

Propensity Score–Based Analysis of Percutaneous Closure Versus Medical Therapy in Patients With Cryptogenic Stroke and Patent Foramen Ovale

The IPSYS Registry (Italian Project on Stroke in Young Adults)

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Background—We sought to compare the benefit of percutaneous closure to that of medical therapy alone for the secondary prevention of embolism in patients with patent foramen ovale (PFO) and otherwise unexplained ischemic stroke, in a propensity scored study.

Methods and Results—Between 2000 and 2012, we selected consecutive first-ever ischemic stroke patients aged 18 to 45 years with PFO and no other cause of brain ischemia, as part of the IPSYS registry (Italian Project on Stroke in Young Adults), who underwent either percutaneous PFO closure or medical therapy for comparative analysis. Primary end point was a composite of ischemic stroke, transient ischemic attack, or peripheral embolism. Secondary end point was brain ischemia. Five hundred

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and twenty-one patients qualified for the analysis. The primary end point occurred in 15 patients treated with percutaneous PFO closure (7.3%) versus 33 patients medically treated (10.5%; hazard ratio, 0.72; 95% confidence interval, 0.39–1.32; $P=0.285$). The rates of the secondary end point brain ischemia were also similar in the 2 treatment groups (6.3% in the PFO closure group versus 10.2% in the medically treated group; hazard ratio, 0.64; 95% confidence interval, 0.33–1.21; $P=0.168$). Closure provided a benefit in patients aged 18 to 36 years (hazard ratio, 0.19; 95% confidence interval, 0.04–0.81; $P=0.026$) and in those with a substantial right-to-left shunt size (hazard ratio, 0.19; 95% confidence interval, 0.05–0.68; $P=0.011$).

Conclusions—PFO closure seems as effective as medical therapy for secondary prevention of cryptogenic ischemic stroke. Whether device treatment might be more effective in selected cases, such as in patients younger than 37 years and in those with a substantial right-to-left shunt size, deserves further investigation. (*Circ Cardiovasc Interv.* 2016;9:e003470. DOI: 10.1161/CIRCINTERVENTIONS.115.003470.)

Key Words: atrial septum ■ follow-up studies ■ patent foramen ovale ■ secondary prevention ■ stroke

WHAT IS KNOWN

- Whether percutaneous closure of patent foramen ovale is an effective treatment option for secondary prevention of otherwise unexplained ischemic stroke (cryptogenic stroke) is still unclear.
- Practitioners need additional patient data to better define optimal treatment in individual cases.

WHAT THE STUDY ADDS

- Overall, there is no significant difference between percutaneous patent foramen ovale closure and medical therapy for secondary prevention of cryptogenic ischemic stroke.
- Percutaneous closure might be more effective in patients younger than 37 years and in those with a substantial right-to-left shunt size.
- Subgroups of patients can be identified in whom device closure is superior to medical therapy.

Up to 61% of cryptogenic stroke patients younger than 55 years of age have patent foramen ovale (PFO), a cardiac interatrial septal abnormality residual of the fetal circulation. Evidence from observational studies suggests an association between cryptogenic stroke and PFO^{1–5} and a 3-fold increased risk of recurrent stroke in PFO carriers.⁶ Therefore, it has been postulated that PFO closure would result in a decreased risk of recurrent neurological events (transient ischemic attack [TIA], stroke, or death because of stroke) through the elimination of the conduit for paradoxical embolism. In spite of this epidemiological evidence, results from 3 recently published randomized controlled trials (RCTs) failed to show a significant benefit of transcatheter PFO closure compared with medical therapy.^{7–9} The 3 trials, however, share several limitations, the most relevant of which were the recruitment rate lower than expected, probably because of off-label closure, the high loss to follow-up, and the small number of recurrent events, raising the possibility of an underpowered comparison. In the absence of definitive results from clinical trials, the precise definition of which patients should be considered for PFO closure is still unclear. Of note, one of the many subgroup analyses of the CLOSURE I trial (Evaluation of the STARFlex

Closure System in Patients with a Stroke and/or Transient Ischemic Attack due to Presumed Paradoxical Embolism through a Patent Foramen Ovale) suggested benefit for device in patients under the age of 40 years.¹⁰ Similarly, PFO closure with Amplatzer device was associated with a decrease in recurrent, nonfatal ischemic stroke (IS), whereas no benefit was observed in the study using the STARFlex device.¹¹ The effectiveness of percutaneous closure of PFO was also greater in patients with a substantial shunt size according to a further stratified analysis.¹² These findings implicate that, perhaps, subgroups of patients can be identified in whom device closure is safe and superior to medical therapy and that additional patient data are needed to better define optimal treatment in individual cases. To further investigate these issues, we tested the effectiveness of percutaneous PFO closure compared with medical treatment in the setting of the IPSYS registry (Italian Project on Stroke in Young Adults).

Methods

Patients and Study Design

IPSYS is a countrywide network of neurological centers with special interest in cerebral ischemia at young age across Italy, aimed at recruiting white patients with first-ever acute stroke who fulfill the following criteria: (1) age 18 to 45 years, (2) computed tomography- or magnetic resonance imaging-proven cerebral infarction, in the setting of a hospital-based, multicenter, observational study. The study was approved by the local Ethics Committee. Informed consent was provided by all study participants. Structure and methods of the IPSYS project have been described in detail previously.¹³ For the purpose of the present analysis, we screened data sets from patients consecutively admitted to 22 hospitals. The recruitment period was January 2000 through January 2012, and follow-up was completed at January 2013.¹⁴ All patients underwent an extensive etiologic work-up aimed at determining the most likely mechanism of stroke in each case (e-Methods for risk factor definition and diagnostic work-up). Patients were categorized according to an etiologic classification based on the Trial of Org 10172 in Acute Stroke Treatment criteria, accommodated and validated for stroke in the young.¹⁵ Only those patients whose cerebral infarct (1) was presumably related to PFO in the presence of cardiac interatrial right-to-left shunt (RLS) and (2) did not meet the criteria for other etiologic categories were included in the present analysis.

Assessment of PFO

Interatrial RLS was assessed in all patients with transesophageal echocardiography with a contrast study and Valsalva maneuver (c-TEE) or transcranial Doppler sonography with intravenous injection of agitated saline (c-TCD). A RLS was considered present if any

microbubble was seen in the left atrium within 3 cardiac cycles from maximum right atrial opacification on echocardiography.⁵ c-TCD was performed according to the Venice Consensus Conference.¹⁶ Briefly, it consists of the injection of 9 mL of saline solution and 1 mL air mixed with a 3-way stopcock by exchange of saline/air mixture between the syringes and injected as a bolus as a contrast-enhancing agent into the right cubital vein 5 seconds before the start of a 10-second Valsalva maneuver, while recording the flow velocity of the middle cerebral artery, insonated through the temporal window on the right side at a depth of 50 to 60 mm, with a handheld probe. The appearance of transient spikes on the velocity spectral curve is considered positive for interatrial RLS. The method has an overall diagnostic accuracy comparable to that of c-TEE.¹⁷ The shunt on c-TCD was graded according to a previously described classification: small (<10 microembolic signals, MES) and moderate to large (>10 microembolic signals, and, in particular, shower pattern if >25 microembolic signals and curtain pattern if uncountable microembolic signal). On c-TEE, the shunt was graded as small if <10 bubbles passed only after Valsalva maneuver, moderate if 10 to 20 bubbles passed only after Valsalva maneuver, and large if there was intense opacification of the left atrium after Valsalva maneuver (>20 bubbles) or if there was any passage at rest.^{18–20} We defined substantial shunts as those shunts that were other than small on c-TCD or c-TEE.

Treatment

The decision in favor of medical therapy rather than treatment with percutaneous catheter-based closure of PFO was at the discretion of the treating physician and the individual preference of the patient. Antithrombotic therapy in the medical treatment group was also left to the discretion of the physician in charge with the patient and included antiplatelet agents (acetylsalicylic acid at the dose of 100–325 mg or clopidogrel at the dose of 75 mg once daily) or oral anticoagulation with warfarin (with a target international normalized ratio of 2.0–3.0). Other treatments for secondary prevention were administered in accordance with published guidelines.²¹ Percutaneous PFO closure was achieved under local anesthesia and fluoroscopic guidance using standardized techniques in all the centers. The choice of the device and its size was left to the preference of the performing physician, based on atrial septal anatomy and patient size. A trans-thoracic echocardiography was conducted within 24 hours after the procedure to document correct device position, and a c-TEE or a c-TCD was performed after 6 months to search for a residual shunt. Antithrombotic treatment in these patients included acetylsalicylic acid at a dose of 100 to 325 mg once daily for 6 months and ticlopidine at a dose of 250 mg twice daily or clopidogrel at a dose of 75 mg daily for 1 to 6 months.

Outcomes

Only patients who survived the index event were entered into the present analysis. Death was considered because of the index stroke if it occurred within 30 days of symptoms onset. Subjects were included in the subgroup of patients who did not experience recurrence if they had at least a 1-year follow-up. Follow-up evaluations were conducted at 3 months and then annually, and outcome events classified using information from interviews (directly during follow-up visits or by telephone) with patients, next of kin, witnesses, and attending physicians or from hospital or general practitioner records.

Long-term vascular recurrence was defined as any event of fatal or nonfatal IS, TIA, or fatal/nonfatal peripheral embolism. Recurrent IS was defined using the same criteria applied for the definition of the index event. Diagnosis of TIA was made when the patient had reliably observed transient (<24 hours) neurological deficit of abrupt onset, without evidence of an underlying nonvascular cause, according to the consulting neurologist or the attending physician who evaluated the event by clinical and imaging methods.²² Deaths were classified using death certificates, medical records, and family interviews. In those cases in which it was difficult to make a precise determination of the cause of death, consensus was reached based on the best available information. If more than one recurrent event occurred, the first was used for calculation of the disease-free survival time.

Primary end point was a composite of IS, TIA, or peripheral arterial embolism. Secondary end points were (1) brain ischemia (IS or TIA) and (2) peripheral embolism.

Statistical Analyses

Duration of follow-up was calculated in person-months by using the follow-up of each participant from baseline examination until recurrent event or most recent, censored, follow-up assessment.

Descriptive group comparisons were performed using χ^2 test for categorical variables and *t* test for continuous variables. Long-term outcomes were examined by using propensity score (PS) methods.²³ The PS is the probability that a patient would have been treated with PFO closure given his pretreatment variables. Equal PS values guarantee equal distribution of measured pretreatment variables at baseline on the sample level; thus, PS is an attempt to create homogeneous groups for comparison when data from a randomization procedure are not available. The individual propensity scores for analyzing PFO closure and medical therapy groups were estimated with a logit model with age, sex, and pretreatment variables that differed between groups in a univariate analysis at 2-side *P* value <0.1. To estimate treatment effects, Cox proportional hazards models were performed on the entire cohort to derive crude, PS-adjusted, and PS-weighted HRs. PS-adjusted HRs were obtained including in the Cox model the PS scores as a covariate with cubic spline functions.²⁴ PS-weighted HRs used inverse probability of treatment weights in all patients, with the inverse PS as weight for patients receiving PFO closure and the inverse of 1 minus the PS as weight for patients with medical therapy.²⁵ The analysis of the primary composite end point was performed overall and stratified by 2 strata according to median age (18–36 years versus 37–45 years), sex (men versus women), shunt size (substantial shunt versus other size), medical treatment (anticoagulant versus antiplatelet), and device type (Amplatzer versus others). All *P* values and 95% confidence intervals were 2 sided. Analyses were performed with R packages (release 3.1.2).²⁶

Results

Of the 1906 patients included in the IPSYS registry, 39 (2.0%) were lost during follow-up. Five hundred and twenty-one patients with PFO and no other potential cause of brain ischemia were entered into the present analysis (Figure 1).

Of these, 315 (60.5%) were treated medically and 206 (39.5%) underwent percutaneous closure. The following devices for percutaneous closure were used: Amplatzer Occluders (AGA Medical Corporation, Golden Valley, MN; 86.8%), CardioSEAL (NMT Medical, Boston, MA; 1.9%), STARFlex (NMT Medical, Boston, MA; 2.4%), GORE HELEX Septal Occluder (Gore Medical, Flagstaff, AZ; 2.4%), BioSTAR (NMT Medical, ; 1.9%), Premere (St. Jude Medical, Maple Grove, MN; 3.3%), Figulla ASD Occluder (Occlutech GmbH, Jena, Germany; 0.9%), and ATRIASSEPT (Cardia Inc, Eagan, MN; 0.4%). The rate of serious adverse events did not differ significantly in the 2 groups (Table I in the [Data Supplement](#)). In particular, new-onset atrial fibrillation was detected in 3 patients (1.5%) in the closure group and in 1 patient (0.3%) in the medical therapy group (*P*=0.306). None of these patients had subsequent end point events. Complete PFO closure was observed in 190 patients (92.2%), whereas a small shunt persisted in 16 (7.8%). Median follow-up time was 36.0 months (25th to 75th percentile, 58.0; 13.5–72.5 months) for the medical treatment group versus 32.0 months (25th to 75th percentile, 37.0; 16–52.25 months) for the PFO closure group. The accumulated (mass) person-months were 14 206 in the medical treatment group and 8483 in the PFO closure group. The 2 groups were

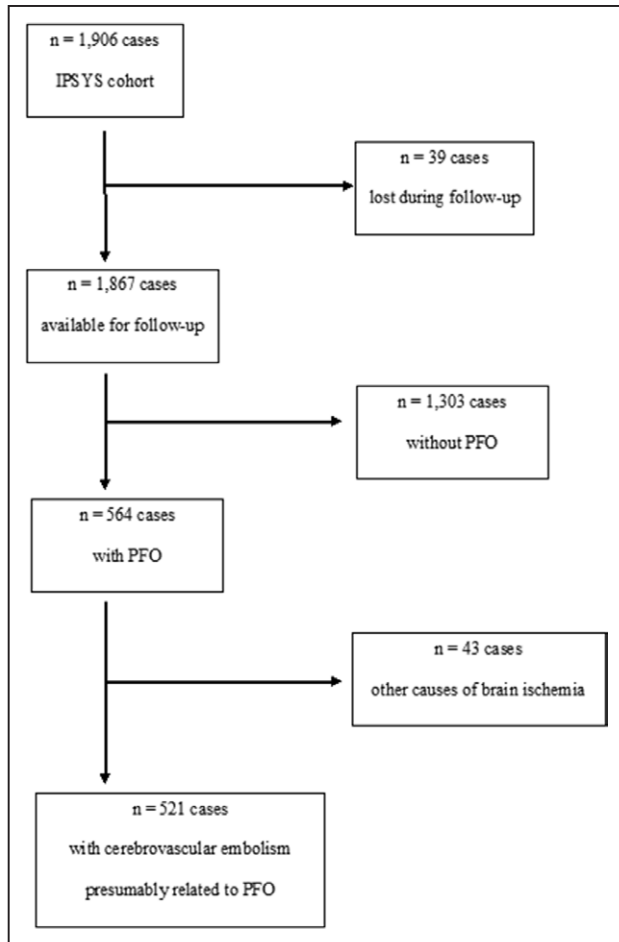


Figure 1. Flow chart of patients selection. IPSYS indicates Italian Project on Stroke in Young Adults; and PFO, patent foramen ovale.

similar with respect to baseline characteristics, except for a greater proportion of hypertensive patients among patients treated medically and of migraine sufferers among those who underwent PFO closure (Table 1).

Antiplatelet drugs were the preferred option for long-term secondary prevention in patients treated medically (259 patients [82.2%] versus 56 [17.8%] who received oral anticoagulants).

Table 2 and Figure 2A and 2B summarize primary and secondary end points of the comparison between patients who received transcatheter PFO closure and those treated medically. The primary composite end point occurred in 15 patients treated with percutaneous PFO closure (7.3%) versus 33 patients who received medical treatment (10.5%; crude hazard ratio, 0.72; 95% confidence interval, 0.39–1.32; $P=0.285$). The rates of the secondary end point brain ischemia were also similar in the 2 treatment groups (6.3% in the PFO closure group versus 10.2%; crude hazard ratio 0.64; 95% confidence interval, 0.33–1.21; $P=0.168$). We then computed propensity scores by using the variables age, sex, hypertension, diabetes mellitus, migraine, and family history as PS predictors. In the entire cohort, adjusted PS and inverse probability of treatment–weighted HRs were compatible to crude HRs (95% confidence intervals overlapped the null [hazard ratio, 1]) for

both the primary end point and the secondary composite end point brain ischemia (IS or TIA; Table 2).

To determine the potential heterogeneity of the treatment effect and identify subpopulations of patients that might benefit most from receiving one therapeutic approach, we performed stratified analyses by splitting patients according to baseline covariates. Findings suggested that closure might provide a greater benefit in younger patients (aged <37 years) and in those with a substantial RLS size (Figure 3).

Discussion

The crucial clinical question on PFO during the past 15 years has been whether percutaneous closure is superior to medical therapy in stroke secondary prevention. Although all of the randomized trials reported to date failed to show any significant differences between the 2 treatments, they are clearly insufficient to draw any conclusion, mainly because of methodological drawbacks, including sample size of the study cohorts smaller than expected and event rates lower than anticipated in the follow-up.²⁷ The other trials currently ongoing (Gore REDUCE trial [NCT00738894] and CLOSE trial [NCT00562289]) are likely to face the same statistical limitations. As a practical implication of this, it seems unlikely that the results of RCTs will change the opinion of clinicians about the use of an approach over the other. In the absence of definitive trial results, therefore, practitioners need more data to consider when deciding whether a stroke patient is likely to have a high-risk PFO and which treatment option is the best in individual cases. The results of our prospective cohort study provide information in this regard. Because, overall, we were unable to detect any benefit of percutaneous PFO closure compared with medical treatment on the primary end point, a composite of stroke, TIA, or peripheral embolism, our findings reinforce the randomized trials evidence of clinical equipoise for the 2 treatments. In contrast, although we cannot exclude a priori the possibility of underpowered comparison, we found differential effect estimates according to subgroups in stratified analyses. In particular, transcatheter PFO closure seemed superior to medical therapy for secondary prevention in stroke patients younger than 37 years and in those with a large RLS. Of note, these findings are in line with those from specific subgroup analyses of RCTs. Actually, benefit for device closure over medical therapy was observed in patients aged ≤ 40 years in CLOSURE I¹⁰ and in those with a substantial RLS size⁸ and aged <60 years in RESPECT²⁸ and in one of the meta-analyses of randomized data.¹² These 2 characteristics have been also associated with an increased likelihood that a stroke is related to PFO in epidemiological studies,²⁹ which provides supportive evidence to the prevailing idea that there might be subgroups of patients in whom device closure is superior to medical therapy.

Because of its characteristic of real-world patient population, the IPSYS cohort is reasonably more representative of patients with PFO and otherwise unexplained IS than those included in controlled studies. Any decision favoring off-label device closure versus medical therapy is likely to reflect clinical practice more closely in this setting, although a formal proof of this generalizability is lacking. On the contrary, collecting data on the basis of a predefined clinical protocol allowed us to collect baseline and follow-up information using

Table 1. Demographic and Clinical Characteristics of the Study Group

Variable	PFO Closure (n=206)	Medical Treatment (n=315)	P Value
	Mean ± SD	Mean ± SD	
Age, y, mean±SD	35.3±7.4	35.7±6.8	0.525
	n (%)	n (%)	
Men	94 (45.6)	154 (48.9)	0.467
Hypertension	20 (9.7)	52 (16.5)	0.028
Diabetes mellitus	0 (0.0)	5 (1.6)	0.069
Current smokers	64 (31.0)	100 (31.7)	0.833
Hypercholesterolemia	39 (18.9)	67 (21.2)	0.494
History of migraine			0.054
No migraine	130 (63.1)	230 (73.0)	
MO	43 (20.9)	46 (14.6)	
MA	33 (16.0)	39 (12.4)	
Oral contraceptives*	44 (39.3)	47 (29.2)	0.082
Family history of stroke	38 (18.4)	79 (25.0)	0.076
Heavy alcohol consumption	16 (7.8)	21 (6.6)	0.633
FV _{G1691A}			0.347
GG	193 (93.7)	301 (95.6)	
AG	13 (6.3)	14 (4.4)	
AA	0 (0.0)	0 (0.0)	
PT _{G20210A}			0.611
GG	197 (95.6)	304 (96.5)	
AG	9 (4.4)	11 (3.5)	
AA	0 (0.0)	0 (0.0)	

FV indicates factor V; MA, migraine with aura; MO, migraine without aura; PFO, patent foramen ovale; and PT, prothrombin.

*In women.

uniform procedures in all patients, thereby reducing the risk of information bias due, for example, to differential intensity and thoroughness of follow-up examinations. Because the present analysis is just one among those we had planned to perform in the setting of the collaborative IPSYS project, any differential outcome ascertainment in medically treated patients compared with closure-treated patients seems unlikely.

There are also some notable limitations of our study that should be considered. First, because of its nonrandomized

design, we cannot exclude that results are subject to confounding by indication. However, although it is theoretically possible that unmeasured characteristics might have differed between patients and confounded our results, the application of propensity score, a robust method of adjustment for confounders that minimizes their effects, is a strength of our analysis compared with others based on traditional, less adequate, methods of multivariate adjustment.³⁰ This makes our findings reliable. To our knowledge, among the comparative observational

Table 2. Clinical Outcomes in the Overall Cohort

	PFO Closure, n (%)	Medical Treatment, n (%)	Crude		PS Adjusted		IPT Weighed	
			HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Primary end point								
IS, TIA, or peripheral embolism	15/206 (7.3)	33/315 (10.5)	0.72 (0.39–1.32)	0.285	0.69 (0.37–1.31)	0.262	0.68 (0.36–1.29)	0.238
Secondary end point								
IS or TIA	13/206 (6.3)	32/315 (10.2)	0.64 (0.33–1.21)	0.168	0.61 (0.31–1.19)	0.149	0.59 (0.30–1.14)	0.114

CI indicates confidence interval; HR, hazard ratio; IPT, inverse probability of treatment; IS, ischemic stroke; PFO, patent foramen ovale; PS, propensity score; and TIA, transient ischemic attack.

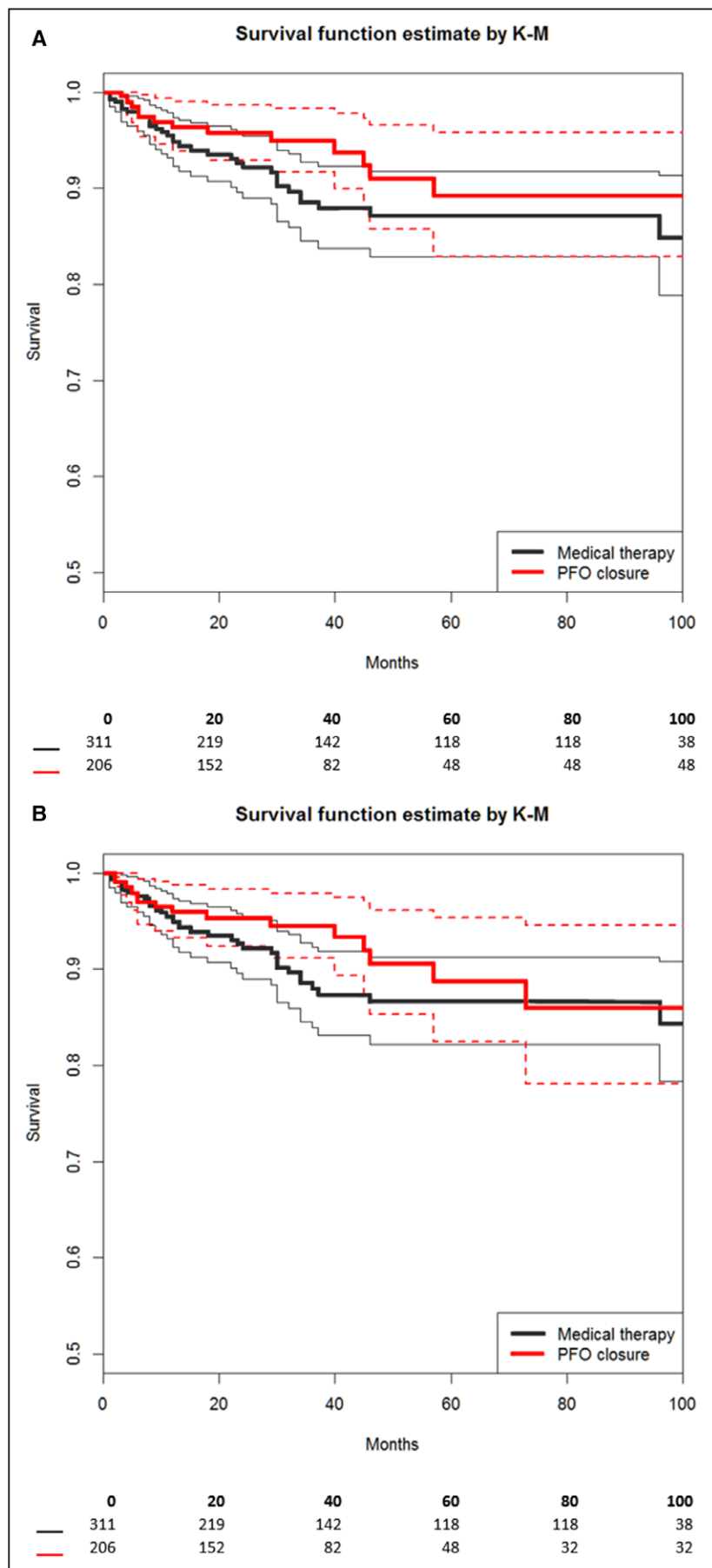


Figure 2. Kaplan–Meier estimates for primary end point events (A) and secondary end point events (B). PFO indicates patent foramen ovale.

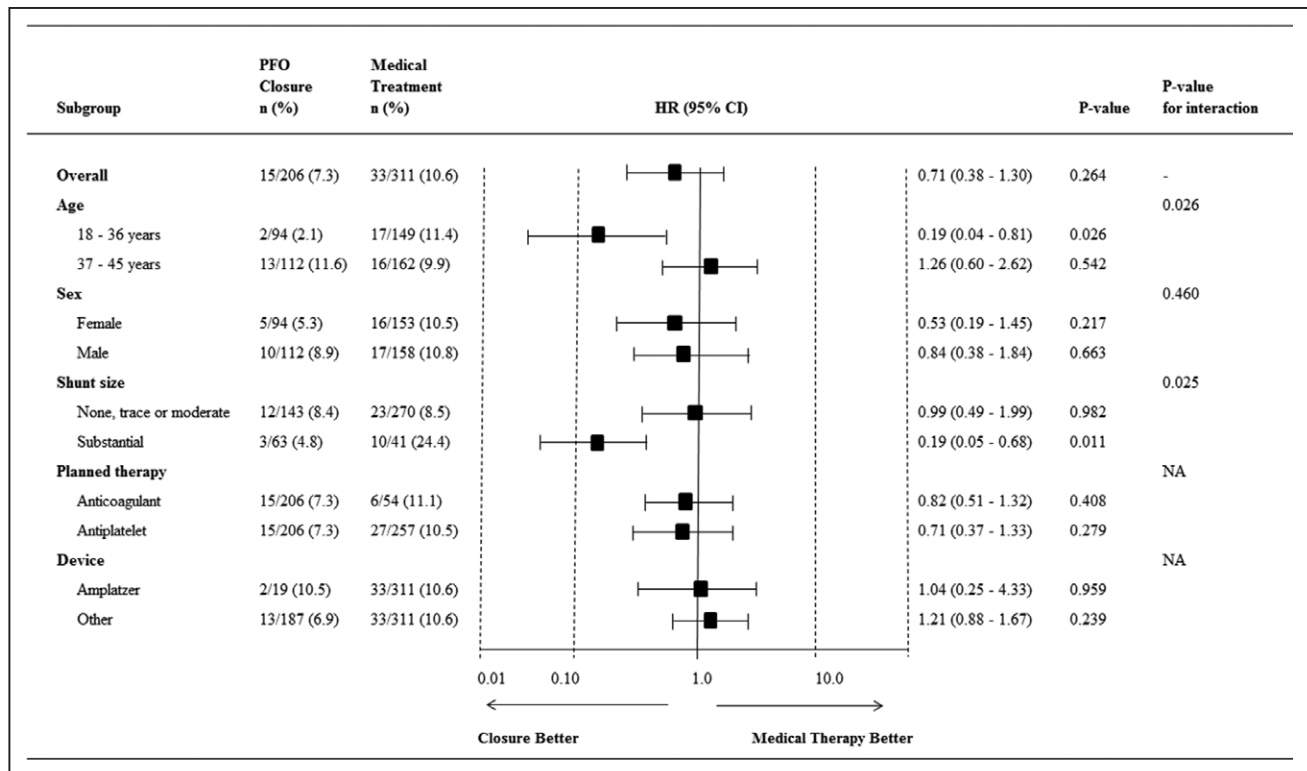


Figure 3. Analysis of the primary end point according to subgroup. Missing values in 4 patients. CI indicates confidence interval; HR, hazard ratio; and PFO, patent foramen ovale.

studies published to date, only one single-center, propensity score–matched analysis was reported, which showed a benefit of percutaneous PFO closure over medical therapy in TIA secondary prevention.³¹ The long-term follow-up duration of that study as compared with other studies including ours is a major strength of the analysis and provides reliable information on the long-term effectiveness and safety of percutaneous PFO closure. Nonetheless, the mean duration of follow-up in our analysis is in line with that of the 3 comparative RCTs on PFO closure. Our observation that covariates were in good balance in 2 treatment groups even before PS matching is a further argument against the possibility that a substantial selection bias might have occurred. Second, our study has a long inclusion period, during which acute treatment and secondary prevention and devices and techniques for PFO closure have improved. Closure devices have changed over time, and different attitudes across centers might theoretically have had an impact on the results as well.¹² This is an unavoidable feature of a multicenter, long-term, longitudinal study with a stringent age cutoff for patient inclusion like ours, whose potential implications are noteworthy. However, although we were unable to perform separate subgroup analyses for each device category because of the low number of patients, we did not detect outcome differences according to the type of device used. Third, patients underwent PFO closure at different time points after the index event, which might represent a further potential drawback. However, as in the device closure arm, we considered person-time after the procedure for the analysis, it seems unlikely that this has significantly altered the results of our study. Fourth, the TCD technique we used

for the diagnosis of interatrial RLS in some cases prevents the assessment of atrial septal aneurysm, which does not allow to achieve precise data on the frequency of atrial septal aneurysm in our series and to perform subgroup analyses on different cohort strata defined by atrial septal aneurysm. Fifth, TIA is a less clear-cut end point than stroke and has several mimicking conditions, particularly in younger individuals. However, at least biologically, TIAs represent reliable markers of failed secondary prevention just as major strokes or any other thrombotic events and, as such, they should not be excluded from long-term comparative analyses. Finally, estimates for some subgroups that contain only a few patients might be unstable and should, therefore, be interpreted with caution. Although the implications of these potential shortcomings should be taken into account, it seems unlikely that they have significantly altered the results of our study.

In conclusion, although the true efficacy of PFO closure in patients with otherwise unexplained ischemic stroke remains to be definitively assessed, the results of the present analysis conducted in patients between 18 and 45 years of age support the evidence from RCTs that this approach provides no significant benefit over medical therapy alone. Whether device treatment might be more effective for secondary stroke prevention in appropriately selected cases, with acceptably low periprocedural complication rates, such as younger patients and patients with a substantial RLS size deserves further investigation.

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Dr Pezzini had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Pezzini and Grassi performed decision making about study

concept and design. All authors played roles in acquisition of data. Drs Pezzini and Grassi helped in analysis and interpretation of data. Dr Pezzini assisted in drafting of the article. All authors took role in critical revision of the article for important intellectual content. Drs Pezzini, Grassi, and Guido performed statistical analysis. Drs Pezzini and Lodigiani obtained funding. Dr Pezzini provided administrative, technical, or material support. Drs Pezzini and Padovani have taken care of study supervision.

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Disclosures

None.

References

- Lechat P, Mas JL, Lascault G, Loron P, Theard M, Klimczac M, Drobinski G, Thomas D, Grosgeat Y. Prevalence of patent foramen ovale in patients with stroke. *N Engl J Med*. 1988;318:1148–1152. doi: 10.1056/NEJM19880503181802.
- Di Tullio M, Sacco RL, Gopal A, Mohr JP, Homma S. Patent foramen ovale as a risk factor for cryptogenic stroke. *Ann Intern Med*. 1992;117:461–465.
- Handke M, Harloff A, Olschewski M, Hetzel A, Geibel A. Patent foramen ovale and cryptogenic stroke in older patients. *N Engl J Med*. 2007;357:2262–2268. doi: 10.1056/NEJMoa071422.
- Mas JL, Arquizan C, Lamy C, Zuber M, Cabanes L, Derumeaux G, Coste J; Patent Foramen Ovale and Atrial Septal Aneurysm Study Group. Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. *N Engl J Med*. 2001;345:1740–1746. doi: 10.1056/NEJMoa011503.
- Webster MW, Chancellor AM, Smith HJ, Swift DL, Sharpe DN, Bass NM, Glasgow GL. Patent foramen ovale in young stroke patients. *Lancet*. 1988;2:11–12.
- Homma S, Sacco RL, Di Tullio MR, Sciacca RR, Mohr JP; PFO in Cryptogenic Stroke Study (PICSS) Investigators. Effect of medical treatment in stroke patients with patent foramen ovale: patent foramen ovale in Cryptogenic Stroke Study. *Circulation*. 2002;105:2625–2631.
- Elmariah S, Furlan AJ, Reisman M, Burke D, Vardi M, Wimmer NJ, Ling S, Chen X, Kent DM, Massaro J, Mauri L; CLOSURE I Investigators. Closure or medical therapy for cryptogenic stroke with patent foramen ovale. *N Engl J Med*. 2012;366:991–999.
- Carroll JD, Saver JL, Thaler DE, Smalling RW, Berry S, MacDonald LA, Marks DS, Tirschwell DL; RESPECT Investigators. Closure of patent foramen ovale versus medical therapy after cryptogenic stroke. *N Engl J Med*. 2013;368:1092–1100. doi: 10.1056/NEJMoa1301440.
- Meier B, Kalesan B, Mattle HP, Khattab AA, Hildick-Smith D, Dudek D, Andersen G, Ibrahim R, Schuler G, Walton AS, Wahl A, Windecker S, Juni P; PC Trial Investigators. Percutaneous closure of patent foramen ovale in cryptogenic embolism. *N Engl J Med*. 2013;368:1083–1091. doi: 10.1056/NEJMoa1211716.
- Furlan AJ. Brief history of patent foramen ovale and stroke. *Stroke*. 2015;46:e35–e37. doi: 10.1161/STROKEAHA.114.007772.
- Stortecky S, da Costa BR, Mattle HP, Carroll J, Hornung M, Sievert H, Trelle S, Windecker S, Meier B, Juni P. Percutaneous closure of patent foramen ovale in patients with cryptogenic embolism: a network meta-analysis. *Eur Heart J*. 2015;36:120–128. doi: 10.1093/eurheartj/ehu292.
- Rengifo-Moreno P, Palacios IF, Junpaparp P, Witzke CF, Morris DL, Romero-Corral A. Patent foramen ovale transcatheter closure vs. medical therapy on recurrent vascular events: a systematic review and meta-analysis of randomized controlled trials. *Eur Heart J*. 2013;34:3342–3352. doi: 10.1093/eurheartj/eht285.
- Pezzini A, Grassi M, Lodigiani C, Patella R, Gandolfo C, Casoni F, Musolino R, Calabrò RS, Bovi P, Adami A, Delodovici ML, Del Zotto E, Rota LL, Rasura M, Del Sette M, Giossi A, Volonghi I, Zini A, Cerrato P, Costa P, Magoni M, Iacoviello L, Padovani A; Italian Project on Stroke in Young Adults Investigators. Predictors of migraine subtypes in young adults with ischemic stroke: the Italian Project on Stroke in Young Adults. *Stroke*. 2011;42:17–21. doi: 10.1161/STROKEAHA.110.592246.
- Pezzini A, Grassi M, Lodigiani C, Patella R, Gandolfo C, Zini A, Delodovici ML, Paciaroni M, Del Sette M, Toriello A, Musolino R, Calabrò RS, Bovi P, Adami A, Silvestrelli G, Sessa M, Cavallini A, Marcheselli S, Bonifati DM, Checcarelli N, Tancredi L, Chiti A, Del Zotto E, Spalloni A, Giossi A, Volonghi I, Costa P, Giacalone G, Ferrazzi P, Poli L, Morotti A, Rasura M, Simone AM, Gamba M, Cerrato P, Miceli G, Melis M, Massucco D, De Giuli V, Iacoviello L, Padovani A; Italian Project on Stroke in Young Adults (IPSYS) Investigators. Predictors of long-term recurrent vascular events after ischemic stroke at young age: the Italian Project on Stroke in Young Adults. *Circulation*. 2014;129:1668–1676. doi: 10.1161/CIRCULATIONAHA.113.005663.
- Johnson CJ, Kittner SJ, McCarter RJ, Sloan MA, Stern BJ, Buchholz D, Price TR. Interrater reliability of an etiologic classification of ischemic stroke. *Stroke*. 1995;26:46–51.
- Jauss M, Zanette E. Detection of right-to-left shunt with ultrasound contrast agent and transcranial Doppler sonography. *Cerebrovasc Dis*. 2000;10:490–496. doi: 10.1159/000016119.
- Job FP, Ringelstein EB, Grafen Y, Flachskampf FA, Doherty C, Stockmanns A, Hanrath P. Comparison of transcranial contrast Doppler sonography and transesophageal contrast echocardiography for the detection of patent foramen ovale in young stroke patients. *Am J Cardiol*. 1994;74:381–384.
- Serena J, Segura T, Perez-Ayuso MJ, Bassaganyas J, Molins A, Dávalos A. The need to quantify right-to-left shunt in acute ischemic stroke: a case-control study. *Stroke*. 1998;29:1322–1328.
- Orzan F, Liboni W, Bonzano A, Molinari F, Ribezzo M, Rebaudengo N, Grippi G, Negri E. Follow-up of residual shunt after patent foramen ovale closure. *Acta Neurol Scand*. 2010;122:257–261. doi: 10.1111/j.1600-0404.2009.01302.x.
- Schuchlenz HW, Weihs W, Horner S, Quehenberger F. The association between the diameter of a patent foramen ovale and the risk of embolic cerebrovascular events. *Am J Med*. 2000;109:456–462.
- European Stroke Initiative: European Stroke Initiative recommendations for stroke management. European Stroke Council, European Neurological Society and European Federation of Neurological Societies. *Cerebrovasc Dis*. 2000;10:335–351.
- Landi G. Clinical diagnosis of transient ischaemic attacks. *Lancet*. 1992;339:402–405.
- D'Agostino RB Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Stat Med*. 1998;17:2265–2281.
- Smith PL. Splines as a useful and convenient statistical tool. *Am Stat*. 1979;33:57–62.
- Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *Am J Epidemiol*. 2008;168:656–664. doi: 10.1093/aje/kwn164.
- R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria: R Development Core Team; 2015. <http://www.R-project.org/>.
- Rhone E, Chung N, Clapp B. Current evidence for closure of a patent foramen ovale for cryptogenic stroke prevention. *Int J Clin Pract*. 2014;68:551–556. doi: 10.1111/ijcp.12312.
- Carroll JD. A Prospective Randomized Trial of PFO Closure in Patients With Cryptogenic Stroke. Long-Term Results. San Francisco: TCT Conference; 2015. Abstract.
- Kent DM, Ruthazer R, Weimar C, Mas JL, Serena J, Homma S, Di Angelantonio E, Di Tullio MR, Lutz JS, Elkind MS, Griffith J, Jaigobin C, Mattle HP, Michel P, Mono ML, Nedelchev K, Papetti F, Thaler DE. An index to identify stroke-related vs incidental patent foramen ovale in cryptogenic stroke. *Neurology*. 2013;81:619–625. doi: 10.1212/WNL.0b013e3182a08d59.
- Kitsios GD, Dahabreh IJ, Abu Dabrh AM, Thaler DE, Kent DM. Patent foramen ovale closure and medical treatments for secondary stroke prevention: a systematic review of observational and randomized evidence. *Stroke*. 2012;43:422–431. doi: 10.1161/STROKEAHA.111.631648.
- Wahl A, Juni P, Mono ML, Kalesan B, Praz F, Geister L, Räber L, Nedelchev K, Mattle HP, Windecker S, Meier B. Long-term propensity score-matched comparison of percutaneous closure of patent foramen ovale with medical treatment after paradoxical embolism. *Circulation*. 2012;125:803–812. doi: 10.1161/CIRCULATIONAHA.111.030494.

Propensity Score–Based Analysis of Percutaneous Closure Versus Medical Therapy in Patients With Cryptogenic Stroke and Patent Foramen Ovale: The IPSYS Registry (Italian Project on Stroke in Young Adults)

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Supplemental Material

Alessandro Pezzini, Mario Grassi, Corrado Lodigiani, et al.

Propensity Score-Based Analysis of Percutaneous Closure versus Medical Therapy in
Patients with Cryptogenic Stroke and Patent Foramen Ovale.

The Italian Project on Stroke in Young Adults (IPSYS)

Supplemental Methods

Supplemental References

Supplemental Table

Serious adverse events

*, hematoma larger than 5 cm in diameter at the access site; *p*-value calculated using Fisher's Exact
Test

IPSYS co-investigators

Supplemental Methods

Centers are included in the network provided that the recruitment process of stroke cases takes place prospectively. Stroke was defined as a sudden loss of global or focal cerebral function that persisted for >24 hours with a probable vascular cause¹. IS due to sinus venous thrombosis, vasospasm after subarachnoid hemorrhage, cardiac surgery, occurring as an immediate consequence of trauma, and iatrogenic strokes were excluded.

Risk factor definition

The following risk factors for premature cerebral ischemia were retained: hypertension, diabetes mellitus, cigarette smoking, hypercholesterolemia, migraine, oral contraceptive use, excessive alcohol consumption, and family history of stroke. These variables were defined and dichotomized as follows: hypertension, systolic blood pressure ≥ 140 mm Hg and diastolic pressure ≥ 90 mm Hg in two separate measurements after the acute phase or use of antihypertensive drugs before recruitment; diabetes mellitus, history of diabetes, use of hypoglycemic agent or insulin, or fasting glucose ≥ 7.0 mmol/l; current smoking, including former smokers who had quit smoking for 6 months before the index event; hypercholesterolemia, cholesterol serum levels ≥ 5.7 mmol/l or use of cholesterol-lowering drugs; migraine (personal history of headache was assessed in all patients by study physicians during a face-to-face interview in both acute phase and follow-up evaluations), as migraine without aura (MO) and migraine with aura (MA) according to the diagnostic criteria of the International Headache Society (IHS)²; heavy alcohol consumption, weekly consumption > 14 drinks for males and > 7 drinks for females; oral contraceptive use, current use (including former users who had quit taking these medications for one month before the index event); and family history of stroke, stroke recorded in first-degree relatives by interviewing probands or family members. We also collected information on atrial fibrillation (medical history or electrocardiographic findings at admission).

Clinical and laboratory investigations

All patients underwent an extensive etiologic workup aimed at determining the most likely mechanism of stroke in each case. The etiologic workup included complete blood cell count, biochemical profile, urinalysis, 12-lead ECG, chest roentgenography, Doppler ultrasonography with frequency spectral analysis and B-mode echotomography of the cervical arteries, transcranial Doppler ultrasonography, and CT and/or MR angiography to investigate extracranial and intracranial vessels. Coagulation testing included prothrombin and activated partial thromboplastin times, circulating anti-phospholipid antibodies [aPL: lupus anticoagulant (LA)³, or IgG anticardiolipin antibodies (aCL)⁴, or IgG anti-β₂-glycoprotein I (anti-β₂ GPI)⁵ or any combination of these], fibrinogen, protein C, protein S, activated protein C resistance, antithrombin III, genotyping to detect factor V Leiden and the G20210A mutation in the prothrombin gene. Transthoracic and/or transesophageal echocardiography were performed to rule out major cardiac sources of emboli.

c-TCD assessment of PFO

Contrast-enhanced TCD procedure for the assessment of PFO consisted of the injection of 9 mL of saline solution and 1 mL air mixed with a three-way stopcock by exchange of saline/air mixture between the syringes and injected as a bolus as a contrast-enhancing agent into the right cubital vein 5 seconds before the start of a 10-second Valsalva maneuver, while recording the flow velocity of the middle cerebral artery, insonated through the temporal window on the right side at a depth of 50 to 60 mm, with a handheld probe. The appearance of transient spikes on the velocity spectral curve was considered positive for interatrial RLS.

Supplemental References

1. Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ.* 1980;58:113-130.
2. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders. *Cephalalgia.* 2004;24(suppl 1):24-36.
3. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost.* 1995;74:1185-90.
4. Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. *Lupus.* 2002;11:269-75.
5. Reber G, Tincani A, Sanmarco M, de Moerloose P, Boffa MC. Standardization group of the European Forum on Antiphospholipid Antibodies. Proposals for the measurement of anti-beta2-glycoprotein I antibodies. Standardization group of the European Forum on Antiphospholipid Antibodies. *J Thromb Haemost.* 2004;2:1860-1862.

Adverse event	PFO Closure (n = 206)	Medical Treatment (n = 315)	<i>P</i>-value
Atrial Fibrillation	3 (1.5)	1 (0.3)	0.306
(Local) Hematoma*	2 (1.0)	0 (0.0)	0.156
Hypotension	1 (0.5)	2 (0.6)	1.000
Convulsion	2 (1.0)	1 (0.3)	0.565
Loss of consciousness	2 (1.0)	3 (1.0)	1.000
Any serious adverse event	10 (4.9)	7 (2.2)	0.129

Supplemental Table. Serious Adverse Events

*, hematoma larger than 5 cm in diameter at the access site; *p*-value calculated using Fisher's Exact Test

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