Statin Pretreatment and Microembolic Signals in Large Artery Atherosclerosis

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Objective—Although statin pretreatment (SP) is associated with better outcomes in patients with acute cerebral ischemia after an ischemic stroke/transient ischemic attack, data on the underlying mechanism of this beneficial effect are limited.

Approach and Results—We sought to evaluate the potential association between SP and microembolic signal (MES) burden in acute cerebral ischemia because of large artery atherosclerosis (LAA). We prospectively evaluated consecutive patients with first-ever acute cerebral ischemia because of LAA in 3 tertiary stroke centers over a 2-year period. All patients underwent continuous 1-hour transcranial Doppler monitoring of the relevant vessel at baseline (≤24 hours). SP was recorded and dichotomized as high dose or low-to-moderate dose. SP was documented in 43 (41%) of 106 LAA patients (mean age, 65.4±10.3 years; 72% men; low-to-moderate dose, 32%; high dose, 8%). There was a significant (P=0.022) dose-dependent effect between SP and MES prevalence: no SP (37%), SP with low-to-moderate dose (18%), and SP with high dose (0%). Similarly, a significant (P=0.045) dose-dependent effect was documented between SP and MES burden: no SP (1.1±1.8), SP with low-to-moderate dose (0.7±1.6), and SP with high dose (0±0). In multivariable logistic regression analysis adjusting for demographics, vascular risk factors, location of LAA, stroke severity, and other prevention therapies, SP was associated with lower likelihood of MES presence (odds ratio, 0.29; 95% confidence interval, 0.09–0.92; P=0.036). In addition, SP was found also to be independently related to higher odds of functional improvement (common odds ratio, 3.33; 95% confidence interval, 1.07–10.0; P=0.037).

Conclusions—We found that SP in patients with acute LAA is related with reduced MES presence and lower MES burden with an apparently dose-dependent association.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37:1415-1422. DOI: 10.1161/ATVBAHA.117.309292.)**

Key Words: arteries ■ atherosclerosis ■ prevalence ■ risk factors ■ stroke

It has been 10 years since the publication of the SPARCL trial (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) that provided high-quality evidence on the role of high-intensity statin treatment for secondary stroke prevention.[1] As the role of statins in both primary and secondary stroke prevention has been consolidated,[2] there is growing number of acute ischemic stroke (AIS) patients who are already treated with statins during the first days of ictus. Recent studies in this subgroup of AIS patients have shown that statin pretreatment (SP) is associated with improved early outcomes after AIS.[3,4] A meta-analysis has indicated a mortality benefit and increased odds of good functional outcome 3 months after stroke onset.[5] In another systematic review, this benefit was more pronounced in patients presenting high vascular risk and those with optimized low-density lipoprotein levels.[6]

See accompanying editorial on page 1261

There has been a plethora of hypotheses trying to explain the beneficial effect of statins in AIS. These effects are distinct to the cholesterol-reducing action of statins and generally referred as pleiotropic: vasodilatatory action through endothelium nitric oxide synthase activation leading to improved vasomotor reactivity and enhanced collateral circulation, anti-inflammatory and antiexcitotoxic effects, inhibition of

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Nonstandard Abbreviations and Acronyms	
AIS	acute ischemic stroke
CI	confidence intervals
cOR	common odds ratio
FFO	favorable functional outcome
HD	high dosage
LAA	large artery atherosclerosis
MES	Microembolic signals
NIHSS	National Institute of Health Stroke Scale
PROVE-IT TIMI22	Pravastatin or Atorvastatin Evaluation and Infection Therapy
RCT	randomized controlled clinical trial
SP	statin pretreatment
SPARCL	Stroke Prevention by Aggressive Reduction in Cholesterol Levels
TCD	transcranial Doppler
TIA	transient ischemic attack

thrombogenesis, enhancement of intrinsic thrombolytic mechanisms, and improved angiogenesis.[7,8] Plaque stabilization is important in diminishing the risk of artery-to-artery embolization, the main underlying mechanism of recurrent stroke in patients with large artery atherosclerosis (LAA). As it has been shown in a small pathology study evaluating carotid plaque specimens in patients with symptomatic carotid stenosis treated with and without statins, the reduction in lipid content and inflammatory activity that constitute the 2 main components of plaque instability may promote plaque stabilization and even regression during the first weeks after acute cerebral ischemia after an AIS or transient ischemic attack (TIA) episode.[9]

Microembolic signals (MES) are solid microparticles or microbubbles represented in the transcranial Doppler (TCD) spectra as high-intensity and short-duration signals.[10–12] MES detection in AIS indicates an embolic cause for stroke, either arterioarterial or cardioembolic. MES detection is frequent in AIS patients harboring >50% carotid stenosis and ulcerated carotid plaques,[13–15] and its presence is associated with a 10-fold increase in stroke recurrence in patients with symptomatic carotid stenosis.[16] Whereas statins have been shown to decrease MES burden in patients with asymptomatic carotid stenosis,[17] their effects on symptomatic LAA stroke in the acute phase has not been studied.

Our collaborative group has recently shown in a prospective, multicenter study that SP in LAA stroke is associated with better early outcomes, including recurrent stroke and functional improvement.[18] Clinical implications of our findings are important considering the fact that LAA stroke carries the highest risk of early recurrent stroke among AIS subtypes.[19,20] In this prospective, international, multicenter study, we aim to investigate the mechanisms behind this beneficial effect by evaluating MES prevalence and burden in patients with acute cerebral ischemia because of LAA with and without SP.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement. In brief, we evaluated consecutive patients with a first-ever acute cerebral ischemic event because of LAA with continuous 1-hour TCD monitoring of the relevant intracranial artery at baseline (<24 hours from the index event) to identify both the presence and number of MES.[21] Previous statin treatments were recorded and dichotomized as high dose (HD) or low-to-moderate dose. The HD was defined as the maximum dose that has been approved by the European Medicines Agency: 80 mg per day for atorvastatin, simvastatin, fluvastatin, lovastatin, or pravastatin, and 40 mg per day for rosuvastatin.[22] Lower doses were defined as low-to-moderate dose.

Results

Baseline Characteristics

A total of 106 patients with acute cerebral ischemia because of LAA stroke (mean age, 65.4±10.3 years; 72% men; 33% TIA; median National Institute of Health Stroke Scale (NIHSS) score of 2 points; interquartile range, 0–4) fulfilled the inclusion criteria and were evaluated during the study period. Medication data were available for all patients, with no missing values. All patients reported to be taking their prescribed medications during the previous days before admission. None of the patients received intra-arterial revascularization procedures. Statin intake before stroke onset was reported in 43 (41%) of these patients.

Baseline characteristics, TCD findings, and outcomes between patients with and without SP are shown in Table 1. Patients with SP before stroke onset were found to have higher rates of antiplatelet administration ($P<0.001$) and increased triglyceride levels on admission ($P=0.049$), when compared with patients who did not receive statins before stroke onset (Table 1). Patients with SP were also found to have lower rates of current smoking on admission ($P=0.042$), milder baseline stroke severity ($P=0.008$), and lower levels of both total ($P=0.047$) and low-density lipoprotein cholesterol on admission ($P=0.05$). Finally, TIA manifestation of acute cerebral ischemia was more common in patients with SP (49% versus 22%; $P=0.004$).

MES Prevalence and Burden

TCD monitoring for 1-hour resulted in the detection of 93 MES and 6 false-positive high-intensity signals (6.1%). Patients pretreated with statins had lower prevalence of MES detection during TCD monitoring (14% versus 37%; $P=0.010$; Table 1). The median number of MES was lower ($P=0.026$ by Mann–Whitney U test) in the SP subgroup (0.6±1.5 versus 1.1±1.8) in comparison to patients without SP (Table 1; Figure 1). In the subgroup analysis by SP dose (Table 2; Figure 2), there was a significant ($P=0.022$) dose-dependent association between SP and prevalence of MES on TCD: no SP (37%), SP with low-to-moderate dose (18%), and SP with HD (0%). Similarly, a significant ($P=0.045$ by Kruskal–Wallis test adjusted for ties) dose-dependent association was documented between SP and MES burden: no SP (1.1±1.8), SP with low-to-moderate dose (0.7±1.6), and SP with HD (0±0).

Table 3 presents the results of univariable and multivariable logistic regression analyses evaluating the association of baseline characteristics with the likelihood of MES detection on TCD monitoring. In univariable analyses, the following variables were associated with MES detection: hypertension ($P=0.070$), current smoking ($P=0.009$), total cholesterol on admission ($P=0.072$), low-density lipoprotein on admission ($P=0.006$), pretreatment with

Table 1. Baseline Characteristics, Clinical Outcomes, and Transcranial Doppler Findings of Study Population Stratified by Statin Pretreatment

Variable	Statin Pretreatment		*P* Value
	No (n=63)	Yes (n=43)	
Baseline characteristics			
Age, y (mean±SD)	65.3±10.5	65.6±10.1	0.907
Male (%)	68	77	0.341
Diabetes mellitus (%)	32	47	0.124
Hypertension (%)	83	88	0.410
Current smoking (%)	40	21	0.042
Coronary artery disease (%)	38	28	0.277
Peripheral artery disease (%)	21	26	0.550
Antiplatelets (%)	30	67	<0.001
Antihypertensives (%)	60	58	0.823
NIHSS on admission (median [IQR])	2 [1–5]	1 [0–4]	0.008
Admission glucose, mg/dL (median [IQR])	114 [102–136]	108 [92–124]	0.054
Admission total cholesterol, mg/dL (median [IQR])	196.5 [165–238]	173 [152–215.5]	0.047
Admission LDL cholesterol, mg/dL (mean±SD)	123.8±38.6	108.1±39.2	0.050
Admission HDL cholesterol, mg/dL (mean±SD)	48.1±15.2	42.1±9.6	0.086
Triglycerides, mg/dL (median [IQR])	112 (89–163)	146 (107–178)	0.049
Intravenous thrombolysis (%)	10	5	0.351
Intracranial location of atherosclerosis (%)	13	16	0.627
Extracranial carotid artery disease (%)	77	74	0.723
Atherosclerosis in posterior circulation (%)	13	14	0.851
TIAs (%)	22	49	0.004
TCD findings			
Presence of MES (%)	37	14	0.010
No. of MES (median, range)	0 (0–6)	0 (0–6)	0.026*
No. of MES (mean±SD)	1.1±1.8	0.6±1.5	NA
Clinical outcomes			
Days of hospitalization (median, IQR)	8 [5–11]	5 [2–10]	0.003
NIHSS at discharge (median [IQR])	1 [0–3]	0 [0–2]	0.008
Absolute NIHSS change (median [IQR])	1 [0–2]	0 [0–2]	0.179
Relative NIHSS change (%)	26	44	0.137
mRS at 1 mo (median [IQR])‡	1 [0–2]	0 [0–1]	0.016†
FFO at 1 mo (%)‡	69	91	0.049
Death at 1 mo (%)‡	2	0	0.406
Recurrent stroke at 1 mo (%)	10	7	0.644
Carotid endarterectomy at 1 mo (%)	62	44	0.072

FFO indicates favorable functional outcome defined as mRS score of 0–1; IQR, interquartile range; LDL, low-density lipoprotein; MES, microembolic signals; mRS, modified Rankin Scale; NA, not applicable; NIHSS, National Institute of Health Stroke Scale; TCD, transcranial Doppler; and TIA, transient ischemic attack.

**P* value was calculated with the Mann–Whitney *U* test.

†*P* value was calculated with the Cochran–Mantel–Haenszel test.

‡Analyses were conducted for the subgroup of patients with acute ischemic stroke (n=71).

antihypertensives (*P*=0.038), and SP (*P*=0.013). In multivariate logistic regression analysis, only current smoking status (odds ratio, 3.09; 95% confidence interval [CI], 1.05–9.07; *P*=0.040) and SP (odds ratio, 0.29; 95% CI, 0.09–0.92; *P*=0.036) were independently associated with MES detection (Table 3).

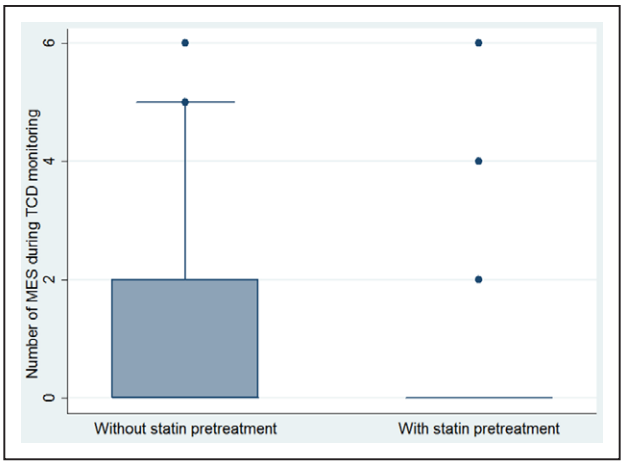

Figure 1. Box and whisker plots depicting the number of microembolic signals (MES) detected on baseline transcranial Doppler (TCD) monitoring in patients with and without statin pretreatment.

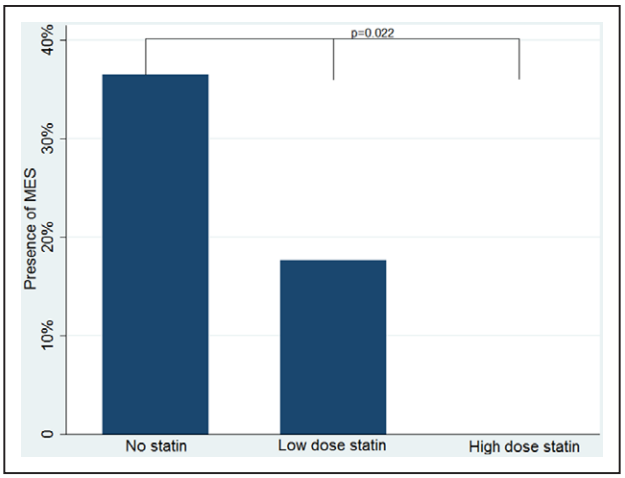

Figure 2. Prevalence of microembolic signal (MES) detection on baseline transcranial Doppler monitoring stratified by statin pretreatment dose (pretreatment with high dose, pretreatment with low-to-moderate dose, and no pretreatment).

Clinical Outcomes

LAA patients pretreated with statins had lower duration of hospitalization stay (P=0.003) and lower $NIHSS_{dis}$ score (P=0.008). The 2 groups did not differ (P>0.05) in terms of recurrent stroke, absolute, and relative NIHSS reduction during hospitalization, as well as 1-month mortality rates (Table 1). Among patients with AIS as the index event, the rate of recurrent stroke tended to be lower in the SP group (0% versus 8%; P=0.168 by Fisher exact test). Patients pretreated with statins had greater functional improvement (P=0.016 by Cochran–Mantel–Haenszel test) according to the distribution of the 30-day modified Rankin Scale scores (Table 1; Figure 3). Moreover, the rate of 1-month favorable functional outcome (FFO) was higher in SP subgroup (91% versus 69%; P=0.049; Table 1).

Table 4 summarizes the associations of baseline characteristics with 30-day modified Rankin Scale shift among patients with AIS in univariable and multivariable ordinal and regression analyses. In univariable analyses, the following variables were associated with 1-month functional improvement: antiplatelet pretreatment (P=0.037), SP (P=0.015), $NIHSS_{adm}$ (P<0.001), and peripheral arterial disease (P=0.069). In the multivariable model only $NIHSS_{adm}$ score, peripheral arterial disease, and SP were retained as independent (P<0.05) predictors of 1-month functional improvement. More specifically, $NIHSS_{adm}$ score (common odds ration [cOR] per 1 point in $NIHSS_{adm}$ score increase, 0.54; 95% CI, 0.44–0.67; P<0.001) and peripheral arterial disease (cOR, 0.25; 95% CI, 0.07–0.93; P=0.038) were independently associated with lower likelihood of functional improvement at 1 month after the index event, whereas SP was related to higher odds of functional improvement (cOR, 3.33; 95% CI, 1.07–10.0; P=0.037).

Table I in the online-only Data Supplement depicts the associations of baseline characteristics of AIS patients with 30-day FFO in univariable and multivariable logistic regression analyses. In univariable analyses, the following variables were associated with 1-month FFO: SP (P=0.065), $NIHSS_{adm}$ (P<0.001), and peripheral arterial disease (P=0.015). In the multivariable model, only $NIHSS_{adm}$ score (cOR per 1 point in $NIHSS_{adm}$ score increase, 0.56; 95% CI, 0.40–0.77; P<0.001) and peripheral arterial (cOR, 0.14; 95% CI, 0.02–0.80; P=0.027) were independently associated with lower likelihood of 1-month FFO. The initial association between SP and 1-month FFO did not retain its statistical significance (cOR, 3.15; 95% CI, 0.53–18.70; P=0.206).

Table 2. Transcranial Doppler Findings in Subgroups of Patients Stratified by Statin Pretreatment Dose

	No Statin Pretreatment (n=63)	Low/Medium Statin Dose (n=34)	High Statin Dose (n=9)	*P* Value
Presence of MES (%)	37	18	0	0.022
No. of MES (median, range)	0 (0–6)	0 (0–4)	0	0.045*
No. of MES (mean±SD)	1.1±1.8	0.7±1.6	0±0	NA

The high dosage was defined as the maximum dose that has been approved by the European Medicines Agency: 80 mg per day for atorvastatin, simvastatin, fluvastatin, lovastatin, or pravastatin, and 40 mg per day for rosuvastatin.[12] Lower doses were defined as low-to-moderate dose. MES indicates microembolic signals; NA, not applicable.

*P value was calculated with the Kruskal–Wallis test, adjusted for ties.

Discussion

Our prospective, multicenter study showed that patients with acute cerebral ischemia pretreated with statins had lower prevalence of MES on TCD monitoring. Moreover, the relationship between MES burden and SP was dose dependent, whereas AIS patients pretreated with statins had greater neurological improvement in 30 days after symptom onset.

MES detection through TCD is a unique modality to assess microemboli in real time, providing diagnostic clues to the cause of stroke.[10–12] The presence of MES has been correlated with brain embolism, and particularly in the case of patients with LAA artery-to-artery embolism.[23] Moreover, it has been reported that among AIS/TIA patients and presence of MES at admission, MES disappearance on follow-up TCD

Table 3. Univariable and Multivariable Logistic Regression Analyses Depicting the Association of Baseline Characteristics With Detection of Microembolic Signals on Transcranial Doppler Examination

	Univariable Analysis		Multivariable Analysis	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Age, y (mean±SD)	1.04 (0.99–1.08)	0.125	…	…
Males (%)	1.73 (0.63–4.81)	0.289	…	…
Diabetes mellitus (%)	1.01 (0.42–2.44)	0.980	…	…
Hypertension (%)	6.77 (0.85–53.8)	0.070	7.76 (0.75–80.40)	0.085
Current smoking (%)	3.27 (1.34–7.99)	0.009	3.09 (1.05–9.07)	0.040
Coronary artery disease (%)	1.27 (0.52–3.09)	0.597	…	…
Peripheral artery disease (%)	1.12 (0.41–3.07)	0.821	…	…
Antiplatelets (%)	0.97 (0.41–2.30)	0.954	…	…
Antihypertensives (%)	2.76 (1.05–7.21)	0.038	1.33 (0.41–4.30)	0.631
Statin pretreatment (%)	0.28 (0.10–0.77)	0.013	0.29 (0.09–0.92)	0.036
NIHSS on admission (median [IQR])	0.90 (0.78–1.05)	0.174	…	…
Admission glucose, mg/dL (median [IQR])	0.99 (0.98–1.01)	0.542	…	…
Admission total cholesterol, mg/dL (median [IQR])	1.01 (0.99–1.02)	0.072	0.99 (0.96–1.01)	0.276
Admission LDL cholesterol, mg/dL (mean±SD)	1.02 (1.01–1.03)	0.006	1.03 (0.99–1.06)	0.063
Admission HDL cholesterol, mg/dL (mean±SD)	0.97 (0.92–1.02)	0.218	…	…
Triglycerides, mg/dL (median [IQR])	1.00 (0.99–1.01)	0.558	…	…
Intravenous thrombolysis (%)	0.88 (0.17–4.61)	0.876	…	…
Intracranial location of atherosclerosis (%)	0.65 (0.17–2.50)	0.531	…	…
Extracranial carotid artery disease (%)	1.61 (0.54–4.81)	0.391	…	…
Atherosclerosis in posterior circulation (%)	0.40 (0.84–1.91)	0.252	…	…
TIAs (%)	1.66 (0.68–4.02)	0.264	…	…

CI indicates confidence interval; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NIHSS, National Institute of Health Stroke Scale; OR, odds ratio; and TIA, transient ischemic attack.

7 days latter is associated with greater neurological improvement.[24] Consequently, our findings lend support to the mounting data highlighting plaque stabilization leading to reduced distal embolization as an essential underlying mechanism for the beneficial effect of statins during the first days of acute cerebral ischemia. The anti-inflammatory effect exerted by statins on atherosclerotic plaques may account for plaque stabilization and decreased artery-to-artery embolization in patients with symptomatic LAA.[25,26]

We documented that AIS patients pretreated with statins experienced greater functional improvement at 1 month, whereas SP was associated with a nonsignificant absolute reduction of 8% in the risk of recurrent stroke in our cohort. This lack of statistical significance could be attributed to the small sample size (n=106 patients) and the limited number of outcome events (n=9). Nevertheless, it should be noted that the absolute risk reduction in this study was similar to previous observations from our multicenter group. We have previously evaluated the potential beneficial effect of SP in a larger (n=516) cohort of AIS patients with LAA and documented a statistically significant (*P*=0.002) absolute risk reduction of 7% in recurrent stroke.[18] These findings also parallel the observations of a recent multicenter study reporting a 9% absolute risk reduction in the 7-day stroke risk among TIA patients with symptomatic carotid artery stenosis who were pretreated with statins.[27]

This reduction in recurrent stroke risk may have in part accounted for the greater improvement in early functional outcomes in the subgroup of patients pretreated with statins in our cohort. An alternative explanation may be related to the potential palliative effect of chronic statin use on cerebral hemodynamics and cerebral autoregulation in AIS patients leading to smaller cerebral infarctions.[8] Interestingly, SP was associated with reduced infarct volume in a cohort of AIS patients with LAA who were treated with intra-arterial reperfusion therapies.[28] Moreover, another magnetic resonance imaging study has demonstrated that impaired vasomotor reactivity in patients with large middle cerebral artery infarctions is correlated to increased prevalence of peri-infarct T2 hyperintensities.[29] Thus, restoration of cerebral autoregulation can potentially limit the effect of ischemic brain damage, and statins may have a role in ameliorating cerebral hemodynamics.

There are limited randomized data on the safety and efficacy of HD statins in the setting of an AIS/TIA.[30,31] These pilot studies provide inconclusive data because of limited sample size,[30,31] incomplete enrollment,[31] or inclusion of AIS with different stroke subtypes (small-vessel disease, cryptogenic

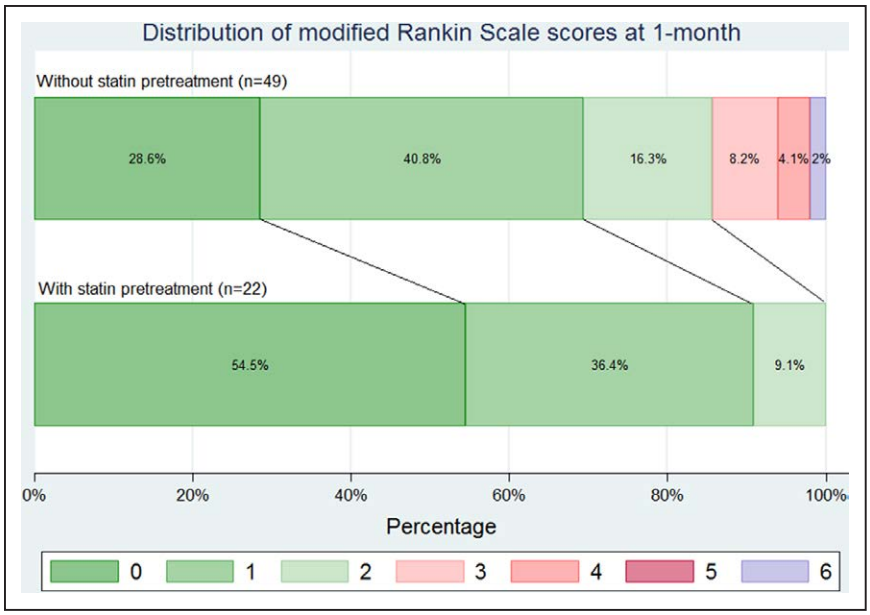

Figure 3. Horizontal (Grotta) bars depicting the distribution of modified Rankin Scale scores at 1 mo in patients with and without statin pretreatment.

stroke, and others).[30,31] In view of the consolidated safety profiles of statins and given the accumulating observational data underscoring a beneficial role of statins especially in patients with LAA,[5,18,32] an adequately powered phase III randomized controlled clinical trial (RCT) seems necessary to provide definitive evidence on statin efficacy in AIS patients with LAA.[33] The design of such a trial should parallel the protocol of PROVE-IT TIMI22 trial (Pravastatin or Atorvastatin

Table 4. Univariable and Multivariable Ordinal Regression Analyses Depicting the Association of Baseline Characteristics With the Improvement in Modified Rankin Scale Scores at 1 Month in Patients With Acute Ischemic Stroke (n=71)

	Univariable Analysis		Multivariable Analysis	
	cOR (95% CI)	*P* Value	cOR (95% CI)	*P* Value
Age, y (mean±SD)	1.00 (0.95–1.05)	0.968	…	…
Males (%)	0.92 (0.36–2.38)	0.861	…	…
Diabetes mellitus (%)	0.98 (0.4–2.38)	0.959	…	…
Hypertension (%)	0.53 (0.13–2.17)	0.379	…	…
Current smoking (%)	1.31 (0.53–3.33)	0.556	…	…
Coronary artery disease (%)	0.91 (0.38–2.17)	0.835	…	…
Peripheral artery disease (%)	0.35 (0.11–1.09)	0.069	0.25 (0.07–0.93)	0.038
Antiplatelets (%)	0.36 (0.14–0.94)	0.037	2.63 (0.84–8.33)	0.097
Antihypertensives (%)	0.65 (0.27–1.56)	0.340	…	…
Statin pretreatment	3.33 (1.27–9.09)	0.015	3.33 (1.07–10.00)	0.037
NIHSS on admission (median [IQR])	0.55 (0.45–0.67)	<0.001	0.54 (0.44–0.67)	<0.001
Admission glucose, mg/dL (median [IQR])	1.00 (0.99–1.02)	0.835	…	…
Admission total cholesterol, mg/dL (median [IQR])	1.00 (0.99–1.01)	0.524	…	…
Admission LDL cholesterol, mg/dL (mean±SD)	1.00 (0.99–1.01)	0.492	…	…
Admission HDL cholesterol, mg/dL (mean±SD)	1.03 (0.98–1.10)	0.241	…	…
Triglycerides, mg/dL (mean±SD)	1.01 (0.99–1.02)	0.491	…	…
Intravenous thrombolysis (%)	0.75 (0.20–2.78)	0.660	…	…
Intracranial location of atherosclerosis (%)	1.67 (0.49–5.55)	0.416	…	…
Extracranial carotid artery disease (%)	0.97 (0.37–2.56)	0.949	…	…
Atherosclerosis in posterior circulation (%)	0.75 (0.23–2.44)	0.636	…	…

CI indicates confidence interval; cOR, common odds ratio; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; and NIHSS, National Institute of Health Stroke Scale.

Evaluation and Infection Therapy) that showed an association between intensive statin treatment and reduction of early recurrent vascular events, including stroke in patients with acute coronary syndromes.[34] The findings of a very recent RCT of early versus late statin initiation in AIS patients also suggest a potential beneficial (although nonsignificant) effect of early statin initiation on adverse functional outcomes of AIS patients with LAA (cOR, 0.63; 95% CI, 0.31–1.27).[35] Given the low dose of statins that were administered in this RCT (atorvastatin 20 mg/d, pitavastatin 4 mg/d, or rosuvastatin 5 mg/d), it may be postulated that a more pronounced benefit could be detected in future RCTs investigating the efficacy of HD statin in AIS patients because of LAA.

Certain limitations of this study need to be acknowledged. First, there was no core laboratory analysis of TCD recordings for MES detection and no central adjudication for outcome events. However, considering that operators were blinded to the SP status of each patient, it is unlikely that this may have led to significant bias. Second, neither specific plaque characteristics (echogenicity, fibrous cap thickness, and ulceration), nor final infarct volume on brain magnetic resonance imaging was documented and could not be included in the analyses. Third, inflammatory biomarkers, including C-reactive protein, were not measured, and the potential association between SP and reduced levels of inflammatory biomarkers was not investigated. Moreover, it should be acknowledged that plasma total homocysteine was not recorded in our study population. Plasma total homocysteine levels have been shown to be higher in patients with MES, suggesting that homocysteine may have a role in plaque instability aggravation and thrombi activation.[36] Consequently, we were unable to account for this potential confounder in our multivariable analyses. Fourth, handheld TCD monitoring of vertebrobasilar arteries is operator dependent and requires extensive experience from the neurosonologist during the 1-hour examination. On the other hand, certain strengths of the present report should be noted, including the strict inclusion and exclusion criteria, the prospective design, the ultrasound evaluation of all patients during the first 24 hours of ictus, the comprehensive neurosonology protocol, and the statistical design adjusting for multiple potential confounders in the multivariable logistic regression models.

To the best of our knowledge, this is the first study reporting an independent association between SP and reduced microembolism in real time among patients with symptomatic LAA. This association seems to be dose dependent and may account for the greater functional improvement at 1 month among AIS patients pretreated with statins. Statin-based interventions in the acute setting of cerebral ischemia need to be tested in future well designed and adequately powered RCTs.

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Disclosures

None.

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Highlights

- We sought to evaluate the association between statin pretreatment and microembolic signal burden in acute cerebral ischemia because of large artery atherosclerosis.
- Acute cerebral ischemia patients with statin pretreatment had lower prevalence microembolic signal on transcranial Doppler monitoring.
- The relationship between microembolic signal burden and statin pretreatment was dose dependent.
- Acute ischemic stroke with statin pretreatment had greater neurological improvement in 30 days after stroke onset.

Arteriosclerosis, Thrombosis, and Vascular Biology

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Statin Pretreatment and Microembolic Signals in Large Artery Atherosclerosis

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Methods and Material

Study Population

We prospectively evaluated consecutive patients with a first-ever after an acute cerebral ischemic event due to LAA presented to three tertiary stroke care centers ("Attikon" University Hospital, Athens, Greece; National University Hospital of Singapore, Singapore; St. Josef-Hospital, Ruhr University, Bochum, Germany) during a two-year study period (January 2014-December 2015).

LAA stroke was diagnosed according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria.[1] Vascular neuroimaging with magnetic resonance angiography (MRA) or computed tomography angiography (CTA) of cervical and cerebral vessels was performed during hospitalization as part of standard diagnostic work-up in all cases, and patients with symptomatic vessel stenosis ≥50% were included.[2-4] All patients underwent complete evaluation for the identification of other sources of embolism with transthoracic echocardiography and ≥24 hours Holter monitoring, as standard of care. Additional details regarding the identification of embolic sources of stroke in our multicenter collaborative group have been previously described.[5] Transesophageal echocardiography was performed in selected patients if indicated by the findings of the initial investigation with transthoracic echocardiography or baseline CT/MRI findings (e.g. the presence of multiple infarcts in different vascular territories).[5] All patients with alternative (aortic or cardiac sources of embolism, paradoxical embolism) of embolism identified during the diagnostic work-up were excluded from further evaluation.

All included patients underwent continuous 1-hour TCD monitoring of the relevant intracranial artery at baseline (<24 hours from the index event) to identify both the presence and number of MES, according to the International Consensus

Group on Microembolus Detection.[6] All TCD examinations in each participating institution were performed by vascular neurologists who were experts in MES detection and accredited in neurosonology. Neurosonologists were blinded to both patients' clinical and imaging data, while were instructed not to ask for information on patients' symptoms and medications or discuss while performing TCD monitoring. More specifically, the ipsilateral proximal middle cerebral artery was monitored using headframe to secure a stable angle of insonation in patients with extracranial carotid artery stenosis as previously described.[7] The ipsilateral intracranial vertebral artery was monitored using hand-held probe fixation in patients with extracranial vertebral artery stenosis.[8,9] When symptomatic intracranial arterial stenosis was identified, the symptomatic vessel was monitored distal to the site of arterial stenosis with headframe fixation for all intracranial vessels, with the exception of vertebrobasilar system that was monitored using hand-held monitoring.[8-10] For the discrimination of true embolic signals from artifacts, apart from ensuring the secure probe placement using a headframe, we used an intensity detection threshold of ≥12 dB and real-time machine calibration by intrapatient analysis of the background signal as proposed by the International Consensus Criteria for Microembolus detection on TCD.[6] Signals with bidirectional identification, above and below the baseline, were considered as potential artifacts and thus excluded from the analysis.[6]

Patients with history of previous stroke, absent temporal windows, co-existing atrial fibrillation/other sources of embolism or who were treated with anticoagulation therapy prior to TCD-monitoring were excluded. The following parameters were prospectively recorded for all patients as previously described in detail by our international collaborative group: 1. Demographic characteristics (age, sex), 2. Vascular risk factors (diabetes mellitus, hypertension, current smoking, coronary

artery disease, atrial fibrillation, peripheral artery disease), 3. Prior medications (statin dose and type, antihypertensives, antiplatelets), 4. Laboratory test values on admission [serum glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides], 5. NIHSS-score (National Institute of Health Stroke Scale) on admission ($NIHSS_{adm}$) and at hospital discharge ($NIHSS_{dis}$), 6. Intravenous thrombolysis (IVT) administered during the first 4.5 hours of symptom onset according to American Heart Association (AHA) recommendations.[11]

Prior statin treatments were recorded and dichotomized as high or low to moderate dose. The high dosage (HD) was defined as the maximum dose that has been approved by the European Medicines Agency: 80 mg per day for atorvastatin, simvastatin, fluvastatin, lovastatin, or pravastatin, and 40 mg per day for rosuvastatin.[12] Lower doses were defined as low to moderate dose (LMD). Patients with missing information on SP were excluded from further evaluation.

In-hospital management

All patients were treated during hospitalization according to current AHA recommendations[11] for management of acute cerebral ischemia in all participating centers. SP patients pursued their treatment to the type and statin dose during hospitalization in all participating centers. Statins were initiated at discharge in the indicated patients without history of SP according to AHA recommendations for secondary stroke prevention.[13] Carotid endarterectomy was performed according to AHA recommendations[14] in all LAA patients with symptomatic extracranial carotid artery stenosis (≥50%) and no or minor residual disability (grade 0-2 on modified Rankin Scale score) within two weeks from symptom onset, as previously described.[15] All remaining LAA patients with extra- or intracranial atherosclerotic

disease were managed conservatively in accordance to AHA[13] recommendations for secondary stroke prevention.

Follow up

We prospectively followed all patients and evaluated their clinical status at 30 days after symptom onset, as previously described.[2-4] We captured the following outcome events during the first 30 days after the index event: 1. neurological improvement during hospitalization, 2. death, 3. recurrent stroke and 4. favorable functional outcome (FFO). Neurological improvement was quantified as both the relative decrease in NIHSS score at hospital discharge in comparison to hospital admission [$(NIHSS_{adm}-NIHSS_{dis})/NIHSS_{adm}$ x100%] for patients with $NIHSS_{adm}>0$, and also as the absolute decrease in NIHSS score at hospital discharge in comparison to hospital admission ($NIHSS_{adm}-NIHSS_{dis}$) for all patients.[16] Recurrent strokes were diagnosed as cerebrovascular events occurring suddenly, lasting >24 hours, and resulting in increased pre-existing neurological deficits or causing new neurological symptoms and signs.[3,4] For the diagnosis of recurrent stroke, along with the clinical findings, the presence of a new lesion on follow-up brain imaging that involved an anatomic site or vascular territory that was unaffected on the admission CT-scan was a prerequisite.[3,4] The modified Rankin Scale (mRS) score at 1 month was estimated for all patients. FFO at 30 days was defined as mRS-score of 0 or 1.[17] All outcome events were assessed by attending-level stroke neurologists at the individual participating centers who were unaware of information regarding SP and MES detection on TCD.

Standard protocol approvals, registrations, and patient consent.

The study protocol was approved by the corresponding ethics committees and informed consent was obtained from all patients (or guardians of patients, when consent could not be obtained directly from the patients) participating in the study.

Sample size calculations

We estimated that a total sample size of 106 patients (40% pretreated with statins and 60% without SP), should undergo 1-hour TCD-monitoring to detect an absolute difference of 30% on MES presence between the two subgroups (with and without SP) with an alpha of 0.05 and 80% power. This assumption was based on the findings of our previous study documenting the prevalence of SP in AIS patients due to LAA (37%, ratio of patients with SP to patients without SP=0.59),[16] a 10% presumed total rate of false positive TCD signals and previously reported MES prevalence in LAA patients randomized to single antiplatelet therapy in a phase IIB randomized controlled clinical trial (Clopidogrel and Aspirin for Reduction of Emboli in Symptomatic Carotid Stenosis).[18]

Statistical analysis

We presented continuous parametric data using their mean values together with their corresponding standard deviations (SDs). We used median values with their corresponding interquartile ranges (IQR) for the presentation of non-parametric data and percentages for all dichotomous variables. Statistical comparisons between different subgroups were performed using the Pearson's χ^2 test, unpaired t-test, Mann–Whitney U test and Kruskal-Wallis test adjusted for ties, where appropriate. The distribution on the mRS-score at 1 month among AIS patients was compared between SP subgroups using both the Cochran Mantel-Haenszel test and univariable/

multivariable ordinal logistic regression (shift analysis).[19] We performed additional subgroup analysis, according to statin dosage prior to the event onset.

We also used univariable and multivariable logistic regression analyses to evaluate the associations between baseline characteristics (including MES detection) and FFO as well as mRS distribution at 1 month following the index event among patients with AIS. In all univariable analyses, a threshold of $p<0.1$ was used to identify candidate variables for inclusion in the multivariable regression models that tested statistical significance hypothesis using the likelihood ratio test with an alpha value of 0.05. We reported all associations as odds ratios (ORs) in logistic regression models and common ORs (cORs) in ordinal regression models, respectively, with their corresponding 95% confidence intervals (95%CI).

The Stata Statistical Software Release 13 for Windows (College Station, TX, StataCorp LP) was used for all statistical analyses.

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SUPPLEMENTAL MATERIAL

Supplemental Tables

Supplemental Table I. Univariable and multivariable logistic regression analysis depicting the association of baseline characteristics with favorable functional outcome at 1-month (mRS-score of 0-1) in patients with acute ischemic stroke (n=71).

	Univariable analysis		Multivariable analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age (years, mean±SD)	0.98 (0.92, 1.04)	0.519	-	-
Males (%)	1.29 (0.41, 4.10)	0.660	-	-
Diabetes mellitus (%)	0.77 (0.25, 2.37)	0.655	-	-
Hypertension (%)	2.06 (0.52, 8.16)	0.300	-	-
Current smoking (%)	1.76 (0.50, 6.17)	0.374	-	-
Coronary artery disease (%)	2.07 (0.59, 7.20)	0.253	-	-
Peripheral artery disease (%)	0.21 (0.06, 0.74)	0.015	0.14 (0.02, 0.80)	0.027
Antiplatelets (%)	1.41 (0.43, 4.60)	0.567	-	-
Antihypertensives (%)	1.11 (0.37, 3.31)	0.850	-	-
Statin pretreatment	4.41 (0.91, 21.30)	0.065	3.15 (0.53, 18.70)	0.206
NIHSS on admission (median [IQR])	0.58 (0.44, 0.76)	<0.001	0.56 (0.40, 0.77)	<0.001
Admission glucose (mg/dl, median [IQR])	0.99 (0.98, 1.01)	0.483	-	-
Admission total cholesterol (mg/dl, median [IQR])	1.00 (0.99, 1.01)	0.776	-	-
Admission LDL cholesterol (mg/dl, mean±SD)	1.00 (0.98, 1.01)	0.938	-	-
Admission HDL cholesterol (mg/dl, mean±SD)	1.05 (0.96, 1.14)	0.294	-	-
Triglycerides (mg/dl, mean±SD)	1.01 (0.99, 1.02)	0.304	-	-
Intravenous thrombolysis (%)	2.00 (0.22, 17.9)	0.535	-	-
Intracranial location of atherosclerosis (%)	1.74 (0.34, 8.88)	0.670	-	-
Extracranial carotid artery disease (%)	1.38 (0.43, 4.41)	0.585	-	-
Atherosclerosis in posterior circulation (%)	0.65 (0.17, 2.46)	0.525	-	-

OR: odds ratio; 95%CI: 95% confidence interval; NIHSS: National Institute of Health Stroke Scale; SD: standard deviation; IQR: interquartile range; mRS: modified Rankin Scale