Thromboembolic complications of recreational nitrous oxide (ab)use : a systematic review

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Abstract

Introduction: The recreatinal use of nitrous oxide has become more common in recent years, especially in adolescents and young adults. It has been mainly associated with medical conditions like megaloblastic anemia and (myelo)neuropathy. We report on the thromboembolic complications, a less known side effect, associated with recreational inhalation of nitrous oxide.

Method: An extensive literature search was performed for publications reporting on the thromboembolic complications associated with recreational nitrous oxide abuse. Data about sex, age, location of thrombosis, laboratory findings, therapy and outcome were collected.

Results: A total of 13 case reports or case series were identified comprising a total of 14 patients. The reported thromboembolic side effects included deep venous thrombosis, pulmonary embolism, mesenterial-, portal and splenic vein thrombosis, cerebral sinus thrombosis, cortical vein thrombosis, stroke, acute myocardial infarction and peripheral artery thromboembolism.

Discussion: These side effects are possibly mediated by the interaction of nitrous oxide with vitamin B12, a cofactor of the methionine synthase complex, which eventually results in elevation of plasma levels of homocysteine. Despite being a known risk factor for cardiovascular disease, the exact pathophysiological mechanism remains unclear. Cessation of nitrous oxide inhalation is necessary to prevent recurrent thrombosis.

Conclusion: Nitrous oxide abuse may result in a wide spectrum of thromboembolic complications. One should be aware of this etiology, especially in a young person with no obvious risk factors for cardiovascular disease. Spreading awareness is important to inform people about the potentially serious side effects associated with nitrous oxide inhalation.

Keywords
Nitrous oxide abuse, thrombosis, embolism, hyperhomocysteinaemia, vitamin B12

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Highlights

- There is a rising trend in the recreational use of nitrous oxide over the past few years.
- A wide spectrum of thromboembolic complications have been linked to nitrous oxide abuse.
- The underlying pathophysiological mechanism is not yet completely understood but may be related to the increase in homocysteine secondary to nitrous oxide abuse.
- More awareness must be spread about the potential dangers of nitrous oxide inhalation.

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Introduction

Nitrous oxide is a colorless and nearly odorless gas that has first been synthesized by Joseph Priestly in 1772[1]. From the 1800s, its anesthetic properties were widely recognized, and it has been utilized as an anesthetic adjunct ever since[1]. It is used both in the hospital and in outpatient care settings for (conscious) sedation and pain relief: e.g., dental and obstetric procedures[2]. The gas is also being investigated as a rapidly acting antidepressant in patients with treatment resistant major depression[3, 4]. Besides its medical use it has also applications in the food industry where it is used in whipped cream canisters among other things [5].

Due to its ability to produce euphoria, hallucinations and relaxation it has also been used in a recreational setting since Victorian times when the British high society had their so called 'laughing gas parties'[6]. The drug has become increasingly popular among adolescents and young adults in the last years. This may be because of its legal status, widespread availability given its applications in the medical and food industry and the presumed safety[6-8]. For recreational use, nitrous oxide is commonly sold in prefilled balloons or small pressurized metal canisters that are normally used for the food industry; the so called 'whippits'. Since a couple of years, the drug is even readily available in large nitrous oxide tanks (e.g., 2 kg, 4 kg or even more) and can be purchased from online shops with delivery in 24 hours[7, 9].

Inhalation of the drug causes euphoria, pleasure and sometimes hallucinations. This effect occurs after a couple of seconds, peaks around one minute after inhaling and then disappears within some minutes without a neurological residual effect[7]. The exact working mechanism is not fully understood yet, but its effect is thought to mainly result from its inhibition of the N-methyl-d-aspartate (NMDA) type glutamate receptor[10]. These effects are comparable to the effects of ketamine, another non-competitive NMDA receptor antagonist[7].

It has long been thought that nitrous oxide is an inert gas with no serious side effects. However, in 1956, the first case of bone marrow depression secondary to prolonged exposure to nitrous oxide was reported[11]. Since then, there has been ample research about the possible hematological, cardiovascular, and neurological effects of nitrous oxide used in anesthesia, both for the patient and the professionals who might get exposed to the gas[5, 12-14]. The most frequently reported side effects in the setting of recreational inhalation of nitrous oxide are headache, dizziness, fainting, and tingling of hands and feet[7, 15]. With higher daily doses or prolonged abuse of the drug, more serious side effects may occur, especially since the introduction of the nitrous oxide tanks. In the last couple of years there have been multiple case reports or small case series that have reported on the medical and neuropsychiatric complications of recreational nitrous oxide (ab)use. Some of these side effects include cytopenias, skin hyperpigmentation, myeloneuropathy, subacute combined degeneration and psychosis[5].

Some less known side effects of nitrous oxide abuse are a couple of major adverse cardiac and vascular events. The aim of this review is to provide an overview of the patient demographics, site of thromboembolic events, laboratory findings and potential therapies for nitrous oxide associated thromboembolic disease.

Method

An extensive literature search was performed in the PubMed and Web of Sience database following Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) guidelines. The latest search was conducted on April 24th, 2022. A combination of the following search terms was used to identify relevant publications on our topic: nitrous oxide, nitrous oxide use, laughing gas, thrombosis, embolism, thromboembolism, infarction, acute coronary syndromes, limb ischemia and stroke. Anesthesiology related studies were excluded by specifying this in the search query or using the available filter in the Web of Science database. Animal studies were excluded. There were no limits to the language or published year. Relevant articles for our review had to meet the following inclusion criteria: 1) the study should report on thromboembolic events that have been linked to the use of nitrous oxide, 2) the use of nitrous oxide needs to be in a recreational setting (i.e., no accidental exposure or use of nitrous oxide in a medical setting). Thromboembolic events were defined as both arterial and venous thrombosis or embolisms. The literature search was performed by SO. Two independent reviewers (SO and BP) screened the articles for inclusion. Reviewer disagreement was resolved by discussion. The references of the selected studies were also screened for relevant publications that may have been missed with the primary literature search strategy. Quality assessment was performed with the JBI critical appraisal checklist for case reports and case series (supplementary material)[16].

The following information was collected from each included study: age, sex, location of thrombosis or embolism, other concomitant substance uses with a known risk for the development of thromboembolisms, relevant laboratory findings at diagnosis and follow-up (especially vitamin B12, folic acid, homocysteine), therapy and outcome. These basic demographic data are presented in table 1 and 2, for respectively arterial and venous thrombosis. Additionally, some summary statistics on the reported counts and proportions of the thromboembolic events are provided in this systematic review.

Results

A search of 341 articles was performed after exclusion of duplicates. Eventually 13 papers were identified that were relevant to the research topic and met the inclusion criteria. No additional studies were added after screening the references of the included papers (figure 1). The selected studies represent all case reports or small case series published from 2015 till 2022, comprising a total of 17 patients. Eventually there remained 14 unique patients after excluding those that were reported on in multiple publications (table 1 and 2).

Most of the patients were men: ten male (71%) and four female (29%). Their age ranged between 16 years and 32 years with a median age of 26,5 years. A wide spectrum of thromboembolic events is being reported with some patients presenting several times with a thrombosis[17] or multiple thromboembolisms in different vascular areas at the same time. There were three cases of deep venous thrombosis in the legs[17-19] and four cases of pulmonary embolism[17-21]. There was one patient with a cortical vein thrombosis[22] and three patients with cerebral sinus thrombosis[23-25]. One patient had a superior mesenteric vein, portal vein and splenic vein thrombosis[21]. A total of three patients presented with an acute myocardial infarction[26-28]. At last, two cases of a stroke[17, 29] and two cases of peripheral artery thrombosis have also been reported[17, 20, 21] (table 3).

Except for one paper, all studies reported the homocysteine levels around the time of presentation which was always elevated except in 2 cases with a high normal value. Both patients were already receiving vitamin B12 suppletion with one patient being tested only 9 days after clinical presentation. The vitamin B12 concentrations were most of the time below the lower limit but not in all the reported cases. Several studies showed that with cessation of nitrous oxide inhalation and vitamin suppletion the homocysteine and vitamin B12 levels normalized (table 1 and 2).

Therapy of the thromboembolism depended on the type of the underlying event and comprised of administration of antiplatelets, anticoagulation, thrombolysis, thrombectomy, stenting or a combination of these therapies. Almost all patients also received vitamin B12 and folate suppletion. There were three cases where only vitamin B12 suppletion therapy was mentioned (table 1 and 2).

There were no deaths reported in the included cases. However, there were several patients in whom the thromboembolic event had significant morbidity. One patient with pulmonary embolism was intubated and cannulated for venous arterial extracorporeal membrane oxygenation for more than 3 weeks. Another patient with bilateral lower limb ischemia was hospitalized for 3 months, required multiple surgeries and eventually a below the knee amputation of the left lower limb. Other long-term sequelae that have been reported are persistent wall motion abnormalities in patients that sustained a myocardial infarction, muscle weakness following an ischemic stroke or cerebral venous thrombosis and even one case of chronic intracranial hypertension after a cerebral venous sinus thrombosis (table 1 and 2).

Discussion

The recreational use of nitrous oxide has become more common in recent years, especially in adolescents and young adults. The exact prevalence of recreational nitrous oxide abuse in the general population remains unclear. However results of the Global Drug Survey (GDS), comprising a total of 74 864 participants in 2014, showed a last year use of 7%[15]. This number has been increasing every year since then with a total of 13% of respondents reporting they had used nitrous oxide in the last 12 months in the GDS of 2020[30]. This prevalence varies between countries, with the United Kingdom and the Netherlands having the highest rate of recreational nitrous oxide users. This prevalence may also be significantly higher in certain subpopulations. A survey among clubbers in Amsterdam showed that 71% had ever used nitrous oxide and 33% had done so in the past month[31]. There is also a heterogeneity in the number of balloons or cannisters used on a single occasion. Results of the GDS showed that 64% of the nitrous oxide users used 5 or less balloons per occasion. The majority (78%) used nitrous oxide on 10 occasions or less in the last year. Only a small proportion (< 3%) inhaled nitrous oxide at least weakly[15].

The serious side effects associated with nitrous oxide abuse have been linked to its interaction with vitamin B12 (cobalamin) which serves as a cofactor for the enzyme methionine synthase and methylmalonyl-CoA mutase (figure 2). There are two forms of vitamin B12, one with a methyl group and the other one with an adenosyl group. Nitrous oxide irreversibly oxidizes methyl-cobalamin from its 1+ to its 3+ valent state[32]. This impairs the function of the vitamin and thus in an inhibition of the methionine synthase complex in the cytosol. Consequently, homocysteine and 5-methyl-tetrahydrofolate will accumulate, and less tetrahydrofolate and methionine will be generated [7]. Reduced methionine further leads to reduced S-adenosylmethionine production, which is associated with defective DNA maturation and megaloblastic changes of red blood cells and impaired myelination of nerve fibers [33-35]. Animal studies have shown that adenosyl-cobalamin is not susceptible to the oxidative action of nitrous oxide. This form of vitamin B12 serves as a cofactor of the mitochondrial methylmalonyl-CoA mutase[32]. However, eventually this form will also be displaced from the mitochondria by a complex interaction with the inactive methyl-cobalamin, and thus disrupt the methylmalonyl-CoA mutase enzyme[20]. This results in the accumulation of methylmalonic acid which will also contribute to the demyelization of neurons via disturbed lipid synthesis[7, 35].

The thromboembolic complications are thought to be related to the accumulation of homocysteine. However, the exact pathophysiological mechanism remains unclear. All patients in our review, except for 2 with high normal homocysteine levels, presented with elevated homocysteine concentrations. It should be noted that one patient was only tested 5 days after presentation and the other even after 9 days. It's thus quite possible that the exact level could have been higher on the day when the thrombosis occurred. The vitamin B12 concentrations were most of the time below the lower limit but not in all the reported cases. These findings differ from the results of another systematic review on the neurologic, psychiatric, and medical manifestations of nitrous oxide abuse where they reported low or low-normal Vitamin B12 across most of the cases and occasionally elevated homocysteine levels[5]. However, it is not clear whether the homocysteine levels were normal in these cases or just not reported.

The majority of the thromboembolic events that have been included in this review are venous thrombosis (63%). Several studies, including three meta-analyses, showed an association between elevated homocysteine levels and the increased risk for the occurrence of venous

thrombosis. The relative risk has been estimated to be 2 to 3 times higher compared to people with normal homocysteine concentrations[36-38]. However, there have also been some recent studies that showed that the effect of homocysteine on the occurrence of venous thrombosis might have been overestimated in the past and that there may even be no association, especially after extensive adjustments for confounding[39].

Elevated homocysteine levels have also been associated with arterial thrombus formation[40, 41]. Several studies, including a large meta-analysis of observational studies have shown that individuals with higher homocysteine levels are at higher risk for the development of ischemic heart disease and stroke[42]. Similar results have also been noted in peripheral arterial disease[43]. One of the possible pathways is due to accelerated atherosclerosis by its adverse effects on vascular endothelium and smooth muscle cells[44]. However, almost none of the studies reported in this review systematically investigated signs of premature atherosclerosis in their patients. Another potential mechanism is the effect of homocysteine on the coagulation pathway where it exerts a prothrombotic effect. Indeed, acute elevations of homocysteine are associated with endothelial dysfunction, oxidative stress, enhanced platelet activation, increased thrombin generation, augmented factor V activity, impaired fibrinolysis and vascular inflammation[45]. Furthermore, animal studies have shown that elevated plasma homocysteine may also lead to abnormal clot formation with thin, tightly packed fibers that are abnormally resistant to fibrinolysis, and prone to recurrent thrombosis[46]. This might be a possible mechanism by which the thrombotic complications occur in these patients with nitrous oxide abuse. Several studies in anesthesia have shown that continual exposure to nitrous oxide in patients leads to progressive increase in plasma homocysteine levels after 1 hour. The Evaluation of Nitrous Oxide in a Gas Mixture for Anesthesia (ENIGMA) trial also suggested that patients who are exposed to anesthetic levels of nitrous oxide may be at increased risk for developing myocardial ischemia due to elevated homocysteine levels[13]. However, the results of the ENIGMA-II trial and long-term follow-up was not able to support this finding[47]. Nevertheless, the use of nitrous oxide in a controlled medical setting may differ from recreational abuse where the patient might be exposed to larger amounts over a longer period.

It should be noted that there were some confounding factors in a couple patients. Two patients were also using cannabis at that time, a known risk factor for the development of cardiovascular disease, including acute myocardial infarction, stroke, and peripheral artery disease[48]. This effect is probably mediated by its effect on the cannabinoid receptor 1 which induces inflammation and oxidative stress and thus has nothing to do with the elevated homocysteine levels encountered in these patients[28]. Nevertheless, it could have attributed to the occurrence of the thrombosis in these patients besides the inhalation of nitrous oxide. On the other hand, there were also 3 patients with a methyl-tetra-hydro-folate-reductase (MTHFR) polymorphism. A mutation in the gene coding for this enzyme has a detrimental effect on the homocysteine metabolism leading to hyperhomocysteinaemia[49, 50]. These mutations are quite common with a prevalence of 20 to 40% in certain populations[49]. However, the case reported by Pratt et al.[23] where the patient had a homozygous MTHFR polymorphism, showed that the homocysteine and methylmalonic acid levels normalized after abstaining from nitrous oxide inhalation suggesting that the elevated homocysteine levels were secondary to the nitrous oxide use.

There is no clear evidence available in the literature to recommend a specific therapy for a thromboembolism associated with hyperhomocysteinaemia. The therapy in the reported cases was in line with the usual treatment of each individual type of thrombosis. However, some authors argued that a low threshold for a more aggressive approach might be necessary

sometimes given the association with the formation of abnormally resistant thrombi as mentioned earlier[28]. Aside from the therapy directed to dissolve the clot, the patients were advised to halt the inhalation of nitrous oxide and most patients were treated with vitamin B12 and folate suppletion to correct its functional depletion and to normalize homocysteine levels. This showed to be effective in all the cases that reported follow-up data after therapy. The case reported by den Uil et al.[17] in which the patient presented three times with a different thromboembolic event nicely illustrates that in a period without nitrous oxide abuse the laboratory results normalized and that they worsened in a renewed period with severe abuse. Even though several randomized trials found no benefit of homocysteine lowering interventions for the prevention of cardiovascular events or death[51], it's still advisable to provide vitamin suppletion given the fact that these patients often have concomitant neurological complications or are at risk to develop these side effects. Administration of vitamin B12 and cessation of nitrous oxide use quickly leads to improvement of these neurological complaints, although complete recovery may take months[7, 52].

This study has several limitations. First, this review is based on case reports or small case series which is subject to potential selection or publication bias. There was also considerable heterogeneity in reporting cases with some providing a detailed description of the clinical course and lab values while this was lacking in some other cases. There were also several cases with confounding factors like other substance abuses with a known cardiovascular risk factor or mutations in the coagulation pathway. It is also very likely that the reported thromboembolic complications of nitrous oxide abuse are an underestimation of the real burden of this condition. This may be because the possible relation between the event and nitrous oxide abuse is not always timely recognized or because of incomplete coagulation work-up at time of diagnosis which will make a possible publication less likely. However, we hope that this review will make clinicians more aware of the condition and eventually to a better understanding of the underlying pathophysiological mechanism.

Conclusion

This systematic review illustrates a wide range of thromboembolic complications that have been linked to the inhalation of nitrous oxide. While making significant conclusions in not possible given the fact that only case reports and small series have been published on this subject, all included studies propose a similar theory in which the prothrombotic properties of homocysteine, which is induced by nitrous oxide, play a central role in a multifactorial process. Cessation of nitrous oxide use and vitamin suppletion is necessary to prevent recurrent thrombosis. At last, more awareness must be spread about the potential dangers of nitrous oxide abuse and the associated long-term morbidity.

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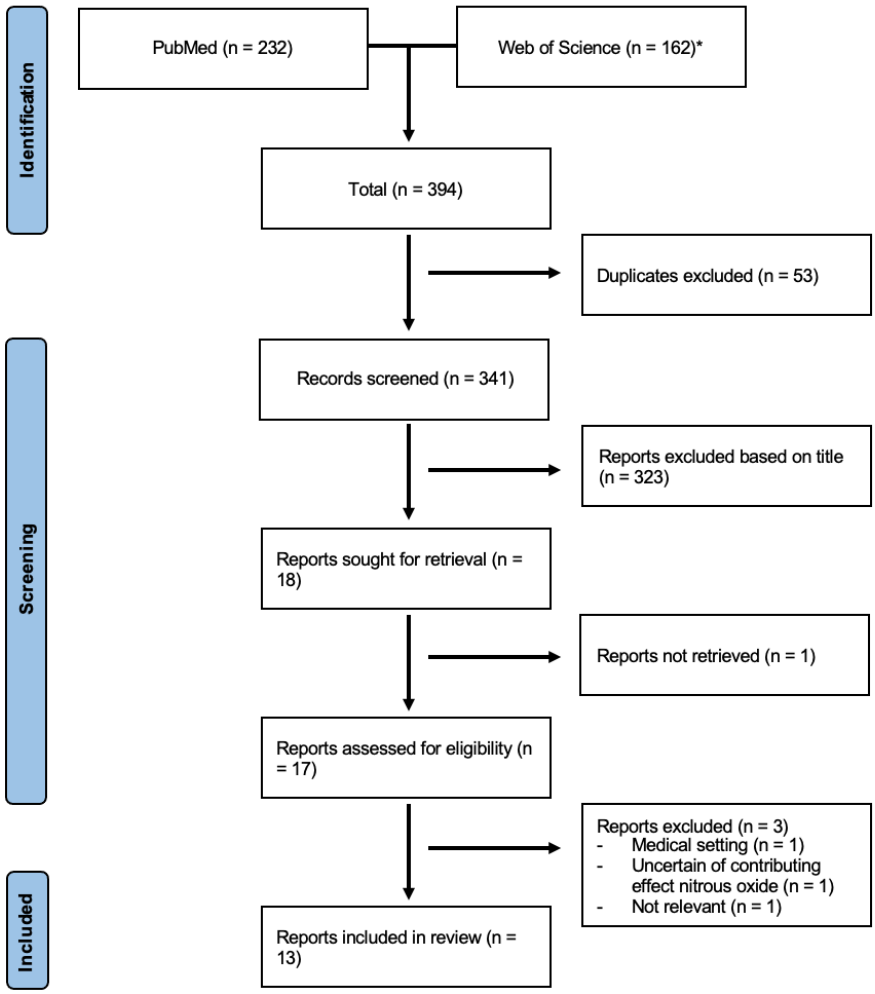
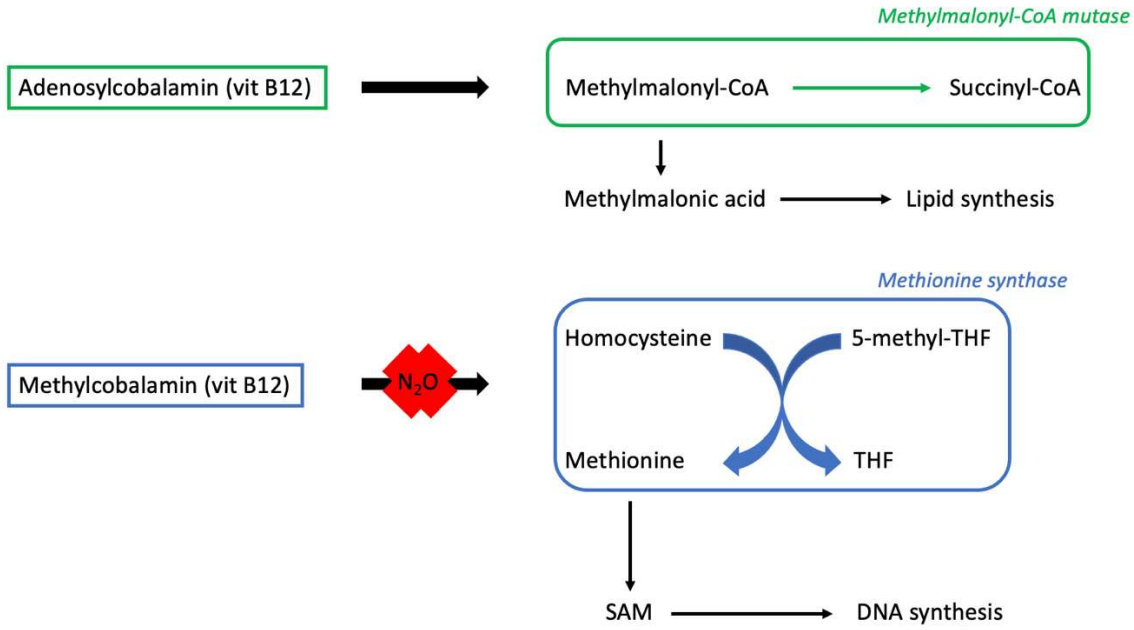
Figure 1 Flow diagram of assessment of studies identified in the systematic review



*The filter 'Anesthesiology' in the Web of Science Categories section was selected to exclude anesthesia related nitrous oxide use.

Figure 2 Metabolic reactions involving vitamin B12



Adenosyl-cobalamin serves as a cofactor of the mitochondrial methylmalonyl-CoA mutase. This form of vitamin B12 is not directly affected by nitrous oxide.

Nitrous oxide irreversibly oxidizes methyl-cobalamin from its 1+ to its 3+ valent state. This r.esults in a functional deficiency of this form of vitamin B12 and an inhibition of the methionine synthase complex in the cytosol. Less tetrahydrofolate and methionine will be generated from homocysteine and 5-methyl-tetrahydrofolate. This results in hyperhomocysteinaemia. Reduced methionine further leads to reduced S-adenosylmethionine production, which is associated with defective DNA maturation and megaloblastic changes of red blood cells and impaired myelination of nerve fibers.

Table 1: Arterial thrombosis

Study	Indraratna et al.[27]	Den Uil et al.[17]	Bajaj et al.[29]	Oomens et al.[28]	Oomens et al.[20]	Bär et al.[26]
Year	2017	2018	2018	2020	2020	2020
Sex	Male	Male	Male	Male	Male	Male
Age (year)	28	32	32	27	26	27
Other abuses	Smoking	None	None	Smoking, cannabis	None	Smoking, cannabis
Thrombus location	Coronary	Aorta, axillary artery, stroke (right middle cerebral artery)	Stroke (right internal carotid and middle cerebral artery)	Coronary	Peripheral artery legs	Coronary
Lab results						
Homocysteine (µmol/L)[1]	65	>55	253	205	229	NR
Vitamin B12 (pmol/L)[2]	NR	116*	146	114	384	NR
Folic acid (nmol/L)[3]	NR	NR	5,7	7,7	<2,7	NR
Therapy						
Antiplatelet	Aspirin, clopidogrel	Clopidogrel	Aspirin	Aspirin, ticagrelor, tirofiban	-	Aspirin, prasugrel
Anticoagulation	Enoxaparin	Heparin, warfarin, rivaroxaban	-	Heparin	Rivaroxaban	-
Thrombolysis	Tenecteplasis	-	-	-	-	-
Other	-	Thrombectomy	-	-	Thrombectomy	Stenting
Vitamin suppletion	NR	NR	NR	Vit B12, folic acid	Vit B12, folic acid	NR
Lab results on follow-up	Normal	Normal**	NR	Normal	NR	NR
Sequelae	Mild hypokinesis anteroseptal wall of left ventricle	Post-stroke epilepsy	Residual left sided motor weakness, mild dysarthria	Anteroseptal wall motion abnormalities	Below knee amputation left knee	No sequelae
Remarks	-	Heterozygous FII mutation	-	MTHFR negative	Heterozygous MTHFR	History of Hodgkin-lymphoma

N_2O: nitrous oxide; NR: not reported; MTHFR: methyl-tetra-hydro-folate-reductase
[1]Reference value: < 15 µmol/L
[2]Reference value: 150 – 800 pmol/L
[3]Reference value: 8,8 – 40 nmol/L
* Although the authors report µmol/L, we assume they meant pmol/L
** Normal lab results at follow-up during cessation of nitrous oxide use, however this patient will present later again with abnormal lab results and venous thrombosis after starting to inhale nitrous oxide again (see also table 2).

Table 2: Venous thrombosis

Study	Den uil et al.[17]	Sun et al.[19]	Molina et al.[18]	Pratt et al.[23]	Liu et al.[22]	Oomens et al.[20]	Vollenbrock et al.[21]	de Valck et al.[25]	Farhat et al.[24]
Year	2018	2019	2020	2020	2020	2020	2020	2021	2022
Sex	Male	Male	Male	Female	Female	Male	Female	Male	Female
Age (year)	32	29	23	21	25	27	22	24	16
Other abuses	None	Smoking	None	None	None	None	Smoking	None	None
Thrombus location	DVT, pulmonary emboism	DVT, pulmonary emboism	DVT, pulmonary emboism	Cerebral sinus	Cortical vein	Pulmonary embolism	Superior mesenteric -, portal - and splenic vein	Cerebral sinus	Cerebral sinus
Lab results									
Homocysteine (µmol/L)[1]	>50	24	104	>65	13,6	88	22	11***	134
Vitamin B12 (pmol/L)[2]	162**	734**	<111	Normal	Normal	108	292	>1476***	68
Folic acid (nmol/L)[3]	NR	Normal	NR	Normal	Normal	3,9	NR	NR	Normal
Therapy									
Antiplatelet	NR	-	-	-	-	-	-	-	-
Anticoagulation	NR	Not specified	-	Heparin, LMWH	LMWH, oral anticoagulation	Heparin, sintrom, enoxaparin	Heparin, rivaroxaban	LMWH, dabigatran	Heparin, LMWH
Thrombolysis	NR	Alteplase	tPA	-	-	Alteplase	-	-	-
Other						Embolectomy	-	-	-
Vitamin suppletion	NR	Vit B12, folic acid	Vit B12	Vit B12, folic acid	Vit B12, folic acid	Vit B12	-	Vit B12	Vit B12, folic acid
Lab results on follow-up	Normal*	Normal	NR	Normal	NR	Normal	NR	NR	Normal
Sequelae	NR	Residual DVT at follow-up	NR	Improvement in gait and weakness	Improvement of right arm paresis to grade 4/5	NR	NR	Chronic intracranial hypertension	No sequelae
Remarks	-	-	COVID	Homozygous MTHFR, pregnancy	MTHFR genotype, oral anticonception	-	-	Heterozygous prothrombin G20210A mutation	-

N_2O: nitrous oxide; NR: not reported; MTHFR: methyl-tetra-hydro-folate-reductase; tPA: tissue plasminogen activator; LMWH: Low-molecular-weight heparin
[1]Reference value: < 15 µmol/L
[2]Reference value: 150 – 800 pmol/L
[3]Reference value: 8,8 – 40 nmol/L
*Normal lab results at follow-up during cessation of nitrous oxide use (same patient as in table 1).
**Patient was receiving vitamin suppletion and the testing was done 5 days after presentation. Although the authors report µmol/L, we assume they meant pmol/L
***Testing was done after 9 days of high dose vitamin B12 suppletion (initial vitamin B12 value was 81 pmol/L).