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The safety of addition of nitrous oxide to general anaesthesia in at-risk patients having major non-cardiac surgery (ENIGMA-II): a randomised, single-blind trial

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Summary

Background Nitrous oxide is commonly used in general anaesthesia but concerns exist that it might increase perioperative cardiovascular risk. We aimed to gather evidence to establish whether nitrous oxide affects perioperative cardiovascular risk.

Methods We did an international, randomised, assessor-blinded trial in patients aged at least 45 years with known or suspected coronary artery disease having major non-cardiac surgery. Patients were randomly assigned via automated telephone service, stratified by site, to receive a general anaesthetic with or without nitrous oxide. Attending anaesthetists were aware of patients' group assignments, but patients and assessors were not. The primary outcome measure was a composite of death and cardiovascular complications (non-fatal myocardial infarction, stroke, pulmonary embolism, or cardiac arrest) within 30 days of surgery. Our modified intention-to-treat population included all patients randomly assigned to groups and undergoing induction of general anaesthesia for surgery. This trial is registered at ClinicalTrials.gov, number NCT00430989.

Findings Of 10 102 eligible patients, we enrolled 7112 patients between May 30, 2008, and Sept 28, 2013. 3543 were assigned to receive nitrous oxide and 3569 were assigned not to receive nitrous oxide. 3483 patients receiving nitrous oxide and 3509 not receiving nitrous oxide were assessed for the primary outcome. The primary outcome occurred in 283 (8%) patients receiving nitrous oxide and in 296 (8%) patients not receiving nitrous oxide (relative risk 0·96, 95% CI 0·83–1·12; p=0·64). Surgical site infection occurred in 321 (9%) patients assigned to nitrous oxide, and in 311 (9%) patients in the no-nitrous oxide group (p=0·61), and severe nausea and vomiting occurred in 506 patients (15%) assigned to nitrous oxide and 378 patients (11%) not assigned to nitrous oxide (p<0·0001).

Interpretation Our findings support the safety profile of nitrous oxide use in major non-cardiac surgery. Nitrous oxide did not increase the risk of death and cardiovascular complications or surgical-site infection, the emetogenic effect of nitrous oxide can be controlled with antiemetic prophylaxis, and a desired effect of reduced volatile agent use was shown.

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Introduction

At least 5% of the 230 million people worldwide who have major surgery each year will have a major perioperative cardiovascular complication. These complications prolong hospital stay, are a threat to disability-free survival, and greatly increase health-care costs,[1] contributing an estimated US$20 billion to costs for hospital care and long-term care annually in the USA alone.[2] The postoperative period is associated with increased myocardial oxygen demand,[3] hypotension, and a procoagulant state.[4] Patients with coronary artery disease are at high risk of cardiovascular complications in this setting.

Nitrous oxide is a commonly used anaesthetic that has been given to billions of patients in the past 150 years. That nitrous oxide increases the risk of postoperative nausea and vomiting is well established, but whether it causes more serious complications is unclear. Concern persists because nitrous oxide increases postoperative plasma homocysteine concentrations and impairs endothelial function.[5–8] Both consequences are exposure-dependent and are probably greater in at-risk patients.[8]

Chronic hyperhomocysteinaemia is associated with cardiovascular disease, but efforts to decrease this risk by reduction of homocysteine concentrations have had mixed results.[9–15] Whether nitrous oxide is associated with myocardial injury during and after surgery is uncertain.[5] In our previous multicentre trial—the Evaluation of Nitrous Oxide in the Gas Mixture for Anaesthesia (ENIGMA) trial[16]—we observed a non-significant increase (from 0·7% to 1·3%, p=0·26) in ischaemic cardiac complications within 30 days of surgery,[16] and a significant increase in late myocardial

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infarction (from 4·5% to 6·4%, p=0·04; median follow-up 3·5 years)[17] in patients receiving nitrous oxide. However, the ENIGMA trial was not designed to assess cardiovascular complications, and enrolled only 2050 patients, most of whom were not at high risk of a cardiovascular complication. The trial was thus underpowered for cardiovascular outcomes.[18]

The aim of the present trial (ENIGMA-II) was to establish whether addition of nitrous oxide to the anaesthetic regimen would increase occurrence of death and cardiovascular complications in at-risk patients having non-cardiac surgery.

Methods

Study design and participants

We have published the design and rationale of the prospective, multicentre, international randomised ENIGMA-II trial.[19] The study was approved by the ethics committee at each site. The steering committee members vouch for the accuracy of the dataset, and adherence to the protocol and analysis plan.

Eligible participants included adults aged at least 45 years who were at risk of cardiovascular complications and who were having non-cardiac surgery under general anaesthesia that was expected to last more than 2 h. Cardiac risk factors included a history of coronary artery disease, heart failure, cerebrovascular disease, or peripheral vascular disease, or older age (≥70 years) with other comorbidities;[19] complete details are reported in the online appendix. Because nitrous oxide administration precludes a high inspired oxygen concentration, we excluded patients in whom intraoperative supplemental oxygen administration was planned, including those having thoracic surgery requiring one-lung ventilation, and patients with substantially impaired gas exchange. We also excluded patients at high risk of postoperative emesis. Written informed consent was provided by all patients who agreed to participate.

Randomisation and masking

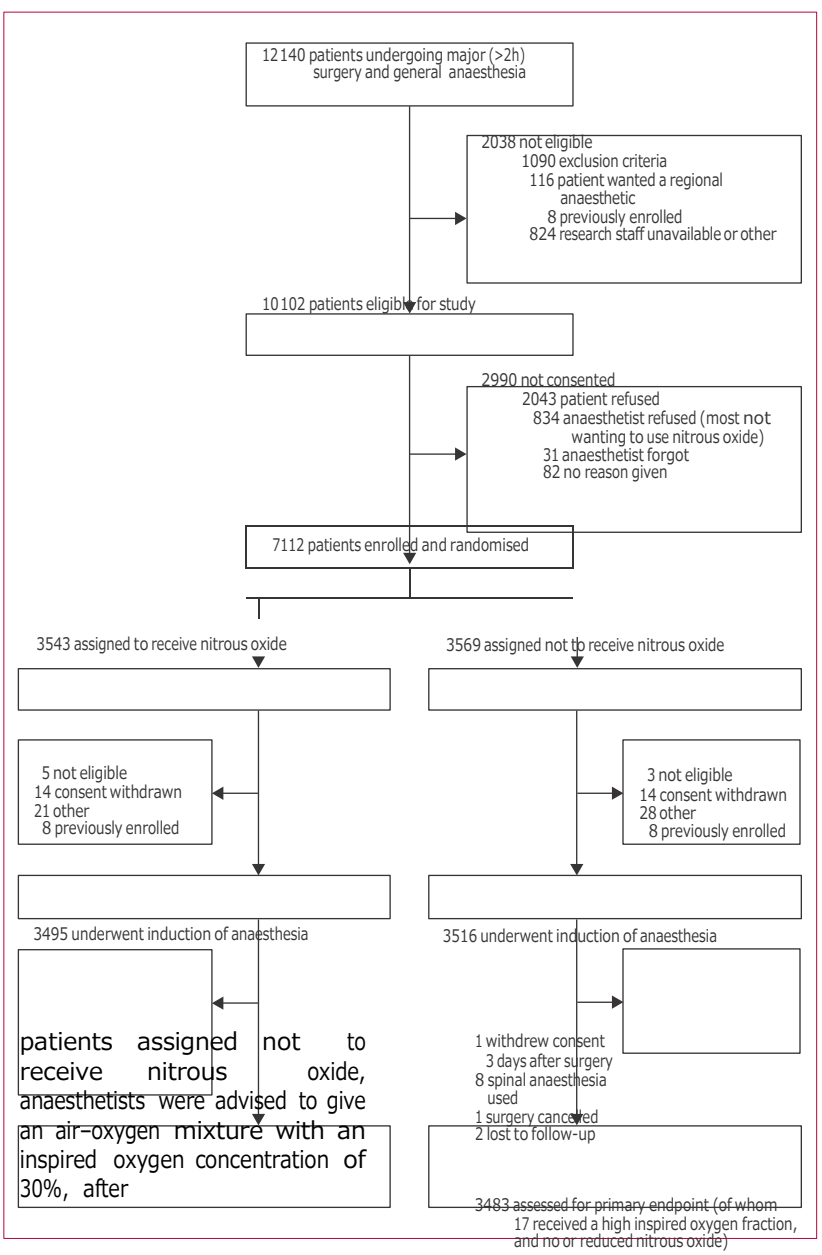
Patients were randomly assigned to receive a general anaesthetic with or without nitrous oxide. Randomisation was done with a computer-generated code, accessed via an automated telephone voice-recognition service. Treatment assignment was stratified by site with permuted blocks. The attending anaesthetists were aware of the patients' group assignments, but the patients, their surgical team, the postoperative interviewers, and endpoint adjudicators were not.

Procedures

For patients assigned to receive nitrous oxide, anaesthetists were advised to give nitrous oxide at an inspired concentration of 70% in 30% oxygen, and for patients assigned not to receive nitrous oxide, anaesthetists were advised to give an air–oxygen mixture with an inspired oxygen concentration of 30%, after induction of anaesthesia and tracheal intubation or laryngeal mask insertion, and until completion of surgery. Arterial desaturation was treated at the anaesthetist's discretion with any airway or ventilatory manoeuvre, including increase of the inspired oxygen concentration.

All patients otherwise received standard anaesthetic and other perioperative care. Anaesthetic depth was adjusted according to clinical judgment; this could include the use of bispectral index monitoring (Covidien, CO, USA) or entropy monitoring (Datex-Ohmeda, Helsinki, Finland). Neuraxial or other regional anaesthetic techniques could be added to general

See Online for appendix



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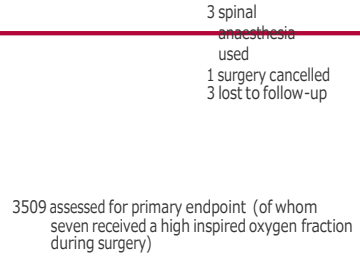

Figure 1: **Trial profile**

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anaesthesia. Anaesthetists were expected to give prophylactic antibiotics as per local routine practice and were advised to avoid intraoperative hypothermia.[20] Patients were reviewed daily while in hospital and were

	Nitrous oxide (n=3495)	No nitrous oxide (n=3516)
Age (years)	69·2 (9·8)	69·5 (9·7)
Age >65 years	2313 (66%)	2359 (67%)
Men	2242 (64%)	2217 (63%)
Bodyweight (kg) - mean	78·3 (20·1)	77·7 (19·1)
Body-mass index (kg/m²)	27·9 (6·5)	27·8 (6·1)
Ethnicity		
White	2587 (74%)	2630 (75%)
Asian	706 (20%)	701 (20%)
Indian/Pakistani	63 (2%)	57 (2%)
Black	19 (1%)	11 (<1%)
Other	43 (1%)	50 (1%)
ASA physical status score		
I	15 (<1%)	11 (<1%)
II	1068 (31%)	1109 (32%)
III	2180 (62%)	2133 (61%)
IV	232 (7%)	262 (8%)
Good exercise capacity ≥4 METS	2620 (75%)	2659 (76%)
Modified nausea and vomiting risk		
0	280 (8%)	229 (7%)
1	1212 (35%)	1199 (34%)
2	1415 (40%)	1551 (44%)
3	571 (16%)	515 (15%)
4	9 (0%)	19 (1%)
Pre-existing medical conditions		
Hypertension	2941 (84%)	2994 (85%)
Coronary artery disease	1257 (36%)	1309 (37%)
Heart failure	268 (9%)	276 (8%)
Previous myocardial infarction	733 (21%)	768 (22%)
Previous CABG or PCI	777 (22%)	825 (24%)
Peripheral vascular disease	1201 (34%)	1213 (35%)
Previous stroke or TIA	637 (18%)	627 (18%)
Hypercholesterolaemia (≥6·2 mmol/L)	1950 (56%)	2018 (57%)
Current smoker (≤6 weeks)	686 (20%)	622 (18%)
Chronic obstructive lung disease/asthma	600 (17%)	645 (18%)
Diabetes	1310 (38%)	1270 (36%)
Current infection or fever	130 (4%)	156 (4%)
Other	1257 (36%)	1422 (40%)
Dietary factors		

	Nitrous oxide (n=3495)	No nitrous oxide (n=3516)
(Continued from previous column)		
Clopidogrel	195 (6%)	188 (5%)
Ticlopidine	9 (0%)	8 (0%)
Warfarin	188 (5%)	174 (5%)
Cyclo-oxygenase-II inhibitor	92 (3%)	89 (3%)
Nitrates	318 (9%)	320 (9%)
Statin	2227 (64%)	2260 (64%)
ACE-inhibitor or angiotensin-receptor blocker	1937 (55%)	1968 (56%)
Amiodarone	41 (1%)	50 (1%)
β blocker	1347 (39%)	1347 (38%)
Heparin or low-molecular-weight-heparin	281 (8%)	277 (8%)
Diuretics	819 (23%)	853 (24%)
Calcium-channel blocker	1148 (33%)	1207 (34%)
Digoxin	103 (3%)	112 (3%)
Insulin	359 (10%)	341 (10%)
Oral hypoglycaemic	928 (27%)	906 (26%)
Current antibiotic therapy	274 (8%)	287 (8%)
Preoperative laboratory tests		
Blood glucose (mmol/L)	7·0 (2·5)	6·9 (2·6)
Haemoglobin (g/L)	131 (19)	131 (19)
Creatinine (mmol/L)	84 (70–105)	85 (70–106)
Dipyridamole-thallium scan	402 (12%)	389 (11%)
If positive, fixed defect*	103 (26%)	94 (24%)
Reversible defect*	133 (33%)	116 (30%)
Type of surgery		
Colorectal	165 (5%)	174 (5%)
Gastrointestinal (non-colorectal)	549 (16%)	521 (15%)
Neurosurgery-spinal	280 (8%)	280 (8%)
Urology-renal	289 (8%)	312 (9%)
Orthopaedic	483 (14%)	481 (14%)
Gynaecology	166 (5%)	151 (4%)
Ear, nose, throat, or faciomaxillary	102 (3%)	101 (3%)
Vascular	1348 (39%)	1369 (39%)
Plastics	50 (1%)	45 (1%)
Other	67 (2%)	82 (2%)
Elective	3357 (96%)	3370 (96%)
Contaminated or dirty (infected)	635 (18%)	672 (19%)
Pre-induction monitoring		

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Vegan or vegetarian	50 (1%)	54 (2%)
Folate or other vitamin B supplementation	654 (19%)	620 (18%)
Vitamin B_{12} (daily oral, or injection ≤3 months)	108 (3%)	94 (3%)
Preoperative medications		
Aspirin within 5 days	1466 (42%)	1459 (42%)
NSAID (excluding aspirin)	168 (5%)	164 (5%)

(Table 1 continues in next column)

Heart rate (beats per min)	73 (14)	73 (14)
Systolic blood pressure (mm Hg)	149 (26)	150 (26)
Duration of surgery (h)	2·6 (1·9–3·7)	2·6 (1·9–3·6)
Duration of anaesthesia (h)	3·2 (2·4–4·4)	3·2 (2·4–4·4)

Data are mean (SD), n (%), or median (IQR). ASA=American Society of Anesthesiologists. METS=metabolic equivalents. CABG=coronary artery bypass graft surgery. PCI=percutaneous coronary intervention. TIA=transient ischaemic attack. NSAID=non-steroidal anti-inflammatory drug. ACE=angiotensin converting enzyme. *Percentage of all scans.

Table 1: **Baseline characteristics of the patients at entry**

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contacted by telephone at 30 days after surgery to ascertain whether they had had any of the prespecified outcomes. Additionally, patients' medical records were reviewed. When outcomes or adverse events were detected, further testing or clinical review was arranged. We recorded patient demographic and perioperative data. The American Society of Anesthesiologists' (ASA) physical status classification (ASA I to IV) score was used to indicate perioperative risk. The risk of postoperative nausea or vomiting was estimated with a modification of validated criteria,[21] which resulted in a score of 0 (low risk) to 4 (high risk).

A 12 lead electrocardiograph was recorded pre-operatively and on days 1 and 3 after surgery. Blood for troponin (or, if unavailable, creatine kinase-myocardial band) measurement was collected at 6–12 h after surgery and on the first 3 postoperative days. Other laboratory tests were ordered if clinically indicated.

Outcomes

We devised a statistical analysis plan with a hierarchical list of prespecified endpoints and published it on a public trial website before completion of the trial (appendix). A masked endpoint adjudication committee assessed all major study outcomes.

The primary outcome of the study was a composite of death and cardiovascular events (non-fatal myocardial infarction, cardiac arrest, pulmonary embolism, and stroke) during the initial 30 postoperative days. Postoperative myocardial infarction was defined according to the third universal definition,[22] requiring raised cardiac biomarker plus at least one of: ischaemic symptoms, pathological Q waves, electrocardiographic changes indicative of ischaemia, coronary artery intervention, or new wall motion abnormality on echocardiography or scanning; or autopsy finding of myocardial infarction. The threshold for significant raised troponin was the local laboratory's 99th percentile of a healthy reference population (upper reference limit), according to recent recommendations.[23]

The prespecified secondary endpoints were non-fatal myocardial infarction and surgical-site infection. Tertiary endpoints were all-cause mortality, stroke, pulmonary embolism, cardiac arrest, severe postoperative nausea and vomiting, duration of stay in the postanaesthesia care unit, unplanned admission to the intensive care unit (ICU), duration of mechanical ventilation, duration of hospital stay, and overall quality of recovery. Explanatory endpoints included the incidence of myocardial ischaemia, fever, need for myocardial revascularisation, and troponin increase at any time during the first 3 postoperative days.[19,23] We recorded adverse events.

Severe nausea and vomiting was defined by the occurrence of at least two episodes of severe nausea or vomiting more than 6 h apart, or if the patient needed more than two doses of any anti-emetic drug. Patient quality of recovery after surgery was measured with a validated nine-item scale score (0=poor recovery to 18=excellent recovery) on the morning after surgery.[24]

Statistical analysis

Using a type I error of 0·05 and a type II error of 0·1, the ENIGMA-II trial needed 7000 patients to detect a clinically important reduction in the primary outcome of death and cardiovascular events from 6% to 8%.

Our modified intention-to-treat population included all patients randomly assigned to groups and undergoing induction of general anaesthesia for surgery. 30 day follow-up was completed for more than 99·7% of patients, and reported results are therefore based on all completed cases without imputation for missing data. The principal analysis produced unadjusted risk ratios with 95% CI using binary regression with a logarithmic link, with the group not

For the **statistical analysis plan** see http://www.enigma2.org.au

	Nitrous oxide (n=3495)	No nitrous oxide (n=3516)	p value
Inspired oxygen concentration	30 (30–36)	33 (30–40)	<0·0001
Bispectral index or entropy monitoring	1396 (40%)	1403 (40%)	0·92
Anaesthetic drugs			
Midazolam	1836 (53%)	1883 (54%)	0·43
Fentanyl	2850 (82%)	2865 (82%)	0·88
Morphine	1622 (47%)	1636 (47%)	0·98
Ketamine	154 (4%)	168 (5%)	0·48
Other opioid	1007 (29%)	1071 (31%)	0·14
Propofol			
Induction	3312 (95%)	3338 (95%)	0·91
Maintenance	111 (3%)	111 (3%)	>0·99
Thiopental	44 (1%)	36 (1%)	0·37
Etomidate	99 (3%)	97 (3%)	0·89
β blocker	355 (10%)	349 (10%)	0·75
Clonidine	173 (5%)	141 (4%)	0·10
End-tidal volatile concentration (MAC equivalents)	0·56 (0·44–0·70)	0·87 (0·72–1·0)	<0·0001
Regional local anaesthetic block	934 (27%)	970 (28%)	0·44
Prophylactic antiemetic used	2088 (60%)	1934 (55%)	<0·0001
Prophylactic antibiotic(s) used	3324 (95%)	3365 (96%)	0·35
Intraoperative haemodynamic monitoring lowest value			
Heart rate (beats per min)	52 (47–60)	54 (48–61)	<0·0001
Systolic blood pressure (mm Hg)	90 (80–98)	88 (80–95)	0·001
Oxygen saturation (%)	96 (95–98)	97 (95–98)	<0·0001
Intraoperative haemodynamic monitoring highest value			
Heart rate (beats per min)	80 (70–90)	80 (70–90)	0·20
Systolic blood pressure (mm Hg)	154 (140–171)	153 (140–170)	0·96
Oxygen saturation (%)	99 (98–100)	99 (99–100)	<0·0001
Body temperature at wound closure (°C)	36·3 (1·3)	36·3 (1·3)	0·76
Mechanical ventilation in PACU (h)	2 (1–3)	1 (1·0–4·0)	0·42
Blood glucose in PACU (mmol/L)	8·2 (2·5)*	8·1 (2·5)†	0·11

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Data are median (IQR), n (%), mean (SD), or p value. P values calculated by either a chi-squared test or a Wilcoxon Rank Sum test. MAC=minimum alveolar concentration, a measure of anaesthetic volatile drug potency; the MACs of sevoflurane, isoflurane, and desflurane are 1·80, 1·15, and 6·0, respectively. PACU=post-anaesthesia care unit. *n=29. †n=28.

Table 2: **Anaesthetic and other intraoperative procedures**

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receiving nitrous oxide as the reference category. Duration of hospital stay was analysed with Cox regression with censoring at 30 days, and in-hospital deaths assigned the highest length of stay.

The total duration of ventilation in the ICU was calculated as a proxy measure for ICU stay, with censoring at 720 h (30 days) and death in the ICU assigned the highest rank in a Wilcoxon censored rank test. Adverse event severity was analysed using ordinal logistic regression. Other secondary endpoints were compared with χ^2 tests for binary outcomes or Wilcoxon rank sum tests for continuous outcomes. We assessed differences in the primary endpoint across specified subgroups by adding a treatment-by-subgroup interaction term to the binary regression models. We did this separately for each subgroup factor. All analyses were done with Stata version 12.1. All reported p values are two-sided and not adjusted for multiple comparisons.

A steering committee provided oversight of the trial, a data quality committee monitored compliance and completeness of the data, and a data and safety monitoring committee advised on whether the trial should be stopped because of clear evidence of benefit or harm.[19] Interim analyses were done after enrolment of 3000 and 5000 patients, adjusted according to an O'Brien and Fleming type I error spending function. The roles and responsibilities of each committee were defined by charters. An independent endpoint adjudication committee, whose members were unaware of the group assignments, reviewed all primary outcome events and sought confirmation of surgical-site infection according to established definitions.[19,22,25] Sites recruiting 35 or more participants were independently audited to review a patient consent, and endpoints using source documents. No discrepancies were identified during the audits.

The study was registered with ClinicalTrials.gov number NCT00430989.

	Nitrous oxide (n=3483)	No nitrous oxide (n=3509)	Risk ratio (95% CI)†	p value
Primary endpoint	283 (8%)	296 (8%)	0·96 (0·83–1·12)	0·64
Death	42 (1%)	57 (2%)	0·74 (0·50–1·11)	0·14
Myocardial infarction	215 (6%)	219 (6%)	0·99 (0·82–1·19)	0·91
Stroke	26 (1%)	19 (1%)	1·38 (0·76–2·49)	0·29
Cardiac arrest	15 (0%)	19 (1%)	0·80 (0·40–1·56)	0·51
Pulmonary embolism	18 (1%)	22 (1%)	0·82 (0·44–1·53)	0·54
Myocardial ischaemia (intraoperative or postoperative within 3 days of surgery)	311 (9%)	325 (9%)	0·96 (0·83–1·12)	0·63
Surgical-site infection	321 (9%)	311 (9%)	1·04 (0·90–1·21)	0·61
Raised troponin, exceeding the upper reference limit				
Day 1	349 (11%)	381 (12%)	0·93 (0·81–1·06)	0·94
Day 2	485 (16%)	473 (16%)	1·03 (0·92–1·16)	0·86
Day 3	446 (16%)	463 (17%)	0·97 (0·86–1·09)	0·92
Severe nausea or vomiting within 3 days of surgery	506 (15%)	378 (11%)	1·35 (1·19–1·53)	<0·0001
Day 1	387 (11%)	267 (8%)	1·46 (1·26–1·70)	<0·0001
Day 2	150 (4%)	115 (3%)	1·31 (1·04–1·67)	0·025
Day 3	109 (3%)	93 (3%)	1·18 (0·90–1·55)	0·24
Fever (≥38°C) within 3 days of surgery	537 (15%)	547 (16%)	0·99 (0·89–1·11)	0·86
Day 1	258 (7%)	301 (9%)	0·86 (0·74–1·01)	0·07
Day 2	277 (8%)	282 (8%)	0·99 (0·84–1·16)	0·89
Day 3	182 (6%)	178 (5%)	1·03 (0·84–1·26)	0·79
Myocardial revascularisation (PCI or CABG)	27 (1%)	32 (1%)	0·85 (0·51–1·42)	0·53
Day of surgery				
PACU stay (h)	1·9 (1·3–3·1)	1·9 (1·3–3·2)	..	0·88
Admitted to HDU	210 (6·0)*	224 (6·4)†	..	0·55
Admitted to ICU	379 (11)*	395 (11)†	..	0·62
Duration of mechanical ventilation (h)	2·2 (0·3–19)	4·6 (0·3–22)	..	0·12

Role of the funding source

The funding source had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to the primary data and have final responsibility to submit for publication.

Results

The 45 participating centres from ten countries in the ENIGMA-II Trial enrolled patients between May 30, 2008, and Sept 28, 2013. Of 10 102 eligible patients, 7112 patients were enrolled and randomly assigned: 3543 to receive nitrous oxide and 3569 not to receive nitrous oxide; 7011 patients underwent induction of anaesthesia and were included at baseline, and 6992 patients were assessed for the primary endpoint (figure 1). Study patient mean age was 69 years, and about two-thirds of patients were men. 62% of patients were classified as ASA III and 7% as ASA IV. The median duration of anaesthesia was 3·2 h. Demographic, dietary, medical, and perioperative characteristics at baseline were similar between groups (table 1). The median inspired oxygen concentration was 30% (IQR 30–36) in patients assigned to nitrous oxide and 33% (IQR 30–40) in those not assigned to nitrous oxide (table 2). 17% of patients in each group were admitted to the ICU or high-dependency unit immediately after surgery. Some differences were recorded in

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Unplanned ICU admission	94 (2·7) ‡	113 (3·2)§	0·84 (0·64–1·10)	0·20
Hospital stay (days)	6·1 (3·3–10) ‡	6·1 (3·3–10)§	..	0·91

Data are n (%) or median (IQR). CABG=coronary artery bypass graft surgery. PCI=percutaneous coronary intervention. PACU=post-anaesthesia care unit. HDU=high-dependency unit. ICU=intensive care unit. *n=3052. †n=3069. ‡n=421. §n=439.

Table 3: **Outcomes**

anaesthetic drug administration as a result of inclusion of nitrous oxide in the inspired gas mixture (table 2).

Death or cardiovascular complications occurred within the first 30 days after surgery in 283 patients (8%) in patients assigned to nitrous oxide and 296 (8%) patients not assigned to receive nitrous oxide (relative risk [RR] for the nitrous group 0·96, 95% CI 0·83–1·12; p=0·64;

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	Nitrous oxide (n=3483)	No nitrous oxide (n=3509)	p value
Any adverse event	624 (17%)	561 (16%)	0·27*
Mild	103 (3%)	104 (3%)	..
Moderate	249 (7%)	215 (6%)	..
Severe	239 (7%)	239 (7%)	..
Category			
Respiratory	103	93	..
Gastrointestinal	106	85	..
Cardiovascular	90	25	..
Urology	72	73	..
Renal failure/dysfunction	13	3	..
Hepatic dysfunction	2	7	..
Vascular	50	38	..
Bleeding complications	54	56	..
Skin	5	1	..
Deep vein thrombosis	18	13	..
Musculoskeletal/arthritis	13	12	..
Sepsis	17	16	..
Lymphatic/oedema	4	4	..
Anaesthetic complications	10	11	..
Unplanned reoperation	15	12	..
Anaemia	19	12	..
Anaphylaxis	1	1	..
Metabolic or electrolyte disturbance	1	13	..
Hospital readmission	16	23	..

Data are n%. *Ordinal logistic regression p value.

Table 4: **Adverse events**

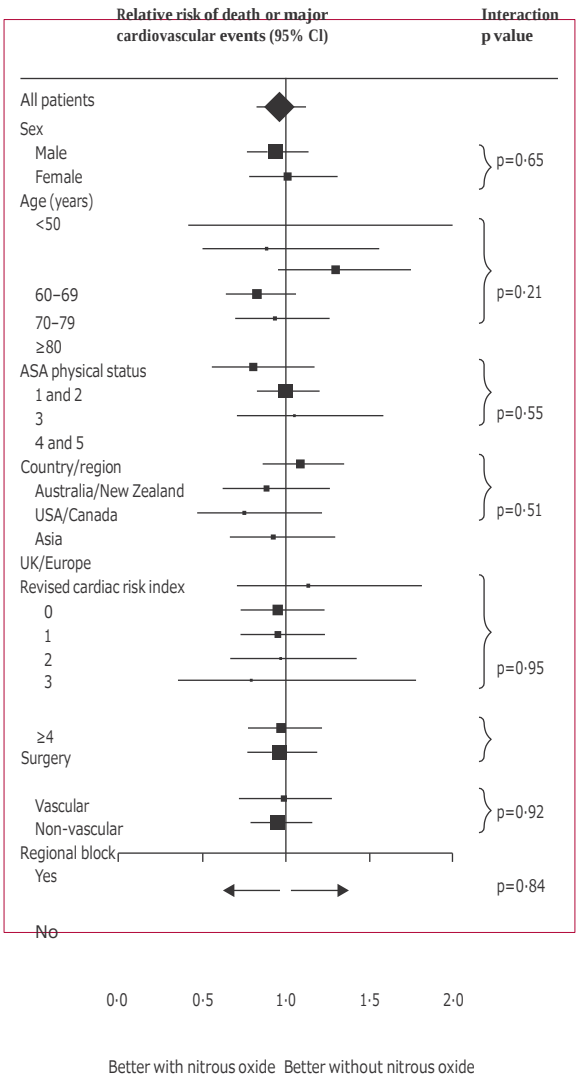

Figure 2: **Relative risk for the primary endpoint (death and cardiovascular complications) associated with use of nitrous oxide in selected subgroups**
ASA=American Society of Anesthesiologists.

table 3). As-treated and per-protocol analyses were not meaningfully affected because of the small fraction of treatment group crossovers (data not shown).

6692 (95%) of patients had at least one electrocardiogram on days 1 or 3 after surgery. Rates of myocardial infarction and surgical-site infection were similar in both groups (table 3). The appendix shows the estimated risks of surgical-site infection for prespecified subgroups.

More patients in the group assigned to receive nitrous oxide than in the group assigned not to receive nitrous oxide had severe nausea or vomiting (table 3). This emetogenic effect was less apparent in those who received prophylactic antiemetics before the end of surgery (RR 1·12, 95% CI 0·95–1·32) than in those who did not, (RR 1·75, 95% CI 1·43–2·13; interaction p=0·001). Mean patient-rated quality of recovery scores were lower in the nitrous oxide group (12·7 [SD 3·3]) than in the no-nitrous oxide group (13·0 [3·3]); p<0·0001.

The proportion of patients who were admitted from the postoperative surgical ward to intensive care, and the cumulative duration of mechanical ventilation in patients admitted to intensive care, were similar between groups

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(table 3). The median hospital stay was about 6 days in both groups (table 3). The appendix shows time-to- discharge curves. The duration of postanaesthesia care unit stay, proportion of patients with postoperative fever, and proportion of patients having adverse events were similar in both groups (tables 3 and 4). No significant interactions were recorded for the primary outcome between treatment group and patient sex, age, ASA physical status, country or region, revised cardiac risk index, and types of surgery (vascular *vs* non-vascular; figure 2).

Discussion

In patients having general anaesthesia for major non-cardiac surgery, addition of nitrous oxide to the gas mixture did not affect the risk of death and major cardiovascular complications (panel). Nitrous oxide did not increase the risk of surgical-site infection, but, consistent with previous studies,[16,26] we noted an increased risk of severe postoperative nausea and vomiting with nitrous oxide administration. Quality of

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Panel: **Research in context**

Systematic review

We searched Medline and the Cochrane controlled trial register (search last done March 16, 2014) for original research and meta-analyses from the past 15 years describing mortality and cardiovascular complication rates with nitrous oxide in patients undergoing non-cardiac surgery. We used combinations of search terms "nitrous oxide", "surgery", "anaesthesia", "mortality", and "complications". A recent relevant meta-analysis had been published,[18] but with most data derived from the first ENIGMA trial.[16] The pooled relative risk of short-term mortality in the nitrous oxide group was 1·38 (95% CI 0·22–8·71). Data for myocardial infarction, stroke, and pulmonary embolism were sparse and could not be reliably pooled for meta-analysis. The conclusion stated that, because of insufficient data, there was no robust evidence to establish whether nitrous oxide affects mortality and cardiovascular complications after surgery. Two trials identified a possible increased risk of surgical-site infection in patients exposed to nitrous oxide,[16,32] but a previous trial had not identified such a risk.[33]

Interpretation

We assessed the safety of nitrous oxide-containing general anaesthesia in adult patients with known or suspected coronary artery disease having major non-cardiac surgery. We have shown that nitrous oxide does not increase the risk of cardiovascular complications or death in this setting. Similarly, we found that nitrous oxide does not increase the risk of surgical-site infection, nor was there any evidence of increased risk of sepsis. Conversely, we found that nitrous oxide clearly increased the risk of nausea and vomiting in the first few days after surgery. Although we found no measurable benefit of nitrous oxide, it is an anaesthetic adjuvant commonly used by many anaesthetists in most parts of the world, partly because it has a long tradition of practice but also because nitrous oxide reduces the dose requirements of other anaesthetic drugs. Nitrous oxide might reduce the risk of persistent pain after surgery; we are doing a follow-up study of ENIGMA-II patients to further assess this hypothesis. The premise of this study was a reduction in the postoperative hyperhomocysteinaemia (and endothelial dysfunction) observed with nitrous oxide. As with many trials in the non-surgical setting, and one in surgical patients,[29] correction of hyperhomocysteinaemia does not seem to reduce cardiovascular risk.

recovery scores was slightly reduced in patients given nitrous oxide anaesthesia, but not by a clinically important amount (2·2%).

Results of our previous ENIGMA Trial[16] suggested that nitrous oxide might increase the risk of cardiovascular complications and surgical site infection, but these were only two of several secondary endpoints in our exploratory analyses. These significant findings could therefore represent a type I error. The design of the earlier trial differed from the present ENIGMA-II trial in two main ways. First, in the ENIGMA Trial, patients not receiving nitrous oxide were given a higher inspired oxygen concentration (80%) than in this trial, which might have confounded the results. Although the effect of hyperoxia on surgical-site infection is controversial,[27,28] the lower rate of infection in the group not assigned to receive nitrous oxide in the ENIGMA Trial compared to the group assigned to nitrous oxide might indicate a potential direct benefit of hyperoxia. In ENIGMA-II, we equalised the inspired oxygen concentrations between groups and did not observe an excess rate of infection with nitrous oxide administration. Second, because we recruited lower-risk patients in the ENIGMA trial than in this trial, cardiovascular events rarely occurred (n=20), which increases the chance of spurious findings. ENIGMA-II focused on moderate-to-high-risk patients, and contained more patients with cardiovascular complications (n=579) than did the previous trial. ENIGMA-II was therefore powered to produce more reliable results.

Through its irreversible inactivation of methionine synthase, exposure to nitrous oxide beyond a few hours induces a state of acute vitamin B_{12} and folate deficiency.

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This deficiency leads to an increase in plasma homocysteine concentration, lasting for at least a week after surgery.[7] The relation between acute hyper- homocysteinaemia after nitrous oxide anaesthesia and endothelial dysfunction, as measured by flow-mediated dilation of the brachial artery, has previously been shown.[6] This suggested a biological rationale for how nitrous oxide could increase the risk of perioperative myocardial ischaemia and cardiovascular complications. Several large trials have not shown a benefit from folate and B_{12} supplements as a means to decrease plasma homocysteine concentrations and to reduce the risk of cardiovascular complications in both medical[9,10,12,13] and surgical settings.[29] Similarly, large, propensity-adjusted, perioperative observational studies did not identify an adverse cardiovascular effect from nitrous oxide.[30,31] Although an increase in plasma homocysteine concen- trations has been consistently reported in previous studies, our ENIGMA-II results suggest that acute hyperhomocysteinaemia with nitrous oxide exposure is of little clinical consequence.

Results of some studies have suggested that nitrous oxide might increase the risk of surgical-site infection,[16,32] but other study results have not accorded with this finding.[30,33] Nitrous oxide exposure might impair DNA synthesis, RNA transcription, and other epigenetic processes.[34,35] Immune suppression might therefore occur with nitrous oxide administration, and some preliminary evidence supports this conjecture. In a trial of 91 patients having colorectal surgery, nitrous oxide inhibited DNA repair and induced genomic instability, which was associated with an increased risk of surgical- site infection.[32] Nevertheless, our results provide reassurance to clinicians that nitrous oxide does not increase the risk of surgical-site infection.

Nitrous oxide has been given to billions of patients since 1844, and, in many parts of the world, it is an integral part of general anaesthesia. In the USA, for example, about 35% of all general anaesthesia cases reporting to the Anaesthesia Quality Institute included nitrous oxide (Richard P Dutton, Anaesthesia Quality Institute, Park Ridge, IL, USA, personal communication). Nitrous oxide provides anaesthetic effects that enable a dose reduction in other anaesthetic drugs, which are usually more expensive and could have other side-effects. Results of our trial showed that inclusion of nitrous oxide in the anaesthetic gas mixture does not increase the risk of death and cardiovascular complications. There is

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therefore no reason to omit nitrous oxide from contemporary anaesthetic practice on the basis of concern about these adverse effects alone. Use of nitrous oxide might confer a long-term analgesic benefit. In a follow-up study[36] (median 4·5 years) of 640 patients randomly assigned to nitrous oxide or no nitrous oxide, persistent pain after surgery was substantially reduced. Nitrous oxide does, however, increase the risk of early postoperative nausea and vomiting, particularly in patients who have not had prophylactic anti-emetic therapy.[16,26] Nitrous oxide should be avoided in those at high-risk of postoperative nausea and vomiting.[26]

Our study had some limitations. Our study cohort consisted mainly of elderly patients with cardiac risk factors having major non-cardiac surgery. We used a pragmatic design that did not control specific anaesthetic or analgesic drug administration, nor standardise management of perioperative haemodynamics or cardiac drugs. Our composite primary endpoint consisted of complications that might or might not be of equal importance to patients and clinicians.

In conclusion, we found no evidence that nitrous oxide increases the risk of death and cardiovascular complications after major non-cardiac surgery, nor that nitrous oxide increases the risk of surgical site infection.

Contributors

PM, KL, and PP contributed to the study design. PM, KL, MC, AF, PP, MP, DS, PD, and BS discussed, critically revised, and approved the final study protocol. PM, KL, and SW organised and did the trial centrally, and KL, MC, PP, MP, WB, DS, PD, BS, and TS in their respective hospitals and regions. PM and SW supervised data management and AF did the analysis. PM, KL, MC, AF, PP, MP, WB, DS, and PD discussed and approved the final strategy for analysis. PM and KL drafted the first version of the Article. MC created the figures. All authors discussed, critically revised, and approved the final version of the Article for publication. ANZCA Trials Group and ENIGMA-II investigators are listed in the appendix.

Declaration of interests

We declare no competing interests.

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