Risk Factors for False-Negative Covid-19 Testing in the Emergency Department: A Retrospective Cohort Study

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Final Project

Scholarly Pursuit and Thesis

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Abstract

Research question

In adults who present to the emergency department for Covid-19 testing, what clinical characteristics are associated with false-negative Covid-19 test results?

Background, significance, and rationale for the question

Covid-19 has made enormous impacts on human health and society. Prior to Covid-19 vaccines and effective treatment strategies, medical and public health officials focused on reducing the morbidity and mortality of infections through early detection and social isolation. These strategies relied on dependable tests that could accurately confirm or exclude the disease. Early in the pandemic, important testing parameters were poorly understood. The lack of complete information on test accuracy had tremendous consequences. Covid-19 test results influence behavior, as those with a positive test result are more likely to socially isolate and cancel in-person plans, thereby limiting disease spread. Negative test results, however, often cause people to participate in their daily activities, like going to work or school in-person. False-negative Covid-19 test results can lead to devastating repercussions, as people with active Covid-19 infection may unknowingly infect others and to forgo other strategies to treat their own disease. The purpose of this study was to identify risk factors for potential false-negative Covid-19 tests. This information could be used to inform patient management, preserve limited resources, and educate patients to reduce the burden of Covid-19.

Materials and methods

This was a multi-center retrospective cohort study of patients who presented to the emergency department at one of 12 study hospitals in Texas, USA from June 1 to August 31, 2020 who were tested for Covid-19. Eligible patients received an initial negative Covid-19 test by reverse transcriptase chain reaction (RT-PCR), followed by a second, positive Covid-19 RT-PCR test within 30 days. We used Wilcoxon signed-rank testing (for categorical variables) and paired *t*-testing (for continuous variables) to examine clinical factors that were different between the first and second visits.

Results

After screening 23,687 emergency department patient encounters, a total of 88 patients were included in the study. Patient-reported symptom duration was shorter for the first ED visit compared to the second (2.6 ± 0.3 days versus 3.6 ± 0.4 days, p-value=0.02). The findings from the first ED encounter that were most commonly associated with Covid-19 in our sample were increased lymphocyte count (35.2%), increased body temperature (32.6%), feeling nauseous (29.5%), and difficulty breathing (27.9%). Compared to the first visit to the ED, patients in our sample had differences in the second ED visit, which included the following: hypoxia (13.6% in the second visit versus 4.6% in the first, p-value: 0.005), abnormal infiltrates on chest radiography (59.7% in the second visit versus 25.9% in the first visit, p-value < 0.001), and elevated aspartate aminotransferase (29.1% in the second visit compared to 9.1% in the first visit, p-value <0.001).

Conclusions

Healthcare providers in the emergency department should understand the factors that may be associated with false negative Covid-19 tests. In our analysis, the biggest factor was a short duration of symptoms (symptoms for less than 3 days). During this window period, the main clinical features were fever, nausea, and difficulty breathing. Additionally, laboratory investigations showing an increased white blood cell count, especially lymphocytes, was also associated with a false-negative result. This information could be used to guide patient management by re-testing in the ED, encouraging patients to re-test at home or another healthcare facility in 1-2 days, and urging patients to social distance while awaiting repeat testing and diagnostic confirmation.

Research Question

PICO Framework:

- Population: Adults with Covid-19-like symptoms presenting to the emergency department with an **first negative test** for Covid-19 followed by a **subsequent positive test** for Covid-19 within 30 days
- Intervention/Indicator: Clinical characteristics from a patient's **first** emergency department visits (when they had a **negative** Covid-19 test)
- Comparison: The same clinical characteristics during the patient's **second** emergency department visit (when they had a **positive** Covid-19 test)
- Outcome: Clinical characteristics, including demographics, history, vital signs, physical exam findings, laboratory investigations, and imaging studies.

Research Questions:

1. In adult patients who presented to the emergency department (ED) twice within 30-days for Covid-19 testing at one of the 12 affiliated study hospitals in north Texas from June to August 2020, how did key clinical characteristics compare between the first ED visits (with a negative Covid-19 test) and the second ED visits (with a positive Covid-19 test)?
2. In this study sample during the study duration, what variables were associated with a false-negative Covid-19 test?

Study Goals:

The purpose of this study was to identify possible variables associated with a false-negative Covid-19 test. This information is valuable because knowing characteristics associated with a false-positive test can change how medical professionals manage patients who test negative for Covid-19 in a variety of settings, including emergency departments, urgent care centers, hospital wards, outpatient clinics, and in the community. Patients with a negative Covid-19 test but certain features that put them at high-risk for receiving a false-negative test result should be advised to act as though they had tested positive, re-test within a short window, avoid close contacts with others, or make other changes to their behavior and daily routines until they receive a more definitive positive or negative Covid-19 diagnosis. Patients can be assured of definitive diagnosis through subsequent testing, evaluation with a different product, or confirmation or an alternative diagnosis.

In order to accomplish this research goal, the specific aim of this thesis was to evaluate clinical characteristics of patients who had a probably false-negative Covid-19 among five domains. We aimed to identify the prognostic utility of factors within these domains that may be associated with a false negative Covid-19 test by comparing clinical characteristics of the first ED visit against those some characteristics at the second visits. The variables we investigated as possibly useful to predict a false-negative Covid-19 test result were organized into the following five domains:

1. Historical features
2. Vital signs
3. Physical exam findings
4. Laboratory investigations
5. Imaging studies

Hypotheses

Prior to starting data collection, I made several predictions about possible variables that would be associated with Covid-19 false-negative test results. It is important to review these hypotheses with the understanding that this work was conducted in the Fall of 2020, when Covid-19 was new, poorly understood, and there were no vaccinations to reduce the likelihood of contacting Covid-19, and no approved monoclonal antibody treatments. Furthermore, these hypotheses were generated at a time when there was fierce debate about scientists, doctors, politicians, and the general public about a variety of topics related to Covid-19, including the origin of the virus and its associated disease, the virology and structure of the Covid-19, the pathophysiology of Covid-19 transmission and illness, and best practices for prevention, diagnosis, and treatment of the Covid-19. This study also took place during a time of immense controversy of public policy in response to Covid-19, with significant disputes on topics such as masking, social distancing, changing schooling and work from in-person to virtual environments, funding for Covid-related activities, enforcement of these policies, and other issues. It is also important to recognize that at the time of this study, there were very few Covid-19 test kits that were available over-the-counter, and so most individuals needed to go to a clinical facility like an emergency department, doctor's office, or urgent care center to receive a Covid-19 test. Finally, the accuracy of various Covid-19 tests (testing modality, brand, best practices for lab methods, etc.) was incompletely understood, and the sensitivity and specificity of said tests were unclear.

With those considerations in place, the hypotheses that myself and the research team working on the project that became my thesis developed were as follows. We included both variables that we suspected **would** and **would not** be associated with a false-negative Covid-19 test. These hypotheses are organized according to the five study domains as outlined above.

1) Historical Features

- The following historical features **would** be associated with a false-negative Covid-19 test result:
    - Recent travel to an area with a high prevalence of Covid-19
    - Recent exposure to an individual with confirmed Covid-19
    - Cough
    - Shortness of breath
    - Ageusia (inability to taste, loss of taste perception)
    - Anosmia (inability to smell, loss of odor perception)
- Other symptoms (headache, earache, sore throat, chest pain, abdominal pain, myalgias) would **not** be associated with a false-negative Covid-19 test result.

2) Vital Signs

- Tachycardia (elevated heart rate), tachypnea (elevated respiratory rate), fever (elevated temperature), and hypoxia (decreased oxygen saturation) **would** be associated with a false-negative test result.
- Hypotension (decreased blood pressure) and hypertension (increased blood pressure) would **not** be associated with a false-negative test result. This hypothesis included predictions for both systolic and diastolic blood pressure measurements.

3) Physical Exam Findings

- Abnormal pulmonary exam findings, including increased work of breathing and abnormal lung sounds **would** be associated with a false-negative Covid-19 test result.
- Other physical exam findings would **not** be associated with a false-negative Covid-19 test result. These including normal and abnormal findings of the head,

4) Laboratory Investigations

- Leukocytosis (an elevated white blood cell count) **would** be associated with a false-negative Covid-19 test result.
    - Specifically, the leukocytosis would be driven by a lymphocytosis (an elevated level of lymphocytes, the cells that are involved in the immune response to viruses and fungi).
    - In contrast, we suspected there would be a normal absolute neutrophil count. Neutrophils are the white blood cell lineage that is most frequently increased in response to bacterial infections.
- Other components of the complete blood count, including hemoglobin, hematocrit, and platelet count would **not** be associated with a false-negative Covid-19 test result.
- Comprehensive metabolic panel components (sodium, potassium, chloride, bicarbonate, creatinine, blood urea nitrogen, glucose, aspartate aminotransferase [AST], alanine aminotransferase [ALT], and bilirubin) would **not** be associated with a false-negative Covid-19 test result.
- Additional laboratory evaluations, including C-reactive protein, lipase, D-Dimer, and others would **not** be associated with a false-negative Covid-19 test result.

5) Imaging Studies

- A pneumonia-like pattern of chest infiltrates **would** be associated with a false-negative Covid-19 test result.
- Other chest X-ray findings, including abnormalities of the clavicles, ribs, sternum, cardiac silhouette, ventricles, atria, great vessels, trachea, esophagus, stomach, gastric bubble, and diaphragm would **not** be associated with a false-negative Covid-19 test result.

Introduction

Covid-19 Background and History

The infectious disease Covid-19 is caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Reports of strange illness that would later be identified as Covid-19 were first noted among individuals living in the Chinese city of Wuhan, in the provide of Hubei, in December of 2019.[2] Over a rapid period of time, the viral illness spread to other parts of China, other countries in Asia, and quickly to many other nations on other continents.[3] On March 11, 2020, the Director-General of the World Health Organization (WHO) officially declared that Covid-19 was a global pandemic.[4] In the wake of this announcement, governments across the world quickly enacted public policies to attempt to reduce the morbidity and mortality associated with Covid-19, which resulted in many companies transitioning their employees to full-time report work, schools transition to only-only learning, mask mandates, and other interventions attempting to reduce the spread of Covid-19.[5] By the spring of 2020, this disease had not only overwhelmed hospitals and healthcare facilities around the world, but also had massive social and financial impacts, which disrupted daily life, economies, schooling, and the fabric of society.[6–10]

By March 2022, over six million people had died from Covid-19, most frequently from the consequences of severe lung disease or as a result of multiple organ failure secondary to pulmonary infection.[11] The mortality rate of Covid-19 has varied significantly across time and space as different populations have been unequally impacted by the disease.[12–15] The case fatality rate has significantly declined over the last 2 years, likely due to the protective effectiveness of vaccination, increased natural immunity due to infection, the benefits of public policy, and advances in medical treatment tools and protocols.[16,17] Despite these gains, the effects of Covid-19 are still very real, as the economic, societal, and educational impacts are still present today. Additionally, many people live with the effects of the condition known as “long covid”, a somewhat poorly-understood syndrome that impacts individuals after recovering from the acute illness of Covid-19 in a variety of ways.[18–20]

It is important to consider the timeline of this thesis in the context of the historical trends of Covid-19. At the time that this project started, the sensitivity and specificity of Covid-19 tests were incompletely known, home test kits were unavailable, vaccines had not been developed, and there was massive public debates about stay-at-home orders, masks mandates, and other public policies. As such, identifying additional information about how to prevent the spread of Covid-19 infections through proper diagnostic testing was a critical component of reducing the overall morbidity and mortality of the disease. The focus of this project was to explore possible risk factors for a “false-negative” Covid-19 test. By clearly defining the clinical characteristics associated with false negative tests, doctors and other healthcare providers could change their patient care strategies to better educate and manage patients while conserving limited resources (like Covid-19 test kits). To accomplish this goal, we collected data on patients who likely had a false-positive Covid-19 test result in the emergency department. Before further describing the specific aims of this thesis, it is important to briefly review diagnostic testing parameters (sensitivity, specificity, positive predictive value, and negative predictive value, and their implications) as well as how testing with reverse transcriptase polymerase chain reaction works.

Review of Diagnostic Testing Parameters

This thesis requires an understanding of diagnostic testing terminology from the fields of epidemiology and biostatistics. In this sub-section, I will briefly review important testing parameters necessary for a comprehensive understanding of the goals and purpose of this project. First, it is important to start with the basic principle that *no diagnostic test is 100% accurate.* A discussion of diagnostic testing parameters assumes a situation in which the disease being tested for is either present or absent, and the device testing for it can be either positive or negative. In reality, there may be more options than these two sets of binary outcomes, but for the purpose of this discussion, we will assume that these are the only possibilities. Additionally, in clinical practice it can be difficult to truly know when the disease is present or absent, and this result is often made by comparing to a "gold standard", some other test or study that may be more expensive or more expensive, but is typically well-studied and believed to provide accurate diagnostic information.

Test results can be correct or incorrect in multiple ways (Table 1).[21–25] A **true positive** result happens when a person who actually has disease receives a positive test result. A **true negative** result occurs when a person who actually lacks the disease receives a negative test result. Both true positive and true negative test results constitute correct test results, in both cases, the diagnostic test has accurately reported the individual's true disease status. However, incorrect test results are also possible in two situations: false negatives and false positives. A **false positive** occurs when someone who lacks the disease receives a positive test result. Finally, a **false negative** occurs when someone with disease receives a negative test result.

Table 1. Diagnostic testing possibilities

	Disease Present	**Disease Absent**
Positive test result	**True Positive** (Correct test result)	**False Positive** (Incorrect test result)
Negative test result	**False Negative** (Incorrect test result)	**True Negative** (Correct test result)

Using these four possible results as an underlying framework, there are several testing parameters that can be derived to describe the accuracy of diagnostic testing tools: sensitivity, specificity, positive predictive value, and negative predictive value (table 2). The **sensitivity** of a test, also called the **true positive rate** is defined as the number of true positive results, divided by all patients who actually have the disease. A highly sensitive test is good at ruling disease out. Sensitivity is calculated as follows:

Sensitivity *= True Positives / (True Positives + False Negatives)*

The specificity of a test, also called the **true negative rate** is defined as the number of true negative test results, divided by all patients who lack the disease. A highly specific test is useful for confidently **ruling disease in.** In other words, if a test is known to have a high specificity, and the test result is positive, it is very likely that the individual does not have the disease. It is calculated as follows:

Specificity *= True Negatives / (False Positives + True negatives).*

Next, the **Positive Predictive Value (PPV)** of a test is used to determine the likelihood of actually having the disease, given a patient has received a positive test result. PPV is defined as the number of true positive test results divided by all patients with a positive test result. PPV can be calculated as follows:

Positive Predictive Value *= True Positives / (True positives + false positives)*

Finally, the **Negative Predictive Value (NPV)** of a test is used to determine the likelihood that an individual truly does not have the disease, given they have already received a negative test result. NPV is defined as the number of true negative test results divided by all patients with a negative test result. NPV can be calculated as followed:

Negative Predictive Value *= True Negatives / (True negatives + false negatives)*

Table 2. Explanation of sensitivity, specificity, positive predictive value, and negative predictive value based on the results of a diagnostic test and an individual's true disease state

	Disease Present	**Disease Absent**	
Positive test result	**True Positive** (TP)	**False Positive** (FP)	**Positive Predictive Value** (TP/TP+FP)
Negative test result	**False Negative** (FN)	**True Negative** (TN)	**Negative Predictive Value** (TN/FN+TN)
	Sensitivity True positive rate (TP/TP+FN)	**Specificity** True negative rate (TN/FP+TN)	

The sensitivity and specificity of a test are intrinsic properties of the test itself. As such, a test's sensitivity and specificity do not change based on the frequency of disease in the population. In contrast, positive predictive value and negative predictive value are influenced by the amount of disease in the underlying population. In any given population, as the prevalence of disease increases, the positive predictive value of a test that is measuring that disease will also increase (the NPV will decrease). Conversely, as the prevalence of said disease decreases, the negative predictive value increases (and the PPV decreases). It is

important to reiterate that the prevalence of disease does *not* impact sensitivity or specificity. Therefore, the sensitivity and specificity of a RT-PCR test for Covid-19 are intrinsic to the test itself, and are not impacted by the prevalence of Covid-19 in a community.

Reverse Transcriptase Polymerase Chain Reaction

There are countless modalities that are used for diagnostic testing in clinical environments, including laboratory investigations and imaging studies. Laboratory investigations include biological assays such as blood counts, serum chemistry analyses, antigen testing, microbiological culturing of the body tissues (blood, urine, feces, skin, organ tissue, etc.), Western-Blots, and others. Imaging studies can be used alongside - or instead of - laboratory studies to support the clinical history and physical exam when making diagnoses in clinical settings. Imaging tests include x-ray radiograph, computed tomography ("CT scans"), ultrasonography, and magnetic resonance imaging, among others. Each modality, both laboratory-based and imaging-focused, contributes to the diagnostic workup of any given patient condition and overall management.

Among the types of laboratory tests is Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). This thesis focuses exclusively on RT-PCR for Covid-19 testing as that was the gold standard diagnostic test employed at the study hospitals during the study period, although other testing modalities exist. This molecular diagnostic technique excels in detecting and amplifying specific regions of the SARS-CoV-2 viral genome, making it possible to identify the virus even in very low quantities. The technical prowess of RT-PCR lies in its multi-stage process, where viral RNA is first converted into complementary DNA (cDNA) and then amplified exponentially. This amplification is achieved through a series of temperature changes, each facilitating different stages of the reaction, from denaturing the viral RNA to extending the DNA strands. The precision and efficiency of RT-PCR are attributes that make it superior in sensitivity and specificity compared to other testing methods, like antigen or antibody tests.

The reliability of RT-PCR in accurately diagnosing Covid-19, particularly in the early stages of infection, is a cornerstone in patient management and infection control in emergency departments. Its high sensitivity ensures that even cases with low viral loads are detected, which is critical in preventing the spread of the virus by asymptomatic or pre-symptomatic individuals. However, its limitations, such as the requirement for specialized equipment and the potential for longer processing times, must be acknowledged. These factors can pose challenges, especially in resource-limited settings or situations demanding rapid turnaround times for decision-making in clinical care.

Our study's focus on RT-PCR's role in Covid-19 diagnosis underscores the importance of understanding and navigating the intricacies of this testing method. It highlights the need for continued innovation and improvement in diagnostic technologies, ensuring they remain robust, accessible, and efficient in responding to evolving public health challenges. In conclusion, while RT-PCR has played a pivotal role in managing the Covid-19 pandemic, the continuous advancement in diagnostic techniques will be vital in our ongoing battle against this and future infectious diseases.

Study Background and Significance

The reliability and accuracy of Covid-19 RT-PCR was highly controversial at the onset of this study. Better understanding the diagnostic parameters of these tests as well as clinical characteristics that may lead to false-negative results had huge implications for individual wellbeing as well as public health and safety. This was especially true in the emergency department, which served as one of the most accessible and frequent locations for individuals to undergo Covid-19 evaluation. Accurate and dependable test results are of utmost importance for guiding patient management, quarantine/social isolation decision-making, public policy, and hospital interventions.

As a result, the possibility of false-negative Covid-19 results could lead individuals with Covid-19 infections to make decisions that they would not make had they received a positive Covid-19 test result instead. If these individuals had been correctly diagnosed, their behavior would likely have been altered to reduce the spread of the infection. Therefore, understanding the risk factors associated with false-negative RT-PCR tests in the ED is critical. Patients presenting with these risk factors, despite testing negative, should potentially be managed as presumptive positive cases.

This gap in knowledge about the risk factors associated with false-negative Covid-19 tests necessitates further investigation. Identifying these factors is imperative to enhance diagnostic accuracy, inform clinical decision-making, and ultimately reduce the transmission of Covid-19.

Thesis Purpose and Goals

The purpose of my thesis was to identify potential risk factors associated with a false-negative Covid-19 RT-PCR test result. By comparing clinical characteristics from patients' initial and subsequent ED visits, this research aims to fill a critical knowledge gap, leading to better resource allocation, reduced disease transmission, and improved patient management and education. The findings of this study have the potential to address a critical knowledge gap, allowing for more efficient use of resources such as Covid-19 tests and ED staffing. Importantly, identifying these risk factors can contribute to reducing disease transmission, lowering morbidity and mortality, and providing targeted patient education and advice. This research endeavors to inform clinical practice by enabling more nuanced approaches to managing patients who test negative for Covid-19 but may still carry the virus.

Materials and Methods

Ethical Statement and Reporting

This study received Institutional Review Board (IRB) approval by the Baylor Scott and White Health IRB (reference ID number: 344143). The Baylor Scott and White IRB also waived the requirement for patients to provide informed consent to enroll in the study because this project was non-interventional and was conducted as a retrospective review of pre-existing electronic medical records. This thesis followed the Strengthening of Observational Studies in Epidemiology (STROBE) guidelines to present information.[26] The authors strictly adhered to the ethical principles for medical research involving human subjects as described in the Declaration of Helsinki.[27]

Study Design and Setting

This was a multi-center retrospective cohort study of patients who were seen at the emergency department of associated study hospitals. These study sites were part of the Baylor Scott & White Health system, the largest health network in the state, and operates hospitals across northern and central Texas, United States of America. In the United States, hospitals can be classified according to their trauma-center level, on a four-point scale, with level I representing the most comprehensive level of care, and level IV offering basic services.[28] The hospitals affiliated with this study include level I trauma centers, level II trauma centers, level III trauma centers, and level IV trauma centers. These hospitals are also located in rural, suburban, and urban geographies.

Study Population

This study sampled from patients who met eligibility criteria (see below) and who were seen in the emergency department at one of the affiliated study hospitals. We screened and sampled patients with visits during the three month period between the study initiation time point of June 1, 2020 through the study termination time point of August 31, 2020.

Eligibility Criteria

To be included in this study, patients had to be 18-years of age or older. Patients had to have two ED visits within a 30-day period. The first ED visit had to include a test for Covid-19 using reverse transcriptase polymerase chain reaction (RT-PCR), with a negative result. The second ED visit had to have a RT-PCR-based Covid-19 test with a positive chart. We excluded pediatric patients, patients with excessive missing data in their EMR charts (missing data on more than 50% of variables analyzed), pediatric patients, and patients who received Covid-19 tests with other testing modalities besides RT-PCR. A summary of the study design is presented in **Figure 1.**

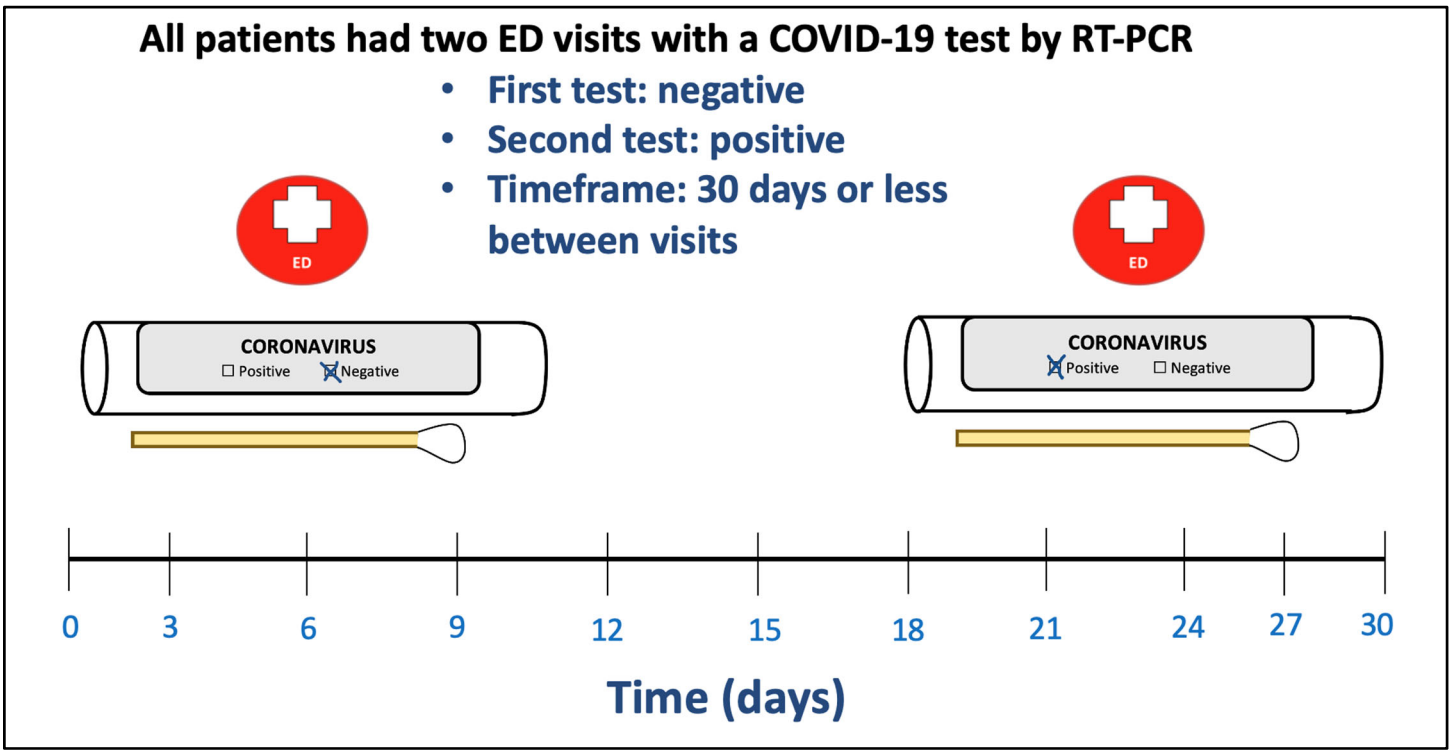

Figure 1. Overview of thesis study design

Definitions and Variables

Researchers extracted clinical data about subjects from the electronic medical record (EMR) system utilized by the health system, Epic (Verona, Wisconsin, USA). This process was facilitated through the employment of an enterprise data warehouse, ensuring accuracy and efficiency. Subsequently, the extracted data underwent a thorough review conducted by the research team. The principal aim of the study focused on conducting a comparative analysis of clinical characteristics observed across two distinct Emergency Department (ED) visits. This comparison encompassed a comprehensive evaluation of clinical symptoms, vital signs, and the results derived from laboratory tests and imaging studies, specifically, chest x-ray Specifically, the study sought to identify the presence of abnormal infiltrates on CXR, which was characterized as any form of unilateral or bilateral lung involvement evident on the CXR images.

Statistical Analysis

In this study, categorical data are reported as frequencies and their corresponding percentages, whereas continuous data are expressed as means accompanied by their standard deviations. The assessment of categorical variables utilized the Wilcoxon signed-rank test, whereas continuous variables were evaluated using the paired t-test. Statistical significance was established at a two-tailed p-value of less than 0.05. All statistical analyses were performed using Stata version 16.1 (provided by Stata Corp LLC, College Station, Texas, USA).

Results

Participant Screening and Inclusion

A total of 23,687 emergency department patient encounters during the three-month study period included at least one RT-PCR test for Covid-19. Of those, 3,640 adults over the age of 18 years-old (15.4% of the total tested) had a positive result. Of those, 128 had received an initial false test before receiving their positive test. However, 40 of those patients had received the false test more than 30 days before the subsequent positive test, and were therefore removed as per the study eligibility criteria. In total, 88 patients were included who had received a false Covid-19 RT-PCR test followed by a positive Covid-19 RT-PCR test within 30 days or less. We assumed that the first test was false-negative and referred to them as such.

Patient Demographics

Baseline patient characteristics of the individuals who were included in this study are summarized in **Table 3.** The mean age of participants in this study was 48.8 years old (± 21.9 years). Our sample was predominately female, with 53 women (60.2%) and 35 men (39.8%). About one quarter (23 patients, 26.1%) of our sample was Hispanic. Almost half of the sample had commercial insurance (43 patients, 48.9%), with Medicaid and Medicare being the insurance of 18 patients (20.4%), and 23 patients (26.1%) having no insurance. Individuals in our sample tended to be overweight and obese, with a BMI of 32.0 (± 8.6).

Table 3. Baseline characteristics of study participants

Characteristic	**Patients (n=88)**
Age (mean, SD)	48.8 (21.9)
Male (n, %)	35 (39.8)
Hispanic (n, %)	23 (26.1)
Insurance status (n, %)	
- Commercial	43 (48.9)
- Medicaid/Medicare	18 (20.4)
- No insurance	23 (26.1)
- Other	4 (4.6)
BMI (mean, SD)	32.0 (8.6)

Comorbid Conditions

In addition to analyzing patient demographics and baseline characteristics, we also reported patient comorbid conditions (**Table 4).** These were conditions other than Covid-19 that the patient had a medical history of experiencing. Out of 88 study subjects, 33 patients (37.9%) had a history of smoking. This included both former and current smokers. Over three-fourths (67 patients, 76.1%) of the sample had at least comorbidity, and over half (35 patients, 51.7%) had more than one comorbid condition. The most common comorbidity was hypertension, with almost half of the sample (42 patients, 47.7%) having this condition. Other frequently occurring comorbidities were diabetes (22.7%), psychiatric disease (14.8%), chronic renal disease (10.2%), depression (10.2%), and congestive heart failure (9.1%). There were three conditions of interest in which no patients in our sample had the disease. These include Hepatitis B, gastroparesis, and sickle cell disease.

Table 4. Comorbid conditions of patients presenting to the emergency department from Covid-19 testing by RT-PCR during their first ED visit

Condition	**Patients (n=88)**
Smoking history (n, %)	33 (37.9)
With any comorbidity (n, %)	67 (76.1)
More than one comorbidity (n, %)	35 (51.7)
COPD (n, %)	6 (6.8)
Asthma (n, %)	6 (6.8)
Diabetes mellitus (n, %)	20 (22.7)
Hypertension (n, %)	42 (47.7)
Coronary artery disease (n, %)	5 (5.7)
Congestive heart failure (n, %)	8 (9.1)
Cerebrovascular disease (n, %)	6 (6.8)
Hepatitis B (n, %)	0 (0)
Hepatitis C (n, %)	2 (2.3)
Cirrhosis (n, %)	3 (3.4)
Cancer (n, %)	7 (8.0)
Current chemotherapy (n, %)	1 (1.1)
Chronic renal disease (n, %)	9 (10.2)

ESRD (n, %)	3 (3.4)
History of solid organ transplant (n, %)	2 (2.3)
Immunodeficiency (n, %)	1 (1.1)
HIV (n, %)	1 (1.1)
Rheumatologic disease (n, %)	7 (8.0)
Dementia (n, %)	4 (4.6)
Peptic ulcer disease (n, %)	1 (1.1)
Gastroparesis (n, %)	0 (0)
Sickle cell disease (n, %)	0 (0)
Migraine (n, %)	3 (3.4)
Fibromyalgia (n, %)	1 (1.1)
Chronic pain syndrome (n, %)	1 (1.1)
Alcohol abuse (n, %)	1 (1.1)
Substance abuse (n, %)	3 (3.4)
Depression (n, %)	9 (10.2)
Psychiatric disease (n, %)	13 (14.8)
Pregnancy (n, %)	4 (4.6)

Differences Between First and Second ED visits

The primary outcome of this study was identifying clinical characteristics that were different between the first and second emergency department visits (**Table 5**). The mean duration for symptoms in the first ED visit was significantly lower compared to the first (2.6 ± 0.3 days for the first visit versus 3.6 ± 0.4 vs. days for the second visit, p = 0.020) (**Table 5, Figure 2**).

In the first ED visit, lymphocytopenia (35.2%), fever (32.6%), nausea (29.5%), and dyspnea (27.9%) are the most common signs of COVID-19 infection during the window period. Leukopenia (5.7%), Hypoxia (4.6%), and thrombocytopenia (2.3%) were the least common in the first ED visits (Table 1). In the second ED visits, cough (54.0% vs. 18.8%, p < 0.001), dyspnea (43.7% vs. 27.9%, p = 0.010), and fever (46.0% vs. 32.6%, p = 0.015) were significantly higher than that in the first ED visit. There were significant increases in hypoxia (13.6% vs. 4.6%, p = 0.005), findings of abnormal infiltrate on CXR (59.7% vs. 25.9%, p < 0.001), and aspartate aminotransferase (AST) elevation (26.1% vs. 9.1%, p < 0.001) in the second ED visit.

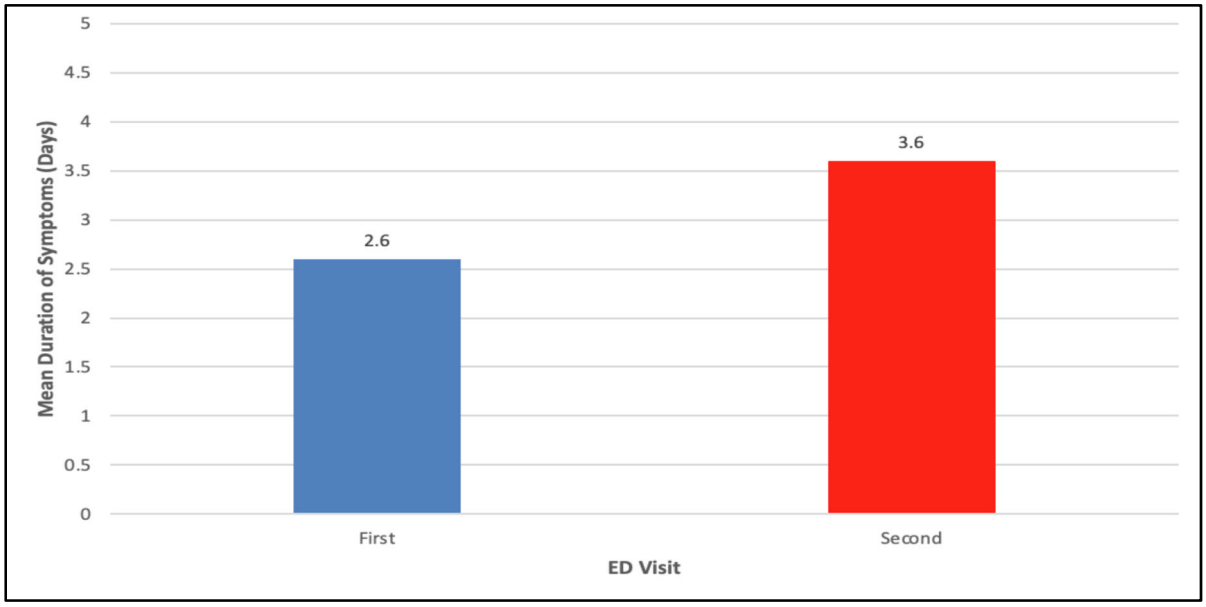

Figure 2. Duration of symptoms between first and second ED visits

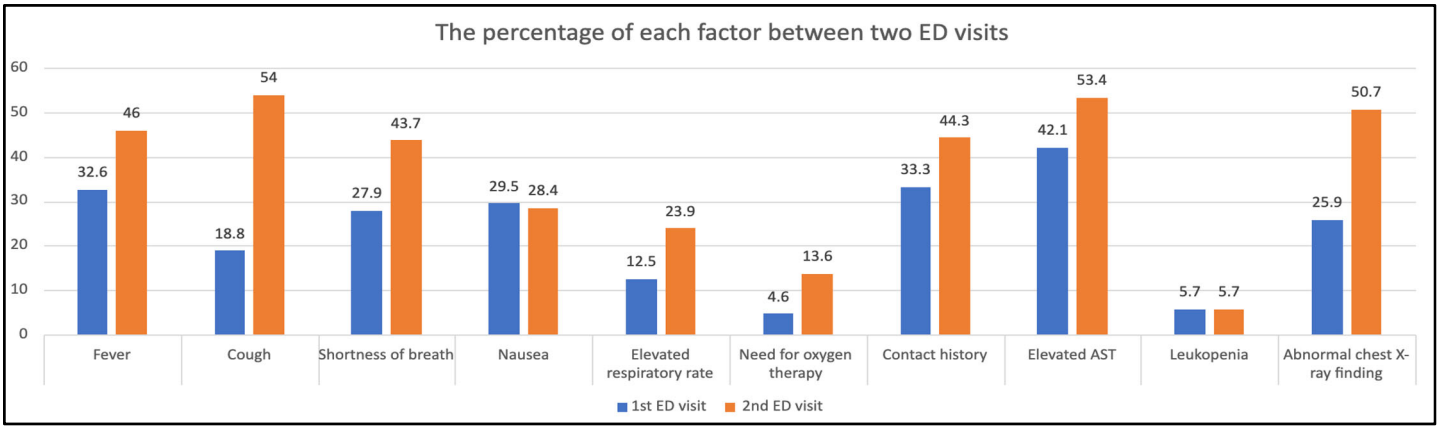

Figure 3. Key clinical characteristics between first and second ED visits

Table 5. Differences in clinical characteristics between patients' first ED visit (with a negative Covid-19 test result) and their second ED visit (with a positive Covid-19 test result).

Characteristic	1st ED visit	2nd ED visit	p-value
Duration of symptoms, mean (SD), unit, days	2.6 (0.3)	3.6 (0.4)	0.020
Symptoms, n (%)			
- Cough	16 (18.8)	47 (54.0)	< 0.001
- Dyspnea	24 (27.9)	38 (43.7)	0.010
- Fever	28 (32.6)	40 (46.0	0.015
- Chills	11 (12.5)	20 (22.7)	0.050
- Myalgias	17 (19.3)	28 (31.8)	0.056
- Diarrhea	11 (12.5)	19 (21.6)	0.108
- Fatigue	22 (25.0)	30 (34.1)	0.194
- Hemoptysis	0 (0.0)	1 (1.1)	0.317
- Sore Throat	10 (11.4)	14 (15.9)	0.371
- Stuffy nose	12 (13.6)	9 (10.2)	0.491
- Nausea	26 (29.5)	25 (28.4)	0.849
- Headache	20 (22.7)	20 (22.7)	1.000
Triage vital signs			
- Hypoxia, n (%)	4 (4.6)	12 (13.6)	0.005
- Tachypnea, n (%)	11 (12.5)	21 (23.9)	0.011
- Body temperature (Fahrenheit), mean (± SD)	98.8 (0.1)	99.1 (0.2)	0.046
Infiltration on chest radiography	14 (25.9)	40 (59.7)	< 0.001
Laboratory values			
- Aspartate aminotransferase > 40 U/liter, n (%)	8 (9.1)	23 (26.1)	< 0.001
- Lymphocyte count < 1,500 cells/mm3, n (%)	31 (35.2)	36 (40.9)	0.317
- Creatinine > 1.2 mg/dL, n (%)	20 (22.7)	22 (25.0)	0.527
- Alanine aminotransferase > 40 U/liter, n (%)	12 (13.6)	10 (11.4)	0.564
- White blood cell count < 4,000 cells/ mm3, n (%)	5 (5.7)	5 (5.7)	1.000
- Thrombocytopenia, n (%)	2 (2.3)	2 (2.3)	1.000

Discussion

Study Summary

The purpose of this thesis was to identify possible risk factors for a false-negative Covid-19 test by reverse transcriptase polymerase chain reaction (RT-PCR). To achieve this goal, we performed a retrospective study of patients who received a negative Covid-19 RT-PCR test result in the ED, followed by a positive test within 30 days. We assumed that the original test result was a false-positive, and that the patient actually had a Covid-19 infection at the time. We then compared clinical characteristics between the first and second ED visits to identify possible variables that are associated with a false-negative Covid-19 test.

We found that the duration of symptoms was significantly longer in the second ED visit (3.6 days) compared to the first (2.6), suggesting that RT-PCR Covid-19 testing too early after symptom onset may lead to a false-negative test. Our data also revealed that the most common early symptoms of Covid-19 infection (based on the prevalence during the first ED visit) were elevated lymphocyte count on blood testing, difficulty breathing, feeling nauseous, and having a fever. Furthermore, between the first and second ED visits, patients had significant differences in occurrence of hypoxia, abnormal lung infiltrates on chest radiography, and an increase in the liver enzyme aspartate aminotransferase.

Testing Implications

This study, encompassing over 23,000 ED patient visits and identifying 88 cases of initial false-negative Covid-19 results, highlights the critical need for heightened vigilance in Covid-19 testing protocols. The significant increase in symptom duration and the prevalence of specific clinical signs like cough, dyspnea, and fever in the second ED visit compared to the first, underscores the dynamic nature of the disease and the potential limitations of early RT-PCR testing. The findings suggest that a single negative RT-PCR result should not be the sole determinant in ruling out Covid-19, especially in patients presenting with suggestive clinical features. The evolution of symptoms like lymphocytopenia, hypoxia, and abnormal chest X-ray findings between the two visits further reinforces the idea that disease progression can affect test sensitivity. These observations emphasize the need for re-evaluation and possibly re-testing of patients who initially test negative but continue to exhibit or develop symptoms consistent with Covid-19.

Clinical Applications in the Emergency Department

From a clinical perspective, the study's findings have significant implications for emergency department protocols. The higher incidence of symptoms like cough, dyspnea, and fever in the second visit implies that these signs might be more predictive of Covid-19 as the disease progresses, which could guide clinicians in their decision-making and threshold for re-testing. The notable increase in hypoxia and abnormal chest X-ray findings in the second visit indicates that radiological and laboratory markers should be considered alongside clinical evaluation in suspected cases of Covid-19, particularly in the setting of an initial negative test. The data suggest that a more cautious approach, perhaps involving serial assessments or a lower threshold for additional testing, might be warranted in specific patient groups.

Implications for Patient Management and Public Health

On a broader scale, the study underscores the importance of continual monitoring and flexible patient management strategies in the context of Covid-19. The significant changes in clinical presentation and laboratory findings between the two visits highlight the disease's unpredictable course and the potential for rapid deterioration. This necessitates a dynamic approach to patient care, where initial negative results are not overly relied upon in the face of evolving clinical pictures. The findings also have implications for public health, as early identification and isolation of Covid-19 cases are crucial in controlling the spread of the virus. The data from this study can inform policy changes, particularly in reinforcing guidelines for repeat testing and the management of patients with persistent or worsening symptoms, even after a negative Covid-19 test. This approach can potentially reduce community transmission and better allocate healthcare resources during the ongoing pandemic.

Study Strengths

This study has meaningful strengths. We conducted this study, and then shared our results as a published abstract, virtual research presentation, and peer-review journal article early in the pandemic when this information was useful to make potentially life-saving decisions and also conserve limited resources.[29,30] This study's primary strength lies in its focused approach to identifying risk factors for false-negative Covid-19 RT-PCR test results by comparing clinical characteristics of patients across two different ED visits.The methodology, which includes a retrospective review of patients who initially tested negative and later tested positive within a short time frame, allows for a direct examination of clinical changes and potential risk factors associated with false-negative results. The use of paired t-tests to compare clinical data strengthens the statistical validity of the findings. Furthermore, the study's setting in the emergency department, a critical point for initial Covid-19 assessment and management, provides valuable insights that are highly relevant to real-world clinical scenarios. This research fills a significant knowledge gap in Covid-19 diagnostics and has the potential to directly impact patient management, resource allocation, and public health strategies.

Study Limitations

Despite its strengths, this thesis also has important limitations that must be acknowledged. First, this study was a retrospective analysis of pre-existing data using electronic medical record data. Inherent to any retrospective study design is the possibility that we failed to fully control for all confounding variables, both measured and unmeasured. It is possible that uncontrolled confounding impacted the ascertainment of Covid-19 testing status and/or the clinical characteristics that we found as possible predictors of a false-negative test result. Second, an additional inherent issue with any retrospective observational study design is the inability to make direct causal inferences. While we found statistically significant associations between false-negative Covid-19 tests as several clinical characteristics (short duration of symptoms, for example), it is impossible to know the causal relationship between those predictor variables and the outcome. Third, this study assumed that the first ED visit Covid-19 test was a false-negative result. While this is likely the case in the vast majority of patients, we cannot know this definitively. It is possible that some patients may have had a true-negative Covid-19 test at their first ED visit, subsequently contracted the virus from another source, and then returned to the ED for their second ED visit where they received a positive Covid-19 test result. Finally,

Future Directions

The prevention, diagnosis, management, and treatment of Covid-19 has improved remarkably since the onset of the pandemic, almost four years ago. The speed and efficiency of these technological advancements and medical discoveries is a testament to human scientific achievement. Building on the findings of this study, future research should aim to prospectively validate the identified risk factors for false-negative Covid-19 RT-PCR tests in a more diverse and larger patient cohort. Such studies would benefit from a multicenter design, encompassing various geographic locations and healthcare settings, to enhance the generalizability of the results. Investigating the temporal dynamics of viral load and immune response in relation to the timing of the test could provide deeper insights into the mechanisms behind false negatives. Additionally, future research should carefully consider how these trends differ based on vaccination status (vaccinated versus unvaccinated, total number of vaccines received, most recent vaccine date, vaccine manufacturer, etc.). These efforts would not only refine diagnostic strategies but also contribute to more personalized patient management approaches, particularly in emergency settings.

From a policy and clinical standpoint, the outcomes of this research should be leveraged to develop updated guidelines for Covid-19 testing in emergency departments. This could include recommendations for repeat testing in patients with specific clinical characteristics or risk factors, even in the face of an initial negative result. Furthermore, the study's implications could extend to public health strategies, advocating for targeted educational campaigns and resource allocation to areas with higher rates of false negatives. On a clinical level, the findings could inform the development of decision-support tools for frontline healthcare workers, aiding in the early identification and isolation of potential Covid-19 cases. Quality improvement initiatives might focus on enhancing record-keeping practices and standardizing clinical assessments to ensure the consistent and accurate capture of data relevant to Covid-19 diagnostics. These future directions not only promise to enhance patient care but also have the potential to significantly impact the overall management of the pandemic.

Conclusions

Healthcare workers must understand the limitations of any diagnostic test when making patient care decisions that are depending on said test results. Our study underscores the profound implications of false-negative Covid-19 RT-PCR test results, which carry the potential for severe consequences, including increased transmission and possibly fatal outcomes. Accurate diagnostic results are pivotal for effective disease management and containment. Recognizing and addressing the risk factors associated with false-negative Covid-19 tests is therefore of utmost importance.

Our research identified a shorter duration of symptoms prior to testing as a significant risk factor for a false-negative result. This insight is particularly relevant for emergency physicians, who often encounter patients in the early stages of symptom development. Our findings also highlighted specific clinical indicators that may suggest a false-negative test, including but not limited to lymphocytopenia, fever, nausea, dyspnea, and changes in radiological and laboratory markers such as hypoxia and abnormal infiltrates on chest X-rays.

In light of these findings, it is recommended that patients who test negative for Covid-19 via RT-PCR but exhibit one or more identified risk factors should be managed with heightened caution. This includes considering strategies such as repeat testing in the ED, advising re-testing at home within 1-2 days, and potentially initiating early quarantine measures. Such patients should be counseled to adopt behaviors as if they are Covid-19 positive, including social distancing, working or studying from home, and postponing or canceling in-person plans. This approach could play a critical role in minimizing the risk of further virus transmission and ensuring the safety of both the patient and the wider community.

The findings from our study contribute significantly to the ongoing efforts to refine Covid-19 management strategies, highlighting the need for continuous vigilance and adaptability in diagnostic and treatment protocols during this global health crisis.

Compliance

My study team and I remained in compliance from the inception of this study up to the point of writing this thesis. As stated in the methods, this study was approved by the Baylor Scott and White Health IRB (reference ID number: 344143). The Baylor Scott and White IRB also waived the requirement for patients to provide informed consent to enroll in the study because this project was non-interventional and was conducted as a retrospective review of pre-existing electronic medical records. All patient data remained confidential using a secure database.

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