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CLINICAL PRACTICE

Nitrous oxide exposure does not seem to be associated with increased mortality, stroke, and myocardial infarction: a non-randomized subgroup analysis of the General Anaesthesia compared with Local Anaesthesia for carotid surgery (GALA) trial

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Editor's key points

- The authors present an important subgroup analysis of the previously reported GALA trial.
- The analysis involves patients receiving general anaesthesia or local anaesthesia during carotid artery surgery.
- Importantly, the analysis took into account the pre-existing risk factors in the subgroups.
- There was no evidence that nitrous oxide increased the risk of mortality, stroke, and myocardial infarction.

Background. Nitrous oxide has been associated with increased vascular risk in the perioperative period. Here, we conducted a secondary analysis of the GALA trial to ascertain the impact of nitrous oxide on outcomes after carotid surgery under general anaesthesia (GA).

Methods. One thousand seven hundred and seventy-three patients underwent GA, but 158 patients were excluded from this analysis as nitrous oxide use was unknown. The decision to use nitrous oxide was at the discretion of the anaesthetist and was not randomized. Six hundred and seventy-one patients received nitrous oxide and 944 patients did not. Logistic regression was used to analyse the same primary outcome as the original trial (risk of death, stroke, or myocardial infarction within 30 days of the operation).

Results. Patients who received nitrous oxide were more likely to have had coronary artery disease, peripheral vascular disease, and atrial fibrillation (all $P<0.05$). Overall, there were 35 (5.2%) primary outcome events in patients receiving nitrous oxide compared with 44 (4.7%) in those who did not [relative risk 1.12, 95% confidence interval (CI: 0.73, 1.73); $P=0.63$]. The adjustment for the imbalanced baseline variables using logistic regression reduced the point estimate of harm for nitrous oxide [adjusted odds ratio 1.09, 95% CI (0.68, 1.74); $P=0.73$].

Conclusions. Given the greater prevalence of vascular risk factors in the nitrous oxide group and the lack of any definite effect on the primary outcome measure, these data do not support a clinically meaningful adverse effect of nitrous oxide on our composite outcome in patients undergoing carotid surgery.

Keywords: anaesthesia; death; myocardial infarction; nitrous oxide; stroke

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Nitrous oxide administration has been associated with adverse perioperative outcomes in small randomized controlled trials (RCTs) and secondary analyses of larger trials. For example, the Evaluation of Nitrous Oxide In the Gas Mixture for Anaesthesia (ENIGMA) group[1 2] and Badner and colleagues[3 4] reported that nitrous oxide exposure was associated with an increased risk of myocardial ischaemia. In particular, in an RCT of 90 patients undergoing carotid endarterectomy, an increased incidence and duration of myocardial ischaemia (diagnosed by the analysis of the ECG) was noted in patients receiving nitrous oxide–isoflurane anaesthesia rather than isoflurane alone.[3] This effect

correlated with increased plasma homocysteine levels.[4] Nitrous oxide exposure has also been associated with an increased incidence of delayed ischaemic neurological deficits reported in a non-randomized secondary analysis of the Intraoperative Hypothermia in Aneurysm Surgery Trial.[5] While controversial, these findings suggest that nitrous oxide may increase vulnerability to ischaemic injury. As nitrous oxide inhibits methionine synthase function leading to the generation of homocysteine,[6] potentially leading to endothelial dysfunction and vasospasm,[7] this hypothesis is plausible. Furthermore, unlike other volatile inhaled anaesthetics, nitrous oxide lacks neuroprotective[6 8] or preconditioning[9 10] effects. Nitrous oxide has also been implicated in neurotoxicity, although any effects in this context appear mild.[6]

We performed a secondary analysis of patients who received general anaesthesia (GA) in the *General Anaesthesia versus Local Anaesthesia for carotid surgery* (GALA) trial[11] to help determine whether intraoperative nitrous oxide exposure was associated with an increased risk of perioperative stroke, myocardial infarction, and death in this large cohort of patients.

Methods

The GALA trial methodology is available elsewhere.[11 12] The trial was registered with Current Control Trials, number ISRCTN00525237. In brief, 3256 eligible patients were randomized to GA or local anaesthesia for carotid surgery. This secondary analysis included patients who actually received GA during the trial, regardless of group allocation creating a cohort of 1773 patients undergoing GA (1629 patients allocated to GA and 144 randomized to local anaesthesia but given GA). Patients in whom nitrous oxide exposure (yes/no) was not recorded were excluded from the analysis. The administration of nitrous oxide was not randomized but rather left to the discretion of the anaesthetist. The inspired nitrous oxide concentration was not recorded on the trial data sheet and so the dose of the drug is unknown. To reduce any sort of expectation bias from this secondary analysis, the primary and secondary outcome measures were not altered from the original trial protocol. The primary outcome was the risk of death, stroke, or myocardial infarction within 30 days of the operation. Secondary outcomes included the risk of stroke and risk of death from stroke.

The *P*-values presented in the patient characteristic tables were calculated excluding those patients where the nitrous oxide status was unknown (i.e. the comparison was definitely nitrous oxide *vs* definitely no nitrous oxide). Where the variables of interest were categorical, they were calculated using the binomial test for comparison of proportions (variables containing two levels) and χ^2 tests (where there were more than two levels). For the continuous variables, a two-sample *t*-test was used to examine the differences between those who received nitrous oxide and those who did not. A Kaplan–Meier survival curve was also produced for primary outcome events within a year of surgery. The analyses were performed as in the GALA trial, with logistic regression and *P*-values presented from the change in log-likelihood on entering the interaction between the subgroup effect and the treatment effect into the model. They were performed using SAS version 9.2 [SAS Institute Inc., Cary, NC, USA].

Results

One thousand seven hundred and seventy-three patients received GA: of these, 671 definitely received nitrous oxide, 944 definitely did not, and for 158, it was unknown. Because this was a non-randomized comparison, it is not surprising that the two groups of interest (definitely *vs* definitely not receiving nitrous oxide) were not well balanced. Patients who received nitrous oxide were more likely to have had volatile, rather than i.v. anaesthesia, neuromuscular block and more likely to have received premedication (Table 1). Patients who received nitrous oxide were also more likely to have had peripheral vascular disease, coronary artery disease, and atrial fibrillation, but also to be ASA category I or II rather than III or IV (Table 2) and be younger (Table 2). Thus, it is likely that patients receiving nitrous oxide were at higher risk of vascular events after operation than in the no nitrous oxide group.[13 14] Patients who received nitrous oxide were also more likely to have a pre-existing infarct on the side of the brain that was being operated on, but the carotid stenosis was less severe; and they were less likely to be on aspirin, clopidogrel, dipyridamole, and warfarin (Table 2). Finally, intraoperatively, patients in the nitrous oxide group were less likely to have had their arterial pressure manipulated upwards (Table 3).

Overall, there were 35 primary outcome events in the 671 patients receiving nitrous oxide (5.2%) compared with 44 out of the 944 (4.7%) (Table 4) who did not [relative risk 1.12, 95% confidence interval (CI: 0.73, 1.73); $P=0.63$]. Given the imbalance in the groups, we conducted logistic regression

Table 1 Anaesthetic management of patients undergoing carotid endarterectomy under general anaesthesia. GA, general anaesthesia; LA, local anaesthesia

	Nitrous oxide				*P*-value
	No		Yes		
	n	%	*n*	%	
Total number of patients given GA	944	100	671	100	
Allocated treatment in original trial					
General anaesthetic	868	92	616	92	0.972
Local anaesthetic	76	8	55	8	
LA used in addition to GA	46	5	23	3	0.171
Premedication used	457	48	430	64	<0.001
Volatile agents used	727	77	625	93	<0.001
Total i.v. anaesthesia	340	36	141	21	<0.001
Neuromuscular blocking agents used	816	86	640	95	<0.001

Table 2 Balance of patient factors between the nitrous oxide and no nitrous oxide groups. GA, general anaesthesia; MR, magnetic resonance; CT, computer tomography. Baseline surgical risk 1 of stroke or death: patients score 1 point each for having systolic arterial pressure higher than 180 mm Hg, being female, and having peripheral arterial disease. Missing arterial pressure values were assumed to be 180 mm Hg or less. Scores of 2 or 3 were combined into one group because of small numbers. Baseline surgical risk 2 of stroke or death, patients score: age 75 yr old or older=2 points, hypertension=3 points, female=4 points, diabetes=5 points, symptomatic cerebral event=6 points, contralateral occlusion=9 points, redo surgery=10 points, emergency surgery=29 points (R. Bond, unpublished data)[11]

	Nitrous oxide				*P*-value
	No		Yes		
	n	%	*n*	%	
Total number of patients given GA	944	100	671	100	
Sex (male)	657	70	477	71	0.544
Hypertension	726	77	524	78	0.573
Peripheral arterial disease	205	22	179	27	0.024
Aortic aneurysm	39	4	25	4	0.7
Coronary heart disease	324	34	266	40	0.032
Cardiac failure	39	4	29	4	0.9
Atrial fibrillation	53	6	65	10	0.003
Diabetes	231	24	172	26	0.6
Chronic lung disease					
Unknown	4	0	—	0	0.277
No	799	85	584	87	
Yes	141	15	87	13	
Never smoked					
Unknown	1	0	1	0	0.205
No	746	79	548	82	
Yes	197	21	122	18	
Baseline surgical risk 1					
Low (score 0)	485	51	341	51	0.855
Medium (score 1)	382	40	270	40	
High (score 2 or 3)	77	8	60	9	
Baseline surgical risk 2					
Very low (score 0–4)	191	20	123	18	0.601
Low (score 5–9)	369	39	283	42	
Medium (score 10–14)	257	27	180	27	
High (score 15 or more)	127	13	85	13	
ASA grade I or II	311	33	252	38	0.057
Randomized artery to be operated on (right)	479	51	339	51	0.96
Indication for surgery					
Asymptomatic carotid stenosis	395	42	280	42	0.114
Carotid stroke only	169	18	143	21	
Cerebral TIA (carotid) only	202	21	127	19	
Retinal infarct only	23	2	6	1	
Amaurosis fugax only	78	8	57	8	
More than one of those above	77	8	58	9	
Contralateral carotid occlusion	81	9	58	9	1
Stenosis assessed by ultrasound	911	97	619	92	
Stenosis assessed by MR angiography	168	18	106	16	
Stenosis assessed by CT angiography	137	15	97	14	
Stenosis assessed by catheter angiography	86	9	113	17	
Infarct on side of brain relevant to operation	172	18	172	26	<0.001
UK patient	439	47	216	32	<0.001
Pre-randomization aspirin					

Continued

Table 2 *Continued*

	Nitrous oxide				*P*-value
	No		Yes		
	n	%	*n*	%	
No	215	23	267	40	<0.001
Unknown	116	12	116	17	
Yes	613	65	288	43	
Pre-randomization clopidogrel					
No	748	79	523	78	0.001
Unknown	116	12	116	17	
Yes	80	8	32	5	
Pre-randomization dipyridamole					
No	776	82	519	77	0.018
Unknown	116	12	116	17	
Yes	52	6	36	5	
Pre-randomization warfarin					
No	817	87	542	81	0.007
Unknown	116	12	116	17	
Yes	11	1	13	2	

to adjust for peripheral arterial disease, coronary heart disease, atrial fibrillation, ASA grade I or II, pre-existing infarct on the side of the brain relevant to operation, patients treated in the UK *vs* overseas, age, and the percentage of stenosis of the operated artery. The unadjusted odds ratio for nitrous oxide on the primary outcome was 1.13 (95% CI 0.71, 1.78; $P=0.61$). After the adjustment for the imbalanced variables, the odds ratio was 1.09 (95% CI 0.68, 1.74; $P=0.73$). A log-rank test showed no difference in the primary outcome at 6 months ($P=0.68$; data not shown) or at 1 yr ($P=0.21$; Fig. 1).

Nitrous oxide did not increase the risk of the secondary outcomes. The risk of stroke was unaffected [relative risk 1.13, 95% CI (0.70, 1.83); $P=0.61$], and while showing a point estimate towards harm, the unadjusted risk of a fatal stroke within 6 months of the operation was not significantly different either [relative risk 2.21, 95% CI (0.86, 5.67); $P=0.10$]. There were too few events to perform an adjusted analysis for this endpoint.

Discussion

Because GALA was a pragmatic large international multicentre trial, the choice of the GA technique was left to the discretion of individual clinicians in each centre. The choice or otherwise of nitrous oxide would have been part of a package of anaesthetic care that involved other agents including opioids, neuromuscular blocking agents, volatile or i.v. anaesthetic agents, varying oxygen concentrations, and the use of other drugs with cardiovascular effects. Not surprisingly, patients who received nitrous oxide were rather different to those who did not. However, given the greater prevalence of vascular risk factors in the nitrous oxide group and the lack of any definite effect on the primary outcome or any other measure, particularly after statistical adjustment to account for the baseline differences, these data do not suggest any clinically meaningful adverse effects of nitrous oxide on vascular outcomes. The non-significant increase in the number of fatal strokes in the nitrous oxide group may be explained by the increased vascular risk inherent in the nitrous oxide group, operation on the side of an existing infarct, nitrous oxide exacerbating an evolving ischaemic injury, or it may simply be due to chance and inadequate adjustment for the imbalances at baseline. Indeed, we must be cautious with the interpretation of our findings because a serious limitation of secondary analyses focusing on non-randomized comparisons within an RCT is that groups are often imbalanced. However, the adjustment for the imbalanced variables did not increase the risk of harm on our primary outcome measure.

With the exception of the secondary analysis of the Intraoperative Hypothermia in Aneurysm Surgery Trial,[5] most previous work has focused on whether there is an increased perioperative risk of myocardial, rather than cerebral ischaemia. With only five myocardial infarctions, our analysis was underpowered to look at myocardial infarction as an independent outcome. At the time we set up the GALA trial, routine troponin analysis was not widespread so it was not adopted for the study, limiting our diagnosis of myocardial infarction to traditional history, clinical examination, and ECG analysis. Therefore, we cannot comment on the association between nitrous oxide exposure and myocardial ischaemia, or indeed silent infarction.

Our postoperative events were predominantly strokes. There was no effect on their overall incidence, although there was a non-significant increase in the unadjusted risk

Table 3 Balance of perioperative factors for the nitrous oxide and no nitrous oxide groups. GA, general anaesthesia; TCD, transcranial Doppler; AP, arterial pressure

	Nitrous oxide				*P*-value
	No		Yes		
	n	%	*n*	%	
Total number of patients given GA	944	100	671	100	
Trainee surgeon					
Missing	1	0	—	0	0.545
No	819	87	590	88	
Yes	124	13	81	12	
Trainee anaesthetist					
Missing	1	0	—	0	0.013
No	819	87	552	82	
Yes	124	13	119	18	
Premedication used					
Missing	484	51	239	36	<0.001
Yes	460	49	432	64	
Type of surgery					
Missing	3	0	1	0	0.322
Conventional	742	79	529	79	
Eversion	190	20	129	19	
Other	9	1	12	2	
Shunt used					
Unknown	2	0	2	0	0.359
No	521	55	386	58	
Yes	421	45	283	42	
Reason for using shunt					
Missing	522	55	387	58	
Low stump pressure	68	7	47	7	
Contralateral stenosis (but not occluded or nearly occluded)	4	0	—	0	
Recent stroke	6	1	—	0	
Unusual or damaged veins/arteries in head or neck	—	0	2	0	
Falling brain oxygen level	—	0	1	0	
Used routinely	250	26	152	23	
Decrease in velocity on TCD	30	3	17	3	
Contralateral occlusion/near occlusion	19	2	19	3	
Unable to use TCD	27	3	36	5	
Arterial pressure decreased	4	0	1	0	
EEG or evoked potentials change	4	0	4	1	
Operation converted to a vein bypass	—	0	1	0	
Irrelevant comment	2	0	1	0	
AP manipulation	—	0	1	0	<0.001
Missing	—	0	1	0	
Up	452	48	261	39	
Down	95	10	118	18	
Up and down	168	18	111	17	
Not manipulated	229	24	180	27	
Patch used	2	0	1	0	0.003
Unknown	2	0	1	0	
No	447	47	368	55	
Yes	495	52	302	45	
Intraoperative heparin	2	0	2	0	0.21
Unknown	2	0	2	0	
No	12	1	14	2	
Yes	930	99	655	98	

Table 4 Outcomes for the nitrous oxide and no nitrous oxide groups. GA, general anaesthesia; MI, myocardial infarction; RI, retinal infarction

	Nitrous oxide			
	No		Yes	
	n	%	*n*	%
Total number of patients given GA	944	100	671	100
Primary outcome event within 30 days	44	5	35	5
Type of primary outcome event within 30 days				
Death	3	0	4	1
MI	2	0	2	0
RI	3	0	—	0
Stroke	36	4	29	4
Stroke				
1—non-fatal	32	3	18	3
2—fatal	7	1	11	2
Type of stroke				
1—infarct	22	2	14	2
2—haemorrhage	6	1	7	1
3—unknown	8	1	8	1
Ipsilateral to surgery	906	96	642	96
Unknown				
No	8	1	4	1
Yes	30	3	25	4
Myocardial infarction				
1—non-fatal	2	0	—	0
2—fatal	—	0	2	0

of fatal stroke. Our data potentially contrast with the similarly non-randomized analysis of the Intraoperative Hypothermia in Aneurysm Surgery Trial[5] group that showed a significant increase in delayed ischaemic neurological deficits with nitrous oxide exposure. The difference may be attributable to differing methods of defining the ischaemic events as we do not have data on delayed ischaemic neurological deficits other than stroke. However, we should emphasize that the Intraoperative Hypothermia in Aneurysm Surgery Trial[5] group also did not find a difference in long-term outcomes. Alternatively, this may be attributable to differing mechanisms of cerebral ischaemia. After carotid surgery, ischaemic events are typically thromboembolic, whereas during intracerebral surgery, vasospasm or brain retraction is a more likely cause. Indeed, our data are entirely from patients undergoing carotid surgery and so may not be generalizable to other surgical procedures where nitrous oxide may exert a greater impact. Carotid surgery is of relatively short duration (a mean procedure time of 95 min in the GALA trial) that could further limit any effects of exposure to nitrous oxide that occur in a time-dependent manner.[6] After 90 min of exposure to nitrous oxide, methionine synthase activity has approximately halved;[6 15] however, it may be that a longer exposure is required for any detrimental effect to be observed.

It may be that particular groups of patients are vulnerable to nitrous oxide, for example, those malnourished or deficient in cobalamin or folate.[6] Indeed, a recent meta-analysis has suggested that homocysteine-lowering therapy may

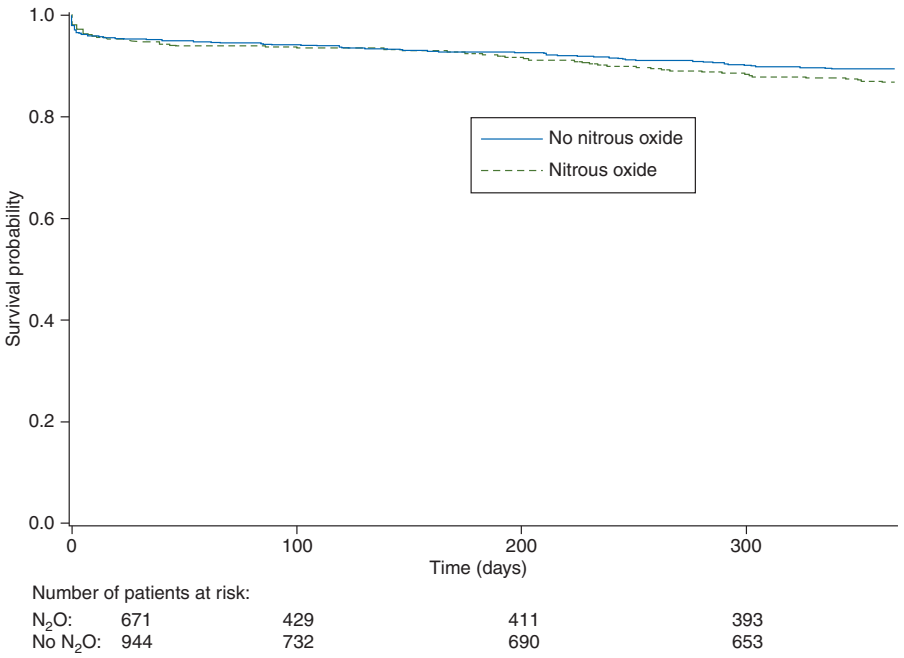

Fig 1 The Kaplan–Meier curve for the primary outcome (mortality, stroke, and myocardial infarction event-free) survival 1 yr after carotid endarterectomy. The log-rank test showed no difference in event-free survival at 1 yr between the two groups (P=0.21).

reduce the risk of stroke in areas where folate deficiency is high, such as Asia.[16] It may be that nitrous oxide exposure in these populations may lead to a particularly raised homocysteine and exacerbations of ischaemia; in turn, this may explain some of the results of the Evaluation of Nitrous Oxide In the Gas Mixture for Anaesthesia (ENIGMA) trial as it was heavily weighted with patients from Asia.[2] Furthermore, certain methylenetetrahydrofolate reductase genotypes [677C>T (rs1801133) or 1298A>C (rs1801131)] incur greater nitrous oxide-induced increases in homocysteine levels than those with wild-type alleles, and thus, these patients may be at greater perioperative risk from exposure to nitrous oxide.[6 17] Future studies should investigate these subgroups of patients. Nonetheless, the lack of effect on the primary outcome in GALA, despite increased vascular risk factors in the nitrous oxide group, adds weight to notion that there is no difference between GA in vascular outcome with or without nitrous oxide.

It is possible that our subgroup analysis lacked power to detect a difference between nitrous oxide treatment or not. However, we have not conducted a *post hoc* power analysis as it is much more informative to look at the CIs around the effect estimates that are observed.[18 19] Indeed, we have discussed the need for future studies to look for vulnerable subgroups of patients as, consistent with the wide confidence limits, there may be patients who are vulnerable to nitrous oxide exposure.

In conclusion, our data from a trial of carotid surgery do not lend support to reports of small RCTs and secondary analyses of other trials that nitrous oxide increases the risk of adverse perioperative vascular events.

Declaration of interest

R.D.S. has acted as a consultant for Air Liquide on the development of medical gases.

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