ISCO3/CLI/00/20
Title: Complementary Treatment of Fibromyalgia with Ozone Therapy

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Index

1. Title ISCO3/CLI/00/20. Ozone Therapy as an Adjuvant Treatment for Fibromyalgia

1.1. Brief background

Fibromyalgia (FM) is a common cause of chronic pain and the most common cause of generalized, musculoskeletal pain in women between ages of 20 and 55 years; in the United States and in other countries, the prevalence is approximately 2 % to 3 % and increases with age.[1-4]

Fibromyalgia is considered the most common cause of chronic widespread musculoskeletal pain (CWP), often accompanied by fatigue, cognitive disturbance, psychiatric symptoms, and multiple somatic symptoms.[5] Ten to 15 % of the general population have CWP and do not have any specific disease or structural abnormality to account for the pain, thus, there is no clear boundary separating those who meet criteria for FM from the larger pool of people with CWP.[4,6,7]

According to the new International Classification of Diseases (ICD-11) FM, is termed both as CWP, as well as conditions such as chronic low back pain, thus, FM may be conceived as diseases in their own right, termed "chronic primary pain".[8] Fibromyalgia is characterized by widespread musculoskeletal pain, accompanied by other somatic symptoms, particularly fatigue and sleep disturbances, as well as cognitive and psychiatric disturbances. Physical examination reveals tenderness in multiple soft tissue anatomic locations. Laboratory testing is normal in the absence of other illnesses.[7]

As many other common chronic pain syndromes, FM has been a controversial condition. Thus, the role of organic illness has been questioned by many, and FM has often been considered by some to be psychogenic or psychosomatic. However, there is evidence that the undefined aetiology probably is caused by an alteration of the immunological and hormonal systems and by psychological conditions, physical trauma, or viral infections. Moreover, ongoing research suggests that FM is a disorder of pain regulation, often classified as a form of central sensitization.[9]

More recently, epigenetics research in chronic pain syndromes as FM has been given much understanding of the real etiopathology of these debilitating conditions.[10,11] Several genes already known for their role in pain (BDNF, HDAC4, PRKG1, IL-17, TNFRSF13B, etc.), and several miRNAs linked to inflammatory regulation, nociceptive signaling and protein kinases functions have been found to differ significantly between people with chronic pain and healthy controls.[12-14] As valuable diagnostic method, epigenetic modifications (such as DNA methylation) should be further investigated.[15]

Unfortunately, the conventional medical therapies that target this pathology produce limited benefits. They remain largely pharmacological in nature and tend to treat the symptomatic aspects of various disorders reported by the patient. The statistics, however, highlight the fact that 90% of people with fibromyalgia also turn to complementary medicine to manage their symptoms.[12]

Due to the failure of traditional therapies, attention has recently been paid to both biological and mental factors even though it now seems established that the pathology is not caused only by psychological conditions. Neuroendocrine disorders, metabolic alterations, and the onset of nociplastic pain have been found in the patient with FM.[16]

The multiple biological activations triggered by Oxygen-Ozone therapy (O_2/O_3) performed with the method of major autohemotherapy (MAH), minor autohemotherapy (MiAH), rectal ozone insufflation therapy (RIO3), Ozonized Saline Solution (OSS3) and ozone local infiltration on critical pain points seem suitable for correcting muscle hypoxia, immunological alteration, inflammation, pain and chronic oxidative stress present in these diseases. Furthermore, inducing a sense of well-being can effectively combat the severe fatigue of many patients.

This guideline "Ozone Therapy as an Adjuvant Treatment for Fibromyalgia" summarizes the previous clinical experience and proposes a clinical protocol based on the most recent published evidence.[17-25] In addition, rating scales are proposed, not only to highlight the progress of therapy in the individual patient, but also to encourage physicians to publish the clinical results of their own experience with FM patients.[17-26]

1.2. Purpose

The purpose of this SOP is to describe the proposed Ozone Therapy protocol as an adjuvant medical treatment to clinically manage fibromyalgia patients.

1.3. Scope

This protocol addressed the diagnosis, clinical presentations, and Ozone Therapy routes of administration, doses, volume of gas, and frequency of application in fibromyalgia and chronic widespread musculoskeletal pain.

1.4. Acronyms, abbreviations, and definitions

ACR, American College of Rheumatology; AFI; Italian Fibromyalgia Association; CWP, Chronic Widespread Musculoskeletal Pain; FAS, Fibromyalgia Assessment Status; FM, fibromyalgia; ICD-11, International Classification of Diseases; IBS, irritable bowel syndrome; IT-FIQR; Italian version of Fibromyalgia Impact Questionnaire; MAH, Mayor Auto Hemotherapy; MiAH, minor Auto Hemotherapy; O_2/O_3, Medical Ozone; RIO3, Rectal Insufflation Ozone Therapy; SF, fibromyalgia syndrome; SSO3, ozonated saline solution; SS, Symptom Scale; SSS, Symptom Severity Score; TP, tender points; WPI, Widespread Pain Index.

2. Responsibility

Physician

Medical Record and anamnesis
Ozone Therapy Indication and contraindication assessment
Explanation of procedure and request of a written informed and privacy consents
Request laboratory testing
Application of therapy and follow up
Recording clinical data in medical records

Nurse

Patient comfort
Patient vital signs control and follow up
Supervision during the procedure
Notification of possible complications

3. Aetiology and Pathogenesis

Despite the increased interest in identifying and successfully treating FM, the aetiology and pathogenesis of this clinical entity are still not fully understood. Several factors such as dysfunction of the central and autonomic nervous systems, neurotransmitters, hormones, immune system, external stressors, psychiatric aspects, and others seem to be involved.[27]

Different mechanisms have been proposed over the last years. However, in this SOP we are going to list the most common factors described in the literature, understanding that there are more to be considered.

1. **Psychosocial factors.** Can be applied to patients with any chronic pain conditions. The concept of resilience, which can be broadly defined as a protective factor, may make people less vulnerable to future adverse life events.[28] In FM patients, genotype and, most importantly, environmental factors may play a major role in the development of a more or less resilient personality.

2. **Immunity.** Plays a pivotal role in the pathogenesis of FM as auto-immunity/ inflammatory syndrome triggers its onset.[29] FM can be temporally related to vaccinations, silicone breast implants or mineral oil injections as part of an auto-immune response induced by adjuvants.[16] A recent study has found a link between autoantibodies and FM as one-third of its FM patients with sicca syndrome and/or xerostomia tested positive for Sjögren's syndrome biomarkers, and the majority of these were also positive for one or more tissue-specific auto-antibodies.[30]

3. **Gut Microbiome imbalance.** Another aspect under investigation is the gut-brain axis, which connects the gut microbiome with the brain through the enteric nervous system. It has been found that FM patients have less diverse gut bacteria and altered serum metabolome levels of glutamate and serine, thus suggesting changes in neurotransmitter metabolism.[31]

4. **Skin increased the number of mast cells**. FM skin biopsies have shown an increased number of mast cells and increased neuronal production of corticotropin-releasing hormone and substance P, which activate mast cells to release neuro-sensitizing pro-inflammatory substances that can exacerbate low-grade inflammation.[32]

5. **Neuromuscular efficiency.** Seems to be impaired in FM patients. Studies of Hyperbaric Oxygen Therapy have found that a central mechanism related to fiber type recruitment order can be modified in such a way as to allow the generation of the same effort using fewer recruited fibers.[33] Genetic polymorphisms of the serotonin receptor gene HTR2A: the GG genotype or G allele is associated with a greater risk of developing the disease and reduced lower limb muscle strength in comparison with controls.[34]

6. **Oxidative stress**. May play an important role in the etiology of FM. It has been found that an alteration in thiol/disulphide homeostasis (a decrease in thiol levels and an increase in disulfide levels) significantly correlated with Fibromyalgia Impact Questionnaire (FIQ) scores.[35]

7. **Neuroendocrine dysregulation.** A recent study has found that hepatic clearance of cortisol is relatively lower in FM patients than in matched controls.[36]

8. **Epigenetics Factors.** More recently, epigenetics research in chronic pain syndromes such as FM has been given much understanding of the real etiopathology of these debilitating conditions.[10,11] Several genes already known for their role in pain (BDNF, HDAC4, PRKG1, IL-17, TNFRSF13B, etc.), and several miRNAs linked to inflammatory regulation, nociceptive signaling and protein kinases functions have been found to differ significantly between people with chronic pain and healthy controls.[12-14]As a valuable diagnostic method, epigenetic modifications (such as DNA methylation) should be further investigated.[15]

4. Symptoms and Signs

Fibromyalgia is characterized by widespread musculoskeletal pain, accompanied by other somatic symptoms, particularly fatigue and sleep disturbances, as well as cognitive and psychiatric disturbances. Physical examination reveals tenderness in multiple soft tissue anatomic locations. Laboratory testing is normal in the absence of other illnesses.[37] The core symptoms of FM are generalized pain, fatigue, and sleep disturbances, present for at least three months, and not explained by any other medical condition.

Other common symptoms, including cognitive disturbances are present in most patients often referred to as "fibro fog", depression and/or anxiety are present in 30 % to 50 % of patients at the time of diagnosis, headaches are present in more than 50 % of patients with FM and include migraine and muscular (tension) types. Patients also often report paresthesias including numbness, tingling, burning, or creeping or crawling sensations, especially in both arms and both legs. Also, FM patients may have a variety of poorly understood pain symptoms, including abdominal and chest wall pain; symptoms suggestive of irritable bowel syndrome (IBS); and pelvic pain and bladder symptoms of frequency and urgency suggestive of the interstitial cystitis/painful bladder syndrome, Symptoms of autonomic nervous system dysfunction, dry eyes, and Raynaud phenomenon are common in FM. Orthostatic hypotension and altered heart rate variability are also common manifestations. Hearing loss was four- to fivefold more often reported in patients with FM than in the general population.[1-5,15,16,37]

5. Diagnosis

FM should be suspected in patients presenting generalized chronic pain that lasts a minimum of three months without another identified cause. The diagnosis is symptom-based and should include a thorough history and physical examination, together with limited laboratory testing to exclude other conditions. Characteristic features and diagnostic evaluation for fibromyalgia:

History
Widespread (multisite) pain
Present for at least 3 months
Fatigue, sleep disturbance
Other symptoms, such as cognitive disturbance, headaches, bowel irritability
Physical examination
Widespread (multisite) tenderness
Absence of join swelling, inflammation
Laboratory testing
Normal acute phase reactants (ESR/CRP)
Normal CBC
In selected cases, muscle enzymes, thyroid testing

Legend: *ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; CBC: Complete blood count.*

The diagnosis of SF up to 2010 was essentially based on the 1990 American College of Rheumatology (ACR) criteria[38] involving the presence of widespread musculoskeletal pain (*i.e.,* affecting both sides of the body both in the upper and lower part and involving the entire spine) for at least 3 months associated with the finding of painful areas with acupressure, TP (at least 11 out of 18 TP) (Table 1). The use of these criteria constituted a very important step forward in the understanding of SF allowing to standardize the diagnosis and to be able to compare scientific works, in particular those of an epidemiological type.

However, these criteria have limitations. There was no agreement on the minimum number and on the precise anatomical localization of TPs: in addition to the 18 TPs described, there are many other painful areas in individual patients; in general, every tendon insertion and every muscle are potentially painful. The tenderness of TPs also varies from day to day in the same patient and establishing a clear limit in the 11 TPs can mean that one day the patient will meet the criteria and the next day it will no longer be the same. Furthermore, there was no agreement on the fact that the evaluation of TPs should be performed manually or with the aid of a pressure algometer, the first method being easier to perform but the second more reproducible and less influenced by the experience of the performing physician. Finally, in the ACR criteria of 1990 they did not consider the presence of other very frequent extra-skeletal accompanying symptoms in SF, such as sleep disturbances, asthenia, or neuro-cognitive alterations. To overcome these criticalities, in 2010 the new ACR criteria for the diagnosis of SF were published, which allow to make a diagnosis in the absence of the evaluation of TP, placing the accent on a list of other symptoms such as fatigue, sleep. non-restorative and cognitive symptoms, as well as headache, depression, and abdominal pain.[39]

Table 1. American College of Rheumatology 1990 classification criteria.[38]

1. History of widespread pain for at least 3 months
Pain is considered widespread when all of the following are present: - Pain in the left side of the body; - Pain in the right side of the body; - Pain above the waist; - Pain below the waist. to the spine (cervical or anterior thorax or dorsal or lumbar). Pain localized to the shoulders or buttocks counts as pain on the affected side.
2. Pain in at least 11 of 18 allogenic areas on digital palpation (the pressure to be exerted in these sites by acupressure should be 4 kg per cm^2)
- Occipital: bilateral, insertion of the sub-occipital muscle - Cervical: bilateral, anterior surface of the C5-C7 intertransversarii ligaments - Trapezius: bilateral, midpoint of the upper margin of the homonymous muscle - Supraspinatus: bilateral, at the origin of the supraspinatus muscle, take this out the above the spine of the scapula, near the medial margin of the scapula - Second rib: bilateral, at the level of the second costo-chondral joint - Lateral epicondyle: bilateral, 2 cm distal to the epicondyle - Gluteus: bilateral, on the upper-extreme quadrant of the gluteus maximus - Great truncation: bilateral, posterior to the trochanteric prominence - Knee: bilateral, medial fat pad, proximal to the joint spacing

These criteria evaluate the painful areas reported by the patient on a body image (Widespread Pain Index - WPI) and the associated symptoms (Symptom Scale - SS). The total score obtained in each of these two scales (WPI ≥ 7 and SS ≥ 5 or WPI 3-6 and SS> 9) allows for the diagnosis of SF. Like the ACR 1990 criteria, any other cause of chronic pain must also be excluded in the 2010 criteria, but the presence of other pathologies does not exclude SF which can coexist with them. The new criteria include, in association with widespread musculoskeletal pain, many associated symptoms that can significantly contribute to the impact of the disease on the patient. Clinical diagnosis is made simpler and is essentially based on clinical symptoms.[40,41]

However, the new criteria, based on the physician's subjective assessment of the extent and severity of the patient's somatic symptoms, do not allow for patient self-assessment of symptoms of the ACR 2010 criteria, in which the areas of pain and the presence / absence of 3 symptoms in the SS (headache, pain or abdominal cramps and depressive symptoms) are self-assessed by the patient.

In 2013, the 2010 criteria were further modified, increasing the localization areas of pain and the number of symptoms the patient assesses for severity, improving the specificity of the criteria and allowing a diagnosis of SF independent of another pain syndrome. It is important to underline, however, that both the 2010 criteria and the subsequent versions 2011 and 2013, although easier and faster to execute, do not provide for the detection of clinical signs highlighted by the doctor through the patient's physical examination, an essential element in the diagnostic process of such a complex pathology as SF.[42]

To further complete the FM diagnosis, blood chemistry tests could be added: ESR, PCR, complete blood count, ANA, ENA, CPK, TSH FT4, ALT / AST / GGT, Anti-HCV, Anti-EBV and haematological tests for Celiac and Gluten Sensitivity: anti-TG / EMA, total IgA, anti-DGP IgG, AGA IgG, instrumental investigations for anatomical damage such as brain MRI (Magnetic Resonance); instrumental investigation for functional damage (PET / SPECT / functional MRI); neurological and rheumatological counselling (differential diagnosis); psychiatric consultancy; psychological consulting.

6. Conventional Treatment / Management

Treatment and management of fibromyalgia is mainly directed at reducing the impact of the major symptoms of this disorder, including chronic widespread pain, fatigue, insomnia, and cognitive dysfunction. Treatment should be tailored and multidisciplinary, involving both nonpharmacologic and pharmacologic measures. Patients without major mood or sleep disturbances, may respond adequately to nonpharmacologic measures alone. However, for most patients, medications will be needed for symptom control, always along with nonpharmacologic treatment. Patient education is pivotal for successful treatment.

The identification and treatment of all pain sources that may be present in addition to fibromyalgia such as peripheral inflammatory or neuropathic pain generators (e.g., comorbid osteoarthritis or neuropathic pathologies) or visceral pain (e.g., comorbid irritable bowel syndrome) are central to the proper clinical management of fibromyalgia.

The American Pain Society (APS) and the Association of the Scientific Medical Societies in Germany (AWMF) gave the highest level of recommendation to aerobic exercise, cognitive-behavioural therapy (CBT), amitriptyline, and multicomponent therapy. The APS guideline and AWMF guideline were completed prior to the approval of pregabalin and duloxetine for the treatment of fibromyalgia by the United States Food and Drug Administration (FDA). The European League Against Rheumatism (EULAR) gave the highest level of recommendation of "A" to a set of pharmacological treatments (i.e., tramadol, amitriptyline, fluoxetine, duloxetine, milnacipran, moclobemide, pirlindol, tropisetron, pramipexole, and pregabalin), a recommendation strength of "B" to aerobic exercise, and a recommendation strength of only "D" to CBT. EULAR did not give any recommendations for cyclobenzaprine, multicomponent treatment, patient education, hypnotherapy, biofeedback, or other complementary and alternative medicine approaches (CAM), such as acupuncture or homeopathy, whereas EULAR gave a "D" recommendation for CBT, and the APS and AWMF decided on an "A" recommendation. Whereas EULAR and AWMF did not recommend strong opioids (expert opinion), APS recommendation strength was a "C". APS and AWMF provided the same strength of recommendation ("B") to tramadol, balneotherapy, hypnotherapy, biofeedback, massage therapy, pregabalin, fluoxetine, and duloxetine. Whereas APS recommended patient education as a single intervention ("B"), acupuncture ("C"), and trigger point injections ("C"), AWMF did not recommend patient education as a single intervention ("A"), acupuncture ("A") (a minority report recommended acupuncture with a strength of "B"), and trigger point injections ("C"). All three guidelines recommend against the use of NSAIDs (as a single intervention) or corticosteroids.

The nonpharmacologic treatments most consistently linked to FM improvements are aerobic exercise and strength training. Effective exercise focuses on stretching, with gradual progression to strengthening and reconditioning exercise. Nutritional assessment and intervention as an Integrative Medical approach in the treatment of FM patients must be addressed. It is well established that nutrition has a pivotal role in generating chronic disease and much has been published in understanding the role of nutritional factors Nutritional Factors Interaction with Chronic Musculoskeletal Pain.[43]

It was found that women with FM showed a lower qualitative and quantitative food intake in comparison with control group, but only vitamin E correlated with quality of life and percentage of protein in the diet with sensation of pain.[44] Moreover, dietary intake of Mg and Ca seems to be substantially lowered by women with FM and has an inverse correlation with pain and direct relation with the pain threshold. It has been suggested that low dietary intake of minerals correlates with worsened pain threshold parameters in FM patients.[45] A comprehensive literature review has concluded

that a plant-based diet might have pain relieving effects on chronic musculoskeletal pain. While patients with chronic rheumatoid arthritis pain can show inadequate intake of calcium, folate, zinc, magnesium, and vitamin B6, whilst patients with FM can show a lower intake of carbohydrates, proteins, lipids, vitamin A-E-K, folate, selenium, and zinc. Moreover, chronic pain severity also shows a positive relation with fat and sugar intake in osteoarthritis, and pain threshold shows a positive association with protein intake in FM.[46]

7. Evidence of Ozone Therapy Efficacy

Level of evidence: Level C (according to the definition of Madrid Declaration ISCO3/QAU/01/03).

Evidence:

Clinical trials (Table 2) give evidence supporting the complementary use of ozone in fibromyalgia as complementary therapy. However, larger studies are required to assess the efficacy and long-term results of this technique.

Table 2. Examples of clinical trials using ozone as complementary treatment in fibromyalgia.

Number of patients	Outcome measures	Ozone protocol	Main Results	References
OT n= 26 PC n= 28	FIQ PSQI SF-12 pre- and post-intervention.	Both groups received OT in the form MAH and MiAH for two sessions per week for a total of 10 sessions.	OT, simultaneously improves the subscale scores (feel good and fatigue) of FM and sleep quality in the treatment period. No changes in FIQ total score post-treatment in both groups.	Hamza Sucuoğlu and Nalan Soydaş (2022).[47]
OT n= 100	Modified 10 points-PI-NRS	MAH 3 to 4 session, two weekly. Blood volume 200 mL, O_3 concentration 45 µg/mL	A quite complete rehabilitation of the musculoskeletal function and of the overall arthralgia was observed in 76% of the patients at one month of follow-up. The number of patients having a reduction in the PI-NRS score from 10 (the maximal observed) to 3 (including 10-1 and 10-2) following only two runs of MAH was 23.5%	U. Tirelli *et al*. (2022).[48]
OT n= 40	FIQ SF-36	MAH 13 session, two weekly the first 5 weeks and 1 session per month for the remaining 3 months	Significant improvement in FIQ and SF-36 scores was observed in all periods compared to the previous period ($P < 0.05$)	Gülçin Gazioğlu Türkyılmaz *et al*, (2021).[49]
OT n= 20	FIQ Serotonin in serum Oxidative stress variables	MAH 10 session, two weekly. Blood volume 150 mL, O_3 increased concentration 30 → 60 µg/mL	Important decline of tender points and FIQ score, as well as a decrease of oxidative stress levels.	Moreno-Fernández A. *et al*, (2019).[24]
OT n=65	Numeric Rating Scale and Fatigue Severity Scale	MAH in 55 patients RiO3 in 10 patients Twice a week for one month and then twice a month as maintenance therapy.	We found a significative improvement (>50% of symptoms) in 45 patients (70%)	U. Tirelli *et al*. (2019).[25]
OT=20 i.m. OT=20 i.m. + RiO3 Control n=10	FIQ VAS HDRE SF-12	2 sessions /week for 5 weeks	OT given by RiO3 together with local tender point's injection seems to be more beneficial than local tender point's injection alone, mainly for the physical symptoms of FM, though anxiety and depression also improved.	Mohammad H. Elgawish *et al*. (2015).[20]

Legend: HDRS, Hamilton depression rating scale; FIQ, The fibromyalgia impact questionnaire; FM, Fibromyalgia; MAH; major autohemotherapy; MiAH, minor autohemotherapy; OT, Ozone therapy; 10-PI-NRS, 10-points Pain Intensity-Numerical Rating Scale; PC, placebo control; PSQI, Pittsburgh sleep quality index; RiO3, rectal insufflation; SF-12, 12-item short-form health survey; SF-36; Quality of Life-short form; VAS, visual analogue scale for pain.

8. Procedure

8.1 Indications

8.1.1 Inclusion Criteria

1. Participant is willing and able to give informed consent for ozone treatment.
2. Male or female, aged 18 - 80 years.
3. According to the 2010 ACR preliminary diagnostic criteria, a patient satisfies FM diagnostic criteria if the following three conditions are met:
   a. Widespread pain index (WPI) >7 and symptom severity (SSS) scale >5 *or* WPI 3 to 6 and SSS scale >9.
   b. Symptoms have been present for at least three months.
   c. There is no other disorder that would explain the patient's symptoms.
4. Participants diagnosed with FM based on the 2010 American College of Rheumatology (ACR) preliminary diagnostic criteria[42] must meet tone of the major criteria and at least 3 of the minor criteria:

Major criteria:

a) Chronic generalized musculoskeletal pain for at least 3 months.
b) Absence of secondary causes (endocrine, tumour, rheumatological disease).
c) Pain in tender points that trigger FM.

Minor criteria

a) Fatigue related to physical activity.
b) Sleep disorder.
c) Morning sensation of joint inflammation and stiffness.
d) Variation of symptoms severity depending on the meteorological changes.
e) Increased symptoms severity under emotional stress or anxiety.
f) Headache and dizziness.
g) Inflammatory bowel disease or other bowel disorder.
h) Genitourinary disorders.
i) Depression.

8.2 Contraindications

Patients who meet any of the contraindications for ozone therapy (ISCO3/QAU/01/03).

8.2.1 Exclusion Criteria

The patient should not be treated with ozone if any of the following conditions are present:

- G6PD glucose-6-phosphate dehydrogenase deficiency (favism, acute haemolytic anemia).
- Hemochromatosis or moderate (Hb: 109 to 80 g/l) to severe anemia (Hb < 80 g/l).[43]
- Coagulation disorders, active bleeding.
- Thrombocytopenia (platelets < 50,000 per µL).
- Intravenous copper or iron treatment.
- Impaired renal or hepatic functions.

- Unstable cardiovascular disease.
- Hyperthyroidism - Basedow Graves' disease.
- Prolonged convulsive states.
- Pregnancy, nursing or planning pregnancy during treatment.
- Hypersensitivity or intolerance to medical ozone.
- Acute alcohol intoxication.
- Any clinical situation that impairs a safe procedure.

8.3 Preliminary Measures to be Evaluated

The health practitioner must be qualified / certified in Ozone Therapy by an Academic/medical Organization.

Fill out the complete medical records of the patient and obtain the written informed consent and the privacy consent. Refer to ISCO3/QAU/00/21.

Record the appropriate FM diagnose and verify the protocol indications and contraindications. Define the appropriate Ozone Therapy protocol for the severity of the disease, affected area and patient conditions.

Using a certified Ozone Generator, set the appropriate concentration of Medical Ozone to achieve the desired dosage. Refer to ISCO3/DEV/00/01.

8.4 Recommended Ozone Therapy Protocol for FM

8.4.1 Diagnosis

Refer to the characteristic features and diagnostic evaluation for fibromyalgia in section 5. Biochemical Diagnostic (In Annex VII highlighted in brown recommended parameters). Consider the measurement of oxidative stress over time and 6 or 12 months.

8.4.2 Informed Consent

Informed consents are mandatory. Refer to (proposed in Annex V) / Privacy consent (proposed in Annex VI).

8.4.3 Inclusion / Exclusion Criteria

Check inclusion and exclusion criteria. Refer to sections 8.1.1 and 8.2.1.

8.4.4 Integrative Treatment

Consider nutritional assessment and intravenous and oral supplementation of trace elements, vitamins, and minerals. Consider conventional treatment and management as described in section 6. Patient follow-up: time 0, 3, 6 and 12 months: repeat IT-FIQR, FAS, WPI and SSS investigations (Annex I-IV) for check evolution.

9. Ozone Therapy Cycles and Routes of Administration

9.1 Ozone Therapy Cycles

Ozone Therapy is applied by cycles and must be tailored to the patients' needs and clinical condition. Each cycle includes between 5 and 15 systemic or local Ozone sessions.

Treatment compliance plays a pivotal role in the success of Ozone Therapy as an adjuvant treatment in FM. When discussing the protocol with the patient the Ozonetherapist must take into consideration the best route of administration and feasibility of compliance.

9.2 Medical Ozone Routes of Administration

The systemic routes of administration advisable for treating FM are Major AutoHemotherapy (MAH), minor AutoHemotherapy (MiAH), Ozonated Saline Solution (O_3SS) and Medical Ozone Rectal Insufflation (RIO_3). Protocols also include concomitant localized infiltrations with Medical Ozone in TPs.

9.2.1 Protocol 1: Ozonated Saline Solution (O_3SS) and Local Infiltration on TPs

Ozonated Saline Solution has become widely used as a first option for systemic Ozone Therapy treatments due to its easy, safe, and well tolerated route. According to ISCO3/QAU/01/03 -Madrid Declaration on Ozone Therapy. 3rd Edition, ISCO3 2020. O_3SS is a systemic route to be administered in cycles of 5 to 15 sessions in a scheme of 2 to 3 sessions a week, starting a low dose and increasing to medium dose every 5 sessions. High doses for this condition are not used (Table 3).

Table 3. Dosage for O_3SS. Adapted from the 3rd Ed. of Madrid Declaration 2020.[50]

Ozonated Saline Solution		
O_2/O_3	**Medium**	**Low**
µg/kg x b.w.	2	1
Saline solution 0.9% Volume (mL)	200	200
O_3 at the generator outlet: (µg /NmL)	3.2	1.6
Total dose per session (µg)*	160	80

Legend: b.w., body weight; O_3SS; ozonized saline solution; * Example for a patient weight 80 kg.

For O_3SS use 200 mL of sterile saline solution for intravenous use at a flow of 20 L / h of Medical Ozone. The saturation time ranges from (10 – 15) min.[51] The protocol provides for 1 monthly maintenance session depending on patients' clinical recovery and relapses.

Intramuscular infiltration in TPs: In a syringe take 3 to 10 mL of O_2/O_3, at 5 µg / mL, infiltrate the TPs twice weekly for 5 weeks. In old surgical scars a subcutaneous injections (0.1 to 0.3) mL from O_2/O_3, 5 µg / mL, twice a week for 5 weeks is advisable.

9.2.2 Protocol 2: Major AutoHemotherapy and Local Infiltration on TPs

Major Auto Hemotherapy according to the ISCO3 / MET / 00/01 SOP,[52] it has been suggested cycles of 2 sessions per week for 5 weeks with increasing dosage, *i.e.,* 5 initial sessions at low dose and 5 -10 following session and medium dose. High doses for this condition are not used[50] (Table 4). Maintenance follow up: 1 session per month at the dose of the week 5 session. It is suggested to repeat this cycle twice a year or every 4 months depending on patients' clinical recovery and relapses.

Table 4. Dosage of the Major Auto Hemotherapy. Adapted from the 3rd Ed. of Madrid Declaration 2020.[50]

Major AutoHemotherapy		
O_2/O_3	**Medium**	**Low**
Concentration (µg/NmL)	20 / 30	10 / 20
Volume (mL)	50-100	
Dose (mg)	1.0-1.5 / 2.0-3.0	0.5-1.0 / 1.0-2.0

Intramuscular infiltration in TPs: In a syringe take 3 to 10 mL of O_2/O_3, at 5 µg / mL, infiltrate the TPs twice weekly for 5 weeks. In old surgical scars a subcutaneous injection (0.1 to 0.3) mL from O_2/O_3, 5 µg / mL, twice a week for 5 weeks is advisable.

9.2.3 Protocol 3: Medical Ozone Rectal Insufflation (RIO_3) and Local Infiltration on TPs

Ozone Rectal Insufflation according to ISCO3 / MET/00/23 SOP,[53] it has been suggested cycles of 5 sessions per week for 4 weeks with increasing dosage *i.e.,* 10 initial sessions at low dose and 10 following session and medium dose.[50] High doses for this condition are not used (Table 5). The week 3 dose is maintained through the rest of sessions. Repeat the RIO_3 cycle at 4 to 5 months after the last session depending on patients' clinical recovery and relapses.

Intramuscular infiltration in TPs: In a syringe take 3 to 10 mL of O_2/O_3, at 5 µg / mL, infiltrate the TPs twice weekly for 5 weeks. In old surgical scars a subcutaneous injections (0.1 to 0.3) mL from O_2/O_3, 5 µg / mL, twice a week for 5 weeks is advisable.

Table 5. Dosage of ozone by RIO_3. Adapted from the 3rd Ed. of Madrid Declaration 2020.[50]

Ozone Rectal Insufflation		
O_2/O_3	**Medium**	**Low**
Concentration (µg/NmL)	20 / 25	10 / 15
Volume (mL)	150	100
Dose (mg)	3.0-3.75	1.0-1.5

In case of Ozone toxicity, use the procedure described in ISCO3/CLI/00/01.[54] To report any side effects use the forms ISCO3/REC/00/03.[55]

9.2.4 Protocol 4: Minor AutoHemotherapy (MiAH) and Local Infiltration on TPs

Minor Auto Hemotherapy is an alternative route that can be considered for patients with poor venous access and may guarantee the treatment compliance. The cycles suggested for MiAH are between 5 to 15 sessions, 2 or 3 times a week with increasing dosage, *i.e.,* 5 initial sessions at low dose and 5 – 10 following session and medium dose.[50] High doses for this condition are not used (Table 6).

Intramuscular infiltration in TPs: In a syringe take 3 to 10 mL of O_2/O_3, at 5 µg / mL, infiltrate the TPs twice weekly for 5 weeks. In old surgical scars a subcutaneous injection (0.1 to 0.3) mL from O_2/O_3, 5 µg / mL, twice a week for 5 weeks is advisable.

Table 6. Dosage of ozone by MiAH. Adapted from the 3rd Ed. of Madrid Declaration 2020.[50]

Minor Autohemotherapy		
O_2/O_3	**Medium**	**Low**
Concentration (µg/NmL)	15/20	15/10
Volume (mL)	5	
Dose (mg)	75-100	25-50

10. Contingencies and Corrective Actions

In case of incidental O_3 inhalation follow procedure ISCO3/CLI/00/01.[54]

11. References

11.1 General References

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11.2 SOP References

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12. Documentation and Attachments

Annex I. Fibromyalgia Impact Questionnaire (IT-FIQR)
Annex II. Fibromyalgia Assessment Status (FAS)
Annex III. Widespread Pain Index (WPI)
Annex IV. Symptom Severity Score (SSS)
Annex V. Informed consent to treatment with O3
Annex VI. Consent to the processing of personal data
Annex VII. Exam Request Form
Annex VIII. Follow-up module

13. SOP Editing History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/CLI/00/20	02/03/2023	Grammatical correction by Dr. Wayne McCarthy. Additional formal input by Dr. Bernardino Clavo	First version
	29/04/2023	Input from Dr. Adriana Schwartz, highlight the recommendation of not use of hight doses in the proposed protocols and minor editing, grammatical corrections.	First version

14. Document Records

	Name	Title	Signature	Date
Author	Carmen Helena Acevedo	M.D. ISCO3 Member		02/03/2023
Co. Authors / Reviewer / working group	Gregorio Martínez-Sánchez	Elected president. Ph.D.; Pharm. D.		02/03/2023
Authoriser / Approved	ISCO3 2020-2024 Members.			01/05/2023

Annex I. Fibromyalgia Impact Questionnaire (IT-FIQR)

Modified: Salaffi F et al. Psychometric characteristics of the Italian version of the revised Fibromyalgia Impact Questionnaire using classical test theory and Rasch analysis Clin Exp Rheumatol. 2013 Nov-Dec; 31 (6 Suppl 79): S41-9. Epub 2013 Jun 26.[41]

Domain 1- Physical Function												
For each of the following NINE questions, please tick the box that best indicates the degree of difficulty you felt, during the last week, in performing the listed activities, due to fibromyalgia: (choose only one number).												
1. BRUSH OR COMB THE HAIR												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
2. WALK UNINTERRUPTED FOR 20 min												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
3. PREPARE MEALS												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
4. PASS THE VACUUM CLEANER WASH THE FLOORS												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
5. LIFT AND CARRY SHOPPING BAGS												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
6. CLIMB A FLOOR OF STAIRS												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
7. CHANGE THE BED SHEETS												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
8. SITTING ON A CHAIR FOR AT LEAST 45 min												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
9. GO SHOPPING												
None Difficulty	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Difficulty
Domain 1 Subtotal:												Total sub / 3 =

Score: Sum the scores of today's single item for the three domains (physical function, general health status and symptoms); Divide the score for the PHYSICAL FUNCTION domain by 3, leave the score unchanged for the GENERAL STATE domain and divide the SYMPTOMS domain score by 2. Add up the results scores of the 3 domains to get the total (FIQR Total Score).

Domain 2 - General health status												
For each of the following TWO questions, please tick the box that best indicates the degree of difficulty you felt, during the last week, in performing the listed activities, due to fibromyalgia: (choose only one number).												
10. Fibromyalgia prevented me from finishing the week's jobs / tasks												
Never	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	All time
11. I was completely overwhelmed by the symptoms of fibromyalgia												
Never	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	All time
Domain 2 Subtotal:												
Domain 3 - Symptoms												
For each of the following TEN questions, please tick the box that best indicates the degree of difficulty you felt, during the last week, in carrying out the activities listed, due to fibromyalgia: (choose only one number).												
12. Score your pain level												
No Ache	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Ache
13. Score your fatigue level												
None Tiredness	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Tiredness
14. Score its stiffness level												
None rigidity	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme rigidity
15. Score the quality of your sleep												
Well rested upon awakening	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extremely tired upon awakening
16. Score your degree of depression												
Not at all depressed	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extremely depressed / a
17. Score your memory problems												
Good memory	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Very scarce memory
18. Score your anxiety level												
Not at all anxious	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extremely anxious
19. Score your pain level (pain plummeting to touch)												
None achiness	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme tenderness
20. Score your balance problems												
No Problem	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Very poor balance
21. Score your sensitivity to noise, light, odors, and cold												
None Sensitivity	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐	7 ☐	8 ☐	9 ☐	10 ☐	Extreme Sensitivity
Domain 3 Sub total:						Total sub / 2 =						

Annex II. Fibromyalgia Assessment Status (FAS)

Salaffi F *et al*. Development and validation of the self-administered Fibromyalgia Assessment Status: a disease-specific composite measure for evaluating treatment effect. Arthritis Res Ther. 2009; 11 (4): R125. doi: 10.1186 / ar2792. Epub 2009 Aug 18.[56]

1. Score your fatigue level (over the last week)

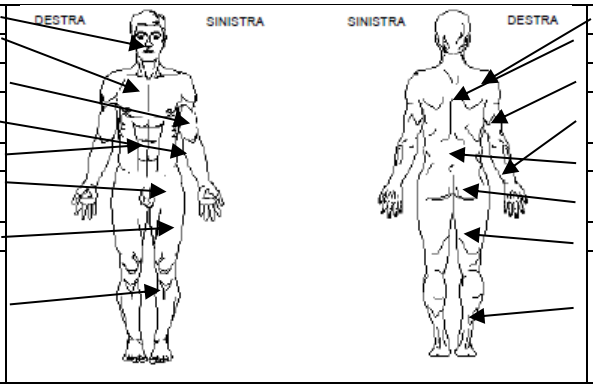
None Tiredness	0	1	2	3	4	5	6	7	8	9	10	Extreme Tiredness
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

2. Score the quality of your sleep (over the last week)

Well rested upon awakening	0	1	2	3	4	5	6	7	8	9	10	Extremely tired upon awakening
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

3. Check the box that best indicates the degree of pain and / or sensitivity you have felt over the last week for each of the body areas that appear in the drawing. Mark with an "X" for both right and left sides, "0" no pain "3" Extreme pain.

Head	0	1	2	3					0	1	2	3	Neck
Chest	0	1	2	3					0	1	2	3	upper back
Arm left	0	1	2	3					0	1	2	3	Arm right
Left forearm	0	1	2	3					0	1	2	3	Right forearm
Abdomen	0	1	2	3					0	1	2	3	Low back
Left buttock	0	1	2	3					0	1	2	3	Right buttock
Left thigh	0	1	2	3					0	1	2	3	Right thigh
Left leg	0	1	2	3					0	1	2	3	Right leg



Nomogram:

1 = 0.2	4 = 0.8	7 = 1.5	10 = 2.1	13 = 2.7	16 = 3.3	19 = 4.0	22 = 4.6	25 = 5.2	28 = 5.8	31 = 6.5	34 = 7.1	37 = 7.7	40 = 8.3	43 = 9.0	46 = 9.6
2 = 0.4	5 = 1.0	8 = 1.7	11 = 2.3	14 = 2.9	17 = 3.5	20 = 4.2	23 = 4.8	26 = 5.4	29 = 6.0	32 = 6.7	35 = 7.3	38 = 7.9	41 = 8.5	44 = 9.2	47 = 9.8
3 = 0.6	6 = 1.3	9 = 1.9	12 = 2.5	15 = 3.1	18 = 3.8	21 = 4.4	24 = 5.0	27 = 5.6	30 = 6.3	33 = 6.9	36 = 7.5	39 = 8.1	42 = 8.8	45 = 9.4	48 = 10

Total Item 3 = _____ Normalized Item 3: _____

FAS = Sum (item 1 + item 2 + normalized item 3) / 3 = _____

Annex III. Widespread Pain Index (WPI)

Wolfe F, Clauw Dj, Fitzcharles Ma *et al.*: Fibromyalgia criteria and severity scales for clinical and epidemiological studies: A modification of the ACR preliminary diagnostic criteria for fibromyalgia. J Rheumatol 2011; 38: 1113-22.[42]

WPI (Diffuse Pain Index)	
Indicate the region of pain. The score should be between 0 and 19	
1. Left shoulder girdle	☐
2. Right shoulder girdle	☐
3. Left arm	☐
4. Right arm	☐
5. Left forearm	☐
6. Right forearm	☐
7. Left hip (gluteus trochanter)	☐
8. Right hip (gluteus trochanter)	☐
9. Left thigh	☐
10. Right thigh	☐
11. Left leg	☐
12. Right leg	☐
13. Right jaw	☐
14. Left jaw	☐
15. Chest	☐
16. Dorsal area	☐
17. Lumbar area	☐
18. Neck	☐
19. Abdomen	☐
Add up the number of areas where the patient has had pain during the past week. How many areas did the patient have pain in?	**WPI =**

Annex IV. Symptom Severity Score (SSS)

Wolfe F, Clauw Dj, Fitzcharles Ma *et al.*: Fibromyalgia criteria and severity scales for clinical and epidemiological studies: A modification of the ACR preliminary diagnostic criteria for fibromyalgia. J Rheumatol 2011; 38: 1113-22.[42]

SSS (Severity of Symptoms Score)

Last week			
1. Score your Tiredness / Asthenia / Fatigue level (over the last week)			
0 No problem	1 Mild or intermittent problems	2 Moderate; significant problems; often present	3 Serious: pervasive, ongoing problems that compromise the life.
☐	☐	☐	☐
2. Score his level of **wake up not rested** (over the last week)			
0 No problem	1 Mild or intermittent problems	2 Moderate; significant problems; often present	3 Serious: pervasive, ongoing problems that compromise the life.
☐	☐	☐	☐
3. Score his level of **cognitive disturbances** (over the last week)			
0 No problem	1 Mild or intermittent problems	2 Moderate; significant problems; often present	3 Serious: pervasive, ongoing problems that compromise the life.
☐	☐	☐	☐
Sum of the severity of the 3 symptoms:			
Last 6 months			
1. Score your level of headache, abdominal pain / cramps, **depression** (over the last 6 months)			
0 No problem	1 Mild or intermittent problems	2 Moderate; significant problems; often present	3 Serious: pervasive, ongoing problems that compromise the life.
☐	☐	☐	☐
SSS *:			

* SSS Score = Sum of the severity of the 3 symptoms (fatigue, unrefreshed wakefulness and cognitive symptoms) plus the sum of the number of the following symptoms that occurred in the previous 6 months: headache, pain or cramps in the lower abdomen and depression. The final score is between 0 and 12.

A patient meets the diagnostic criteria for fibromyalgia if they meet the following 3 conditions:
1. Widespread Pain Index (WPI) ≥7 and symptom severity (SSS) scale score ≥5 or WPI 3-6 and SSS scale score ≥9
2. Symptoms have been present with the same intensity for at least 3 months
3. The patient does not have a pathology that could explain the pain differently

Annex V. Informed consent to treatment with O3

Name / Surname: _____

Birthplace: _____ Date of birth): _____

Address _____

Identity number _____ Phone _____

Medical Facility Address: _____

a) Pathology description: Fibromyalgia
b) Therapeutic alternatives related to the symptoms presented by the patient: Palliative, Pharmacological.
c) The medical or surgical treatment that will be performed (explain why and what you want to achieve): __ Major auto-hemotherapy _____ Minor auto hemotherapy ____ Ozonized Saline Solution ___Rectal therapy _____ Ozone infiltration.
d) The therapy before the main treatment: it is a complementary treatment.
e) Method of intervention: Sessions for a cycle of therapies. Certified medical ozone generators are used.
f) Possible treatments or interventions that may be needed in addition to the main treatment: Supportive care, diet, supplements, diagnostic.
g) Complications: ozone allergy, vagal syndrome.
h) Side effects that may appear after the treatment: pain or hematoma in the application site.
i) Therapy or indications that must be followed after the main surgery: rest.

The undersigned (taking into consideration the above, with full awareness and freedom) **DECLARES.**

- ✓ Be fully aware,
- ✓ I have carefully read the entire document,
- ✓ That the doctor who will perform the treatment has explained everything necessary for my full understanding of what has been indicated in the document,
- ✓ I understood the content of the document,
- ✓ I authorize the doctor to perform the above medical treatment,
- ✓ That I can leave the treatment when I want,
- ✓ Which can be compensated if unexpected damages appear following the procedure,
- ✓ From now on, authorize the doctor to carry out the procedure described in point f).
- ✓ I understand that I will be given a copy of this consent form.
- ✓ I authorize that my clinical data serve to study the effect of ozone in fibromyalgia.
- ✓ Authorize the use of me medical records / data, for research purposes.

Date (dd / mm / yyyy) ____/____/_____ Patient's signature* _____

*Representative or guardian (in case the patient does not cooperate or is a minor).

Statement from the physician in charge of informing the patient.

The undersigned Dr. _____ confirm and certify, by signing this document, that, in my opinion, the patient understands, point by point, the content of this document.

Date (dd / mm / yyyy) ____/____/ 2023_____ Doctor's signature * _____

* Everything previously reported in this document offers concise information on the procedure and is not intended to replace the doctor / patient dialogue.

Annex VI. Consent to the processing of personal data

CONSENT TO THE PROCESSING OF PERSONAL DATA in the health sector and methods of communication on the state of health. Pursuant to the EU GDPR 2016/679

Having taken note of the information pursuant to art. 13 of the EU GDPR 2016/679, the undersigned

Mr./Mrs. Surname name:

DECLARES

☐ To have been informed about 1) the purposes and methods of the processing for which the data are intended, connected with the prevention, diagnosis, treatment and rehabilitation activities carried out by the doctor to protect their health. 2) the subjects or categories of subjects to whom the personal data may be communicated (substitute doctors, laboratory analyzes, specialist doctors, pharmacists, hospitals, private nursing homes and tax specialists) or who can learn about them as appointees. 3) the right to access personal data, the right to request updating, rectification, integration and cancellation as well as to oppose the sending of commercial communications. 4) the name of the doctor who will be the owner of the personal data processing as well as the address of the relative professional office. 5.

☐ I have received the information on the processing of personal data in the health sector.

☐ To give consent to the processing of their personal data produced and used by the Outpatient Clinic □ Center _____) □ Other_____, for treatment purposes;

☐ To acknowledge that the consent is valid for all health services performed in this clinic;

☐ To be aware that the consent, once manifest, may be modified or revoked, at any time, in whole or in part.

☐ To give consent for information on your state of health to be provided to the following people:

- ☐ Spouse_____ Tel_____□ Children_____ Tel ._____
- ☐ Parents _____ Tel ._____□Others_____ Tel ._____
- ☐ Nobody □ Anyone

☐ To give consent that the clinical data, compressed photographic or filmed images relating to health services, object of the treatment, made anonymous, are used for research, epidemiology, teaching, training and disease studies purposes. □ YES □ NO

_____, date_____ Signature of the declarant _____

In the case of minor or interdicted patients or in cases where the manifestation of consent is given by a person other than the interested party, the person exercising parental authority or the person entitled to grant consent or in any case the person, other than the patient, called to express consent to the processing of personal data, sign the following:

REPLACEMENT STATEMENT OF CERTIFICATION

the undersigned_____, born in_____, on_____,

Pursuant to articles 46 and 47 of the Presidential Decree of 28 December 2000, aware of the penal sanctions envisaged for the case of untruthful declaration, formation and use of false documents, as established by art. 76 of the same Presidential Decree 445/200, with reference to

the undersigned_____, born in_____, on_____,

Declares under its own responsibility: □ To exercise parental authority over the indicated minor; □ To be a guardian; □ To be a curator; □ To be a support administrator; □ To be living with the patient indicated above (1); □ To be familiar with the patient indicated above (1,2); □ To be responsible for the facility where the interested party lives (1,3).

SIGNATURE OF THE DECLARANT:_____

(1) Consent is given by a person other than the interested party in cases where the patient is unable to give his consent and the failure to provide the health service would jeopardize his state of health. (2). Specify the degree of kinship or marriage relationship, if any. (3). Specify the name of the facility where the patient is staying.

Annex VII. Exam Request Form

Requesting Doctor: Dr. _____ Pick-up date: / / 202_ No: _____
Send date: / / 202_ No: _____

Sample sent: ☐ Peripheral blood ☐ EDTA peripheral blood ☐ Citrated peripheral blood ☐ Peripheral blood heparin ☐ Plasma ☐ Serum ☐Urine ☐ Other:_____

Patient:

Surname name:_____ Name: _____

Fiscal Identity: _____ Date of birth: _____ / _____ / _____

LAB. Test REQUIRED

X COMPLETE HEMOCHROME:☐ Leukocyte count ☐ Erythrocyte count ☐ Hemoglobin ☐ Hematocrit ☐ Mean corpuscular volume (MCV) ☐ Average Hb content (MCH) ☐ Average Hb concentration (MCHC) ☐ Red blood cell volume distribution (RDW) ☐ Platelets (PLTS) ☐ Neutrophil granulocytes ☐ Lymphocytes ☐ Monocytes ☐ Eosinophilic granulocytes ☐ Basophilic granulocytes ☐ RETICULOCYTES (Research and counting)
X GLYCOSYLATED HEMOGLOBIN (HbA1) ☐ HEMOGLOBIN ELECTROPHORESIS (HbA2) ☐ APTOBLOBINA
X EMAZIE SEDIMENTATION SPEED (VES) ☐ Serum protein electrophoresis
☐ PROTHROMBIN TIME PT (INR International Ratio) ☐ THROMBOPLASTIN TIME PTT
X TRIGLYCERIDES X CHOLESTEROL X HDL CHOLESTEROL X LDL CHOLESTEROL
☐ GLYCEMIA ☐ LOADING GLUCOSE CURVE ☐ INSULINEMIC CURVE ☐ POST-PRANDIAL GLUCOSE☐INSULIN
☐ TOTAL BILIRUBIN ☐ DIRECT BILIRUBIN X ALBUMINA ☐ TOTAL PROTEINS
X LDH (lactic dehydrogenase) X CK (CPK) X GOT (AST) X GPT (ALT)
☐ CERULOPLASMINE ☐ TOTAL TRANSFERRINA ☐ UNSATURATED TRANSFERRIN (UIBC)
X REACTIVE PROTEIN C (PCR) X CREATININE ☐ CREATININE CLEARANCE
X T4 FREE X TSH (thyroid stimulating hormone) ☐ FSH (follicle-stimulating hormone) ☐ LH (Luteotropic hormone) ☐ PRL (prolactin) ☐ DHT5α-DIHYDROTESTOSTERONE (5α-DHT) ☐ 3-ALPHA. ANDROSTANEDIOL ☐ DHEA (Dehydroepiandrosterone) ☐ ANDROSTENEDIONE ☐ SHBG (Glob. sex hormone binding)
☐ TESTOSTERONE (T) ☐ FREE TESTOSTERONE ☐ ADENOCORTICOTROP (ACTH) ☐17 - OH - PROGESTERONE
☐ LITHIUM ☐ COPPER ☐ SODIUM ☐ MAGNESIUM ☐ SOCCER ☐ ZINC ☐ VITAMIN B12
☐ PSA (Specific Prostate Antigen) ☐ PSA FREE
☐ other: ☐Uricemia ☐ alkaline phosphatase X γ glutamin-transferase (γ GT)
☐ URINE ☐ COMPLETE URINE (Chemical-physical examination) ☐ URINOCULTURE
☐ URIC ACID ☐ URINARY CREATININE ☐ POTASSIUM ☐ MAGNESIUM ☐ SODIUM ☐ ZINC
X other: __ X Serotonin

☐ **Microbiology**

☐ Antibiogram (Kirby Bauer)	☐ Research Microscop. Microbial Flora	☐ Skin Cytohistopathology
☐ Antibiogram (MIC)	☐ Vaginal Exudate Culture	☐ Needle Aspirated Cytological
☐ Microscopic Research (Gram)	☐ Culture Expectorate	

☐ Other:_____

☐**Immunological**

☐ NATIVE DNA (Antibodies)	☐ MITOCHONDRI (AMA Antibodies)	X Anti EBVCA (AntiCapside Epstein–Barr virus) IgM and IgG (quantitative) X AntiEBVNA (AntiNucleo EBV) IgM and IgG (quantitative)
☐ ENA SCREENING (Antibodies)	X REUMATEST (rheumatoid factor)	☐ HIV 1 - 2 (Antibodies)
☐ MICROSOMIALS (Antibodies) MAB	☐ SMOOTH MUSCLE (ASTHMA Antibodies)	X HAC IgG / IgM (Hepatitis C virus antibodies)
X IgA anti-transglutaminase antibodies (tTG)	☐ HELICOBACTER PYLORI (IgG Antibodies)	☐ HbsAb (Hepatitis B virus surface antibodies)
X ENDOMYSIUM (EMA Antibodies)	☐ Tg-TPO (Anti Thyroid Acid)	☐ HAV (Hepatitis A virus antibodies)
X Anti-DGP IgG (gliadin peptide epitopes)	☐ HERPES 1 - 2 (IgM Antibodies)	☐ Total Ig ☐ IgM
X AGA-IgG (gluten ataxia)	☐ HERPES 2 (IgG Antibodies)	X AntiVZV IgM and IgG (quantitative) X AntiCMV IgM and IgG (quantitative)

☐ Other:_____

☐ FAECES (CHEMICAL AND MICROSCOPIC EXAMINATION)☐I SEARCHED FOR HIDDEN BLOOD ☐ Coproculture

☐ Other:_____

Annex VIII. Follow-up module

For Acceptance and Follow-up Date:(dd / mm / yyyy): / /2023	**Folder no**	

TO. PATIENT INFORMATION □ Preventive protocol □ Intervention protocol

Name surname Patient's or identification number (if confidential)	**Date of birth** (dd / mm / yyyy) / /	**Sex** □ Male □ Female	**Patient n °:**
	Time of evolution of the disease _____months	Fiscal Code: Assigned Group ☐ Local infiltration ☐MAH ☐ RiO3 ☐ MiAH ☐ SSO3	

Dose or Quantity	Frequency	Protocol	Generator Brand O3	O3 Generator Model

B. Clinical Variables

□ Other:

C. Hematology

White blood cell count		MCH			
Erythrocyte count		MCHC			
Hematocrit		Platelets			
MCV		Hemoglobin			

D. Biochemistry

G6PD		ALT		CPK	
C-reactive protein		AST		T4	
Albumin		Creatinine		TSH	
HDL		Lactate dehydrogenase		γ GT	
Triglycerides		HbA1		Serotonin	
Total cholesterol		Homocysteine		Oxidative stress	
LDL		VES			

E. Immunology

IgA tTG		Anti-AGA IgG		Reumatest	
Anti EMA		IgM HAC		AntiEBVCA, EBVNA, VZV, CMV, HSV1 and 2	
Anti-DGP		IgG HAC			

Brain MRI image data:
Brain PET / SPECT / MRI data:

Concomitant therapies: ☐ _____

F. Other relevant information:	
G. Name and signature of the doctor:	