Review

Oxygen-ozone autohaemotherapy in fibromyalgia: safety profile and adverse events. A scoping review

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ABSTRACT

Objective. *Oxygen-ozone autohaemotherapy (O_2-O_3-AHT) has gained clinical interest as an adjunctive treatment for fibromyalgia (FM) based on its anti-inflammatory and anti-oxidative properties. However, comprehensive data on safety and adverse events remain limited. This scoping review aimed to systematically evaluate documented adverse events associated with O_2-O_3-AHT and assess risk stratification.*

Methods. *Following PRISMA-ScR guidelines, we searched Medline, EMBASE, AMED, Cochrane Library, CINAHL, Web of Science, TRIP, Clinical Evidence, and ROAD databases for relevant articles published in the last decade. Search terms included "ozone autohaemotherapy," "GAET," "autologous blood transfusion," "systemic ozone therapy," combined with "adverse effects," "side effects," "contraindications," and "iatrogenic complications." Studies involving local injections, hyperbaric oxygen therapy, veterinary applications, or non-systemic routes were excluded.*

Results. *The literature search identified predominantly case reports documenting rare but potentially serious adverse events. Major categories included: haemolysis and renal failure (associated with excessive ozone concentrations >60 μg/mL), hyperkalaemia in patients with complex comorbidities (hypertension, diabetes, chronic kidney disease), myocardial infarction and ischaemic events (attributed to vasoconstrictive and pro-thrombotic effects), cerebral gas embolism in patients with patent foramen ovale, autonomic reactions related to rapid reinfusion rates, anaphylactic reactions (linked to equipment materials), and infectious complications due to protocol breaches. The overall incidence of serious adverse events cannot be reliably quantified given the absence of prospective registries and reliable denominator data: A frequent cited historical low estimation of uncertain methodology should be interpreted with considerable caution. Most safety data were derived from mixed clinical populations and not specifically from fibromyalgia cohorts, although they are relevant for clinical decision-making in this setting.*

Conclusion. *Current evidence does not identify a pattern of frequent serious unexpected harm when standardised protocol is followed. Key safety measures include mandatory glucose-6-phosphate dehydrogenase screening, adherence to recommended ozone concentrations (10–40 μg/mL), controlled reinfusion rates (<50 drops/minute), pre-treatment cardiovascular evaluation in selected cases, and strict aseptic technique. The estimated incidence of serious complications derived from historical global data on O2-O3 autohaemotherapy may be only inferred in a fibromyalgia population. Standardisation of treatment protocols and prospective adverse event registries are needed to further optimise safety.*

Introduction

Fibromyalgia (FM) is a disabling chronic pain condition affecting 2-4% of the population, predominantly women (female-to-male ratio 7:1), characterised by widespread musculoskeletal pain, fatigue, sleep disturbances and cognitive impairment ('fibro fog') (1-3). The clinical complexity reflects multiple pathophysiological mechanisms including central sensitisation, neuroinflammation, mitochondrial dysfunction, and oxidative stress (4, 5). Current pharmacological therapies, including pregabalin, duloxetine and milnacipran, provide inadequate relief for many patients, with response rates below 50% (6).

Table I. Contraindications to oxygen-ozone autohaemotherapy.

Category	Contraindication	Rationale
Absolute	G6PD deficiency (favism)*	Acute haemolytic crisis
	Active hyperthyroidism (Graves' disease)	Metabolic stimulation risk
	Severe thrombocytopenia (<50,000/μL)	Bleeding risk
	Severe coagulopathy (*e.g.* Factor V Leiden homozygous)	Thrombotic risk
	Pregnancy	Insufficient safety data
	Acute myocardial infarction	Haemodynamic instability
	Massive acute haemorrhage	Hypovolaemia, instability
	Active alcohol/drug intoxication	Unpredictable responses
	Uncontrolled seizure disorder	Neurological risk
Relative	Advanced chronic kidney disease (stage 4-5)	Hyperkalaemia risk, reduced clearance
	Severe cardiovascular instability	Haemodynamic stress
	Haemochromatosis	Iron overload complications
	Active malignancy	Individualised assessment
	Inability to provide informed consent	Ethical requirement

*Mandatory G6PD testing required before any O2-O3-AHT treatment.
Prevalence varies by ethnicity: sub-Saharan Africa 7.5%, Middle East 6.0%, Asia 4.7%, Europe 3.9%, Americas 3.4%, Pacific 2.9%.
Modified from: Madrid Declaration on Ozone Therapy (ISCO3), 4th Edition, 2025.

Non-pharmacological interventions supported by clinical guidelines include physical therapies, rehabilitation programs and cognitive-behavioural strategies (7, 8). Among emerging rehabilitation approaches, oxygen-ozone therapy (O_2-O_3) has attracted clinical interest due to recognised anti-inflammatory effects, immune modulation, and ability to reduce pro-inflammatory cytokines and oxidative stress (9, 10). Oxidative stress is increasingly recognised as a pivotal factor in nociplastic pain conditions including FM, as well as chronic neuropathic pain (11-14).

Oxygen-ozone therapy is delivered as a gas mixture with ozone concentrations typically ranging from 10 to 40 μg/mL, depending on clinical protocols and regional guidelines (15, 16). The mixture exerts dual actions: enhancing tissue oxygen levels and inducing mild oxidative stress that activates endogenous antioxidant responses through the Nrf2 pathway (16, 17). Clinical applications span multiple fields including pain medicine and rehabilitation, with documented antibacterial, immunomodulatory and anti-inflammatory properties (18).

In rehabilitation and pain medicine, two major administration routes predominate: local injection into affected tissues and systemic administration via autohaemotherapy. Oxygen-ozone autohaemotherapy (O_2-O_3-AHT), also termed major autohaemotherapy, involves extracorporeal treatment of autologous blood. Blood (50–200 mL) is withdrawn into a sterile collection system containing anticoagulant, mixed with medical-grade oxygen-ozone gas at specified concentrations, and reinfused intravenously (15, 16).

While local ozone injections have documented rare but serious adverse events including transient neurological deficits (19), systematic data on adverse events specific to O_2-O_3-AHT remain sparse. Historical references dating to the 1980s reported extremely low complication rates (0.0007%), with only transient non-specific effects (nausea, headache, fatigue) that were indistinguishable from control groups and attributable primarily to venipuncture (20). Cuban experience spanning 25 years across multiple treatment centres documented only minor adverse effects (17).

Current guidelines addressing O_2-O_3-AHT provide lists of contraindications (Table I) but lack comprehensive discussion of documented adverse events, risk stratification, or evidence-based safety protocols (15, 21-23). This knowledge gap is particularly concerning given that systemic administration theoretically poses higher risk than localised injections. Although several studies have suggested clinical benefit of oxygen-ozone autohaemotherapy in several pain related pathologies, no structured review focusing specifically on its safety profile is currently available. Considering the typical multimorbidity of fibromyalgia patients, including cardiovascular and metabolic risk factors and frequent polypharmacy, evaluating safety is particularly relevant for rheumatologists and pain specialists.

This scoping review maps available evidence on adverse events and contraindications related to systemic O_2-O_3 autohaemotherapy, with a focus on practical implications for clinical decision-making in fibromyalgia.

This scoping review aimed to:

i. Systematically identify and characterise documented severe unexpected clinical adverse events associated with O_2-O_3-AHT; ii. Evaluate potential mechanisms underlying reported complications; iii. Critically appraise emerging safety concerns and propose risk stratification strategies; iv. Inform evidence-based patient selection criteria and protocol optimisation.

Methods

Search strategy

Following the PRISMA Extension for Scoping Reviews (PRISMA-ScR), we conducted a comprehensive literature search across nine electronic databases: Medline (PubMed), EMBASE, AMED, Cochrane Library, CINAHL, Web of Science, TRIP database, Clinical Evidence, and ROAD. Searches covered publication from 2014 to 2025; classical safety reports published before 2014 were also retrieved through citation tracking and included when historically relevant (Fig. 1).

Search terms utilised Boolean operators combining: "ozone autohaemotherapy"

OR "GAET" OR "autologous blood transfusion" OR "systemic ozone therapy" OR "oxygen ozone systemic therapy" OR "O2O3 systemic treatment" OR "ozonated autohaemotherapy" AND "iatrogenic complications" OR "side effects" OR "contraindications" OR "adverse effects" OR "adverse events" OR "safety."

Inclusion and exclusion criteria

- Inclusion criteria

1. Studies describing adverse events, side effects, or complications associated with systemic oxygen-ozone autohaemotherapy; 2. Case reports, case series, observational studies, clinical trials and systematic reviews; 3. Human subjects; 4. Any language with English abstract available.

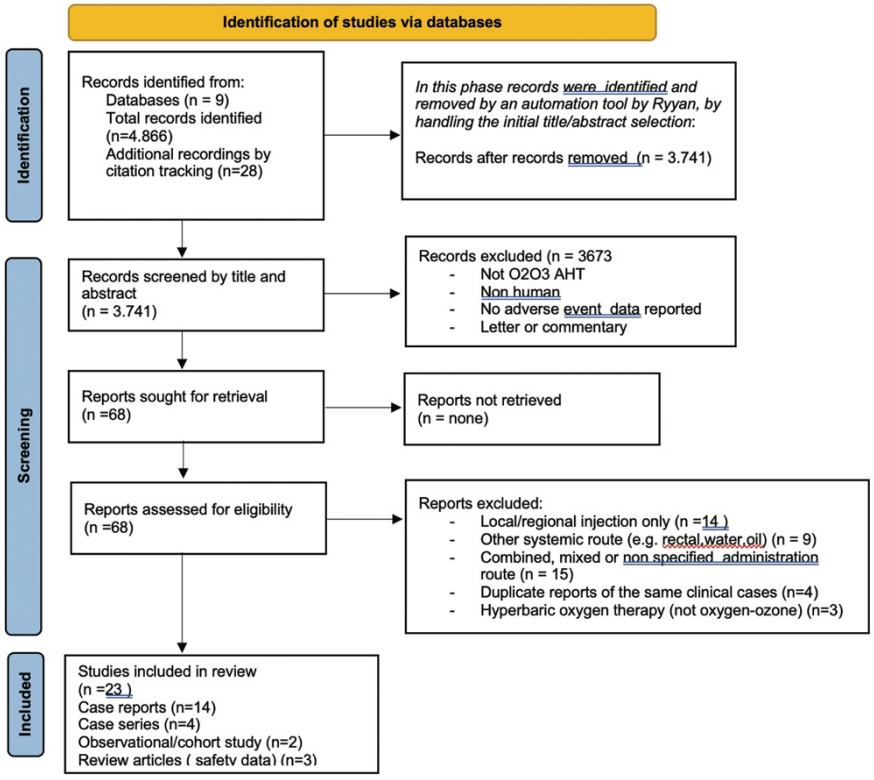
- Exclusion criteria

1. Studies focusing exclusively on local/regional ozone injections; 2. Hyperbaric oxygen therapy (HOT); 3. Veterinary or studies on animals; 4. Non-systemic routes (rectal insufflation, ozonated water, oil, or cream) or direct *i.v.* administration; 5. Studies without clear description of administration route.

It is worth of note that as O2-O3-AHT is defined as extracorporeal treatment of autologous blood, direct intravenous gas injection, a fundamentally different and dangerous procedure, is excluded from scope and should never be confused with properly performed autohaemotherapy. One recent case report describing gas embolism was excluded as the patient received multiple ozone modalities concurrently (local injections, rectal insufflation, and autohaemotherapy), precluding attribution to O_2-O_3-AHT specifically, though cited for completeness (24).

Data extraction

For each included case, we extracted: patient demographics and comorbidities, O_2-O_3-AHT protocol details (ozone concentration, blood volume, treatment frequency), adverse event description and timing, proposed mechanism, interventions required, and outcome. Cases are presented thematically by adverse event category.



Source: Page MJ, et al. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

Fig. 1. Identification of studies across electronic databases from 2014 to 2025.

Results

The initial search yielded approximately 4,866 publications using broad ozone therapy terms. After applying focused search criteria and exclusion filters, we identified a limited number of case reports and review articles specifically documenting adverse events from O_2-O_3-AHT. Results are organised by adverse event category with analysis of proposed mechanisms and preventive strategies. Table II summarises categories of documented adverse events with estimated frequency and severity based on the available literature, here in reported.

Haemolysis and renal complications

A case report documented haemoglobinuria and acute renal failure following O_2-O_3-AHT administered at ozone concentrations equal to or exceeding 60 µg/mL (25). This concentration substantially exceeds the 10–40 µg/mL range recommended by international guidelines (ISCO3), which explicitly warn that concentrations of 70–80 µg/mL and above carry significantly increased risks including haemolysis, depletion of erythrocyte 2,3-diphosphoglycerate, exhaustion of antioxidant reserves and consequent inability to activate immune-competent cells (15).

Recent research demonstrates that even therapeutic ozone doses induce statistically significant increases in microparticles derived from erythrocytes and endothelial cells, though changes in coagulation factors remain within normal ranges (26). With escalating ozone exposure, progressive erythrocyte damage occurs. Haemolysis risk is particularly elevated in patients with pre-existing anaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, or renal impairment. *Key prevention*. Strict adherence to recommended ozone concentrations (10-40 µg/mL), mandatory pre-treatment G6PD screening, baseline haemoglobin assessment, and avoidance in patients with significant anaemia or renal dysfunction.

Hyperkalaemia and cardiac arrhythmia

Tang *et al*. reported a complex case involving a patient with hypertension, diabetes mellitus, and chronic kidney dis-

Table II. Documented unexpected adverse events associated with O_2-O_3-AHT.

Adverse event category	Frequency estimate	Severity	Key risk factors	Prevention strategy
Haemolysis with renal failure	Very rare	Severe	Excessive ozone dose (>60 µg/mL), G6PD deficiency, pre-existing anaemia	Adhere to 10-40 µg/mL range, mandatory G6PD screening, baseline Hb assessment
Hyperkalaemia/arrhythmia	Very rare	Severe	Advanced CKD, diabetes, hypertension	Renal function assessment, electrolyte monitoring, caution in CKD stage 4-5
Myocardial infarction	Rare	Severe	Coronary artery disease, multiple CV risk factors	Pre-treatment CV assessment, defer if unstable angina or recent MI
Cerebral gas embolism	Rare	Severe	Patent foramen ovale (25-30% population)	Consider echocardiography in high-risk patients, meticulous technique
Autonomic reactions	Uncommon (1-5%)	Mild	Rapid reinfusion, volume load	Controlled reinfusion rate (<50 drops/min), supine position
Anaphylaxis	Very rare	Moderate	Previous anaphylaxis, material sensitivity	Modern equipment, allergy history, emergency medications available
Infectious complications	Very rare	Severe	Protocol breach, inadequate sterility	Strict aseptic technique, single-use equipment, quality assurance
Minor effects	Common (5-15%)	Mild	Individual variability	Patient education, reassurance, symptomatic management

Frequency estimates based on historical data (Jacobs 1986).
The 0.0007% figure (Jacobs, 1986) pertains to serious adverse events only and is not comparable to the minor symptom frequencies in Table IV, which reflect procedural tolerability data from clinical observations, not systematic surveillance. All frequency estimates in this table are qualitative clinical impressions based on case reports and non-systematic observations, not epidemiologically derived incidence figures.

ease who developed acute symptomatic hyperkalaemia following O_2-O_3-AHT (27). The patient experienced sudden dizziness, visual disturbance (described as black haze lasting five hours), brief loss of consciousness (2–3 seconds) and profuse diaphoresis. Physical examination revealed blood pressure 158/76 mmHg, respiratory rate 28 breaths/minute, heart rate 40 beats/minute, and oxygen saturation 98%. Electrocardiography demonstrated sinus arrest with junctional escape rhythm, T-wave changes, and laboratory testing confirmed hyperkalaemia.

The mechanism likely relates to blood transfusion-associated hyperkalaemia, particularly in patients with advanced chronic kidney disease, compounded by the autohaemotherapy procedure itself (28). This case underscores potential for serious adverse events in patients with multiple comorbidities.

Key prevention. Careful evaluation of renal function, electrolyte monitoring in high-risk patients, and consideration of relative contraindication in advanced chronic kidney disease.

Thromboembolic and ischaemic events

While thromboembolic complications have been documented following local ozone injections (29), our search identified no definitive cases directly attributable to O_2-O_3-AHT alone. However, mechanistic concerns remain valid. Ozone may transiently activate platelets and endothelial cells, potentially increasing thrombotic risk in predisposed individuals (30). This theoretical risk is particularly relevant for patients with inherited thrombophilia (*e.g.* Factor V Leiden, prothrombin G20210A mutation), antiphospholipid syndrome, or active malignancy. Despite the theoretical prevalence of patent foramen ovale in 25–30% of the general population, only a handful of cerebral gas embolism cases have been reported over decades of systemic O_2-O_3 use. This suggests that clinically significant events are extremely rare when protocols are correctly followed and blood reinfusion is performed without residual gas.

Key prevention. Pre-treatment coagulation assessment in high-risk patients, detailed personal and family history of thromboembolism, and consideration of thrombophilia screening in selected cases.

Cardiovascular events: myocardial infarction

Case reports describe acute myocardial infarction occurring in temporal association with O_2-O_3-AHT. Ureyen *et al*. reported acute myocardial infarction in a 46-year-old male following O2-O3-AHT, postulating vasoconstrictive and pro-thrombotic mechanisms (31). Bingham and Platt described non-ST elevation myocardial infarction in a 50-year-old woman with cervical disc disease receiving what was described as 'ozone infusion' (32). Coronary catheterisation revealed no atherosclerotic disease or angiographic abnormalities, and investigations excluded gas embolism, leading authors to attribute the event to myocardial oxidative stress.

However, this report was subsequently challenged in the same journal for insufficient procedural details, specifically unclear whether the intervention constituted properly performed O_2-O_3-AHT or potentially direct intravenous gas injection, which represents an inappropriate and dangerous route of administration (33). This controversy highlights the critical importance of precise procedural documentation in adverse event reporting.

Key prevention. Cardiovascular risk assessment before O_2-O_3-AHT initiation, caution or deferral in patients with unstable angina or recent myocardial infarction (<3 months), and avoidance of

Table III. Recommended pre-treatment safety screening.

Assessment	Test/Evaluation	Rationale	Priority
Haematological	G6PD activity	Detect deficiency causing haemolysis	Mandatory (guidelines)
	Complete blood count	Assess anaemia, thrombocytopenia	Mandatory (guidelines)
	Baseline haemoglobin/haematocrit	Establish baseline for monitoring	Mandatory (guidelines)
Metabolic	Comprehensive metabolic panel	Renal and hepatic function	Mandatory
	Serum electrolytes	Baseline potassium in CKD	Mandatory
	Thyroid function (TSH, free T4)	Detect hyperthyroidism	Mandatory
Cardiovascular	Detailed CV history	Identify CAD, recent MI, arrhythmia	Mandatory
	Resting ECG	Baseline cardiac status	Recommended
	Echocardiography with bubble study	Detect PFO in high-risk patients	Selected cases*
Coagulation	PT/INR, aPTT, platelet count	Detect coagulopathy	Mandatory
	Thrombophilia screening†	Genetic/acquired thrombophilia	Selected cases**
Medical history	Detailed interview	Comorbidities, medications, allergies	Mandatory
	Family history	Thromboembolism, bleeding disorders	Mandatory

Items marked 'Mandatory (guidelines)' reflect requirements explicitly stated in one or more of references (14, 22, 23). If not specified they reflect the authors' expert opinion based on the pathophysiological rationale described in the text and in relation to its use in fibromyalgia, not a formal requirement of current guidelines.
*High-risk patients: cryptogenic stroke, documented cardiac shunt, frequent diving/altitude exposure, pulmonary hypertension.
†Factor V Leiden, prothrombin G20210A, antiphospholipid antibodies.
**Personal/family thrombosis history, recurrent miscarriage, young stroke/MI.

direct intravenous gas injection under all circumstances.

Autonomic reactions and vasoconstriction

Sporadic reports describe perioral paraesthesia, sensations of warmth, tachycardia, chest coolness, and vasovagal reactions occurring during or immediately after O_2-O_3-AHT (34). Rimini *et al*. investigated these phenomena using transcranial Doppler (TCD) and near-infrared spectroscopy (NIRS) to assess haemodynamic changes during blood reinfusion at different rates (34).

Rapid reinfusion (>80 drops/minute) produced measurable increases in peripheral vascular resistance, attributed to volume-induced autonomic nervous system stimulation. This vasoconstrictive reflex likely underlies reported symptoms. In contrast, slow reinfusion (<50 drops/minute) significantly reduced adverse reactions (35).

Key prevention. Controlled reinfusion rates not exceeding 50 drops/minute (approximately 1 drop per second), ensuring patient is supine or semi-recumbent during procedure, and monitoring for early signs of autonomic dysregulation.

Cerebral gas embolism and neurological complications

Cerebral gas embolism represents one of the most serious documented complications, though exceedingly rare. Multiple case reports describe cerebral gas embolism following O2-O3-AHT in patients with patent foramen ovale (PFO) (36-40). The proposed mechanism involves microbubbles formed during ozone-blood mixing that bypass normal pulmonary filtration via right-to-left cardiac shunts, entering systemic arterial circulation and ultimately cerebral vasculature.

Patent foramen ovale affects approximately 25–30% of the general population, representing a clinically silent anatomical variant in most individuals but conferring risk during procedures generating microbubbles. Reported neurological manifestations include: I) Generalised tonic-clonic seizure (3-minute duration) in one patient, with neuroimaging revealing multiple ischaemic infarcts in left thalamus and right cerebellum consistent with embolic events (37, 38). Motor recovery was nearly complete; however, significant cognitive deficits in speech, memory, and comprehension persisted for six months, requiring intensive speech and occupational therapy. II) Acute hemiplegia in a patient with complex medical history including chronic lymphocytic leukaemia, chronic migraines, hypertension, and diabetes mellitus, emphasising vulnerability in patients with multiple comorbidities (40).

Key prevention. Consideration of echocardiography with agitated saline (bubble study) in patients with history of cryptogenic stroke, known cardiac shunts, frequent diving or high-altitude exposure, significant pulmonary hypertension, or multiple vascular risk factors. Meticulous technique minimising microbubble formation during ozone-blood mixing.

Anaphylactic reactions

Bocci documented four female patients, including one with previous documented anaphylaxis, who developed acute hypersensitivity reactions following O_2-O_3-AHT (41). Manifestations included urticaria, pruritus, nausea, flushing, and mild hypotension. Symptoms resolved within two hours following intravenous methylprednisolone (1 g). Investigation attributed these reactions to sensitisation from phthalates or other polyvinyl chloride (PVC) additives present in older-generation infusion equipment (41).

Key prevention. Use of modern, medical-grade equipment free from sensitising plasticisers, pre-treatment allergy history, and availability of emergency medications (antihistamines, corticosteroids, epinephrine) in treatment areas.

Infectious complications

A concerning outbreak of hepatitis C

Table IV. Common minor side effects of O_2-O_3-AHT.

Symptom	Frequency	Timing	Duration	Management
Transient fatigue	5-10%	Hours after treatment	12-24 hours	Reassurance, rest, hydration
Perioral paraesthesia	5-8%	During/immediately after	Minutes to hours	Slow reinfusion rate
Mild headache	4-8%	During/after treatment	Hours	Hydration, slower reinfusion, analgesics if needed
Nausea	2-4%	During/after treatment	Hours	Slower reinfusion, antiemetics if needed
Metallic taste	2-3%	During treatment	Minutes to hours	Transient, no intervention
Dizziness	1-3%	During/after treatment	Minutes to hours	Supine position, slower reinfusion
Chest tightness/palpitations	<2%	During reinfusion	Minutes	Slower reinfusion, monitor vital signs

Adapted from Bocci V, 1994 (20) and Bocci V, 2006 (16).

virus (HCV) infection was documented among patients receiving ozone autohaemotherapy (42). The overall incidence reached 9.7% (3 of 31 patients), with 25% (3 of 12) among those specifically undergoing autohaemotherapy. This represented a clear breach of infection control protocols.

Key prevention. Strict adherence to aseptic technique throughout procedure, use of single-use disposable equipment where applicable, proper sterilisation protocols for reusable equipment, regular training in infection control, and quality assurance auditing.

Unexpected mortality in cancer patients

Cassileth and Yarett reported five terminal cancer patients who died unexpectedly while receiving O_2-O_3-AHT as complementary therapy (43). No specific mechanisms were identified, and the report acknowledged the inherent difficulty in attribution given patients' advanced disease states. This report, published in the context of discussing cancer quackery, emphasised concerns about unproven complementary therapies in vulnerable populations (43).

Interpretation. While direct causality could not be established, this report underscores the need for careful patient selection, informed consent emphasising experimental status of O_2-O_3-AHT in cancer, and enhanced monitoring in seriously ill patients.

Discussion

It is essential to acknowledge at the outset that all frequency and incidence estimates discussed below are derived from a literature base consisting almost entirely of case reports and non-systematic clinical series. Therefore, although of clinical relevance, they do not constitute epidemiologically valid incidence figures and must not be interpreted as such. With these premises this scoping review documented that adverse events associated with O_2-O_3-AHT, while occasionally serious, occur in literature with low frequency. The historical incidence of 0.0007% reported by Jacobs (20) and subsequent extensive Cuban experience (17) seem to be coherent with the herein reported data and suggest an overall favourable profile when procedures follow established protocols.

Several limitations constrain definitive conclusions as this scoping review is limited to published case reports and case series; prospective cohort studies with systematic adverse event surveillance are lacking and if unusual or severe outcomes are preferentially reported, minor complications may be likely underreported. Other factors limiting this review are the heterogeneity of protocols precluding precise safety estimates.

As far as the calculation of incidence is concerned, the incomplete denominator data, namely the total number of O_2-O_3-AHT procedures globally performed, prevent accurate incidence calculations. Moreover, most reports document acute or subacute events while potential delayed complications are inadequately characterised as data on long-term follow-up are missing in literature, particularly in non-English language literature and clinical practice settings outside academic medicine.

In this systematic review the overwhelming majority of reported unexpected serious events were reported in population of unspecified diagnosis, and it can be only inferred in fibromyalgia. Moreover, it is worth of note that fibromyalgia patients are predominantly women aged 40–60 years and frequently present comorbid conditions such as metabolic syndrome, hypertension, dysautonomia, anxiety disorders and chronic use of antidepressants or analgesics. These characteristics may increase susceptibility to autonomic reactions (including vasovagal responses or transient dizziness) and justify careful pre-treatment assessment, gradual dosage titration and close monitoring during early sessions.

Despite these limitations, the nature and severity of documented adverse events, particularly those requiring hospitalisation or resulting in persistent morbidity, would be expected to reach medical literature if occurring with substantial frequency. Although the rarity of such reports, combined with widespread clinical use over decades current evidence, though of limited methodological quality, does not identify a pattern of frequent serious harm when standardised protocols are followed. However, safety conclusions remain provisional pending higher-quality prospective data.

Analysis of documented adverse events reveals three primary categories of risk factors requiring systematic attention:

a. Technical and procedural variables. *Dosing precision*. Haemolytic reactions and hepatocellular injury demon-

strate clear dose-response relationships. Prevention requires individualised dosing based on patient body weight, baseline haematological parameters (haemoglobin, haematocrit, red cell indices), hepatic function markers, and treatment frequency (15, 21). Recommended ozone concentrations of 10-40 μg/mL should not be exceeded; concentrations above 60 μg/mL carry unacceptable haemolysis risk.

Mechanical factors. Mechanical haemolysis represents a preventable iatrogenic complication. Gentle mixing using a tilting blood scale combined with controlled reinfusion rates (gravitational flow or calibrated infusion pumps at <50 drops/minute) maintain haemolysis rates at 2.8±0.7%, within clinically acceptable parameters and significantly below thresholds causing physiological consequences (26, 35).

Infection control. Breaches in sterile protocol represent entirely preventable sources of infectious complications. Mandatory measures include strict aseptic technique, single-use disposable equipment preferentially, proper sterilisation of reusable components, and regular quality assurance auditing.

b. Patient-specific risk factors

Haematological conditions. Glucose-6-phosphate dehydrogenase deficiency represents an absolute contraindication due to life-threatening haemolysis risk (15, 21-23). G6PD deficiency prevalence varies by ethnicity: lower in Americas (3.4%), Europe (3.9%), and Pacific (2.9%) *versus* higher in sub-Saharan Africa (7.5%), Middle East (6.0%), and Asia (4.7%) (44). Mandatory pre-treatment G6PD screening is non-negotiable.

Coagulopathies. Undiagnosed or inadequately characterised thrombophilias present significant thromboembolic risk. Screening should include complete blood count with platelet count, coagulation profile (PT, aPTT, INR) and detailed personal/family thrombosis history. In high-risk populations, consider genetic thrombophilia testing (Factor V Leiden, prothrombin G20210A, antiphospholipid antibodies).

Cardiovascular anatomy. Patent foramen ovale, present in 25–30% of the population, creates potential for paradoxical gas embolism. Risk stratification requires cardiovascular history assessment and consideration of echocardiography with bubble study in patients with cryptogenic stroke history, documented cardiac shunts, frequent diving/altitude exposure, or significant pulmonary hypertension (36-40).

Comorbidity burden. Cases in patients with multiple conditions (diabetes, hypertension, chronic kidney disease) highlight vulnerability in complex medical scenarios (27). Enhanced caution, dose reduction, or deferral may be appropriate in patients with advanced multisystem disease.

c. Protocol standardisation

Current practice demonstrates substantial heterogeneity in key parameters as ozone concentration (eight-fold variation 10 to 80 μg/mL) across protocols, with some exceeding safety thresholds) and gas mixture ratio. Moreover, also blood volume as a very broad variation ranging from 50/100 mL to 200/250 ml with a variable treatment frequency (single session to multiple weekly treatments). This heterogeneity creates challenges for comparative effectiveness research, safety meta-analysis and optimal therapeutic dosing. International consensus efforts should prioritise establishing evidence-based standard operating procedures (15, 21-23).

The critical importance of appropriate dosing and route selection is exemplified by contrasting effects of pulmonary *versus* systemic ozone exposure. Direct inhalational ozone exposure causes dose-dependent alveolar epithelial damage, respiratory tract inflammation, pulmonary surfactant oxidation and documented pulmonary function impairment, recognised as well-established effects of environmental ozone pollution (45).

In striking contrast, systemic O_2-O_3-AHT at controlled concentrations (10-40 μg/mL) demonstrates improved pulmonary function parameters in asthma patients: increased FEV_1, FVC, and peak expiratory flow; improved symptom scores; reduced exacerbation frequency; and decreased bronchodilator requirement (46). This apparent paradox reflects fundamental pharmacological principles related to dose-response relationship and to the route of administration: dose-response relationships govern therapeutic *versus* toxic effects with a direct oxidative damage at high concentrations. This underscores that O_2-O_3-AHT safety and efficacy depend critically on adherence to evidence-based protocols.

Conclusions

The conclusion of this scoping review regarding safety are necessarily provisional, given the low quality and largely based on case reports and anecdotical nature of the available evidence base. However**,** it indicates that O_2-O_3-AHT has a safety profile when administered according to established protocols with appropriate patient selection and technical expertise. Few single cases are reported in literature documenting severe unexpected adverse events with only an historical estimation suggesting an incidence of approximately 0.0007%. Most adverse events are preventable through proper risk management strategies.

Essential safety measures include mandatory G6PD screening before treatment initiation, adherence to recommended ozone concentrations of 10–40 μg/mL, regulation of reinfusion rates to a maximum of 60 drops per minute, comprehensive pre-treatment patient assessment to identify contraindications or high-risk conditions, strict aseptic technique, and availability of emergency intervention capabilities. While serious complications such as haemolysis, cardiac arrhythmias, myocardial infarction, and cerebral gas embolism are rare, their potential occurrence underscores the critical importance of physician expertise, careful patient selection, and strict protocol adherence. Further improvements in safety can be achieved through development of international standardised protocols, implementation of prospective adverse event registries, and establishment of formal operator certification programs.

As the typical fibromyalgia patient presents a distinctive comorbidity profile, including female sex predominance, dysautonomia, central sensitisation, chronic polypharmacy, physical deconditioning (1, 3, 8, 47), that may modulate both procedural tolerance and ad-

verse event risk in ways not captured by the existing literature more attention should be taken in the patient's selection when treating fibromyalgia. When appropriate precautions are implemented, O_2-O_3-AHT may serve as a valuable component of comprehensive multidisciplinary fibromyalgia management.

Based on the spectrum of reported adverse events and their underlying mechanisms, a comprehensive safety protocol is recommended. Pre-treatment assessment should include G6PD activity testing, complete blood count, comprehensive metabolic panel, and detailed medical history review. For patients with elevated risk profiles, additional investigations should be considered, including coagulation studies, echocardiography with bubble study if cardiovascular concerns exist, and thrombophilia screening if personal or family history warrants such evaluation.

Protocol standardisation is critical to maintaining safety. Ozone concentrations must be maintained between 10–40 μg/mL and should never exceed 60 μg/mL. Blood volume should be adjusted from 50–150 mL based on patient body size, and reinfusion rates must be limited to a maximum of 50 drops per minute. Vital signs should be monitored continuously throughout the procedure, and only modern, medical-grade equipment should be employed, with preference given to single-use disposable systems.

Patient selection must exclude individuals with absolute contraindications, which include G6PD deficiency, and as authors indication, also active hyperthyroidism, severe coagulopathy, pregnancy, and recent myocardial infarction within the preceding three months. Relative contraindications such as advanced chronic kidney disease, significant multimorbidity, or active malignancy require individualised risk-benefit assessment before proceeding with treatment.

To further optimise the safety profile of O_2-O_3-AHT, several research initiatives require prioritisation. The establishment of prospective international multi-centre registries is essential for systematic adverse event monitoring and reduction of publication bias across diverse clinical settings. Protocol standardisation trials are needed to conduct comparative studies evaluating safety outcomes across different ozone concentrations and treatment regimens, thereby informing evidence-based international consensus guidelines. The identification and validation of patient-specific biomarkers would enable prediction of individual susceptibility to adverse events, facilitating personalised risk stratification and tailored therapeutic approaches.

Detailed mechanistic investigations are required to elucidate the biological processes underlying both therapeutic efficacy and risk in O_2-O_3-AHT. These studies should focus on platelet activation, endothelial interactions, and immunomodulatory effects. The development and validation of comprehensive operator certification programs are imperative to establish technical competency standards that minimise procedural errors and enhance patient safety. Finally, long-term outcome studies are necessary to assess delayed complications and evaluate the cumulative effects of repeated treatments over extended follow-up periods. High-quality prospective studies including fibromyalgia-specific cohorts are needed to better quantify risk and support guideline development.

Take home messages

- Current evidence though of limited methodological quality, does not identify a pattern of frequent serious harm when standardised protocols are followed.
- Mandatory glucose-6-phosphate dehydrogenase screening before any treatment is non-negotiable.
- Strict adherence to recommended ozone concentrations (10-40 μg/mL) prevents haemolytic complications.
- Controlled reinfusion rates (<60 drops/minute) to minimise autonomic reactions.
- The first minutes of reinfusion should be conducted at a slow rate to promptly identify any citrate intolerance.
- Pre-treatment cardiovascular screening in selected high-risk patients may prevent serious embolic complications.
- Prospective adverse event registries and protocol standardisation would further enhance safety.

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