# 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 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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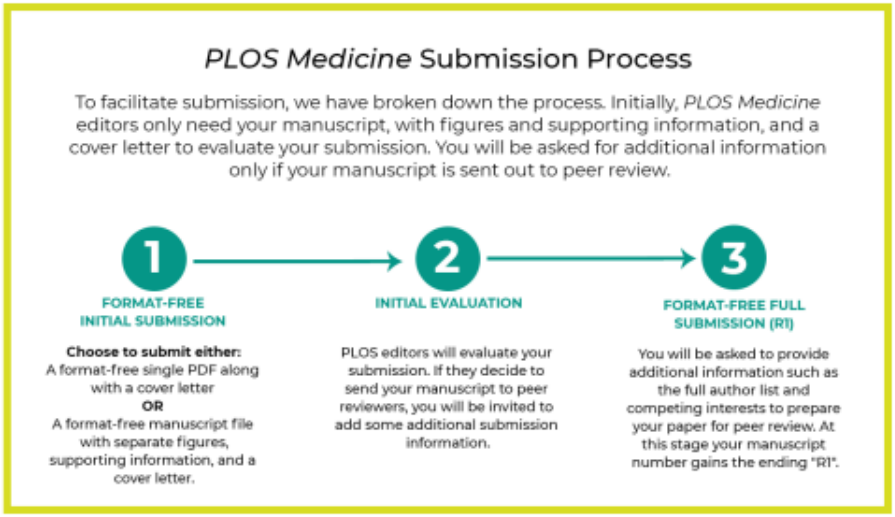


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**Acknowledgments**

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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:**

1. S Singh[a]
2. B Jacobson[b]
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a. Department of Internal Medicine, School of Health Sciences, University of the Witwatersrand, Johannesburg, Gauteng, South Africa
b. Department of Internal Medicine, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, Gauteng, South Africa
c. Private practice, Mediclinic Milnerton, Cape Town, Western Cape, South Africa

**Conflicts of interest:** Nil

**Corresponding author:** Sharise Singh

**Email:** sharisesingh@gmail.com

**Telephone:** 083 387 1380

**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 x$10^9$/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**

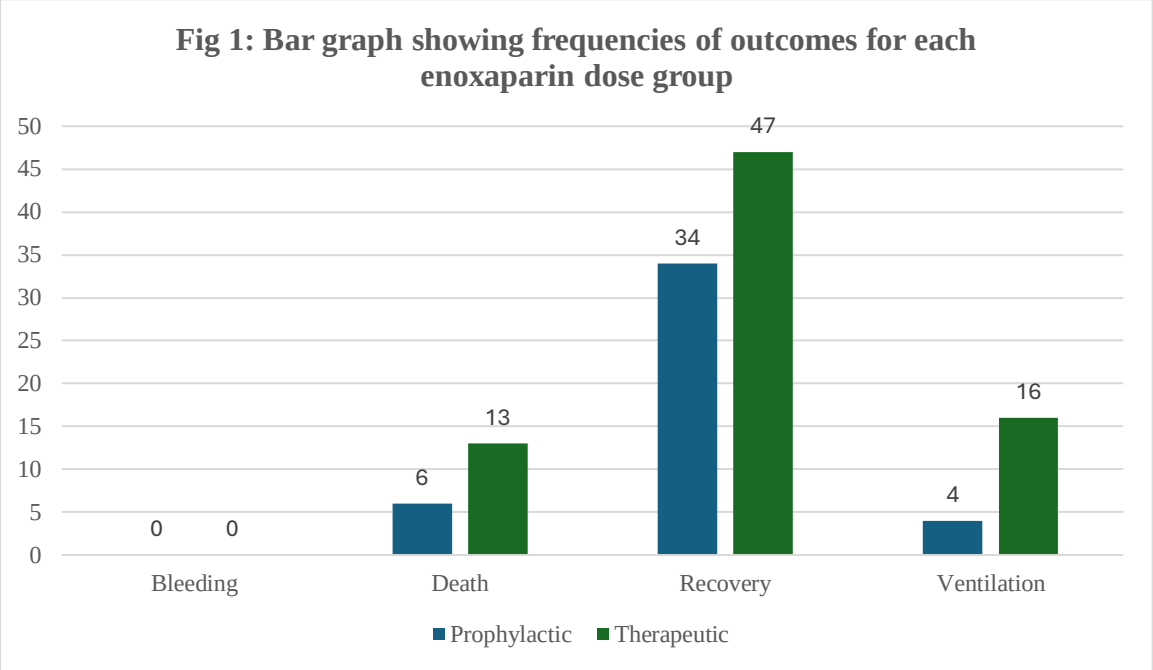
This study included 100 patients. Demographic characteristics and the 5 most common comorbidities are tabulated in Table 1. Most patients were male (n=56) and the most predominant age group was >50 years (n=42). African patients accounted for 81% of the study participants. The most common comorbidity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

**Table 1: Demographic features and most common comorbidities**

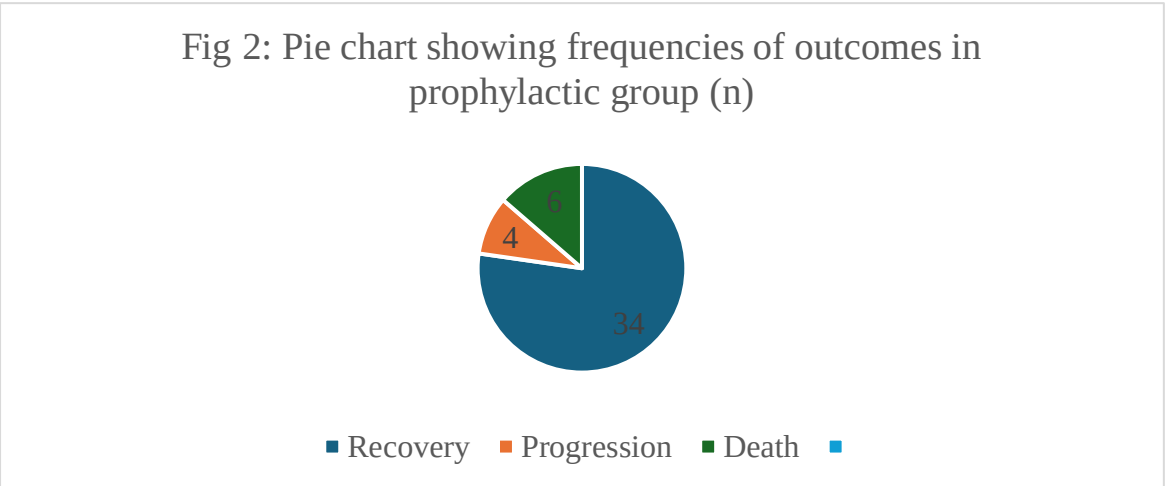
| Characteristic | Number |
|---|---|
| **Gender** | |
| Female | 44 |
| Male | 56 |
| **Race** | |
| African | 81 |
| Asian | 12 |
| Caucasian | 6 |
| Mixed | 1 |
| **Age (years)** | |
| <30 | 8 |
| 30-40 | 27 |
| 40-50 | 23 |
| >50 | 42 |
| **Top 5 comorbidities** | |
| Obesity | 75 |
| Type 2 diabetes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

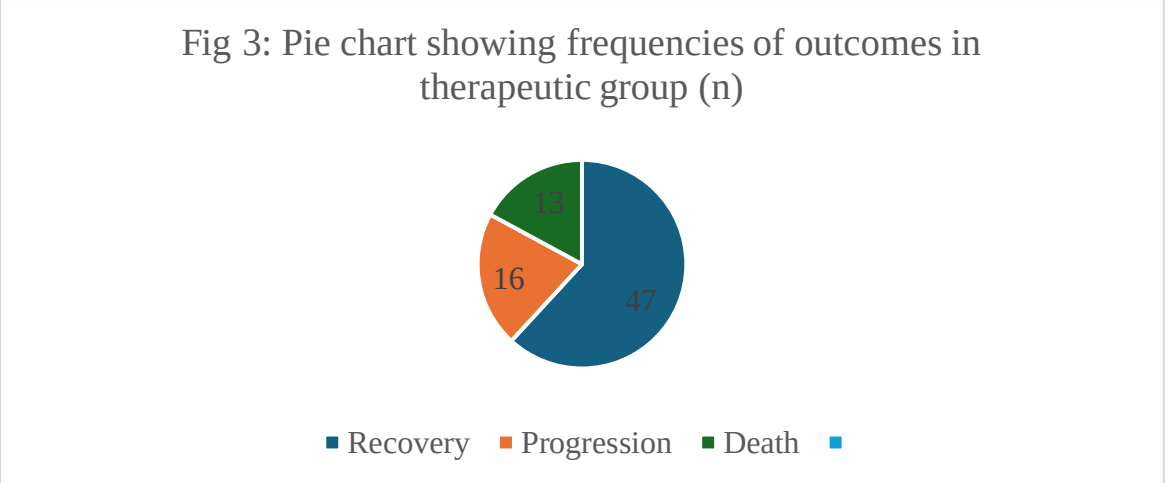
The prophylactic dose group was comprised of 40 patients and the therapeutic group consisted of the remaining 60 participants. Since dosage types and outcomes were categorical variables, the

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)

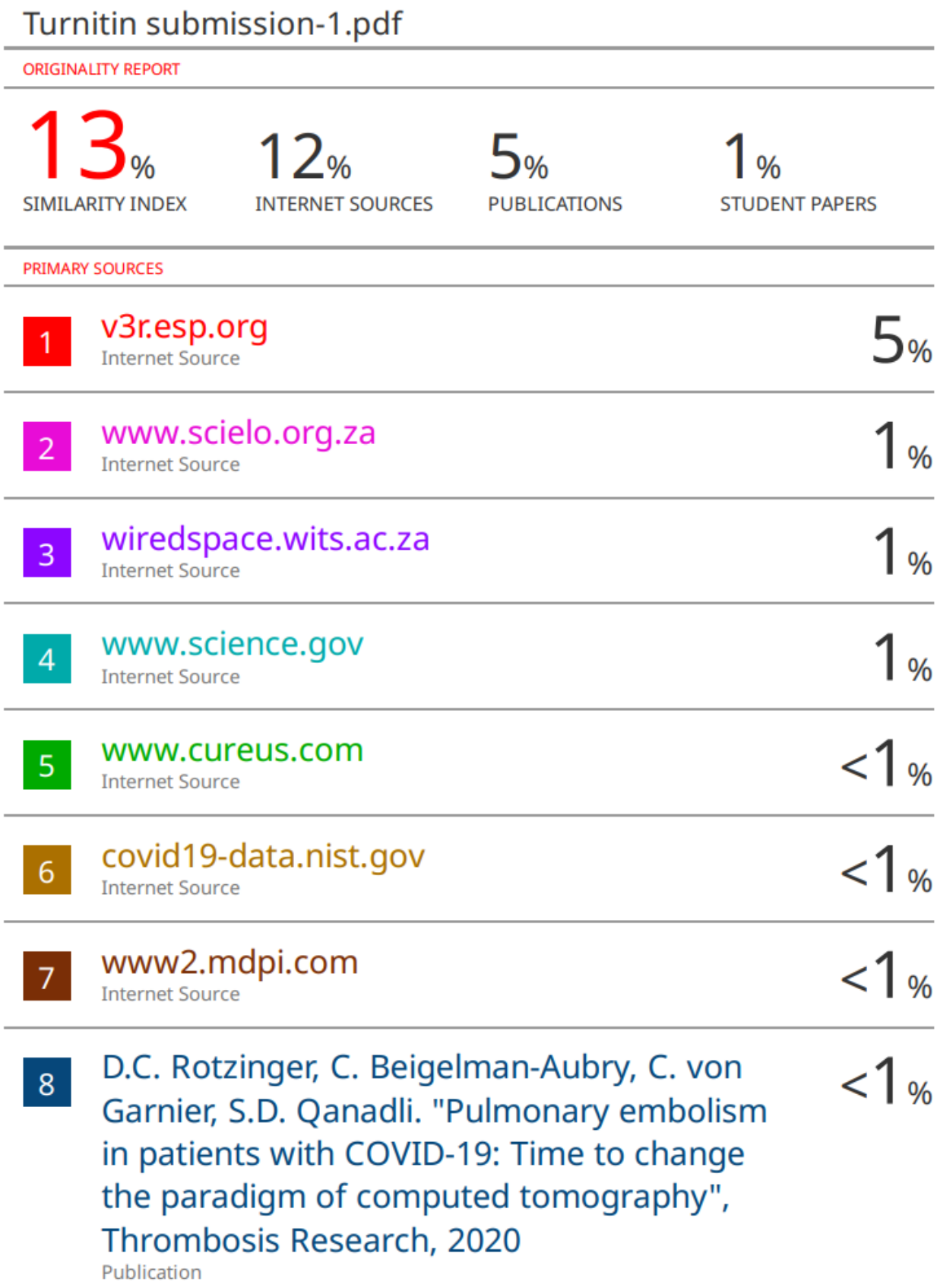
# CLEARANCE CERTIFICATE NO. M210204

| | |
|---|---|
| NAME: (Principal Investiqator) | Dr Sharise Singh |
| DEPARTMENT: | Internal Medicine<br>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].