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The Hematological Effects of Nitrous Oxide Anesthesia in Pediatric Patients

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Abstract

Background—Prolonged administration of nitrous oxide causes an increase in plasma homocysteine in children via vitamin B₁₂ inactivation. However, it is unclear if nitrous oxide doses used in clinical practice cause adverse hematological effects in pediatric patients.

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Methods—This retrospective study included 54 pediatric patients undergoing elective spinal surgery: 41 received nitrous oxide throughout anesthesia (maintenance group), 9 received nitrous oxide for induction and/or emergence (induction/emergence group), and 4 did not receive nitrous oxide (nitrous oxide-free group). Complete blood counts obtained before and up to 4 days after surgery were assessed for anemia, macro-/microcytosis, anisocytosis, hyper-/hypochromatosis, thrombocytopenia and leucopenia. The change () from preoperative to the highest postoperative value was calculated for mean corpuscular volume (MCV) and red cell distribution width (RDW).

Results—No pancytopenia was present in any patient after surgery. All patients had postoperative anemia; none had macrocytosis. Postoperative MCV (mean [99% CI]) peaked at 86 [85 to 88] fL, 85 [81 to 89] fL, and 88 [80 to 96] fL, and postoperative RDW at 13.2 [12.8 to 13.5] %, 13.3 [12.7 to 13.8] %, and 13.0 [11.4 to 14.6] % for the maintenance group, the induction/emergence group, and the nitrous oxide-free group. Two patients in the maintenance group (5 %) developed anisocytosis (RDW>14.6%), but none in the induction/emergence group or in the nitrous oxide-free group (P = 0.43). Both MCV (P=0.52) and RDW (P=0.16) were similar across all groups.

Conclusions—Nitrous oxide exposure for up to eight hours is not associated with megaloblastic anemia in pediatric patients undergoing major spinal surgery.

Introduction

Nitrous oxide irreversibly inactivates vitamin B₁₂ and causes a dose-dependent increase in plasma homocysteine concentrations.¹ In a previous report we showed that, among pediatric patients who undergo major spinal surgery, nitrous oxide-induced homocysteine increase could be fairly pronounced.² Some children experienced a several-fold increase in plasma homocysteine concentrations. Yet despite the profound effect of nitrous oxide on plasma homocysteine concentrations, the clinical relevance of this aberration is unclear.^{3,4} Is this simply a biochemical aberration without clinical relevance or indicator, perhaps even cause, of important clinical outcomes?^{5,6}

Prolonged nitrous oxide exposure administration for several days, as seen during the polio epidemic in Denmark in the 1950s, can cause severe hematological side effects including bone marrow failure, agranulocytosis, thrombocytopenia, and aplastic anemia.⁷ Given the prolonged duration of nitrous oxide exposure and profound homocysteine increase observed in our previous study, we asked whether we could detect signs of hematological complications such as megaloblastic anemia in these patients. To answer this question, we retrospectively studied a cohort of 54 children undergoing major spinal surgery, which included the 27 children from our previous cohort.

Methods

Design and Setting

We performed a retrospective analysis of pediatric patients enrolled in a study of methadone in pediatric anesthesia.⁸ Washington University in St. Louis' IRB approved both the parent study and our retrospective analysis. All participants and their parents/legal guardians provided written assent/consent for the original study, and a waiver of consent was approved

for this retrospective analysis. In the parent study, except for the use of methadone, the anesthetic regimen was at the discretion of the anesthesia providers. Nitrous oxide was administered to many patients as part of their anesthetic plans.

Study population

The parent study enrolled 61 pediatric patients (age 5 to 18 years) who had spinal surgery under general anesthesia, a scheduled postoperative inpatient stay of 4 days, no history of kidney or liver disease, and were not pregnant or nursing. All patients underwent posterior spinal fusion, predominantly for idiopathic scoliosis or kyphosis. This retrospective analysis excluded patients who had no preoperative or postoperative complete blood count analyses available. There were no cases of preoperative pancytopenia, active hematopoietic disease (e.g., leukemia), or drug treatment with significant hematopoietic action.

Measurements

Subjects' demographic and surgical data, medical history and home medication including over-the-counter vitamins were available from the parent study.

Complete blood counts (collected during the preoperative visit and up to 4 days after surgery) were retrieved from medical records. All samples were assessed for anemia (defined as hemoglobin < 13.8 g/dL in male or < 12.1 g/dL in female), macro- and microcytosis (defined as mean corpuscular volume (MCV) < 80 fL or >97.6 fL), anisocytosis (defined as red cell distribution width (RDW) > 14.6 %), hypo- and hyperchromatosis (defined as mean corpuscular hemoglobin concentration < 32.7 g/dL or > 35.5 g/dL, respectively), thrombocytopenia (defined as platelet count < 140,000/mm³) and leucopenia (defined as white cell count < 3,800/mm³).⁹ Institutional reference values were applied as normative values.

In addition, data were collected on red cell transfusion, the cumulative nitrous oxide dose and, if available from our previous report², on the total plasma homocysteine levels. Methylmalonic acid and folic acid concentrations were not available. Cumulative nitrous oxide exposure was calculated as the product of the applied nitrous oxide concentration and the duration of exposure using the following formula:

$$\frac{\text{Mean Inspired N}_2\text{O}\%}{100} \times (\text{Minutes of N}_2\text{O Exposure})$$

Statistical Analysis

The sample size of this study was limited to sample size of our previous reports. The sample size basis of the parent studies^{2,8} is not related to this study. Subjects were categorized into 3 groups based on nitrous oxide use: patients who received nitrous oxide for the entire duration of anesthesia (maintenance group), patients who received nitrous oxide only during induction and/or emergence (induction/emergence group), and patients who did not receive nitrous oxide (nitrous oxide-free group).

For each group, the fraction of patients with anemia, macro- or microcytosis, anisocytosis, hyper- or hypochromatosis, thrombocytopenia or leucopenia before and after surgery, was calculated and Fisher exact test (3×2) was used to determine significant differences in the postoperative incidence among groups. Preoperative prevalence was considered. If multiple blood samples of a patient were taken within a 24-hour period, results were averaged for that day. For MCV and RDW, 99% confidence intervals (CI) of the mean of the highest postoperative values were calculated. The Kruskal-Wallis test was used to compare the peak change from the preoperative (baseline) to the highest postoperative value (MCV, RDW) among the three groups. Median and 99% CI were calculated for the groups' MCV,

RDW, and the perioperative decrease in platelet count.¹⁰ To determine the association between cumulative nitrous oxide exposure and MCV, as well as cumulative nitrous oxide exposure and RDW, we calculated Spearman's correlation coefficient and 95% CI. Furthermore, the change in plasma homocysteine available from a subset of patients² was correlated with MCV and RDW. IBM® SPSS® version 22 (Armonk, NY) was used for statistical analysis, and a two tailed P-value of < 0.05 was considered significant.

Results

Fifty-four patients had pre- and postoperative complete blood counts and were included in this study. There were 41 patients in the maintenance group (> 80 nitrous oxide*min), 9 patients in the induction/emergence group (< 30 nitrous oxide*min), and 4 patients in the nitrous oxide-free group. Table 1 shows the demographic and surgical characteristics of the patient population.

Twenty-five (46%) pediatric patients were transfused perioperatively. Intraoperatively, 35% of patients (n=19), 39% (n=16) in the maintenance group, 22% (n=2) in the induction/emergence group, and 25% (n=1) in the nitrous oxide-free group, received either exclusively autologous (22%, n=12) (Cell Saver®, Haemonetics, Braintree, MA), exclusively allogeneic (6%, n=3), or both types (7%, n=4) of red cell transfusion (Table 2). Postoperatively, 11 (20%) pediatric patients were transfused of which 5 (9%) were already intraoperatively transfused. One patient was transfused on postoperative day (POD) 0, 3 patients were transfused on POD 1, 3 on POD 2, 2 on POD 3, and 2 on POD 4.

No significant differences were found in the incidence of postoperative anemia, macro- or microcytosis, anisocytosis, hyper- or hypochromatosis, thrombocytopenia or leucopenia among the maintenance group, the induction/emergence group, and the nitrous oxide-free group (Table 3). All 54 patients developed postoperative anemia. No macrocytosis (high MCV) was present before or after surgery, regardless of nitrous oxide exposure. Before surgery, no patient had anisocytosis (high RDW). However, after surgery, two patients in the maintenance group had high RDW, indicating anisocytosis (RDW > 14.6%). No patient in the induction/emergence group, or in the nitrous oxide-free group, developed signs of anisocytosis. No pancytopenia was present after surgery in any patient, regardless of nitrous oxide exposure.

There was a trend (P=0.09) for differences in the incidence of postoperative thrombocytopenia among groups. The range (min – max) of the lowest postoperative platelet

count in thrombopenic patients was similar in the maintenance group (110,000 – 137,000/mm³, n=10) and the nitrous oxide-free group (103,000 – 122,000/mm³, n=2).

The perioperative decrease in platelet count was as follows (median [99% CI]): –123,000 [–221,000 to –8,000] for the nitrous oxide-free group, –82,000 [–148,000 to –46,000] for the induction/emergence group, and –103,000 [–124,000 to –75,000] for the maintenance group (Kruskal-Wallis test: P=0.6).

Postoperative MCV peaked (mean [99% CI]) at 86 [85 to 88] fL in patients who received nitrous oxide for maintenance of anesthesia, 85 [81 to 89] fL in patients who received nitrous oxide for induction and/or emergence, and 88 [80 to 96] fL in patients who did not receive nitrous oxide. Postoperative RDW peaked (mean [99% CI]) at 13.2 [12.8 to 13.5] % in patients who received nitrous oxide for maintenance of anesthesia, 13.3 [12.7 to 13.8] % in patients who received nitrous oxide for induction and/or emergence, and 13.0 [11.4 to 14.6] % in patients who did not receive nitrous oxide.

Figure 1 shows data of perioperative MCV and RDW. The relative MCV (median [99% CI]) was 1.2 [–0.2 to 1.7] % in patients who received nitrous oxide for maintenance of anesthesia, 1.2 [–0.7 to 3.4] % in patients who received nitrous oxide for induction and/or emergence, and –0.4 [–5.9 to 5.6] % in patients who did not receive nitrous oxide (Figure 1C). The relative RDW (median [99% CI]) was 0.8 [–0.1 to 4.0] % in patients who received nitrous oxide for maintenance of anesthesia, 0.7 [–1.7 to 1.4] % in patients who received nitrous oxide for induction and/or emergence, and 1.6 [–10.8 to 12.9] % in patients who did not receive nitrous oxide (Figure 1D). Both, MCV (P = 0.52) and RDW (P = 0.16), were similar across all groups. No correlation was observed between cumulative nitrous oxide exposure and MCV (n=54, r = –0.04, 95% CI: –0.30 to 0.23, P = 0.8), cumulative nitrous oxide exposure and RDW (n=54, r = 0.09, 95% CI: –0.18 to 0.35, P = 0.5), plasma homocysteine change and MCV (n=26, r = –0.05, 95% CI: –0.43 to 0.34, P = 0.8), and plasma homocysteine change and RDW (n=26, r = –0.03, 95% CI: –0.41 to 0.36, P = 0.9).

Discussion

The goal of this study was to evaluate the hematological effects of prolonged nitrous oxide anesthesia among pediatric patients undergoing spinal surgery. We observed no severe or life-threatening hematological aberrations, such as pancytopenia or leucopenia. In fact, we found no clinically significant changes in blood cell count or morphology related to nitrous oxide exposure.

Several facts support our findings. (1) Our cohort was exposed to a large dose of nitrous oxide without significant hematological changes. Most interventions in pediatric patients requiring anesthesia are of short duration,¹¹ and therefore result in less nitrous oxide exposure than that of patients undergoing major spinal surgery. Because the side effects of nitrous oxide are dose-dependent, it is unlikely that shorter procedures would result in significant changes.^{2,3,12} (2) We showed previously that very long nitrous oxide exposure in pediatric patients causes a several-fold increase in plasma total homocysteine concentration,

which indicates vitamin B₁₂ inactivation.² The current study however, suggests that nitrous oxide-induced vitamin B₁₂ inactivation that increases plasma total homocysteine levels does not automatically translate into clinically important hematological changes.³ (3) Our sample size derived narrow 99% CIs for the postoperative RDW and MCV peak, the most sensitive red cell markers of vitamin B₁₂ deficiency.⁹ Both markers were within reference limits regardless of nitrous oxide exposure, indicating regular erythropoiesis in pediatric patients who were exposed to a large dose of nitrous oxide. (4) Vitamin B₁₂ is the coenzyme of methionine synthase. When Vitamin B₁₂ is oxidized by nitrous oxide, methionine synthase irreversibly loses function. To recover function, the enzyme methionine synthase must be *de novo* synthesized, which requires up to 4 days.¹³ During this period of impaired methionine synthase function abnormal DNA synthesis can occur. Therefore, hematoproliferation could be affected until methionine synthase function fully recovers within 4 days after nitrous oxide exposure. We investigated hematological changes for up to 4 days and had blood counts available in most patients for 4 postoperative days. Red blood cells have an average transit time of 5 days from the proerythroblast to emergence of the erythrocyte into the circulation, which is accelerated by anemia to 1–2 days. The observed postoperative period should therefore be sufficient to capture the egression of misshapen red cells.¹⁴ However, we detected no hematological changes during this period, and clinically significant aberrations are unlikely more than 4 days after one nitrous oxide exposure for up to 8 hours. We suppose that regeneration and egression of normocytic red cells was rapid enough to obviate megaloblastosis.

All pediatric patients underwent major surgery with inherent blood loss and postoperative anemia. Hematopoiesis, when stimulated by blood loss, might be especially susceptible to abnormal DNA synthesis caused by nitrous oxide.¹⁵ We suppose that microcytic erythrocytes due to iron deficiency after blood loss and megaloblastic erythrocytes due to nitrous oxide exposure may be present simultaneously during the early postoperative period. The microcytic changes may possibly disguise the nitrous oxide-induced macrocytic changes when MCV is investigated and result in a confounded, normal MCV. However, we also investigated RDW, which would have increased if undersized and oversized red cells had been simultaneously present. Similar to a study of adults,¹⁵ our study of pediatric patients finds no correlation between nitrous oxide exposure and anemia. However, it is unclear whether fluid management, blood loss, and transfusion during the peri- and postoperative period may have affected our results. Fluid management would alter red cell and hemoglobin concentrations, and should be accounted for. Exact measurement of blood loss is also critical to determining nitrous oxide's contribution to anemia. If blood loss is overestimated, the resulting degree of anemia may be wrongly attributed to blood loss only. Transfusions attenuate the degree of anemia. If nitrous oxide aggravates hemorrhagic anemia this may result in larger transfusions. We were unable to retrieve exact data to account for fluid management, blood loss, and transfusion. Hence, we cannot determine whether nitrous oxide might have contributed to the degree of anemia. Allogeneic transfusions may have also influenced the postoperative morphology of red cells due to storage time-dependent changes of their shape.¹⁶ However, 63% of intraoperative red cell substitution was immediate auto-transfusion. Furthermore, pediatric patients always receive the freshest available banked red blood cells to minimize the risk of reduced function and

irreversible changes of shape.¹⁶ We therefore suggest that the fraction of allogeneic transfusion insignificantly confounded the perioperative red cell morphology.

We also investigated changes in red cell morphology, which are less biased and more specific for vitamin B₁₂ inactivation than for postoperative anemia. MCV and RDW are two specific and sensitive markers of megaloblastic anemia, the predominant hematological sign of vitamin B₁₂ deficiency.⁹ If cytoplasm and nuclei mature abnormally, erythrocytes enlarge or become misshapen.⁹ Although macrocytosis is the eponymous marker of megaloblastic anemia, it may be less well known that anisocytosis is another important marker of vitamin B₁₂ deficiency and should raise suspicion on its own. Vitamin B₁₂ inactivation can cause concurrent production of macrocytic and fragmented microcytic red cells.⁹ The resulting heterogeneity in red cell volume is characterized by high RDW or anisocytosis. However, the mean volume of red cells (MCV) may remain unchanged if the number of undersized red cells counterbalances the number of oversized red cells.⁹ In this study we found no sign of megaloblastic anemia. Neither RDW nor MCV increased with the use of nitrous oxide, or was correlated with homocysteine levels.

Nitrous oxide may cause major adverse side effects such as pancytopenia or agranulocytosis when given for several days, as shown in the seminal report of Lassen et al.⁷ But despite its wide use in pediatric anesthesia, there is surprising uncertainty about the frequency and magnitude of side effects on pediatric patients in the perioperative setting.²⁻⁶ This is of concern since nitrous oxide exposure for more than 2 hours has been reported to result in hematological changes in adults.^{13,17,18} However, other reports found no hematological changes in adults after 3 to 10 hours of nitrous oxide exposure.^{13,15,19}

Although our study shows no hematological effects of nitrous oxide anesthesia in pediatric patients, some limitations should be addressed. First, generalizability is limited to the age and nutrition of our patients, and findings might differ in breast-fed and younger infants or in the populations of countries with a higher risk of silent vitamin B₁₂ or folic acid deficiency.^{9,20,21} Second, we could not retrieve iron status, methylmalonic acid levels, MTHFR C677T polymorphism and, as discussed above, exact data on fluid management, and transfusion, which could have confounded our findings.^{21,22} Third, we did not investigate for deteriorated function of leukocytes and thrombocytes associated with nitrous oxide exposure, which would possibly translate to outcomes such as infection or thrombosis.¹⁸

In conclusion, our results suggest that pediatric patients with no suspected Vitamin B₁₂ deficiency can be exposed to nitrous oxide for several hours during surgery without developing signs of megaloblastic anemia, pancytopenia, thrombocytopenia or leucopenia. Future trials investigating the hematological effects of nitrous oxide in pediatric patients should focus on the functionality of blood (e.g., wound infection, thrombosis).

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Figure 1. Perioperative changes in MCV and RDW

Whether patients were exposed to nitrous oxide or not, some experienced a slight increase in MCV, while others had either a small decrease or no change (A). On average, no change in MCV was observed among the three groups (C).

A few patients, who received nitrous oxide for the whole duration of surgery, experienced a marked increase in RDW (B), indicating the occurrence of erythrocytes with irregular shape or size (anisocytosis). On average, no change in RDW was observed among the three groups (D).

MCV = mean corpuscular volume,

RDW = red-cell distribution width,

RDW = % change of peak postoperative compared to preoperative red-cell distribution width,

MCV = % change of peak postoperative compared to preoperative mean corpuscular volume,

Preop = preoperative blood sample,

POD = day of postoperative blood sample.

Table 1

Patient characteristics and perioperative data

	maintenance group	induction/emergence group	nitrous oxide- free group
N	41	9	4
Male N (%)	14 (34)	3 (33)	1 (25)
Caucasian N (%)	35 (85)	7 (78)	4 (100)
Age (years)	14 [13–16]	14 [13–17]	15 [13–16]
Weight (kg)	55 [50–67]	60 [40–72]	59 [56–68]
Height (cm)	164 [156–168]	160 [151–175]	164 [152–174]
Vitamin user	4 (10)	1 (11)	0
Duration of anesthesia (min)	345 [303–406]	365 [315–434]	345 [278–365]
Cumulative N ₂ O dose (N ₂ O*min)	150 [128–178]	20 [10–26]	0
N ₂ O inspiratory concentration (%)	46 [39–50]	n.a.	n.a.
Length of stay (days)	6 [5–6]	5 [5–7]	6 [5–6]
N (%) with available tHcy levels	22 (54)	3 (45)	1 (25)
Absolute change in tHcy μ mol/L	11.2 [7.8–14.0]	4.5 [0.7– n.a.]	0 [n.a.]
Relative change in tHcy (%)	214 [172–265]	52 [14 – n.a.]	0 [n.a.]

Continuous variables are presented as median [25th–75th percentile]. N = count, N₂O = nitrous oxide, tHcy = plasma total homocysteine, n.a. = not applicable due to low count

Table 2**Intraoperative blood loss and perioperative red cell transfusion**

	maintenance group (n=41)	induction/emergence group (n=9)	nitrous oxide- free group (n=4)
Intraoperative blood loss (mL)	500 [300–650]	450 [300–750]	425 [238–725]
Intraoperative red cell transfusion			
Exclusively autologous red cell transfusion			
N (%) of pediatric patients	11 (27)	1 (11)	0
Volume (mL)	203 [125 – 300]	68 [n.a.]	0
Exclusively allogeneic red cell transfusion			
N (%) of pediatric patients	2 (5)	0	1 (25)
Volume (mL)	500 [n.a.]	0	250 [n.a.]
Combined autologous and allogenic red cell transfusion			
N (%) of pediatric patients	3 (7)	1 (11)	0
Volume (mL)	650 [480 – 1250]	678 [n.a.]	0
Postoperative red cell transfusion			
N (%) of pediatric patients	8 (19)	1 (11)	2 (50)
Volume (mL)	250 [250 – 250]	250 [n.a.]	250 [n.a.]

Continuous variables are presented as median [25th–75th percentile]. Postoperative red cell transfusions were exclusively allogeneic. N = count.

Incidence of postoperative aberrations of complete blood cell analysis up to 4 days after surgery

Table 3

	Macrocytosis MCV > 97.6 fL	Microcytosis MCV < 80 fL	Anisocytosis RDW > 14.6 %	Anemia ♂: Hb < 13.8 g/dL ♀: Hb < 12.1 g/dL	Hypochromatosis MCHC < 32.7 g/dL	Hyperchromatosis MCHC > 35.5 g/dL	Thrombocytopenia Plt < 140,000/mm ³	Leucopenia WBC < 3,800/mm ³
<i>Count of pediatric patients with postoperative aberrations / Count of pediatric patients without preoperative aberrations (%)</i>								
maintenance group	0/41 (0)	0/39 (0)	2/41 (5)	33/33 (100)	0/39 (0)	10/36 (28)	10/39 (26)	0/39 (0)
induction/emergence group	0/9 (0)	0/7 (0)	0/9 (0)	8/8 (100)	0/9 (0)	0/7 (0)	0/9 (0)	0/9 (0)
nitrous oxide-free group	0/4 (0)	0/4 (0)	0/4 (0)	4/4 (100)	0/4 (0)	1/4 (25)	2/4 (50)	0/4 (0)
P-value	1.00	1.00	0.43	1.00	1.00	0.23	0.09	1.00

This table shows the incidence of pediatric patients with anemia, macro- or microcytosis, anisocytosis, hyper- or hypochromatosis, thrombocytopenia, or leucopenia, after surgery for each group. Incidence rates of novel postoperative aberrations were compared among the 3 groups using Fisher-exact test, and none were significantly different. MCV = mean corpuscular volume, RDW = red-cell distribution width, Hb = hemoglobin, MCHC = mean corpuscular hemoglobin concentration, Plt = platelet count, WBC = white cell count