ISCO3/MET/00/01 Major Autohemotherapy

Index

Title

ISCO3/MET/00/01 Major Autohemotherapy

1.1. Brief background

Major Autohemotherapy (MAH) was originally developed by Dr. Hans Wolff, in Frankfurt, Germany, in the late 1960s. It is one of the preferred systemic ways to administer ozone to a patient. It is very safe, virtually free of negative side effects, and permits an ozone dosage range from very low to very high. It is recommended, either as only treatment or in combination with other forms of ozone therapy, or other medication, for a rather large number of diseases or conditions.

1.2. Purpose

The purpose of this SOP is to describe the procedure for a Major Autohemotherapy (MAH) session with ozone.

1.3. Scope

This procedure specifies the blood collecting technique, doses, volume of gas and frequency of application of ozone.

1.4. Acronyms, abbreviations and definitions

G6PD	Glucose 6 phosphate dehydrogenase
Gamma	Not appropriate but frequently word to designate microgram
MAH	Major Autohemotherapy
SOP	Standard Operation Procedure
Phthalates	*Phthalates* or *phthalate esters*, are esters of phthalic acid and are mainly used as plasticizers (substances added to plastics to increase their flexibility, transparency, durability, and longevity)
Total dosis	Total amount, in micrograms, of ozone given per session, calculated as volume in mL multiplied by concentration in µg/mL

2. Responsibility

Physician
Clinical records registration
Assessment of the indication, contraindications
Request the informed consent (ISCO3/QAU/00/21) and the privacy consent
Applications and monitoring
Recording all data on medical records
Prescription of investigations to assess the effectiveness of the treatment (e.g. biochemical or immunological assays)

Reporting any late complications
Patient follow-up

Nurses Accommodate the patients
Preparation of the clinical procedure
Supervision of patients, and vital signs control (temperature and pressure)
Detect and alert the doctor to anomalies due to possible reactions
Notification of possible complications

A MAH session should be done by a physician, adequately trained in ozone therapy. Also a trustworthy assistant, nurse, or paramedical professional, may do the procedure, provided this person is adequately trained for this work. It is the physician's responsibility to see that all steps of the procedure are done in the correct manner, in order to always avoid errors, accidents, and to prevent incidents.

3. Procedure

3.1 Indications

Arterial circulatory disturbances
- ✓ Peripheral arterial circulatory disturbance
- ✓ Cerebral circulatory disturbance (stroke)
- ✓ Ocular circulatory disturbances (retinopathies)
- ✓ Inner ear circulatory disturbances (acute hearing loss AHL), tinnitus
- ✓ Arterial insufficiency

Angiopathy
- ✓ Diabetic angiopathy
- ✓ Trophic skin lesions

Viral diseases
- ✓ Viral Hepatitis type A, B and C
- ✓ Virus infections: eg. Herpes simplex, Herpes zoster

General immune deficiency
- ✓ As complementary therapy in general weakness, geriatric, environmental medicine
- ✓ As complementary therapy during bacterial infections

Chronic inflammatory processes in orthopedics and rheumatology
- ✓ Chronic inflammatory processes
- ✓ Chronic pain conditions
- ✓ Arthrosis of large joints

Complementary concept in oncology
Pre-conditioning for patients who plan to undergo major surgery

3.2 Contraindications

Absolute contraindication: Favism: Glucose-6-phosphate dehydrogenase deficiency (favism, acute haemolytic anaemia).*

Relative contraindications / special situations:

- ✓ Uncompensated diabetes
- ✓ Pregnancy, especially in the first 3 months (use it with precaution)[1-4]
- ✓ Uncompensated toxic hyperthyroidism - Basedow Graves status
- ✓ Thrombocytopenia less than 50.000 and serious coagulation disorders
- ✓ Severe Cardiovascular instability
- ✓ Acute alcohol intoxication
- ✓ Acute infarction of myocardium
- ✓ Massive and acute hemorrhage
- ✓ During convulsive states
- ✓ Hemochromatosis
- ✓ Patients receiving treatment with copper or iron.

* The prevalence of Glucose 6 phosphate dehydrogenase (G6PD) deficiency varies among ethnic groups with overall lower frequency in the Americas (3.4%), Europe (3.9%), and the Pacific (2.9%) as compared to sub-Saharan Africa (7.5%), the Middle East (6.0%), and Asia (4.7%).[5] Test of G6PD is recommended prior to O_3 therapy in order to avoid complications.

3.3 Recommended doses and intervals

MAH may be given at most variable intervals, from daily to weekly or monthly. Also as cycles to be given once or more times per year (Table 1). The total dose of ozone to be given at each session will vary according to the disease to be treated and the general condition of the patient. It may vary from less than 500 µg to 4 000 µg (Table 2). This procedure is in line with the Madrid Declaration (ISCO3/QAU/01/03). The ozone concentration in the O_2-O_3 gas mixture must not exceed 78 µgN/mL due to the risk of hemolysis.

The **volume of blood** to use varies between 50 mL and 100 mL. Blood volumes greater than 200 mL must be avoided to prevent any risk of hemodynamic disturbances, especially in elderly or unbalanced patients. Approximate ranges of a safe blood collection are: 1.2 mL / kg to 1.3 mL / kg. Example: a person of 85 kg; 1.2 · 85 = 102 mL blood to be extracted.

Frequency of treatment: The number of treatment sessions and the ozone dosage administered will depend on the general condition of the patient, age and main disease. From the clinical point of view, a patient's improvement can be expected to occur between the fifth and tenth session, and it is generally considered that after the twelfth session the antioxidant defense mechanism has already been activated and optimized. The treatment may be given daily if necessary. It may also be administered two to three times a week. Cycles can be repeated two or three times per year.

Table 1. Indicative recommended ozone doses for Major Auto Haemotherapy.[6]

Indication	O_3 quantity (µg)	Treatment frequancy	Number of treatments
ARTERIAL CIRCULATORY DISORDERS			
Cerebral and peripheral, stage II	(800 - 1000) µg per 50 mL, 15–20 µg /mL	2 per week	Series of 10 treatments 2-3 per year
Stage III and IV	(1000 - 1500) µg per 50 mL, 20–30 µg /mL	Daily at first, then 2 per week	
IMMUNOACTIVATION			
Geriatrics	(800 - 1 500) µg, 15–20 µg /mL, 50 mL	2 per week	Series of 10 treatments 2 per year
Preventive vs. infection	(1 000 - 1 500) µg, 20–25 µg/mL, 50 mL	2 per week	Series of 6 treatments 2 per year
Adjuvant in cancer therapy	(500 µg - 1 000) µg, 10 –15 µg /mL, 50 mL	2 per week	Series of 10 treatments several times per year or: 2 treatments per month after the 1st treatment series (continuously)
INFECTIONS			
Hepatitis, A, B, C			Several series
Acute	2 000 µg, 30-40 µg /mL, in 70 -100 ml blood	Daily	As per control
Subsiding	1500 - 2000 µg	2 per week	As per control
Chronic (B/C)	500 -1 000 µg, 10–20 µg /mL 50 mL / 3000 - 4 000 µg, 10–20 µg /mL 100 mL	1 - 2 per week	6 -12 months
Herpes zoster			
Acute stage	2000 µg, 40 µg/mL 50 mL	daily in the 1st week	1 series of 10 treatments
Post acute	1000 -1500 µg, 20–30 µg /mL in 50 mL	2 x per week	As per control
INFLAMMATORY PROCESSES			
Rheumatoid arthritis			
Acute stage	30 -35 µg /ml, 50 mL 1 500 -1 750 µg (100 mL, 3 000 -3 500 µg).	Daily	As per control
Chronic stage	20–25 µg /mL, 50 mL 1 000 -1 250 µg	2 per week, then 2 per month	As per control
Angiopathia, diabetic angiopathia	20–25 µg /mL, 50 mL 1 000 -1 250 µg	2x per week, later 2x per month	In compliance with the patient

The total ozone dose per session as described in the table above is according to the author mentioned in the reference No. 2. **Many other ozone therapists all over the world may recommend different - usually higher - doses of ozone and also blood volume of 100 mL or higher.**[7]

Table 2. Range of doses for Major Authohemotherapy according to Madrid Declaration.

MAJOR AUTO HEMO				
O_3	Levels			**Remarks**
	High	**Medium**	**Low**	
C. (µg/mL)	30-40	20-30	10-20	[a] In some cases one may consider the use of up to 60 µgN/mL which has proved to be safe and with greater capacity of induction of cytokines. Venous blood volume 50 mL-100 mL.
V. (mL)	50 - 100			
Dose (mg)	1.5-4.0 3.0-4.0	1.0-3.0 2.0-3.0	0.5-2.0 1.0-2.0	

Legend: C, concentration; V, volume.

Ozone concentrations for systemic uses range from 10 µg/NmL to 40 µg/NmL, concentrations above 60 µgN/mL should be avoided because of the increased risk of hemolysis, reduction of 2,3 DPG and anti-oxidant and a consequent inability to activate immune-competent cells.

3.4 Clinical evaluation

A clinical and/or laboratory evaluation is necessary to establish a precise diagnosis and to permit comparisons between the patient's status before, during and after ozone therapy. **Much value should be given to the subjective data the patient refers**, as for example "I feel more energy", etc. The main variable should be the marker of the specific disease. Because the intervention of ozone in the oxidative system, variable of oxidative stress should be taken into consideration (e.g. SOD, CAT, NO, GSH, AOPP, TH, etc.), in addition markers of immune function can be measure (IL1, IL6, TNFα, etc.).

3.5 Preliminary operations

The patient must be fully informed in advance about the method itself, about all the steps of the procedure, about the desired effect(s) and also about the possible unwanted side effects. Also a written Term of Informed Consent should be read, understood and signed by the patient or the person responsible for the patient before the procedures.

Device: must fit the standard requirement ISCO3/DEV/00/01

Perfusion set: Plastic-based devices, intended to contain blood, must meet the ISO 15747 standard: 2005 (This is the European Union regulation). All containers and devices used in O3x must be ozone-resistant and must not release phthalates. For that reason, it is preferable to use glass for MAH.

Anticoagulant: it is most advisable to use ACD-A *Anticoagulant Citrate Dextrose* Solution A, USP (2.13% free citrate ion), or Citrate Sodium 3.8 % 10 mL per 100 mL of blood. Generally, heparin is not advisable because it can induce thrombocytopenia[8] and Platelet aggregation,[9] but

it could be acceptable or even preferred in some pathologies and Citrate Sodium chelates Calcium. The quantity of ACD-A ranges from 7 mL -10 mL per 100 mL of blood.

Nevertheless heparin is regularly used during EBOO (Extra corporeal Blood Oxygenation - Ozonation) and dialysis and, bearing in mind the above indicated restrictions, may be useful in vascular diseases and cancer because of the increased release of a number of growth factors from platelets[4] and cytokines from leukocytes[10, 11]. Thus, only after a careful analysis of the patient, the ozone therapist can select the most idoneous anticoagulant.

3.6 Main procedure

3.6.1 In a calm and clean professional environment, put the patient on a comfortable armchair or table, preferably one which can have the back rest inclined and the leg support raised. Make sure there is enough space around the armchair, for persons and material carts or tables.

3.6.2 Prepare and assemble the necessary materials: scalp ("butterfly") needle gauge 19 G x 3/4 (1.01 mm • 19 mm) when using a plastic pouch or 21 G x 3/4 (0.80 mm • 19 mm) when using a glass vacuum bottle to draw. Fill a glass syringe, to which an antibacterial filter ("Milipore" or similar 0.2 µm) is attached, with the desired volume and concentration of oxygen / ozone gas mixture coming out of the ozone generator.

3.6.3 Use a disposable 5 mL syringe and a 25 G x 2 (0.508 mm • 5.08 mm) disposable needle to inject the necessary volume of anticoagulant into the plastic pouch or glass vacuum bottle, keeping about 1 ml in the syringe for later use. Instead of natrium citrate, Heparin at 5000 IU/mL (Liquemine® by ROCHE, or equivalent) may also be used (see specifications for the use of heparin). In this case, 1 mL of this solution will suffice to prevent clotting of up to 100 mL of blood in the bottle or pouch. In the case of ACD-A the ranges are from 7 mL to 10 mL per 100 mL of blood. Make sure that all the line of extraction of blood has been anti-coagulated before extracting the blood.

3.6.4 Connect the butterfly scalp to a tubing and insert the other end into the pouch or vacuum bottle. Caution: when using vacuum bottle the tubing must be clamped closed, in order not to lose the vacuum.

3.6.5 Install a venous access. The veins in the area of elbow or forearm are to be preferred. The small veins on wrist and back of hand are mostly too small and have easy to rupture walls. Veins in the lower limbs and on the neck must not be punctured.

3.6.6 Draw the desired amount of venous blood into the recipient. Usually a total volume of 100 mL will suffice. This recipient is ideally a vacuum bottle, of 250 mL to 350 mL volume. This bottle may be placed on a nearby surface at the same horizontal level as the patient's arm, as the vacuum will pull the blood from the vein. If there are no vacuum bottles available, the recipient may also be a disposable sterile plastic bag / pouch, made of ozone-resistant material, into which the blood will flow from the vein moved mainly by gravity. In this case, it is better to place the recipient in such a way as to have the largest possible difference in altitude between arm vein and recipient, i.e. lower than the table surface.

3.6.7 Clamp closed the tubing, disconnect the tubing from the butterfly needle, connect the 5 mL syringe to the butterfly needle and inject part of the remaining anticoagulant, just enough to prevent the clotting of blood in the needle, and leave the syringe connected. Then, by slowly opening the clamp, see that all blood in the tubing flows into the bottle

our pouch, closing the clamp immediately when all blood is in the recipient, especially when using vacuum bottle.

3.6.8 Using a 25 G x 2 (0.508 mm • 5.08 mm) needle, inject the desired volume of the O_2/O_3 mixture into the pouch or vacuum bottle and shake or invert the recipient *very gently* once or twice.

3.6.9 Insert new tubing with a drip and filter into the recipient and clamp closed the lower extremity of this tubing.

3.6.10 Hang recipient onto a drip stand, assuring it is in a position high enough to assure a good and easy flow of the now ozonized blood back into the same vein by force of gravity.

3.6.11 Let the blood flow out from the recipient, filling the new tubing until immediately before the connection tip.

3.6.12 Disconnect the syringe with anticoagulant from the butterfly needle, connect the new tubing and make sure all connections are tight enough.

3.6.13 Slowly open the clamp and see the blood flowing through the tubing and butterfly scalp back into the patient's vein.

3.6.14 Adjust the flow speed to approx. 1 drop per second. The whole procedure may last 20 min to 30 min. The procedure should NEVER be made in a hurry.

3.6.15 When all blood has been reinfused, clamp closed the end of the tubing just before the butterfly needle connection, remove the needle from the patient's arm and apply a small cover unto the skin. Caution: as a very small amount of anticoagulant has been used, the puncture in the vein may take one or two minutes longer than usually to close. Make sure the puncture site is gently pressed for two or three minutes before the patient is allowed to get up.

3.6.16 Especially during the first two or three MAH sessions, it is recommendable not to leave the patient alone. A professional should always be around and observe the patient, preferably engaging him in an easy conversation.

This procedure must be slightly modified depending of the auto hemo kit and the anticoagulant used.

3.7 Alternatives

In poor countries it is fairly common practice to draw the blood into a 60 mL disposable syringe into which some anticoagulant and the O_2-O_3 gas mixture have been previously introduced, vigorously shake the syringe and its contents, and re-inject the thus ozonized blood immediately. **This technique must not be used**, as it bears several important risks, such as infections, blood clotting, gas embolism, blood embolism, vein damage.

Use of bottles or plastic bags: Plastic bags should be used only if have been made of phthalates free materials. The main advantage of vacuum glass bottle versus plastic bag is that the extraction speed of venous blood may be accelerated by the reduced pressure inside the bottle.

3.8 Frequent side effects

Very discrete taste of metal at start of reinfusion, tiredness on next day, need to adjust the antidiabetic medication to lower doses, need to adjust the anti-hyperthryroidism medication to lower doses, need to adjust the Digitalis heart medication to lower doses, need to adjust anti-hypertensive medication.

3.9 Warning, safety assessment

The blood reinfusion during a MAH should not be made under pressure, in order to avoid unnecessary risks. The resulting difference in time is only a matter of a few minutes.

MAH is a low-risk treatment method. The benefit/risk ratio is by far on the benefit side. Risk ratio is estimated in 0.52 % at occur when nonadherence to hygiene rules during treatment take place. Hygiene guidelines, such as described for every kind of work with blood in laboratory, clinic and practice, must be adhered to at all times; the relevant measures to be taken as well as the materials to be used are described in the Guidelines and apply for all users.[12]

3.10 Patients Follow-up

Patients may be followed and re-evaluated from time to time, from the clinical / subjective point of view and/or with laboratory and/or image examinations.

3.11 Effect Mechanism

Action mechanism: Chronic inflammatory processes are always accompanied by: high oxidative stress, reactive oxygen species, such as radical and nonradical oxidants, a suppressed antioxidant capacity and immunologic disbalance, each of which in turn promotes and maintains the inflammatory process.[12] At low doses, systemically applied ozone in the form of MAH acts as a bioregulator, ozone intermediary (H_2O_2, 4-hydroynonenal, etc.)[13] induce a signal transduction via the oxidation of glutathion or cysteine residues and the corresponding nuclear factors, resulting in a regulation of the antioxidants via Nrf2 information,[13, 14] or an immunomodulation via NFkB.[12]

3.12 Clinical evidences

More than 11 000 systemic ozone treatments in the form of MAH in 577 patients in various clinical studies has been reported. The main study has been done in: 206 patients with vascular inflammatory diseases and arterial circulatory disturbances, 203 patients with chronic hepatitis, and 122 patients with chronic inflammation and immune deficiency. Statistically significant improvement of clinical and biochemical parameters, and no side effects was reported. According to evidence level (Cochrane library 1992):[15] In total, two controlled randomized

clinical studies (N = 97) have evidence level Ib (at least 1 high-quality randomized controlled trial), 10 controlled studies IIa (at least 1 high-quality non randomized trial) (N = 261), 3 clinical studies with nontreated control groups (N = 192) level IIb (at least 1 high-quality trial without control group). 2 controlled case studies with 14/19 patients and 3 case reports (N = 13) can be assigned to level IIIb (High quality noncontrolled case study).[12]

4. Contingencies; Corrective Actions

Some patients react very sensitively to the pain caused by the insertion of a needle. They may feel dizzy, may sweat profusely, and even faint. It is very convenient to have an electric table which allows a Trendelemburg position. Emergency bottles of saline, injectable glucose, injectable vitamin C should be at hand. In extreme cases, an oxygen mask might be required. The patient should not have fasted too long before the MAH, but also should not come immediately after a large and heavy meal. The ingestion of alcoholic beverages should be discouraged for the 24 h after the MAH.

In case of other side effects follow the instructions of ISCO3/CLI/00/01 "Fist Aids in ozone therapy (Inhalatory exposition and accidental over dose)" and report the side effect using ISCO3/REC/00/03 "The ISCO3 Safety Information and Adverse Event Reporting Program Form".

5. References

5.1 SOP References

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6. Documentation and Attachments

6.1 List of recommended medical disposables

Siliconated Luer lock syringe of 50 mL
5 ml syringe
Gloves
Disinfectant solution
Major Autohemotherapy kit
Lines for blood extraction
Lines for blood reinfusion
Scalps (butterfly) 19 G or 21 G
19 G x 3/4 (1.01 mm • 19 mm) or 21 G x 3/4 (0.80 mm • 19 mm) needles
25 G x 2 (0.508 mm • 5.08 mm) needles
Adhesive tape
Surgical (Kelly) clamp

7. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/MET/00/01	22/12/2015	Draft.	First version
	11/07/2016	Draft 2 with main corrections suggested by Heinz, Adriana, Nasarow and Bernardino. Was modified the following aspects: Type of anticoagulant, recommendations during pregnancy, type of blood container. Was deleted the recommendations of peristaltic pumps during re infusion.	2 Draft
	02/10/2016		Version 1

8. Document Records

	Name	**Title**	**Signature**	**Date**
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Authoriser / Approved	ISCO3 Board and members 2015-2020	All members		02/10/2016