Statistical Analysis Plan

(SAP)

HIGH-FLOW vs. CONVENTIONAL OXYGEN THERAPY IN PATIENTS WITH ACUTE RESPIRATORY FAILURE DUE TO SARS-CoV-2: The HiFLo-COVID RANDOMIZED CLINICAL TRIAL

The HiFLo-COVID collaborative group

This document includes:

- Statistical Analysis Plan (SAP) VERSION 2.0 (corresponding to the Protocol Version 3.0). Date: 02nd January 2.021.

  Approved by: Ethical and Biomedical Research Committee (EBRC) from the Fundación Valle del Lili, Cali, Colombia

- Summary of Changes to the SAP

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FUNDACION VALLE DEL LILI – UNIVERSIDAD ICESI

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1 INTRODUCTION

Arterial hypoxemia is the leading feature of severe cases of Covid-19. In general, management of hypoxemic respiratory failure relies on oxygen supplementation aiming to improve oxygenation and to support respiratory effort.
High-flow oxygen therapy through a nasal cannula is a technique whereby a mixture of heated and humidified oxygen and air are delivered to the nose at high flow rates. Data suggest that high flow oxygen through a nasal cannula might decrease the need for tracheal intubation and might reduce the risk of escalation of oxygen therapy in patients with acute respiratory hypoxemic failure, with no apparent impact on mortality rates. Nevertheless, there is no information about if such data are applicable to Covid-19. Accordingly, we conducted a trial to assess the impact of high-flow oxygen therapy through a nasal cannula vs. conventional oxygen therapy on the need for intubation and the time to clinical recovery in patients with severe Covid-19.

2 STUDY DESIGN / INTERVENTIONS

Open (non-blind), randomized, controlled, Phase IIb clinical trial including adults with acute respiratory failure and partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio < 200 due to suspected or confirmed infection by SARS-CoV-2. Patients will be randomly allocated to high-flow oxygen through a nasal cannula vs. conventional oxygen therapy within the 6 hours of fulfilling acute respiratory failure and prespecified hypoxemia threshold.

3 STUDY MAIN OBJECTIVE

To evaluate the impact of using high-flow oxygen through a nasal cannula vs. conventional oxygen therapy on the need for intubation/support with invasive mechanical ventilation and clinical status as assessed by a 7-category ordinal scale in patients with acute hypoxemic respiratory failure secondary to severe Covid-19.

4 OUTCOMES

4. 1 Co-Primary Outcomes

- Need for intubation / invasive mechanical ventilation support (time frame: 28 days).
- Time to clinical recovery as assessed by a 7-category ordinal scale (time frame: 28 days).

4.2. Secondary - tertiary outcomes and Prespecified Subgroup Analysis

Secondary Outcomes

Efficiency

- Early requirement of intubation / invasive mechanical ventilation support (time frame: 7 days – 14 days).
- Mechanical ventilation-free days (time frame: 28 days).
- Renal replacement therapy-free days (time frame: 28 days)
- Length of ICU stay (time frame: 28 days)
- Length of hospital stay (time frame: 28 days)
- Hospital mortality – all causes (time frame: 14 and 28 days)

Safety

- Occurrence / proportion of patients with serious adverse events (time frame: 28 days)
- Occurrence / proportion of bacterial - fungal infections (time frame: 28 days).

Tertiary Outcomes

- Time-course of oxygen flow and PaO_2/FiO_2 ratio among the groups under study (time frame: 7 days)

- Time elapsed from randomization to intubation / invasive mechanical ventilation support in patients failing to high-flow oxygen therapy and conventional oxygen therapy (time frame: 28 days).
- Clinical condition at day-28 (time frame: 28 days).
- Evolvement of multiorgan dysfunction as assessed by SOFA score (time frame: 14 days)
- Evolvement of extra-pulmonary organ dysfunction as assessed by extra-pulmonary SOFA score (time frame: 14 days).
- HACOR and ROX scores at 2- and 4-hours post-randomization and their relation with requirement of intubation (time frame: 28 days)
- Differences in time-course of IL-6 and IL-8 between study groups (time frame: 7 days)
- Differences in time-course of ferritin, LDH, leukocyte count, neutrophil to lymphocyte count relationship, platelet count, and D-dimer among the groups under study (time frame: 7 days)
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Subgroup Analysis

- Time to intubation / invasive mechanical ventilation and clinical recovery in subgroups with baseline PaO_2/FiO_2 > and < 100 mmHg (time frame: 28 days)
- Time to intubation / invasive mechanical ventilation and clinical recovery in subgroups with baseline IL-6 > and < 100 pg/mL (time frame: 28 days)
- Time to intubation / invasive mechanical ventilation and clinical recovery in subgroups aged > and < 60 years (time frame: 28 days)

5 Study Population

5.1. Inclusion criteria

- Age 18 years or older;
- Suspected or confirmed infection by SARS-CoV-2;
- Acute respiratory distress with a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO_2/FiO_2) < 200;

- Clinical signs of respiratory failure: laborious breathing, use of accessory muscles and respiratory rate greater than 25/min;
- Less than 6 hours from fulfilling the criteria of acute respiratory failure;
- Having a progression < 6 hours since fulfilling definition of moderate or severe acute respiratory failure due to suspected or confirmed SARS-CoV-2 infection.

5.2. Exclusion criteria

- < 18 years.
- Indication for immediate tracheal intubation.
- Pregnant woman / positive pregnancy test at the time of potential inclusion.
- Chronic liver disease / liver cirrhosis Child-Pugh C.
- Confirmation of active bacterial or fungal infection.
- Uncontrolled HIV/AIDS disease (defined by presence of viral load > 200 copies/mL).
- Previous history of COPD Gold C – D.
- History of COPD requiring hospitalization or ICU admission during the last year.
- History of congestive heart failure NYHA III – IV.
- History or actual left ventricular ejection fraction < 45%
- Highly suspected or confirmed cardiogenic pulmonary edema.
- Hypercapnic respiratory failure ($PaCO_2$ > 55 mmHg).
- History or high suspicion of central or peripheral demyelinating disorders at the time of potential inclusion.
- Imminence of death within the next 24 hours (according to investigator´s clinical judgment)
- Any serious medical condition or clinical laboratory test abnormality that, at the investigator's judgment, prevents safe patient participation and completion of the study.
- Participation in another clinical trial (except other related to SARS-CoV-2. These criteria will be always discussed among steering committee members

6 Sample Size Calculation

Sample size was calculated under the assumption of an intubation rate of 60%, according to the data obtained from the first 75 patients with Covid-19-related moderate and severe hypoxemic respiratory failure treated in the coordinating center. Such proportion of intubation events was in agreement with previous data from a randomized controlled trial testing high-flow oxygen through nasal cannula in a mixed population of patients with acute hypoxemic respiratory failure (1). In such a trial, the maximal value of the 95% confidence interval for intubation at day 28 in the groups subjected to standard oxygen therapy and non-invasive mechanical ventilation were 57% and 59% independently of basal oxygenation, and 64% and 68% for those with an initial PaO_2/FiO_2 ratio < 200. Consequently, estimating an intubation rate around 60% in conventionally-treated patients, we calculated that enrollment of 196 patients would be necessary to demonstrate an absolute reduction of 20% in the proportion of intubation and requirement of invasive mechanical ventilation with an 80% power and two-side alpha level of 0.05. In addition, data obtained from the initial cohort of patients with Covid-19-related severe hypoxemic respiratory failure treated in the coordinating center revealed a time to recovery of 14 (± 4.5) vs. 12 (± 4.0) days for the conventional oxygen and high-flow oxygen therapy groups respectively. Thus, it was estimated that 160 patients (80 by arm) would be necessary to demonstrate such a difference with an 80% power and two-side alpha level of 0.05. Consequently, the sample size of 196 patients was retained as the sample size target.

Nevertheless, due to the particular situation during pandemic, an important number of participants (n=18, representing the 9.2% of the total sample size) were transferred to other hospitals within 72 hours from randomization at the time in which the HiFLo-Covid protocol amendment 2.0 was constructed. After an extensive discussion with the Ethical Committee and trying to favor the possibility that results of this trial keep sufficient power and consequently, more reliable results, the number total of randomized patients is newly adjusted up to complete a total of 220 participants.

7 Type of analysis

Main analysis will be performed on an intention-to-treat basis with no exclusion after randomization except exclusions for withdrawn consent, according to the local regulations. Baseline characteristics will be displayed as the shown Table 1.

7.1. Primary Outcome Analysis

The first primary outcome is to evaluate the effect of high-flow oxygen through a nasal cannula on the requirement of intubation and invasive mechanical ventilation within 28-days from randomization. The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the cumulative incidence of intubation / invasive mechanical ventilation will be calculated with a Cox proportional hazard model with stratification by age (< or ≥ 60 years old), initial hypoxemia severity (PaO_2/FiO_2 ratio as continuos variable), and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B). The proportional hazards assumption will be tested with the Grambsch and Therneau method (2). Results will be reported as hazard ratios with 95% confidence intervals and represented in Kaplan-Meier curves.

The other primary outcome is the clinical improvement within 28 days from randomization. Time to clinical improvement is defined as the time elapsed from randomization until the first day, during the 28 days after enrollment, on which a patient attained a reduction in two or more points in the modified ordinal 7-category scale. The effect size of the allocated therapy treatment (high-flow oxygen vs. conventional oxygen therapy) on the time to recovery will be assessed by computing the hazard ratio with its 95% confidence interval (CI) as estimated from Cox proportional hazard model adjusted by age (< or ≥ 60 years old), initial hypoxemia severity (PaO_2/FiO_2 ratio as continuos variable), and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B). Such analysis will be constructed for the overall population and also stratified according to baseline 7-category ordinal scale at enrollment (i.e., scores of 4 or 5), and plotted in Kaplan-Meier curves.

Hazard ratios calculated for the outcome clinical recovery greater than 1, will indicate benefit with the high-flow oxygen therapy. Meanwhile, hazard ratios calculated for need for intubation less than 1 will indicate benefit with the use of high-flow oxygen therapy.

7.2. Secondary outcomes, terciary outcomes, and predefined subgroups analysis

Continuous variables will be described as medians (interquartile ranges) and categorical variables as proportions.

Secondary Outcomes analysis

- Proportion of patients requiring early intubation and mechanical ventilation support: defined as the proportion of patients requiring intubation for mechanical ventilatory support by days 7 and 14. Differences between groups will be compared by using a Mann Whitney test.
- Mechanical ventilation free-days within 28 days: defined as the number of days from day 2 up to 28 days after randomization in which the patients remained without mechanical ventilation support. A Proportional odds model adjusted for age (≥ or < 60 years old), PaO_2/FiO_2 ratio at the randomization, and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B) will be used to determine differences between groups. Odds ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Renal replacement therapy-free days: defined as the number of days from day 2 up to 28 days after randomization in which the patients remained without renal replacement support. A Proportional odds model adjusted for age (≥ or < 60 years old), PaO_2/FiO_2 ratio at the randomization, and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B) will be used to determine differences between groups. Odds ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Hospital length of stay: number of days in which the patient remained hospitalized. A Proportional odds model adjusted for age (≥ or < 60 years old), PaO_2/FiO_2 ratio at the randomization, and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B) will be used to determine differences between groups. Odds ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Hospital length of ICU stay: number of days in which the patient remained hospitalized in the Intensive Care Unit. A Proportional odds model adjusted for age

(≥ or < 60 years old), PaO_2/FiO_2 ratio at the randomization, and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B) will be used to determine differences between groups. Odds ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.

- Mortality at days- 14 and 28: defined as mortality within the 14 and 28 days from randomization. A Proportional odds model adjusted for age (≥ or < 60 years old), PaO_2/FiO_2 ratio at the randomization, and comorbidities (a composite of arterial hypertension, diabetes, obesity [body mass index > 30], chronic obstructive pulmonary disease, end-stage renal failure, heart failure, cirrhosis Child-Pugh A-B) will be used to determine differences between groups. Odds ratios < 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Proportion of major events: defined as the proportion of patients developing some defined major adverse event. Differences between groups will be compared by using a Mann Whitney test

Tertiary Outcomes analysis

- Time-course of oxygen flow and PaO_2/FiO_2 ratio among the groups under study. Differences between groups will be estimated by using a mixed linear regression considering patient as random effect and adjusting for baseline value of the variable.
- Time elapsed from randomization to intubation / invasive mechanical ventilation support: defined by the number of hours between randomization up to intubation (when required). Differences between groups will be compared by using a Mann Whitney test
- Clinical condition at day-28 according to 7-category ordinal scale: defined as the patient clinical condition at day-28 after randomization according to such scale. Differences between groups will be compared by using a Mann Whitney test
- Multiple organ dysfunction during the first 7 and 14-days: time-course of multiorgan dysfunction as assessed by the Sequential Organ Failure Assessment Score (SOFA) score (3). Differences between groups will be estimated by using a mixed linear regression considering patient as random effect and adjusting for baseline value of the variable.
- Extra-pulmonary organ dysfunction during the first 7 and 14-days: time-course of extra pulmonary organ dysfunction as assessed by the Sequential Organ Failure Assessment Score (SOFA) score (3), but excluding the corresponding pulmonary points. Differences between groups will be estimated by using a mixed linear

regression considering patient as random effect and adjusting for baseline value of the variable.

- HACOR and ROX scores at 2- and 4-hours post-randomization and their relation with requirement of intubation (time frame: 28 days)
- Time-course of IL-6 and IL-8 between study groups (time frame: 14 days): defined as the evolvement in time of IL-6 and IL-8 levels within the first 14 days from randomization. Differences between groups will be estimated by using a mixed linear regression considering patient as random effect and adjusting for baseline value of each variable.
- Time-course of ferritin, LDH, leukocyte count, neutrophil/lymphocyte count relationship, platelet count, and D-dimer among the groups under study (time frame: 14 days): defined as the evolvement in time of such markers within the first 14 days from randomization. Differences between groups will be estimated by using a mixed linear regression considering patient as random effect and adjusting for baseline value of each variable.

Predefined subgroups analysis

- Requirement of intubation / invasive mechanical ventilation in subgroups with baseline PaO_2/FiO_2 > and < 100 mmHg (time frame: 28 days). Differences between groups will be compared by using a Mann Whitney test
- Time to intubation / invasive mechanical ventilation in subgroups with baseline PaO_2/FiO_2 > and < 100 mmHg (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the cumulative incidence of intubation / invasive mechanical ventilation will be calculated with a Cox proportional hazard model according to baseline PaO_2/FiO_2 > and < 100 mmHg. Hazard ratios < 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa
- Requirement for intubation / invasive mechanical ventilation in subgroups with baseline IL-6 > and < 100 pg/mL (time frame: 28 days). Differences between groups will be compared by using a Mann Whitney test
- Time to intubation / invasive mechanical ventilation in subgroups with baseline IL-6 > and < 100 pg/mL (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the cumulative incidence of intubation / invasive mechanical ventilation will be calculated with a Cox proportional hazard model according to baseline IL-6 > and < 100 pg/mL. Hazard ratios < 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa

- Requirement intubation / invasive mechanical ventilation in subgroups aged > and < 60 years (time frame: 28 days). Differences between groups will be compared by using a Mann Whitney test
- Time to intubation / invasive mechanical ventilation in subgroups aged > and < 60 years (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the cumulative incidence of intubation / invasive mechanical ventilation will be calculated with a Cox proportional hazard model according to aged > and < 60 years. Hazard ratios < 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Time to clinical recovery in subgroups with baseline PaO_2/FiO_2 > and < 100 mmHg (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the time to clinical recovery will be calculated with a Cox proportional hazard model according to baseline PaO_2/FiO_2 > and < 100 mmHg. Hazard ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Time to clinical recovery in subgroups with baseline IL-6 > and < 100 pg/mL (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the time to clinical recovery will be calculated with a Cox proportional hazard model according to baseline IL-6 > and < 100 pg/mL. Hazard ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.
- Time to clinical recovery in subgroups aged > and < 60 years (time frame: 28 days). The effect of the allocated treatment (high-flow oxygen vs. conventional oxygen therapy) on the time to clinical recovery will be calculated with a Cox proportional hazard model according to age > and < 60 years. Hazard ratios > 1 will indicate benefit with the use of High-Flow Oxygen Therapy and vice versa.

7.3. Additional analysis (Post-Hoc Analysis)

Sensitivity analysis

We will consider to perform some sensitivity analyses:

- Including just patients with confirmed SARS-CoV-2 infection by means of RT-PCR test.
- Including patients receiving the randomly allocated treatment (per-protocol analysis)

- Imputation analysis including patients excluded because early transfers to other hospitals because administrative reasons

7.4. Handling missing data

For Primary outcomes

The primary outcome is expected to be available in all patients. If death occurs during follow-up, data will be censored until last observation. In the case of lost of follow-up, the last report on the 7-category scale will be considered as the outcome.

For Covariates

It is expected to collect information about all covariates for both primary and secondary outcomes. Nevertheless, in the case of missing data of variables needed to analyze effect modification, and this may have an effect the primary outcomes, then simple imputation will be performed using the median of the treatment group to which the observation belongs based on the information collected with complete data.

Data will not be imputed for secondary outcomes.

8 INTERIM ANALYSIS

A first and only interim analysis was conducted when the 28-day follow-up had been completed for the first 100 randomized patients. Database will be prepared and sent to the members of the Data Safety Monitoring Board (DSMB). An independent statistician will perform the analysis and will discuss it with the DSMB, who later will communicate results to the Steering Committee. Haybittle–Peto stopping boundaries (4) are a priori proposed as rules to continue or stop the trial. Thus, a P-value threshold of less than 0.001 will be considered to interrupt the trial for safety and a P-value threshold of less than 0.0001 to interrupt the trial for efficacy.

9 TABLES

9.1 Table 1. Patients characteristics at randomization

Characteristic	High-Flow Oxygen Therapy (n=xxx)	Conventional Oxygen Therapy (n=xxx)
Demographics		
Age, median (IQR) – years	xx (xx – xx)	xx (xx – xx)
Sex - No. (%)		
Men	xx (xx)	xx (xx)
Women	xx (xx)	xx (xx)
Body Mass Index, median (IQR) †	xx (xx – xx)	xx (xx – xx)
APACHE II	xx (xx – xx)	xx (xx – xx)
Testing for COVID-19, No. (%)		
Positive on RT-PCR	xx (xx)	xx (xx)
Negative on RT-PCR or unavailable	xx (xx)	xx (xx)
Comorbidities, No. (%)		
Hypertension	xx (xx)	xx (xx)
Diabetes	xx (xx)	xx (xx)
Chronic Obstructive Pulmonary Disease	xx (xx)	xx (xx)
Heart Failure	xx (xx)	xx (xx)
Chronic Renal Disease	xx (xx)	xx (xx)
Cancer	xx (xx)	xx (xx)
Cirrhosis Child A-B	xx (xx)	xx (xx)
Previous medications, No. (%)		
Steroids	xx (xx)	xx (xx)
ACE inhibitor	xx (xx)	xx (xx)
Angiotensin II Receptor Antagonist	xx (xx)	xx (xx)
Statins	xx (xx)	xx (xx)
Characteristics at randomization		
Median time from symtom onset to randomization (IQR) - days	xx (xx – xx)	xx (xx – xx)
Median time from admission to	xx (xx – xx)	xx (xx – xx)

randomization (IQR) - days		
SOFA score at randomization	xx (xx – xx)	xx (xx – xx)
Respiratory status just before randomization		
Respiratory Rate, median (IQR), /min	xx (xx – xx)	xx (xx – xx)
PaO_2/FiO_2 ratio, median (IQR)	xx (xx – xx)	xx (xx – xx)
Received standard oxygen therapy before randomization		
Oxygen flow, median (IQR), L/min	xx (xx – xx)	xx (xx – xx)
PaO_2 with standard oxygen, median (IQR), mmHg	xx (xx – xx)	xx (xx – xx)
$PaCO_2$ with standard oxygen, median (IQR), mmHg	xx (xx – xx)	xx (xx – xx)
Score on Seven-Level Ordinal Scale – No. (%) ††		
4. Hospitalized and receiving supplemental oxygen	xx (xx)	xx (xx)
5. Hospitalized in ICU and receiving oxygen supplementation	xx (xx)	xx (xx)

453 **9.2 Table 1S. Other patient characteristics at randomization**

Characteristic	**High-Flow Oxygen Therapy (n=xxx)**	**Conventional Oxygen Therapy (n=xxx)**	**P**
Symptoms			
Fever - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Odynophagia - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Cough - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Vomiting / diarrhea - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Asthenia / adynamia - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Anosmia / dysgeusia - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Odynophagia - no. (%)	XX (XX.X)	XX (XX.X)	.XX
Vital signs at admission, median (IQR)			.XX
Systolic arterial pressure, mmHg	XX (XX – XX)	XX (XX – XX)	.XX
Diastolic arterial pressure, mmHg	XX (XX – XX)	XX (XX – XX)	.XX
Mean arterial pressure, mmHg	XX (XX – XX)	XX (XX – XX)	.XX
Heart rate, beats/min	XX (XX – XX)	XX (XX – XX)	.XX
Respiratory rate, breaths/min	XX (XX – XX)	XX (XX – XX)	.XX
Laboratory, median (IQR)			.XX
pH	X.XX (X.XX – X.XX)	X.XX (X.XX – X.XX)	.XX
$PaCO_2$, mmHg	XX (XX – XX	XX (XX – XX	.XX
PaO_2, mmHg	XX (XX – XX	XX (XX – XX	.XX
HCO_3^-, mmol/L	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	.XX

Base excess, mmol/L	X.XX (X.XX – X.XX)	X.XX (X.XX – X.XX)	.XX
PaO_2/FiO_2 ratio	XX (XX – XX	XX (XX – XX	.XX
Lactate, mmol/L	X.XX (X.XX – X.XX)	X.XX (X.XX – X.XX)	.XX
Hemoglobin, gr/dL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Hematocrit, %	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Platelets, /mm^3	XXX,XXX (XXX,XXX – XXX,XXX)	XXX,XX (XXX,XXX – XXX,XXX)	.XX
Protrombin Time – PT, sec	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Activated Partial Thromboplastin Time – aPTT, sec	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Fibrinogen, mg/dL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Leucocytes, /mm^3	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Neutrophils, /mm^3	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Lymphocytes, /mm^3	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Macrophages, /mm^3	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Neutrophil: Lymphocyte Ratio	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Bilirubin, mg/dL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Aspartate Amino Transferase – AST, IU/L	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Alanine Amino Transferase – ALT, IU/L	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
C-Reactive Protein – CRP, mg/L	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Lactate dehydrogenase – LDH, U/L	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Ferritin, ng/mL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
D-dimer, µg/mL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
IL-6	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
IL-8	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Creatinine, mg/dL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Blood Nitrogen Urea – BUN, mg/dL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX
Troponin, ng/mL	XX.XX (XX.XX – XX.XX)	XX.XX (XX.XX – XX.XX)	.XX

9.3. Table 2. Primary and Secondary Outcomes

	Overall		Ordinal Score at Baseline			
			4		5	
	High-Flow Oxygen Therapy (n=XXX)	**Conventional Oxygen Therapy (n=XXX)**	**High-Flow Oxygen Therapy (n=XX)**	**Conventional Oxygen Therapy (n=XX)**	**High-Flow Oxygen Therapy (n=XX)**	**Conventional Oxygen Therapy (n=XX)**
Primary Outcomes						
Intubation over first 28 days						
No. of intubations by day 28	XX	XX	XX	XX	XX	XX
Hazard ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Clinical Recovery §						
No. of recoveries	XX	XX	XX	XX	XX	XX
Median time to recovery (95%CI) - days	XX (XX –XX)	XX (XX –XX)	XX (XX –XX)	XX (XX –XX)	XX (XX –XX)	XX (XX –XX)
Hazard ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	

Secondary Outcomes						
Ordinal score at day 28 – no. (%) ∫∫						
1	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
2	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
3	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
4	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
5	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
6	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
7	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Odds ratio (95%CI)	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Mortality						
At day 14						
No. of patients (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Hazard Ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
At day 28						

No. of patients (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Hazard Ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Intubation						
At day 7						
No. of patients (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Hazard Ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Intubation						
At day 14						
No. of patients (%)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Hazard Ratio (95%CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Time elapsed from randomization to Intubation, hours	X.XX (X.XX–X.XX)	X.XX (X.XX–X.XX)	X.XX (X.XX–X.XX)	X.XX (X.XX–X.XX)	X.XX (X.XX–X.XX)	X.XX (X.XX–X.XX)
Adjusted odds ratio (95% CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Ventilation-free days at day-28, days (IQR)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)

Adjusted odds ratio (95% CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Renal replacement therapy-free days, , days (IQR)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)
Adjusted odds ratio (95% CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Length of Stay – Intensive Care Unit, , days (IQR)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)
Adjusted odds ratio (95% CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	
Length of Stay – Hospital, days (IQR)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)	XX (XX – XX)
Adjusted odds ratio (95% CI) for data through day 28	X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]		X.XX (X.XX–X.XX) [p=X.XX]	

9.4 Table 3. Time from randomization to intubation / invasive mechanical ventilation support / additional respiratory support maneuvers

	High-Flow Oxygen Therapy (n=XXX)	**Conventional Oxygen Therapy (n=XXX)**	**P value**
No. of intubations by day 28, (%)	XX (XX.X)	XX (XX.X)	0.026 †
Time elapsed from randomization to Intubation, hours	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	0.69
Time elapsed with FiO_2 > 0.70 from randomization to Intubation, hours	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	< 0.001
Awake prone position, n (%)	XX (XX.X)	XX (XX.X)	0.12
Time in awake prone position, hours (interquartile range)	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	0.35
Prone position during mechanical ventilation, n (%)	XX (XX.X)	XX (XX.X)	0.08
Time in prone position during mechanical ventilation, hours (interquartile range)	XX.X (XX.X – XX.X)	XX.X (XX.X – XX.X)	0.90
Neuromuscular paralysis, n (% from total populatin)	XX (XX.X)	XX (XX.X)	0.04

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Statistical Analysis Plan

(SAP)

HIGH-FLOW vs. CONVENTIONAL OXYGEN THERAPY IN PATIENTS WITH ACUTE RESPIRATORY FAILURE DUE TO SARS-CoV-2: The HiFLo-COVID RANDOMIZED CLINICAL TRIAL

The HiFLo-COVID collaborative group

SUMMARY OF CHANGES

Versions and dates

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Statistical Analysis Plan version 1.0

Version date: 16th July, 2020

Refers to protocol Version 1.0

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Statistical Analysis Plan version 2.0

Version date: 02nd January, 2021

Refers to protocol Version 3.0

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Summary of changes from SAP version 1.0 to version 2.0

1. Sample Size

Changes: Increasing of sample size

In the Protocol amendment 1.0, sample size had been increased from 196 to 200 patients (i.e., 2 more by arm) aiming to compensate a potential 2% of losses. Nevertheless, due to the particular heatlh system conditions during pandemic, an important number of participants (n=18, representing the 9.2% of the total sample size) had been transferred to other hospitals within 72 hours from randomization at the time in which Protocol amendment 3.0 was being constructed. After an extensive discussion with the Ethical Committee and trying to favor the possibility that results of HiFLo-Covid trial keep sufficient power and consequently, more reliable results, the

number total of randomized patients was newly adjusted up to complete a total of 220 participants.

2. Objectives and Outcomes

Changes: Re-arrangement of Objectives and Outcomes list

Primary and secondary outcomes were preserved as initially conceived in the Protocol version 1.0 and Statistical Plan Analysis version 1.0. Nevertheless, for a better understanding, secondary and tertiary outcomes, and predefined subgroups were listed separately

Time-course for labs was shortened to 7 days because median length of stay < 14 days observed during interim analysis.