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Sonolysis during carotid endarterectomy: randomised controlled trial

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ABSTRACT

OBJECTIVE

To evaluate the effectiveness and safety of sonolysis using a low intensity 2 MHz pulsed wave ultrasound beam during carotid endarterectomy.

DESIGN

Multicentre, phase 3, double blind, randomised controlled trial.

SETTING

16 European centres.

PARTICIPANTS

1004 patients (mean age 68 years; 312 (31%) female) were enrolled in the study between 20 August 2015 and 14 October 2020 until the interim analysis was performed.

INTERVENTIONS

Sonolysis (n=507) versus sham procedure (n=497).

MAIN OUTCOME MEASURES

The primary endpoint was the composite incidence of ischaemic stroke, transient ischaemic attack, and death within 30 days. The incidence of new ischaemic lesions on follow-up brain magnetic resonance imaging was the main substudy endpoint, and incidence of intracranial bleeding was the main safety endpoint.

RESULTS

The results favoured the sonolysis group for the primary endpoint (11 (2.2%) v 38 (7.6%); risk difference −5.5%, 95% confidence interval (CI) −8.3% to −2.8%; P<0.001), as well as in the substudy for magnetic resonance imaging detected new ischaemic lesions (20/236 (8.5%) v 39/224 (17.4%); risk difference −8.9%, −15% to −2.8%; P=0.004). Sensitivity analysis resulted in a risk ratio for sonolysis of 0.25 (95% CI 0.11 to 0.56) for ischaemic stroke and 0.23 (0.07 to 0.73) for transient ischaemic attack within 30 days. Sonolysis was found to be safe, and 94.4% of patients in the sonolysis group were free from serious adverse events 30 days after the procedure.

CONCLUSION

Sonolysis was safe for patients undergoing carotid endarterectomy and resulted in a significant reduction in the composite incidence of ischaemic stroke, transient ischaemic attack, and death within 30 days.

TRIAL REGISTRATION

Clinicaltrials.gov NCT02398734.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Stroke and silent brain infarctions are important complications associated with carotid endarterectomy

Prolonged low power, pulsed wave ultrasound insonation using a 2 MHz probe (sonolysis) positively affects fibrinolytic system activation

Pilot studies have shown a potential positive effect of sonolysis on reduction in the risk of stroke or brain infarction during various interventions including carotid endarterectomy

WHAT THIS STUDY ADDS

Periprocedural sonolysis during carotid endarterectomy significantly reduced the risk of ischemic stroke, transient ischaemic attack, and new brain infarction detected using brain magnetic resonance imaging

No increase in the risk of any adverse events was seen

A multivariable model identified only female sex as an independent factor increasing the risk of the primary composite endpoint (ischemic stroke, transient ischaemic attack, and death)

Introduction

The possibilities for using therapeutic ultrasound are constantly expanding.[1-3] Enhancement of the lysis of thrombus is one of the investigated effects of ultrasound. Since the 1970s, in vitro and animal model studies have shown acceleration of thrombus dissolution by use of an ultrasound beam.[4-7] Many animal model studies have shown acceleration of spontaneous or pharmacologically induced thrombolysis with an ultrasound beam (sonothrombolysis), with frequencies ranging between 20 kHz and 2 MHz and various intensities; frequencies of 1 MHz and higher are also used in clinical diagnostics.[8-13] This is a unique condition in which a 2 MHz pulsed wave ultrasound beam can be used for both diagnostic and therapeutic purposes.

Experimental studies have shown the potential mechanical effect of low power, pulsed wave ultrasound on disruption of thrombus structure, as well as its possible effect on activation of the fibrinolytic system through use of 2 MHz ultrasound probes, transient peripheral vasodilatation, elimination of air microemboli, and improvement of microvascular patency.[14 15] Prolonged ultrasound insonation of the middle cerebral artery by using a 2 MHz ultrasound probe caused a significant decrease of plasminogen activator inhibitor-1, plasminogen, and α-2-antiplasmin activity in both healthy volunteers and patients with acute stroke.[14 15] This combined effect of continuous ultrasound application (sonolysis) was tested in patients with a high risk of stroke during interventions.[16-18] Pilot studies have shown a potential positive effect of sonolysis on reduction of the risk of stroke or brain infarction during interventions, such as carotid endarterectomy, carotid stenting, coronary artery stenting, or cardiac surgery.[16-18]

Carotid endarterectomy is an intervention that is frequently used worldwide in patients with severe carotid artery stenosis. However, stroke and silent brain infarction are important complications associated with carotid endarterectomy[19]; thromboembolism is responsible for most of these events. Minimising perioperative vascular complications after carotid endarterectomy is essential for its safe use. Therefore, we hypothesised that activation of the fibrinolytic system and a direct mechanical effect on flowing thrombi in intracranial arteries by use of sonolysis during carotid endarterectomy could reduce the risk of cerebrovascular events, such as ischaemic stroke, transient ischaemic attack, and silent brain infarction. We did a randomised controlled clinical trial to evaluate the effectiveness and safety of sonolysis during carotid endarterectomy.

Methods

Trial design

The Sonolysis in Prevention of Brain Infarctions During Internal Carotid Endarterectomy (SONOBIRDIE) trial is a randomised, double blind, sham controlled study designed to demonstrate the safety and effectiveness of sonolysis. It involved continuous transcranial Doppler monitoring using a 2 MHz probe with maximal adjustable power for reducing the risk of ischaemic stroke, transient ischaemic attack, and silent brain infarctions detected using brain magnetic resonance imaging by activating the endogenous fibrinolytic system during carotid endarterectomy in patients with ≥70% symptomatic or asymptomatic internal carotid artery stenosis.[20] Lists of the committee members, participating centres, and principal investigators are provided in supplementary table A in web appendix 1.

The chair of the steering committee (DS) designed the protocol in collaboration with all steering committee members. The full trial protocol and statistical analysis plan are available in web appendix 2 and web appendix 3, respectively. Members of the steering committee and the Data and Safety Monitoring Board had unrestricted access to the data (after data unblinding). They also approved the manuscript, made the decision to submit it for publication, and vouched for the accuracy and completeness of the data and for the reliability of the trial protocol. Data analyses were done by statisticians from the University of Bern, Switzerland.

Enrolment, randomisation, and follow-up

Eligible patients had a confirmed diagnosis of internal carotid artery stenosis of ≥70% made using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria as detected by duplex sonography and confirmed by computed tomography, magnetic resonance imaging, or digital subtraction angiography. A list of the inclusion and exclusion criteria is provided in appendix 1. Carotid endarterectomy was indicated in all patients and was performed under standard conditions described in appendix 1. Recommended best medical treatment is described in appendix 1.

A certified stroke neurologist who was blinded to study conditions conducted standard physical and neurological examinations before and 24 hours after carotid endarterectomy, as well as 30 days and one year after randomisations. Neurological status was evaluated using the National Institutes of Health Stroke Scale and modified Rankin scale.

Brain magnetic resonance imaging was done in patients enrolled in the substudy (supplementary table B2), in which the protocol consisted of four sequences: transverse T2 weighted spin echo; diffusion weighted imaging; T2* weighted gradient recalled echo sequence for detection of bleeding (including microbleeds); and fluid attenuated inversion recovery. See appendix 1 for more details.

Consecutive patients were assigned to the sonolysis group or the control group by computer generated one-to-one randomisation using an independent online randomisation system. A full description of the trial inclusion and exclusion criteria and further details of eligibility, enrolment, randomisation, and follow-up are provided in appendix 1. A trial flowchart is available in supplementary figure A.

Investigational device

Sonolysis in the SONOBIRDIE trial was performed using a standard ultrasound machine and 2 MHz transcranial Doppler probe. The criteria for selecting ultrasound devices for the study and a list of the ultrasound machines used for sonolysis are provided in supplementary table B.

Sonolysis (sonothrombolysis)

In patients randomised into the sonolysis group, the main stem of the middle cerebral artery ipsilateral to the intervened artery at a depth of 55 mm was continuously monitored during the intervention by using a 2 MHz transcranial Doppler probe with maximal power allowable for acoustic output intensity of a diagnostic ultrasound device (thermal index for cranial bone 1.2-2.0; mechanical index 1.2-1.9; spatial peak pulse average intensity 120-190 mW/cm^2) and a sample volume of 10 mm. The probe was fixed in the required position by using a headband (supplementary figure B). Sonolysis was started before the first skin incision and stopped after the last suturing of the skin at the end of the intervention, but no later than after 120 minutes. The sound of the device was turned off and the display of Doppler waves was available only to the sonographer. Only the sonographer was not blinded to the application of sonolysis or sham procedure. Information on blood flow parameters in the middle cerebral artery was communicated to the operator team on request only during carotid clamping and declamping. The sonographer was not authorised to exchange information about the detected microembolic signals with anybody on the surgery team during the intervention.

Sham procedure

In patients randomised to the control group, the transcranial Doppler probe was fixed in the required position by using a special headband as in the sonolysis group patients, but only the middle cerebral artery segment ipsilateral to the intervened artery was localised at a depth of 55 mm using a 2 MHz transcranial Doppler probe with maximal power allowable for a diagnostic ultrasound device. Transcranial Doppler monitoring was stopped afterwards and restarted only when the operator needed information on flow parameters in the middle cerebral artery during clamping and declamping of the carotid artery for the minimum necessary time not exceeding two minutes. No information about microembolic signals was accessible to anyone on the surgery team during the intervention.

Trial endpoints

The primary endpoint was a composite incidence of ischaemic stroke, transient ischaemic attack, and death over the course of 30 days after randomisation. Secondary efficacy endpoints included occurrence of any stroke within 30 days (including ischaemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage, and unspecified stroke), myocardial infarction within 30 days, death within 30 days, and death within one year. All endpoints were evaluated by a certified stroke neurologist.

Endpoints of the magnetic resonance imaging substudy were appearance of at least one new ischaemic lesion on a post-procedural brain scan, number of new ischaemic lesions on a post-procedural brain scan, appearance of at least one new ischaemic lesion ≥0.5 mL in volume on post-procedural diffusion weighted brain imaging, and appearance of at least one new ipsilateral ischaemic lesion on post-procedural diffusion weighted brain imaging. All endpoints of the magnetic resonance imaging substudy were assessed 24 hours (within four hours either side) after carotid endarterectomy.

Safety endpoints included adverse events, serious adverse events, and incidence of haemorrhagic stroke, including subarachnoid haemorrhage, within 30 days after carotid endarterectomy and incidence of intracranial bleeding (including brain microbleeds) on a control T2* weighted gradient recalled echo sequence in the magnetic resonance imaging substudy.

All magnetic resonance imaging data were assessed at an independent core laboratory by radiologists blinded to the study conditions. Adverse events and serious adverse events were evaluated by an independent Clinical Adverse Events Committee, and clinical endpoints were reviewed by an independent Data and Safety Monitoring Board (supplementary table A).

Statistical analysis

We determined the sample size by using estimates of the treatment effect from previous studies.[16-18] We assumed the risk of the composite of ischaemic stroke, transient ischaemic attack, and death during the 30 day postoperative period in the control group to be 4%. A clinically relevant absolute risk reduction would be 2.5%, leading to a risk of 1.5% in the sonolysis group. A χ^2 test indicated that 1342 patients (671 per group) were needed to detect such a difference with a two sided α level of 0.05 and a power of 80%. To account for a loss to follow-up of 10%, we planned to recruit 1492 patients (746 per group) to the study.

For the substudy, we assumed the risk of new ischaemic lesions on diffusion weighted magnetic resonance imaging in the control group to be 25%. A clinically relevant absolute risk reduction would be 15%, leading to a risk of 10% in the sonolysis group. A χ^2 test indicated that 200 patients (100 per group) were needed to detect such a difference with a two sided α level of 0.05 and a power of 80%. To account for a loss to follow-up of 10%, we planned to recruit at least 224 patients (112 per group) to the study. However, we recruited patients for the substudy continuously as long as the main study was ongoing, even after the targeted sample size was reached. A total of 296 patients (148 per group) resulted in a power of 90%.

Efficacy analyses were done primarily for the intention-to-treat population. We also did secondary analysis for the per protocol population. The intention-to-treat population consisted of all randomised participants who signed the informed consent form. The per protocol population consisted of all participants in the intention-to-treat population who received the allocated treatment and did not have any major protocol deviations (supplementary tables C and D). Efficacy analyses for the magnetic resonance imaging substudy were done for the intention-to-treat population—participants with an indication for carotid endarterectomy, who were randomised and had the procedure, and who had magnetic resonance imaging before and 24 hours after carotid endarterectomy.

For the analysis of the primary outcome, we calculated the proportion of patients experiencing the composite of ischaemic stroke, transient ischaemic attack, or death within 30 days in both groups with a 95% Wilson score confidence interval. We used a χ^2 test to compare the groups.

We represented mortality graphically by using Kaplan-Meier curves for each treatment group. We compared groups by using the log rank test. We calculated mortality at 30 days for both groups and the difference between them with a 95% confidence interval obtained from the Kaplan-Meier estimator. We calculated a risk difference with a 95% confidence interval on the log scale by using Greenwood standard errors and a normal approximation.

We calculated the proportion of patients experiencing any stroke and myocardial infarction within 30 days for each group by using the non-parametric cumulative incidence function estimator with death as the competing event and a 95% confidence interval according to Choudhury and colleagues.[21] We compared groups by using the cumulative incidence difference with a 95% confidence interval and a

z test based on the delta method standard errors and a normal approximation. We also reported the cumulative incidence of the competing event (death without readmission) for each group in addition to the risk difference between groups.

We compared binary substudy outcomes (appearance of at least one new lesion) by using χ^2 tests. We presented the effects as absolute risk differences with Newcombe hybrid score 95% confidence intervals.

Furthermore, we used a first sensitivity analysis to calculate the risk ratios with Koopman asymptotic score 95% confidence intervals and odds ratios with Gart adjusted logit 95% confidence intervals for all binary outcomes.[22 23] A second sensitivity analysis focused on the primary endpoint by using survival methods similar to the mortality calculation.

Finally, we used multivariable logistic regression analyses to evaluate the predictors of the possible primary outcome. Candidate predictors included age, sex, side of stenosis, symptomatic stenosis, percentage of ipsilateral/contralateral internal carotid artery stenosis, arterial hypertension, diabetes mellitus, hyperlipidaemia, smoking, alcohol misuse, coronary heart disease, atrial fibrillation, type of anaesthesia, shunt use, antiplatelet drug use, anticoagulant drug use, antihypertensive drug use, insulin use, oral antidiabetic drug use, statin use, and type of plaque in ipsilateral carotid stenosis. The model included the treatment group. Predictors were incorporated into the model such that the P value obtained from the Wald χ^2 test of coefficient nullity was <0.2. Results were reported as odds ratios with 95% confidence intervals based on the robust estimator of variance.

Table 1 | Baseline characteristics. Values are numbers (percentages) unless stated otherwise

Characteristics	Sonolysis (n=507)	Control (n=497)
Demographics		
Age, years:		
Mean (SD)	68 (8.0)	68 (7.7)
Median (IQR)	69 (63-73)	69 (63-74)
Female sex	149 (29)	163 (33)
Side of carotid stenosis—left	245 (48)	236 (47)
Percentage of stenosis:		
Mean (SD)	80 (8.9)	80 (8.8)
Median (IQR)	80 (75-90)	80 (75-85)
Symptomatic stenosis	215 (42)	235 (47)
Medical history		
Arterial hypertension	432 (85)	448 (90)
Diabetes mellitus	199 (39)	170 (34)
Coronary heart disease	134 (26)	168 (34)
Atrial fibrillation	30 (5.9)	42 (8.5)
Hyperlipidaemia	399 (79)	424 (85)
Smoking status:		
No	239 (47)	268 (54)
Former	66 (13)	49 (10)
Yes	202 (40)	180 (36)
Alcohol consumption, units/day:	(n=504)	(n=496)
0	228 (45)	243 (49)
1	193 (38)	175 (35)
2	58 (12)	55 (11)
≥3	25 (5.0)	23 (4.6)
Type of plaque		
Soft	139 (47)	106 (40)
Calcificated	88 (30)	104 (39)
Other	71 (24)	58 (22)
Unclassified	209	229
Medication		
Statin	405 (80)	420 (85)
Antiplatelet drug	452 (89)	451 (91)
Anticoagulant	57 (11)	67 (13)
Antihypertensive drug	418 (82)	435 (88)
Insulin	53 (10)	52 (10)
Oral antidiabetic drug	128 (25)	128 (26)
Clinical data		
Modified Rankin score:		
0	390 (77)	380 (76)
1	81 (16)	79 (16)
2	33 (6.5)	36 (7.2)
≥3	3 (0.6)	2 (0.4)
National institutes of Health Stroke Scale:		
Mean (SD)	0.64 (1.8)	0.68 (1.7)
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)

IQR=interquartile range; SD=standard deviation.

Patient and public involvement

Patient and public involvement is increasingly recognised as important, but this research was initiated before formal patient and public involvement procedures became common in Europe. Although no patients or members of the public were directly involved in the planning, design, or conduct of this study and no resources were allocated for formal patient involvement, informal interviews with patients undergoing carotid endarterectomy and members of the public were conducted and focused on the technology used in the study. We asked members of the public to read the manuscript before submission.

Results

Trial population

A total of 1004 patients from 16 centres in three European countries (Czech Republic, Slovakia, and Austria) were enrolled between 20 August 2015 and 14 October 2020 until interim analysis in the randomised phase of the trial. Members of the Data and Safety Monitoring Board evaluated the interim analysis results for the first 1000 enrolled patients and unanimously recommended stopping the SONOBIRDIE trial early owing to clear evidence of efficacy. Recruitment per centre, cumulative and monthly recruitment, and participant disposition are listed in supplementary tables B and E and supplementary figure C. The Statistical Analysis Plan was updated after randomisation of 50% of planned patients, resulting in a reduction in the number of patients randomised between September 2018 and February 2019.

Of the 1004 enrolled patients, 507 were assigned to the sonolysis group and 497 to the control group. The mean age of patients was 67.9 (standard deviation (SD) 7.8) years (range 31-86 years), and 31% of patients were female (table 1). A total of 450 (45%) patients had symptomatic carotid stenosis, and the mean degree of carotid stenosis was 79.9% (SD 8.9%). Baseline characteristics seemed to be well balanced between the groups (table 1). We observed no important differences

Table 2 | Summary of primary outcome in full analysis set and proportion of any stroke or TIA within 30 days calculated using non-parametric cumulative incidence function estimator with death as competing event

Outcome	Sonolysis (n=507)	Control (n=497)	Risk difference (95% CI)	P value
Primary outcome (composite of ischaemic stroke, TIA, or death within 30 days)*	11 (2.2, 1.2 to 3.8)	38 (7.6, 5.6 to 10.3)	−5.5 (−8.3 to −2.8)	<0.001
Ischaemic stroke or TIA within 30 days†	1.8 (0.6 to 2.9)	7.4 (5.1 to 9.8)	−5.7 (−8.2 to −3.1)	<0.001
Death without stroke/TIA within 30 days†	0.4 (0.0 to 0.9)	0.2 (0.0 to 0.6)	0.2 (−0.5 to 0.9)	0.57

CI=confidence interval; TIA=transient ischaemic attack.
*Values in sonolysis and control group are numbers (percentages, 95% Wilson CI). 95% CIs for proportions are calculated using Wilson method and P values are calculated from χ^2 test; 95% CI for risk difference is calculated using Newcombe hybrid score.
†Values in sonolysis and control group are proportions (Choudhury CI). CIs are computed according to Choudhury's method; cumulative incidence of competing event (death without readmission) for each group and risk difference between groups are also reported.

between the randomisation groups with respect to used ultrasound devices (supplementary table B). Table 1 provides information on medical history, medication use, sonographic findings, and neurological status. Methods used for diagnosis and evaluation of the carotid artery stenosis are in supplementary table F. Procedural data are provided in supplementary table G.

Primary endpoint

All 1004 randomised patients had carotid endarterectomy 0-9 days after randomisation (mean 0.9 (SD 1.1); median 1 (interquartile range 0-1)). Crossover between groups occurred in seven patients. Six patients in the sonolysis group had sonolysis lasting for <10 minutes, and one patient in the control group had sonolysis exceeding 10 minutes.

The primary composite outcome, including ischaemic stroke, transient ischaemic attack, and death, occurred significantly less often in the sonolysis group than in the control group (2.2% v 7.6%; P<0.001) in the full analysis set (table 2). In the sonolysis group, ischaemic stroke occurred in seven (1.4%) patients, transient ischaemic attack occurred in three (0.6%) patients, and three (0.6%) patients died within 30 days. In the control group, 27 (5.4%) patients had ischaemic stroke, 13 (2.6%) patients had transient ischaemic attack, and one (0.2%) patient died within 30 days (table 3).

Sensitivity analysis showed that significant differences (P<0.05 in all cases) in the odds ratios and risk ratios were present not only for the composite outcome (risk ratio 0.28; odds ratio 0.27) but also for stroke (risk ratio 0.25; odds ratio 0.24) and transient ischaemic attack (risk ratio 0.23; odds ratio 0.22) (table 3). Figure 1 shows the cumulative incidence of the primary outcome.

Secondary endpoints

Mortality within 30 days and cumulative mortality over the course of one year did not differ significantly between groups (table 2; table 3; fig 2). Figure 2 shows the cumulative incidence of death over the course of the one year follow-up. Stroke as a cause of death was recorded only in one patient in the sonolysis group. Ischaemic stroke or transient ischaemic attack within 30 days was detected in nine (1.8%) patients in the sonolysis group and in 37 (7.4%) patients in the control group (table 2 and table 3), with a risk difference of −5.7 (95% confidence interval (CI) −8.2 to −3.1). In the sonolysis group, two patients had a transient ischaemic attack, six patients had a stroke, and one patient had both a transient ischaemic attack and a stroke within 30 days. In the control group, 10 patients had a transient ischaemic attack, 24 patients had a stroke, and three patients had both a transient ischaemic attack and a stroke within 30 days. Myocardial infarction within 30 days was diagnosed in one (0.2%) patient in both groups (P=1.00). No unspecified stroke was recorded.

In addition to the sonolysis/sham procedure, the multivariable model for the primary outcome identified only female sex as an independent risk factor that increased the risk of the primary composite endpoint, with an odds ratio of 2.0 (supplementary table H). A post hoc analysis evaluated the effect of shunt use, symptomatic carotid stenosis, ultrasound device used, or centre on risk difference between groups (supplementary table I), finding no statistically significant effect.

Magnetic resonance imaging substudy

A total of 460 (45.8%) patients underwent brain magnetic resonance imaging examination before and one day after carotid endarterectomy: 236 in the

Table 3 | Risk ratios with Koopman asymptotic score 95% CI and odds ratios with Gart adjusted logit 95% CI for each binary outcome

Outcome	Sonolysis (n/N)	Control (n/N)	Risk ratio (95% CI)	Odds ratio (95% CI)
Primary outcome (composite of ischaemic stroke, TIA, or death within 30 days)	11/507	38/497	0.28 (0.15 to 0.54)	0.27 (0.14 to 0.54)
Death within 30 days	3/507	1/497	2.94 (0.42 to 20.49)	2.95 (0.34 to 15.61)
Ischaemic stroke within 30 days	7/507	27/497	0.25 (0.11 to 0.56)	0.24 (0.11 to 0.58)
TIA within 30 days	3/507	13/497	0.23 (0.07 to 0.73)	0.22 (0.08 to 0.81)
Death within 365 days	12/499	17/491	0.69 (0.34 to 1.42)	0.69 (0.33 to 1.45)

CI=confidence interval; n=number of events; N=number of patients; TIA=transient ischaemic attack.

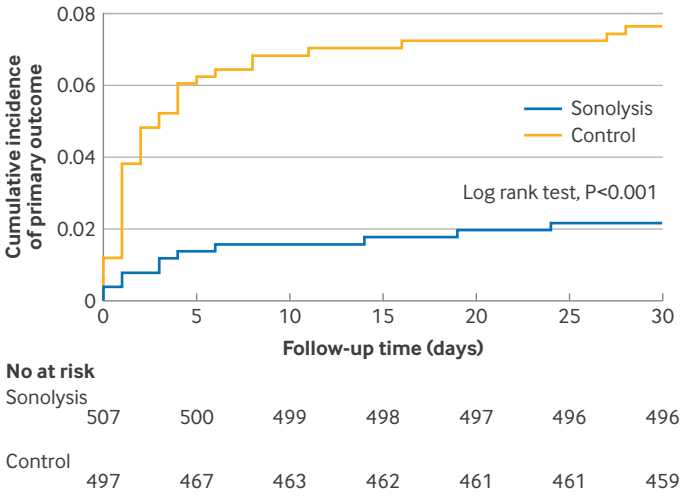

Fig 1 | Cumulative incidence of primary outcome (composite of ischaemic stroke, transient ischaemic attack, or death within 30 days)

sonolysis group and 224 in the control group. New brain ischaemia was detected in 20 (8.5%) patients in the sonolysis group, with five of them having a volume of ≥5 mL, and in 39 (17.4%) patients in the control group, with 13 of them having a volume of ≥5 mL. All ischaemic lesions were only ipsilateral to the intervened internal carotid artery. The risk difference for new brain ischaemia of −8.9% (95% CI −15.0% to −2.8%) was statistically significant (P=0.004; table 4). However, the risk difference of 8.3% (95% CI −29% to 17%) for new brain ischemia ≥5 mL in volume did not reach statistical significance (P=0.51).

Safety

Protocol violation was recorded in a total of 80 patients (supplementary table C). Violation in age limit was recorded in three patients and violation in self-sufficiency measured using the modified Rankin scale was detected in five patients. Four patients in the sonolysis group had sonolysis lasting for >120 minutes (125 min twice, 150 min, and 186 min), although no serious adverse event was recorded in these patients. A total of 53 patients completed the visit on day 30, outside the time interval.

Intracerebral bleeding as well as subarachnoid haemorrhage occurred in only one (0.2%) patient in the sonolysis group and none in the control group (P=0.32). No intracranial bleeding, including microbleeds, was detected in any patient.

The percentages of patients on site reporting serious adverse events and the incidence rates for serious adverse events expressed as events per 100 000 patient years were reported. The incidence rate ratios are shown in supplementary table J.

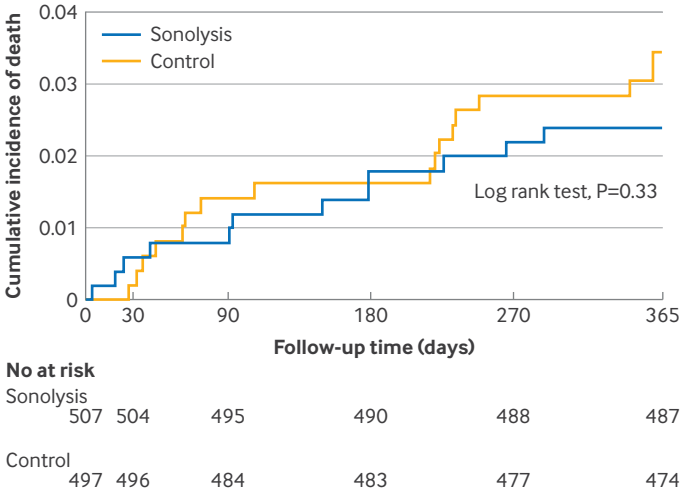

Fig 2 | Cumulative incidence of death over one year of follow-up

Discussion

In this double blind, randomised, sham controlled trial involving patients with an indication for carotid endarterectomy, we found periprocedural sonolysis to be safe and the 30 day risk of the composite primary endpoint, including ischaemic stroke, transient ischaemic attack, and death, was statistically significantly lower by 5.5% compared with sham, with a risk ratio of 0.28. The risk reduction was similar for ischaemic stroke at (risk ratio 0.25) and for transient ischaemic attack at (risk ratio 0.23).

Comparison with existing evidence

We detected no significant difference between groups in terms of mortality and serious adverse events. Because a higher incidence of intracranial bleeding was reported when lower than diagnostic ultrasound frequencies were used,[24] our study focused primarily on the occurrence of bleeding from a safety perspective. However, we observed no increased risk of haemorrhagic stroke, including subarachnoid haemorrhage, or intracranial bleeding, including microbleeds, using magnetic resonance imaging in the substudy. These results were in accordance with those of other studies using sonolysis or sonothrombolysis with a standard 2 MHz transcranial Doppler probe.[25]

The exact mechanism of the therapeutic effect of sonolysis—that is, continuous pulsed wave Doppler insonation of an artery for prevention and treatment of brain ischaemia—remains unclear, although it has been studied for more than 50 years.[4-7] Mechanical effects of ultrasound, such as radiation force and acoustic cavitation, have been implicated in sonolysis.[26 27] Cavitation is defined as the growth and collapse of a microbubble in response to ultrasound insonation. It creates a mechanical shear force that acts on both thrombi and endothelial cells when they occur in the area of insonation. Stable cavitation generates non-linear microbubble oscillation, whereas inertial cavitation results in microbubble destruction and greater shear from the fluid jets caused by microbubble collapse.[28] A threshold for inertial cavitation was observed with a mechanical index of ≥0.5.[29]

In addition to the mechanical effect, ultrasound waves are assumed to accelerate enzymatic fibrinolysis via direct activation of fibrinolytic enzymes and by

Table 4 | Analysis of appearance of new lesions in full analysis set

Lesion	Sonolysis (n=507)*	Control (n=497)*	Risk difference (95% CI)	P value
New ischaemic lesion on control DW-MRI (only for substudy)	20/236 (8.5, 5.6 to 12.7)	39/224 (17.4, 13.0 to 22.9)	−8.9 (−15 to −2.8)	0.004
Ischaemic lesion ≥0.5 mL	5/20 (25.0, 11.2 to 46.9)	13/39 (33.3, 20.6 to 49.0)	−8.3 (−29 to 17)	0.51

Values for sonolysis and control group are number of events/number of non-missing observations (percentages, 95% Wilson CI). 95% CI for proportions are calculated using Wilson method, and P values are calculated from χ^2 test; 95% CI for risk difference is calculated using Newcombe hybrid score.
CI=confidence interval; DW-MRI=diffusion weighted magnetic resonance imaging.

increasing the transport of fibrinolytic agents, such as plasmin, into the thrombus.[14 15] Additionally, transient peripheral vasodilatation may also play a role in arterial reperfusion.[30] Activation of fibrinolytic enzymes and disruption of thrombo-emboli may account for reduced incidence and volume of lesions in the outside regions that are directly exposed to ultrasound energy.[15-18]

To confirm the effect of sonolysis, we selected new ischaemic lesions detected using diffusion weighted magnetic resonance imaging as the endpoint in the substudy. We showed that sonolysis significantly reduced the risk of new brain infarction by 8.9% and observed a similar trend for the reduction in the incidence of brain infarction ≥0.5 mL in volume. This is in accordance with previous studies.[16-18]

The multivariable model in the SONOBIRDIE trial showed that only female sex was an independent risk factor for the composite primary outcome, with an odds ratio of 2.0, which was in agreement with other studies. A systematic review of 25 studies showed that female patients had a higher rate of operative stroke and death, with an odds ratio of 1.31.[31] Similarly, female sex was associated with a 29% significantly increased risk of stroke 30 days after surgery in a recent meta-analysis, with significant increases in operative risks among patients without symptoms (odds ratio for stroke risk 1.51).[32]

Although the primary composite outcome in the control group was higher than we assumed in the sample size calculation, the 5.4% perioperative risk of stroke in the SONOBIRDIE trial was comparable to that seen in other published randomised controlled trials. In the meta-analysis including 3157 participants who had carotid endarterectomy for symptomatic carotid stenosis, 222 perioperative strokes or deaths were recorded (7.0%, 95% CI 6.2% to 8.0%).[19] For asymptomatic carotid stenosis, 102 (3.2%; range 2.4-10.0%) perioperative strokes, myocardial infarctions, or deaths were recorded among the 3198 participants.[33]

Strengths and limitations of study

The strengths of this study include the relatively large number of randomised patients and the use of a sham procedure in the control group. One limitation of the study was that no study specific ultrasound device was used and only standard ultrasound (transcranial Doppler) machines were used, which did not allow blinding for the sonographer. However, all patients, surgeons, radiologists describing the brain magnetic resonance imaging, and neurologists doing the follow-up examinations were blinded to the study conditions and had no information about inclusion of patients into the sonolysis or sham procedure group. The study used different, centre specific approaches for antithrombotic treatment, application of heparin and protamine sulphate during carotid endarterectomy, use of general or local anaesthesia, ultrasound device, and shunt indication. However, the distribution of baseline demographic, medical, and periprocedural characteristics was balanced between the groups, with only slightly more women and symptomatic stenosis in the control group. The multivariable model showed that the effect of sonolysis was independent of age, sex, symptomatic stenosis, type of antithrombotic treatment, and surgical technique. Because part of the study was carried out during the covid-19 pandemic, the total number of adverse events could have been influenced by restrictions during hospital admission and difficulty with adherence to the time intervals for individual follow-up visits.

Clinical implications

The results of the SONOBIRDIE trial suggest that sonolysis should be used to reduce the risk of periprocedural cerebrovascular events during carotid endarterectomy in all patients with sufficient temporal bone window for transcranial Doppler. It has the potential to make carotid endarterectomy safer with a higher benefit for patients with carotid stenosis compared with the best medical treatment, especially in patients with asymptomatic carotid stenosis.

Conclusion

The SONOBIRDIE trial showed that periprocedural sonolysis during carotid endarterectomy significantly reduced the risk of ischaemic stroke, transient ischaemic attack, and new brain infarction detected using magnetic resonance imaging of the brain, with no increase in the risk of any adverse events.

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Contributors: DŠ was responsible for study conception and design. TH, DN, JF, VB, and MK acquired the data. JBR and MB analysed and interpreted the data. DŠ and DN drafted the manuscript. TH, JF, VB, MK, and JBR critically revised the manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. DŠ and DN are the guarantors. See web appendix 1 for trial committees and subcommittees.

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Ethical approval: The protocol was approved by the multicentre ethics committee of Vítkovice Hospital (2 June 2015) and local institutional review boards of the participating centres. All patients provided written informed consent.

Data sharing: Data will become available to interested investigators on submission of a reasonable research request by email to the corresponding author (skoloudik@email.cz) and approval by the steering committee of the trial.

Transparency: The lead authors (the manuscript's guarantors) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained.

Dissemination to participants and related patient and public communities: Participating institutions and surgical departments were informed of study results through grand round conferences. The results were presented to the neurological and stroke community at the European Stroke Organisation Conference—ESOC 2023 in Munich, Germany, in May 2023 (https://eso-stroke.org/events/esoc-2023/), the 43rd Annual Meeting of the Japan Academy of Neurosonology, and the 27th Annual Meeting of the Japan Society of Embolus Detection and Treatment, Chiba, Japan, in June 2024 (https://www.jstage.jst.go.jp/article/neurosonology/37/2/37_69/_article/-char/en). The study results will be internally disseminated through the media departments and websites of the participating institutes, shared with public health organisations, and presented at regional and national events with health, lay, and government representation.

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Web appendix 1: Supplementary materials
Web appendix 2: Study protocol
Web appendix 3: Statistical analysis plan