn-lines observed in panel C, which can be seen in both venous and arterial thrombi indicate that part of the thrombus was formed under flowing blood conditions. This is not specific for DVT thrombi. Hematoxylin Eosin stain (B-D). Bars indicate magnification.

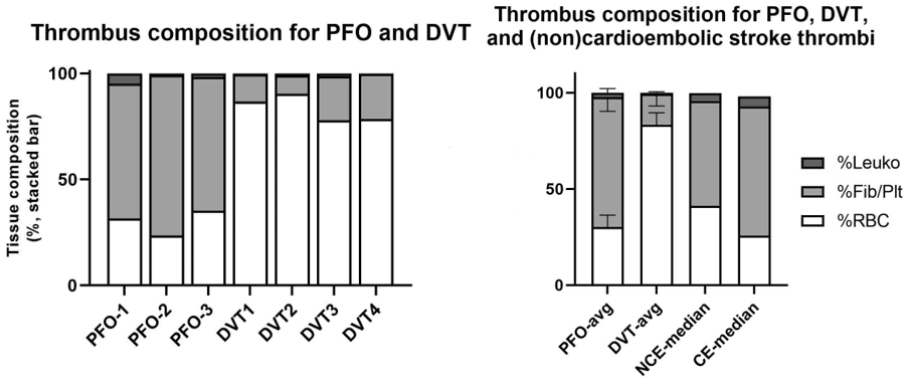


Figure 3. Differences in thrombus composition. Tissue composition= histological composition of the thrombus, depicted as a percentage of %Leuko= Leukocytes, %Fib/Plt= Fibrin and platelets and %RBC= Red blood cells. PFO= Patent foramen ovale thrombus, DVT= Deep venous thrombus, avg= average, NCE= non-cardioembolic, CE= cardioembolic, *Data on the groups: NCE-median and CE-median are derived from Hund et al.(7)

Discussion

In our study, PFO-associated stroke thrombi differed histologically from DVT thrombi and were most comparable to the composition of thrombi retrieved from patients with a different cardioembolic cause of stroke.

The correlation between DVT and PFO-associated stroke remains unclear, although the paradoxical embolism theory is widely accepted. Studies report a wide frequency of DVT between 7-27% in patients with a PFO-associated stroke. Based on the current literature, routine screening for DVT in PFO-associated stroke patients is not justified.[46] This approach is in contrast with the standard care for diagnosing pulmonary embolism, where routinely searching for DVT is a common practice. This appears contradictory, as the paradoxical embolism theory assumes that the thrombus causing a PFO-associated stroke shares the same pathophysiology as a pulmonary embolism. Identifying the thrombus of PFO-associated stroke is crucial, as the treatment involves percutaneous closure of the orifice and potentially discontinuing antithrombotic therapy after closure in young stroke patients.[60] Consequently, patients who are incorrectly diagnosed with a PFO-associated stroke risk receiving erroneous treatment and are exposed to potential surgical complications after closure of PFO like atrial fibrillation.[61]

Previous research has indicated that stroke-causing thrombi exhibit a broad spectrum of composition, ranging from predominantly fibrin and platelets to primarily RBC.[56, 58] With our study, we wanted to pilot the future role of histological analyses in determining which patients could benefit from percutaneous closure. In our cohort, PFO-associated stroke thrombi did not differ from cardioembolic thrombi in terms of RBC, Fib/Plt, and Leuko composition. Other parameters, such as thrombin levels of the clot, may have the potential to distinguish PFO-associated stroke from other etiologies. [62]

Percutaneous closure of the PFO, despite having a high number needed to treat, has been proven beneficial in preventing recurrent PFO-associated stroke. There are several potential explanation for this: 1) percutaneous closure prohibits the passage of thrombi originating from the tunnel, as is supported by Yan et al. [55], 2) percutaneous closure restores the atrial integrity and therefore the atrial function[63], or 3) alternatively, percutaneous closure may prevent thrombus originating from the venous circulation passing through the tunnel, which could not be proven by our study as the PFO-associated stroke thrombus composition differed from DVT thrombus. Another possibility could be that PFO-associated strokes have different etiologies, as mentioned

above, and that some thrombi indeed originate within the venous system. Future studies are necessary to compare thrombi from PFO patients who have had a stroke and undergone mechanical thrombectomy, with and without DVT. This approach could potentially be of value in identifying patients who would benefit from a PFO closure.

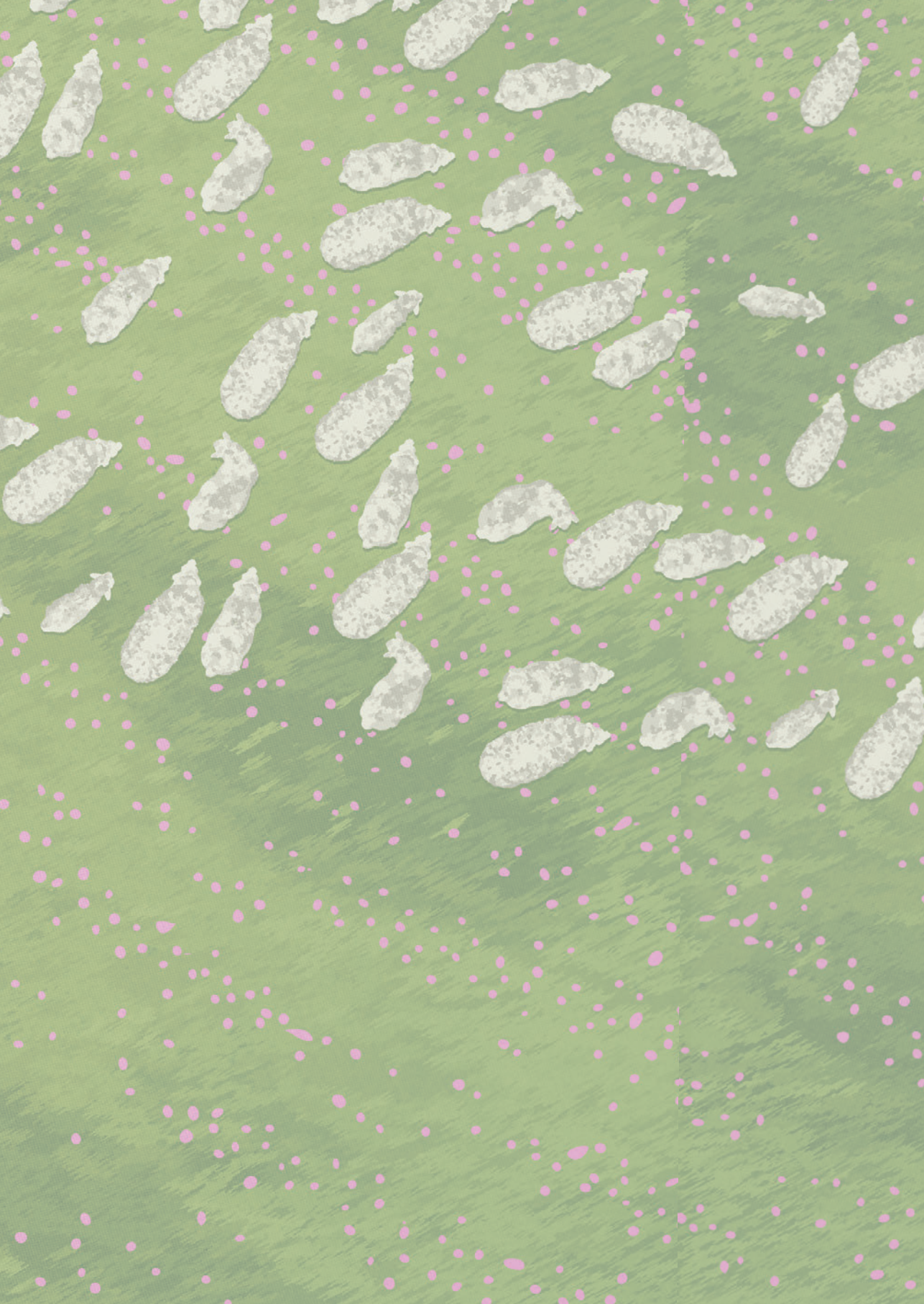
Our results are in contradiction with the results of another study that reported a significantly higher percentage of RBC and lower percentage of Fib+Plt in thrombi of patients with a PFO and with a cryptogenic stroke compared to patients without a PFO and with a cryptogenic stroke.[64] However, that study does not report whether these PFOs were considered the cause of stroke or if they were incidental findings. In the previous study, patients with a PFO, cryptogenic stroke and proven (asymptomatic) DVT (N=5) the histological composition was still remarkable different from the composition of the DVT from our cohort (RBC 64% vs. 83%) again questioning whether DVT has any causality with PFO-associated stroke. The histology of DVT thrombi in our cohort also differed from that of thrombi defined as non-cardioembolic. [58] As described in the study by Hund et al., the non-cardioembolic group includes large artery atherosclerosis and other identified causes (e.g., carotid artery dissection). All with an arterial origin. Since DVT thrombi are formed in the venous vasculature and hence under lower flow, we anticipated that the histology of DVT thrombi would differ from that of non-cardioembolic clots.

The main limitation of our study is the small amount of thrombi available for analysis. This is largely due to the fact that we could only include patients who were identified with a clear PFO-associated stroke during initial admission, whereas most PFO-associated strokes are usually diagnosed later during outpatient clinic follow-up.

This is the first study to compare the composition of PFO-associated stroke thrombi with those derived from DVT and other cardioembolic causes of stroke. Further prospective studies into the histopathological aspects of thrombi and the cause of stroke are needed to optimize the personalized treatment strategies and to distinguish pathological PFOs from incidental PFOs.

Conclusion

We observed that the composition of the three thrombi from patients with PFO-associated stroke differs from that of the four DVT thrombi in our cohort. Prospective studies are needed to determine whether thrombi in PFO-associated stroke share a similar pathophysiology with venous thrombi.



Part V

Risk factors

5

Trigger factors in patients with a patent foramen ovale associated stroke: a case-crossover study

Published as

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Trigger factors in patients with a patent foramen ovale-associated stroke: A case-crossover study. *International Journal of Stroke. 2024 August.*

Abstract

Background

Patent foramen ovale (PFO) is a congenital anatomical variant which is associated with strokes in young adults. Contrary to vascular risk factors and atherosclerosis, a PFO is present from birth. However, it is completely unknown how an anatomical structure that is already present at birth in a large proportion of the population, can convert into a PFO that causes stroke in a few. Recent studies reported a significant association between certain trigger factors and ischemic stroke in young adults. This study aims to investigate these triggers in PFO-associated stroke.

Methods

The ODYSSEY Study, a multicenter prospective cohort study between 2013 and 2021, included patients aged 18-49 years experiencing their first-ever ischemic event. Participants completed a questionnaire about exposure to potential trigger factors. A case-crossover design was used to assess the relative risks (RR) with 95% confidence intervals (95% CI). The primary outcome was the relative risk of potential trigger factors for PFO-associated stroke.

Results

1043 patients completed the questionnaire and had an ischemic stroke of which 124 patients had a PFO-associated stroke (median age 42.1 years, 45.2% men). For patients with PFO-associated stroke the RR was 26.0 (95% CI 8.0-128.2) for fever, 24.2 (95% CI 8.5-68.7) for flu-like disease and RR 3.31 (95% CI 2.2-5.1) for vigorous exercise.

Conclusion

In conclusion, flu-like disease, fever and vigorous exercise may convert an asymptomatic PFO into a stroke causing PFO in young adults.

Data access statement

The raw and anonymized data used in this study can be made available to other researchers on request. Written proposals can be addressed to the corresponding author and will be assessed by the ODYSSEY investigators for appropriateness of use, and a data sharing agreement in accordance with Dutch regulations will be put in place before data are shared.

Introduction

The foramen ovale allows for right-to-left shunting prebirth and usually spontaneously closes after birth. However, in about 25% of individuals it remains patent.[34] This patent foramen ovale (PFO) is a possible cause of ischemic stroke in selected patients, without any other cause of stroke. PFO closure is proven beneficial in these selected patients over medical therapy in preventing recurrent ischemic stroke.[65, 66] Given the high prevalence of PFO it will most often be an innocent bystander, as the majority of patients with a stroke, also those with a PFO, will have another cause of stroke.

Contrary to vascular risk factors and atherosclerosis, a PFO is present from birth. However, it is completely unknown how an anatomical structure that is already present at birth in a large proportion of the population, can convert into a PFO that causes stroke in a few. The relatively new concept of trigger factors may elucidate this pathological conversion. A trigger factor is a short-lasting exposure to a trigger (i.e. toxins, caffeine, sexual activity, physical exercise or infection), that may subsequently create a (short-lasting) condition (for example a prothrombotic state or increase in blood pressure) that may predispose to stroke.[12-14] Recent studies reported a significant association between trigger factors (cola consumption, vigorous physical exercise, sexual activity, illicit drug use, fever and flu-like disease) and ischemic stroke in young adults (<50 years).[18, 19] To date, only one, case series (n=4) investigated the relation between a trigger factor (exercise-induced-Valsalva) and PFO-associated stroke.[15, 16] In practice, only a small percentage of patients who experience a PFO-associated stroke reportedly performed a Valsalva-like maneuver prior to the event. Besides, more than 50% of patients with a PFO have a permanent right-to-left shunting, also without a Valsalva-like maneuver.[17]

There is evidence suggesting that a PFO, unlike atrial fibrillation or atherosclerotic plaques, is not a direct cause of stroke; instead, it facilitates the passage of a venous thrombus into the arterial circulation.[67] We hypothesize, therefore, that other trigger factors create environmental conditions leading to a prothrombotic environment, causing a PFO-associated stroke. Hence, we investigated the relationship between potential trigger factors (caffein-containing coffee consumption, caffein-containing cola consumption, alcohol consumption, cigarette smoking, illicit drug use, vigorous physical exercise, sexual activity, fever and flu-like disease) and the risk of stroke at young age in patients with a PFO-associated stroke.

Methods – study population

We performed a case-crossover study as part of the Observational Dutch Young Symptomatic StrokE studY (ODYSSEY), a multicenter prospective cohort study on the prognosis and risk factors of patients aged 18 to 49 years with a first-ever ischemic stroke or intracerebral hemorrhage (ICH).[29] Ischemic stroke was defined according to a tissue based definition as acute onset of a neurologic deficit with imaging proof of ischemia. We included patients between May 2013 and February 2021. Exclusion criteria were any type of subarachnoid hemorrhage (SAH), cerebral venous sinus thrombosis or a history of a clinically symptomatic TIA, ischemic stroke or ICH.

Baseline data collection

We systematically collected information including stroke characteristics and severity (National Institutes of Health Stroke Scale, NIHSS), diagnostic laboratory- and cardiac tests, (vascular) risk factors, causes of stroke according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification and medication at discharge.[3, 29]

Diagnosis of a PFO and its role in stroke

Most patients underwent transthoracic echocardiography (TTE) with a bubble test using agitated saline to assess the presence of a PFO. In cases where it remained unclear whether the patient had a PFO or further evaluation was required, they underwent a transesophageal echocardiography (TEE). If the cause of stroke was evident early in the diagnostic process (e.g. carotid artery dissection, significant carotid artery stenosis), and additional information regarding the presence of PFO would be of no value, the patients would not receive an echocardiography. In June 2018 we established a multidisciplinary Heart-Stroke Team (HST) at the Radboud University Medical Centre to evaluate the relationship between PFO and stroke.[49] Patients evaluated in this multidisciplinary team underwent a thorough work-up (cardiac evaluation, prothrombosis screening and neuroimaging). Afterwards, the HST decided whether the stroke was most likely to be caused by the PFO. Prior to the HST patients were classified by their neurologist based on the available knowledge of that time, derived from the randomized control trials (RCTs) (2016) on PFO closure (a cryptogenic stroke, below the age 60 years and with any type of PFO).[20-22] As the understanding of PFO-associated stroke rapidly evolved during the inclusion period of the ODYSSEY trial, because of the appearance of the major PFO closure trials[20-22] that reported a benefit of closure in patients with no other cause of stroke, all patients that were included in our cohort before these trials were published were re-evaluated to assess whether there was a relation between the PFO and the occurrence of stroke. These patients were re-evaluated by a panel of five researchers/ medical doctors (MI, ME, NH, EV, JV) to determine if the patients had a PFO-associated

stroke. In the re-evaluation patients were classified as having a PFO-associated stroke when they had a genuine cryptogenic stroke with no signs of other (more likely) causes of stroke after a complete work-up. This includes, no lacunar infarction with signs of white matter hyperintensity and not more than 1 cardiovascular risk factor (e.g. hypertension, diabetes, hyperlipidemia, smoking and obesity). All cases that were questionable were discussed with a specialized stroke neurologist (FE). If there was another potential (competing) cause of stroke besides the PFO, and it was more likely to be the primary cause, the patient would be classified within that category. If the cause of the stroke was attributed to another etiology (Table 1) rather than the PFO, the PFO was considered to be an innocent bystander.

Table 1 Baseline demographics.

Demographics	**PFO-associated stroke (n=124)**	**Bystander PFO (n=48)**
Men, n (%)	56 (45.2)	21 (43.7)
Median age (IQR)	42.1 (31.1- 53.1)	43.9 (36.0-51.8)
Vascular risk factors, n (%)		
Smoking	35 (28.2)	30 (62.5)
Hypertension	9 (7.3)	11 (22.9)
Diabetes mellitus	5 (4.0)	4 (8.3)
Hypercholesterolemia	62 (50)	37 (77.1)
Territorial infarction, n (%)	109 (87.9)	40 (83.3)
Percutaneous PFO closure, n (%)	83 (66.9)	x
RoPE score, n (%)*		
0-5	0 (0.0)	2 (6.9)
6	7 (5.9)	3 (10.3)
7	27 (22.9)	14 (48.3)
8	50 (42.4)	8 (27.6)
9	22 (18.6)	2 (6.9)
10	12 (10.2)	0 (0.0)
Median RoPE score (IQR)	8 (6-10)	7 (6-8)
TOAST classification, n (%)		
Large artery atherosclerosis	x	2 (4.2)
Likely atherothrombotic stroke	x	21 (43.8)
Small vessel disease	x	6 (12.5)
Cardio-embolic	x	0 (0.0)
Other determined cause	x	16 (33.3)
Multiple causes	x	1 (2.1)
Cryptogenic	x	2 (4.2)

*Missing data on RoPE score: 6 in the "PFO-associated stroke" group, 19 in the "Bystander PFO" group.

Assessment of Trigger factors

Patients completed a structured, standardized questionnaire on exposure to potential trigger factors within predefined hazard periods (Supplementary material). Based on previous studies, we recorded validated trigger factors including caffein-containing coffee consumption, caffein-containing cola consumption, alcohol consumption, cigarette smoking, any illicit drug use (cocaine, heroin, methadone, amphetamine, ecstasy and d-lysergic acid diethylamide (LSD) were considered hard drugs, cannabis products and mushrooms containing psilocin considered soft drugs), vigorous physical exercise, sexual activity, fever and flu-like disease.[12, 18, 68-72] Illicit drug use was combined due to the small sample size. The cross-over design presumes that exposure to a trigger factor within a predefined period of time increases the risk of stroke compared to non-exposure. Patients were asked about their frequency of exposure to each trigger factor in the previous year and exposure during a predefined hazard period which was specific for each trigger factor based on its estimated duration of the trigger effect.[68, 70-75] Different grades of vigorous physical exercise were classified based on their metabolic equivalent of task (MET), an objective measure of the ratio of the rate at which a person expends energy, relative to the mass of that person, while performing specific physical activity compared to the resting metabolic rate. One MET is equivalent to the resting metabolic rate, e.g. sitting quietly, and results in burning 1 kcal/kg/hour. As an example, a patient with a weight of 70 kg performing a 1 MET activity (sitting) for 1 hour will use 70 kcal. We analyzed the following subcategories: heavy exercise [MET]=6, severe exercise [MET]=7, extreme exercise [MET]=8.

To minimize recall bias, patients were asked to complete the questionnaire as soon as possible after stroke. In the sensitivity analysis we excluded patients that completed the questionnaire >4 weeks after stroke (Table 3). Furthermore, unreliable answers were excluded (e.g. inconsistent answers, unmistakable false answers), before analyses were done.

Table 2 Trigger factors for patients with PFO and stroke.

	PFO-associated stroke (n=124)	Exposed* Year (hazard period)	Bystander PFO (n=48)	Exposed* Year (hazard period)
Trigger factor	RR (95% CI)	N (n)	RR (95% CI)	N (n)
Alcohol consumption	0.86 (0.22-3.44)	56 (2)	-	13 (0)
Cigarette smoking	0.74 (0.33-1.68)	26 (14)	0.72 (0.33-1.57)	21 (13)
Coffee Consumption	0.92 (0.48-1.77)	89 (13)	0.92 (0.35-2.44)	29 (6)
Cola Consumption	2.08 (0.86-5.02)	41 (5)	1.23 (0.12-12.75)	12 (1)
Heavy exercise	3.4 (2.16-5.36) [a]	99 (21)	2.84 (1.05-7.68) [a]	27 (4)
Severe exercise	5.03 (2.59-9.79) [a]	77 (10)	1.72 (0.26-11.53)	18 (1)
Extreme exercise	6.38 (2.82-14.43) [a]	39 (7)	-	10 (0)
Combined vigorous exercise	3.31 (2.16-5.08) [a]	105 (27)	1.76 (0.67-4.60)	28 (4)
Sexual activity	2.0 (0.64-6.20)	87(3)	2.7 (0.37-19.94)	30 (1)
Illicit drug use	-	8 (0)	3.9 (0.28-54.74)	4 (1)
Fever	25.98 (7.96-84.78) [a]	30 (3)	-	12 (0)
Flu-like disease	24.2 (8.53-68.70) [a]	47 (4)	22.75 (3.02-171.33) [a]	14 (1)

RR: relative risk, CI: confidence interval

[a] RR and 95% CI turned out to be significant

* The number of patients exposed to the trigger factor during the previous year (the number of patients exposed within the hazard period)

Data analyses

We examined the exposure of potential trigger factors in a hazard period compared to a control period using a case-crossover design.[12, 14] In this design patients serve as their own control thereby minimizing the occurrence of confounding bias. Relative risk (RR) with 95% confidence interval (CI) was calculated for each potential trigger factor for ischemic stroke using the Mantel-Haenszel case-crossover method. The RR was determined by calculating the ratio of exposure in the hazard period and the reported yearly exposure frequency based on patients weekly or daily average frequency.[14] The RRs should be interpreted as for a short-term period and not as cumulative risks for a long-term period.

Data were reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines and analyzed using SPSS Software version 22 (IBM) and R version 4.1.2 (R Project for Statistical Computing).

Standard Protocol Approvals, Registrations, and Patient Consents

The Medical Review Ethics Committee region Arnhem-Nijmegen approved the study (NL41531.091.12). All patients signed informed consent.

Results

Demographics are depicted in Table 1. There were 1322 patients with an ischemic stroke of which 1043 who completed the trigger questionnaire. There were 124 (11.9%) patients with a PFO-associated stroke of which 83 (66.9%) were closed. The median age of patients with a PFO-associated stroke was 42.1 years (IQR 31.1-53.1) and 45.2% were male. Patients with a bystander PFO and stroke of other etiology (4.6%) had a median age of 43.9 years (IQR 36.0-51.8) and 43.7% were male.

In total, 870 (83.4%) patients underwent TTE, 30 (2.9%) patients underwent TEE and 169 (16.2%) patients underwent both TTE and TEE. 143 (13.7%) patients did not undergo echocardiography because the cause of stroke was evident early in the diagnostic process and the demonstration of a PFO would not alter our treatment strategy.

Table 3 Sensitivity analysis.

	PFO associated stroke (n=124)	**Excluded patients that filled in questionnaires > 4 weeks after index event (n=74)**
Trigger factor	RR (95% CI)	RR (95% CI)
Alcohol	0.86 (0.22-3.44)	0.68 (0.10-4.84)
Cigarette smoking	0.74 (0.33-1.68)	0.54 (0.21-1.42)
Coffee	0.92 (0.48-1.77)	1.15 (0.53-2.48)
Cola	2.08 (0.86-5.02)	2.09 (0.80-5.47)
Heavy exercise	3.4 (2.16-5.36) [a]	3.14 (1.75-5.64) [a]
Severe exercise	5.03 (2.59-9.79) [a]	6.29 (2.35-16.81) [a]
Extreme exercise	6.38 (2.82-14.43) [a]	9.92 (3.47-28.34) [a]
Sexual activity	2 (0.64-6.20)	3.52 (1.12-11.08) [a]
Illicit drug use	-	-
Fever	25.98 (7.96-84.78) [a]	41.96 (12.47-141.20) [a]
Flu-like disease	24.2 (8.53-68.70) [a]	44 (14.75-131.24) [a]

RR: relative risk, CI: confidence interval

[a] RR and 95% CI turned out to be significant

Trigger factors of PFO-associated stroke

Fever and flu-like disease that occurred within 24 hours prior to stroke increased the risk of PFO-associated stroke significantly (RR 26.0, 95% CI 8.0-84.8 and RR 24.2, 95% CI 8.53-68.7, respectively) compared with periods without fever or flu. Furthermore, heavy and severe vigorous physical exercise within one hour before ischemic stroke increased the risk of having a PFO-associated stroke significantly with a RR of 3.31 (95% CI 2.2-5.1) for all vigorous physical exercise combined (Table 2). Our sensitivity analysis, which excluded all patients who completed the questionnaire four weeks or more after their index event, showed a RR increase for fever, flu-like disease and vigorous exercise (Table 3). No increased risk was found after exposure to caffein-containing coffee consumption, caffein-containing cola consumption, alcohol consumption, cigarette smoking, illicit drug use or sexual activity.

Discussion

We found that vigorous exercise, fever and flu-like disease may act as a potential trigger factor for PFO-associated stroke. Ekker et al. (2022) elaborate on the influence of trigger factors in distinct non-PFO associated stroke causes.[18] Given the fact that PFO is a life-long persisting anatomical variant, our findings may shed light on which (trigger) factors play a role in conversion to a PFO causing stroke.[76] There are several biological mechanisms explaining how an infection may convert a PFO into an stroke causing PFO. First, pathogens can cause an endotheliopathy by direct invasion of the arterial wall when present in the systemic circulation, therefore causing a prothrombotic state.[68]

It is possible that the endocardium of the PFO tunnel is particularly vulnerable for pathogens. Previous research using optical coherence tomography found in situ thrombus formation and abnormal endocardium within the PFO tunnel in PFO-associated stroke patients.[7]

Infection and inflammation is known to be associated with venous thrombo-embolism (VTE), especially the first two weeks after infection onset, gradually declining thereafter.[77] This may partly explain PFO-associated strokes, as the concept is that a (paradoxical) embolism emerges from the venous circulation, entering the arterial circulation when passing through the PFO.

A possible explanation for the association between vigorous exercise and PFO-associated stroke could be the acute activation of the sympathetic nervous system

and therefore increase in heart rate and blood pressure, resulting in increased shear stress.[72] This may result in platelet deposition, with an accompanying risk of thrombi.[78] Furthermore, high norepinephrine levels may lead to increased platelet aggregation and oxygen demand.[79] Hypothetically, increased blood pressure due to exercising could increase the intracardiac pressure and therefore increase right-to-left shunting. Besides increasing blood pressure, several sports (for example weight lifting) could also increase the intrathoracic pressure. Therefore, causing a Valsalva-like maneuver during exercise which is a known risk factor for PFO-associated strokes.[16]

A major strength of this study is the large sample size with ischemic stroke patients based on a tissue based definition, minimizing the inclusion of stroke mimics. In addition, all echocardiographic images were re-evaluated by an experienced cardiologist. Due to the case-cross over design patients serve as their own control thereby minimizing the occurrence of confounding bias. All patients with a stroke and PFO who were not found eligible for PFO closure or were included before the instigation of percutaneous closure, were re-evaluated by five independent researchers/ medical doctors (MI, ME, NH, EV, JV) to determine if the patient had a PFO-associated stroke.

Our study also has limitations. Recall bias may have played a role as all trigger factors were self-reported by means of a questionnaire. It may be difficult for patients to remember or make an estimated guess how often a trigger occurred in the past year. Several trigger factors could be defined more accurately. For example, we could quantify caffeine consumption in milligrams or number of drinks and separate the different types of exercise instead of using the MET. Furthermore, we have no consecutive data on PFO characteristics like diameter or tunnel length. Another limitation of the study is the small number of patients with each of the exposures therefore creating a large confidence interval. Due to the small number of patients exposed to illicit drug use, we were not able to assess whether different types of drugs could serve as a trigger factor for stroke. There could be a bias in reporting, patients may not have been willing to share information about physical activity, the use of drugs or smoking. Of all patients with a PFO-associated stroke, 33.1% did not undergo percutaneous closure. This could (partially) be explained by the fact that a significant number of patients were enrolled prior to the existence of positive RCTs demonstrating the superiority of percutaneous closure over best medical treatment. Unfortunately, there is no available data regarding the reasons for acceptance or rejection of percutaneous closure.

Furthermore, the time window between the event and the trigger may be difficult to define. We tried to minimize recall bias by using a sensitivity analysis. A possible solution to address this in future research is to objectify data by using health record data and information from wearables. Second, especially in fever and flu-like disease the width of the 95% confidence interval is quite wide due to the smaller subgroups. Unfortunately, the sample size of patients with an innocent bystander was too small to calculate a reliable RR for fever and possible also for flu-like disease regarding the large confidence interval. A larger cohort of patients with a bystander PFO and a stroke of other etiology is needed to explore the role of trigger factors in these patients. Another limitation in PFO-associated stroke research is the uncertainty about the role of PFO in strokes with competing causes. As there is no reliable method to definitively determine which of the competing causes is the actual culprit. We attribute the stroke to the most prevalent cause, considering that most competing etiologies are more likely to cause a stroke than a PFO. Since several patients did not undergo echocardiography due to an evident cause of stroke being identified early in the diagnostic process, the prevalence of patients with a bystander PFO is likely to be much higher than represented in our study. Future research should focus on which patients based on their characteristics, in combination with PFO characteristics, are at the highest risk for PFO-associated stroke. They should also evaluate why most patients with a PFO experience a stroke only once in their life, despite the fact that most trigger factors are present multiple times throughout life. Another interesting question for future research concerns the age aspect of PFO-associated stroke, since it occurs more often in young adults, but it is very rare in children, whilst trigger factors such as fever and flu-like disease are abundantly present in childhood. Furthermore, it remains unclear why, for example, labour does not seem to be a major risk factor for PFO-associated stroke, despite the facts that women during childbirth are in a pro-thrombotic state and need to exert a significant Valsalva maneuver. Lastly, imaging studies could detect if patients with a PFO have more "silent infarctions" which lack clinically overt stroke symptoms or if the PFO-associated strokes are indeed sporadic.

Conclusion

In conclusion, flu-like disease, fever and vigorous exercise may convert an asymptomatic PFO into a stroke causing PFO in young adults.

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Disclosures

There was no role of any sponsor or funder in the design and conduct of the study, in the collection, analysis or interpretation of the data. Neither was there a role in the preparation of the manuscript.

Supplemental material

Trigger factor questionnaire English

1. Smoking

1.1 Have you ever smoked?
o Yes, I still smoke
o Yes, I have smoked and quit less than 6 months ago
o Yes, I have smoked and quit more than 6 months ago
o No, I have never smoked

1.2 At what age did you start smoking?
o Age:……..
o I don't know

1.3 How many cigarettes did you smoke on average per day the week before the stroke/ TIA?
o Number:……..
o I don't know

1.4 How many cigarettes did you smoke 1 hour before the onset of the stroke/TIA?
o Number:……..
o I don't know

2. Alcohol

2.1 Do you drink alcohol?
o Yes
o No

2.2 How many units of alcohol did you consume on average per week in the year before the stroke/TIA?
o Number of units:……
o I don't know

2.3 How many units of alcohol did you consume the week before the stroke/TIA?
o Number of units:……..
o I don't know

2.4 How many units of alcohol did you consume 24 hours before the onset of the stroke/TIA?
o Number of units:……..
o I don't know

2.5 When was the last time you drank alcohol? (number of hours before the stroke/TIA)
o Number of hours:……
o I don't know

2.6 How many units of alcohol were consumed?
o Number of units:……
o I don't know

3. Drugs

3.1 Have you ever used drugs?
o Yes
o No

3.2 Which drugs have you used? (circle what applies)

Cocaine:	Yes	No
Heroin:	Yes	No
Methadone:	Yes	No
Amphetamines:	Yes	No
Cannabis:	Yes	No
Hallucinogens:	Yes	No
Inhalants:	Yes	No
XTC:	Yes	No
Anabolic steroids:	Yes	No
EPO:	Yes	No

Other, namely: .....

If it concerns multiple types of drugs, please answer the following questions about the substance you used the shortest time before the stroke/TIA.

3.3. How often did you use this drug on average per week in the year before the stroke/TIA?
o Number of times per week: .....
o I don't know

3.4. How often did you use this drug 1 week before the onset of the stroke/TIA?
o Number of times per week: .....
o I don't know

3.5. Did you use these drugs 4 hours before the onset of the stroke/TIA?
o Yes
o No
o I don't know

3.6. When was the last time you used these drugs? (number of hours before the stroke/TIA)
o Number of hours: .....
o I don't know

4. Coffee

4.1. Do you ever drink coffee?
o Yes
o No

4.2. How many units of coffee did you consume on average per day in the year before the stroke/TIA? (units per day)
o Number of cups of coffee: .....
o I don't know

4.3. How many units of coffee did you consume on average per day 1 week before the onset of the stroke/TIA? (units per day)
o Number of cups of coffee: .....
o I don't know

4.4. How many units of coffee did you consume 1 day before the onset of the stroke/TIA?
o Number of cups of coffee: .....
o I don't know

4.5. How many units of coffee did you consume 1 hour before the onset of the stroke/TIA?
o Number of cups of coffee: .....
o I don't know

4.6. When was the last time you drank coffee? (number of hours before the stroke)
o Number of hours before the stroke/TIA: .....
o I don't know

4.7. How many units were consumed?
o Number of cups of coffee: .....
o I don't know

5. Cola

5.1. Do you ever drink cola?
o Yes
o No

5.2. How many units of cola did you consume on average per week in the year before the stroke/TIA? (units/week)
o Number of glasses of cola: .....
o I don't know

5.3. How many units of cola did you have 1 week before the onset of the stroke/TIA?
o Number of glasses of cola: .....
o I don't know

5.4. How many units of cola did you have 1 day before the onset of the stroke/TIA?
o Number of glasses of cola: .....
o I don't know

5.5. How many units of cola did you have 1 hour before the onset of the stroke/TIA?
o Number of glasses of cola: .....
o I don't know

5.6. When was the last time you drank cola? (number of hours before the stroke/TIA)
o Number of hours before the stroke/TIA: .....
o I don't know

5.7. How many units were consumed?
o Number of glasses of cola: .....
o I don't know

6. Fever

6.1. How often did you have a fever in the year before the onset of the stroke/TIA?
o Number of times with fever: .....
o I don't know

6.2 Did you have a fever 1 week before the onset of the stroke/TIA?
o Yes
o No
o I don't know

6.3. Did you have a fever 24 hours before the onset of the stroke/TIA?
o Yes
o No
o I don't know

7. Flu

7.1. How often did you have the flu in the year before the onset of the stroke/TIA?
o Number of times: .....
o I don't know

7.2. Did you have the flu 1 week before the onset of the stroke/TIA?
o Yes
o No
o I don't know

7.3. Did you have the flu 24 hours before the onset of the stroke/TIA?
o Yes
o No
o I don't know

8. Sexual Activity

8.1. How often per month were you sexually active in the year before the onset of the stroke/TIA?
o Number of times per month: .....
o I don't know

8.2. Were you sexually active the day before the onset of the stroke/TIA?
o Yes
o No
o I don't know

8.3. Were you sexually active twhours before the onset of the stroke/TIA?
o Yes
o No
o I don't know

9. Exercise

The following questions are about the number of hours of heavy, very heavy, and extreme exercise you have engaged in during the year, 1 week, and 1 hour before the onset of the stroke/TIA. The table below provides examples of heavy, very heavy, and extreme exercise.

PLEASE NOTE, you do not need to fill in the example below, please answer the questions on the next page.

MET nr	Description	Type of activity	Number of hours per week
1	Sleeping	Resting, Sunbathing, lying on the couch watching TV	X
2	Sitting	Reading, desk work, sitting watching TV, highway driving, eating	
3	Very light exercise	Office work, city driving, personal care, standing in line, strolling in the park	X
4	Light exercise	Mopping, slow walking (shopping), bowling, gardening with tools, golfing, sweeping	X
5	Moderate exercise	Normal walking, golfing, slow biking, downhill skiing, picking up leaves, washing windows, fishing, dancing, painting, wallpapering, light restaurant work	X
6	Heavy exercise	Jogging, brisk walking, tennis, swimming, cross-country skiing, hoeing, fast biking, heavy household repairs, climbing up and down ladders, ice hockey, softball, bricklaying, heavy restaurant work	X
7	Very heavy exercise (lots of sweating and out of breath)	Running, non-stop tennis, pushing car in snow, changing tires, making cement, basketball, climbing ladders with heavy weight	X
8	Extreme exercise	Extreme exertion Sprinting, uphill running, pushing with all your strength, unusually heavy work or sports	X

9.1. How much exercise did you engage in the year before the onset of the stroke/TIA?

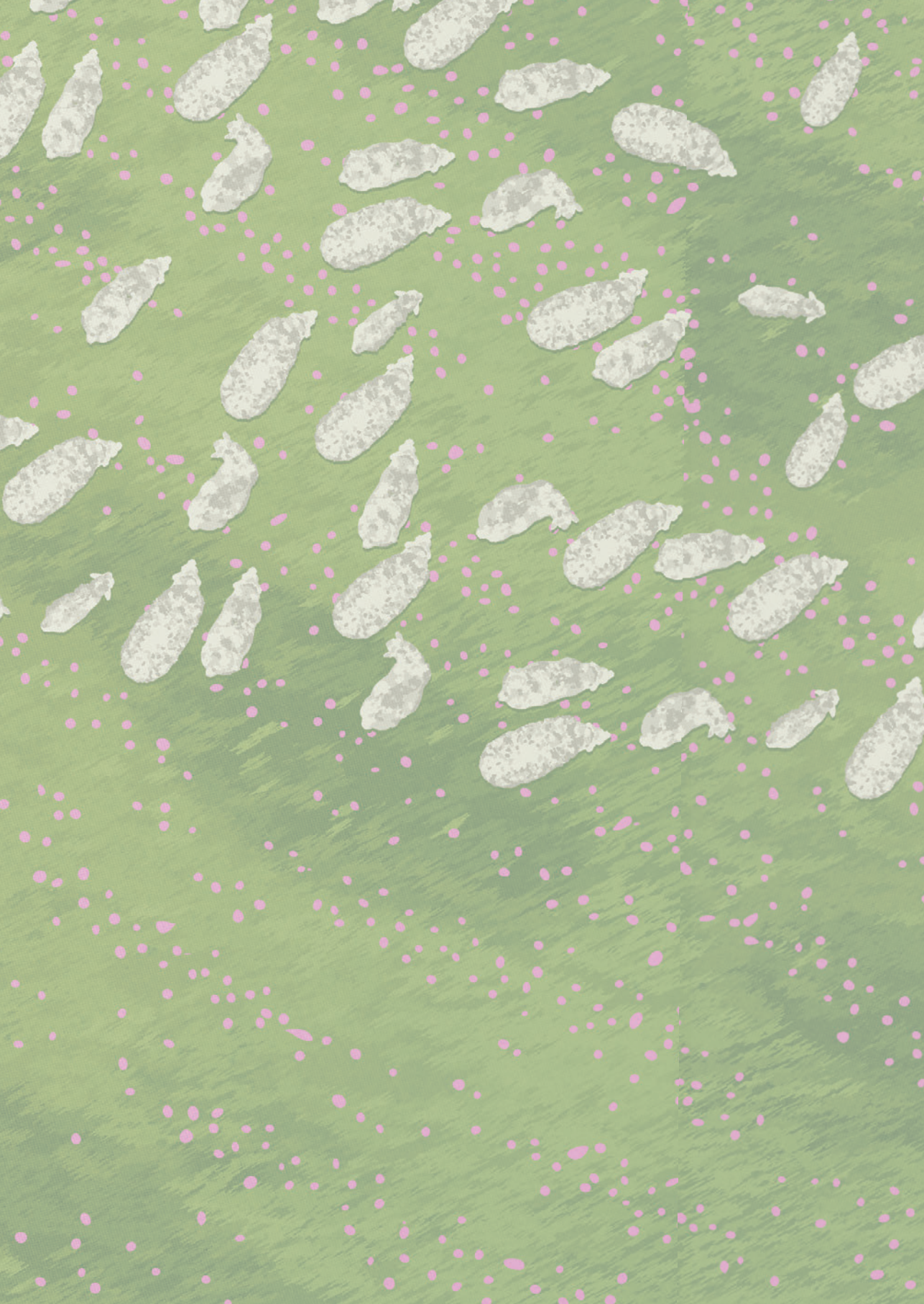
a. Average number of hours of heavy exercise per week .....
b. Average number of hours of very heavy exercise per week .....
c. Average number of hours of extreme exercise per week .....

9.2. How much exercise did you engage in one week before the onset of the stroke/TIA?

a. Average number of hours of heavy exercise per week .....
b. Average number of hours of very heavy exercise per week.....
c. Average number of hours of extreme exercise per week.....

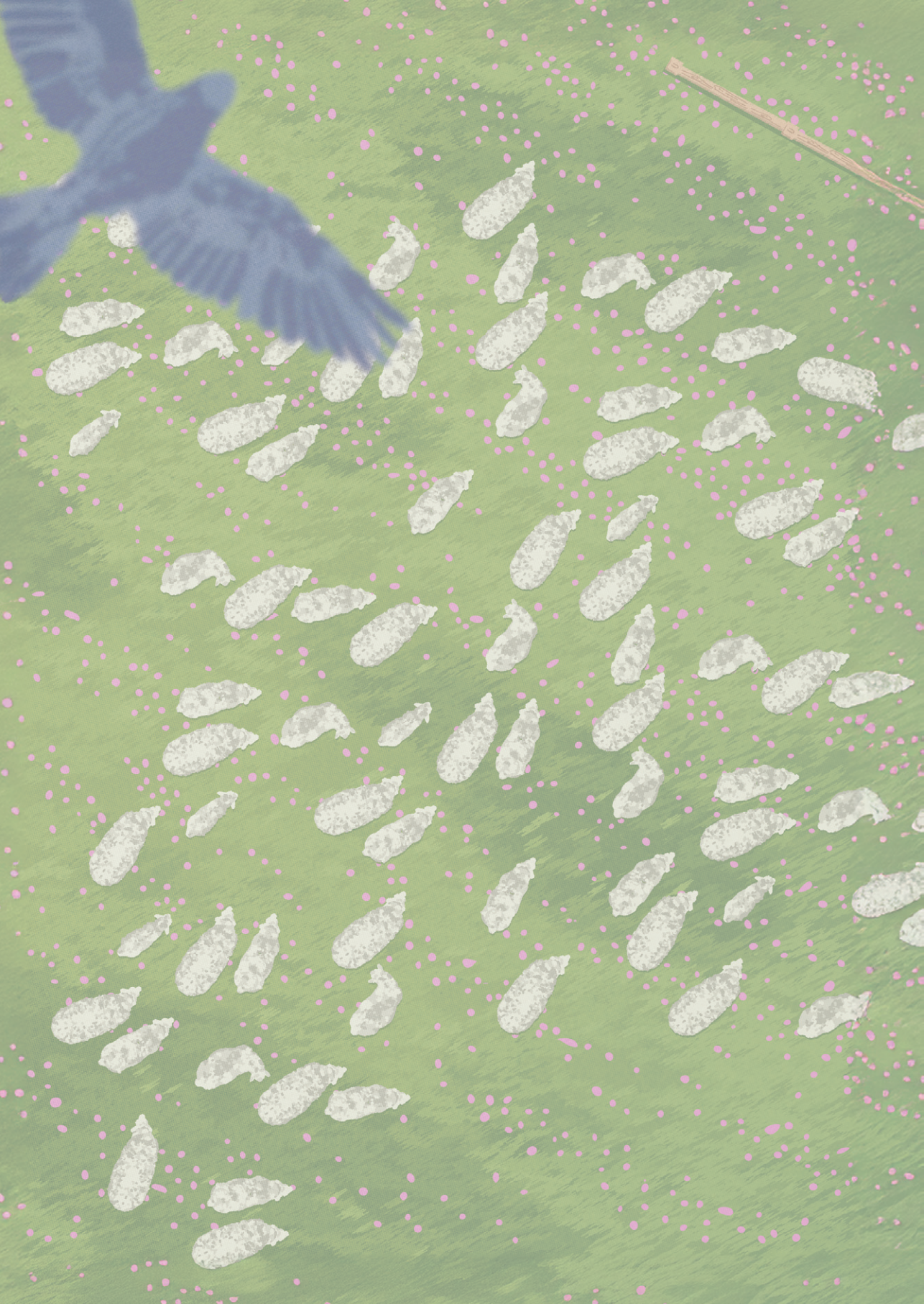
9.3. How much exercise did you engage in 1 hour before the onset of the stroke/TIA?

a. Number of minutes of heavy exercise .....
b. Number of minutes of very heavy exercise.....
c. Number of minutes of extreme exercise.....



Part VI

Making a decision



6

Heart-stroke team: a multidisciplinary assessment of patent foramen ovale-associated stroke

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Abstract

Introduction

Patent foramen ovale (PFO) closure prevents recurrent ischemic stroke in selected patients with a cryptogenic stroke. Trial results tend to be generalized to daily practice, often extending original trial inclusion criteria. This may result in unnecessary closure without benefit, but with risk of complications. We therefore introduced a standardized and structured evaluation by an interdisciplinary Heart-Stroke Team (HST). Our aim was to investigate the proportion of actual PFO closure of all referred patients with a cryptogenic stroke, after evaluation by the HST.

Patients and methods

We conducted a single-center, retrospective cohort study. Patients with an assumed cryptogenic ischemic stroke or transient ischemic attack (TIA) and a PFO who were referred for PFO closure were analyzed. As part of the HST approach, all patients underwent a standardized work-up, first to demonstrate the ischemic event on neuroimaging, second to evaluate all potential causes of stroke and finally, to assess the possible relation between the PFO and stroke. Outcome was the proportion of patients treated with PFO closure after referral.

Results

A total of 195 patients were included. In 124 patients (64%) PFO closure was advised. Fourty-two (22%) patients had a clear alternative cause of stroke and in 13 (7%) patients the initial stroke diagnosis could not be confirmed.

Conclusion

After careful analysis of patients referred for PFO closure a relationship between the PFO and stroke could not be demonstrated in 32% of referrals, and 3% preferred best medical treatment over percutaneous closure. This stresses the need for a complete neurovascular work-up and multidisciplinary assessment.

Introduction

A PFO is a common finding, with a prevalence of up to 25% in the general population.[34] In young patients with a cryptogenic stroke a prevalence as high as 40%–50% has been reported.[80, 81] Randomized controlled trials (RCT) demonstrated a lower risk of recurrent ischemic stroke after PFO closure in selected patients,[20-22] however it is difficult to demonstrate a causal relation between highly prevalent findings in healthy individuals, such as PFO and stroke. In the randomized clinical trials, this relation was operationalized as evidence of a cortical infarct on imaging without any other cause of the stroke than a PFO. Over time, trial results tend to be generalized, with the risk of indications for PFO closure becoming extended. This is illustrated in a study that reported that 33% of all PFO closures were performed in patients over the age of 60 years, even though such patients had been excluded from the RCTs.[82] Extrapolating trial results to the general population is a cautious, deliberate and inevitable process. This requires a standardized work-up and individualized assessment to determine in which patients the PFO is deemed the cause of stroke and consequently which patients will benefit most from PFO closure, since unnecessary closure has no benefits and may cause complications.[83]

The European Association of Percutaneous Cardiovascular Interventions (EAPCI) and The Society for Cardiovascular Angiography and Interventions (SCAI) have each published position papers on the management of patients with PFO and stroke.[5, 84] These papers advocate the installment of an interdisciplinary Heart-Stroke Team (HST) including at least a neurologist and an interventional cardiologist, to investigate whether the stroke can be attributed to the PFO. However, HSTs have not been implemented in every stroke unit and limited data are available on the effects of the introduction of an HST on the proportion of PFO closures of all referred patients. Only one previous study, conducted after the positive closure RCTs, investigated the added value of an HST.[20-22] That study showed that in 20% of PFO-patients, the HST diagnosis was discordant from that of referring physicians. Fourteen percent were stroke misdiagnoses and the authors advised against closure in 53% of patients. However the investigators did not describe in detail the reasons for rejection of PFO closure, neither specifying why patients with a cryptogenic stroke and PFO did not have a PFO-associated stroke, nor showing how echocardiographic data influenced the decision-making on PFO closure.[85] We therefore investigated what proportion of patients with a cryptogenic stroke referred to a tertiary stroke center (Radboudumc, Nijmegen, The Netherlands) for PFO closure, were actually closed after HST evaluation. Furthermore, we planned to state clearly the reasons for rejection and show the value of variables like RoPE

Score, anatomical-, stroke- and patient characteristics in deciding on PFO closure. Additionally, we explored group differences between the patients who underwent PFO closure before versus after the instigation of the HST.

Methods

Study design and patients

We conducted a single-center, retrospective cohort study. Patients with a cryptogenic ischemic stroke or transient ischemic attack (TIA) and a PFO, who were referred to the Radboudumc (Nijmegen, the Netherlands) for consideration of PFO closure between November 2016 and December 2021, were included. Patients were referred because in the referring centers the PFO was concluded to be the cause of stroke.

Before the HST

Before the instigation of the HST (2016 until 2018) all patients who were referred for PFO closure were accepted for closure when criteria of the RCTs (a cryptogenic stroke, below the age 60 years and with any type of PFO) were fulfilled. All investigation for the cause of stroke was done within the referring hospitals, and according to their local guidelines.

After the introduction of the HST

The Radboudumc HST started in June 2018. It comprises a congenital interventional cardiologist, a vascular internal medicine specialist, stroke neurologist and a specialized nurse. This composition is based on the Dutch guideline for PFO closure.[86] The inclusion criteria for evaluation by the HST are: (1) a presumed cryptogenic stroke according to the referring neurologist, (2) demonstration of a PFO, and (3) a probable relation between the PFO and the stroke. Patients were excluded when another, more likely, cause of stroke was present.

The HST evaluates all data collected during the standardized work-up to determine the relationship (or its absence) between the PFO and the stroke.

Standardized work-up after installment of the HST

Relevant data on prothrombotic state, cardiac arrythmia, neuroimaging (including cervical artery angiography) and vascular risk factors were retrieved from the patient files. If the available information was considered insufficient, additional testing or imaging was performed. Before a decision regarding PFO closure, all

patients underwent the following standardized assessment on the cause of their stroke: imaging proof of the ischemic lesion (preferably an MRI), prothrombotic testing (Supplemental Table 1), electrocardiogram, a minimum of 72 hours' heart rhythm registration, a transthoracic echocardiogram (TTE) with agitated saline contrast to demonstrate a right-to-left shunt and preferably measurements of PFO characteristics (e.g. presence of an atrial septal aneurysm, tunnel length), imaging of the cervical arteries and a comprehensive cardiovascular risk profile. Any tests missing were subsequently performed in our hospital or in the referring hospital if considered of importance for the decision-making. Following the SCAI guideline, PFO closure is performed in most patients with thrombophilia, except for those with uncertain benefits of PFO closure and a concomitant higher risk of periprocedural risks. Taking into account the lifelong need for oral anticoagulation in patients with anti-phospholipid syndrome and the low level of evidence for PFO closure in these patients, non-closure of the PFO can be considered and would always be discussed with the patient.

The relation between stroke and the PFO was deemed causal when the stroke diagnosis was verified by the HST without any other cause of stroke, except the PFO. If the stroke was not attributed to the PFO, the etiology was classified according to the TOAST-criteria.[3, 87]

Vascular risk factors

Classical cardiovascular risk factors were obtained from the medical files. The first LDL-value available was used for analysis. This was preferably a statin naive value; if this was not available the first available LDL-value was used. Hypertension was defined as a systolic blood-pressure of >130 mmHg on at least two occasions during out-patient visits. When a 24 h blood-pressure recording was available this was used. Diabetes mellitus was defined as the need for medication to control blood glucose levels.

Cardiac work-up

All patients underwent a minimum of 72 hours' heart rhythm registration, but preferably 7 day heart rhythm monitoring for atrial fibrillation. Atrial fibrillation as cause of stroke was defined as any atrial fibrillation on heart rhythm monitoring lasting more than 15 min, independent of patient symptoms.

Echocardiographic work-up

All patients underwent a TTE with agitated saline contrast to demonstrate a right-to-left shunt. An additional transesophageal echocardiogram (TEE) was left to the

discretion of the referring neurologist and cardiologists. All TTE and TEE were re-evaluated by the same cardiologists (TtC/AvD). When the available images from the referring center were inconclusive, a TTE with agitated saline contrast was repeated in the Radboudumc. At least three contrast runs without and with Valsalva maneuvers were conducted. When the patient was unable to perform a proper Valsalva maneuver, a TEE was performed to visualize the atrial septum.

PFO was defined as a right-to-left shunt when the contrast was visible in the left atrium within five heartbeats after its administration. Atrial septal aneurysm (ASA) was defined in three different ways: (1) at least 10 mm of excursion from the midline as used by the Systematic Collaborative, PFO closure Evaluation (SCOPE), (2) at least 15 mm protrusion of interatrial septum used by Hanley et al., and (3) protrusion of saccular redundant tissue.[43, 88] Shunt size was defined as (0) None, (1) Small, <5 bubbles, (2) Moderate, 5–30 bubbles, (3) Large, >30 bubbles, (4), Opacification, near immediate opacification of the left ventricle.[50]

Statistical analyses

Statistical analysis was done using IBM SPSS Statistics 26. Data are shown as number (%) for categorical data or as mean ± SD or median and range for continuous data depending on the Gaussian distribution. Categorical data was analyzed using the Chi-squared test. Two-sided independent Student's *t*-test and Mann-Whitney *U* test were used to analyze differences between the groups for continuous data. Differences within the groups were analyzed by repeated measures ANOVA.

A *p*-value of <0.05 was considered statistically significant. In general, data were considered missing if the item was not explicitly mentioned in the medical files.

Results

Patient characteristics

We included 240 patients, of whom 45 were evaluated before the introduction of the HST (2016–2018) and all 45 had PFO closure.

After the introduction of the HST, 195 patients were included with a mean age of 46 years (SD 12.2); 96 (49%) were female. Patients accepted for PFO closure were younger (mean age 43 years (SD 10.6)), had fewer classical cardiovascular risk-factors and less often a history of stroke or TIA (Table 1) than those rejected for closure.

Table 1 Baseline characteristics.

Characteristics	All patients N = 195	Accepted for closure N = 124	Rejected for closure N = 71	p-Value
Mean age at time of referral, years [SD]	45.9 [12.2]	42.7 [10.6]	51.5 [12.8]	<0.001¤
Age groups, N (%)				
18–29	21 (10.8)	18 (14.5)	3 (4.2)	0.026
30–39	31 (15.9)	23 (18.5)	8 (11.3)	0.181
40–49	68 (34.9)	48 (38.7)	20 (28.2)	0.137
50–59	49 (25.1)	31 (25.0)	18 (25.4)	0.956
60–65	16 (8.2)	4 (3.2)	12 (16.9)	0.001¤
>65	10 (5.1)	0 (0.0)	10 (14.1)	xΩ
Male, *N* (%)	99 (50.8)	63 (50.8)	36 (50.7)	0.989
Mean BMI, kg/m^2 [SD]	25.4 [4.0]	25.1 [4.0]	25.9 [4.0]	0.232
History of smoking,**N* (%)	82 (42.7)	48 (39.0)	34 (49.3)	0.168
Mean LDL-value without medication, mmol/L [SD],*	2.42 [0.81]	2.36 [0.87]	2.54 [0.65]	0.408
History of stroke or TIA,**N* (%)	34 (17.6)	10 (8.1)	24 (34.3)	<0.001¤
History of hypertension, *N* (%)	41 (21.0)	18 (14.5)	27 (38.0)	<0.001¤
Mean systolic blood pressure without medication,* mmHg [SD]	124.1 [15.1]	122.6 [14.2]	127.6 [16.5]	0.063
History of diabetes mellitus, *N* (%)	8 (4.1)	1 (0.8)	7 (9.9)	0.004¤
Family history of vascular disease,**N* (%)	74 (38.5)	43 (35.0)	31 (44.9)	0.173
Antithrombotic therapy at evaluation,*N (%)				
Anticoagulant	13 (6.7)	11 (8.9)	5 (7.1)	0.654
Antiplatelet	157 (80.9)	112 (90.3)	62 (88.6)	0.516
None	3 (1.5)	1 (0.8)	3 (4.3)	0.105

BMI: Body Mass Index; SD: standard deviation; LDL: low-density lipoprotein; TIA: transient ischemic attack; HST: Heart-Stroke team.

Five patients were deemed eligible for PFO closure but did not undergo PFO closure due to their own preference (these patients are included in the "Accepted for closure" group).

* Information was missing in the following number of patients: 8 patients in "LDL-value before medication," 2 patients in "History of stroke or TIA," 3 patients in "Family history of vascular diseases," 3 patients in "Smoking," 11 patients in "systolic blood pressure," 1 patient in "Antithrombotic therapy at evaluation." Missing variables have been excluded from the calculated percentages.

Ω Could not be calculated due to presence of "0."

¤ Reached significance level of 0.05. *p*-Values were calculated two-sided using a chi-squared test to assess the equality of proportion.

Patients' characteristics with PFO closure prior to the existence of the HST

Patients who underwent PFO closure before the presence of a HST were younger (mean age 38 years SD 9.1vs 43 years SD 10.6; $p = 0.005$), had a higher RoPE Score (8–10, 67% vs 39%; $p = 0.001$) and fewer high-risk PFO features on echocardiography (Supplemental Table 2) than those who underwent PFO closure after HST installment.

Echocardiographic features of PFO

Patients accepted for PFO closure, compared to those who were rejected, had a significantly larger shunt, both with (3 [IQR 2–4] vs 2 [IQR 1.25–3]; $p = 0.028$) and without Valsalva maneuver (2 [IQR 1–3] vs 1 [IQR 0.25–2]; $p = 0.022$). Their tunnel lengths were longer (8.8 mm SD 3.3vs 7.7 mm SD 2.0; $p = 0.027$) and the opening of the PFO was larger (5.4 mm SD 2.6vs 3.6 mm SD 1.5; $p < 0.001$) (Supplemental Table 3).

Reasons not to close the PFO

In total, 76 (39%) patients did not undergo PFO closure. Seventy-one (36%) patients were rejected for closure and five (3%) patients were accepted but preferred best medical treatment over surgery. The most common reasons included rejection of stroke diagnosis (13 patients (7%)), another cause of stroke (large artery atherosclerosis (5%), small vessel disease (4%), atrial fibrillation (1%), antiphospholipid syndrome (2%), cervical artery dissection (1%) and vasculitis (1%)) or the presence of multiple classical cardiovascular risk factors that made the PFO less likely the cause of stroke (9%). Finally, in 6 (3%) patients the stroke – HST evaluation time-interval was considered too long, by both patients and the HST (mean 8.5 years; range of 5–16 years) to proceed with PFO closure, also because of a lack of data on the efficacy on PFO closure several years after index event (Supplemental Table 4).

Missing data

Due to the retrospective design there was a considerable amount of missing data. All missing data are noted in the legends below each table.

Discussion

After installment of the HST, 71 (36%) patients were rejected for PFO closure as the team found no relation between the PFO and the stroke. Patients who might have been accepted for PFO closure prior to the installment of the HST were now rejected based on several reasons, especially the presence of another, more likely stroke diagnosis.

Conversely, patients who in the past would have been excluded for PFO closure because they would not match the inclusion criteria of the RCTs may now be accepted after careful HST evaluation. In the past, patients older than 60 years and patients with a TIA as index event were excluded, while in our cohort 37 (19%) patients who were accepted for closure either had a TIA or were older than 60 years (Table 2).

Table 2 Clinical characteristics.

Characteristics	All patients N = 195	Accepted for closure N = 124	Rejected for closure N = 71	p-Value
Index event at referral				
Ischemic stroke	130 (66.7)	91 (73.4)	39 (54.9)	0.009¤
TIA	65 (33.3)	33 (26.6)	32 (45.1)	0.009¤
Etiology based on TOAST criteria PFO excluded				
Large artery atherosclerosis	18 (9.9)	0 (0.0)	18 (25.4)	xΩ
Small-vessel occlusion	12 (6.6)	0 (0.0)	12 (16.9)	xΩ
Cardio embolic stroke	2 (1.1)	0 (0.0)	2 (2.8)	xΩ
Other	12 (6.6)	4 (3.2)α	8 (11.3)	0.016¤
Undetermined	16 (8.8)	0 (0.0)	16 (22.5)	xΩ
Multiple affected vascular territories*	42 (24.0)	26 (21.7)	16 (29.1)	0.491
Cortical infarction*	95 (52.5)	70 (56.5)	25 (43.9)	0.115
RoPE score*				
0–5	35 (19.1)	6 (4.9)	29 (48.3)	<0.001¤
6	51 (27.9)	37 (30.1)	14 (23.3)	0.339
7	44 (24.0)	33 (26.8)	11 (18.3)	0.207
8–10	53 (29.0)	47 (38.2)	6 (10.0)	<0.001¤
PFO-related medical history				
Pulmonary embolism*	12 (7.8)	12 (10.5)	0 (0.0)	0.033¤
Deep venous thrombosis*	6 (4.9)	6 (7.3)	0 (0.0)	0.079
Migraine*	52 (40.9)	35 (41.7)	17 (39.5)	0.817
Use of oral contraceptives*~	37 (38.1)	24 (40.0)	13 (35.1)	0.632

PFO: patent foramen ovale; TIA: transient ischemic attack; TOAST: trial of org in acute stroke treatment; RoPE: risk of paradoxical embolism.

* Information was missing in the following amount of patients: 20 patients in "Multiple affected vascular territories," 14 patients in "Cortical infarction," 12 patients in "RoPE Score," 41 patients in "pulmonary embolism," 73 in "deep venous thrombosis," 68 in "migraine," 98 patients in "use of oral contraceptive medication." Missing variables have been excluded from the calculated percentages.

α In four patients who underwent PFO closure an additional possible cause for stroke was present: antiphospholipid syndrome in three patients and atherosclerosis in one patient.

~ Male patients have been excluded from the calculated percentages.

Ω Could not be calculated due to presence of "0."

¤ Reached significance level of 0.05. *p*-Values were calculated two-sided using a chi-squared test to assess the equality of proportion.

PFO closure in patients with a cryptogenic stroke is cost-effective compared to medical therapy, based on the RCTs on PFO closure.[89] Avoiding unnecessary PFO closure will not only diminish surgical risks but also reduce healthcare costs. In addition, there is a high risk of overlooking the true cause of stroke (which will then be left untreated) in cases where PFO closure is performed before completing a comprehensive work-up and evaluation by an HST.

Patients accepted and rejected for PFO closure have different characteristics. Patients who underwent PFO closure after HST evaluation were significantly younger than those without closure, supporting the notion that PFO-associated stroke is predominantly prevalent in young adults.[33] Apart from being older, patients rejected for PFO closure were more likely to have hypertension and diabetes. These factors subsequently increase the risk of developing (subclinical) atherosclerosis, which is more likely to be a risk factor or cause of stroke than the PFO.[90] Patients accepted for PFO closure were more likely to have a history of pulmonary embolism (PE) compared to patients who were rejected, which supports the notion that the presence of a venous embolism may convert a PFO into a stroke causing PFO. The guideline published by EAPCI also stresses careful history taking, including enquiries on PE.[5, 22, 86] Several studies have identified anatomical features which increase the risk of a causal relationship between PFO and stroke.[91] A High-risk PFO includes the presence of an atrial septal aneurysm and a large right-to-left shunt.[36] Our study added a large diameter of the opening and long tunnel length to that. However, in contrast to previous studies[36, 38] we could not replicate the presence of ASA as a factor that converted a PFO into a stroke causing PFO, perhaps due to our small sample size. (High risk) PFO characteristics played a minor role in the decision-making on PFO closure, but recent studies indicate that this could be a useful approach as illustrated by the PASCAL classification which combines the RoPE Score with high-risk PFO features.[26, 27, 38]

Prior to the HST (June 2018), patients received PFO closure based on the evaluation of the referring centers. The decision to refer patients was based on their age, a high RoPE Score, the presence of PFO and an apparent cryptogenic stroke. The patients who underwent PFO closure prior to the HST did have a significant higher RoPE Score and were younger compared to the patients accepted for PFO closure by the HST. Furthermore, the patients accepted for closure after HST evaluation had more high risk PFO features (e.g. larger shunt size and longer tunnel) compared to patients accepted for PFO closure prior to the HST. Taking into account the number of patients rejected for PFO closure after additional work-up and HST evaluation it is possible that several patients referred for PFO closure (and actually closed) before HST installment would nowadays not be considered eligible for PFO closure.

PFO closure can be considered in patients who do, apart from older age, fulfill the RCT inclusion criteria as recent studies suggests PFO closure to be beneficial also in patients older than 60 years of age,[92-94] provided that the PFO is deemed causal to the stroke by the HST. On the contrary, patients who are being referred for PFO closure may get rejected after a thorough work-up and evaluation by the HST, for example when misclassification or other causes of stroke are identified. Nevertheless, caution is warranted when deviating from the inclusion criteria on which the RCTs are based and future research should indicate if extension of the inclusion criteria is justifiable.

A strength of our study is the re-evaluation of all echocardiographic data by the same experienced interventional cardiologist to prevent measurement bias and to look in detail at the anatomical echocardiographic characteristics of PFO. Three studies have evaluated the added value of a multidisciplinary approach to PFO closure. Two were executed (mainly) before the results of the positive RCTs, showing PFO closure to be superior over best medical treatment, and before the existence of the RoPE Score.[95, 96] One more recently performed study did not clearly state the reasons for rejection of PFO closure.[85] Our study is the first to evaluate the added value of a multidisciplinary HST in the decision process of PFO closure or not, combining RoPE Score, re-evaluated echocardiographic data, stroke- and patient characteristics.

A limitation of this study is the considerable amount of missing data due to the retrospective design. Also, outcome regarding the recurrence of stroke has not been noted.

Future research should therefore preferably entail long-term follow-up of patients rejected and accepted for PFO closure, collecting all outcomes and adverse events like atrial fibrillation.[97, 98]

In conclusion, we found that a thorough, standardized assessment complemented by an evaluation in a multidisciplinary team is paramount in deciding on PFO closure. Furthermore, centralizing the HST in a tertiary stroke center builds expertise on the decision-making. We advise to evaluate all patients in a centralized HST when PFO closure is considered.

Ethical approval

The Ethics Committee of the Radboudumc, Nijmegen, Netherlands waived the need for ethics approval.

Informed consent

The Ethics Committee of the Radboudumc, Nijmegen, Netherlands waived the need to obtain informed consent for the collection, analysis and publication of the retrospectively obtained and anonymized data for this non-interventional study.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary tables and figures

Supplementary table 1 Laboratory tests for the diagnosis of thrombotic disorders.

Laboratory test
Antinuclear Antibody
Antineutrophil Cytoplasmic Antibodies
Anti-beta 2 glycoprotein
Anti-cardiolipin antibodies
Lupus anticoagulant
Factor V Leiden
Homocysteine
Methylmalonic acid
Protein C
Protein S
Antithrombin
Factor VIII activity
Factor II mutation 20210A

Supplementary table 2 Accepted for PFO closure prior to HST compared to patients accepted after HST evaluation.

Characteristics	All patients N=169	After HST N=124	Before HST N=45	P-value
Mean age at time of referral, years [SD]	41.3 [10.5]	42.7 [10.6]	37.6 [9.1]	0.005¤
Male, N(%)	83 (49.1)	63 (50.4)	20 (44.4)	0.465
Mean BMI, kg/m2 [SD]	25.6 [4.3]	25.1 [4.0]	26.9 [5.0]	0.020¤
History of stroke or TIA, N(%)	27 (16.1)	10 (8.1)	17 (38.6)	<0.001¤
RoPE Score*				
0-5	6 (3.6)	6 (4.9)	0 (0.0	xΩ
6	40 (24.2)	37 (30.1)	3 (7.1)	0.003¤
7	44 (26.7)	33 (26.8)	11 (26.2)	0.936
8-10	75 (45.5)	47 (38.2)	28 (66.7)	0.001¤
Median shunt without Valsalva [IQR]*α	1 [1-3]	2 [1-3]	1 [1-2]	0.048¤
Median shunt with Valsalva [IQR]*α	3 [2-3]	3 [2-4]	2 [1-3]	0.126
Excursion of septum, mm [SD]*	8.90 [5.49]	9.02 [5.26]	8.25 [5.36]	0.567
10mm excursion, N(%)	42 (33.3)	36 (34.0)	6 (30.0)	0.730
15mm excursion, N(%)	19 (15.1)	17 (16.0)	2 (10.0)	0.489
Protruding redundant septal tissue, N(%)	15 (8.9)	10 (8.1)	5 (11.1)	0.538
Mean tunnel length, mm [SD]*	8.48 [3.10]	8.76 [3.28]	7.68 [2.36]	0.033¤
Mean diameter opening, mm [SD]*	5.13 [2.49]	5.35 [2.60]	4.51 [2.08]	0.081

HST: Heart-Stroke team, BMI: Body Mass Index, SD: Standard deviation, TIA: Transient ischemic attack, RoPE: Risk of paradoxical embolism.

* Information was missing in the following number of patients: 1 patient in 'History of stroke or TIA', 4 patients in 'RoPE Score', 26 patients in 'Mean shunt without Valsalva', 35 patients in 'Mean shunt with Valsalva', 43 patients in 'Excursion of septum', 23 patients in 'Mean tunnel length', 28 patients in 'Mean diameter opening'. Missing variables have been excluded from the calculated percentages.

Ω Could not be calculated due to presence of "0".

¤ Reached significance level of 0.05. P-values were calculated two-sided using a chi-squared test to assess the equality of proportion or an independent-samples t-test.

α Shunt size was defined as (0) None, (1) Small, <5 bubbles, (2) Moderate, 5-30 bubbles, (3) Large, >30 bubbles, (4), Opacification, near immediate opacification of the left ventricle.

Supplementary table 3 Echocardiographic characteristics.

Characteristics	All patients N=195	Accepted for closure N=124	Rejected for closure N=71	P-value
Median shunt grade without Valsalva [IQR]*α	1 [1-2]	2 [1-3]	1 [0.25-2]	0.022¤
Median shunt grade with Valsalva [IQR]*α	3 [2-3]	3 [2-4]	2 [1.25-3]	0.028¤
Excursion of septum, mm [SD]*	8.98 [5.40]	9.02 [5.53]	8.83 [4.98]	0.866
10mm excursion, N(%)	45 (33.3)	36 (33.9)	9 (31.0)	0.767
15mm excursion, N(%)	22 (16.3)	17 (16.0)	5 (17.2)	0.876
Protruding redundant septal tissue, N(%)	16 (8.2)	10 (8.1)	6 (8.5)	0.925
Mean tunnel length, mm [SD]*	8.53 [3.08]	8.76 [3.28]	7.66 [2.02]	0.027¤
Mean diameter opening, mm [SD]*	4.98 [2.50]	5.35 [2.60]	3.61 [1.47]	<0.001¤

* Information was missing in de following amount of patients: 28 patients in 'Mean shunt without Valsalva', 38 patients in 'Mean shunt with Valsalva', 60 patients in 'Excursion of septum', 58 patients in 'Mean tunnel length', 63 patients in 'Mean diameter opening'.

¤ Reached significance level of 0.05. P-values were calculated two-sided using an independent-samples t-test.

α Shunt size was defined as (0) None, (1) Small, <5 bubbles, (2) Moderate, 5-30 bubbles, (3) Large, >30 bubbles, (4), Opacification, near immediate opacification of the left ventricle.

Supplementary table 4 Reasons for not receiving PFO closure.

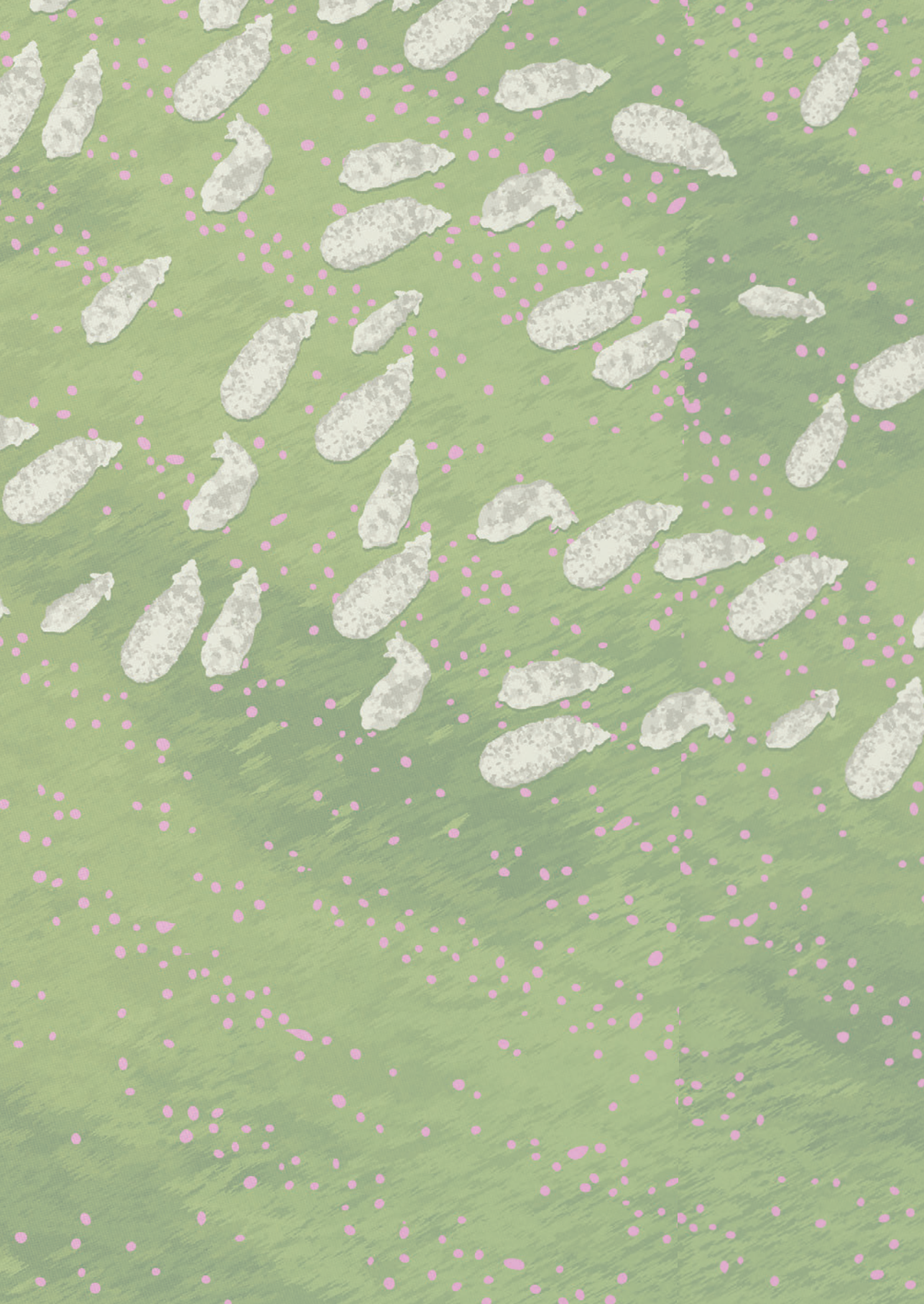
Reason	N(%)
Other stroke etiology	24 (31.6)
Large artery atherosclerosis	9 (11.8)
Small vessel occlusion	7 (9.2)
Atrial fibrillation	2 (2.6)
Antiphospholipid syndrome	3 (3.9)
Cervical artery dissection	2 (2.6)
Vasculitis	1 (1.3)
Presence of classic cardiovascular risk factors	18 (23.7)
Presence of classic risk factors alone	9 (11.8)
Presence of classic risk factors + RoPE Score <6	4 (5.3)
Presence of classic risk factors + RoPE Score <6 + not cardioembolic*	2 (2.6)
Presence of classic risk factors + low-risk PFO	3 (3.9)
Low-risk PFO~	4 (5.3)
Low-risk PFO alone	3 (3.9)
Low-risk PFO + not cardioembolic*	1 (1.3)
Stroke diagnosis rejected or doubted	13 (17.1)
Accepted for closure but patient preferred best medical treatment	5 (6.6)
PFO technically not feasible for closure (small shunt size)	1 (1.3)
Long interval between qualifying stroke and first opportunity to close PFO (without interval ischemic stroke) Ω	6 (7.9)
ASD instead of PFO	1 (1.3)
Possible fat embolism syndrome after surgery	1 (1.3)
Asymptomatic cerebellar infarction (not included in the trials)	1 (1.3)
Retinal artery occlusion (not included in the trials)	2 (2.6)
Total	76 (100.0)

PFO: Patent foramen ovale, RoPE: Risk of paradoxical embolism, ASD: Atrial septal defect

* Single affected vascular territory and absence of cortical infarction.

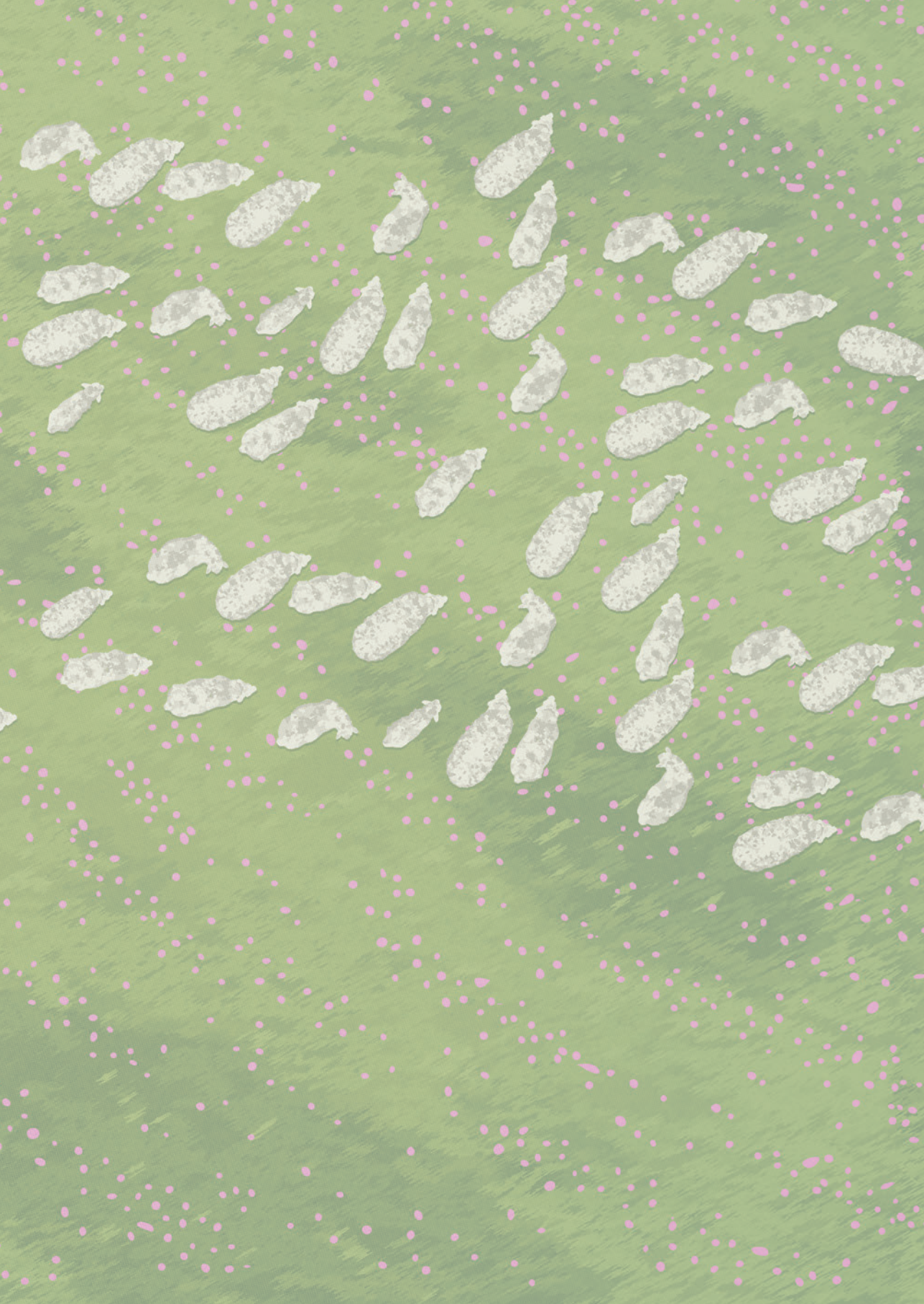
~ Small interatrial shunt and absence of an interatrial septal aneurysm.

Ω Mean interval without stroke = 8.5 years.



Part VII

General discussion and summary



7

General discussion

Overview

The aim of this thesis was to investigate which PFOs can be considered vulnerable and how they can be distinguished from innocent bystander PFOs. Furthermore, we aimed to gain a deeper understanding of the pathogenesis of PFO-associated stroke. Ultimately, the goal was to improve the selection process for patients with a presumed PFO-associated stroke and thereby attributing to adequate treatment of these patients. The results described in this thesis are based on five different cohorts: the ODYSSEY-study, the MR-CLEAN Registry, a registry-based study with data of the Radboud- and Amsterdam University Medical Center combined and two registry-based studies with data of the Radboud University Medical Center. This chapter will focus on methodological considerations first. Thereafter, I will focus on the main findings of the studies and end with clinical relevance of the studies in this thesis and future perspectives.

Methodological considerations

In the next paragraphs I will discuss the study design, internal validity, precision and external validity since these are important parameters for assessing the quality of a study.

Study design

The study in **chapter 2** uses data from two congenital heart disease institutions in the Netherlands (Radboudumc and Amsterdam UMC). We retrospectively collected data on all consecutive patients that underwent percutaneous PFO closure, to prevent a recurrent stroke, between 2016 and 2022 (n=223). All patients underwent standardized work-up to rule out other potential causes of stroke. Following this assessment, the Heart-Stroke Team (HST) reviewed all cases to determine the potential contribution of the PFO to the stroke. We gathered all information on the anatomical characteristics of the PFO. Unfortunately, it was not possible to compare the PFO diameters between the two groups (Radboudumc and Amsterdam UMC) due to differences in measurement techniques. In one group, the PFO diameter was assessed at rest or during Valsalva maneuver, while in the other group, it was measured under stretch using a probe. It would be of particular interest to investigate whether PFOs associated with stroke have a greater stretchability. This ability to stretch could be compared to that of a floppy atrial aneurysm, which is recognized as a significant anatomical feature that enhances stroke risk. Furthermore, there may be a larger number of patients with a severe shunt size, as 15% of the patients with a

shunt grade lower than severe (opacification) did not undergo a Valsalva maneuver. This could result in an underrepresentation of shunt size.

In **chapter 3 and 6** we used data from our HST cohort, a single-center, retrospective cohort study. Patients with a cryptogenic ischemic stroke or transient ischemic attack (TIA) and a PFO, who were referred to the Radboudumc (Nijmegen, the Netherlands) for consideration of PFO closure between November 2016 and December 2021, were included. For chapter 3 we only used data of patients after the instigation of the HST (after June 2018). All patients underwent standardized assessment on the cause of their stroke including a transthoracic echocardiography (TTE) with agitated saline contrast to demonstrate a right-to-left shunt and preferably measurements of PFO characteristics (e.g. presence of an atrial septal aneurysm). In **chapter 3** we looked at the left atrial strain (LAS) as a potential diagnostic marker in PFO-associated stroke. In total, valid echocardiography was available for 64 patients with a PFO-associated stroke and 12 patients with a PFO and stroke of other etiology. Unfortunately, due to the imbalance in groups no reliable statistical analysis was performed. Furthermore, we were only able to measure the LAS in patients from our own hospital due to software limitations. Additionally, we lacked data on patients with a PFO but without a stroke, which would have allowed for an interesting comparison. It would be valuable to investigate whether patients with benign PFOs also have LAS changes in the presence of risk factors. Lastly, the group of patients with stroke of other etiology (n=12) was too small to reliably assess the impact of risk-enhancing anatomical features on LAS.

In **chapter 6**, we evaluated the added value of the HST by examining the proportion of patients who were declined for PFO closure. A limitation of this study is the considerable amount of missing data due to the retrospective design. Also, outcome regarding the recurrence of stroke has not been noted. It would be interesting to see if patients discussed by the HST have a lower recurrent stroke risk compared to patients who received treatment before the instigation of the HST.

For **chapter 4** we used data from the MR CLEAN Registry. In short, the MR Clean registry is a Dutch nationwide, multicenter, prospective registry of patients who underwent endovascular treatment (EVT) for ischemic stroke between March 2014 until June 2016. For the purpose of our particular substudy we only selected patients between 18 and 55 years, as PFO-associated stroke mainly occurs in younger patients and beneficial effects of PFO closure have been demonstrated in this age group. Stroke etiology was determined based on the diagnosis at discharge. In this exploratory study we aimed to compare the histological composition of thrombi

of patients with PFO-associated stroke (n=3) with venous thrombi from patients with iliofemoral deep venous thrombosis (DVT) (n=4). Unfortunately, we did not have follow-up data on these patients; only those with a clearly diagnosed PFO-associated stroke (either prior to or during initial admission) were included. As a result, our analysis was limited to a small proportion of the thrombi.

Chapter 5 uses data from the ODYSSEY registry, a prospective cohort study conducted between 2013 and 2021, which focused on the prognosis and risk factors of patients aged 18–49 years with a first-ever ischemic stroke, TIA or intracranial hemorrhage (ICH), in 17 centers in the Netherlands. Ischemic stroke was defined according to a tissue-based definition as acute onset of a neurologic deficit with imaging proof of ischemia. Since the diagnostic work-up varied slightly between hospitals, the amount of data available for each patient differed. We aimed to investigate the role of trigger factors in PFO-associated stroke. Patients completed a structured, standardized questionnaire on exposure to potential trigger factors within predefined hazard period. Unfortunately, some trigger factors are too uncommon to reliably determine their potentially risk-enhancing effect (e.g. illicit drug use).

Internal validity

Internal validity examines whether the study design, conduct, and analysis answer the research questions without bias. It examines the extent to which systematic error (bias) is present. Such systematic error can arise through selection bias, information bias and confounding.[99]

Selection bias

Bias is colloquially defined as any tendency that limits impartial consideration of a question or issue.[100] Bias occurs if the study population does not closely represent a target population due to errors in study design or implementation, termed selection bias.[100]

In the ODYSSEY study, we included all young patients with stroke, regardless of stroke severity. However, some patients who experienced severe strokes died early and were therefore not included in the study. This may have introduced selection bias, favoring the inclusion of patients with less severe strokes and consequently representing a cohort with slightly better outcomes than would be expected in the overall population. Furthermore, in **chapter 5**, trigger factors were assessed only in survivors and patients without aphasia, potentially leading to selection bias.

In **chapter 3 and 6**, all patients referred for PFO closure to Radboudumc were included. And in **chapter 2** all patients referred to Radboudumc and Amsterdam UMC who underwent PFO closure were included. Investigations to determine the cause of stroke were conducted at the referring hospitals, following their respective local guidelines. The decision to refer a patient was made at the discretion of the referring neurologist and cardiologist. There was notable heterogeneity in the diagnostic work-up performed by the referring physicians, this work-up was not standardized. Depending on the thoroughness of the initial diagnostic work-up and the referring physician's familiarity with PFO-associated stroke, patients with a doubtful or less certain diagnosis may not have been referred. Furthermore, some patients may not have undergone echocardiographic evaluation at all. As a result, our study population may not be fully representative of all patients with PFO-associated stroke.

From the MR CLEAN Registry, **chapter 4**, we were only able to include patients who were identified with a clear PFO-associated stroke during their initial admission. Since only the admission letter was available, with no follow-up data, this posed a limitation, as most PFO-associated strokes are typically diagnosed later during outpatient follow-up. We were only able to select the patients with a prestated PFO-associated stroke in whom the relationship between PFO and stroke was clear. This is a type of sampling bias, since our sample does not contain patients whom have a more questionable relationship between PFO and stroke. Furthermore, we were only able to investigate larger thrombi retrieved through EVT, which limited our ability to examine a broader spectrum of thrombi, such as those associated with lacunar strokes, thereby narrowing our sample.

Information bias

Information bias is a type of error that occurs when key study variables are incorrectly measured or classified. Information bias can affect the findings of observational or experimental studies due to systematic differences in how data is obtained from various study groups. Types of information bias are recall bias, observer bias, reporting bias and misclassification bias.

As the trigger factors were self-reported in **chapter 4**, recall bias might have occurred, particularly among patients with limited recollection of the event and the period preceding it. It is possible that patients who were more severely affected by the stroke have a poorer recollection of the event, potentially introducing information bias. Limited recollection may lead to underreporting of trigger factors preceding the event, thereby underestimating their influence on stroke occurrence. In the ODYSSEY study, it is unlikely that misclassification of the index

event has occurred, since every TIA, ischemic stroke and ICH was confirmed by neuroimaging. Misclassification of the event could have occurred in the HST cohort since this cohort also included presumed TIA's without imaging proof. In the MR CLEAN cohort misclassification is unlikely when it comes to the diagnosis of ischemic stroke since they were all treated with endovascular thrombectomy. For all patients with an ischemic stroke or TIA, misclassification of the etiology remains possible, as direct causality cannot be definitively established. The same applies to patients with a presumed PFO-associated stroke. Since there is no gold standard to determine whether a PFO is causally related to a stroke, misclassification bias is likely to affect all research on PFO-associated stroke. The implementation of the HST aims to minimize this risk of misclassification.

The inclusion period of **chapter 3 and 4** spanned five years, during which knowledge of PFO-associated stroke evolved. As a result, it is plausible that the quality of referral by the local physicians and decision-making by the HST improved over the years. This could be seen as temporal bias.

Confounding

Confounding can occur when a variable is associated with both the determinant and outcome of interest, but not in the causal chain. It might influence the result or association found between determinant and outcome.

A potential confounder in **chapter 5** relates to exercise. In this chapter we assessed the relationship between trigger factors and PFO-associated stroke, one of these trigger factors was exercise. It is plausible that individuals who engage in more frequent exercise, including vigorous activity, have fewer cardiovascular risk factors. Patients with a cryptogenic stroke, no cardiovascular risk factors, and a PFO are more likely to have their stroke attributed to the PFO. In our study, we identified vigorous exercise as a potential trigger for PFO-associated stroke. Therefore, it is possible that patients exposed to vigorous exercise have fewer cardiovascular risk factors and are consequently more likely to have their stroke attributed to a PFO.

External validity

External validity is a construct that attempts to answer the question of whether we can use the results of a study in patients other than those enrolled in the study.[101] The cohorts included in our studies primarily consist of Dutch patients of Caucasian ethnicity, treated in both academic and non-academic hospitals. Moreover, these cohorts predominantly comprise patients younger than 60 years of age. An additional factor influencing external validity is the involvement of a

HST, which played an important role in several of our studies. We recognize that access to a similar multidisciplinary team may not be universally available in other healthcare settings. Consequently, we consider our findings to be generalizable to most European countries with comparable healthcare infrastructures, particularly for relatively young patients with stroke. However, extrapolation of these results to populations with different ethnic backgrounds, older patients or healthcare systems should be approached with caution.

The baseline characteristics of patients with PFO-associated stroke in the ODDYSEY study cohort - with a median age of 42 years and 56% male - are comparable to those reported in a meta-analysis comparing PFO closure with best medical treatment in patients with cryptogenic stroke including all relevant RCT's on PFO closure.[98] In contrast, the patients discussed in the HST study, presented in **chapter 3 and 6**, were slightly older, with a median age of 46 years. This is likely explained by the inclusion of patients up to 60 years of age, a group for whom PFO closure has recently been shown to be both beneficial and safe.[92] We expect these findings to be generalizable to most predominantly Caucasian populations.

We expect the trigger factors for PFO-associated stroke identified in **chapter 5**, fever, flu-like disease, and vigorous exercise, to be applicable across populations. However, the incidence of these trigger factors varies globally. In rural regions and in countries with limited access to basic hygiene or lower public health awareness, fever and flu-like disease are more prevalent, potentially leading to a higher incidence of PFO-associated strokes.

The proportion of PFO-associated stroke relative to other stroke etiologies also differs worldwide. For instance, hypertension is a significantly more common risk factor in Asian countries than in European countries.[102] Consequently, young patients in Asian populations are more likely to experience stroke due to hypertension compared to their European counterparts.

Previous research has found no significant differences in the prevalence of PFO and atrial septal aneurysm (ASA) across racial and ethnic subgroups. However, large PFOs were found to be significantly less prevalent among Black individuals compared to White individuals.[103]

Discussion of main findings

Histology of a PFO-associated thrombus

To develop effective treatment strategies, it is essential to understand the underlying pathophysiology. Although percutaneous closure of the PFO has a high number needed to treat, it has been demonstrated to be beneficial in preventing recurrent PFO-associated strokes.[20-23] However, the precise mechanisms by which percutaneous closure is effective remains unclear. The most widely accepted theory is that PFO closure prevents thrombi from the venous circulation from crossing the PFO into the arterial circulation, a mechanism referred to as paradoxical embolism. Another potential explanation is that percutaneous closure may prevent the formation or passage of thrombi originating within the PFO tunnel, as supported by Yan et al.[55] Additionally, closure may restore atrial integrity and thereby improve atrial function. [63]

The paradoxical embolism hypothesis is widely accepted as the most likely explanation. In such cases, a PFO-associated stroke thrombus may be conceptualized as a misdirected pulmonary embolism (PE), given the shared pathophysiological mechanisms. However, unlike pulmonary embolism, PFO-associated stroke does not exhibit a consistent association with DVT. Accordingly, based on the current literature, routine screening for DVT in PFO-associated stroke patients is not justified.[46]

Furthermore, PFO-associated stroke remains a rare condition, unlike DVT and PE. The annual incidence of DVT in the Netherlands is several tens of thousands, and according to *Het Longfonds*, the leading institute for pulmonary diseases in the Netherlands, the incidence of PE is estimated at 10,000–12,500 cases per year. If we assume that PFO-associated stroke shares a similar pathophysiological mechanism with PE, and given that a PFO is present in approximately 25% of the general population, we would expect the annual incidence of PFO-associated stroke to be at least several thousand cases. Nevertheless, the estimated incidence in the Netherlands is only 300–1,500 cases per year.[4, 15] Another observation that challenges the concept of paradoxical embolism as the primary mechanism is the age distribution of DVT cases. PFO-associated stroke is more common in younger patients, whereas DVT is rare in younger individuals (approximately 1 per 10,000 person-years between the ages of 25 and 30) and increases exponentially with age (about 8 per 8,000 person-years in individuals aged 85 years and older).[47] This inverse age relationship makes it less likely that DVT plays a causal role in PFO-associated stroke. An alternative mechanism is that certain PFOs may be inherently vulnerable (e.g. mobile atrial septum) and require an additional trigger, such as infection, to

become pathological. This pathological state could facilitate thrombus formation within the PFO, potentially promoted by turbulent flow within the tunnel.[7]

The histological composition of a thrombus serves as a blueprint, providing information about its age and site of origin.[56] Mapping these features and linking them to specific etiologies may, in the future, facilitate rapid and accurate identification of thrombus origin and appropriate treatment selection.[58] In **chapter 4**, we demonstrate that thrombi associated with PFO-related stroke differ histologically from those originating from DVT and most closely resemble thrombi retrieved from patients with other cardioembolic sources of stroke. To our knowledge, this is the first study to directly compare the composition of PFO-associated stroke thrombi with those derived from DVT. These findings suggest that, in our cohort, the thrombi are more likely to have originated within the atrial circulation rather than the venous system.

Since intra-arterial thrombectomy is a widely used and highly effective therapy for acute stroke, it enables direct retrieval of the causative thrombus. Using samples, stained with hematoxylin-eosin and digitized via the Hamamatsu scanner, we performed histological analyses to quantify the proportions of red blood cells (RBC), fibrin (Fib), and platelets (Plt). These analyses are sufficient to distinguish venous from arterial thrombi due to their distinct compositions.

However, a recent systematic review and meta-analysis demonstrated that classifying thrombi based solely on the proportions of RBC, Fib, and Plt is insufficient to differentiate among various arterial causes of stroke; more sophisticated histological techniques, such as single-cell RNA sequencing, are required.[104] If histological markers specific to PFO-associated stroke can be identified, they may ultimately aid in determining which patients are most likely to benefit from PFO closure.

Risk factors for PFO-associated stroke

Given the fact that PFO is a lifelong persisting anatomical variant, it is intriguing to consider why some individuals experience a stroke at a specific point in time. In **chapter 5**, we show that trigger factors may contribute to the pathological transformation of an innocent bystander PFO into a stroke-causing one. Vigorous exercise, fever and flu-like disease may act as potential triggers for PFO-associated stroke. While the concept of trigger factors in young patients with stroke has previously been explored by Ekker et al.,[105] it was not yet investigated in the context of PFO-associated stroke. The only recognized trigger factor for PFO-associated stroke has been Valsalva-like maneuver (e.g. during weight lifting).[16]

Several biological mechanisms could explain how an infection might convert a PFO into a stroke-causing PFO. First, pathogens in the systemic circulation can induce endotheliopathy by directly invading the arterial wall, thereby causing a prothrombotic state.[68] Moreover, infection and inflammation are well-established risk factors for venous thromboembolism (VTE), particularly within the first 2 weeks of onset, with the risk gradually declining thereafter.[77] If PFO-associated thrombi do indeed originate from the venous system, then infection-triggered VTE could plausibly lead to a PFO-associated stroke.

The link between vigorous exercise and PFO-associated stroke may be explained by acute activation of the sympathetic nervous system, leading to elevated heart rate and blood pressure.[71] This hemodynamic pressure increases shear stress. This may promote platelet deposition and thrombogenesis.[78] Additionally, elevated norepinephrine levels during intense physical activity may enhance platelet aggregation and oxygen demand.[79] Hypothetically, increased intrathoracic pressure due to exercising (e.g. heavy lifting) could facilitate right-to-left shunting.

It is understandable that a trigger factor alone, such as fever or physical exertion, is insufficient to cause a stroke in patients with a PFO. The PFO must possess certain vulnerabilities before it can become stroke-causing. Fever and flu-like illnesses are extremely common, as is the presence of a PFO, yet PFO-associated strokes remain rare. Moreover, these potential trigger factors are prevalent in children, but data on PFO-associated stroke in childhood are lacking, given its exceptional rarity.[106] Therefore, it is likely that among the billions of individuals with a PFO, only a small proportion have a vulnerable PFO. Such vulnerability may be related to specific anatomical characteristics.

Anatomy of a pathological PFO

The role of PFO anatomy in clinical decision-making remains challenging. **Chapter 2** demonstrates that in 20% of patients who underwent PFO closure to reduce the risk of recurrent stroke, across one of the two congenital heart disease centers in the Netherlands (Amsterdam UMC and Radboudumc), no risk-enhancing anatomical features were present. This suggests that PFO anatomy may not be a decisive factor in the selection process for closure.

We therefore assume that a PFO can be pathogenic even in the absence of established risk-enhancing features, such as an ASA or a large shunt. Conversely, there are individuals with a PFO, large shunt, and ASA who will never experience a stroke. It is possible that physiological factors, particularly hemodynamics, rather

than morphological features, play a more decisive role in determining which PFOs are vulnerable and capable of causing stroke. Similar high-risk anatomical features may give rise to different hemodynamic patterns and potential atrial adaptations. Anatomical features do play a significant role in scoring systems such as the PASCAL score. In this system, the presence of even a single risk-enhancing anatomical feature can shift the classification of a PFO-related stroke from 'unlikely' to 'possible'. For example, patients with a Risk of Paradoxical Embolism (RoPE) score below 7 who have a small-shunt PFO without an ASA are classified as having a PFO unlikely to be related to stroke. However, if the same individual has a large shunt, defined as more than 20 bubbles crossing the PFO (e.g. 21 bubbles instead of 20) the classification changes to PFO possibly related to stroke.[27] If this patient also has an ASA, the causal relationship between the PFO and stroke is considered 'probable'. The interpretation of these terms remains complex. In our study, more patients were classified as having a 'possible' rather than a 'probable' PFO-related stroke, despite all undergoing percutaneous PFO closure.

Therefore, the decision to close a PFO cannot rely solely on scoring systems. Multidisciplinary assessment by a HST is essential to identify patients most likely to benefit from closure. In **chapter 6**, we describe the selection process for PFO closure. Overall, 4% of patients were declined closure primarily due to low-risk PFO anatomy, and in 11% of cases, low-risk anatomy contributed to the decision not to proceed with closure. The selection process described in our study occurred prior to the introduction of the PASCAL scoring system. Our findings indicated that, when considering all relevant factors (e.g. PFO characteristics, patients profile, type of stroke), anatomical features alone were decisive in only a small proportion of cases. Currently, the PASCAL scoring system is the predominant tool used to guide decisions on PFO closure and relies heavily on anatomic criteria. In hospitals without a dedicated multidisciplinary team, where neurologists or cardiologists may base closure decisions on the PASCAL score, mistreatment is lurking. These findings highlight the importance of a comprehensive evaluation, in which anatomical features play only a minor role in the final decision.

Among risk-enhancing anatomical features, the most prominent remains the ASA.[41] However, it remains unclear how the presence of ASA affects the risk of stroke in patients with a PFO. One hypothesis is that the presence of ASA facilitates hemodynamic flow from the right to the left atrium.[107] ASA can be seen as a hypermobile septum, which is more mobile during changes in intracardiac pressure. This increased mobility may allow the PFO to open more widely during pressure fluctuations.[38]

In **chapter 2**, we demonstrated that the mean diameter of the PFO orifice in patients who underwent closure was relatively large (5.4 ± 2.6 mm), compared to the bystander PFO diameters reported in previous studies.[40] Furthermore, stroke-associated PFOs were shown to stretch to nearly double the diameter (11.0 ± 3.5 mm). Unfortunately, because assessing PFO stretch requires an invasive procedure, comparisons with non-stroke-associated PFOs are not feasible. Nonetheless, this stretchability, similar to the mobility of an ASA, may support the hypothesis that such anatomical characteristics enable thrombus passage across the PFO or causes hemodynamic changes in the left atrium.

Physiology of the left atrium in PFO-associated stroke

Dysfunction of the left atrium is one of the alternative mechanisms of PFO-associated stroke. Left atrial function can now be assessed using standard TTE, including measurements of left atrial strain (LAS). LAS measures the atrial wall deformation within the cardiac cycle.[10] LAS was demonstrated to be lower in the presence of a PFO and an ASA, similar to the atrial function in chronic atrial fibrillation.[9] This suggests that impaired LAS may be a factor that turns an asymptomatic PFO into a possible stroke-causing PFO.

In **chapter 3**, we showed that the left atrial function is impaired in patients with a PFO-associated stroke with an ASA and large shunt (both risk-enhancing anatomical features). Hypothetically, an ASA in combination with a large shunt could lead to remodeling of the left atrium due to altered blood flow, thereby causing shear stress, impairing its function and increasing the risk of subsequent local thrombus formation. This is similar to the elevated stroke risk observed in patients with dilated cardiomyopathy.[108]

Furthermore, LAS below 33.4% is associated with paroxysmal atrial fibrillation which could be an alternative cause of stroke.[48] It is possible that this atrial dysfunction could mimic a so called "atrial fibrillation like" pathogenesis for stroke.[9] A recent study identified in situ thrombus formation within the PFO tunnel.[55] Similar to the left atrial appendage in atrial fibrillation, the PFO tunnel may serve as a preferred site for thrombus formation in patients with impaired atrial function. It is possible that multiple mechanisms contribute to PFO-associated stroke and that these causes coexist simultaneously. The simultaneous presence of a trigger factor, high-risk anatomical characteristics, and atrial dysfunction may render the PFO more vulnerable. PFO closure may protect against recurrent stroke by preventing right-to-left shunting, reducing the risk of in situ thrombus formation from entering the arterial circulation, and restoring left atrial integrity. Notably, LAS appears to recover following PFO closure.[9]

Deciding on PFO closure

Deciding to close a PFO has several risks and disadvantages. First, there are procedural and post-procedural risks associated with the intervention. For example, PFO closure is linked to a significantly increased risk of AF compared to medical management (odds ratio, 5.3; 95% CI, 2.5-11.41).[61] Six months after PFO closure, patients may discontinue antithrombotic therapy.[60] Consequently, patients who are incorrectly diagnosed with a PFO-associated stroke risk receiving inappropriate treatment. Furthermore, unnecessary PFO closure contributes to increased health care costs. Therefore, it is of utmost importance to accurately identify patients who are most likely to benefit from PFO closure. In this thesis, the HST approach is evaluated. This evaluation revealed that 36% of all patients referred to our center for PFO closure were ultimately rejected, as the multidisciplinary team found no causal relationship between the PFO and the stroke. The most common reasons included: rejection of stroke diagnosis (7%), another cause of stroke (large artery atherosclerosis (5%), small vessel disease (4%), atrial fibrillation (1%), antiphospholipid syndrome (2%), cervical artery dissection (1%) and vasculitis (1%)) or the presence of multiple classical cardiovascular risk factors that made the PFO less likely the cause of stroke (9%). The patients accepted for closure were significantly younger, had fewer cardiovascular risk factors, and more often had a history of pulmonary embolism. These findings support the notion that PFO-associated stroke is predominantly observed in young adults[33], typically without vascular disease and may occasionally occur in individuals at risk of VTE. Rejecting a substantial amount of referred patients by thoroughly examining all aspects of the diagnostic process and discussing them in a multidisciplinary manner, the HST is able to prevent unnecessary risks and reduce health care costs.

Clinical relevance

Our research on PFO-associated stroke addressed several important knowledge gaps. We observed that the histological composition of PFO-associated stroke thrombi differs from that of DVT and more closely resembles that of thrombi classified as cardioembolic. The main limitation of this study is the small number of thrombi available for analysis, which precludes definitive conclusions about their true origin or the potential clinical implications. If future studies on the histological composition of PFO-associated stroke thrombi were to identify a venous origin, the rationale for percutaneous closure would be strengthened. Conversely, if no venous origin is found in a large, representative sample, the effectiveness of percutaneous closure could be called into question. This is especially relevant if the true origin of the thrombi lies in the left atrium.

Vulnerable- versus Innocent Bystander PFOs

Because PFO is a highly prevalent anatomical variant in the general population, an incidental, benign PFO is far more likely to be encountered when investigating stroke etiology than a PFO that has actually caused a stroke. Distinguishing between the two remains challenging and is a subject of ongoing debate. Factors associated with pathological PFOs are derived from studies showing that certain PFO, patient, or stroke characteristics occur more frequently in individuals with cryptogenic stroke compared to those without stroke. However, given the circumstantial nature of this evidence, it remains difficult to determine which features are most important, and ultimately decisive, in identifying pathological PFOs. Former studies have showed us that the absence of vascular risk factors and the presence of cortical infarction in patients younger than 60 years suggest that a PFO is likely to have caused the stroke. Especially PFOs with a large shunt or ASA and a stroke that occurred during Valsalva maneuver or in the presence of a DVT.[109]

Although a large shunt and ASA are prominently cited as important markers for pathological PFOs in the PASCAL classification, we found that 20% of patients with PFO-associated stroke had a small shunt and no ASA. This finding implies that these anatomical features are not essential for classifying a PFO as stroke-causing.

In our study, we also identified vigorous exercise and recent flu-like illness as potential trigger factors for PFO-associated stroke, both in comparison to patients with an innocent bystander PFO and stroke of other etiologies. In other words, patients who had experienced a flu-like illness or engaged in intense exercise prior to their stroke were more likely to have a PFO as the underlying cause. Regarding the work-up for stroke etiology, the presence of trigger factors preceding the stroke may tilt the balance toward PFO as the underlying cause.

Furthermore, we observed that patients with stroke-causing PFOs exhibited a broad spectrum of left atrial function, as measured by left atrial strain (LAS). Notably, left atrial function was most impaired in patients with high-risk PFOs (large shunt and/or ASA). This finding suggests a possible role for left atrial dysfunction in the pathogenesis of stroke. Previous studies support this hypothesis, having reported that left atrial enlargement, associated with permanent right-to-left shunting, is a predictor of PFO-associated stroke. The observed reversal of left atrial diameter after PFO closure further supports the hypothesis that right-to-left shunting may induce cardiopathy, which could contribute to stroke.[110] Taken together, these findings suggest that reduced left atrial function may be linked to pathological PFOs. LAS could eventually be developed as a novel diagnostic marker in deciding on PFO closure.

Lastly, in our research we stressed the importance of the HST. Showing that a substantial amount of patients are rejected for PFO closure after a thorough work-up. These were all patients who potentially undergo percutaneous closure if the HST would not have interfered. Centralizing the HST in a tertiary stroke center builds expertise on the decision-making. We advise to evaluate all patients in a centralized HST when PFO closure is considered.

Future perspective

PFO-associated stroke predominantly occurs in young adults, and as the incidence of stroke in this population continues to rise,[2] the likelihood of encountering young adults with PFO-associated stroke is also increasing. Given that these patients have their entire lives ahead of them, it is crucial to provide optimal treatment and to minimize the risk of recurrent stroke.

A major issue surrounding PFO closure after stroke is that this treatment is based on a hypothesis that remains unproven. In the first randomized controlled trial on PFO closure, the CLOSURE I trial (2010), the researchers stated: *"Some strokes of unknown etiology may be the result of a paradoxical embolism traversing through a nonfused foramen ovale."* The concept of paradoxical embolism, however, predates this trial by several years. Nevertheless, a causality between venous emboli and PFO-associated stroke has not been proven.

This thesis presents the first histological comparison between thrombi retrieved from patients with PFO-associated stroke and those from deep vein thrombosis, the presumed source of emboli in the paradoxical embolism hypothesis. Demonstrating that these thrombi are histologically different raises questions about the validity of this hypothesis. In the future, prospectively retrieving thrombi via endovascular thrombectomy and histologically comparing them with thrombi from strokes of other etiologies and from venous thromboembolism (e.g. deep vein thrombosis, pulmonary embolism) may provide clearer insights into the underlying mechanisms of PFO-associated stroke. Understanding its origin may facilitate the development of personalized treatment strategies and improve our ability to differentiate pathological PFOs from incidental ones. Some studies have reported a correlation between DVT and PFO-associated stroke, with the reported frequency ranging from 7% to 27%.[46] Ideally, we would conduct histological examinations of PFO-associated stroke thrombi (retrieved via intra-arterial thrombectomy) in parallel with venous thrombi (e.g. from the deep veins of the leg) obtained from

the same patient at the same time. Such an analysis could help determine whether these thrombi are histologically compatible, or whether the presence of a venous clot is merely coincidental. Asymptomatic venous thromboembolism is common in the general population and even more prevalent among hospitalized patients. For instance, studies have shown that asymptomatic venous thrombemboli are relatively frequent in patients with psychiatric disorders: 25.3% in catatonic inpatients, 11.5% in restrained inpatients, and 2.3% in psychiatric patients overall.[111]

For physicians, it is important to recognize that the scoring systems such as the PASCAL or RoPE classification are merely tools to estimate the probability that a PFO is causally related to a stroke. **Chapter 1** shows that only a minority of the patients that underwent PFO closure have a "Probable PFO-associated stroke" (45%) according to the PASCAL classification. **Chapter 6** of this thesis demonstrates how many patients are denied for PFO closure after undergoing a standardized work-up and multidisciplinary assessment. If treatment decisions were based solely on these scoring systems, a significantly larger number of patients would have received PFO closure, potentially unnecessarily. Therefore, we advocate for evaluation by a dedicated HST prior to making decisions regarding PFO closure. A future study on the long-term outcomes of these patients is warranted to shine light on the effectiveness of such decision-making strategies.

In general, caution is warranted when administering treatments that may be potentially harmful or of questionable benefit, particularly when only a small proportion of patients are likely to achieve a meaningful benefit. Furthermore, financial considerations play an important role in clinical decision-making. Choosing an expensive treatment inevitably diverts resources away from other therapeutic or diagnostic options. These issues are highly relevant in the context of percutaneous PFO closure. The number needed to treat (NNT) to prevent one stroke over two years, compared with best medical therapy, remains relatively high, 21 for patients with high-risk anatomical PFO features and 37 overall.[24] This implies that a substantial proportion of patients could be managed effectively with medical therapy alone. It would be valuable to investigate how patients with PFO-associated stroke who received best medical therapy but subsequently develop a recurrent stroke differ from those who do not experience another event. The rationale for PFO closure is to prevent recurrent events in patients for whom medical therapy alone is insufficient. It is plausible that PFOs vary in their pathophysiological relevance, and that some are only appropriately treated through percutaneous closure. There may be multiple mechanisms underlying PFO-associated stroke, with only those patients experiencing a true paradoxical embolism having significant benefit from closure. Histological

research may be key to differentiating between these potential mechanisms. If all thrombi associated with PFO-related stroke could be examined histologically, and those with a clear venous origin identified, it would be possible to directly determine which patients are most likely to benefit from PFO closure assuming that PFO closure is most beneficial in patients with a genuine paradoxical embolism.

PFO-associated stroke has traditionally been considered a *diagnosum per exclusionem*. The challenge with this approach is that the diagnosis heavily depends on the precision of the clinical assessment and the diagnostic work-up. Circumstantial evidence related to PFO-associated can help to tip the scale in favor of PFO closure. In this thesis we looked into trigger factors as an additional clinical parameter of PFO-associated stroke and left atrial strain as a potential diagnostic parameter.

Future studies with long-term, structured follow-up of patients with PFO-associated stroke, compared to those with a PFO and stroke of other etiologies, are needed to clarify whether LAS can identify young patients with PFO who would benefit from closure. In our research, we observed that LAS decreases in patients with PFO-associated stroke in combination with a large shunt or ASA. It would be of interest to determine whether LAS also decreases in patients with a large shunt and/or ASA who have not experienced a stroke. If changes in left atrial function are observed only in the context of stroke, this would support our hypothesis that left atrial dysfunction plays a significant role in the pathogenesis of PFO-associated stroke.

Furthermore, we should evaluate why most patients with a PFO experience a stroke only once in their life, despite the fact that most trigger factors are present multiple times throughout life. Another interesting question for future research concerns the age aspect of PFO-associated stroke since it occurs more often in young adults, but it is very rare in children, while trigger factors, such as fever and flu-like disease, are abundantly present in childhood. Furthermore, it remains unclear why, for example, labor does not seem to be a major risk factor for PFO-associated stroke, despite the fact that women during childbirth are in a prothrombotic state and need to exert a significant Valsalva maneuver.

Even today, many questions remain unanswered regarding PFO-associated stroke. Rather than relying on circumstantial evidence to judge whether a stroke is likely related to a PFO, we need a deeper understanding of the underlying pathophysiology. In this field, research has bypassed the fundamentals and moved directly towards developing treatments, treatments whose mechanisms of action and rationale remain poorly understood.

8

Dutch summary
Nederlandse samenvatting

Een herseninfarct blijft wereldwijd de belangrijkste oorzaak van invaliditeit. Hoewel de incidentie toeneemt met de leeftijd, treedt naar schatting 10–15% van all e herseninfarcten op bij personen tussen de 18 en 50 jaar. Aangezien deze jongvolwassenen zich veelal in de bloei van hun leven bevinden - carrière maken, bezig zijn met gezinsvorming en persoonlijke ontwikkeling - kan de impact van een herseninfarct bijzonder ingrijpend zijn en ernstige sociaaleconomische gevolgen hebben. Om recidiverende beroertes te voorkomen is het van essentieel belang de onderliggende oorzaak zo nauwkeurig mogelijk te identificeren, aangezien verschillende oorzaken ook een verschillende therapeutische aanpak vereist. De oorzaak van een herseninfarct wordt doorgaans geclassificeerd volgens de TOAST-criteria (Trial of Org 10172 in Acute Stroke Treatment), waarin vijf hoofdsubtypes worden onderscheiden. Atherosclerose van de grote vaten is hiervan de meest voorkomende. Bij jongvolwassenen daarentegen wordt een herseninfarct minder vaak veroorzaakt door traditionele cardiovasculaire risicofactoren aangezien dit tijd nodig heeft om schade te veroorzaken. Een mogelijke oorzaak van een herseninfarct in deze leeftijdsgroep is een persisterend foramen ovale (PFO); een congenitale cardiale afwijking die ontstaat door het niet volledig sluiten van het atriumseptum na de geboorte. Een PFO komt relatief vaak voor in de algemene populatie, met een geschatte prevalentie van 15% tot 31%. Dit zorgt ervoor dat het lastig te concluderen is welke PFO's wel en welke niet gerelateerd zijn aan het herseninfarct.

De studies in dit proefschrift bevatten verschillende resultaten van de ODYSSEY studie en MR CLEAN Registry. Daarnaast is er data verzameld van de patiënten die besproken zijn in het Heart-Stroke Team (HST) van het Radboudumc.

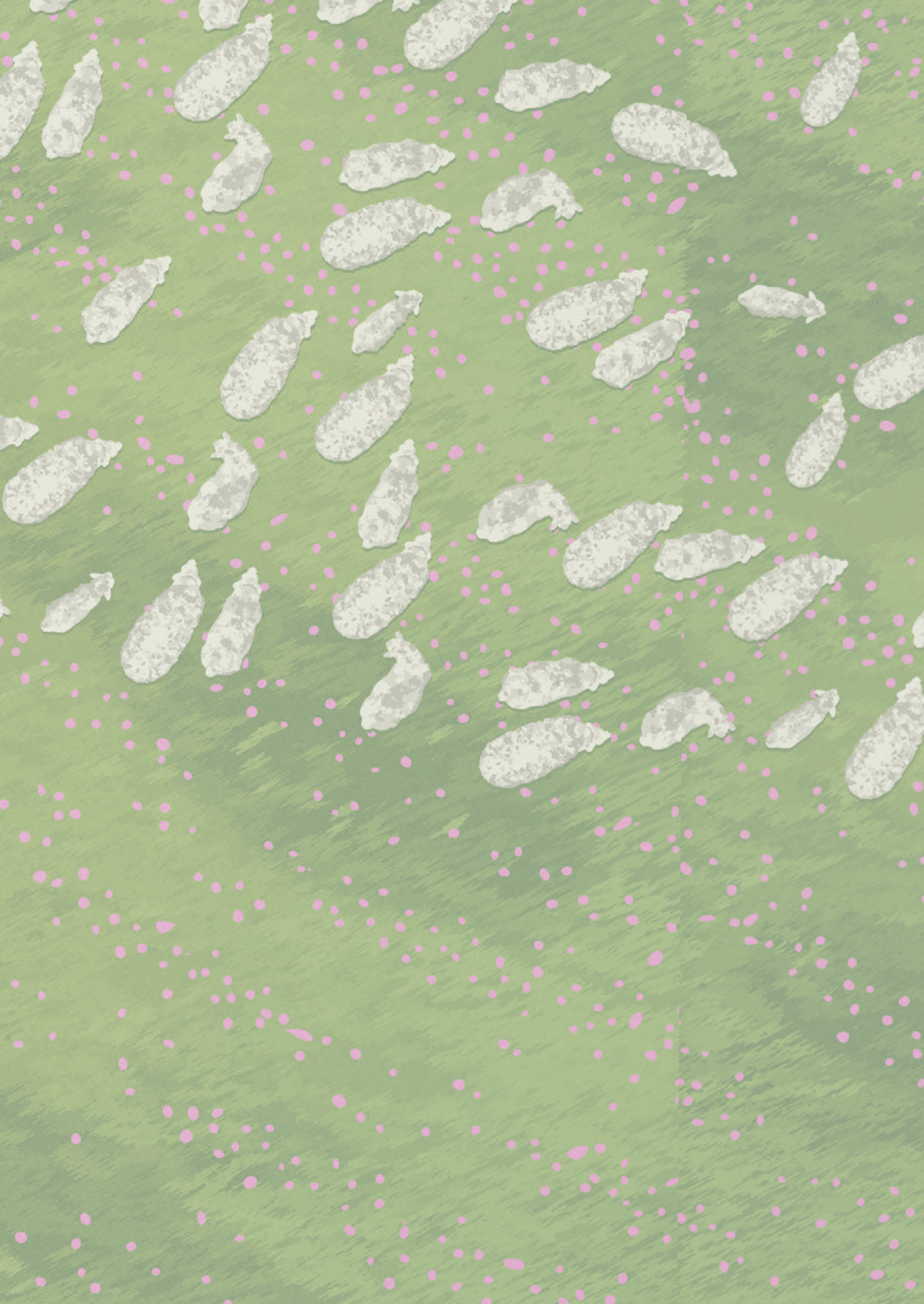
Om te achterhalen welke PFO's pathologisch zijn wordt er gekeken naar bepaalde kenmerken van de patiënt en het PFO. Anatomische karakteristieken van het PFO worden in toenemende mate gebruikt als een parameter om te bepalen of een PFO gerelateerd is aan een herseninfarct. Neem bijvoorbeeld de PASCAL (PFO-Associated Stroke Causal Likelihood) classificatie, waarin wordt meegenomen of het PFO risicoverhogende anatomische kenmerken heeft. In **hoofdstuk 2** hebben we gekeken naar de anatomische kenmerken van de PFO's van alle patiënten die tussen 2016 en 2022 besproken zijn in het HST van het Radboudumc en Amsterdam UMC, waarna ze PFO sluiting hebben ondergaan. We zien dat bij 20% van de patiënten die een PFO sluiting hebben ondergaan geen risicoverhogende anatomische kenmerken van het PFO worden gezien. Dit laat zien dat de aan- of afwezigheid van deze anatomische kenmerken geen doorslaggevende rol kan spelen in het wel of niet sluiten van het PFO.

Een andere manier om naar het PFO te kijken is niet vanuit een anatomisch, maar vanuit een fysiologisch oogpunt. Een van de theorieën over hoe een PFO de oorzaak kan zijn van een herseninfarct, is dat het PFO voor dysfunctie zorgt van het linker atrium. Doordat er bloed van het rechter atrium naar het linker atrium stroomt ontstaat er verandering in hemodynamiek, wat weer kan zorgen voor een verminderde functie van het atrium. Een vrij nieuwe manier om deze functie te beoordelen is middels het berekenen van de "left atrial strain" (LAS). Een verlaagde strain past bij een minder goed functionerend linker atrium. In **hoofdstuk 3** hebben wij middels "2D speckle-tracking" echocardiografie gekeken naar de functie van het linker atrium in patiënten met een PFO-geassocieerd herseninfarct en dat vergeleken met patiënten met een PFO dat niet gerelateerd is aan het herseninfarct. Patiënten die besproken zijn in het HST en in het Radboudumc en transthoracale echocardiografie hebben ondergaan tussen juni 2018 en december 2024 werden geïncludeerd. Er werd geen lagere LAS gezien bij patiënten met een PFO-geassocieerd herseninfarct in vergelijking met patiënten met een PFO en een herseninfarct van andere origine. Wel zagen we dat de LAS verlaagd was bij hoog risico PFO's (grote shunt en de aanwezigheid van een atriumseptumaneurysma). Het lijkt er dus op dat risicoverhogende anatomische structuren ook de functie van het atrium aantasten, mogelijk dus dat atriale dysfunctie een rol speelt in de ontwikkeling van het herseninfarct. Grotere groepen patiënten zijn nodig om te bepalen of LAS een rol kan spelen in het differentiëren tussen onschuldige en pathologische PFO's.

Op dit moment is de "paradoxale embolie hypothese" de meest geaccepteerde hypothese als oorzaak van PFO-geassocieerde herseninfarcten. Deze hypothese stelt dat het stolsel ontstaat in het veneuze systeem (bijvoorbeeld een diep veneuze trombose in het been), via het foramen ovale in het arteriële systeem terecht komt en uiteindelijk een herseninfarct veroorzaakt. In **hoofdstuk 4** hebben we gekeken naar de histologische samenstelling van stolsels die via intra-arteriële trombectomie verkregen zijn en waarvan achteraf bepaald is dat het PFO hiervan de oorzaak is. Deze data is verzameld in het MR CLEAN register. De histologische samenstelling van deze stolsels is vergeleken met de histologische samenstelling van stolsels die middels trombectomie uit de diep veneuze vaten van patiënten met een symptomatisch trombosebeen zijn verkregen. Ons onderzoek laat zien dat de PFO-geassocieerde stolsels histologisch niet vergelijkbaar zijn met de veneuze stolsels, maar eerder een vergelijkbare samenstelling hebben als stolsels afkomstig uit het hart. Dit pleit voor een arteriële herkomst van het stolsel in PFO-geassocieerde herseninfarcten.

Het blijft onduidelijk waarom patiënten op een bepaald moment in hun leven een PFO-geassocieerd herseninfarct krijgen terwijl het PFO vanaf de geboorte aanwezig is. Het is mogelijk dat een externe factor, een zogenaamde trigger factor, doorslaggevend kan zijn in het ontstaan van het herseninfarct. Tot dusver is alleen een Valsalva manoeuvre een bekende trigger factor voor PFO-geassocieerde herseninfarcten. Met data van de ODYSSEY studie hebben we in **hoofdstuk 5** gekeken naar verschillende (potentiële) uitlokkende factoren voor een PFO-geassocieerd herseninfarct. Het bleek dat zeer intensief sporten, koorts en griepachtige symptomen uitlokkende factoren zijn voor het krijgen van een PFO-geassocieerd herseninfarct.

In de huidige richtlijnen staat dat wanneer de verdenking op een PFO-geassocieerd herseninfarct gesteld is, de patiënt besproken dient te worden in een HST. Er wordt dan multidisciplinair gekeken naar de uitslagen van de onderzoeken en de patiëntkarakteristieken. Uiteindelijk wordt door het HST bepaald of de patiënt in aanmerking komt voor percutane sluiting van het PFO. In **hoofdstuk 6** hebben wij gekeken wat de meerwaarde is van het HST. Alle patiënten die voor het bestaan van het HST (voor juni 2018) PFO sluiting hebben ondergaan en alle patiënten die besproken zijn in het HST (tussen juni 2018 en december 2021) werden bekeken. Dit zijn alleen patiënten waarvan de verwijzend specialist veronderstelt dat de patiënt een PFO-geassocieerd herseninfarct heeft. Uiteindelijk blijkt dat in 32% van de patiënten geen relatie tussen het PFO en het herseninfarct gevonden wordt. Dit laat zien hoe essentieel het HST is in de besluitvorming.



Appendices

References

List of abbreviations

About the author

List of publications

PhD Portfolio

Research data management

Donders Graduate School for Cognitive Neuroscience

Dissertations of the Cerebrovascular Research Program

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*

List of abbreviations

ASA	atrial septal aneurysm
ASD	atrial septal defect
BMI	body mass index
2D-STE	2D speckle-tracking echocardiography
DVT	deep vein thrombosis
CE	cardioembolic
EAPCI	European association of percutaneous cardiovascular interventions
EVT	endovascular treatment
Fib	fibrin
HST	heart stroke team
ICH	intracerebral hemorrhage
LAS	left atrial strain
Leuko	leukocytes
LDL	low-density lipoprotein
LSD	d-lysergic acid diethylamide
MET	metabolic equivalent of task
MR CLEAN	multicenter randomized clinical trial of endovascular treatment for acute ischemic stroke in the Netherlands
MRI	magnetic resonance imaging
NCE	non-cardioembolic
NIHSS	national institutes of health stroke scale
ODDYSEY	observational dutch young symptomatic stroke study
PASCAL	PFO-associated stroke causal likelihood
PE	pulmonary embolism
PFO	patent foramen ovale
Plt	platelets
RBC	red blood cells
RCT	randomized control trial
RLS	right-to-left shunt
RoPE	risk of paradoxical embolism
SAH	subarachnoid hemorrhage
SCAI	society for cardiovascular angiography and interventions
SCOPE	systematic collaborative, PFO closure evaluation
TEE	transesophageal echocardiography
TIA	transient ischemic attack
TOAST	trial of org 10172 in acute stroke treatment
TTE	transthoracic echocardiography
VTE	venous thromboembolism

About the author

Maikel Immens was born on October 21, 1994, in Ottersum, the Netherlands. He attended the bilingual VWO program at the Kandinsky College in Nijmegen. In 2012, he moved to Nijmegen to begin medical school at Radboud University. As part of his curriculum, Maikel first came into contact with medical research by conducting a functional MRI study at the Donders Institute under the supervision of Rick Helmich.

He obtained his medical degree in October 2019 and started as a resident not in training at the Neurology Department of the Jeroen Bosch Hospital in 's-Hertogenbosch for one year. He continued his clinical work at Rijnstate Hospital in Arnhem. He eventually started his PhD project in November 2021 under the supervision of Prof. Frank-Erik de Leeuw and Dr. Tim ten Cate, focusing on patent foramen ovale associated stroke. After eight months, he began combining clinical work with his PhD project and started his residency in January 2023.

Besides research, Maikel focuses on developing and improving education for healthcare professionals. In 2029, Maikel hopes to complete his residency.

List of publications

* Shared authorship

This thesis:

1. **Immens, M. H. M.**, Witte, L. S.*, El Bouziani, A., Duijnhouwer, A., Bouma, B. J., Tijssen, J. G. P., de Leeuw, F. E., de Winter, R. J., & Ten Cate, T. J. F. (2025). Anatomical features of percutaneously closed patent foramen ovale in patients with cryptogenic stroke. *Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation*, 33(10), 313–318. https://doi.org/10.1007/s12471-025-01983-y
2. **Immens, M. H. M.**, Stam, M., Dippel, D. W. J., Nijeholt, G. J. L. À., van der Worp, H. B., Jenniskens, S., van Rijn, M. J., de Leeuw, F. E., Cate, T. J. F. T., van Beusekom, H. M. M., & Tuladhar, A. M. (2025). Histology of PFO-associated stroke thrombus compared to iliofemoral deep vein thrombus: an explorative study. *Neuroradiology*, *67*(9), 2321–2326. https://doi.org/10.1007/s00234-025-03693-z
3. **Immens, M. H. M.**, Ekker, M. S., Verburgt, E., Verhoeven, J. I., Schellekens, M. M., Hilkens, N. A., Boot, … de Leeuw, F. E. (2024). Trigger factors in patients with a patent foramen ovale-associated stroke: A case-crossover study. *International journal of stroke : official journal of the International Stroke Society*, *19*(7), 809–816. https://doi.org/10.1177/17474930241242625
4. **Immens, M. H. M.**, van den Hoeven, V., van Lith, T. J., Duijnhouwer, T. D., Ten Cate, T. J., & de Leeuw, F. E. (2024). Heart-Stroke Team: A multidisciplinary assessment of patent foramen ovale-associated stroke. *European stroke journal*, *9*(1), 219–225. https://doi.org/10.1177/23969873231214862

Articles submitted in this thesis:

5. **Immens, M. H. M.**, C de Wildt, A.L. Duijnhouwer, H.F. de Leeuw, T.J.F. ten Cate. Left atrial strain as a diagnostic marker in PFO-associated stroke.

Other publications:

6. Verburgt, E., Fellah, L., Ekker, M. S., Schellekens, M. M. I., Boot, E. M., **Immens, M. H. M.,** …de Leeuw, F. E. (2025). Risk of Poststroke Epilepsy Among Young Adults With Ischemic Stroke or Intracerebral Hemorrhage. *JAMA neurology*, *82*(6), 597–604. https://doi.org/10.1001/jamaneurol.2025.0465
7. Verburgt, E., Hilkens, N. A., Ekker, M. S., Schellekens, M. M. I., Boot, E. M., **Immens, M. H. M.**, …Verhoeven, J. I. (2024). Short-Term and Long-Term Risk of Recurrent

Vascular Event by Cause After Ischemic Stroke in Young Adults. *JAMA network open*, *7*(2), e240054. https://doi.org/10.1001/jamanetworkopen.2024.0054

8. Boot, E. M., Meijer, F. J. A., Pegge, S., Teeselink, S., Schellekens, M. M., Ekker, M. S., Verhoeven, J. I., Verburgt, E., **Immens M. H. M.**, M., Hilkens, N., de Leeuw, F. E., & Tuladhar, A. M. (2025). Prevalence of vessel wall abnormalities and the risk of recurrent vascular events in young patients with stroke. *European stroke journal*, 23969873251343828. Advance online publication. https://doi.org/10.1177/23969873251343828
9. **Immens, M. H. M.**, Verstraete, E., Klein Bleumink, G., & Pisters, R. (2023). Outpatient Check-In Using an Online Portal. *Telemedicine reports*, *4*(1), 336–342. https://doi.org/10.1089/tmr.2023.0026
10. **Immens M. H. M**., Boerman R.H., Koonen L.S.P. (2022). Het syndroom van Trousseau. *Tijdschrift voor Neurologie en Neurochirurgie.*
11. Schellekens, M. M., Boot, E. M., Verhoeven, J. I., Ekker, M. S., Verburgt, E., **Immens, M. H. M.**, …Tuladhar, A. M. (2025). Cognitive performance is associated with return to work after ischemic stroke in young adults: The ODYSSEY study. *European stroke journal*, *10*(3), 784–795. https://doi.org/10.1177/23969873251324400
12. Evi J. van Kempen, Mijntje M.I. Schellekens, Jamie I. Verhoeven, Merel S. Ekker, Esmée Verburgt, **Immens, M. H. M.**, …Tuladhar, A.M. (2025). Prevalence and Factors Associated With Atrial Fibrillation Among Young Patients With Ischemic Stroke. *JAHA*.https://doi.org/10.1161/JAHA.125.043996

PhD portfolio

Activity	Organizer	Year	ECTS*
Conference Lyon, France	ESO[α]	2022	2.5
'Basiscursus Regelgeving en Organisatie voor Klinische onderzoekers' (BROK) certificate	NFU BROK Academie	2022	1.5
Presenting and Poster Pitching	Radboud University	2022	1.8
Grant Writing and Presenting for Funding Committees	Radboud University	2022	0.7
Writing Scientific Articles	Radboud University	2022	3.4
Education in a Nutshell	Radboud University	2022	1.0
Presentation on "Paradoxale embolus en work up bij young stroke", Breukelen, Netherlands	Abbott	2022	0.5
Conference Munich, Germany	ESO	2023	2.5
Conference Basel, Switzerland	ESO	2024	2.5
Conference Helsinki, Finland	ESO	2025	2.5
Conference Seoul, South Korea	WCN[β]	2025	2.5
Scientific integrity course	Coursera	2025	0.7

*1 ECTS (European Credit Transfer System) equals a workload of 28 hours

[α] European Stroke Organization

[β] World Congress of Neurology

Research Data Management

All research presented in this thesis that involved human participants was conducted in accordance with the principles of the Declaration of Helsinki and Dutch legislation on human research (WMO). The handling of data complied with the General Data Protection Regulation of 2018.

Data presented in this thesis are collected and archived according to the FAIR principles: Findable, Accessible, Interoperable and Reusable.

Ethics and privacy

The data presented in **chapter 2** are based on a database containing collected data from patients at both Radboudumc, Nijmegen, and Amsterdam UMC, Amsterdam. The data used in **chapters 3 and 6** are based on two databases consisting of patients from Radboudumc who were discussed by the multidisciplinary heart-stroke team. These studies were not subject to the Dutch Medical Research Involving Human Subjects Act (WMO) and therefore did not require additional informed consent.

Participant privacy was ensured through pseudonymization using a unique subject number assigned to each participant. The pseudonymization key was stored separately from the research data on a secure departmental server accessible only to the principal investigator.

The data presented in **chapter 4** are based on results from the MR CLEAN Registry. In short, the MR CLEAN Registry is a Dutch nationwide, multicenter, prospective registry of patients who underwent endovascular treatment (EVT) for ischemic stroke between March 2014 and June 2016. The central medical ethics committee of Erasmus Medical Center, Rotterdam, approved the study protocol and granted permission to conduct the study as a registry (MEC-2014–235). Written informed consent was waived by the Institutional Review Board.

The pseudonymization key for this data was stored separately from the research data on a secure departmental server at Erasmus Medical Center, accessible only to members of the Research Data Management team involved in the project.

Participant privacy was protected by pseudonymizing patient data and storing a separate key document containing the information required to re-identify individuals. This key document was accessible only to the principal investigators.

The data presented in **chapter 5** are based on the ODYSSEY study (file number: NL41531.091.12), which was approved by the local medical ethics committee Region Oost-Nederland. Informed consent was obtained from all participants (or their legal representatives, if applicable) to collect and process their data for this research project.

Participant privacy was maintained through pseudonymization using unique subject numbers. The pseudonymization key was stored separately from the research data on a secure departmental server, accessible only to authorized project members based on their roles.

Data Collection and Storage

Data from the ODYSSEY study presented in **chapter 5** were collected from existing health records via electronic Case Report Forms (eCRFs) as part of a prospective data collection in Castor EDC. These data were transferred from (electronic) health records or Castor EDC to SPSS (IBM) and R (R Project for Statistical Computing).

Data from **chapters 2 and 3** were collected from health records, transferred to SPSS (IBM) and stored on a server at Radboudumc.

Data from **chapter 4** were collected using Excel and stored at Erasmus Medical Center. Access to this data is restricted to investigators who submitted a data request for a CONTRAST research proposal.

Processed data and related documentation have been archived in a Research Documentation Collection (RDC) within the Radboud Data Repository (DOI 10.34973/a8w8-w45). These secure storage solutions ensure the availability, integrity, and confidentiality of the data.

Data sharing according to the FAIR principles

The chapters 2, 4, 5 and 6 have been published, of which none with open access. Chapter 2 is submitted for publication and is currently under review. Chapter 2 is based on existing data, which was obtained from the database *PFO_AMC_RADBOUD* that is maintained by the Radboudumc. Questions regarding use of the data from this database van be addressed to Maikel.immens@radboudumc.nl. The data used for chapter 4 are not owned by Radboudumc. The data are archived by Erasmus Medical Center. Questions about the data can be addressed to dawc.contrast@contrast-consortium.nl. Chapter 5 is based on existing data, which was obtained from the ODYSSEY study. Questions regarding use of the data from the

ODYSSEY study can be addressed to frankerik.deleeuw@radboudumc.nl. The data used in the unpublished chapter 3 are archived at the department server. Upon publication of the chapter the data will be archived via the Radboud Data Repository (DOI 10.34973/a8w8-w45) with closed access. Questions regarding use of the data from this database van be addressed to Maikel.immens@radboudumc.nl. Processed data and related documentation have been archived in a Research Documentation Collection (RDC) within the Radboud Data Repository (DOI 10.34973/a8w8-w45), where it will be stored for 10 years.

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- Karlijn F. de Laat. Motor performance in individuals with cerebral small vessel disease: an MRI study. Radboud University Nijmegen, 29 November 2011
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