ISARIC (International Severe Acute Respiratory and Emerging Infections Consortium)

A global federation of clinical research networks, providing a proficient, coordinated, and agile research response to outbreak-prone infectious disease

Analysis Plan for ISARIC International COVID-19 Patients

Please complete the following sections:

Title of proposed research
Lymphopenia in severe COVID19 patients: are they a unique immunologically compromised population?
Version: (Date: Day/Month/Year)
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Final draft SAPs will be circulated to all ISARIC partners for their input with an invitation to participate. ISARIC can help to set up collaborator meetings; form a working group; support communications; and accessing data. Please note that the details of all approved applications will be made publicly available on the ISARIC website. Please complete all sections of this form fully and return to ncov@isaric.org

Introduction

COVID19 is a complex new form of respiratory disorder, with several pathological pathways, leading to frequent progression to multiorgan failure and admission to intensive care to ventilatory, hemodynamic and kidney supports. It is well known the importance of cytotoxic T lymphocytes and natural killer cells to the control of viral infections, and functional exhaustion of antiviral lymphocytes and lymphopenia have been already documented in COVID19 patients [1].

However, the prognostic value on patients' outcome and mortality rate is still eluding, and could potentially impact the clinical approach and foresee the severity of COVID19 and its outcomes.

We aim to determine the association between lower lymphocyte count and a poor prognosis, analyze its statistical correlation and verify if lower lymphocyte count is independently correlated to COVID19 infection outcomes. We also aim to ascertain how lymphocyte total count influence poor prognosis variables and in which magnitude, define if lymphocyte count could be a strong predictor of combined outcomes using a propensity score and even if its correlation is explained partially by an increased nosocomial infections during hospital length of stay, in these patients.

Participatory Approach

All contributors to the ISARIC database are invited to participate in this analysis through review and input on the statistical analysis plan and resulting publication. The outputs of this work will be disseminated as widely as possible to inform patient care and public health policy, this will include submission for publication in an international, peer-reviewed journal. ISARIC aims to include the names of all those who contribute data in the cited authorship of this publication, subject to the submission of contact details and confirmation of acceptance of the final manuscript within the required timelines, per ICMJE policies and the ISARIC publication policy.

Research Plan

Summary of Research Objectives
• Determine the rate of lymphopenia in severe COVID19 patients • Characterize COVID19 patients with lymphopenia in what concerns previous comorbidities, clinical presentation and survival rates. • Ascertain the prognostic value of the ratio Neutrophil/Lymphocyte ratio on ICU admission and Mortality rate of COVID19 patients [3]

- Ascertain the prognostic value and magnitude of effect of lymphopenia on vasopressor free days (see additional notes section below – Note 1), vasopressor dosage, ventilator free days, SOFA score and Mortality rate
- Document the rate of intercurrent nosocomial infections on COVID19 patients with lymphopenia and its possible association with altered inflammatory serum markers profiles (C-reactive Protein and Procalcitonin, ferritin, LDH, D dimer)

Proposed Target Population

All patients admitted to hospital care, regardless of ICU admission, with severe COVID19 registered in ISARIC data set.

Clinical Questions/Descriptive Analyses

1. What is the rate of lymphopenia within COVID19 patients?
2. What are the characteristics of severe COVID19 patients with lymphopenia?
3. Does lymphopenia predict the outcome and survivability of these patients?
4. Does lymphopenia predict a higher rate of nosocomial infections in COVID19 patients?
5. Do COVID19 patients with lymphopenia have different inflammatory serum markers profiles (different C-reactive Protein and Procalcitonin levels between groups) ?

Planned Statistical Analyses, Methodology and Representation

1. Overall frequencies of key demographic variables and frequencies stratified by patients' comorbidities. A clustering matching will be made considering age, sex, ICU admission and SOFA score at admission , reducing the potential bias inherent to the data and variable behavior.
2. SOFA score at admission, daily minimum lymphocyte serum levels registered, daily maximum vasopressor dosage, Vasopressor-free days, ventilator-free days, ICU length of stay, KDIGO acute renal failure criteria and Mortality rate will be ascertained and compared between groups (see additional notes section below – Note 2)
3. Rate of nosocomial infections registered as well as antibacterial-dependent days in ICU will be ascertained between groups (see additional notes section below – Note 3).
4. Bar plots for displaying frequencies of categorical variables.
5. Linear mixed models to ascertain differences between C-reactive Protein and Procalcitonin levels between patients with lymphopenia vs patients without lymphopenia
6. Qui-square test for categorical variables and Kruskal-Wallis and multivariable logistic regression for continuous variables for statistical assessment of outcomes between groups.
7. Kaplan-Meier survival curve and Cox regression test for survivability.

Handling of Missing Data

Preliminary analysis would be performed to ascertain a detailed overview of the extent of missingness in the data. This should enable the identification of variables which lack sufficient data to allow for any useful analysis to be performed on them. Type of missingness shall be considered including whether data are not missing at random and follow-up with sites will be conducted if appropriate. Variables with greater than 30% missingness will be excluded from analysis. Where appropriate, imputation will be performed using Multiple Imputation by Chained Equations (MICE). We aim to include as many sites as possible considering that the data provided does not bias the statistical analysis we are trying to accomplish. Therefore, we may apply some imputation strategies to missing data, but if not possible, certain variables or data may be excluded from the analysis.

Additional Notes

NOTE 1 - Considering the range of possible form “answers” to the pretended factor of vasopressor support, we will consider:

1. an initial categoric variable with yes/no (to all patients with the need of either of the following treatments: dobutamine, dopamine, epinephrine, Noradrenaline, adrenaline or vasopressin)
2. A further continuous variable reflecting a vasopressor score (a dimensionless variable calculated as follows: dopamine dose (mcg/kg/min) × 1) + (dobutamine dose (mcg/kg/min) × 1) + (epinephrine dose (mcg/kg/min) × 100) + (norepinephrine dose (mcg/kg/min) × 100) + (phenylephrine dose (mcg/kg/min) × 100 + (vasopressin dose (mcg/kg/min) × 100))

This variable will allow us to include the vasopressor in account for a multivariable logistic regression and to compose different vasopressor therapies in a single variable. This vasopressor score has been successfully used in the PROWESS-Shock trial subanalysis [4]

NOTE 2 - The major groups for this analysis would be defined as COVID19 patients (diagnosed using clinical and radiologic criteria with a SARS-CoV-2 positive RT-PCR test), admitted to hospital care, with a total lymphocyte count below 1000 per microliter of blood in the first 48h of admission vs with a total lymphocyte count higher than 1000 per microliter in the first 48h of admission.

- We will also define a subanalysis of lymphocyte count by severity splitting the patients with less than 500 lymphocyte count and between 500-1000 lymphocyte count (Reference 2 below).
- We believe that after the 48h time lapse, the total count of lymphocyte may be influenced by too many factors including COVID19 therapies (corticosteroids, for example) or other biases. Regardless, we may artificially study and split patients with a total lymphocyte count below 1000

per microliter of blood during their hospital stay vs those who didn't present such lymphocyte count.

NOTE 3 - We aim to define nosocomial infections as nosocomial infection as an infectious event that is diagnosed >48 hours after admission without evidence that the pathogen was already in
the incubation phase. The main inclusion criteria for a nosocomial infection event would be:

- Clinical criteria of a new infection after 48h of patients' admission (not promptly available in ISARIC form but that undoubtedly is connected to (see next criteria))

AND

- A significant increase of inflammatory serum markers (defined as an increase of at least 50% of CPR serum levels in the first 48h of clinical suspicion of an event of nosocomial infection)

AND

- Motivates a new course of antibacterial, antifungal or antiviral therapy

References

1 - Zheng M., Gao Y., Wang G. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020

2 - Lee J, Park SS, Kim TY, Lee DG, Kim DW. Lymphopenia as a Biological Predictor of Outcomes in COVID-19 Patients: A Nationwide Cohort Study. Cancers (Basel). 2021 Jan 26;13(3):471. doi: 10.3390/cancers13030471. PMID: 33530509; PMCID: PMC7865511.

3 - Imran MM, Ahmad U, Usman U, Ali M, Shaukat A, Gul N. Neutrophil/lymphocyte ratio-A marker of COVID-19 pneumonia severity. Int J Clin Pract. 2021 Apr;75(4):e13698. doi: 10.1111/ijcp.13698. Epub 2021 Jan 12. PMID: 32892477.

4 - (Póvoa P, Salluh JI, Martinez ML, Guillamat-Prats R, Gallup D, Al-Khalidi HR, Thompson BT, Ranieri VM, Artigas A. Clinical impact of stress dose steroids in patients with septic shock: insights from the PROWESS-Shock trial. Crit Care. 2015 Apr 28;19(1):193. doi: 10.1186/s13054-015-0921-x. PMID: 25928214; PMCID: PMC4456711.)

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