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A retrospective safety study evaluating differences of safety and outcomes of prophylactic versus therapeutic doses of enoxaparin in patients admitted to Charlotte Maxeke Johannesburg Academic Hospital with confirmed COVID-19 pneumonia presenting with disease of mild to moderate severity

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A retrospective safety study evaluating differences of safety and outcomes of prophylactic versus therapeutic doses of enoxaparin in patients admitted to Charlotte Maxeke Johannesburg Academic Hospital with confirmed COVID-19 pneumonia presenting with disease of mild to moderate severity.

Sharise Singh (BHSc, MBBCh)

A research report submitted to the University of the Witwatersrand, Johannesburg in fulfilment of the requirements of the degree of Master of Medicine, 2024

DECLARATION

I, Sharise Singh, declare that this research report is my own work which is being submitted for the degree Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature: 

Date: 10 September 2024

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ABSTRACT

Background: Coagulopathy is a common finding in coronavirus disease 2019 (COVID-19) infection and contributes towards disease severity and mortality. International treatment protocols advocate for the early use of anticoagulation therapy for patients admitted with COVID-19 pneumonia.

Objectives: In patients admitted with COVID-19 infection, this study served as a safety study to identify any increased risk of major bleeding in those treated with therapeutic doses (1mg/kg/twice daily subcutaneously) of a low-molecular weight heparin (LWMH, i.e. enoxaparin) compared to those treated with prophylactic doses (0.5mg/kg/daily subcutaneously). In addition, it sought to identify differences in outcomes (other than bleeding) when comparing different enoxaparin doses in patients hospitalised with COVID-19 infection.

Methods and findings: This retrospective study included 100 patients (over the age of 18 years) who were admitted to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with confirmed COVID-19 pneumonia of mild to moderate severity between 01 March 2020 and 31 August 2020.

Data was captured using the Research Electronic Data Capture (REDCap) database and National Health Laboratory Services (NHLS)-Labtrak. The following outcomes were measured: recovery, progression to invasive ventilation, death, and bleeding.

Of the patients included in this study, 40 were treated with prophylactic doses of enoxaparin and 60 were treated with therapeutic doses. Outcomes were as follows: 81% recovered, 20% progressed to invasive ventilation, and 19% demised. No patients experienced episodes of bleeding during their admission.

Of the recovery group, 42% (n=34) of patients received prophylactic doses of enoxaparin, and 58% (n=47) received therapeutic doses.

With regards to patients who progressed to requiring invasive ventilation and admission to the intensive care unit (ICU), prophylactic enoxaparin doses were administered in 4 patients and 16 patients received therapeutic doses.

Of the patients who demised, 32% (n=6) had been treated with prophylactic enoxaparin doses and 68% (n=13) were treated with therapeutic doses.

Prophylactic enoxaparin was associated with a higher rate of recovery (p-value = 0.001) but also with progression of disease (p-value = 0.0495). Death was approximately twice more common in those treated with therapeutic enoxaparin.

Conclusion: The findings of this study revealed no increased risk of bleeding when using therapeutic doses of enoxaparin as compared to prophylactic doses in the treatment of non-severe COVID-19. Current literature shows that once COVID-19 patients progress to severe disease, use of therapeutic anticoagulation results in a higher risk of bleeding. Our study did not demonstrate such risks. In addition, our study showed that use of prophylactic enoxaparin was associated with increased survival rates when compared to therapeutic doses. This contrasts to existing literature. We recognise the limitations of a small sample size and a unicentric population.

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ABBREVIATIONS

ACE-2	Angiotensin converting enzyme-2
ARDS	Acute respiratory distress syndrome
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
COVID-19	Coronavirus Disease 2019
DIC	Disseminated intravascular coagulation
HIV	Human Immunodeficiency Virus
ICU	Intensive care unit
IL-6	Interleukin-6
ISTH	International Society on Thrombosis and Haemostasis
ISTH-SCC BAT	International Society on Thrombosis and Haemostasis-Scientific and Standardisation Committee Bleeding Assessment Tool
LMWH	Low-molecular weight heparin
NHLS	National Health Laboratory Services
NIH	National Institutes of Health
NO	Nitric oxide
PLOS	Public Library of Science
PT	Prothrombin time
REDCap	Research Electronic Data Capture

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TNF	Tumour necrosis factor
UFH	Unfractionated heparin
VTE	Venous thromboembolism
WHO	World Health Organisation

CHAPTER 1 – PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1 INTRODUCTION

In December 2019, a novel coronavirus emerged in the Chinese province Hubei.[1] It was given the name severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was the causal agent of the disease entity coronavirus disease 2019 (COVID-19).[2]

COVID-19 spread rapidly around the world, leaving devastating rates of hospitalisation and mortality in its wake, and prompting the World Health Organisation (WHO) to declare a global health emergency.[2,3,4] South Africa, a country home to over 50 million people and more than half of those stricken by poverty, had less than 10 000 critical care beds at the start of the pandemic.[5] By September 2020, the number of COVID-19 deaths in South Africa far exceeded 10 000.[5]

The need for hospital admission and critical care support overwhelmed available healthcare facilities and services, elucidating the importance of risk stratification strategies to facilitate early intervention, improve clinical outcomes, and promote efficient allocation of resources.[6,7]

Coagulopathy is associated with severe disease and adverse outcomes in patients hospitalised with COVID-19.[7,8] Risk stratification strategies include identification of risk factors for severe disease, assessment of clinical severity, and laboratory investigations.[7] To prevent complications and mortality related to coagulopathy, the International Society on Thrombosis and Haemostasis (ISTH) recommended that all COVID-19 hospitalised patients should receive low-molecular weight heparin (LMWH) viz. enoxaparin as part of standard treatment protocols unless contraindicated.[9]

Much research has been undertaken to determine the efficacy of therapeutic versus prophylactic anticoagulation with regards to improving outcomes in hospitalised COVID-19 patients and reducing the need for critical care.[10] Studies have also sought to observe rates of bleeding when using therapeutic anticoagulation to treat COVID-19 and thus establish its safety profile.[10,11]

1.2 CLINICAL FEATURES OF COVID-19

The SARS-CoV-2 virus is an enveloped betacoronavirus that is mainly transmitted via contact with respiratory droplets.[4,12] Most people are asymptomatic or only display mild symptoms such as cough, fever, and malaise.[12] However, approximately 10% can develop severe pneumonia and progress to acute respiratory distress syndrome (ARDS).[16] A study in Wuhan reported that ARDS was more common in older patients and those with hypertension or diabetes mellitus.[12]

Risk factors for severe disease and mortality include male sex, older age (above 65 years), cardiovascular disease, diabetes mellitus, hypertension, obesity, and chronic obstructive pulmonary disease.[2,12] Infection with the Human Immunodeficiency Virus (HIV) has been shown to be an independent risk factor for severe disease and mortality.[13] Approximately two-thirds of the world's HIV population live in Sub-Saharan Africa.[13]

1.3 INFLAMMATION AND THROMBOSIS IN COVID-19

The virus infects the host cell by utilising the angiotensin converting enzyme-2 (ACE-2) receptor.[14] This receptor is expressed by many cell types throughout the body, including (but not limited to) the lungs, heart, kidneys, and endothelium.[14] Infection of pneumocytes in alveoli invokes a profound inflammatory response with a significant increase in production of pro-inflammatory cytokines, a phenomenon termed the "cytokine storm".[15,16] In a healthy individual, inflammation results in activation of the coagulation system to limit systemic spread of an infectious agent.[17] However, in COVID-19 infection, the normal balance between coagulation and anticoagulation pathways is disrupted, and the resultant hypercoagulable state portends a poorer prognosis.[2,18] Immunothrombosis and its sequalae in COVID-19 is multifactorial.[7,19]

Infection of endothelial cells disrupts the normal function of endothelium.[17] Consequences of this include vasoconstriction, increased production of thrombin without concomitant fibrinolysis, altered

interactions with immune cells, abnormal platelet aggregation, and altered vascular permeability.[1,14,17,19,20] Enhanced platelet activation further mediates the inflammatory response.[2]

Endothelial dysfunction in COVID-19 leads to ischaemic injury and dysfunction in multiple tissues and organs, further enhancing inflammatory responses and thrombosis.[14,16]

In the lungs, endothelial injury and inflammation contribute to the development of ARDS, which is associated with a high mortality rate.[1,21] This is worsened by pneumocyte infection and damage, which results in localised thrombin generation and platelet activation, and decreased fibrinolysis.[18]

Lung damage, inflammation and microvascular thrombosis all impair pulmonary gaseous exchange and cause hypoxia.[18] Hypoxia contributes to thrombosis by increasing blood viscosity, activating certain transcription pathways, and promoting further inflammation.[1,16,17]

Disseminated intravascular coagulation (DIC) can develop due to activation of coagulation pathways by cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF).[21] It is postulated that DIC may contribute to the development of ARDS by promoting a consumptive coagulopathy localised in the pulmonary vasculature.[21]

Conventional risk factors for development of thromboembolism also play an important role in hospitalisation with COVID-19.[22] These include prolonged immobilisation, use of central venous catheters, comorbidities (e.g. malignancy) surgical procedures, and sepsis.[6,22]

1.4 PREDICTORS OF DISEASE SEVERITY

Typical laboratory findings of COVID-19 patients include thrombocytopaenia, prolonged prothrombin time (PT), and raised D-dimer levels.[2] D-dimer levels serve as a marker of formation and lysis of fibrin, and thus reflect activation of the coagulation system.[22]

After the emergence of COVID-19, it soon became evident that raised D-dimer levels (defined by the ISTH as a rise by 3-4-fold above normal) was a risk factor for organ dysfunction, ARDS, need for

intensive care, and mortality.[15,22] A Chinese study of over 300 hospitalised patients showed that D-dimer levels greater than 2.0mcg/ml on admission was an accurate predictor of mortality.[6] Another study also showed that patients who required intensive care had higher D-dimer levels on admission.[6]

Evidence suggests that D-dimer levels could be an early predictor of complications and mortality in COVID-19, and recognition of such can be used to improve clinical outcomes.[6] The ISTH recommends the use of D-dimer measurements to risk stratify patients and facilitate early intervention.[9]

Research has shown that patients with severe COVID-19 infection tend to have lower platelet counts.[7] One systematic review reported that thrombocytopaenia was observed in 57.7% of patients with severe disease, whilst only 31.6% of patients with non-severe illness had low platelet counts.[23] A meta-analysis done by Lippi and colleagues reported that thrombocytopaenia was associated with an increased risk of severe disease by 5-fold.[7]

Possible mechanisms for thrombocytopaenia in COVID-19 include:

- Viral infection of platelets[23]
- Antibody-mediated destruction and clearance of platelets due to molecular mimicry[24]
- Platelet consumption due to microthrombi formation[24]
- Infection of bone marrow and decreased thrombopoiesis[24]
- Damage to pulmonary capillaries and lung tissue, leading to impaired breakdown of megakaryocytes and decreased release of platelets into the systemic circulation.[24]
- Increased cytokines and activation of macrophages, causing a decrease in the number of platelet progenitor cells.[23]

In cases of viral infection, thrombocytopaenia due to increased destruction or immune clearance of platelets tend to occur earlier in the disease process, whilst mechanisms of decreased production tend to occur later.[16]

Despite the association between thrombocytopaenia and the severity of COVID-19, research has shown that the degree of thrombocytopaenia is generally mild (counts = 100-150 x 10^9 cells/L), and episodes of bleeding are rare.[7,16]

The ISTH recommends monitoring of coagulation parameters (i.e. D-dimer, PT, platelet count) to identify patients who are at risk of developing severe complications from COVID-19, and thus prompt early escalation of care.[7] Similarly, if those same parameters show improvement (in conjunction with an improved clinical response), clinicians may consider de-escalating level of care.[7]

1.5 ANTICOAGULATION AND COVID-19

The ISTH recommends that prophylactic enoxaparin be considered in all hospitalised COVID-19 patients with platelet count > 25 x 10^9 cells/L and in the absence of contraindications.[7] Benefits of enoxaparin in COVID-19 may include:

- Protection against development of venous thromboembolism (VTE)[7]
- Anti-inflammatory effects, which may mitigate the cytokine response and recruitment of leukocytes, thereby reducing lung damage.[3,15]
- Improvement of pulmonary gaseous exchange by reducing the cycle of immunothrombosis in the lungs.[21]
- Decreasing the effects of thrombin and thus curbing inflammation[18]
- Limiting endothelial damage caused by histones (released from damaged cells)[18]
- Decreasing organ damage by lessening endothelial damage[18]
- Possible antiviral effects due to its polyanionic properties[18]

- Diminishing the contribution of thrombosis towards the development of ARDS and providing time for additional therapies to take effect.[16]
- Immunomodulatory effects[15]

Overall, enoxaparin therapy has proved beneficial in improving outcomes, preventing complications such as ARDS, and lessening the need for critical care support.[10,18]

1.6 PROPHYLACTIC VERSUS THERAPEUTIC ANTICOAGULATION IN COVID-19

Despite evidence supporting the beneficial effects of enoxaparin therapy when treating COVID-19, evidence has also shown that patients may still develop thrombotic complications despite receiving prophylactic anticoagulation.[16,19] Results from one study revealed that 49% of patients developed thromboembolism despite receiving prophylactic anticoagulation therapy.[19] A report by Rannuci et al. suggested that increased doses of enoxaparin may improve outcomes in COVID-19.[16] Explanations for this may include mitigating the progression to DIC and reducing the effects of thrombosis on respiratory compromise.[16] The report suggests that since COVID-19 seems to invoke a hypercoagulable state that is more remarkable than other hospital illnesses, higher doses of anticoagulant therapy are needed.[16]

One large clinical trial (which incorporated findings from over 300 hospitals across the world) found that therapeutic anticoagulation decreased the need for critical care in patients hospitalised with COVID-19 of moderate severity.[20] Contrastingly, patients with severe disease and requiring intensive care did not show improved outcomes when treated with therapeutic doses of heparin.[20] Another study found that use of therapeutic anticoagulation increased the risk of major bleeding events by 5.7% in patients hospitalised with COVID-19.[11] However, this study did not compare this risk with those who received prophylactic doses.[11] A multiplatform trial published in 2021 that involved over 2000 patients reported that when treating COVID-19 of non-critical severity, therapeutic-dose heparin decreased the need for vital organ support and increased survival rates

when compared to prophylactic doses. However, major bleeding occurred more often in the therapeutic (1.9%) group than the prophylactic (0.9%).[10]

It is postulated that the timing as well as doses of anticoagulation is important when treating COVID-19.[3,16] Studies have found that once patients develop severe COVID-19, therapeutic anticoagulation offers no benefit and in fact increases the risk of bleeding.[20] It is possible that once a patient progresses to severe disease, which is associated with dysfunctional coagulation and ARDS, therapeutic enoxaparin fails to halt the inflammatory cascade and thrombosis.[10]

Previous studies indicate that hypercoagulability develops early in COVID-19.[3] Thus, by initiating higher doses of anticoagulant therapy earlier, progression to critical illness and DIC may be mitigated.[3] In doing so, episodes of major bleeding (associated with severe COVID-19) may also be avoided by halting disease progression.[3]

The ISTH recommends prophylactic doses of LMWH or unfractionated heparin (UFH) in all hospitalised patients with COVID-19 of non-critical severity.[9] In select patients hospitalised with non-severe illness (those at increased risk of adverse outcomes), therapeutic LMWH/UFH may be considered to improve outcomes (class A evidence).[9] The guidelines do not recommend escalation of anticoagulation in patients with critical COVID-19 illness unless a specific indication exists (e.g. atrial fibrillation).[9] A commentary published by Connors et al. proposed that the introduction of other therapies such as IL-6 antagonists may be beneficial when treating severe COVID-19, rather than increasing anticoagulant therapy.[16]

1.7 RATIONALE FOR RESEARCH

Coagulopathy and thrombotic events are associated with adverse outcomes in COVID-19.[8] Early and accurate predictors are paramount to identify those at risk of severe disease and initiate treatment urgently.[6] International studies report improved survival rates and reduced need for critical care when therapeutic enoxaparin therapy is initiated early in hospitalised COVID-19 patients with

moderate disease in the absence of contraindications.[20] In severe cases, not only does therapeutic anticoagulation not seem to improve outcomes but it also raises the concern of bleeding.[20]

In contrast, studies regarding thrombocytopaenia in COVID-19 report that incidence of bleeding is rare.[7,16] A paper describing the treatment protocols at South African hospital Groote Schuur Hospital reported that COVID-19 patients who were critically ill received intermediate doses of heparin during their admission, in contrast with the ISTH recommendations.[25] This article does not report on any comparison with patients who receive prophylactic doses, and it makes no mention of increased of bleeding when using higher doses.[25]

In a country like South Africa, with its unique burden of HIV infection and limited healthcare resources, it may prove valuable to evaluate both the efficacy and safety of therapeutic versus prophylactic enoxaparin when treating hospitalised COVID-19 patients, to optimise outcomes and promote efficient use of resources.[5,13]

2. AIMS AND OBJECTIVES

The aim and primary objective of this study is to evaluate the safety of therapeutic enoxaparin (1mg/kg/twice daily subcutaneous) in the treatment of patients admitted to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with COVID-19 of mild-moderate severity on admission. We intend to identify any episodes of bleeding that may occur in the therapeutic group and compare the incidence rate with the prophylactic enoxaparin (0.5mg/kg/daily subcutaneous) group.

Our secondary objective is to compare outcomes other than bleeding between the 2 dosage groups, namely: recovery, progression to invasive ventilation and death.

3. METHODS

3.1 Study type:

Retrospective study.

3.2 Study participants:

One hundred patients admitted to CMJAH with confirmed COVID-19 that meet the inclusion criteria (see below). Each treatment group (prophylactic enoxaparin versus therapeutic enoxaparin) will include a minimum of 30 patients each. These numbers were decided upon following the advice of the protocol assessors.

Patients will remain anonymous and will be allocated random numbers unrelated to their personal details or hospital numbers.

3.3 Inclusion criteria:

- Confirmed COVID-19 of mild-moderate severity as classified by the National Institutes of Health (NIH; see appendix 3) on admission to CMJAH between 01 March 2020 and 31 August 2020.
- All ages 18 years and above

3.4 Exclusion criteria:

- Pre-existing bleeding diathesis
- Chronic anticoagulant medication such as vitamin K antagonists or novel oral anticoagulants
- Patients receiving invasive ventilation or non-invasive ventilation such as high-flow oxygen or continuous positive airway pressure therapy on admission.

3.5 Data collection:

Data will be collected from the REDCap database, NHLS-Labtrak, and information from patient hospital files accessible from the online database Medicom.

The following data will be captured:

- Age
- Race
- Gender
- Height and weight
- Comorbidities and chronic medications
- Date of admission
- Oxygen saturation at room air on admission
- Enoxaparin dose
- Final outcomes
- Episodes of bleeding

3.6 Statistical analysis:

A statistician will be consulted to assist with statistical interpretation of the data. To assess for a relationship between enoxaparin doses and outcomes, data will be organised in contingency tables prior to statistic testing. Tests such as the Chi-square analysis and likelihood ratios will be used to test the null hypothesis that there is no relationship between enoxaparin doses and outcomes in hospitalised COVID-19 patients. The strength of association between dosage types and outcomes will be tested using Cramer's V correlation coefficient.

4. ETHICAL CONSIDERATIONS:

Permission to conduct this study will be obtained from the Internal Medicine department at CMJAH, the Chief Executive Officer of CMJAH, the REDCap and NHLS-LABTRAK databases, and the Human Research Ethics Committee of the University of the Witwatersrand.

5. FUNDING:

As all tests and interventions will form part of standard routine care, need for additional funding is not anticipated for this study.

6. STRENGTHS:

- All data will be captured by one researcher so methods will remain consistent.
- Data collected from REDCap and NHLS-Labtrak will be standardised.

7. LIMITATIONS:

- Small sample size
- Unicentric population
- Patient comorbidities which may influence outcomes independent of enoxaparin dose.
- Additional therapies such as corticosteroids which may influence outcomes independent of enoxaparin dose.

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CHAPTER 2: SUBMISSIBLE ARTICLE

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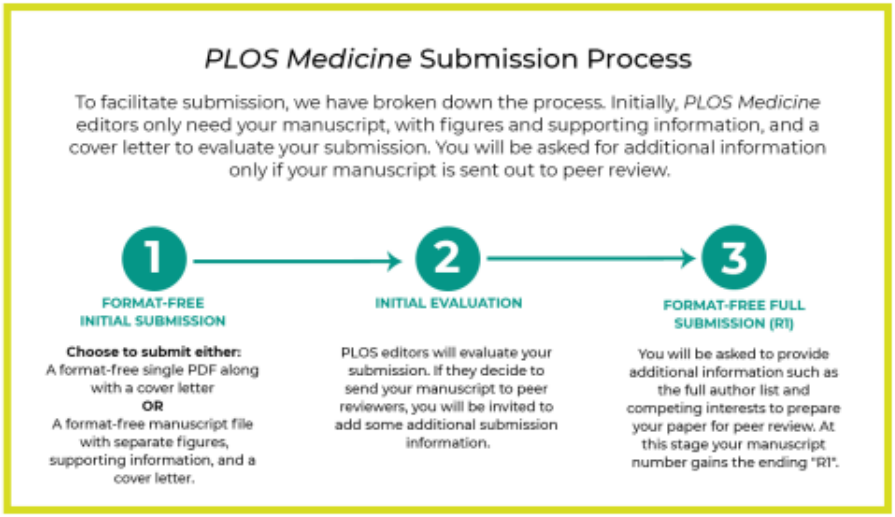

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Submission format	› A single PDF file containing manuscript, figues and supporting information files, with a separate cover letter (recommended for ease of initial submission) **OR** › If preferred, upload figure files alongside a PDF or Word manuscript file, with a separate cover letter

Creating an initial submission is the most efficient way to obtain feedback from the journal. Authors who email with an inquiry will be asked to submit the manuscript as an initial submission.

Format-free full submission

If you receive an initial decision from the journal committing to peer review, you will be asked to complete a full submission with additional information including:

- Full author list and author details
- Details of the availability of data generated in the study
- Details of any ethical approvals
- Details of clinical trials registration, if applicable

Style and Format

The Style and Format criteria below are required only if the manuscript is pursued for publication with a positive decision after peer review. Authors invited to submit a revision will be prompted to format their manuscript in line with these requirements. We do not require specific formatting of manuscripts for Full Submission.

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Style and Format

File format

Submit the manuscript file in DOC, DOCX, RTF, or PDF format. Your file should not be locked or protected.

If you have written your manuscript in LaTeX, please submit as a PDF. Read the LaTeX guidelines.

Length

Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information.

We encourage you to present and discuss your findings concisely.

Font

Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.

Headings

Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.

Layout and spacing

Manuscript text should be double-spaced.

Do not format text in multiple columns.

Page and line numbers

Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).

Footnotes

Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.

Language

Manuscripts must be submitted in English.

You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.

Abbreviations

Define abbreviations upon first appearance in the text.

Do not use non-standard abbreviations unless they appear at least three times in the text. List all non-standard abbreviations (with definitions) in alphabetical order in a separate section at the beginning of the manuscript.

Keep abbreviations to a minimum.

Reference style

PLOS uses "Vancouver" style, as outlined in the ICMJE sample references.

See reference formatting examples and additional instructions below.

Equations

We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable. Please do not embed equations as images.

Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ' [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values.

Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.

Nomenclature

Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Name (rINN).
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).

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Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HGNC for human genes; we strongly recommend using this tool to check against previously approved names). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-committee prior to manuscript publication. Examples of the systematic allergen nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

Manuscript Organization

Most manuscripts should be organized as follows. Instructions for each element appear below.

- Title
- Authors
- Affiliations
- Abstract
- Introduction
- Methods (or Methods and Materials)
- Results
- Discussion
- Acknowledgments
- References
- Supporting information captions

Other elements

- Upon revision, figure files should be uploaded separately from the manuscript, and each figure caption should be inserted in read order after the first paragraph where the figure is cited. Read more information about our figure requirements during each stage of editorial review.
- Tables are inserted immediately after the first paragraph in which they are cited.
- Supporting information files are uploaded separately.

Refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

- Download sample title, author list, and affiliations page (PDF)
- Download sample manuscript body (PDF)

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	200 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	70 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

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Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-committee prior to manuscript publication. Examples of the systematic allergen nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

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satisfy the other remaining contributions; however, these alone will not qualify them for authorship.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at full submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

All authors will be contacted via email upon Full Submission to ensure that they are aware of and approve the submission of the manuscript, its content, authorship, and order of authorship. Articles will not be published unless all authors have provided their assent to publication.

Cover letter

Upload a cover letter as a separate file in the online system.

The cover letter should address the following questions:

- Why is this manuscript suitable for publication in *PLOS Medicine*?
- Why will your study inspire researchers or clinicians, and how will it improve patient care or public health, or drive the understanding of disease forward?

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

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Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The research article Abstract is divided into the following three sections: Background, Methods and Findings, and Conclusions. It should contain all the following elements (items in square brackets are needed only for some study types).

PLOS Medicine prefers abstract submissions not exceed 300 words, with a maximum of 500 words allowed.

Background

- This section should clearly describe the rationale for the study. It should end with a statement of the specific study hypothesis and/or study objectives.

Methods and Findings

- Describe the study participants or what was studied (e.g., patient population, cell lines; be as specific as possible, including numbers of individuals studied). Describe the study design, intervention if applicable, main methods used, primary outcome measure(s), and length of follow up if applicable.
- [If appropriate, include how many participants were assessed out of those enrolled. For survey research, include the response rate.]
- [If critical to the understanding of the paper, describe how results were analyzed, i.e., which specific statistical tests were used.]
- Describe the main outcomes and quantify the results using a measure of precision (e.g., 95% confidence interval). Describe any adverse events.
- Describe the main limitations of the study.

Conclusions

- Provide a general interpretation of the results with any important recommendations for future research.
- [For a clinical trial, provide any trial identification number(s) and name(s) (e.g., trial registration number, protocol number or acronym).]

Introduction

The introduction should put the focus of the manuscript into a broader context. As you compose the Introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can delve into these issues further. The Introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

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Methods

The Methods should provide enough detail for reproduction of the findings. Protocols for new methods should be included, but well-established methodological procedures may simply be referenced. A full description of the methods should be included in the manuscript itself rather than in a supplemental file.

Methods should also include a section with descriptions of any statistical methods used. The description should conform to the criteria outlined by the Uniform Requirements, as follows:

> *Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as P values, which fail to convey important information about effect size and precision of estimates. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the statistical software package(s) and versions used. Distinguish prespecified from exploratory analyses, including subgroup analyses.*

Submit detailed protocols for newer or less established methods. Well-established protocols may simply be referenced. Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information.

We recommend and encourage you to deposit **laboratory protocols** in protocols.io, where protocols can be assigned their own persistent digital object identifiers (DOIs).

To include a link to a protocol in your article:

1. Describe your step-by-step protocol on protocols.io
2. Select **Get DOI** to issue your protocol a persistent digital object identifier (DOI)
3. Include the DOI link in the Methods section of your manuscript using the following format provided by protocols.io: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]

At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time by selecting **Publish** on the protocols.io site. Any referenced protocol(s) will automatically be made public when your article is published.

PLOS ONE offers an option for publishing peer-reviewed Lab Protocol articles, which describe protocols hosted on protocols.io articles. Read more information on Lab Protocol articles.

Results

The Results section should include all primary and secondary outcome measures analyzed. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. The Results section should be written in past tense.

PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

Large data sets, including raw data, may be deposited in an appropriate public repository. See our list of recommended repositories.

For smaller data sets and certain data types, authors may provide their data within Supporting Information files accompanying the manuscript. Authors should take care to maximize the accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

As outlined in the Uniform Requirements:

> *Give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical significance attached to them, if any. Restrict tables and figures to those needed to explain the argument of the paper and to assess supporting data. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample."*

Discussion

The Discussion should be concise and tightly argued. It should start with a brief summary of the main findings. It should include paragraphs on the generalizability, clinical relevance, strengths, and limitations of your study.

You may wish to discuss the following points also:

- How do the conclusions affect the existing knowledge in the field?

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- How can future research build on these observations and what are the key experiments that must be done?

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
- Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article's retracted status in your article's text and reference list.

Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article's submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

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Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]: 1954. Los Angeles: MGM.

Supporting Information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

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Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and Tables

Figure files

If you are submitting an **Initial** or **Full Submission** and would prefer to embed each figure in the manuscript, do so in read order, immediately following the paragraph where the figure is first mentioned and above the related figure caption.

Upon revision, prepare and submit each figure as an individual file, removing all embedded figures.

Figure citations

Cite figures in ascending numeric order upon first appearance in the manuscript file.

For detailed instructions, read the guidelines for figures.

Figure captions

Insert figure captions in the manuscript text, immediately following the paragraph where the figure is first cited (read order). Don't include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

Read more about figure captions.

Avoiding image manipulation

As part of our efforts to improve published figure quality, we routinely and thoroughly check all main and supporting figures for all papers editorially accepted for publication in *PLOS Medicine*. In doing so, we not only ensure that all figure files meet our requirements for publication and are available to publish under our CC BY license, but also that we remain vigilant to image manipulation of photographic images.

Image files should not be manipulated or adjusted in any way that could lead to misinterpretation of the information present in the original image. For full details on best practices regarding your figures, read our figure guidelines.

If evidence is found of inappropriate manipulation, we reserve the right to ask for original data and, if that is not satisfactory, we may decide not to accept the manuscript, and may also contact the authors' institutions to ask them to assist with investigation.

In checking for manipulation, we may request higher resolution versions of your images, or the original images, so that we can efficiently and accurately check all figures.

If you ever need to email files to the journal office, our system has a 10 MB attachment limit, meaning that we will not receive any emails larger than this size. If your files are larger than 10 MB, please either send them one email at a time, or look into reducing the size of the files. If you are having problems sending us large files, please contact the journal office for details of how we can help you transfer your files.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

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Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS Full Submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
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2.2 Manuscript for submissible article

Title: A retrospective safety study evaluating differences of safety and outcomes of prophylactic versus therapeutic doses of enoxaparin in patients admitted to Charlotte Maxeke Johannesburg Academic Hospital with confirmed COVID-19 pneumonia presenting with disease of mild to moderate severity.

Authors:

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Conflicts of interest: Nil

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Abstract word count: 438

ABSTRACT

Background: Coagulopathy is a common finding in coronavirus disease 2019 (COVID-19) infection and contributes towards disease severity and mortality. International treatment protocols advocate for the early use of anticoagulation therapy for patients admitted with COVID-19 pneumonia.

Objectives: In patients admitted with COVID-19 infection, this study served as a safety study to identify any increased risk of major bleeding in those treated with therapeutic doses (1mg/kg/twice daily subcutaneously) of a low-molecular weight heparin (LWMH, i.e. enoxaparin) compared to those treated with prophylactic doses (0.5mg/kg/daily subcutaneously). In addition, it sought to identify differences in outcomes (other than bleeding) when comparing different enoxaparin doses in patients hospitalised with COVID-19 infection.

Methods and findings: This retrospective study included 100 patients (over the age of 18 years) who were admitted to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) with confirmed COVID-19 pneumonia of mild to moderate severity between 01 March 2020 and 31 August 2020.

Data was captured using the Research Electronic Data Capture (REDCap) database and National Health Laboratory Services (NHLS)-Labtrak. The following outcomes were measured: recovery, progression to invasive ventilation, death, and bleeding.

Of the patients included in this study, 40 were treated with prophylactic doses of enoxaparin and 60 were treated with therapeutic doses. Outcomes were as follows: 81% recovered, 20% progressed to invasive ventilation, and 19% demised. No patients experienced episodes of bleeding during their admission.

Of the recovery group, 42% (n=34) of patients received prophylactic doses of enoxaparin, and 58% (n=47) received therapeutic doses.

With regards to patients who progressed to requiring invasive ventilation and admission to the intensive care unit (ICU), prophylactic enoxaparin doses were administered in 4 patients and 16 patients received therapeutic doses.

Of the patients who demised, 32% (n=6) had been treated with prophylactic enoxaparin doses and 68% (n=13) were treated with therapeutic doses.

Prophylactic enoxaparin was associated with a higher rate of recovery (p-value = 0.001) but also with progression of disease (p-value = 0.0495). Death was approximately twice more common in those treated with therapeutic enoxaparin.

Conclusion: The findings of this study reveal no increased risk of bleeding when using therapeutic doses of enoxaparin as compared to prophylactic doses in the treatment of non-severe COVID-19. Current literature shows that once COVID-19 patients progress to severe disease, use of therapeutic anticoagulation results in a higher risk of bleeding. Our study did not demonstrate such risks. In addition, our study showed that use of prophylactic enoxaparin was associated with increased survival rates when compared to therapeutic doses. This contrasts to existing literature. We recognise the limitations of a small sample size and a unicentric population.

ABBREVIATIONS

ACE-2	Angiotensin converting enzyme-2
ARDS	Acute respiratory distress syndrome
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
COVID-19	Coronavirus Disease 2019
DIC	Disseminated intravascular coagulation
HIV	Human Immunodeficiency Virus
ICU	Intensive care unit
IL-6	Interleukin-6
ISTH	International Society on Thrombosis and Haemostasis
ISTH-SCC BAT	International Society on Thrombosis and Haemostasis-Scientific and Standardisation Committee Bleeding Assessment Tool
LMWH	Low-molecular weight heparin
NHLS	National Health Laboratory Services
NIH	National Institutes of Health
NO	Nitric oxide
PLOS	Public Library of Science
PT	Prothrombin time
REDCap	Research Electronic Data Capture

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TNF	Tumour necrosis factor
UFH	Unfractionated heparin
VTE	Venous thromboembolism
WHO	World Health Organisation

INTRODUCTION

In December 2019, a novel coronavirus named severe acute respiratory syndrome virus (SARS-CoV-2) emerged as the causal agent of coronavirus disease 2019 (COVID-19).[1] The pandemic that followed resulted in high rates of hospitalisation and mortality, overwhelming healthcare services and facilities.[2,3] Prompt recognition of predictors for severe disease was vital for early intervention, lessening disease progression and the need for critical support, improvement of survival rates, and optimal resource allocation.[3,4]

Clinical presentation ranges from asymptomatic or mild symptoms to severe pneumonia, acute respiratory distress syndrome (ARDS), and multiorgan failure.[5,6] Adverse outcomes in COVID-19 are directly related to the virus' ability to evoke a remarkable inflammatory response ("cytokine storm") and procoagulant state, a phenomenon termed "immunothrombosis".[7,8]

Risk factors for severe disease and mortality include older age (>65 years), and comorbidities such as hypertension, type 2 diabetes mellitus, obesity, chronic obstructive pulmonary disease, etc.[1,5] Infection with the Human Immunodeficiency Virus (HIV) has proven an independent risk factor for adverse outcomes in COVID-19.[9] Sub-Saharan Africa is home to nearly two-thirds of the world's HIV population.[9]

SARS-CoV-2 is an enveloped betacoronavirus that is mainly transmitted via respiratory droplets.[5,10] Infection of the host cell is facilitated by the angiotensin converting enzyme-2 (ACE-2) receptor, which is expressed by multiple tissues throughout the body, such as endothelial cells and pneumocytes.[11]

Normal vascular endothelium regulates coagulation and the inflammatory response via the synthesis and release of nitric oxide (NO).[12] Infection of endothelium with SARS-CoV-2 results in unregulated inflammation, thrombosis, and vasoconstriction.[11] Subsequent organ ischaemia and dysfunction propagates inflammation and thrombosis.[6,11]

In the lungs, inflammation and thrombosis contribute to the development of ARDS, which is associated with high mortality.[13,14] The resultant hypoxia exacerbates hypercoagulability and inflammation.[13,15,16]

Hypercoagulability and thrombotic events in COVID-19 are associated with poor outcomes and mortality.[17,18] Studies report that raised D-dimer levels correlate with disease activity and thus severity.[1] Similarly, thrombocytopaenia (platelet count <150 cells x 10^9/L) is also a common finding in severe cases of COVID-19.[4] Probable mechanisms include direct viral infection, bone marrow suppression, and excessive consumption secondary to microthrombi formation.[19,20] The degree of thrombocytopaenia is typically mild (100-150 cells x10^9/L).[6] Evidence suggests that despite abnormal coagulation parameters, bleeding in COVID-19 is rare.[11]

The International Society on Thrombosis and Haemostasis (ISTH) advocates for the use of D-dimer levels to risk stratify patients and guide therapy, thereby promoting efficient use of limited resources.[4] The ISTH also recommends the use of prophylactic heparin anticoagulant therapy for all patients admitted with COVID-19 infection in the absence of contraindications.[4] In addition to halting the overactive coagulation cascade, other benefits may include limiting endothelial damage and organ dysfunction, as well as antiviral properties.[4,21] Evidence has shown that early use of low-molecular weight heparin (LMWH) in patients admitted with COVID-19 pneumonia aided in reducing the incidence of organ dysfunction, need for intensive care and mortality.[2,8]

However, despite the use of prophylactic anticoagulation, research shows that COVID-19 patients still have a high risk of developing thromboembolism.[6,12] Several international trials have sought to demonstrate the superiority of therapeutic doses of anticoagulant therapy over prophylactic doses.[2] Findings of such trials show that in patients hospitalised with non-critical COVID-19 illness, therapeutic anticoagulation improves outcomes.[2] However, once patients progress to severe disease and require organ support, these studies show that use of therapeutic anticoagulation poses no benefit

and in fact increases the risk of bleeding and harm.[2] Thus, the ISTH recommends that therapeutic heparin be used for non-critical COVID-19 patients (provided there are no contraindications) but does not recommend such doses for patients who are critically ill.[19]

Contradicting international guidelines, South African Groote Schuur Hospital instituted treatment protocols involving the use of intermediate doses of heparin when treating COVID-19 pneumonia.[22] There are no comments as to whether this proved advantageous over prophylactic doses, and it does not report any increased incidence of bleeding when using higher doses in their patients.[22]

In South Africa, with its limited resources and burden of HIV, investigating the efficacy and safety of therapeutic anticoagulation may prove valuable in risk stratification, resource allocation, and improving outcomes in COVID-19.[9,23]

METHODS

Ethical considerations:

Ethics approval was granted by the Human Research Ethics Committee of the University of the Witwatersrand (clearance certificate number M210204; Appendix 1). This study has also been submitted to the National Health Research Database (GP_202404_088).

Study population and design:

We conducted a retrospective review. The study included 100 patients (over the age of 18 years) who were admitted to CMJAH with confirmed COVID-19 pneumonia of mild-moderate severity on presentation between 01 March 2020 and 31 August 2020.

Illness severity was classified according to criteria as per National Institutes of Health (NIH) guidelines (Appendix 3). Patients already on anticoagulant therapy (i.e. vitamin K antagonists or novel oral anticoagulant agents) were excluded.

Data was captured using the REDCap database and NHLS-Labtrak. The following information was recorded: age, gender, comorbidities, dose of enoxaparin received, and outcomes. The following outcomes were recorded: recovery, progression, death, and episodes of bleeding (defined as per the International Society on Thrombosis and Haemostasis-Scientific and Standardisation Committee Bleeding Assessment Tool (ISTH-SCC BAT).[24] Progression was defined as deterioration of clinical status leading to requirement of high flow oxygen, or continuous positive airway pressure, or invasive ventilation. Personal details of patients were not recorded.

Statistical data analysis:

Captured data was entered into a Microsoft Excel l (Microsoft Corp, USA) ® spreadsheet. Statistical analysis was done with the assistance of a statistician. Descriptive analysis of participants included use of non-parametric statistics including mean, median and mode. Tests for normality were conducted using the Kolmogorov-Smirnov test. The Chi-squared test, Mann-Whitney test and Kruskal-Wallis tests were used to demonstrate any statistically significant differences between groups.

Data was sorted into contingency tables and Chi-squared analysis and likelihood ratios were used to assess any significant associations. The strength of these associations was then tested using Cramer's V correlation coefficient.

RESULTS

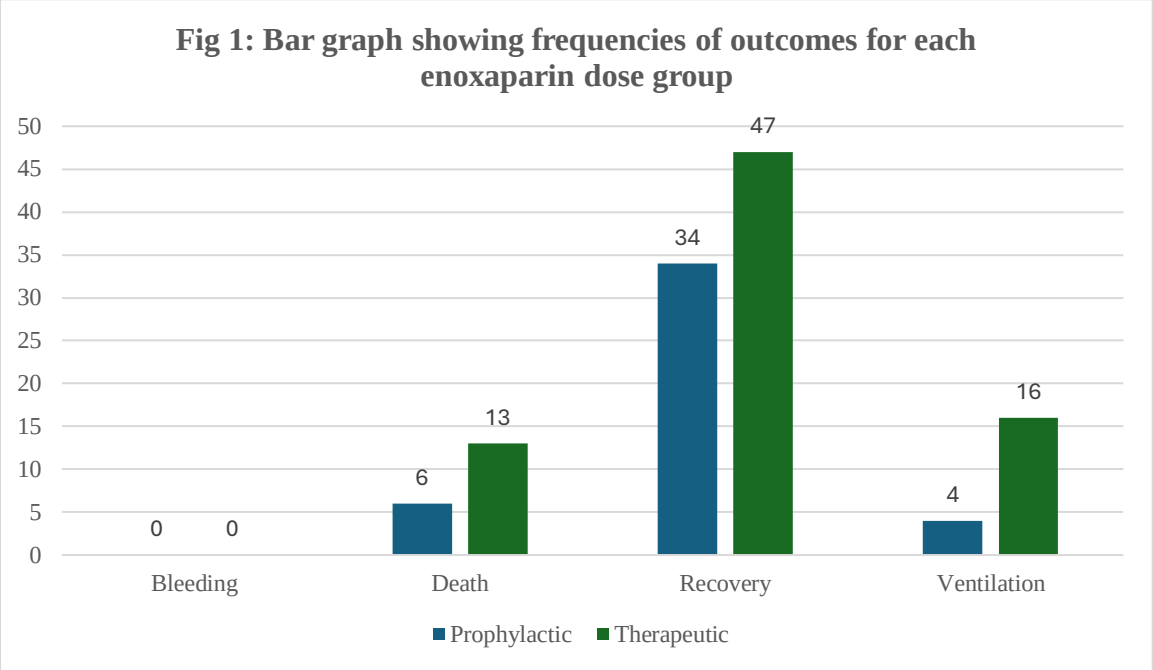
This study included 100 patients. Demographic characteristics and the 5 most common comorbidities are tabulated in Table 1. Most patients were male (n=56) and the most predominant age group was >50 years (n=42). African patients accounted for 81% of the study participants. The most common comorbidity was obesity (n=75), followed by type 2 diabetes mellitus, hypertension, dyslipidaemia, and HIV.

Table 1: Demographic features and most common comorbidities

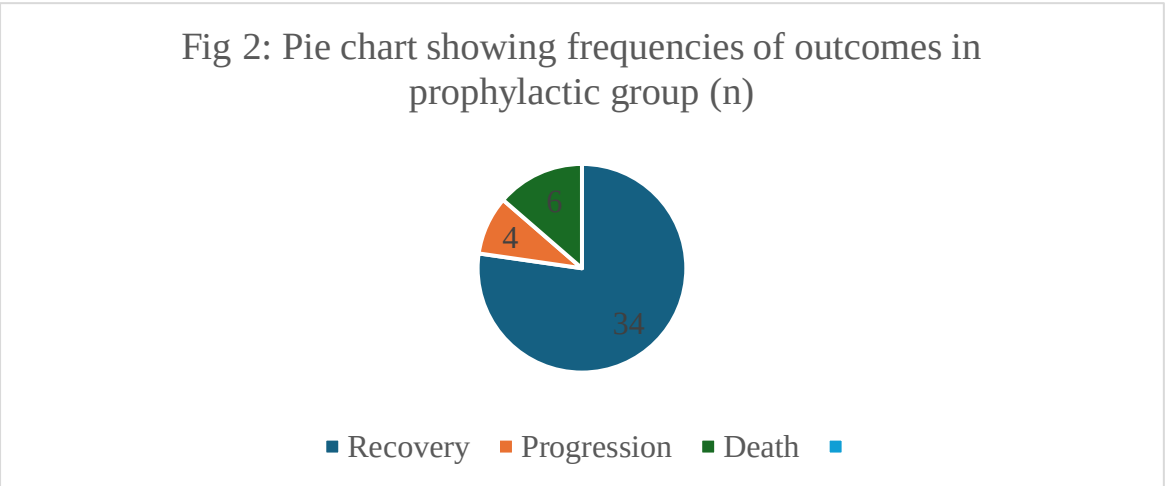
Characteristic	Number
Gender	
Female	44
Male	56
Race	
African	81
Asian	12
Caucasian	6
Mixed	1
Age (years)	
<30	8
30-40	27
40-50	23
>50	42
Top 5 comorbidities	
Obesity	75
Type 2 diabetes mellitus	46
Hypertension	35
Dyslipidaemia	16
HIV	11

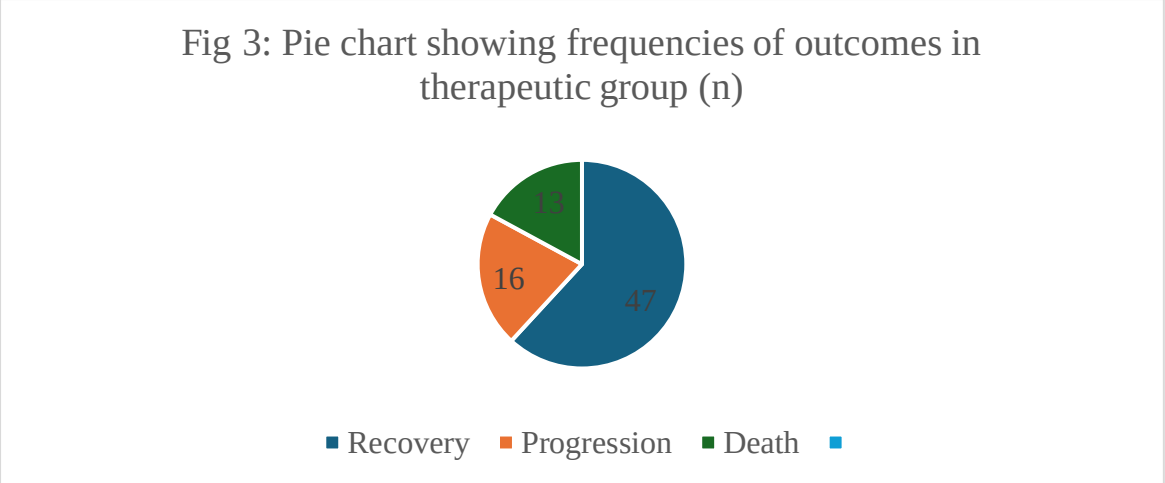
The prophylactic dose group was comprised of 40 patients and the therapeutic group consisted of the remaining 60 participants. Since dosage types and outcomes were categorical variables, the

relationships between them were measured using the Chi-squared test. Prior to this, data was organised into contingency tables to assess the relationship using frequencies. The frequencies of dosage types and outcomes are illustrated in Figure 1.



Within the prophylactic dose group (n=40): 85% were discharged home, 10% progressed to ventilation, and 6% demised. Comparatively, findings of the therapeutic dose group were as follows: 78% were discharged, 27% progressed to ventilation, and 22% demised. These findings are depicted in Figures 2 and 3 below. Regardless of enoxaparin dose, no patients in this cohort experienced any bleeding events.





The Chi-square test was used to assess the relationship between different enoxaparin doses and outcomes. Statistical analysis shows that there is a strong association between prophylactic enoxaparin and recovery (p-value = 0.001). In addition, there is an association between prophylactic enoxaparin and disease progression requiring invasive ventilation (p-value = 0.0495). The incidence of death in the therapeutic group exceeded that of the prophylactic by >50%.

DISCUSSION

Males accounted for most patients included in this study. This is in keeping with current evidence which states that males are more prone to COVID-19.[1] Majority of patients were over the age of 50 years old, supporting current literature which shows that older patients are at greater risk of developing severe disease.[1] The predominant racial category was African, reflecting the racial makeup of South Africa where African people account for approximately 80% of the population.[25] The most common comorbidities found in patients in this study were those associated with cardiovascular disease and obesity, and these findings are consistent with existing studies of COVID-19 risk factors.[9,11]

Poor outcomes in COVID-19 infection are heralded by a florid inflammatory host response and disruption of normal coagulation, shifting the balance to favour thrombosis.[1] Consequences include a progression to ARDS and other thrombotic events, as well as a higher risk of mortality.[21]

Studies show that D-dimer levels correlate with disease severity and can be used to predict outcomes and tailor treatment.[1] Interestingly, raised admission D-dimer levels did not seem to correlate with disease outcomes in this cohort. Indeed, the patient with the highest level (45.5mcg/ml) on admission was successfully discharged from hospital, despite requiring ICU level of care. This contrasts to the literature, suggesting that other factors may play an important role in morbidity and mortality in COVID-19. These may include timing of presentation and intervention of care, comorbidities, and other treatment modalities such as corticosteroids.

International guidelines recommend thromboprophylaxis therapy to mitigate these complications, prevent progression of disease, avoid need for invasive ventilation in high care/ICU settings, and improve patient outcomes.[19] The ISTH recommends the use of therapeutic enoxaparin in non-pregnant hospitalised COVID-19 patients with non-critical illness, in the absence of contraindications.[19] Several studies have noted that use of therapeutic heparin correlates with improved survival in COVID-19 patients who are not critically ill.[2] They also show that in COVID-19 patients who are severely ill, therapeutic heparin seems to offer no benefit and instead confers a risk of harm in the form of bleeding.[2] In contrast, other literature asserts that bleeding in COVID-19 is rare, even in the setting of abnormal coagulation parameters.[4,6] Our study supports the latter: none of our patients experienced bleeding even if they received therapeutic anticoagulation.

None of the patients in this study experienced bleeding, suggesting that use of therapeutic enoxaparin in COVID-19 is safe and does not increase the risk of bleeding in those admitted with mild-moderate illness. However, this may simply be due to the small sample size of this study. Comparison of other outcomes when evaluating differences between prophylactic and therapeutic enoxaparin in this study do not correlate with other similar studies. Where other studies have shown that therapeutic heparin improves survival and prevents disease progression in select COVID-19 patients when compared to prophylactic heparin, our study indicates that prophylactic enoxaparin is in fact advantageous over therapeutic doses in terms of recovery (p-value = 0.001).[2] This was unexpected, but may be

explained by the fact that most of these patients were less than 65 years of age, and thus may have an inherently lower risk of adverse outcomes due to COVID-19.[5] Even when these patients did require escalation of ventilation, they tended to have a higher rate of recovery and hospital discharge.

Our study did show an increased risk of disease progression in the prophylactic group, but this association is weaker than that of recovery (p-value = 0.0495). Possible explanations include the following:

- Risk of thrombotic complications in COVID-19 are high even when prophylactic anticoagulation is administered, highlighting the possible consideration for higher doses in select cases.
- Data review revealed a lack of body weight measurements of patients, which may indicate ineffective anticoagulant doses in some patients.
- Other patient factors contributing towards morbidity and mortality, including but not limited to older age, pre-existing comorbidities, and in-hospital complications such as sepsis.
- Possible lack of high care/ICU facilities, making it difficult to escalate care when indicated and leading to poorer outcomes.
- Administration of additional therapies such as IL-6 antagonists or colchicine.

Death was more common in the therapeutic group than the prophylactic group. Again, this did not correlate with existing literature. Analysis of the patients in the therapeutic group who demised revealed that most were obese and had other comorbidities that may have influenced outcomes.

CONCLUSION

Coagulopathy is common in COVID-19 and is associated with severe disease and mortality.[17,18] Anticoagulant therapy in the form of heparin (preferably low-molecular weight) is an integral pillar of treatment of COVID-19.[19] Several studies show that therapeutic anticoagulation improves outcomes in non-critical patients but harmful in patients with severe disease.[2] In contrast to the

literature, this study showed that prophylactic enoxaparin to be advantageous over therapeutic in the treatment of COVID-19 pneumonia of mild-moderate severity. It did, however, support the current evidence that bleeding is a rare complication of COVID-19.[4,6] Thus, while our study suggests that use of therapeutic enoxaparin may be safe when treating COVID-19 patients, it may not confer any advantage over prophylactic doses. We do, however, recognise the limitations of a small and unicentric population sample and that other therapies (e.g. corticosteroids) may have had unmeasured impacts on outcomes. Larger and more diverse studies are necessary to tailor guidelines for South Africa, a resource-limited country with a unique HIV burden, to improve patient outcomes and optimise resources.

ACKNOWLEDGEMENTS

I would like to express gratitude towards my supervisors, Prof Barry Jacobson and Dr Jason Edgar for their advice and guidance. I also would like to thank Mr Mduduzi Moyo, the statistician who assisted with the statistical analysis in this paper.

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APPENDIX 1: Human Research and Ethics Committee approval

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Dr Sharise Singh

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

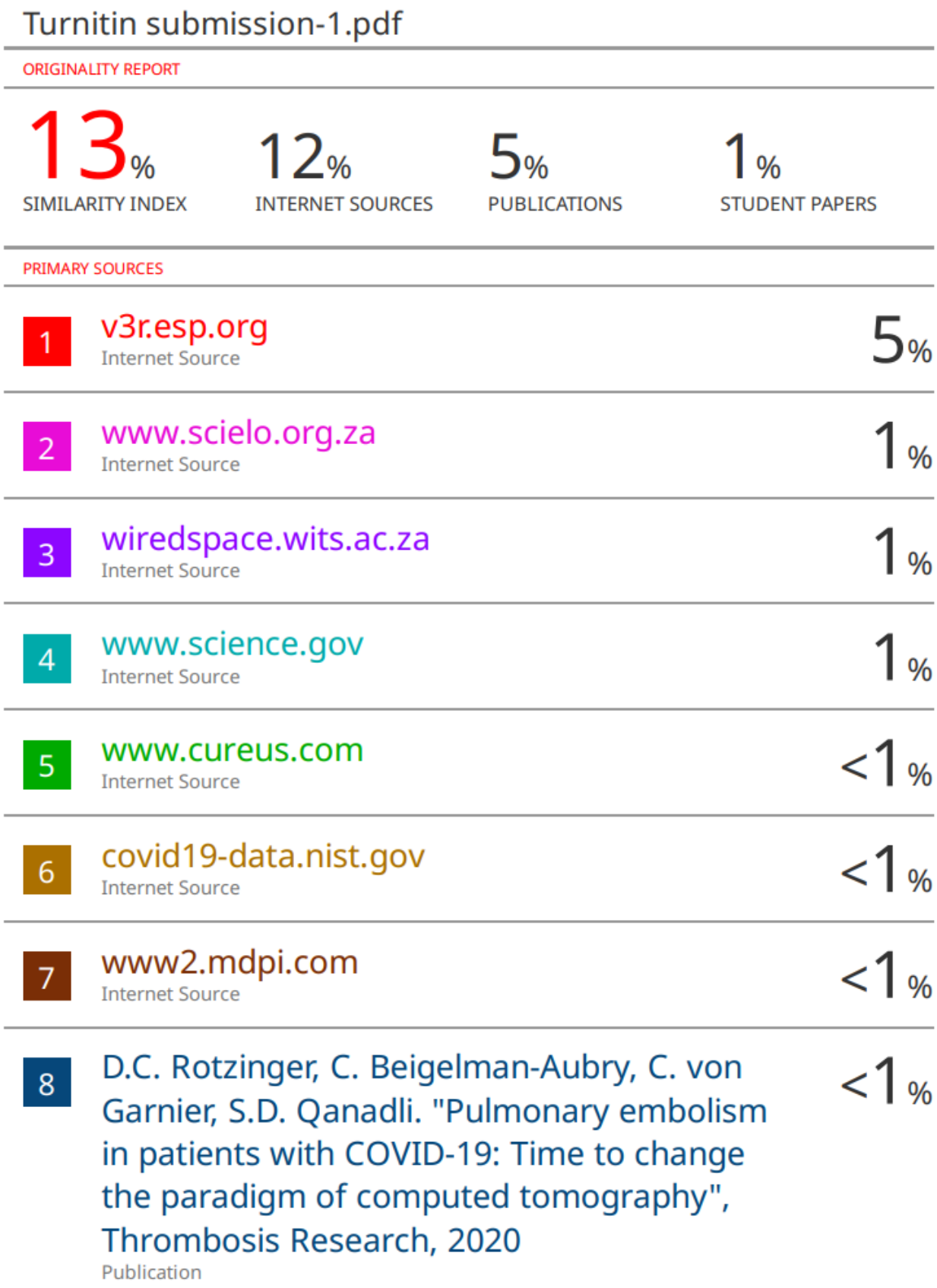
CLEARANCE CERTIFICATE NO. M210204

NAME: (Principal Investiqator)	Dr Sharise Singh
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A retrospective safety study evaluating differences of safety and outcomes of prophylactic versus therapeutic doses of enoxaparin in patients admitted to Charlotte Maxeke Johannesburg Academic Hospital with confirmed Covid-19 pneumonia of moderate-critical severity on presentation (a safety study for the Proper Study)
DATE CONSIDERED:	26/02/2021
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Barry Jacobson and Dr Jason Edgar
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL:15/06/2021

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

APPENDIX 2: TurnItIn originality report



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APPENDIX 3: National Institutes of Health illness severity classification in adults

- Asymptomatic/pre-symptomatic infection
- Mild:
    - Mild symptoms but no dyspnoea
    - Normal chest radiographs
- Moderate:
    - Evidence of pneumonia either on clinical examination or imaging
    - Room air oxygen saturation levels ≥ 94% at sea-level
- Severe:
    - Respiratory rate > 30 breaths/minute
    - Room air oxygen saturation levels < 94% at sea-level
    - Arterial partial pressure of oxygen to fraction of inspired oxygen ratio < 300mmHg
    - Chest imaging showing > 50% infiltrates
- Critical:
    - Respiratory collapse
    - Haemodynamic instability
    - Multiorgan failure

REFERENCE: COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed [21 December 2021].