TITLE

Conservative vs conventional oxygen administration in critically ill patients: effects on ICU mortality. A multicentre randomized open label clinical trial**.**

1.1 Running Title

ICU-Conservative O2 trial

1. TRIAL REGISTRATION

The trial will be registered on ClinicalTrials.gov.

2. PROTOCOL VERSION

Protocol version 1.2, 21, February, 2018

3. FUNDING

Partially supported by “Clinical Trial Award 2017” by European Society of Intensive CareMedicine (EISICM).

4. ROLES AND RESPONSIBILITIES

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5.2 Steering Committee (provisional)

Antonelli Massimo, University Cattolica, Rome, Italy

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5.3 Role of the funders

The steering committee will take responsibility for study design and oversight. The funders will have no role in study design, data collection, management, analysis, data interpretation, manuscript writing, or in the decision to submit manuscripts for publication.

5.4 Coordinating Centre

The Coordinating centre and the data management centreare the Department of Anesthesiology and Intensive Care and the Department of Medical Statistics of the University of Modena and Reggio Emilia, ITALY

5. INTRODUCTION

6.1. Background and rationale

Hypoxemia is a frequent condition in hospitalized patients and it is caused by several pathologies not only of respiratory origin. Oxygen supplementation of the inspired gases is commonly used as a first-line support treatment for counteracting absolute or relative hypoxia. Critically ill patients are a particular subset of hospitalized patients where baseline co-morbidities are associated to various life-threatening clinical conditions, which can result in high complexity situations. Because of their severity, they appear to be particularly vulnerable to hypoxia and oxygen debt. Some observational studies have shown that in this subset of patients consistent supplementation of oxygen is often administered exposing patients to even prolonged periods of supernormal values of PaO_2 (hyperoxia). However, exposure to inhaled oxygen-enriched mixtures is widely recognized as potentially harmful and cause of multiple organs damage [1-4]. For these reasons, oxygen should be considered to all effects a drug with a limited therapeutic window and many considerations have to be made in order to provide the appropriate dose to support the vital function but, also, to avoid the harmful effects of the overdosing. Many recent experimental and clinical trials have tried to explain the pathophysiologic mechanisms that leads to the oxygen-related damage on various cellular pathways. The production of reactive oxygen species (ROS), due to the excess of oxygen availability and its incomplete metabolism and uncoupling of mitochondrial respiration, is the first responsible of a dangerous chain of events. Structural alterations of proteins and DNA, activation of apoptotic pathways, expression of pro and anti-inflammatory mediators cause cell death and could end in innate immunity alteration, exposing the patients also to a higher risk for infections development [5-8]. Moreover, increased ROS leads to a reduction of nitric oxide release, formation of reactive nitrogen species (RNS) and induction of alteration in microcirculatory reactivity and tissue perfusion [9]. Even if the pulmonary oxygen toxicity is the best recognized and characterized form of oxygen-related damage, many other organs and systems are showed to be affected from iatrogenic oxidative stress, leading to severe dysfunction and poor outcome.

The PROXI trial reported an association between peri-operative administration of a high inspired oxygen concentration (FiO_2) with an increase in long-term mortality [10]. Similarly,

the recent AVOID trial showed that, in patients with ST-elevation myocardial infarction but without hypoxemia, supplemental oxygen therapy may increase early myocardial injury and is associated with larger myocardial infarct size at six months [11]. Prolonged hyperoxia has also been investigated as a potential cause of increased risk of cognitive dysfunction in the post-operative setting (POCD) [12]. During anaesthesia and surgical procedures, the maintenance of a proper cerebral oxygenation is of paramount importance. However, an excess of oxygen supplementation leads to an increasing of cerebral vascular resistances and, consequently, to a reduced cerebral blood flow independently from CO2 and vascular reactivity [13]. The cerebral desaturation, enhanced from this inappropriate systemic hyperoxia, is proved to be associated to a higher incidence of POCD, longer hospitalization and long-term consequences on cognitive function.Conflicting data have been published on the role of hyperoxia in the normalization of oxidative metabolism in patients with brain trauma or stroke, in preconditioning of the ischemia-reperfusion injury in cardiac surgery and the onset of the infection site surgery [14-18].

Despite these numerous suggestions of potential harm from hyperoxia, both treatment guidelines and standard clinician behavior promote prompt administration of high flow, high concentration O_2 therapy, especially in emergency rooms and ICU settings, being more concerned about deleterious effects of hypoxia than hyperoxia. In these setting, supra-physiologic values of arterial oxygen partial pressure (PaO_2) are frequently achieved and maintained for prolonged periods, increasing risk of toxicity. The amount of oxygen administrated in the ICUs remain, thus, quite variable and not regulated, as demonstrated by a recent observational study by Suzuki et al. [19]. Moreover, an Australian observational trial shows how the level of attention of the clinicians and nurses in a way more focused on hypoxia than on hyperoxia; lower limits of allowed peripheral oxygen saturation (SpO_2) are widely more often prescribed than upper limits and those last ones are more often disregarded than lower limits, when prescribed [20].

The specific level of PaO_2 considered harmful, or the dangerous duration of hyperoxia, is not determined yet as there are no clinical trials on humans that evaluate the appropriate percentage of oxygen considered safe to maintain an adequate tissue oxygen availability. In patients suffering from acute respiratory distress syndrome (ARDS) a number of volumetric and pressure parameters in the ventilation have been studied [21], leading however to the

recommendation to use the smallest fraction of inspired oxygen for the maintenance of normoxia. [22] The toxicity of hyperoxia in patients undergoing mechanical ventilation was the subject of a recent retrospective Dutch study, in which inspiratory fractions of oxygen and high PaO2 values were associated with a higher mortality in ICU [23]. A recently published randomized open label controlled clinical trial on a general mixed ICU population, showed that the application of a protocol for conservative oxygen administration lead to a significant decrease in ICU mortality if compared with conventional clinical practice [24]. However, this study was prematurely stopped because of a low inclusion rate, leading to the need for a further multicentre clinical trial to better elucidate the potential benefits of this conservative strategy.

6.2 Objectives

Primary Objective:

To verify the hypothesis that strict maintenance of normoxia improves survival in a wide population of mechanically ventilated critically ill patients compared to the application of conventional more liberal strategies of oxygen administration. Survival will be measured at ICU discharge.

Secondary objectives

To verify the hypothesis that, in a wide population of mechanically ventilated critically ill patients, the strict maintenance of normoxia compared to the application of conventional more liberal strategies of oxygen administration reduces the 90-day mortality, onset of new organ failures (respiratory, cardiovascular, renal and hepatic after 48 hours from ICU admission) during ICU stay, occurrence of nosocomial infections during ICU stay (only microbiologically documented bloodstream, respiratory and surgical site infections will be considered), the length of mechanical ventilation, vasopressor use and ICUstay, the occurrence of ICU acquired weakness and cognitive dysfunction

The confirmation of the efficacy of a conservative strategy for oxygen administration in reduce the mortality rate among critically ill patients will lead to a profound revision of the current clinical practice and a rationale revision of the current recommendations would be mandatory, maybe also in other clinical scenarios such as emergency departments.

6.3 Trial design

The study is designed as multicentre, open label, two parallel groups, randomized superiority clinical trial. The included patients will be randomized in 1:1 ratio in conservative and conventional group. It will be single blinded (patient); after the information provided by the clinician in order to ask the informed consent, the patients (and their family members) will ignore the randomization group and any detail about the oxygen administration strategy.

7 METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

7.1 Study setting

The study will involve 10 European intensive care units and will recruit adult critically ill patients requiring non-invasive (NIMV) or invasive mechanical ventilation (IMV) with an expected length of stay > 72 hours admitted to the participant ICUs.

List of centers (provisional):

1. ICU, Modena University Hospital, Italy
2. ICU, Ancona University Hospital, Italy
3. ICU, Cattolica University Hospital, Rome, Italy
4. ICU, University College London, UK
5. ICU, Humanitas University Hospital, ITALY
6. ICU, Hospital Valld'Hebron, Spain
7. ICU, Milano Ca' GrandaUniversity Hospital
8. ICU, ParisDiderot University Hospital Saint-Louis

7.2. Eligibility criteria

Inclusion Criteria:

- Critically ill patients admitted to participant ICUs,
- Age ≥ 18 years;
- Expected length of ICU stay of more than 72 hours.
- Need of any respiratory support (IMV or NIMV) at admission and with an expected length of respiratory support ≥ 6 hours

Exclusion Criteria:

- Pregnancy;
- Admission to ICU after elective surgery
- ICU readmission (after a first discharge) in the study period;
- IMV or NIMV greater than 12 hours in the 28 days before study inclusion;
- Clinical decision to withhold life-sustaining treatment or "too sick to benefit" or patients with a life expectancy of less than 28 days due to a chronic or underlying medical condition.
- Previous enrolment in other interventional studies of targeted oxygen therapy;

- Acute respiratory failure on chronic obstructive pulmonary disease
- Acute respiratory distress syndrome (ARDS) with a PaO2/FiO2 ratio < 150.
- Long-term supplemental oxygen therapy.
- Patients candidate to hyperoxia treatment for reasons including (but not limited to) carbon monoxide poisoning or to hyperbaric oxygen therapy;

7.3 Interventions

On the ICU admission, or later when they fulfil eligibility criteria, patients will be randomly assigned to a group of liberal conventional oxygen administration (CONVENTIONAL) or a group of conservative experimental oxygen administration (CONSERVATIVE). The study is conceived as single blinded: only the patients will not be aware of the received treatment. (see APPENDIX 1)

CONVENTIONAL: Within the conventional group(Control Group), patients will receive a FiO_2 aiming to maintain a $SpO_2 \geq 98\%$, accepting an upper limit of PaO_2 of 150 mmHg and a lower limit of 60 mmHg. The use of an FiO2 of less than 0.3 whilst ventilated is discouraged. According to standard ICU practice, control patients will receive a FiO_2 of 1.0 during endotracheal intubation maneuver, airway suction or in-hospital transfers.

CONSERVATIVE: To minimise unnecessary exposure to hyperoxaemia and reduce the exposure to unnecessary high FiO2, patients in the conservative (Treatment Group) group will receive the lowest FiO_2 to maintain SpO_2 between 94% and 98%, or when available a PaO_2 between 60 mmHg and 100 mmHg. A SpO2 alarm limit of 99% will apply whenever supplemental oxygen is being administered. The changes in oxygen therapy will be according to a nurse order-set. In particular, the FiO2 will be reduced or oxygen supplementation discontinued whenever the SpO2 or PaO2 exceeded 98% or 100 mm Hg. An oxygen supplementation will be given only if SpO_2 falls below 94%. Pre-oxygenation with FiO2 1.0 will not be performed during in-hospital transports or in anticipation of diagnostic and therapeutic maneuvers.

The blood gas analysis (BGA) control, laboratory and microbiological tests will be performed according to clinical indications in both groups in order to avoid biases induced by unplanned and unnecessary sampling. Extra blood samples will be obtained only in the patients included in the IMMUNE-O2 sub-study (see APPENDIX 5).

The indications to NIV or tracheal intubation and IMV (and to the most appropriate mode of ventilatory support) will be guided by principles of the Good Clinical Practice and by the clinical judgement of the attending physician. No other pharmacological therapy or treatment will be influenced from the study protocol.There are no restrictions to concomitant treatments provided to patients in this study.

7.4Criteria for discontinuing or modifying allocation

The duration of study therapy will be until discharge from the study ICU regardless the type of ventilation (e.g. mechanically or spontaneous). Patients may be prematurely discontinued from study protocol at the discretion of the Investigator, should any untoward effect occur (including an AE or clinically significant laboratory abnormality that, in the opinion of the Investigator, warrants the subject's permanent discontinuation of study protocol-directed care)

7.5Strategies to improve adherence to protocols

The site PIs will take primary responsibility for training local staff and for ensuring that protocol compliance is achieved.

7.6 Outcomes

Primary endpoint:

ICU mortality rate, defined as the number of deaths for any cause occurred during the ICU stay (censored at 90 days)

Secondary endpoints:

- 90-day mortality for any case,
- The occurrence of new organ dysfunction defined as a Sequential Organ Failure Assessment (SOFA) score ≥3 for the corresponding organ), occurring ≥48 hours after ICU admission
- bloodstream, respiratory and surgical site infections (defined according to CDC definitions[20]). Only microbiologically documented bloodstream and respiratory tract infections were considered.
- Ventilation-free hours (VFHs) during the ICU stay. VFHs is defined as the total number of hours of unassisted breathing between randomisation and ICU discharge. Periods of assisted breathing lasting less than 24 hours for surgical procedures will not count against the VFH calculation. Patients who are receiving extracorporeal membrane

oxygenation (ECMO) will be defined as receiving assisted breathing irrespective of whether they are ventilated or not.

- Vasopressor free-hours (VasoFHs) during the ICU stay. VasoFHs is defined as the total number of hours without vasoactive drugs infusion used for hypotension between randomisation and ICU discharge.
- ICU free days at 28-day and at 90-day
- Cognitive Dysfunction at ICU discharge (censored at 90 days) by Mini-Mental State Examination Test (MMSE) [26](APPENDIX 2)
- ICU acquired weakness at ICU discharge (censored at 90 days) by Medical Research Council (MRC) Scale[27] (APPENDIX 3)

<u>Physiological and Processes of care outcomes (all parameters censored at day 90)</u>

- Daily FiO2 and PaO2 time-weighted calculated as the mean value of 2 consecutive measurements multiplied by the time (hours) between the measurements and divided by 24 hours. If only 1 value was available within a 24-hour period, the PaO2 time-weighted average was equal to that value;
- The proportion and the total number of hours in ICU when the SpO2 is greater than or equal to 98%. These measures will be based on all values (up to a maximum of one per hour) recorded on the ICU flow chart;
- The proportion and the total number of hours in ICU when the SpO2 is less than 91%. These measures will be based on all values (up to a maximum of one per hour) recorded on the ICU flow chart;
- Highest daily PaO2: the highest daily PaO2 will be recorded daily from randomisation until ICU discharge.
- Lowest daily PaO2: the lowest daily PaO2 will be recorded daily from randomisationuntil ICU discharge.
- The proportion of hours in ICU when the FiO2 is 0.21. This will be based on all values (up to a maximum of one / 2 hour) recorded on the ICU flow chart.
- Highest daily FiO2: the highest daily FiO2 will be recorded daily from randomisation until ICU discharge.
- Lowest daily FiO2: the lowest daily FiO2 will be recorded daily from randomisation until ICU discharge.

- Ventilation mode: the predominant mode of ventilation used on each day will be recorded daily from enrollment to ICU discharge
- Time to hospital discharge (censored at 90 days);
- Proportion of patients requiring renal replacement therapy in ICU;
- Proportion of patients requiring a tracheostomy in ICU;
- Proportion of patients who received each of the following respiratory co-interventions: Extracorporeal membrane oxygenation, extracorporeal CO2 removal, ongoing neuromuscular blockade, nitric oxide, prone positioning and prostaglandins in ICU;
- Proportion of patients who received PRBC transfusions in ICU and the number of units per patient.

7.7. Participant Timeline

(SEE APPENDIX 4)

7.8 Sample size

Assuming a two-sided alpha level of <0.05 and a power of 80%, we calculated that 460 patients are needed per arm to detect a relative risk reduction of 40% (absolute risk reduction of 6%) compared to a mortality of 15% observed in conventional group patients in previous studies. The global sample size is established to 1000 patients. This includes an additional 80 participants to allow for drop-outs and an expected temporal trend of reducing mortality at ICU discharge.

7.9 Recruitment

All centres participating in this study are well experienced in large scale, pragmatic investigator-initiated studies. On the basis of a conservative estimation, the 10 participating sites admit an average of 25 eligible patients per months per centre(250 patients/months). Assuming that35 % of eligible patients are enrolled, recruitment of 1000 participants will be completed in around one year. A PIs meeting will be held before study starting (within 2 weeks from approval) to discuss practical and operational issues. Every month, recruitment status will be evaluated, and a newsletter will be disseminated, including any practical, clinical or scientific issue arisen.

8.METHODS: ASSIGNMENT OF INTERVENTIONS, DATA MANAGEMENT, COLLECTION AND ANALYSIS

8.1 Allocation and blinding

A block randomisationwill be used with variable block sizes (block size 4-6-8), stratified by center. Central randomisation will be performed using a secure, web-based, randomisation interface. The allocation sequence will be generated by the study statistician using computer generated random numbers. The attending physician, according to the protocol and the randomization, will note the inclusion of the patient in the study. Both nurses and clinicians will receive all the instructions to attend the protocol and paper printed instructions will be distributed in the participating centres. The local coordinator will be responsible of the formation and information of the personnel about the study protocol. As the healthcare staff can not be blinded to the treatment provided, the study is conceived as single blinded: only the patients will be not aware of the group allocation.

8.2 Data collection and management

Every patient who meets the inclusion criteria will be included after ICU admission and randomised in the treatment or control group. The study data will be collected along the entire ICU stay in a dedicated Case Report Form (CRF).The CRF will be provided by the steering committee with proper options to minimize data entry errors: the datasheet will incorporate unmodifiable fixed intervals of values (for continuous variables) and pre-defined coding system (for binary or categorical variables). Data entry will be performed and double-checked from a dedicated researcher in each centre; in order to limit collection errors, 10% of all records will be randomly re-checked from the PI in each participating centre. Data will be collected and stored in double copy on hardware supports in every participant centre and sent to the coordinating centre every 6 months in order to prevent data losses and protected by password to prevent unintentional modifications or deletion. Each satellite centre will monthly communicate and report via e-mail with the coordinating centre about number of recruited patients, eventual missing data or missing visit or any kind of problem correlated to data collection. Data related to the study will be stored for eventual further analysis or study purpose for 5 years after the end of the study.

All the data about the included patients will be extrapolated from the clinical documentation and recorded in an CRFfrom adequately formed researcher. Demographic information (gender, age), co-morbidities, reason of ICU admission, type of ICU admission (medical, elective surgery, emergency surgery) will be registered at the inclusion; severity of critical illness (quantified by the Simplified Acute Physiology Score II, SAPS II) will be calculated by the data from the first 24 h of ICU stay. During the entire ICU stay, SOFA score will be calculated and registered daily. Every partial SOFA score will be registered separately (Nervous System, Respiratory, Cardiovascular, Liver, Renal and Coagulative). BGA results will also be reported: FiO_2, PaO_2, $PaCO_2$, pH, lactates, HCO^{3-}, Base Excess. Other daily parameters: duration of ventilatory support in hours, need and dose of vasoactive drugs (doses reported in μg/Kg/min), need of renal replacement therapy (intermittent haemodialysis or continuous veno-venous hemofiltration) subsequent to the first 24 hours of ICU stay. The occurrence of ICU-acquired respiratory, blood and surgical site infections (from 48 hours after ICU admission) and the implicated microorganisms will be registered. Data from routine laboratory test will be reported: haemoglobin, platelets count, white blood cells count, coagulative parameters, parameters for liver and renal function; $ScvO_2$ and arterial lactates will be reported when available.

The patient will be followed-up until 90 days in order to register ICU mortality, ICU length of stay (LOS), hospital LOS, 90-day mortality.

8.3 General methods for statistical analysis

The intention to treat population will be considered for primary analysis. A descriptive statistical analysis will be performed to describe every relevant variable. Kolmogorov-Smirnov normality test will be performed in order to verify the variables distribution. Results will be expressed in mean ± standard deviation or median and interquartile range as appropriate. Baseline and outcome variables will be compared between the two groups using Mann-Whitney U test or t-test as appropriate. Categorical variables will be compared using Fisher's Exact test. The effect of conservative O_2 therapy on ICU and long-term mortality will be explored in the intention to treat population by a Kaplan-Meier analysis and Log-Rank (or Tarone-Ware test as appropriate) for the hazard ratio. The primary and secondary outcomes will be also evaluated in pre-defined subgroups: quartile distribution of SAPSII and SOFA score (total and for single organ) at admission, patients with respiratory

SOFA score ≥ 3 (respiratory dysfunction), patients with cardiovascular SOFA score ≥ 3 (shock), surgical admissions compared to non-surgical admissions, documented infections at admissionand distribution of length of stay in ICU. The relationship between O_2 exposure and ICU mortality will be evaluate according to the quartile distribution of the median value of the daily ICU time-weighted PaO_2 values. Every test will be performed considering a two-sided p value < 0,05 for statistical significance.

9. METHODS: MONITORING

9.1 Data monitoring:

An independent Data Monitoring Committee (DMC), consisting of 2 experts in clinical research in intensive care and 1biostatistic will be established before patient enrolment. The DMC Charter will be prepared by the steering committee and signed by the members of the DMC before the trial commences.The DMC will have access to all results and make the appropriate considerations about the appropriateness of the sample size, the efficiency and quality of data collection system, eventual occurrence of suspected protocol-related adverse event.

Analyses ad interim

During the study period, an interim analyses is planned after the randomization of 500 patients, for the double objective of monitoring safety and verifying the accuracy of the assumptions made for sample size estimation regarding the primary end-point event rate in relation to the anticipated survival benefit. No stopping rules are foreseen, either for utility or for efficacy.

9.2. Safety

All the patients, regardless the inclusion in the study and the randomization group, will benefit from the best standard of care following the principles of Good Clinical Practice. Previous safety studies did not identify specific risks correlated to the application of a conservative oxygen administration; however, all the included patients will be intensively monitored following the standard procedures of the Intensive Care and any suspected protocol-related adverse event will be reported. Intensive care patients frequently develop life-threatening organ failure unrelated to study interventions and despite optimal management. Therefore, events that are part of the natural history of the primary disease

process or expected complications of critical illness [28] will not be reported as serious adverse events in this study.

9.3. Auditing

The principal investigator and the steering committee will provide all the necessary information and material to the participating centresin order to standardize all the protocol-related procedures and to avoid unexpected variability between centres. A preliminary audit between the coordinating centre and the satellite centres will be performed before to start the recruitment phase to offer the major consensus and homogeneity possible. Printed and electronical informative material (complete original protocol, informed consent modules, informative modules for patients and relatives, recruitment check-list, graphic timeline of interventions and visits, order-list for physicians and nurses) will be distributed to every centre.

10. ETHICS AND DISSEMINATION

10.1. Research ethics approval:

The entire study protocol, including informative material for the patients and modules for the informed consent, will be evaluated from the Local Ethics Committee from the coordinating centre and from all the collaborating centres. Every intention to modify any element of the original protocol after the first approval will be promptly notified to the Ethics Committee and will be applicate only after its written authorization.

10.2. Consent and confidentiality

A written informed consent will be asked to each eligible patient before enrolment.

If the patient will be unable to comprehend or to give his consent (because of compromised neurological status), the following consent options are acceptable: (i) A priori consent by a substitute decision maker; (ii) delayed consent from a substitute decision maker; (iii) Delayed consent from the patient; (iv) waiver of consent; (v) consent provided by an ethics committee or other legal authority. Which options are available at individual participating sites will be determined by the relevant ethics committee and subject to applicable laws.

In Italy, the approach to patients unable to provide an informed consent before enrolment will be to consider whether participation is in the best interests of each individual patient

and as soon as it is practical and reasonable to do so, to seek the advice of persons interested in the patient's welfare (e.g. family member) to establish that study participation is consistent with the patient's wishes. All participants who recover sufficiently will be given the opportunity to provide informed consent for ongoing study participation and for the use of data collected for the study.

Every patient is free to leave the study protocol at any state of the study and can request to retire his consent and, consequently, to ask the elimination of all his data from the database.. Data about personal and private information, included sensible data, will be treated following current legislation on data protection; patients will be identified with a coding system and data registered in anonymous form.

10.3 Declaration of interest

The study participants declare no financial and/or other conflicts of interest related to the study.

10.4. Dissemination policy:

The Circ. Min. Health N° 6 of 09/02/2002 obliges each researcher who get any results of interest to public health, to publish the results within 12 months from the end of the study. All the patients will freely agree or disagree to participate to the study in the belief that the results will be useful to improve knowledges about their pathologies, for health benefit from themselves or other patients. To respect their will and in the maximum interest of honest clinical research, the investigators agree on the need to ensure the wide publication and diffusion of their results in a consistent and responsible way under their responsibility.The steering committee is the official data owner and ithas the right to present methods and results of the study at public symposia and conferences. The principal publications from the trial will be in the name of Investigators with full credit assigned to all collaborating investigators and institutions.

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APPENDIX 1:

Figure 1. Graphic protocol for management of O2 therapy in CONVENTIONAL GROUP

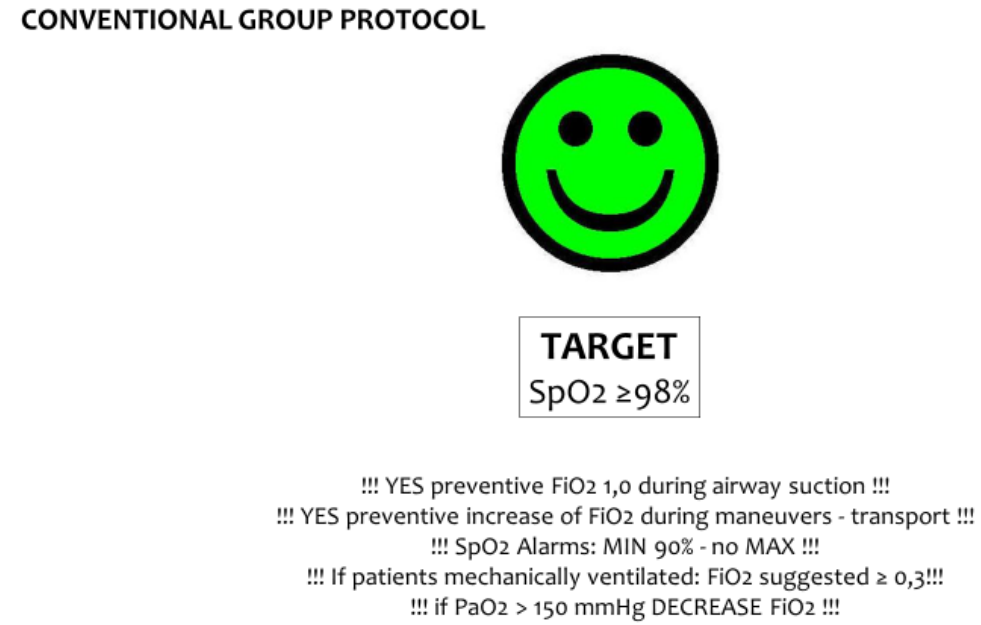
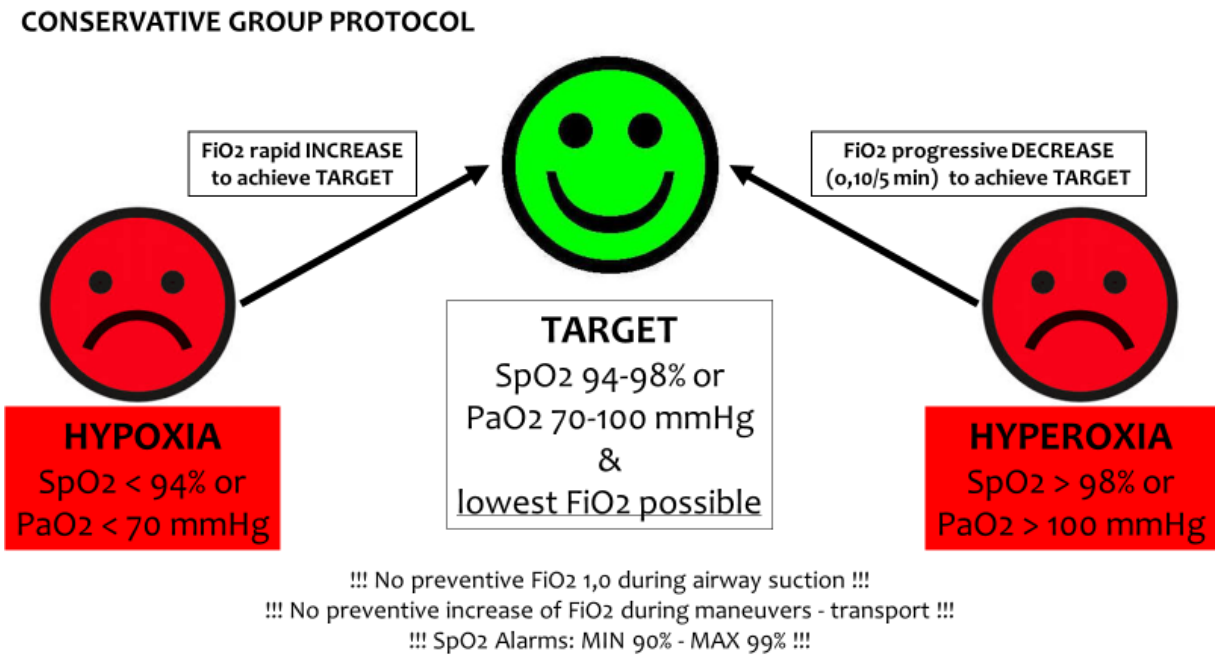
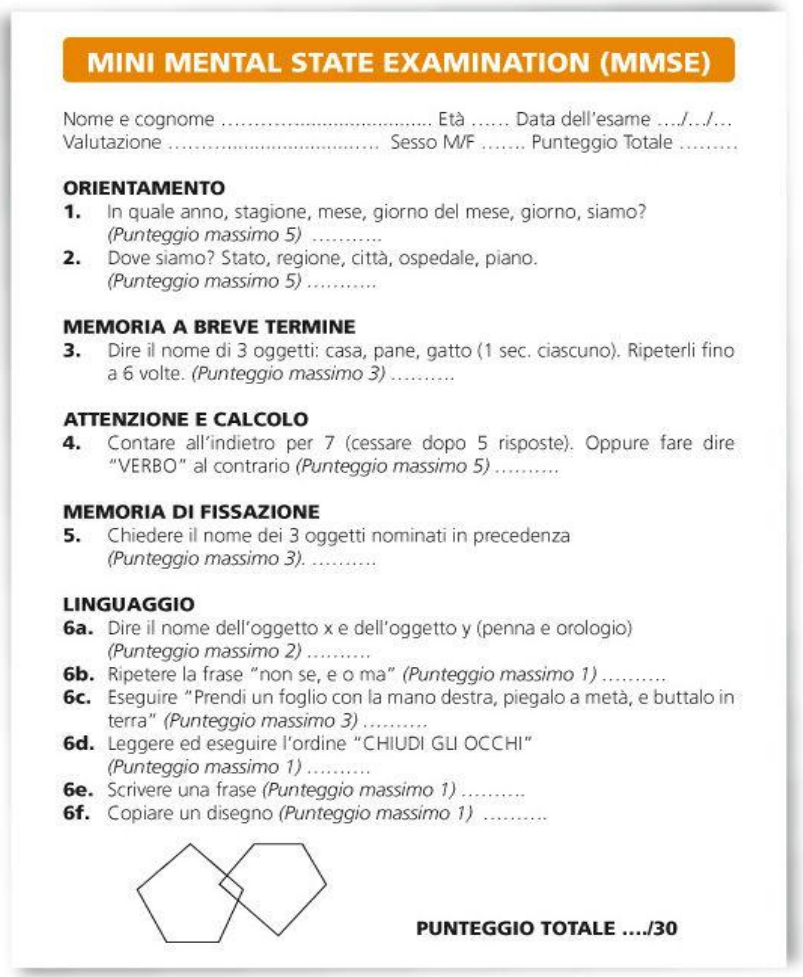


Figure 2. Graphic protocol for management of O2 therapy in CONSERVATIVE GROUP



APPENDIX 2: Mini Mental State Examination (MMSE)



MINI MENTAL STATE EXAMINATION (MMSE)

Nome e cognome ..... Età ..... Data dell'esame ..../.../...
Valutazione ..... Sesso M/F ..... Punteggio Totale .....

ORIENTAMENTO

1. In quale anno, stagione, mese, giorno del mese, giorno, siamo? *(Punteggio massimo 5)* .....

2. Dove siamo? Stato, regione, città, ospedale, piano. *(Punteggio massimo 5)* .....

MEMORIA A BREVE TERMINE

3. Dire il nome di 3 oggetti: casa, pane, gatto (1 sec. ciascuno). Ripeterli fino a 6 volte. *(Punteggio massimo 3)* .....

ATTENZIONE E CALCOLO

4. Contare all'indietro per 7 (cessare dopo 5 risposte). Oppure fare dire "VERBO" al contrario *(Punteggio massimo 5)* .....

MEMORIA DI FISSAZIONE

5. Chiedere il nome dei 3 oggetti nominati in precedenza *(Punteggio massimo 3).* .....

LINGUAGGIO

6a. Dire il nome dell'oggetto x e dell'oggetto y (penna e orologio) *(Punteggio massimo 2)* .....

6b. Ripetere la frase "non se, e o ma" *(Punteggio massimo 1)* .....

6c. Eseguire "Prendi un foglio con la mano destra, piegalo a metà, e buttalo in terra" *(Punteggio massimo 3)* .....

6d. Leggere ed eseguire l'ordine "CHIUDI GLI OCCHI" *(Punteggio massimo 1)* .....

6e. Scrivere una frase *(Punteggio massimo 1)* .....

6f. Copiare un disegno *(Punteggio massimo 1)* .....

PUNTEGGIO TOTALE ..../30

APPENDIX 3: Medical Research Council (MRC) Scale

MRC SIMPLIFIED scale

In this evaluation is considered the strength of 12 muscolar groups, with a score from 0 to 5 assigned to each one, and a sumscore of 60. We considered as ICUAW (Intensive Care Unit Acquired Weakness) a sumscore ≤ 48, indicating that average strength is limited to movement against gravity and partial resistance.

Grade 0	No contraction visible or palpable

Grade 1	Flicker of contraction visible or palpable, although no limb movement
Grade 2	Movement with gravity eliminated over almost full range of motion
Grade 3	Movement against gravity over almost full range of motion
Grade 4	Movement against moderate resistance over full range of motion

Chart we will use to calculate the score:

	LEFT	RIGHT	Notevaluable	
Abduction of the arm				
Flexion of the forearm				
Extension of the wrist				
Flexion of the leg				
Extension of the knee				
Dorsal flexion of the foot				
Subtotal				
TOTAL SCORE				_____

If not evaluable the reason must be chosen between the following:

- GCS ≤ 10
- HemiplegiaAfterStroke
- Paraplegia SpinalCordInjury
- ProhibitedOrthopaedicsReason
- PeripheralNerveInjury
- Amputation
- Others

APPENDIX 4: Participant timeline

Timepoint	T0	From D1 to ICU discharge	ICU-Discharge	D90
Eligibility Screen	X			
Informed consent	X or As soon as feasible			
Allocation	X			
Treatment: Conventional or Conservative O2 therapy		X		
Baseline characteristics	X			
Physiological and Processes of care outcomes		X		
Primary outcome			X	
Secondary outcomes		X	X	X

APPENDIX 5: IMMUNE-O2 Sub-study

OBJECTIVE

The objectives of the IMMUNE-O2 sub-study are to evaluate the relationship between different levels of arterial PO2 and the immune immune-system in critically ill patients and to verify the hypothesis that strict maintenance of normoxia compared to the application of conventional more liberal strategies of oxygen administration reduces the impairment of immune-system in critically ill patients.

STUDY SETTING

The study will be conducted in Modena ICU, as coordinating centre, collaborating with Modena University Immunology Laboratory (LAB IMMUNO).

SAMPLE SIZE

A sample of approximately 100 patients, 50 assigned to a conventionalgroup and 50 to the treatment group,has 80% power to detect a difference of 0.57 standard deviations at 5% type I error rate.

METHODS

Markers of oxidative stress and of immune system function will be measured at the baseline and after 72 hours and 7 day from ICU admission. A maximum of thirty ml of blood will be collected in ethylenediaminetetraacetic acid (EDTA) for each data collection. Samples will be kept cold (on ice or at 4°C) at all stages and processed as rapidly as possible to avoid artifactual oxidation of the biomarkers.

<u>Biomarkers assessment:</u>

Oxidative stress will be assessed by measuring plasmatic total GSH and GSH/GSSG ratio , plasma levels of Nitric Oxide, high Reactive Oxygen Species (hROS)plasma content of mitochondrial DNA , plasma levels of LPS and cytokines (such as TNF-α, IL-1, IL-6, IL-8, IL-10), lymphocyte and monocytes immunophenotyping, quantification of the activation of inflammasome in peripheral blood mononuclear cells (PBMC) and monocytes, Immunoglobulins sub-classes plasma concentration.