TITLE PAGE

Clinical Study Protocol

Study Protocol Number:	CCAM 20-01
Protocol Title:	Prophylactic Corticosteroids to Prevent Covid-19 Cytokine Storm
Sponsor Name/ Address:	Hospital Español Auxilio Mutuo Auxilio Mutuo Ave**.** Ponce de León #715, San Juan, Puerto Rico 00918
Phase:	2
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INVESTIGATOR SIGNATURE PAGE

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, Institutional Review Board (IRB)/ Ethics Committee (EC) procedures, the Declaration of Helsinki, International Conference on Harmonisation (ICH), Good Clinical Practices (GCP) guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

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1.0 Study synopsis

This is a Phase II pilot exploratory study designed to investigate if prophylactic treatment with short term steroids administered to high risk Covid-19 patients might prevent cytokine storm and progression to respiratory failure. It is also designed to determine if it is feasible to identify a subset of cases with low risk for complications that can be observed as outpatients without treatment. High risk is defined based on serologic markers of inflammation that include abnormalities of IL-6, ferritin, D-dimer, LDH, as well as lymphopenia and impaired O_2 saturation prior to or on the 7^{th} -10^{th} day of first symptom of Covid-19.

1.1 This is a non-randomized study which will be carried out at Auxilio Mutuo and possibly at other institutions to be recruited.

2.0 Schedule of Study Assessments

2.1 At, or prior to, entry into study:

- 2.1.1 History and Physical exam
- 2.1.2 Labs: CBC, CMP, LDH, arterial blood gases, serum IL-6, serum ferritin, CRP, D-dimers, troponin
- 2.1.3 Radiologic studies: CT chest without IV contrast preferably prior to treatment with methylprednisolone.
- 2.1.4 Other studies:
  - 2.1.4.1 Either PCR for molecular detection of SARS-Cov-2 virus and serologic rapid test (IgG, IgM), or both (see figure 2). Cases who are IgG positive but IgM negative, will not be considered eligible unless they are positive by molecular PCR test or they are acutely ill with symptoms typical of Covid-19 (see section 6.2 for typical symptoms) .
  - 2.1.4.2 O_2 saturation by pulse oximetry
  - 2.1.4.3 Influenza rapid test if considered necessary.

2.2 During study (only applies to those eligible for treatment with methylprednisolone):

- 2.2.1 For patients admitted to hospital: Vital signs q 8 hours (not q 4 hrs) to reduce chance of nursing staff contamination
- 2.2.2 Day 7-10 from first symptom of illness: CBC, CMP, LDH, serum IL-6, serum ferritin, CRP, d-dimers
- 2.2.3 Day 14 from registration on trial:

2.2.3.1 repeat CBC, CMP, LDH, serum IL-6, serum ferritin, CRP, d-dimers
2.2.3.2 repeat PCR for molecular detection of SARS-Cov-2 virus

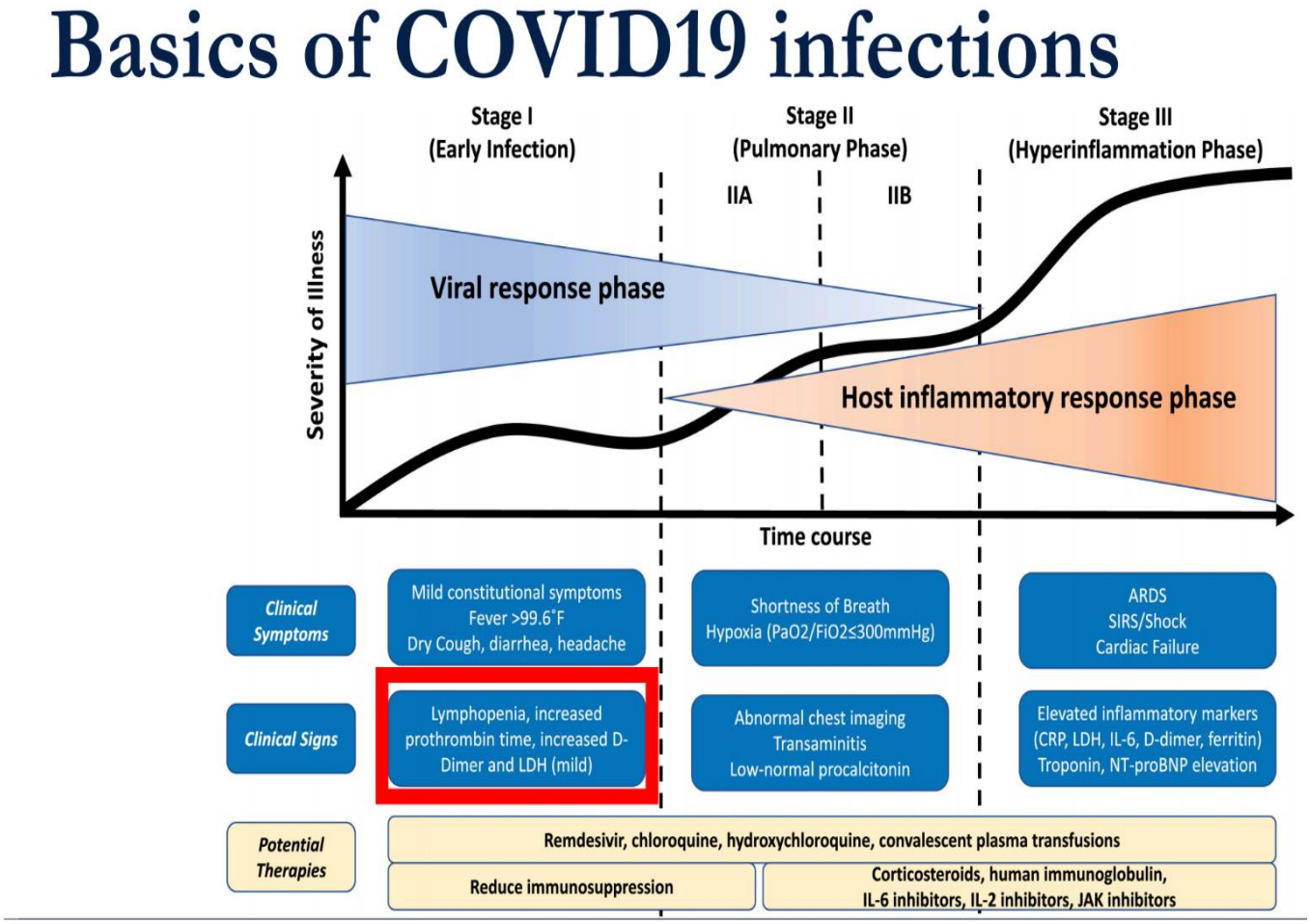
2.2.4 Day 28 from registration on trial:
2.2.4.1 repeat CBC, CMP, LDH, serum IL-6, serum ferritin, CRP, d-dimers
2.2.4.2 repeat PCR for molecular detection of SARS-Cov-2 virus
2.2.4.3 CT chest without IV contrast

3.0 Background and Rationale

The management of Covid-19 with mild manifestations has usually been conservative. Traditionally, treatment has consisted of observation at home provided there is no shortness of breath or evidence of pneumonia. Front line therapy with Hydroxychloroquine (Plaquenil) combined with Azithromycin has been used with preliminary positive results[1]. Plasma therapy has been successfully used in a small number of cases[2]. Vitamin C has also been utilized, although no definitive results are available[3].

Covid-19 is a triphasic disorder typified by a first infectious phase that lasts from the first onset of symptoms until 7-10 days later when it is followed by a second phase considered as the inflammatory or pulmonary phase with appearance of lung infiltrates which is later followed by a hyperinflammation phase characterized by Acute Respiratory Distress Syndrome (ARDS), shock and cardiac failure. For unclear reasons, patients do not always proceed to develop the second or third phase and are spontaneously cured after the first phase. Figure 1

Figure 1

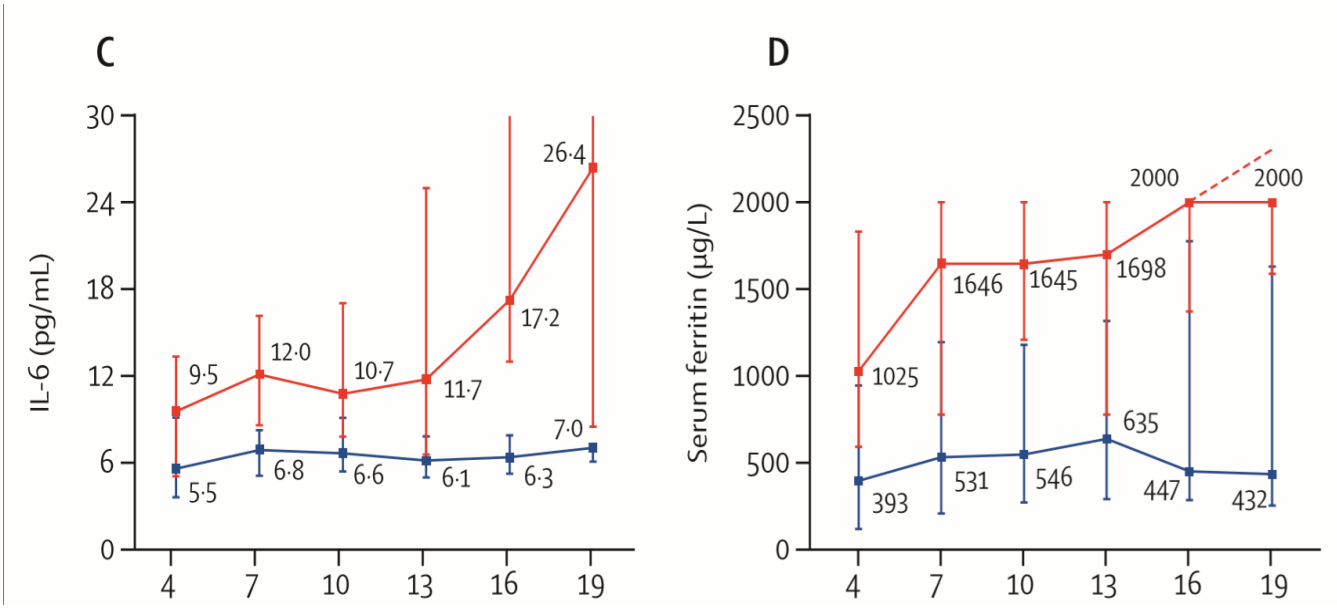


5 Siddiqi & Mehra, *J Heart Lung Transpl* (2020). Epub ahead of print: https://doi.org/10.1016/j.healun.2020.03.012

This second phase is heralded by an elevation of certain serologic markers of inflammation such as IL-6, CRP, LDH and ferritin as well as D-dimers[4, 5]. See figure 2

It is not clear why some patients enter this second phase of the disease while others do not, but what is clear is that the second or inflammatory phase is caused by pulmonary macrophage activation with subsequent release of the inflammatory mediators. These molecules will damage the capillary-alveolar membrane eventually causing respiratory insufficiency. These are the patients that frequently will require ventilatory support and eventually most will die. This second and third phase are in great part due to activation of the patient's immune system while the first phase is mostly mediated by the viral infection itself.

Figure 2 *



*Red line represents patients who died while blue line represents survivors. The x axis represents number of days after onset of illness.

Usually anti-inflammatory therapy with an anti-IL-6 antibody, Tocilizumab, is reserved for patients who are evolving poorly, especially patients with severe pneumonia with elevated IL-6 serum levels [6]. However, once these patients have pneumonia, anti-inflammatory treatment is usually not very successful. Nevertheless, the use of methylprednisolone has been associated with substantial reduction of risk of death even if administered late, when patients already have ARDS[7]. However, if given late, the probability of dying is 52%[7].

Dr. Angel Atienza, from Hospital Peset, in Valencia, Spain has developed an approach designed to anticipate the second, or so-called inflammatory phase. He has proposed that when the pulmonary infiltrates appear, this is no longer an infectious process, but it is purely an inflammatory or immune issue which causes anatomical injury to the capillary alveolar membrane resulting in respiratory distress. He uses corticosteroid therapy starting on the sixth day of the onset of symptoms, given for 5-7 days, with the goal of preventing the inflammatory phase. Steroids are used specifically to treat those cases who on day 6 are febrile and show elevation of serologic inflammatory markers without advanced radiologic pulmonary manifestations. His initial results reportedly are excellent and admissions to the intensive care unit decreased, with shortened hospital stays and radiological and favorable clinical evolution that he has described as “spectacular” (personal communication)

In Spain and Italy this approach is gaining acceptance but at present time it is not considered standard of care. The use of steroids has been criticized because there is no evidence from randomized clinical trials to support its use for COVID-19. In addition, the anti-inflammatory medications, may delay the elimination of virus and increase the risk of secondary infection.

However, there is no evidence that, as proposed by Dr. Atienza, a short course of steroids given shortly before the second phase of the disease to patients at high risk of entering the inflammatory phase, has had any unfavorable impact on the disease. Thus, the concern against its use is totally based on theoretical grounds. The few data available against the use of steroids come from trials in which they have used them either too late or too early[8].

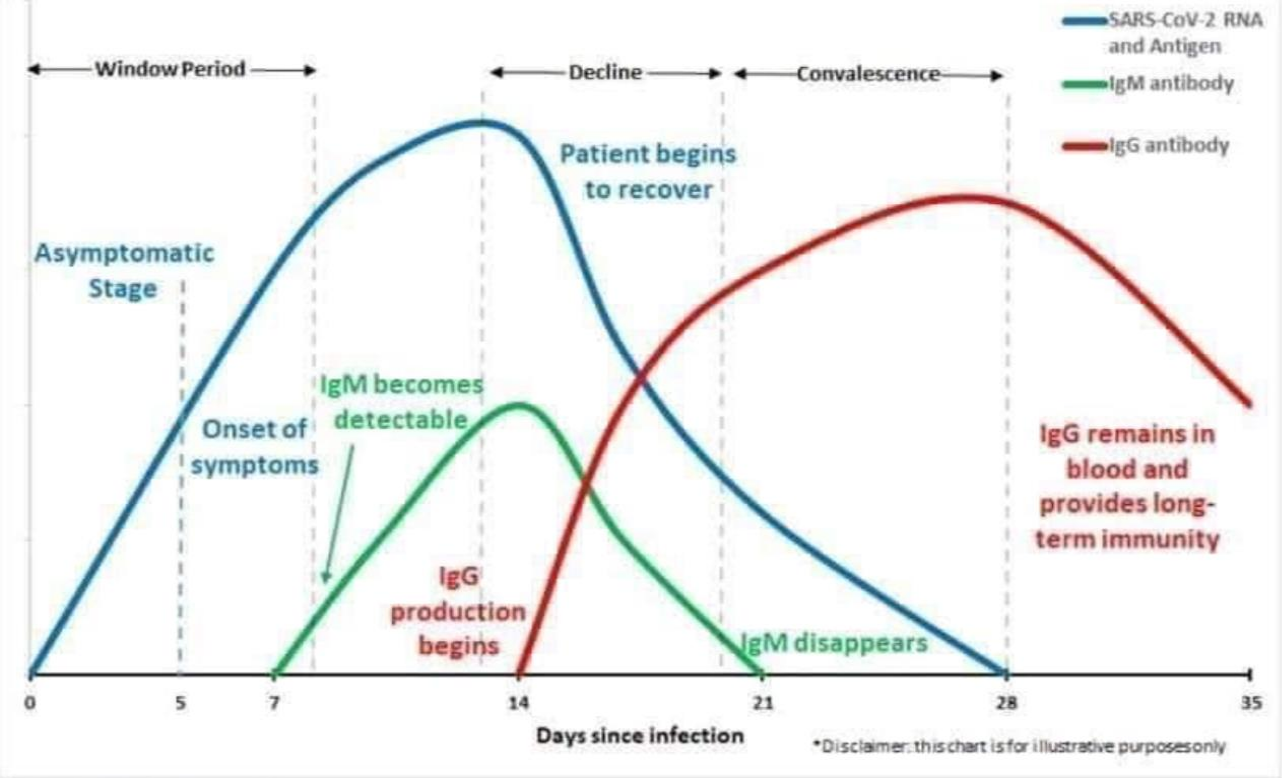
An elevated interleukin-6 (IL-6) has been strongly associated with the need for mechanical ventilation (p=.00005). In addition, the maximal IL-6 level (cutoff 80 pg./ml) for each patient during disease predicted respiratory failure with high accuracy (p=.000005, AUC=0.98). The risk of respiratory failure for patients with IL-6 levels of ≥ 80 pg./ml was 22 times higher compared with patients who have lower IL-6 levels[9].

In this protocol, we propose to identify patients with Covid-19 who are at very high risk of developing cytokine storm and consequently respiratory failure. The goal is to treat them early with corticosteroids in order to determine if we can avoid the cytokine storm.

Diagnosis of Covid-19 will be established by means of either the PCR molecular test or with the rapid serologic test or both (see figure 3 below). Cases who are IgG positive but IgM negative, will not be considered eligible unless they are positive by molecular PCR test.

In addition, we propose to identify a subset of patients with low risk of complications by analyzing their markers of inflammation as well as other prognostic variables. Due to the large number of cases currently admitted to our hospital, the situation now has reached a critical point. If we could generate data that can provide guidance as to which patients can be safely managed on an outpatient basis and which ones could be discharged with little risk of complications, it could help relieve this burden.

Figure 3



Test results			Clinical Significance
PCR	IgM	IgG	
+	–	–	Patient may be in the window period of infection.
+	+	–	Patient may be in the early stage of infection.
+	+	+	Patient is in the active phase of infection.
+	–	+	Patient may be in the late or recurrent stage of infection.
–	+	–	Patient may be in the early stage of infection. PCR result may be false-negative.
–	–	+	Patient may have had a past infection, and has recovered.
–	+	+	Patient may be in the recovery stage of an infection, or the PCR result may be false-negative.

4.0 Study Objectives and End Points

Primary Objective:

1- To decrease the rate of progression to hypoxemic respiratory failure in high risk patients with Covid-19, treated with prophylactic steroids during the first phase of the disease.

   a. Hypoxemic respiratory failure is defined as:

      1. O_2 saturation of < 91% or $p0_2$ <60 on room air

2- To evaluate the rate of complications of patients observed without treatment. Complications include progression to respiratory failure or any other complication leading to hospitalization.

Secondary Objectives:

1. To determine % survival at 28 days from registration on this clinical trial
2. To determine if there is any improvement in inflammatory markers following treatment with corticosteroids.
3. To define the length of hospitalization
4. To determine the frequency of admissions to ICU.
5. To determine the percentage of patients who require ventilatory support.
6. To determine the rate of complications and progression to respiratory failure in patients with favorable features (i.e., those eligible for observation and not for therapy with methylprednisolone.)
   a. To validate these results in an independent cohort of cases diagnosed as Covid-19 from our general hospital population.
7. We will evaluate the proportion of patients with clinical improvement, as defined by live discharge from the hospital, a decrease of at least 2 points from baseline on a modified ordinal scale (as recommended by the WHO R&D Blueprint Group), or both. [10]

   7.1 The six-point scale consists of the following categories:
   (1) not hospitalized;

(2) hospitalized, not requiring supplemental oxygen;

(3) hospitalized, requiring supplemental oxygen;

(4) hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both;

(5) hospitalized, requiring invasive mechanical ventilation, ECMO, or both; and

(6) death.

5.0 Investigational Plan

5.1 Overall Design: This is a pilot phase II exploratory study. It is a non-randomized study which will be carried out at Auxilio Mutuo Hospital and possibly at other institutions to be recruited.

5.2 We plan to enter a total of 100 - 200 patients with the expectation that at least

40-60 will be eligible for therapy with methylprednisolone in order to determine whether the risk of progressing to respiratory failure can be reduced by administering corticosteroids.

5.3 We assume that >50% of patients who meet the criteria for entry into this trial will develop respiratory insufficiency if left untreated.

5.4 If $\leq$ 50% of patients with high risk develop respiratory failure we will consider the treatment as successful.

6.0 Inclusion Criteria

6.1 **All patients diagnosed with Covid-19 will be registered on the study** regardless of their severity, but only those who meet high risk criteria will be treated with steroids. Those who do not meet the criteria will only be registered with no need to collect further data except baseline labs and CT chest. They should sign the consent form and the data manager can collect the rest of the information.

6.2 Male or female patients of 21 years or older diagnosed with Covid-19 by PCR or by rapid serological test will be eligible. Diagnosis of Covid-19 will be established by means of either the PCR molecular test or with the rapid serologic test or both. (see figure 3)

We have learned recently that some cases produce IgG very early and presence of IgG doesn't mean that infection is late. Those who are IgM positive but PCR negative, will be considered as "suspicious for Covid-19"; however, if their clinical presentation is typical for Covid-19, (see below) they will be counted as a positive diagnosis. Those who are IgM positive but PCR negative, for purposes of analysis will be divided as "polysymptomatic" (2 or more symptoms characteristic of Covid-19) or "not polysymptomatic". The symptoms considered as characteristic are fever, cough, dyspnea, myalgia, diarrhea, anosmia/dysgeusia, malaise, headache, abdominal pain, neurological symptoms (paresthesiae, delirium etc).

6.2 a. Patients who have negative Covid IgG and IgM but whose PCR results are not available yet and who have typical symptoms of Covid-19 (see section 6.2 above), or who have typical CT chest findings such as ground glass opacities, will be considered eligible. If PCR later on turns to be negative, the patient will be retrospectively considered as ineligible.

6.3 Eligible patients will be registered on study on or before 10 days from first onset of symptoms. Patients whose symptoms started > 10 days ago; but who are not in respiratory failure are eligible if they are still symptomatic.

6.4 In order to be treated with methylprednisolone, **they must meet at <u>least two</u> of the following criteria <u>on or shortly before day 10 (but not before day 7) from first onset of symptoms, in order to avoid treatment during viral phase:; if</u>** <u>two criteria are met before day 7, patient is eligible but treatment should not start before day 7 of illness</u>:

6.4.1 Serum IL-6 <u>≥</u> 10 pg/ml before day 10.

6.4.2 Ferritin> 500 ng/ml before day 10.

6.4.3 D-dimer > 1 mg/L (1,000 ng/ml) before day 10.

6.4.4 CRP > 10 mg/dL (100 mg/L) before day 10.

6.4.5 LDH above normal before day 10.

6.4.6 Lymphopenia (absolute lymphocyte count <900) before day 10.

6.4.7 O_2 saturation <94% by pulse oximetry, but more than 91%, before day 10.

6.4.8 CT chest with evidence of infiltrate(s).

6.5 Interpretation of results of rapid and PCR molecular tests:

6.5.1 Those with serological positive test but negative molecular PCR test will be analyzed based on the clinical context. See below.

6.5.2 Those who present with at least 2 of the following typical symptoms of Covid-19 (cough, fever, dyspnea, diarrhea, anosmia) will be considered as likely cases of Covid-19 while those with one or less symptoms will be considered as unlikely cases but will still be considered evaluable. They will be analyzed separately.

6.5.3 Those who are PCR positive and serology positive or negative will be considered as definitive cases.

6.6 The CALL score will be used in the analysis of all cases (11). This score represents a composite index based on presence of comorbidities, age >60, lymphopenia and elevated LDH. Those cases with a score >6 have greater than 50% risk for serious complications.

6.7 Simultaneous treatment will be allowed with the following drugs: remdesivir, hydroxychloroquine, azithromycin, vitamin C, doxycycline, colchicine, zinc and ivermectin. Patients who have received 1 single dose of dexamethasone or methylprednisolone will be eligible. **Their use is not considered as a basis for exclusion** from this trial.

7.0 Exclusion Criteria

7.1 Any patient with life expectancy <1 month

7.2 Any patient who is oxygen dependent

7.3 Any patient with long standing history of severe COPD

7.4 Any patient who is chronically oxygen dependent because of previously existing lung disease

7.5 Anyone with severely uncontrolled diabetes despite adequate management.

7.6 Anyone with active serious bacterial infection such as septicemia or pneumonia.

7.7 Anyone receiving Tocilizumab (anti-IL-6 therapy) or plasma therapy

7.8 Any patient already receiving steroids for another pre-existing illness

7.9 Cases who are IgG positive but IgM negative, will not be considered eligible unless they are positive by molecular PCR test or symptoms are highly suggestive of Covid-19 ($\geq$ 3 of the following: cough, dyspnea, anosmia, diarrhea, myalgia, ground glass infiltrate(s) in CT chest) within first 10 days of illness.

8.0 Management and Dosing Regimen

8.1 Patients will be admitted to a regular room in the hospital (**not** ICU)

8.2 They will be monitored closely with vital signs every 8 hours to ensure their respiratory and cardiovascular status do not deteriorate.

8.3 Methylprednisolone 80 mg IV bolus injection will be given daily x 5 days starting upon day 1-3 of registration on trial. Patients who are stable with O2 sat >94% and no other indication for hospitalization will be treated as outpatient with methylprednisolone 80 mg p.o. x 5 days.

9.0 Adverse Events

9.1 Toxicity of steroids will be recorded.

9.2 The more common side effects are:

- headache
- nausea and vomiting
- weight gain.
- confusion, excitement, and restlessness.
- swelling of ankles, feet, or hands.
- skin problems, such as acne, thin skin, and shiny skin.
- increased thirst.
- Infection
- Hyperglycemia
- Gastrointestinal bleeding

- Hypertension (increased blood pressure)

10.0 Primary Response Criteria for patients receiving methylprednisolone

10.0.1 **Clinical complete response criteria require all the following:**

10.0.1.1 No need for ventilatory support at any point

10.0.1.2 Not oxygen dependent by day 28 of registration

10.0.1.3 Alive by day 28 from registration on trial

10.0.1.4 CT chest with minimal or no evidence of disease by day 28 from registration

10.0.2 **Clinical partial response criteria require that 2 of the following be present by day 14 of therapy:**

10.0.2.1 No need for ventilatory support at any point

10.0.2.2 Not oxygen dependent by day 28 from registration on trial

10.0.2.3 CT chest stable to improve over baseline by day 28 from registration

10.1 Secondary Response Criteria:

10.1.1 Decrease of at least 25% in anyone of the following markers, if elevated at baseline: IL-6, ferritin, D-dimer, CRP or LDH by day 14

10.1.2 Improvement of absolute lymphocyte count in those presenting with lymphopenia. Improvement is defined as increase by 25 % or more by day 14.

11 Data Management

11.1 Patients will be registered after obtaining signed consent form.

11.2 **Registration will be performed at Auxilio Mutuo by calling 787-758-2000 x 3569.**

12 Statistical Analysis

12.0 Analysis will focus in comparing pre-treatment and post treatment values. Results will be analyzed using the intent to treat principle which in this case means that those cases who receive at least one dose of methylprednisolone will be considered evaluable.

12.1 Evaluable cases are considered as those who have clinical data for at least 1 subsequent day.

12.2 Due to the small sample size, non-parametric tests for matched data will be used.

13.0 REFERENCES:

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APPENDICES

Appendix I: EASTERN COOPERATIVE ONCOLOGY GROUP SCALE

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

Appendix II: NCI CTC Version 5.0

Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (published 27 Nov 2017). Available from: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0/CTCAE_v5.0_2017-11-27.xlsx.