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# *Effect of Nitrous Oxide on Neurologic and Neuropsychological Function after Intracranial Aneurysm Surgery*

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***Background:*** **Laboratory studies suggest that nitrous oxide augments brain injury after ischemia or hypoxia. The authors examined the relation between nitrous oxide use and outcomes using data from the Intraoperative Hypothermia for Aneurysm Surgery Trial.**

***Methods:*** **The Intraoperative Hypothermia for Aneurysm Surgery Trial was a prospective randomized study of the impact of intraoperative hypothermia (temperature = 33°C) *versus* normothermia (temperature = 36.5°C) in patients with aneurysmal subarachnoid hemorrhage undergoing surgical clipping. Anesthesia was dictated by a limited-options protocol with the use of nitrous oxide determined by individual anesthesiologists. All patients were assessed daily for 14 days after surgery or until hospital discharge. Neurologic and neuropsychological testing were conducted at 3 months after surgery. Outcome data were analyzed *via* both univariate tests and multivariate logistic regression analysis correcting for factors thought to influence outcome. An odds ratio (OR) greater than 1.0 denotes a worse outcome in patients receiving nitrous oxide.**

***Results:*** **Outcome data were available for 1,000 patients, of which 373 received nitrous oxide. There was no difference between groups in the development of delayed ischemic neurologic deficit. At 3 months after surgery, there were no significant differences between groups in any outcome variable: Glasgow Outcome Score (OR, 0.84; 95% confidence interval [CI], 0.63–1.14; $P = 0.268$), National Institutes of Health Stroke Scale (OR, 1.29; 95% CI, 0.96–1.73; $P = 0.087$), Rankin Disability Score (OR, 0.84; 95% CI, 0.61–1.15; $P = 0.284$), Barthel Activities of Daily Living Index (OR, 1.01; 95% CI, 0.68–1.51; $P = 0.961$), or neuropsychological testing (OR, 1.26; 95% CI, 0.85–1.87; $P = 0.252$).**

***Conclusions:*** **In a population of patients at risk for ischemic brain injury, nitrous oxide use had no overall beneficial or detrimental impact on neurologic or neuropsychological outcomes.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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MANY studies have evaluated the influence of different anesthetics on the impact of cerebral ischemia in various animal models.[1–3] These studies have focused largely on metabolic-depressant or excitatory amino acid–antagonist anesthetics. Even when using the highest standards (*i.e.*, anatomical and functional endpoints) for assessing ischemic brain injury, there is ample evidence that select inhaled and intravenous anesthetics, such as isoflurane and barbiturates, can alter outcome and may provide some measure of protection.

In recent years, attention has been directed toward the effects of anesthetic supplements on the progression of ischemic brain injury. This research has focused largely on the narcotics[4] and nitrous oxide.[5,6] In many of these studies, the endpoints may be of limited clinical relevance (*e.g.*, electrophysiologic endpoints in contrast to the aforementioned accepted standard endpoints of anatomy and function).[1] Some of these have concluded that nitrous oxide is capable of augmenting ischemic brain injury or conversely reversing or attenuating the cerebroprotective effects of other anesthetics.[7–11] Even without corresponding human data, these laboratory findings have led some clinicians to recommend avoiding nitrous oxide in patients experiencing, or at risk for, ischemic brain injury.[12,13]

Given the immense costs and logistic challenges associated with conducting a prospective randomized clinical trial of complete anesthetics on outcome, it is not surprising that few studies exist in which functional outcome has been quantified,[14–16] and in only one of these studies has long-term outcome after surgery been examined.[16] Consistent with these challenges, it is even less surprising that, to date, no study has evaluated the

effect of anesthetic supplements on long-term outcome in humans.

The Intraoperative Hypothermia for Aneurysm Surgery Trial (IHAST) was a large, multicenter project intended to examine the impact of intraoperative cooling on neurologic outcome in patients with aneurysmal subarachnoid hemorrhage.[17] In this study, anesthetic technique was guided by a limited-options protocol, and the decision to use nitrous oxide was left to the discretion of the attending anesthesiologist. The ability to analyze the effect of nitrous oxide use on long-term neurologic outcome data in a large patient population at risk for the development of cerebral ischemic episodes allows new insights about whether nitrous oxide affects outcome in patients experiencing or at risk for ischemic neurologic injury.

## Materials and Methods

Our study was based exclusively on a *post hoc* analysis of the IHAST database. IHAST was a large (1,001 patient), international, multicenter, randomized, and partially blinded prospective clinical trial. Details regarding trial design are described elsewhere.[17] In brief, nonpregnant adults with a World Federation of Neurological Surgeons score of I, II, or III, who had aneurysmal subarachnoid hemorrhage no more than 14 days before surgery, were eligible for enrollment. Specific exclusion criteria included a body mass index of 35 $kg/m^2$ or greater, any cold-related disorder (*e.g.*, Raynaud disease), and the presence of an endotracheal tube at the time of enrollment. Extensive information regarding the patients' presubarachnoid hemorrhage health status and events occurring between the time of hospital admission and surgery were collected. The study was approved by each center's local human studies committee (see appendix), and informed consent was obtained from each patient or his legal representative. All study personnel, except the anesthesiologists involved in intraoperative care, were blinded to treatment assignment.

Anesthesia was limited to either thiopental or etomidate for induction of anesthesia, and isoflurane or desflurane for maintenance, supplemented by either fentanyl or remifentanil. Nitrous oxide use was at the discretion of the anesthesiologists, and no limitations were imposed by the study protocol on the concentration of nitrous oxide administered.

After the induction of anesthesia, an esophageal temperature probe was inserted, and the patient was positioned for surgery. In patients randomized to hypothermia, esophageal temperature was reduced as quickly as possible, with the goal of achieving a temperature between 32.5° and 33.5°C at the time a clip was applied to the first aneurysm. Temperature in patients randomized to normothermia was kept between 36° and 37°C. Rewarming of hypothermic patients began after the last aneurysm had been secured, and was continued until normothermia was achieved. No attempt was made to control postoperative care, but all adverse events, procedures, and other aspects of treatment were monitored either for 14 days or until hospital discharge (if this occurred before 14 days). Of particular note, a clinical diagnosis of delayed ischemic neurologic deficit (DIND) was made if there was a decrease in the Glasgow Coma Score with alteration in level of consciousness or the development of a new or worsening focal neurologic deficit was present after the exclusion of other causes (*e.g.*, drug effect, hydrocephalus, aneurysmal rebleeding, intracranial hematoma, cerebral edema, or metabolic disturbances such as hypoxia, hyponatremia, or aberrant glucose homeostasis).

A final follow-up examination was conducted approximately 3 months after surgery. Outcome measures included the (1) modified Glasgow Outcome Score (GOS; this was the primary outcome measure for the trial)[18,19]; (2) Rankin Disability Score[20]; (3) Barthel Activities of Daily Living Index[21]; (4) National Institutes of Health Stroke Scale (NIHSS) score[22]; (5) site to which the patient was discharged from the hospital where surgery was performed (*e.g.*, to home, an acute care hospital, or a chronic care/rehabilitation facility); and (6) a five-test neuropsychological battery, which included the Benton Visual Retention Test,[23] Controlled Oral Word Association,[24] Rey-Osterrieth Complex Figure Test,[25] Grooved Pegboard, and Trail Making Tests.[25,26] Details regarding neuropsychological testing and scoring can be found elsewhere.[27] T scores for individual tests (after adjustment for age and education) were averaged to obtain a single composite score; a composite score of 30 or less (2 SDs below the population norm of 50) was considered evidence of neuropsychological impairment. We also determined the number of subjects who were impaired (T score $<30$) on at least one test in the battery, regardless of the composite score. In addition, a Mini-Mental State Examination[28] was performed; impairment was defined according to the data reported by Crum *et al.*[29] All evaluations were performed by trained examiners who were certified by the University of Iowa Steering Committee.

### *Statistical Analysis*

All data analysis was conducted by the Data Management Center at the University of Iowa, Iowa City, Iowa, using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). Univariate comparisons of various measures in patients who did or did not receive nitrous oxide were performed using the Student *t* test, Pearson chi-square test, or Fisher exact test depending on the characteristics and distribution of the data. It was not possible to structure the analysis according to nitrous oxide dose because

nitrous oxide use was reported in the IHAST database as either used or not used.

All neurologic and neuropsychological outcomes were analyzed using both univariate and multivariate logistic regression. For binary outcomes, standard logistic regression analyses were performed, and for ordered categorical outcomes with more than two categories, cumulative logistic (proportional odds) models were used. Because the use of nitrous oxide was not based on random assignment, multivariate analyses were performed to assess the effect of nitrous oxide on outcomes after adjusting for a standard set of covariates, determined by the IHAST Coordinating Center to be important covariates to include in all *post hoc* analyses of neurologic and neuropsychological outcomes of the IHAST trial. The covariates for the multivariate analysis include race (white *vs.* nonwhite), age, sex, baseline World Federation of Neurological Surgeons score, baseline NIHSS score, Fisher grade, history of hypertension, time from subarachnoid hemorrhage to surgery, largest aneurysm size (1–11, 12–24, ≥25 mm in greatest dimension), aneurysm location (posterior *vs.* anterior), use of cerebroprotective drugs intraoperatively (thiopental or etomidate), and IHAST treatment assignment (normothermic *vs.* hypothermic). For analysis purposes, GOS was treated as an ordered categorical variable using all possible responses (1 = minor or no disability, 2 = moderate disability, 3 = severe disability, 4 = vegetative state, 5 = death) and also using a binary response (1 *vs.* others). DIND was treated as a binary response (yes *vs.* no), NIHSS score was analyzed using five ordered categories (0 = no deficit, 1–7 = mild deficit, 8–14 = moderate deficit, 15–42 = severe deficit, death), Rankin score was treated as a binary variable (0–1 = minimal or no deficit, >1 = significant deficit), and Barthel Activities of Daily Living Index was treated as a binary variable (95–100 = minimal to no impairment, <95 = impairment).[17] Specific details related to the scoring of neuropsychological tests can be found elsewhere.[27] Briefly, the results of each test were compared with normative data (adjusted for age, sex, and years of education), with a binary outcome (presence or absence of impairment) determined for each test. For the current report, two binary neuropsychological outcomes are included: impairment for the composite score and impairment on any individual test.

Because the IHAST study was a randomized trial evaluating whether intraoperative hypothermia would improve neurologic outcomes, initial analyses were performed to evaluate whether the effect of the randomized treatment (normothermic *vs.* hypothermic) differed for patients who received nitrous oxide *versus* not. These analyses were performed using models that included nitrous oxide use (no *vs.* yes), IHAST treatment assignment (normothermic *vs.* hypothermic), and the nitrous oxide–by–treatment assignment interaction effect. After confirming that there were no significant interaction effects, subsequent logistic regression models that included nitrous oxide use as the only explanatory variable were used to assess the univariate association of nitrous oxide use on outcomes. Because the explanatory variable of interest for this investigation was nitrous oxide use, the findings from the multiple logistic regression models are summarized by presenting the odds ratio (OR) and corresponding 95% confidence interval (CI) for nitrous oxide use. For all logistic regression analyses, the models are parameterized so that an odds ratio significantly greater than 1.0 would indicate an increased likelihood of a worse outcome in patients receiving nitrous oxide. In all cases, two-sided tests were performed with $P \leq 0.05$ used to denote statistical significance.

## Results

Details regarding the primary IHAST trial results can be found elsewhere.[17,27] To briefly review, between February 2000 and April 2003, 3,966 patients underwent surgery at 30 participating centers. Of these, 2,856 had experienced an acute subarachnoid hemorrhage. Of these patients, 1,183 were eligible, and 1,033 were enrolled. Because of changes in status after enrollment, 32 patients were not randomized, resulting in a total of 1,001 subjects. Three-month GOSs were obtained in 1,000 patients (499 hypothermia, 501 normothermia), and all analysis reported in this article are based on these 1,000 individuals. Sixty-one patients died (29 in the hypothermic group, 32 in the normothermic group). Sixty-six percent of hypothermic patients *versus* 63% of normothermic patients were classified as having "good outcomes" by the GOS (*i.e.*, GOS = 1; $P = 0.32$). Similarly, no significant intergroup differences (*i.e.*, by temperature assignment) were seen in Rankin score, NIHSS score, Barthel Activities of Daily Living Index, or on neuropsychological testing. Three hundred seventy-three patients received nitrous oxide, and the remaining 627 did not. A large degree of variability in nitrous oxide use existed among the 30 testing centers (fig. 1). Specifically, the percentage of cases in which nitrous oxide was used as part of a balanced anesthetic ranged from 0 to 100% among the various centers. Further, at 24 of the 30 centers, nitrous oxide use was outside the interquartile range (*i.e.*, <25% or >75% of cases).

Demographic and intraoperative data as well as baseline neurologic function scores from the two groups are provided in tables 1 and 2. Preoperative medical history (*e.g.*, the incidence of hypertension, diabetes, or smoking), the time from subarachnoid hemorrhage to induction of anesthesia, and various characteristics of the aneurysms (*i.e.*, size, location, and number) were well matched between groups. There were statistically significant, but probably clinically inconsequential, differences

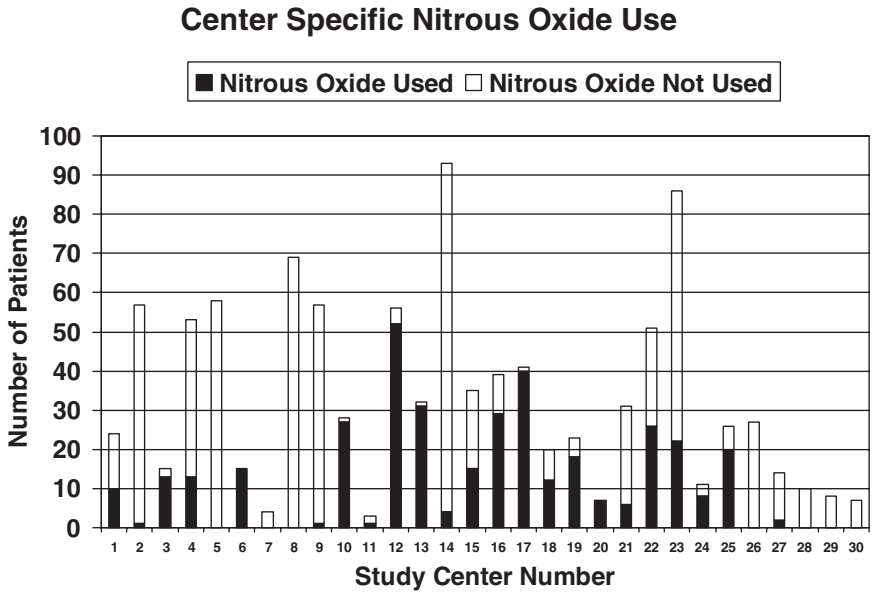


**Fig. 1. Test center–specific nitrous oxide use. The number of cases in which nitrous oxide was (*filled bars*) and was not (*open bars*) used are represented for 30 individual testing centers.**

between groups with regard to many demographic, preoperative neurologic status, and intraoperative data. The average ages were 53 ± 13 and 50 ± 12 yr for the no nitrous oxide and nitrous oxide groups, respectively ($P < 0.001$). There was significantly greater baseline neurologic impairment in patients who did not receive nitrous oxide based on the World Federation of Neurological Surgeons score ($P = 0.006$) and the NIHSS score ($P = 0.001$). There was a difference between groups with regard to the intraoperative use of pharmacologic neuroprotective agents (thiopental or etomidate), 15.9% for no nitrous oxide *versus* 30.3% for nitrous oxide ($P < 0.001$), and use of a temporary clip, 39.1% for no nitrous oxide *versus* 53.6% for nitrous oxide ($P < 0.001$).

The extent of subarachnoid hemorrhage and the interval between aneurysm rupture and surgery were comparable between groups. Specifically, the distribution of Fisher grades was similar (table 1; $P = 0.291$), and the time interval from subarachnoid hemorrhage to surgery was comparable (mean, 3 ± 3 days; median, 2 days; and range, 0–14 days for both the no nitrous oxide group and the nitrous oxide group; $P = 0.088$ for comparison of means).

Postoperative data can be found in table 3. After both univariate and multivariate logistic regression analysis correcting for variables thought to influence outcome, a greater proportion of patients who received nitrous oxide had an intensive care unit duration of stay greater than 5 days (OR, 2.34; 95% CI, 1.72–3.18; $P < 0.001$), but there was no difference in the proportion of patients with a hospital duration of stay of 15 days or more (OR, 1.19; 95% CI, 0.89–1.60; $P = 0.241$). A greater proportion of patients who received nitrous oxide were discharged from the hospital to home *versus* other destinations (*i.e.*, other acute care hospital, chronic care facility, death; OR, 0.62; 95% CI, 0.45–0.86; $P = 0.004$).

Table 4 presents findings from both the univariate and multivariate logistic regression analysis of early (<14 days after surgery) outcome data (DIND) and late (3 months after surgery) outcome data (GOS, Rankin score, Barthel Index, NIHSS score, and psychological data at 3 months after surgery). Overall, a number of patients in each group experienced neurologic deterioration between hospital admission and 3 months after surgery, as has been described previously.[30] Postoperative data revealed that of those patients who received nitrous oxide, 93 of 373 (25%) developed neurologic deterioration with a diagnosis of DIND, and 126 of 627 (20%) patients who did not receive nitrous oxide also experienced a new neurologic deficit diagnosed as DIND (univariate $P = 0.074$; adjusted OR, 1.29; 95% CI, 0.91–1.83; $P = 0.157$) by 14 days after surgery or hospital discharge, whichever came first. Two additional patients in the group that did not receive nitrous oxide experienced DIND during the time interval between hospital discharge and the 3-month assessment. Although the statistical results reported in this article do not reflect these two additional patients, the conclusion does not differ when they are included in data analysis.

From both univariate and multivariate (adjusted) logistic regression analysis, there was no significant association between nitrous oxide use and outcome at 3 months after subarachnoid hemorrhage as measured by GOS (either as a binary variable [univariate $P = 0.073$; adjusted OR, 0.82; 95% CI, 0.60–1.13; $P = 0.222$] or as an ordered categorical variable [univariate $P = 0.066$; adjusted OR, 0.84; 95% CI, 0.63–1.14; $P = 0.268$]), Rankin Disability Score (univariate $P = 0.305$; adjusted OR, 0.84; 95% CI, 0.61–1.15; $P = 0.284$), NIHSS score (univariate $P = 0.335$; adjusted OR, 1.29; 95% CI, 0.96–1.73; $P = 0.087$), or Barthel Index (univariate $P = 0.314$; adjusted OR, 1.01; 95% CI, 0.68–1.51; $P = 0.961$). Fur-

**Table 1. Baseline Characteristics**

| | Nitrous Oxide Use | | |
|---|---|---|---|
| | No | Yes | *P* Value |
| n | 627 | 373 | |
| Demographics | | | |
| Age, mean ± SD, yr | 53 ± 13 | 50 ± 12 | <0.001* |
| % Female | 64.8 | 66.8 | 0.519† |
| % White, not of Hispanic origin | 84.7 | 71.6 | <0.001† |
| Preoperative medical history | | | |
| % with diabetes mellitus | 4.0 | 4.3 | 0.816† |
| % with hypertension | 39.2 | 40.8 | 0.636† |
| % current smokers | 54.4 | 52.5 | 0.572† |
| Preoperative neurologic status | | | |
| Preoperative WFNS score | | | 0.006† |
| % with GCS 15 and no motor deficit or aphasia | 62.8 | 71.3 | |
| % with GCS 13–14 and no motor deficit or aphasia | 31.6 | 24.4 | |
| % with GCS 13–14 and motor deficit or aphasia | 5.6 | 4.3 | |
| NIHSS score at baseline, % | | | 0.001‡ |
| 0 | 50.4 | 58.7 | |
| 1–7 | 41.3 | 31.1 | |
| 8–14 | 1.9 | 3.8 | |
| 15–42 | 1.3 | 0 | |
| Missing | 5.1 | 6.4 | |
| Preoperative Fisher grade, % | | | 0.291† |
| 1 | 5.1 | 5.9 | |
| 2 | 34.8 | 33.2 | |
| 3 | 45.8 | 50.1 | |
| 4 | 14.4 | 10.7 | |
| Interval between subarachnoid hemorrhage and induction of anesthesia, days | | | |
| Mean ± SD | 3 ± 3 | 3 ± 3 | 0.088* |
| Median | 2 | 2 | |
| % of patients with hydrocephalus on first computed tomographic scan | 40.7 | 37.5 | 0.327† |

* Based on Student *t* test, † Based on chi-square test, ‡ Based on Fisher exact test.

GCS = Glasgow Coma Score; NIHSS = National Institutes of Health Stroke Scale; WFNS = World Federation of Neurological Surgeons.

thermore, there was no difference between groups with respect to impairment of at least one neuropsychological test (univariate $P = 0.485$; adjusted OR, 0.81; 95% CI, 0.59–1.10; $P = 0.168$) or on the neuropsychological composite score (univariate $P = 0.103$; adjusted OR, 1.26; 95% CI, 0.85–1.87; $P = 0.252$) measured at 3 months after subarachnoid hemorrhage.

## Discussion

In this *post hoc* investigation involving 1,000 patients having aneurysmal subarachnoid hemorrhage necessitating aneurysm clipping, the intraoperative use of nitrous oxide was not associated with the development of (1) postoperative DIND occurring within 14 days of surgery or hospital discharge (whichever came first), (2) long-term gross neurologic deficits, or (3) long-term neuropsychological dysfunction.

Nitrous oxide has many properties that are theoretically detrimental to the brain at risk for ongoing injury. These properties, evaluated in a variety of *in vitro* studies, animal models, and human studies, include (1) increased cerebral metabolic rate, cerebral blood flow, and intracranial pressure[31–40]; (2) increases in intracranial air volume[41,42]; and (3) later effects on homocysteine and vitamin $B_{12}$ metabolism.[43–47] In a variety of investigations in animals, nitrous oxide exacerbated ischemic brain injury[7,9,48]; however, these effects have never been demonstrated in human studies. Offsetting these potential detriments, nitrous oxide use during procedures in which the brain is at risk for ischemia may pose several advantages. In the setting of neuronal ischemia, glutamate excitotoxicity is known to exacerbate neurologic injury,[49,50] and blockade of *N*-methyl-D-aspartate receptors may attenuate this injury.[51] Nitrous oxide is a known *N*-methyl-D-aspartate receptor antagonist[52,53] and has been shown to reduce infarct size after focal cerebral ischemia in animals.[54,55] Also, nitrous oxide offers the advantage of facilitating rapid emergence from anesthesia.

Our study design provided a rich opportunity to evaluate the clinical manifestations of these detrimental and beneficial processes. The failure of this research to confirm harm by nitrous oxide to the ischemic brain, as reported in some animal models, may be due to a variety of factors. Not all laboratory studies used the accepted standards—*i.e.*, anatomical changes or changes in neurologic function in an intact animal—to assess outcome. Instead, some claims of detriment by nitrous oxide were based on biochemical[56] or electrical markers,[9] both of which may not correlate with functional outcome in an intact mammal.[1,57–59] Further, even in the few whole animal experiments in which accepted standard endpoints were used,[6,9] the studies were performed under highly controlled conditions, using homogeneous study subjects, and short-duration assessment of outcome. Hence, it is not at all surprising that results from our long-term study did not demonstrate the detrimental short-term outcomes reported in some,[5–7,9–11] but not all,[54,55] laboratory experiments.

Our research did evaluate one metric of short-term outcome by nitrous oxide: DIND. This pathologic state has some relevance to nitrous oxide's effects on basal brain metabolism. The amino acid methionine, critical to anatomic and physiologic well-being, is produced *in vivo* by methylation of homocysteine *via* the action of methionine synthase. Nitrous oxide is known to inhibit methionine synthase, resulting in significant increases in plasma homocysteine concentrations.[60] Homocysteine, in turn, is reported to increase production of the platelet-derived vasoconstrictor and aggregant thromboxane $A_2$, aid in the formation of thrombin, and increase neutrophil-endothelial adhesion.[61] Because of these physio-

**Table 2. Aneurysm and Intraoperative Characteristics**

| | Nitrous Oxide Use | | |
|---|---|---|---|
| | No | Yes | *P* Value |
| n | 627 | 373 | |
| Aneurysm characteristics | | | |
| Largest aneurysm diameter, mm | 8.2 ± 5.1 | 7.8 ± 5.0 | 0.248* |
| Aneurysm location, % in anterior circulation of first aneurysm clipped‡ | 92.0 | 90.9 | 0.532† |
| % with one aneurysm treated | 90.6 | 89.0 | 0.420† |
| Intraoperative factors | | | |
| Time from induction of anesthesia to placement of first clip, min | 205 ± 80 | 230 ± 77 | <0.001* |
| Time from placement of last clip to arrival in recovery area, min | 94 ± 36 | 114 ± 33 | <0.001* |
| Temperature, °C | | | |
| On arrival in operating room | 36.9 ± 0.6 | 36.7 ± 0.7 | <0.001* |
| At placement of first clip | 35.0 ± 1.7 | 34.9 ± 1.0 | 0.475* |
| 2 h after surgery | 36.7 ± 1.0 | 36.9 ± 0.9 | 0.001* |
| Temporary clip placement | | | |
| Temporary clips applied, % of cases | 39.1 | 53.6 | <0.001† |
| Temporary clips applied for ≥ 20 min, % of cases | 4.5 | 8 | 0.019† |
| Duration of temporary clipping, min | 9.3 ± 7.7 | 11.9 ± 13.1 | 0.012* |
| % with moderate or severe brain swelling at dural opening | 35.7 | 38.6 | 0.371† |
| % with aneurysm exposure judged difficult or very difficult | 36.3 | 33.8 | 0.417† |
| % in which cerebroprotective drugs were used intraoperatively | 15.9 | 30.3 | <0.001† |
| % in which intraoperative controlled hypotension was used | 5.6 | 2.9 | 0.055† |
| % in which unintended hypotension occurred up to 2 h postoperatively | 4.1 | 2.4 | 0.149† |
| Vasopressor use, % | 22.6 | 17.4 | 0.049† |
| Intraoperative leak or rupture of aneurysm, % | 31.3 | 32.4 | 0.740† |
| Estimated intraoperative blood loss, ml | 419 ± 385 | 427 ± 402 | 0.743* |
| Intraoperative blood loss ≥1,000 ml, % | 5.6 | 7.5 | 0.229† |
| Intraoperative crystalloid administration, ml | 3,632 ± 1,685 | 3,432 ± 1,424 | 0.045* |
| Erythrocyte transfusion, % | 2.1 ± 1.6 | 1.8 ± 1.1 | 0.282* |
| Intraoperative urinary output, ml | 1,934 ± 1,275 | 1,749 ± 981 | 0.010* |
| New cardiac arrhythmia intraoperatively, % | 3.8 | 3.8 | 0.953† |

Largest aneurysm diameter, temperature data, and estimated intraoperative blood loss, crystalloid administration, and urinary output expressed as mean ± SD.

* Based on Student *t* test, † Based on chi-square test, ‡ Anterior aneurysms include those involving the carotid, ophthalmic, anterior choroidal, middle cerebral, anterior communicating, posterior communicating, and anterior cerebral arteries. Posterior aneurysms include those involving the vertebrobasilar and posterior-inferior cerebellar arteries.

logic effects, long-term increased plasma homocysteine concentrations are associated with an increased risk incidence of both coronary artery and cerebrovascular disease.[62–65] It is currently unknown whether short-term elevations in plasma homocysteine concentrations, as may occur with the use of nitrous oxide, have any adverse effects. Further, given that homocysteine is known to promote vasoconstriction (*i.e.*, *via* enhancing the production of platelet-derived thromboxane $A_2$), it is unknown whether short-term exposure to nitrous oxide increases the risk of developing cerebral vasospasm in patients already at risk for this devastating disorder.

In our investigation, the overall incidence of DIND was similar to that reported in the literature[66] and did not differ significantly between nitrous oxide groups (20.1% for no nitrous oxide *vs.* 24.5% for nitrous oxide; $P = 0.157$). The two factors that seem to have the highest association with the development of cerebral vasospasm are the amount of subarachnoid blood and the time interval after subarachnoid hemorrhage.[66] Our study groups did not differ in the amount of subarachnoid blood, as assessed by the Fisher grade at the time of surgery (table 1). Regarding the timing of vasospasm, it is unlikely to develop during the initial 3 days postictus, has a peak incidence at 7 days, and is unlikely to begin beyond 14 days.[66] Hence, given that all patients in this investigation underwent nitrous oxide exposure during surgical intervention a median of 2 days after initial subarachnoid hemorrhage (range, 0–14 days), they were an ideal group for studying the effects of an intervention which can theoretically exacerbate vasospasm. Therefore, within the limits of our study, we conclude that short-term exposure to nitrous oxide did not increase the risk for developing cerebral vasospasm in the overall IHAST population, nor did it influence functional outcome in this population at high risk for developing vasospasm. However, such analysis and conclusions do not preclude the possibility that nitrous oxide may influence the incidence of vasospasm, or the consequences of that vasospasm, in select subpopulations of IHAST patients.

It is clear from years of empirical data that, in the exploration of cerebroprotective agents or cerebrotoxins, results from *in vitro* and animal studies are poorly predictive of outcomes noted from human studies.[1] Hence, nitrous oxide would not be the first intervention

**Table 3. Postoperative Data: Nitrous Oxide *versus* No Nitrous Oxide**

| Metric | No Nitrous Oxide Group | Nitrous Oxide Group | Univariate Analysis *P* Value | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | | | | Odds Ratio | 95% CI | *P* Value |
| Hospital duration, total days | | | | | | |
| n | 622 | 373 | | | | |
| <15 days (%) | 295 (47) | 170 (46) | 0.572 | 1.19 | 0.89–1.60 | 0.241 |
| Duration of intensive care unit stay | | | | | | |
| n | 626 | 373 | | | | |
| <5 days (%) | 326 (52) | 126 (34) | <0.001 | 2.34 | 1.72–3.18 | <0.001 |
| Discharge destination | | | | | | |
| n | 622 | 373 | | | | |
| Discharged to home (%) | 338 (54) | 255 (68) | <0.001 | 0.62 | 0.45–0.86 | 0.004 |

Values in "No Nitrous Oxide Group" and "Nitrous Oxide Group" columns represent numbers of patients (% within group).

*Discharge destination* refers to the facility to which patients were sent upon discharge from the center where surgery was performed and included locations such as the patient's home, another acute care hospital, or chronic/rehabilitation facility.

Both unadjusted (univariate) and adjusted (multivariate) analyses were performed using standard logistic regression for binary outcomes and cumulative logistic regression for ordered categorical outcomes. For the multivariate analysis, the findings are summarized by presenting the odds ratio corresponding to the increased (or decreased) likelihood of the given outcome for patients receiving nitrous oxide compared with patients not receiving nitrous oxide. In all cases, the models are parameterized so that an odds ratio significantly greater than 1.0 would indicate an increased likelihood of a worse outcome in patients receiving nitrous oxide.

The odds ratios are adjusted for treatment assignment (normothermia, hypothermia), age, sex, race (white *vs.* other), baseline World Federation of Neurological Surgeons score, Fisher grade, baseline National Institutes of Health Stroke Scale score (0, 1–7, 8–14, 15–42), aneurysm location (anterior, posterior), aneurysm size, history of hypertension, time from subarachnoid hemorrhage to surgery, and use of cerebroprotective drugs.

known to provide some evidence of effect on outcome in some laboratory models while having a divergent effect on outcome in other models or in the clinical setting. Indeed, studies of treatment with cyanide, glucose, corticosteroids, and other interventions fit this scenario.[4,67–70]

In our analysis of the IHAST data, there was a large difference between the nitrous oxide and no nitrous oxide groups with respect to the use of neuroprotective drugs (*i.e.*, thiopental or etomidate) and a temporary vessel occlusion intraoperatively. One would imagine that these two factors are related. Use of neuroprotective drugs was left up to the discretion of the individual neurosurgical and anesthesia providers, and in almost 80% of cases where these drugs were administered at the time of clipping, the indication was not recorded. In most of the cases where the indication for these drugs was recorded, placement of a temporary vascular clip was planned to remain on the artery for more than 10 min. Although we reported the statistical values with correction for the use of cerebroprotective drugs, we also conducted the analysis without correcting for this technique as a covariate (secondary analysis is not tabulated) and found that the final results were not altered (*i.e.*, no significant difference between groups with respect to all metrics of neurologic function). Such results seem logical because it is likely that ischemic events in our population occur over a long interval of time (*i.e.*, occurring preoperatively, intraoperatively, and postoperatively) and cerebroprotective drugs provide their effects for only a small fraction of this high-risk period. In addition, there was a difference among groups in the fraction of patients in whom a temporary clip was applied to a major artery.

We found that nitrous oxide use among the 30 testing centers was quite variable. Specifically, most centers either used nitrous oxide on most cases (*i.e.*, >75% of cases at 9 centers) or limited the use of nitrous oxide (*i.e.*, <25% of cases at 13 centers, with 7 of 30 centers not using nitrous oxide for any case). This effect is probably multifactorial and may represent local attitudes toward nitrous oxide use in neurosurgical patients in general and also in the extremes of illness severity (*i.e.*, nitrous oxide avoidance or inclusion in patients perceived as having the most severe disease).

Variation of center volume may, in theory, have an impact on outcome. Specifically, patient care teams at centers that perform a great deal of aneurysm clipping cases may be more experienced in dealing with these patients and potential complications, and this may result in improved outcome, compared with centers with a low volume caseload. However, preliminary analysis of the relations between center volume and primary outcome of data from the IHAST trial showed no effect of center volume of the primary outcome measure, GOS at 3 months after treatment (untabulated data).

Local difference may also have had some bearing on the numbers and types of patients who remained in the intensive care unit for care for more than 5 days after surgery. Despite these possible differences in the approach to care in patients having cerebral aneurysm surgery, our research identified no evidence of nitrous oxide effect on either duration of hospital stay or 3-month neurologic function or neuropsychologic outcome. Despite a greater fraction of patients in the group that did not receive nitrous oxide having an intensive care unit duration of stay of less than 5 days, significantly more patients in the nitrous oxide group were dis-

**Table 4. Gross Neurologic and Neuropsychometric Outcome Results: Nitrous Oxide *versus* No Nitrous Oxide**

| Metric | No Nitrous Oxide Group | Nitrous Oxide Group | Univariate Analysis *P* Value | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | | | | Odds Ratio | 95% CI | *P* Value |
| GOS at 3 months: 1 *vs.* >1 | | | | | | |
| n | 627 | 373 | | | | |
| 1: Minor or no disability | 389 (62) | 253 (68) | 0.073 | 0.82 | 0.60–1.13 | 0.222 |
| GOS at 3 months: 1, 2, 3, 4, 5 | | | | | | |
| n | 627 | 373 | | | | |
| 1: Minor or no disability | 390 (62) | 253 (68) | 0.066 | 0.84 | 0.63–1.14 | 0.268 |
| 2: Moderate disability | 140 (22) | 73 (20) | | | | |
| 3: Severe disability | 54 (9) | 28 (8) | | | | |
| 4: Vegetative state | 1 (0) | 0 (0) | | | | |
| 5: Death | 42 (7) | 19 (5) | | | | |
| Rankin score at 3 months: 0 or 1 *vs.* >1 | | | | | | |
| n | 627 | 373 | | | | |
| 0 or 1: Mild or no neurologic disability | 394 (63) | 256 (69) | 0.305 | 0.84 | 0.61–1.15 | 0.284 |
| NIHSS score at 3 months: 0, 1–7, 8–14, >14, death | | | | | | |
| n | 611 | 363 | | | | |
| 0: No deficit | 385 (63) | 212 (58) | 0.335 | 1.29 | 0.96–1.73 | 0.087 |
| 1–7: Mild deficit | 158 (26) | 119 (33) | | | | |
| 8–14: Moderate deficit | 16 (3) | 8 (2) | | | | |
| 15–42: Severe deficit | 10 (2) | 5 (1) | | | | |
| Death | 42 (7) | 19 (5) | | | | |
| Barthel Index at 3 months: 95–100, <95 | | | | | | |
| n | 627 | 372 | | | | |
| 95–100 | 508 (81) | 311 (84) | 0.314 | 1.01 | 0.68–1.51 | 0.961 |
| <95 | 77 (12) | 42 (11) | | | | |
| Death | 42 (7) | 19 (5) | | | | |
| DIND: yes or no | | | | | | |
| n | 627 | 373 | | | | |
| | 126 (20) | 93 (25) | 0.074 | 1.29 | 0.91–1.83 | 0.157 |
| Impairment on ≥1 neuropsychological test: yes or no | | | | | | |
| n | 585 | 354 | | | | |
| | 348 (60) | 203 (57) | 0.485 | 0.81 | 0.59–1.10 | 0.168 |
| Impairment on neuropsychological composite score: yes or no | | | | | | |
| n | 585 | 354 | | | | |
| | 105 (18) | 79 (22) | 0.103 | 1.26 | 0.85–1.87 | 0.252 |

Values in "No Nitrous Oxide Group" and "Nitrous Oxide Group" columns represent numbers of patients (% within group).

Both unadjusted (univariate) and adjusted (multivariate) analyses were performed using standard logistic regression for binary outcomes and cumulative logistic regression for ordered categorical outcomes. For the multivariate analysis, the findings are summarized by presenting the odds ratio corresponding to the increased (or decreased) likelihood of the given outcome for patients receiving nitrous oxide compared with patients not receiving nitrous oxide. In all cases, the models are parameterized so that an odds ratio significantly greater than 1.0 would indicate an increased likelihood of a worse outcome in patients receiving nitrous oxide.

The odds ratios are adjusted for treatment assignment (normothermia, hypothermia), age, sex, race (white *vs.* other), baseline World Federation of Neurological Surgeons score, Fisher grade, baseline National Institutes of Health Stroke Scale score (0, 1–7, 8–14, 15–42), aneurysm location (anterior, posterior), aneurysm size, history of hypertension, time from subarachnoid hemorrhage to surgery, and use of cerebroprotective drugs.

DIND = Delayed Ischemic Neurologic Deficit; GOS = Glasgow Outcome Score; NIHSS = National Institutes of Health Stroke Scale.

charged to home. The reasons for this are unclear but may be related to differences in the initial patient population before surgery. Specifically, those in the nitrous oxide group were significantly younger and had better World Federation of Neurological Surgeons and NIHSS scores (table 1). Further, our finding may also reflect difference in practices among the various study centers.

There are several elements of our study design that deserve comment. Our study evaluated long-term neurologic function using a time frame far beyond that of laboratory studies, and using established tools for neurologic outcome assessment. Further, our study evaluated a large number of patients at demonstrated risk for perioperative and postoperative neurologic injury.

Offsetting these benefits was the fact that ours was a *post hoc* analysis and, because nitrous oxide was not randomized, covariate adjustments were necessary. Given the *post hoc* nature of the current analysis, the potential for a type II statistical error exists since the effective sample sizes (*i.e.*, number of patients receiving *vs.* not receiving nitrous oxide) were not determined as part of the study design. The sample size for IHAST (n = 1,000, with approximately 500 per treatment group) was selected to permit detection of a 10% point difference in GOS between groups with statistical power of 91% using a two-sided, $\alpha = 0.05$ level test.[17] The analyses presented in the current report compare patients who received nitrous oxide (n = 373) *versus* those who

did not receive nitrous oxide (n = 627). In general, when comparing two groups with a total sample size of n = 1,000, the statistical power provided by effective sample sizes of n = 373 and n = 627 is only slightly less than the statistical power provided with sample sizes of n = 500 in each group. For example, to detect a 10% point difference between groups for the GOS outcome (60% *vs.* 70%), sample sizes of n = 500 in each group will provide statistical power of 91%, whereas sample sizes of n = 373 and n = 627 will provide statistical power of 89%. Therefore, the statistical power for the current *post hoc* analysis is only slightly less than that provided in the original investigation, which was assumed to have sufficient statistical power to detect clinically relevant differences between treatment groups.

Of more concern is the fact that nitrous oxide use was not randomized and, therefore, a number of potential biases may impact the findings. To address this concern, we used a multivariate analysis to compare groups after adjusting for a number of covariates. However, the possibility of bias still exists. For example, baseline neuropsychological testing was not conducted on subjects before surgery, so it is unknown whether differences in cognitive function existed between nitrous oxide groups before surgery. In a study of this design, baseline neuropsychological testing is not feasible, nor would testing in the acute postsubarachnoid hemorrhage period provide a meaningful baseline for subsequent comparison.

Our research also had limited ability to examine the possibility that, within the overall IHAST patient population, there were subgroups of patients who might experience an enhanced effect of nitrous oxide. For example, in the subset of patients who had temporary vessel occlusion before clipping a cerebral aneurysm, a combination of transient nitrous oxide–associated increases in serum homocysteine concentrations plus any direct arterial trauma due to vessel occlusion might predispose patients to a worse outcome. However, performing analyses to assess for potential interaction effects of nitrous oxide use and other patient or procedural characteristics is problematic. As the number of variables added to the original matrix for our nitrous oxide analysis increases, the power of that analysis decreases, and our ability to make any meaningful conclusions regarding our original hypothesis are diminished. Further, attempts to single out subgroups independent of a larger matrix analysis and subject those subgroups to the same type analysis used in the current investigation (*e.g.*, as might occur with nitrous oxide use or nonuse in patients having temporary vessel occlusion) are problematic. Specifically, repeating the analysis on the smaller IHAST subgroups introduces its own problems with decreased statistical power and an increased likelihood of type I error due to multiple comparisons. Despite this, at the completion of the aforementioned research, we indeed performed an analysis of the 445 patients who had temporary clip placement, 200 of whom received nitrous oxide and 245 of whom did not. The results of this subgroup analysis (untabulated data) were not meaningfully different from the results of the overall analysis of IHAST patients. Because of the complexities of reporting and interpreting such subgroup analyses related to nitrous oxide use, they are the subject of ongoing research.

Although the IHAST study was designed to investigate the effect of hypothermia on outcome in cerebral aneurysm patients, it was also intended that the data would be used to answer other questions pertaining to the treatment of this disease. Further, we used a patient population in which both nitrous oxide groups had been divided into treatments of either intraoperative hypothermia or normothermia. Although the original IHAST study found no hint of modification of outcome by temperature management,[17] we nevertheless cannot conclusively rule out a temperature–and–nitrous oxide interaction on our data. Unfortunately, definitive answers to these questions would require a new trial of a size and expense similar to that of IHAST—something that is highly unlikely given these negative findings.

In summary, the current analysis of long-term neurologic outcome in 1,000 patients having general anesthesia for surgical treatment of aneurysmal subarachnoid hemorrhage found that the use of nitrous oxide was unrelated to neurologic outcome. Although our study had several methodologic limitations that may inhibit a direct comparison with prospective randomized laboratory studies, it is nevertheless the largest and most clinically relevant investigation to date to determine whether nitrous oxide affects outcome in the brain at risk for ongoing ischemic injury. Unless our results are refuted by other human trials with stronger study designs than ours, or in other patient populations, we conclude that there is no scientific evidence for categorically avoiding nitrous oxide in the patient at risk for ischemic brain injury.

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## Appendix

### *Members of the Intraoperative Hypothermia for Aneurysm Surgery Trial*

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