



ISCO3/MET/00/02 Minor Autohemotherapy

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Title

ISCO3/MET/00/02 Minor Autohemotherapy

1.1. Brief background

Minor AHT without ozone, only injecting whole blood through I.M. route, is a very old, traditional treatment as unspecified immunomodulatory therapy. Later Dr. Hans Wolff, suggested to add ozone for activating blood components.

It is one of the methods to apply ozone therapy. It is very safe, has almost no 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 Minor Autohemotherapy (MiAHT) 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	Non appropriate but frequently word to designate microgram
MiAHT	Minor Autohemotherapy
SOP	Standard Operation Procedure
Total doses	Total amount, in micrograms, of ozone given per session, calculated as volume in mL multiplied by concentration in $\mu\text{g/mL}$

2. Responsibility

Physician	Patients' 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
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Nurses	Accommodate the patients Preparation of the clinical procedure Supervision of patients, and vital signs control (temperature and pressure) Detects and alerts the doctor to anomalies due to possible reactions Notification of possible complications
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A MiAHT 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

Mainly benefits in immune system; either boost or suppression like Acne vulgaris (common acne), allergies, adjuvant in cancer therapy, unspecific immune activation, infections, rheumatoid arthritis, Herpes infections, Herpes zoster, Post herpetic neuralgia etc. Basically indications of using Antibiotic, Antiviral, Antifungal agents or Corticosteroids in conventional medicine can be indication reasons for MiAHT as well.

3.2 Contraindications

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

Relative contraindications / special situations:

- ✓ Uncompensated diabetes
- ✓ Acute myocardial infarction
- ✓ Pregnancy in the first 3 months
- ✓ 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%).¹ Test of G6PD is recommended prior to O₃ therapy in order to avoid complications (Nkhoma *et al.* 2009).



MiAHT approach must be carefully balanced in patients under anticoagulant or antiaggregating treatment. If MiAHT is finally performed, special and prolonged compression has to be applied at the injection site.

3.3 Recommended doses intervals

MiAHT 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.

The **volume of blood** to use varies between 2 mL and 10 mL.

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.

Ozone concentrations for MiAHT

The ISCO3 recommended dose for MiAHT are range from 10 µg/NmL to 40 µg/NmL (see table below), concentrations above 60 µg/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. This is the dose ranges used by German, Russian and Madrid Declaration (Viebahn-Hänsler *et al.* 2012; Maslennikov *et al.*, 2008; ISCO3/QAU/01/03). 2). theoretically, the vigorous mix of blood and gas leads to hemolysis. So, hemolysis for high O₃ concentration (and small blood volume) is not relevant here and very small concentrations could be not useful (Bocci, 2005).

O ₃		High	Medium	Low
Minor Auto Hemotherapy	C. (µg/mL)	40-30	15-20	5-10
	V. (mL)	5		
	Dose (µg)	200-150	75-100	25-50

Note: German Guideline: Vol. 10 mL blood + 10 mL O₃ at a concentration of 10-20 µg/mL (Viebahn-Hänsler *et al.* 2012); Russian Guideline Vol. 5-10 mL blood + 10-15 mL O₃ at a concentration of 10-40 µg/mL (Maslennikov *et al.*, 2008); Madrid Declaration as shown in the table (ISCO3/QAU/01/03); Bocci Vol. 5 mL blood + 5 mL O₃ at a concentration 40-80 µg/mL (Bocci, 2005).

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.

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.

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

Syringes: 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

3.6 Main procedure

Its principle is similar to major auto hemotherapy. Medical ozone gas and patient's own whole blood are mixed in a sterile, pyrogen-free disposable syringe and then reinjected to the patients. Comparing to Major Auto Hemotherapy, Minor Auto Hemotherapy has some differences.

- 1- **Volume:** In general 2 - 10 mL (mostly 5 mL) blood
- 2- **Injection Site:** Intramuscular mostly (or Subcutaneous)
- 3- **Anti Coagulant:** It is not necessary to use in Minor Auto Hemotherapy
- 4- **Ozone Volume:** It can be equal or a little more than the blood volume.
- 5- **Ozone Dose:** It must be set by the physician depending on the case but in general dose should be in therapeutic dose ranges.

In minor autohemotherapy (MiAHT), under aseptic conditions, patient's whole blood is mixed with ozone in desired amount and at necessary concentration. Mixing procedure can be done two ways:

- 1- Drawing blood into a syringe and taking ozone into another syringe (dose and amount of ozone vary depending on the treatment). Then inserting ozone into the blood containing syringe or the opposite – blood into the ozone syringe.
- 2- Ozone can be taken into a syringe and then blood directly can be drawn from patients venous and then ozone – blood are mixed at the same syringe.

Both of the methods above, have the same effect. But inserting ozone into blood in syringe results serious bubbling which means either blood loss or injecting air – oxygen into the muscle. Also there will be two syringes used. First to take ozone into the syringe and then drawing blood to the same syringe, mixing gently, drawing the residual gas out and injecting is the best recommended way. When medical ozone is mixed with whole blood, ozone reacts with blood in seconds. The residual gas in the syringe is left over oxygen and it is meaningless to inject this volume to the patient.

After mixing ozone and blood gently for about 1 min, it can be injected via I.M. route. Some physician can prefer S.C. route. There is no clinical data comparing I.M. or S.C. route but in general I.M. route is preferred. Because in S.C. method, later some color changes can occur on the skin.

3.7 Side effects

No systematic side effect is reported. Only light local side effects can be observed. Several processes, such as serum reabsorption, fibrinolysis, via lymphatic vessels and a mild sterile



inflammatory reaction, are likely to take place as occasionally suggested by a slight swelling at the injection site reported by some patients during the next few days.

3.8 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.

3.9 Effect Mechanism

The hypothetical action mechanism of minor autohemotherapy in infectious diseases will involve that ozone can fragment most virions present in blood, without regard to preserving cellular elements. This treated blood, injected intramuscularly, carries fragments of viral envelope and nucleic acids which find their way into the general circulation and to the immune network. The latter, if still relatively operational, begins to manufacture appropriate antibodies which in turn, serve to counter the evolution of the infection. The interesting feature of this technique is that antibodies thus manufactured are individualized to the particular patient receiving the treatment, since they are derived from their own viral stock. In view of the high mutability of retroviruses, each patient carries a unique viral strain. Minor autohemotherapy can thus be conceptualized as a method of auto vaccination providing a high degree of antibody specificity (V. Bocci 1999).

The minor autohemotherapy specifically stimulates the body's second line of defense that is important in autoimmune diseases and long-time chronic illness. After a period of time when the body needs to fight a long-term illness, the first line of defense becomes weakened. Ozone therapy, especially the minor autohemotherapy has the ability to reactivate the second line of defense, especially the natural killer cells (V. Bocci 1999).

Chemotactic compounds released at the site may stimulate the local infiltration of monocytes and neutrophils which take up hemolyzed erythrocytes and denatured proteins. Activated monocytes and lymphocytes may release interferons and interleukins either in loco or along the lymphatic system, upregulating the physiological cytokine response (Bocci, 1981; 1988). Thus it would be quite interesting to evaluate some immunological parameters and some other heat shock proteins (Tamura *et al.*, 1997) that may enhance immune reactivity and explain the beneficial effects (V. Bocci 1999).

Ozonated, hence primed, leukocytes may either infiltrate the tissue or/and may return via lymphatics into the blood pool or into other lymphoid microenvironments. Most of the erythrocytes will be slowly broken down locally and will provide substrates for rebuilding the extracellular matrix but, most importantly, heme will induce the synthesis of stress proteins, particularly heme oxygenase I (HO-I or HSP-32). This is a most protective enzyme that, by enhancing the release of CO and bilirubin, facilitates the local circulation and neutralizes oxidant compounds. Finally, the twice ozonated platelets will be activated and release locally a wealth of growth factors (platelet-derived growth factor-PDGF, basic-fibroblast growth factor, b-FGF, transforming growth factor b1, TGFb1), which will greatly help tissue reconstruction (Gracer and Bocci, 2005).



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 Trendelenburg position. Emergency bottles of saline, injectable glucose, injectable vitamin C should be at hand. In extreme cases, an oxygen mask might be required.

In case of other side effects follow the instructions of ISCO3/CLI/00/01 "Fist Aids in ozone therapy (Inhalator 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 20 mL
Gloves and disinfectant solution
Sterile antimicrobial filter

7. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/MET/00/02	19/01/2016	Draft.	First version
	31/03/2016	Final version	First version

8. Document Records

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Authoriser / Approved	ISCO3 Board and members 2015-2020	All members		