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Peer Review of “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years”

Heikki Vapaatalo, MD, PhD
Department of Pharmacology, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Related Articles:
Companion article: http://preprints.jmir.org/preprint/45220
Companion article: https://med.jmir.org/2023/1/e45280/
Companion article: https://med.jmir.org/2023/1/e45220/

(JMIRx Med 2023;4:e45278) doi:10.2196/45278

KEYWORDS
aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is a peer-review report submitted for the paper “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years.” [1]

Round 1 Review

General Comments
The study is interesting, and the title promises more than the manuscript finally contains. The background, question, and the aim are relevant as explained in the Introduction.

The major concerns the small size of the material (6 subjects), the small age difference (64-67 years), and the lack of younger controls.

Specific Comments
Title: ACE seems better than ACE1; or, does the sophisticated, elegant method include both ACEs? The same should be explained and taken into consideration throughout the text.

Introduction: in the last chapter, the author should explain in more detail how Pawelec et al [2], Alves et al [3], Alves and Bueno [4], and Bueno et al [5] suggest that “ACE1 plays an important role in the aging process.” Does “ACE1 plays” mean, that ACE1 is somehow regulating the aging process or are ACE1 levels changed with age?

Methods: The N value of the subjects should be mentioned here, as well the relation of females and males. Do the authors really regard 64-67 years “older age” nowadays? The study lacks younger controls. Why were the initial assays done many years after the collection of blood samples? Are the samples still usable and not destroyed? Did the subjects have some diseases or were taking drugs because they possibly were from a hospital sample bank? Provide the companies’ details.

Results: “Table 1 shows that older adults…..” The comparison between the present data and historical studies belongs to the Discussion. Also, provide individual ages and genders of the subjects in Table 1. What do P values mean here—what is being compared, or are interindividual differences being highlighted in the particular variables? This should be explained. The numbering of tables and the text seems confusing to me. Only 3 tables, but in the text, 4 are mentioned. Table 4 does not exist. It would be good to have a list of abbreviations used in the description of the cell types for an unfamiliar reader.

Discussion: A major part of the discussion deals with previous publications and not meaning or clinical significance of the present findings and comparison between the present and earlier studies. In those previous studies, ACE2 has also been reported; why is it not studied here? In the limitations paragraph, the authors fairly mention the real problem—the small sample size, and I would like to add a lack of younger subjects. The point regarding the COVID-19 pandemic, seemingly worth mentioning, is too far from this study and unnecessary. Linguistic checking would improve the manuscript.

In summary, I recommend the acceptance of the manuscript for publication after the authors carefully rethink the message of the Results and correct per the minor comments. I hope that in the future, possible age-related correlations to old age of up to >80 years would be possible.

Decision
Verified with reservations: The content is scientifically sound but has shortcomings that could be improved by further studies and minor revisions.

Decision Changed
Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.
Round 2 Review

I read with pleasure the very detailed answers to my comments. I very warmly recommend the acceptance of this manuscript for publication without any further notes.

Decision Changed

Verified manuscript: the content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

References

1. Bueno V, Destro PH, Teixeira D, Frasca D. Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years. JMIRx Med 2023 Jan;4:e45220 [FREE Full text]

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Peer Review of “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years”

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University of Palermo, Palermo, Italy

Related Articles:
Companion article: http://preprints.jmir.org/preprint/45220
Companion article: https://med.jmir.org/2023/1/e45280/
Companion article: https://med.jmir.org/2023/1/e45220/

(JMIRx Med 2023;4:e45279) doi:10.2196/45279

KEYWORDS
aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is a peer-review report submitted for the paper “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years.” [1]

Round 1 Review

General Comments
The paper is essentially anecdotal because it studies the cells of 6 subjects without any comparison with other age groups. There is also a serious limitation because beyond the age and sex, there is no information on the donors (how and why they were recruited, what drugs they took, etc). To infer that chronological and biological ages do not match is inappropriate in the absence of the above information.

However, the paper is of some interest because there are few studies on the topic.

Specific Comments
Essential revisions that are required to verify the manuscript

Although we do not have data on donors, placing an age and gender column in all tables adds a minimum of useful information for the reader.

Inflammaging means low grade of inflammation. The CRP value of 23.1 suggests acute inflammation (also because albumin has high values, while in chronic inflammation its values decrease). Therefore the averages do not have to take this subject into account.

Other suggestions to improve the manuscript

The authors write that their findings suggest that ACE1 could play a role in several processes linked to aging including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE suppress the autoimmune process in a number of autoimmune diseases such as EAE, arthritis, autoimmune myocarditis [2]. They do not appear to have these findings in their paper. So, they need to change the sentence.

Decision
Requires revisions: The manuscript contains objective errors or fundamental flaws that must be addressed and major revisions are suggested.

Decision Changed
Verified manuscript: The content is scientifically sound, only minor amendments (if any) are suggested.

Round 2 Review

Decision Changed
Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest
None declared.

Editorial Notice
This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.
References

1. Bueno V, Destro PH, Teixeira D, Frasca D. Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years. JMIRx Med 2023 Jan;4:e45220 [FREE Full text]

Peer-Review Report

Peer Review of "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"

Dacre Knight¹, MD
Mayo Clinic, Jacksonville, FL, United States

Related Articles:
Companion article: https://preprints.jmir.org/preprint/43880
Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45304) doi:10.2196/45304

KEYWORDS
COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper “Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review.”

Round 1 Review

Serious Concerns
Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?
No.

Have authors’ provided the necessary ethics approval (from authors’ institution or an ethics committee)?
Not applicable.

Language Quality
How would you rate the English language quality?
High quality.

Validity and Reproducibility
Is the reasons for conducting the study and its objectives clearly explained?
Yes.

Are sufficient details provided so that the method can be replicated?
Yes.

Are datasets available so that others could use them?
Not applicable.

Suggestions
Based on your answers in section 3 how could the author improve the protocol?
There is a more specific definition of PASC that should be included (with a reference). There is a need to list specific medical databases to search and not just mention “various” [1]. PECO criteria need to be listed and not only implied that they will be used.

Do you have any other suggestions, feedback, or comments for the Author?
The GRADE approach will be useful, as is mentioned along with a narrative synthesis if needed. Strengths and limitations seem accurate and are good to list.

Decision
Verified with reservations: The content is scientifically sound but has shortcomings that could be improved by further studies and minor revisions.

Conflicts of Interest
None declared.

Editorial Notice

https://med.jmirx.org/2023/1/e45304
Reference

Peer-Review Report

Peer Review of "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"

Yin Qianlan¹, MD
Navy Medical University, Shanghai, China

Related Articles:
Companion article: https://preprints.jmir.org/preprint/43880
Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45306) doi:10.2196/45306

KEYWORDS
COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper “Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review.”

Round 1 Review

Serious Concerns
Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?
No.

Have authors’ provided the necessary ethics approval (from authors' institution or an ethics committee)?
Yes.

Language Quality
How would you rate the English language quality?
High quality.

Validity and Reproducibility
Is the reasons for conducting the study and its objectives clearly explained?
No.

Are sufficient details provided so that the method can be replicated?
Yes.

Are datasets available so that others could use them?
Not applicable.

Suggestions
Based on your answers in section 3 how could the author improve the protocol?
As an important part of a review is the declaration of the purpose of the protocol [1], the Introduction section should be the core of the article. However, after reading the beginning of the paper, I realized the seriousness of COVID-19, but I could not see the key point of the research. There are a lot of data to emphasize the worse outcomes, but I do not know how these data contributed to the relationship between the major topic of postacute sequelae of COVID-19 and adverse psychiatric outcomes; for example, the narrative on the effect of therapies. Hence, a more organized structure for the Introduction section with more conciseness would be easier for the readers.

Decision
Requires revisions: The manuscript contains objective errors or fundamental flaws that must be addressed and major revisions are suggested.

Conflicts of Interest
None declared.

Editorial Notice

https://med.jmirx.org/2023/1/e45306
Reference

Peer-Review Report


Daniel Griffin, MD, PhD
Columbia University, New York City, NY, United States

Related Articles:
Companion article: https://preprints.jmir.org/preprint/43880
Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45308) doi:10.2196/45308

KEYWORDS
COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper “Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review.”

Round 2 Review

General Comments
The authors lay out a reasonable protocol for this type of investigation [1].

Serious Concerns
Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?
No.

Have authors’ provided the necessary ethics approval (from authors’ institution or an ethics committee)?
Yes.

Language Quality
How would you rate the English language quality?
High quality.

Validity and Reproducibility
Is the reasons for conducting the study and its objectives clearly explained?
Yes.

Is the study design appropriate?
Yes.

Are sufficient details provided so that the method can be replicated?
Yes.

Are datasets available so that others could use them?
Not applicable.

Suggestions
Based on your answers in section 3 how could the author improve the protocol?
It is appropriate as it is.

Do you have any other feedback or comments for the Author?
The authors lay out a reasonable protocol for this type of investigation that is based on a fairly standard approach with the standard GRADE grading approach.

Decision
Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest
None declared.

Editorial Notice
This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.
Reference

Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Ramesh Poluru¹, PhD
The INCLEN Trust International, New Delhi, India

Related Articles:
Companion article: https://preprints.jmir.org/preprint/34598
Companion article: https://med.jmirx.org/2023/1/e46944/
Companion article: https://med.jmirx.org/2023/1/e34598/

(JMIRx Med 2023;4:e46906) doi:10.2196/46906

KEYWORDS
COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

The manuscript [1] attempts to investigate the impact of SARS-CoV-2 lineages in South African COVID-19 epidemiology. I would like to congratulate the authors on this useful attempt. The manuscript is well written, and the subject addressed in this manuscript is worth investigating; however, the manuscript partly failed to present a clear picture of its analytical methodology and presentation of results. The following are some minor concerns for consideration. I suggest that the authors (a) extend the study to include the recent Omicron variant, (b) present results with complete models, (c) avoid excessive references (~71).

In conclusion, the subject addressed in this manuscript is worth investigating and acceptable after taking into account the abovementioned minor issues.

Conflicts of Interest
None declared.

Reference

Edited by E Meinert; submitted 01.03.23; this is a non–peer-reviewed article; accepted 01.03.23; published 03.07.23.
Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Round 1 Review

General Comments

This paper [1] discusses the impact of the SARS-CoV-2 lineage in the South African COVID-19 epidemiology because it is important to investigate the evolution of distinct SARS-CoV-2 lineage that dominates among three epidemic waves in South Africa. The authors begin by recalling the background of the COVID-19 global pandemic and introducing the SARS-CoV-2 lineage and its variants. In section 2, their methodology is introduced. The data were obtained from public sources. Descriptive statistics, paired sample \( t \) test, and regression analysis with new variables such as active cases, deaths, and daily patient discharge are provided. The authors interpret the results of statistical analyses and discuss their findings from the data in section 3. However, the manuscript should be polished. Here are some comments.

Specific Comments

Major Comments

1. Throughout the manuscript, the notation of numbers is not consistent. For example, in the middle of the second paragraph in section 1, Introduction, “The genome of SARS-CoV-2 is a single positive-stranded RNA approximately 29 903 bases (nucleotides) pairs in length [2-5].” It looks like a space between numbers indicates a digit of a thousand, and a comma is omitted. However, in the middle of the paragraph in section 2.2.1, “Table 2 shows that the mean COVID-19 daily tests in the first, second and third South African COVID-19 epidemic wave period were 20 575±14 062, 31 046±14 115 and 46 822±18 460 respectively.” A space between numbers indicates a decimal point, not a comma.

2. Sections 2 and 3 are extremely difficult to read because they are too lengthy, although subsections indicate each statistical analysis that was performed. I believe that the authors do not need to provide outputs copied from SPSS directly. Are all columns in each table meaningful? Should readers know both standard deviation and variance for each statistic, for example? I strongly suggest that the authors get rid of unnecessary columns in each table and move unnecessary tables from sections 2 and 3 to the appendix.

3. I believe that the \( P \) values in the manuscript do not need to be specific. For example, Table 3 displays Pearson and Spearman correlation coefficients and \( P \) values. Many people may not understand what 9.94E-79 means. It can be simplified to “<0.001” or 0.

Minor Comments

4. The font style and size are not consistent throughout the manuscript.

Round 2 Review

General Comments

The authors have tried to improve the quality of the manuscript. However, the manuscript still needs substantial improvement. Please see my comments.
Specific Comments

Major Comments

1. This issue has not been resolved. The authors said that the space between numbers indicates a digit of a thousand. However, according to JMIR house style and editorial guidelines, numbers greater than 999 have a comma to separate thousands, millions, etc. Please see [6] and update the style of numbers throughout the manuscript.

2. The authors have reduced unnecessary columns. However, the JMIR production team suggests no more than 5 tables per manuscript. There are still unnecessary tables in the manuscript, that do not provide meaningful information and are just the same outputs of SPSS. What is the purpose of including so many tables without interpretation? Should Table 1 really be placed in the main manuscript? Why? Please see [7].

3. The authors have updated the representation of P values according to the suggestion of the editorial director [8].

4. The font style is still not consistent throughout the manuscript. Please revise the font style.

5. The Introduction in the manuscript is too long. I would suggest reducing the Introduction in the manuscript.

6. There are 13 equations in the manuscript. I believe that the authors can reduce the number of equations in the manuscript by combining similar equations. Listing all equations is unnecessary. Also, reference numbers for equations could be a number in the parenthesis such as (1) instead of Equation 1.

7. Detailed information about the paired test (what pairs to what) will be placed in the footnote in the corresponding table or figure.

8. Why do the authors think that the following text or Table 3 is needed in the manuscript?

“Table 3 shows that the Pearson (Spearman) Correlation Coefficients between COVID-19 daily tests (Independent Variable) and cases (Dependent Variable) in the first, second, third and fourth COVID-19 epidemic wave in South Africa were 0.910 (0.955), 0.877 (0.751), 0.893 (0.847) and 0.854 (0.812) respectively.”

This text and Table 3 are the same information.

9. What is the reason to provide Pearson correlation and Spearman rho together? Do the authors want to show a linear relationship or an ordinal relationship?

Minor Comments

10. The footnotes in Tables 3 and 4 are redundant. Where are the superscripts a, b, or c in the tables?

11. There is an inconsistent number of digits in all tables in the manuscript.

12. From Tables 1 to 16, why do the authors think that the minimum and maximum provide meaningful information in 2?

13. Please use “95% confidence interval” instead of “95 % confidence interval.”

Round 3 Review

General Comments

The authors have improved the manuscript’s quality compared to the previous version. However, I would assume that the quality could be improved more if the authors addressed the following comments.

Major Comments

1. In “Covariance and Regression of South African Epidemiological Data,” the authors stated that the 2-tailed Pearson correlation above 0.850 with P<.001 was considered as having a high degree of linearity. Pearson correlation coefficient has a value between –1 and 1. A negative value (eg, –0.850) could also be considered as a strong negative relationship between two variables. Was a negative relationship included in the determination of linearity?

2. In “Normalisation and Paired T-tests on South African Epidemiological Data,” the authors considered only 7 pairs among 5 periods. Normalized parameter 2 and 4, normalized parameter 2 and 5, and normalized parameter 3 and 5 were not included in pairing. Was there a specific reason to exclude these three pairs in the paired t test?

3. In the Discussion, the authors stated that the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson/Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 88.9%, respectively. However, two terms, vaccine efficacy and effectiveness, are used in different settings. According to [9], Pfizer demonstrated their COVID-19 vaccine efficacy based on randomized controlled trials. However, Johnson & Johnson did not show their COVID-19 vaccine efficacy according to [10]. Instead, Johnson & Johnson demonstrated their COVID-19 vaccine effectiveness based on observational studies, which is in a real-world setting. Could you please clarify this? (Please see [11].)

Minor Comments

4. The authors did not explain what the special characters after SARS-CoV-2 variants mean (eg, BA.4# or BA.2.75***). Could you please provide details on what the special characters after SARS-CoV-2 variants indicate?

5. The authors used unnecessary abbreviations throughout the manuscript. Could you please review the manuscript and remove some unnecessary abbreviations that are not used in a section of the manuscript?

Round 4 Review

Specific Comments

Major Comments

1. It is difficult to understand what Tables 2 and 3 show. Table 3 provides the mean difference between two daily positive COVID-19 tests in a percentage. If we look at the paired differences mean of pair 5 (daily positive COVID-19 test 2 – daily positive COVID-19 test 3), the difference is −1.20. However, the mean of the daily positive COVID-19 test 2 is
11.5 and the mean of the daily positive COVID-19 test 3 is 13.3 in Table 2. Could you please clarify what you compare between the two groups? How do we understand Tables 2 and 3 together? The same comment will be applied to Tables 4 and 5.

**Minor Comments**

2. The notation of $P$ values throughout the manuscript is inconsistent.

On page 5, “with Pearson correlations above 0.850 or below -0.850 with $P<.001$ considered as having a high degree of linearity.” On page 8, “The Spearman’s correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth and fifth COVID-19 epidemic wave in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916) and 0.811 (95% CI 0.673-0.895) respectively with $P$-values<.001. While the Spearman’s correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 ($P=.79$ 95% CI -0.207-0.266), -0.014 ($P=.93$ 95% CI -0.341-0.316) and -0.077 ($P=.62$ 95% CI -0.374-0.233) respectively.” Could you please make an update on the notation?

**Round 5 Review**

**General Comments**

The authors’ responses are clear. However, this paper still needs cosmetic improvement. I have some minor comments to improve the quality of this manuscript.

**Specific Comments**

**Minor Comments**

1. In Tables 1 and 2, some minimum values are “-.” Does this mean zero or unknown? Could you please specify what “-” is?

2. The format of $P$ values in Table 3 and the tables in the appendix is incorrect. Please edit based on [8].

3. Tables 6, 7, and 8 show both standard deviation and variance. Are there any specific reasons that the authors display both? If there is no reason, it is sufficient to show the standard deviation only.

**Conflicts of Interest**

None declared.

**References**


7. How many tables and figures can I include in my article? JMIR Publications Knowledge Base and Help Center. URL: https://support.jmir.org/hc/en-us/articles/360021623072 [accessed 2023-03-24]


10. Johnson and Johnson COVID-19 vaccine demonstrates 85 percent effectiveness against hospitalization in South Africa when Omicron was dominant. Johnson & Johnson. 2022. URL: https://tinyurl.com/uptnyv7 [accessed 2023-03-24]

Edited by E Meinert; submitted 01.03.23; this is a non–peer-reviewed article; accepted 01.03.23; published 03.07.23.

Please cite as:
Anonymous
Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”
JMIRx Med 2023;4:e46908
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Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Anonymous

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Companion article: https://med.jmirx.org/2023/1/e34598/

(JMIRx Med 2023;4:e47384) doi:10.2196/47384

KEYWORDS
COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

General Comments
In this article [1], the authors study the emerging variants of SARS-CoV-2 at the immune and epidemiological levels. The authors conclude that the Delta, Beta I VOC SARS-CoV-2, and lineage cluster, predominantly B.1.1.54, B.1.1.56 C.1 SA SARS-CoV-2 were observed to cause similar cases of COVID-19 hospital mortality and discharge rates in South African hospitals.

Specific Comments
The article seems good to me but too complex and difficult to follow, it should be “lightened.”

Major Comments
When talking about COVID-19 and its variants, some important points should be clarified that inform and prepare the reader well to deal with the specifics. Therefore, to make this paper more complete and interesting for the readers of this important journal, the authors should expand a bit of the discussion on cytokines. On this subject, three important articles have recently been reported. Below I list these interesting articles that should be studied, incorporated into the meaning, and reported briefly in the discussion and in the list of references.


Minor Comments
Some legends should be expanded.
I believe these suggestions are important for improving this paper. Without these corrections, the paper cannot be published. So I recommend minor revision.

Conflicts of Interest
None declared.
Reference


Edited by E Meinert; submitted 17.03.23; this is a non–peer-reviewed article; accepted 17.03.23; published 03.07.23.

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Authors' Response to Peer Review Reports for "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years"

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KEYWORDS
aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is the authors’ response to peer-review reports for “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years” [1].

Round 1 Review

Reviewer Heikki Vapaatalo [2]

Dear reviewer, thank you very much for the insightful suggestions; the manuscript improved a lot with the suggested changes. Please find our point-by-point answers to the raised questions. In the main text, all changes are highlighted in yellow. I hope that with the changes made, the new version is suitable for publication.

General Comments
1. The study is interesting, and the title promises for me more than the manuscript finally contains.

Answer: The manuscript is part of a project aiming to study ACE1 and ACE2 expression in cells from the immune system of aging and young adults. These initial results suggest that ACE1 (and probably ACE2) somehow plays a role in the process of aging.

2. The background, question, and the aim are relevant as explained in the Introduction.

Answer: We included some information in the Introduction, trying to link ACE1 expression in tissue cells and age-related diseases, as follows:

"ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. ACE1 polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain
homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between ACE1 polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20].

3. The major concerns the small size of the material (6 subjects), the small age difference (64-67 years), and the lack of younger controls.

Answer: We agree that the small number of studied subjects is a limitation of this study. In spite of the interesting results suggesting that ACE1 expression could be linked to the health status, it was not possible to perform correlation analysis due to the small sample size. Even though there is a small chronological difference among the subjects, the biological aging is very different among them and reflects the genetics, lifestyle, nutrition, and comorbidities. Another limitation is the lack of younger controls to compare with the subjects studied. Our next steps are to include younger controls, to increase the number of studied subjects, and, if possible, to obtain samples from older subjects (ie, aged 70-80, 80, and >80 years).

Specific Comments

1. Title: ACE seems better than ACE1; or, does the sophisticated, elegant method include both ACEs?

Answer: We evaluated only ACE1 expression, and thus, the title, abstract, and main text were changed to indicate ACE1 instead of ACE. We decided to change the title to “Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years.”

2. Introduction: in the last chapter, the author should explain in more detail how Pawelec et al [3], Alves et al [4], Alves and Bueno [5], and Bueno et al [6] suggest that “ACE1 plays an important role in the aging process.” Does “ACE1 plays” mean, that ACE1 is somehow regulating the aging process or are ACE1 levels changed with age?

Answer: These cited studies show that age-related diseases occurring in older adults are associated with changes in the immune system. To complete the text, we added the following:

“ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. ACE1 polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between ACE1 polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20].”

Methods:

1. The N value of the subjects should be mentioned here, as well the relation of females and males.

Answer: Text was corrected as suggested: “Blood was collected from adults (n=6, four females and 2 males) aged 64-67 years in 2015.”

2. Do the authors really regard 64-67 years “older age” nowadays?

Answer: Nowadays, the most common term used for individuals older than 65 years is “older adults.”

3. Why were the initial assays done many years after the collection of blood samples? Are the samples still useable and not destroyed?

Answer: Samples are part of UNIFESP Biobank and have been maintained in adequate conditions. We wanted to test cells from a period before the COVID-19 pandemic and those samples were the only ones that served our purpose. We compared samples used in this study with fresh blood samples (cell viability and percentage of CD4?, CD8?, and CD19? cells) and the results showed good preservation of the cells.

4. Did the subjects have some diseases or were taking drugs because they possibly were from a hospital sample bank?

Answer: The samples are part of UNIFESP Biobank, but unfortunately, we do not have information about diseases and medicaments.

5. Provide the companies’ details.

Answer: Changes were made as required: “ACE CD143 fluorescein isothiocyanate (R&D Systems).”

Results:

1. “Table 1 shows that older adults…..” The comparison between the present data and historical studies belongs to the Discussion.

Answer: Changes were made as required.

2. Also, provide individual ages and genders of the subjects in Table 1.

Answer: The manuscript version sent to medrxiv@medrxiv.org had age and gender on tables, but due to their request, any possible variable that could identify the study participant had to be removed. Hence, the present version these variables are not shown.

3. What do P values mean here—what is being compared, or are interindividual differences being highlighted in the particular variables? This should be explained.

Answer: We used P values for interindividual differences in each variable, since individuals age differently (biological aging); thus, physiological parameters could be affected by genetics, lifestyle, nutrition, and comorbidities. It is now explained in the Methods section.

4. The numbering of tables and the text seems confusing to me. Only 3 tables, but in the text, 4 are mentioned. Table 4 does not exist.

Answer: For some reason, Table 2 is missing in the main text. Please find the new version with Table 2 included.
5. It would be good to have a list of abbreviations used in the description of the cell types for an unfamiliar reader.

Answer: In each figure and table, we are now providing a description of cells evaluated.

Discussion:

1. A major part of the discussion deals with previous publications and not meaning or clinical significance of the present findings and comparison between the present and earlier studies.

Answer: The discussion was changed as suggested:

“Our results show that for the studied population, chronological aging and biological aging are asynchronous. Even among individuals with a small chronological difference (64 to 67 years), there is heterogeneity in physiological parameters such as glucose, urea, glycated hemoglobin, and CRP. Changes in the same functional parameters have been reported by Carlsson et al [22] and Helmersson-Karlqvist [23] in healthy older adults. Carlsson’s [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24].

The link between the RAS and inflammation has been suggested but its role is not completely clear under physiological and pathological conditions [25,26]. In addition, the association between altered ACE1 expression in tissues (brain, muscle, heart, and vessels) and the development and progression of age-related conditions such as Alzheimer disease, sarcopenia, and cardiovascular disease has been suggested, but results are controversial [17,27-30].

There are few studies showing the association between ACE1 expression in cells from the immune system (monocytes and T cells) and the progression of kidney and cardiovascular disease [8,9,31,32]. Therefore, considering the lack of information on this issue, we questioned whether ACE1 (CD143) was highly expressed in cells from the immune system during the aging process. We found that ACE1 was expressed in almost 100% of T (CD4+ and CD8+) and B cells, and nonlymphoid cells, in addition to the lowest percentage of CD4+ naïve cells, and the highest percentage of CD8+ terminally differentiated (EMRA) and DN B cells. However, due to the small sample size, it was not possible to associate the high expression of ACE1 in immune cells with inflammaging and immunosenescence. Correlation of physiological parameters and health status with ACE1 expression and investigating whether age and associated chronic diseases could lead to increased ACE1 expression would yield important information.”

4. The point regarding the COVID-19 pandemic, seemingly worth mentioning, is too far from this study and unnecessary.

Answer: Our point was to emphasize the negative impact of chronic diseases for the outcome of the aging population during a viral infection and how ACE1/ACE2 expression could provide information regarding diagnosis and treatment. Therefore, we would like to maintain this information.

5. Linguistic checking would improve the manuscript.

Answer: We checked for possible language errors.

Reviewer Calogero Caruso [7]

Dear reviewer, thank you very much for suggesting revisions to our manuscript. It is a privilege to have a manuscript reviewed by a researcher with high expertise on the field of ageing. Please find our responses to your questions and corresponding revisions made to the main text.

General Comments

1. The paper is essentially anecdotal because it studies the cells of 6 subjects without any comparison with other age groups. There is also a serious limitation because beyond the age and
sex, there is no information on the donors (how and why they were recruited, what drugs they took, etc).

Answer: It is really a limitation to have only 6 individuals for the study, but they were the only ones meeting the criteria of the proposed study. The samples were from a central bank of cells at the UNIFESP, and participants were considered “healthy” but there was no further information in addition to what we displayed in the tables in the manuscript. They were not living on homecare or hospitalized.

Our aim was to evaluate samples from individuals aged 60-69 years before the COVID-19 pandemic or vaccination. In addition, there were no samples maintained under the same conditions (PBMCs at –80 °C), obtained from young individuals, and using fresh blood could yield a result that could not be compared mainly for myeloid cells and B cells as shown in Braudeau et al [8]. Our goal from now on is to expand this study with young and older adults’ samples, since it is important to understand whether ageing is associated with an increase in ACE expression in immune cells.

2. To infer that chronological and biological ages do not match is inappropriate in the absence of the above information.

Answer: This information regarding chronological and biological age was required by another reviewer. I agree that the concept does not match without more information on the donors. However, the information is now provided in Vasto et al [9] and should be considered when older adults are studied.

3. However, the paper is of some interest because there are few studies on the topic.

Answer: Thank you for this positive comment. Few studies on the topic are the reason why we decided to send the manuscript for publication, even though there some important information on the donors is missing and a limited number of individuals was included.

Specific Comments

Essential revisions that are required to verify the manuscript

1. Although we do not have data on donors, placing an age and gender column in all tables adds a minimum of useful information for the reader.

Answer: The first table was submitted with age, but per requirement of MedRxiv, gender and age could not be linked to the metabolic results to preserve the anonymity of the donors.

2. Inflammaging means low grade of inflammation. The CRP value of 23.1 suggests acute inflammation (also because albumin has high values, while in chronic inflammation its values decrease). Therefore the averages do not have to take this subject into account.

Answer: Thank you for this comment. In a review of the literature, Heumann et al [10] found a CRP variation from 0.1 to 19.8. There is also an article from your group [11] showing that a CRP level of <5 g/dL and >5 g/dL will be considered to investigate how ageing impacts CRP levels. Considering the already small number of donors, data were maintained and statistics (mean and SD) with and without 23.1 mg/dL are now shown.

This will be the new version (Discussion) with respect to CRP: “Carlsson’s [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24].”

However, an individual presented CPR 23.1 mg/dL, suggesting acute inflammation instead, but as all donors were not hospitalized or living on homecare, this sample was considered a part of the study. Another study [12] evaluating gait speed found CRP levels varying from 0.1 to 19.8 mg/dL. Our study has an important limitation, that is, the lack of data on donors such as the use of continuous medicaments or sarcopenia, hypertension, and cognition, among others, and thus it was not possible to correlate CRP with age-related conditions.

Table 1. Updated

Other suggestions to improve the manuscript

1. The authors write that their findings suggest that ACE1 could play a role in several processes linked to aging including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE suppress the autoimmune process in a number of autoimmune diseases such as EAE, arthritis, autoimmune myocarditis [13]. They do not appear to have these findings in their paper. So, they need to change the sentence.

Answer: The sentence has been changed as follows: “Our findings suggest that ACE1 could play a role in several processes linked to aging, including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE suppress the autoimmune process in a number of autoimmune diseases such as EAE, arthritis, autoimmune myocarditis [49].”

References

1. Bueno V, Destro PH, Teixeira D, Frasca D. Angiotsin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years. JMIRx Med 2023 Jan;4:e45220 [FREE Full text]
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Authors’ Response to Peer Reviews of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

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KEYWORDS
COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is authors’ response to peer-review reports for “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

Reviewer AA [1]

The manuscript [2] is well written, and the subject addressed in this manuscript is worth investigating; however, the manuscript partly failed to present a clear picture of its analytical methodology and presentation of results.

Response: The authors have revised the methodology and presentation of the results.

The following are some minor concerns for consideration. I suggest that the authors (a) extend the study to include the recent Omicron variant.

Response: The authors have extended the study to include the Omicron variant.

The following are some minor concerns for consideration. I suggest that the authors (b) present results with complete models.

Response: The authors have presented results with complete model methodologies.

The following are some minor concerns for consideration. I suggest that the authors (c) avoid excessive references (~71).
Response: The authors have tried to reduce the references in the manuscript to critical references.

Reviewer BQ [3]

General Comments
This manuscript is well written, comprehensive, and filled with detail. This is both a strength and a possible weakness. The strength is that the data included have been analyzed in depth, and one can be fairly certain that the results obtained are likely to be accurate. On the other hand, depending on the audience, some readers may struggle to engage with the data appropriately: the dissemination of data and reporting has not been formatted and simplified in a manner that improves readability without compromising on accuracy.

Response: The authors have reworded most of the sections, particularly the Results and Discussion to make the manuscript more reader friendly.

The use of scientific notation for P values to the 11th power, use of 3 or 4 decimal places for proportions, etc, and extensive reporting of findings instead of picking a few of the most relevant findings with reference to the table for other findings are a few examples of this.

Response: The presentation of P values in the manuscript has been reformatted as required.

Specific Comments

Major Comments
1. I have not seen whether time was included as a potential confounder/covariate in any of the regression models that were conducted. Increasing immunity, the initiation of vaccination campaigns halfway through the third wave, and movement restrictions have not been discussed adequately.

Response: The authors have included nonpharmaceutical interventions and COVID-19 vaccination as confounders in the study analysis.

2. Please provide brief details on how data used to assess movement restriction were obtained and analyzed.

Response: Data on community movement was obtained from the Google Community Mobility reports. The regression of movement and the daily COVID-19 effective contact rate was then conducted through a literature review of earlier work done by the authors on this.

3. Please comment on the appropriateness of using means and standard deviations for the description of the majority of some of these data, which may or may not have been normally distributed.

Response: The authors have addressed this key question in the manuscript. For comparative inferential statistical analysis of continuous variables using the magnitude of the mean and variance, the distribution of the continuous variable must be the same in the periods being compared.

4. Please provide ethical considerations in the manuscript for the data and analysis, whether approval was required or not, and justify.

Response: Information used in this study was from public sources with creative commons licenses. The authors ensured reference data sources and acknowledged relevant institutions. The data used was blinded regarding patient personal information.

Minor Comments

1. “While, there is global consensus on the health risk posed by COVID-19, ground-breaking vaccine developments, and a great drive towards the vaccination of the world population against COVID-19.”

This sentence is fragmented. Please revise.

Response: The sentence was revised to “There is global consensus on the health risk posed by COVID-19, ground-breaking vaccine developments, and a great drive towards the vaccination of the world population against COVID-19. However challenges still persist in controlling the Global COVID-19 transmission and severity.”

2. “emergent.” Possible typo error, consider using “emergence.”

Response: Typo corrected.

3. National Coronavirus Command Council: A one-liner describing the National Coronavirus Command Council would be beneficial to the reader.

Response: The authors felt this would be unnecessary considering the word limit. The relevant reference has been added for the reader interested in looking for more information. The background has relatively low relevance to the study.

Response: The authors wish to guide you to the following paper for more information. This background has relatively low relevance to the study, particularly the information on the reagent used. Cele S, Gazy I, Jackson L, Hwa SH, Tegally H, Lustig G, et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. Nature. 2021;593(7857):142-146. doi:10.1038/s41586-021-03471-w.

Response: The author team can check submission guidelines, and the editor can confirm, but I believe that P values <.001 should be stated as such.

Response: The presentation of P values in the manuscript has been reformatted as required.

7. Table entries with variable names that have underscores and spaces between words, were corrected. Labeling could be cleaned up to improve readability.
Response: The use of the underscore was left unchanged as the authors feel this is the best method of referencing the epidemic waves in multiple variables of the study. This is also described in the methodology for the reader to understand their meaning (underscore and number).

8. As noted above, the use of 3 or 4 decimal places and exponential notation of extremely small P values reduces the clarity and readability. Consider reviewing.

Response: The presentation of P values in the manuscript has been reformatted as required.

Reviewer Anonymous [4]

Specific Comments
The article seems good to me but too complex and difficult to follow, it should be “lightened.”

Response: The authors have restructured the paper for easier readability.

Major Comments
When talking about COVID-19 and its variants, some important points should be clarified that inform and prepare the reader well to deal with the specifics. Therefore, to make this paper more complete and interesting for the readers of this important journal, the authors should expand a bit of the discussion on cytokines. On this subject, three important articles have recently been reported. Below I list these interesting articles that should be studied, incorporated into the meaning, and reported briefly in the discussion and in the list of references.


Response: The authors found the suggested papers interesting. The following paper “Mast Cells Activated by SARS-CoV-2 Release Histamine Which Increases IL-1 Levels Causing Cytokine Storm and Inflammatory Reaction in COVID-19” was included in the paper as a reference; however, the authors, due to the word limit, did not expand on this topic. Though interesting, it has low relevance to the study.

Minor Comments
Some legends should be expanded.

I believe these suggestions are important for improving this paper. Without these corrections, the paper cannot be published. So I recommend minor revision.

Response: Legends in the paper were expanded.

Reviewer Anonymous [5]

Specific Comments
1. Throughout the manuscript, the notation of numbers is not consistent. For example, in the middle of the second paragraph in section 1, Introduction, “The genome of SARS-CoV-2 is a single positive-stranded RNA approximately 29 903 bases (nucleotides) pairs in length 9 [6-9].” It looks like a space between numbers indicates a digit of a thousand, and a comma is omitted. However, in the middle of the paragraph in section 2.2.1., “Table 2 shows that the mean COVID-19 daily tests in the first, second and third South African COVID-19 epidemic wave period were 20 575±14 062, 31 046±14 115 and 46 822±18 460 respectively.” A space between numbers indicates a decimal point, not a comma.

Response: The authors have corrected this error. A space between numbers in the manuscript represents a digit of a thousand.

2. Sections 2 and 3 are extremely difficult to read because they are too lengthy, although subsections indicate each statistical analysis that was performed. I believe that the authors do not need to provide outputs copied from SPSS directly. Are all columns in each table meaningful? Should readers know both standard deviation and variance for each statistic, for example? I strongly suggest that the authors get rid of unnecessary columns in each table and move unnecessary tables from sections 2 and 3 to the appendix.

Response: The authors have reduced the columns in the tables and moved some of the tables to the appendix. The authors have also rewritten these sections for easier readability.

3. I believe that the P values in the manuscript do not need to be specific. For example, Table 3 displays Pearson and Spearman correlation coefficients and P values. Many people may not understand what 9.94E-79 means. It can be simplified to “<0.001” or 0.

Response: The presentation of P values in the manuscript has been reformatted as required.

Minor Comments
4. The font style and size are not consistent throughout the manuscript.

Response: The font and style have been made consistent throughout the manuscript.

Round 2 Review

Reviewer BQ [3]

The manuscript has been improved based on previous reviewer comments but is still unnecessarily too long, dense, and bloated. I believe that the adage “simpler is better” would have suited
the objectives of this paper well. The average reader may find it difficult to read to the end, and some readers may have difficulty fully engaging with the content as a result. Five pages on the virology of SARS-CoV-2 as an introduction is likely unnecessary for a manuscript whose data focus on the epidemiology and statistics of COVID-19 rather than its virology.

Response: The authors agree with this review note and have cut down the Introduction (to 2.25 pages) to focus on the background of detected SARS-CoV-2 and COVID-19 vaccination in South Africa to prepare the reader for the study objectives.

There are many statistical tests conducted here; however, the authors do not appear to have performed any adjustments for the multiple tests conducted. The familywise error rate is bound to be higher than 0.05, so some of your conclusions based on the statistical probability may be inaccurate.

Response: Each descriptive and inferential statistical analysis conducted/applied on the analysis data sets and conclusions drawn from each inference were done independently as per the objective of the statistical analysis method. However, type I error are noted and covered in the limitations stated in the manuscript under Data Handling and Limitations.

Finally, there are some statements that have been made based on the Discussion and Conclusion sections that I do not believe are adequately supported by the data presented, and these may need to be reconsidered/softened. Please see specific comments below.

Response: Thank you for this review. The authors agree with your statements below.

1. Methods: Many hypothesis tests are conducted in this paper. Was adjustment for multiple testing performed? Otherwise, the possibility of making type I errors is quite high. This should either be reviewed or listed as a key limitation.

Response: The limitations of the manuscript have been listed under Data Handling and Limitations. Statistical tests were applied independently; however, the potential for type I or II errors has been noted.

2. South Africa community mobility data: How is movement in these data measured? Kilometers? Significant movement out of the house? The number of people in an area? Please describe.

Response: The Google Mobility reports are created with aggregated data from users who have turned on their Location History in their Google accounts. The baseline in these reports is the median values of movement in the respective locations from January 3 to February 6, 2020. This movement unit is the percentage from baseline (number of people in that location per time relative to the number observed at baseline).

3. “The mean daily positive COVID-19 tests in South Africa’s first and second COVID-19 epidemic wave had no statistically significant difference.”

Response: Each descriptive and inferential statistical analysis conducted/applied on the analysis data sets and conclusions drawn from each inference were done independently as per the objective of the statistical analysis method. However, type I error are noted and covered in the limitations stated in the manuscript under Data Handling and Limitations.

4. Please insert a legend for the figures (eg. Figures 7 and 8).

Response: Legends inserted for the figures.

5. Table 1: The maximum COVID-19 hospitalized intensive care unit percentage of 7 and 814.1 is unclear.

Response: This statement was removed in the writing of the Discussion section.

6. Discussion: “The values of the Pearson and Spearman Correlation Coefficients obtained between the daily COVID-19 tests and cases in this study indicated a strong positive correlation between daily COVID-19 tests and cases with more than 95% confidence in the four COVID-19 epidemic waves in South Africa.”

Response: The statement was changed to “The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the five COVID-19 epidemic waves in South Africa,” and the “95% confidence” was removed.

As a suggestion, you may leave the 95% confidence part out altogether and just say that testing was significantly related to case incidence in the 4 COVID-19 waves.


Response: The statement was changed to “The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the five COVID-19 epidemic waves in South Africa,” and the “95% confidence” was removed.

7. These data, as presented, do not allow you to make this conclusion as you have not made a relationship of causality, but rather have demonstrated an association, as you rightly say in the following lines. Please revise to describe this as a significant association rather than a causal relationship.

Response: Instead of relationship, the word “association” was used to avoid an interpretation of causality instead of correlations.

8. “To understand the causality of relationships between two or more variables, statistical theory must be applied.” Text like this is unnecessary and contributes to the bloating of your manuscript. Consider removing.

Response: This statement was removed in the rewriting of the Discussion section.

9. “Daily COVID-19 tests in South Africa were observed to be normally distributed while the daily COVID-19 cases were positively skewed with a lognormal distribution (Galton distribution).”

Response: The statement was changed to “The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the five COVID-19 epidemic waves in South Africa.”

I do not recall the data distributions being assessed or described in the Results, so it is surprising that they are now included in the Discussion. Consider including or revising the need to

https://med.jmirx.org/2023/1/e46944
discuss the data distributions (a similar comment applies to the following paragraph).

Response: The discussion of variable normal distributions was removed from the manuscript.

10. I have reservations about the use of the word “confounder” in this discussion. While the movement is most likely a potential contributing factor in the detection rate of COVID-19, this was not analyzed or demonstrated using appropriate statistical methods such as multiple regression or interaction tests.

Showing that there was a correlation between population movement and COVID-19 detection does not automatically demonstrate that movement is a significant confounder. The messaging may have to be altered to suggest a possible confounding effect, or alternatively, this would need to be demonstrated by conducting appropriate data analysis.

Response: The words “possible” and “association” were used since there were not enough multivariable statistical methods applied in the manuscript to avoid conclusive statements.

11. “The values of the Spearman Correlation Coefficients obtained between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases in the half period of the third and fourth COVID-19 epidemic wave in this study indicated a low correlation between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases with this correlation statistically insignificant.”

This statement should be reconsidered. If vaccination does indeed have a significant effect on daily infection rates, there is bound to be a lag between exposure and effect, and this would need to be demonstrated in a robust time series analysis. Correlating the vaccination rate with the COVID-19 case rate without adjustment for time periods would not adequately demonstrate the effect of vaccination if such an effect existed. This is particularly important because the statement “These results suggest that COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19” would be a controversial conclusion to come to without solid evidence to support this statement that may be seen as inflammatory in the politically charged topic of vaccines and vaccine hesitancy in South Africa.

Response: This statement was removed from the Discussion. The authors agree that there is not enough evidence generated in the results of the manuscript to make this conclusion.

12. “This result can be explained by the percentage of the population per age group who had received at least one dose of the COVID-19 vaccine by the end of the fourth COVID-19 epidemic wave.”

This statement appears to contradict your earlier statement that vaccines did not appear to have an impact on COVID-19 transmission in South Africa. Please review and reconcile. Also, natural immunity and potentially reduced virulence of the Omicron variant are important factors to consider in the reduced mortality in the fourth wave.

Response: The statements on the impact of the COVID-19 vaccine on transmissibility were retracted in the manuscript due to insufficient evidence from the available data. Including this conclusive analysis will require data that captures COVID-19 daily cases and their vaccination status.

13. “Showed statistical significant indifferences at 95% confidence.” Unusual wording and terminology such as indifference at 95% confidence. Please revise.

Response: The wording has been revised.

14. “While COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19 within the South African population.”

Again, this statement is not supported by the data provided and should be reviewed and reconsidered.

Response: The statements on the impact of the COVID-19 vaccine on transmissibility were retracted in the manuscript due to insufficient evidence from the available data. Including this conclusive analysis will require data that captures COVID-19 daily cases and their vaccination status.

15. Table A.1: Consider formatting these large sums of square and mean square values including thousand separators for readability.

Response: Commas to separate thousands were included in the formatting of all numbers in the manuscript.

Reviewer Anonymous [5]

General Comments
The authors have tried to improve the quality of the manuscript. However, the manuscript still needs substantial improvement. Please see my comments.

Response: Thank you for this review. The authors agree with your statements below.

Specific Comments

Major Comments
1. This issue has not been resolved. The authors said that the space between numbers indicates a digit of a thousand. However, according to JMIR house style and editorial guidelines, numbers greater than 999 have a comma to separate thousands, millions, etc. Please see [10] and update the style of numbers throughout the manuscript.

Response: Commas to separate thousands were included in the formatting of all numbers in the manuscript.

2. The authors have reduced unnecessary columns. However, the JMIR production team suggests no more than 5 tables per manuscript. There are still unnecessary tables in the manuscript, that do not provide meaningful information and are just the same outputs of SPSS. What is the purpose of including so many tables without interpretation? Should Table 1 really be placed in the main manuscript? Why? Please see [11].

Response: The authors have moved unnecessary tables and figures to the appendix.

3. The authors have updated the representation of P values according to the suggestion of the editorial director [12].
Response: No updates required.

4. The font style is still not consistent throughout the manuscript. Please revise the font style.

Response: The font style has been revised and made consistent throughout the manuscript.

5. The Introduction in the manuscript is too long. I would suggest reducing the Introduction in the manuscript.

Response: The authors agree. The Introduction in the manuscript was reduced.

6. There are 13 equations in the manuscript. I believe that the authors can reduce the number of equations in the manuscript by combining similar equations. Listing all equations is unnecessary. Also, reference numbers for equations could be a number in the parenthesis such as (1) instead of Equation 1.

Response: The authors have removed unnecessary equations in the manuscript.

7. Detailed information about the paired test (what pairs to what) will be placed in the footnote in the corresponding table or figure.

Response: This was removed from the captions of the tables and described in the methodology.

8. Why do the authors think that the following text or Table 3 is needed in the manuscript?

“This table shows that the Pearson (Spearman) Correlation Coefficients between COVID-19 daily tests (Independent Variable) and cases (Dependent Variable) in the first, second, third and fourth COVID-19 epidemic wave in South Africa were 0.910 (0.955), 0.877 (0.751), 0.893 (0.847) and 0.854 (0.812) respectively.”

This text and Table 3 are the same information.

Response: Table 3 was moved to the appendix and the text was used instead for the Results section.

9. Why do the authors think that the following text or Table 3 is needed in the manuscript?

Response: Table 3 was moved to the appendix and the text was used instead for the Results section.

10. The footnotes in Tables 3 and 4 are redundant. Where are the superscripts a, b, or c in the tables?

Response: Footnotes in Tables 3 and 4 were removed, and the tables were moved to the appendix.

11. There is an inconsistent number of digits in all tables in the manuscript.

Response: The authors agree and have resolved all formatting of numbers according to JMIR guidelines.

12. From Tables 1 to 16, why do the authors think that the minimum and maximum provide meaningful information in Table 2?

Response: The minimum and maximum provide the lowest and highest values observed in the epidemic wave period, which corresponds to the start/end and the peak of the epidemic wave period.

13. Please use “95% confidence interval” instead of “95 % confidence interval.”

Response: “95% confidence interval” was used instead, and all percentage values were formatted accordingly.

Round 3 Review

Reviewer BQ [3]

Comments

1. Table 8: Consider having the case-fatality age risk ratio value for the reference group as “Ref” for reference. It may be confusing to have a risk ratio for the reference category.

Response: Updated the caption of Table 8 and the values of the case-fatality age risk ratio reference group to make the case-fatality age risk ratio reference clearer.

2. Table 9: Case-fatality rate is abbreviated as “CRF” at times (and in subsequent text) and as “CFR” at times.

Response: The abbreviation of case-fatality rate in Table 9 and Table A12 was corrected to “CFR.” The in-text reference to the case-fatality rate abbreviation was checked to ensure they are all abbreviated as “CFR.”

Reviewer Anonymous [5]

Major Comments

1. In “Covariance and Regression of South African Epidemiological Data,” the authors stated that the 2-tailed Pearson correlation above 0.850 with P<.001 was considered as having a high degree of linearity. Pearson correlation coefficient has a value between –1 and 1. A negative value (eg, –0.850) could also be considered as a strong negative relationship between two variables. Was a negative relationship included in the determination of linearity?

Response: Thank you for this comment. The authors agree with the reviewer. Indeed, a value of less than –0.850 implies a strong negative relationship/association between two variables. The authors did conduct their analysis in this manner; however, unfortunately, the wording was omitted in the methodology. The Methods section Covariance and Regression of South African Epidemiological Data has been updated to include “or below -0.850.”

2. In “Normalisation and Paired T-tests on South African Epidemiological Data,” the authors considered only 7 pairs among 5 periods. Normalized parameter 2 and 4, normalized parameter 2 and 5, and normalized parameter 3 and 5 were not included in pairing. Was there a specific reason to exclude these three pairs in the paired t-test?
Response: The authors initially did consider having all possible test pairings; however, it would have complicated the analysis. We, therefore, chose two analysis groupings in terms of test pairing. The first one was comparing all COVID-19 epidemic waves to the first COVID-19 epidemic wave (pair 1 to pair 4). This would help us understand the impact of the evolution of SARS-CoV-2 (inclusive of other factors: nonpharmaceutical interventions, vaccination, etc) against the ancestral SARS-CoV-2 lineage (and initial conditions). The second analysis grouping was understanding the evolution per consecutive waves (pairs 5, 6, and 7). This would help us understand the impact of the evolution of SARS-CoV-2 (including changing conditions) between each consecutive wave. This simplified the analysis and allowed us valid inference between test pairings and an overview based on the two analysis test pairing groupings.

3. In the Discussion, the authors stated that the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson/Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 88.9%, respectively. However, two terms, vaccine efficacy and effectiveness, are used in different settings. According to [13], Pfizer demonstrated their COVID-19 vaccine efficacy based on randomized controlled trials. However, Johnson & Johnson did not show their COVID-19 vaccine efficacy according to [14]. Instead, Johnson & Johnson demonstrated their COVID-19 vaccine effectiveness based on observational studies, which is in a real-world setting. Could you please clarify this? (Please see [15].)

Response: Thank you for this comment, and it touches on an important discourse regarding the implications of using different methodologies to infer efficacy, with of course, randomized clinical trials being the standard. Certainly, the authors accept the reviewer’s point; for the Discussion, the authors wanted to highlight these studies for reference in terms of the efficacy against severe COVID-19. Unfortunately, there are, of course, limitations in the inference of efficacy, as it does depend on the methodology of those studies. The authors in the manuscript used the reference to allow the reader to understand the current work regarding the association, which is highlighted by the manuscript (increasing vaccination, decreasing hospitalization). In light of the reviewer’s point, we have updated reference [14] to Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against COVID-19. N Engl J Med. 2021;384:2187-2201. PMID:33882225 doi:10.1056/NEJMoa2101544.

Minor Comments

4. The authors did not explain what the special characters after SARS-CoV-2 variants mean (eg, BA.4# or BA.2.75**). Could you please provide details on what the special characters after SARS-CoV-2 variants indicate?

Response: The naming of these lineages with special characters “#” or “**” appeared due to an error in rendering our document. We have updated to remove these from the naming of the lineages.

5. The authors used unnecessary abbreviations throughout the manuscript. Could you please review the manuscript and remove some unnecessary abbreviations that are not used in a section of the manuscript?

Response: The authors reviewed the abbreviations used in the manuscript and removed unnecessary abbreviations.

Round 4 Review

Reviewer Anonymous [5]

Specific Comments

Major Comments

1. It is difficult to understand what Tables 2 and 3 show. Table 3 provides the mean difference between two daily positive COVID-19 tests in a percentage. If we look at the paired differences mean of pair 5 (daily positive COVID-19 test 2 – daily positive COVID-19 test 3), the difference is –1.20. However, the mean of the daily positive COVID-19 test 2 is 11.5 and the mean of the daily positive COVID-19 test 3 is 13.3 in Table 2. Could you please clarify what you compare between the two groups? How do we understand Tables 2 and 3 together? The same comment will be applied to Tables 4 and 5.

Response: Table 2 shows the descriptive statistics for the COVID-19 active cases and daily positive COVID-19 tests (%), ie, what percentage of the total COVID-19 tests were positive) for each epidemic wave. The descriptive statistics include the number of valid observations (n), minimum, maximum, mean, and standard deviation (std deviation).

While, Table 3 shows the paired sample t test results between test pairing (ie, between epidemic waves), showing the paired differences of the mean and standard deviation, the student t test value, degrees of freedom (df), and the P value.

Now discussing the pairings you are comparing, pair 5 in Table 3 is the comparison between the daily positive COVID-19 tests in the COVID-19 epidemics 2 and 3. The paired mean difference was –1.20; however, the actual mean difference (13.3 – 11.5) is 1.80 as you have stated. The discrepancy between Tables 2 and 3 is due to the degrees of freedom (df) in Table 3 and observations (n) in Table 2. Test pairing was done based on the epidemic day; therefore, the epidemic wave with the lowest observations will always be the df of the t test. We have to compare like with like; due to this, some of the observations in Table 2 are not included in the t test. This concept is the same for Tables 4 and 5.

Minor Comments

2. The notation of P values throughout the manuscript is inconsistent.

On page 5, “with Pearson correlations above 0.850 or below -0.850 with P<.001 considered as having a high degree of linearity.” On page 8, “The Spearman’s correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth and fifth COVID-19 epidemic wave in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916) and 0.811 (95% CI 0.673-0.895)
respectively with P-values<.001. While the Spearman’s correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 (P=.79 95% CI -0.207-0.266), -0.014 (P=.93 95% CI -0.341-0.316) and -0.077 (P=.62 95% CI -0.374-0.233) respectively.” Could you please make an update on the notation?

Response: The authors have updated the notation of P values in the manuscript. The authors have followed the recommendations in [12].

References


11. How many tables and figures can I include in my article? JMIR Publications Knowledge Base and Help Center. URL: https://support.jmir.org/hc/en-us/articles/360021623072 [accessed 2023-03-24]


The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study

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Abstract

Background: Emerging SARS-CoV-2 variants have been attributed to the occurrence of secondary, tertiary, quaternary, and quinary COVID-19 epidemic waves threatening vaccine efforts owing to their immune invasiveness. Since the importation of SARS-CoV-2 in South Africa, with the first reported COVID-19 case on March 5, 2020, South Africa has observed 5 consecutive COVID-19 epidemic waves. The evolution of SARS-CoV-2 has played a major role in the resurgence of COVID-19 epidemic waves in South Africa and across the globe.

Objective: We aimed to conduct descriptive and inferential statistical analysis on South African COVID-19 epidemiological data to investigate the impact of SARS-CoV-2 lineages and COVID-19 vaccinations in South African COVID-19 epidemiology.

Methods: The general methodology involved the collation and stratification, covariance, regression analysis, normalization, and comparative inferential statistical analysis through null hypothesis testing (paired 2-tailed t tests) of South African COVID-19 epidemiological data.

Results: The mean daily positive COVID-19 tests in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 11.5% (SD 8.58%), 11.5% (SD 8.45%), 13.3% (SD 9.72%), 13.1% (SD 9.91%), and 14.3% (SD 8.49%), respectively. The COVID-19 transmission rate in the first and second COVID-19 epidemic waves in South Africa was similar, while the COVID-19 transmission rate was higher in the third, fourth, and fifth COVID-19 epidemic waves than in the aforementioned waves. Most COVID-19 hospitalized cases in South Africa were in the general ward (60%-79.1%). Patients with
COVID-19 on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the intensive care unit (8.07%-16.7%). Most patients hospitalized owing to COVID-19 in South Africa’s first, second, third, and fourth COVID-19 epidemic waves were aged between 40 and 49 years (16.8%-20.4%) and 50 and 59 years (19.8%-25.3%). Patients admitted to the hospital owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%). In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, while for age groups between 30 and 79 years, hospital admissions decreased. Most COVID-19 hospitalization deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic waves were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%).

Conclusions: The relaxation of COVID-19 nonpharmaceutical intervention health policies in South Africa and the evolution of SARS-CoV-2 were associated with increased COVID-19 transmission and severity in the South African population. COVID-19 vaccination in South Africa was strongly associated with a decrease in COVID-19 hospitalization and severity in South Africa.

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KEYWORDS
COVID-19; SARS-CoV-2; vaccines; variants; lineages; South Africa; epidemiology; statistics

Introduction

Background

On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic [1]. The COVID-19 pandemic has resulted in >6,490,817 deaths in the reporting period until August 30, 2022 [2]. Public health measures, such as nationwide lockdowns aimed at reducing the transmission of COVID-19, have come at a great cost to the global economy [3]. There is a global consensus on the health risk posed by COVID-19, groundbreaking vaccine developments, and a great drive toward vaccination of the world population against COVID-19. However, challenges persist in controlling the global COVID-19 transmission and severity. One challenge is the large disparity in access to vaccines between low-income and high-income countries [4]. Another challenge is the emergence of SARS-CoV-2 lineages and sublineages (variants) with increased transmissibility [5]. Lineages and sublineages are a series of entities (in this case, genetic) forming a single line of direct ancestry and descent [6]. Emerging SARS-CoV-2 variants have been attributed to the occurrence of secondary, tertiary, quaternary, and quinary COVID-19 epidemic waves and threatening vaccine efforts owing to their immune invasiveness [7].

SARS-CoV-2 Variants of Concern and Interest

SARS-CoV-2 is the virus that causes COVID-19 upon infecting a human host. Whole-genome sequencing of 104 strains of SARS-CoV-2 from patients with COVID-19 symptom onset from December 2019 to mid-February 2020 showed 99.9% homology, without major mutations [8]. However, the rapid spread of SARS-CoV-2 has allowed the virus opportunity replications to evolve into lineages and sublineages. To prioritize global monitoring and research and to inform the ongoing response to the COVID-19 pandemic, SARS-CoV-2 variants have been characterized as either variants of concern (VOCs) or variants of interest (VOIs). The main characteristics of VOCs are that they have evidence of an increase in transmissibility and more severe disease that leads to increased hospitalization or deaths, thereby reducing the effectiveness of public health and social measures [9]. In addition, VOIs substantially reduce the neutralization of antibodies generated during previous infection or vaccination which ultimately reduces the effectiveness of treatments, vaccines, or diagnostic detection [10]. VOIs are lineages whose changes have predicted genetic markers that are known to affect virus characteristics, such as transmissibility, disease severity, immune escape, and diagnostic or therapeutic escape [9]. They are also identified to have a predictable increase in transmissibility or disease severity, thus having an apparent epidemiological impact to suggest an emerging risk to global public health [9,10]. The SARS-CoV-2 lineages that have been characterized as VOCs by the WHO are the alpha (B.1.1.7), beta (B.1.351, B1.351.2, and B.1.351.3), gamma (P.1, P.1.1, P.1.2, P.1.4, P.1.6, and P.1.7), delta (B.1.617.2, AY.1, AY.2, AY.3, and AY.3.1), and omicron (B.1.1.529, BA.4, BA.5, BA.2.12.1, and BA.2.75) SARS-CoV-2 lineages [9,10]. The variants that have been characterized as VOIs are eta (B.1.525), iota (B.1.526), kappa (B.1.617.1), lambda (C.37), epsilon (B.1.427, B.1.429), zeta (P.2), theta (P.3), and mu (B.1.621) SARS-CoV-2 lineages [9].

SARS-CoV-2 Lineages in South Africa

Of interest in this study is the impact of the evolution of SARS-CoV-2 lineages and COVID-19 vaccination in the COVID-19 epidemiology in South Africa. Since the importation of SARS-CoV-2 in South Africa, with the first reported COVID-19 case on March 5, 2020, South Africa has observed 5 consecutive COVID-19 epidemic waves [2,11]. The response by the Government of South Africa toward the COVID-19 epidemic was the establishment of a National Coronavirus Command Council to oversee the epidemic; the use of health policy measures, including nonpharmaceutical interventions (NPIs) to try to mitigate the transmission of COVID-19; and the implementation of COVID-19 vaccination programs to try to vaccinate the South African population against COVID-19 [12-17].

“Globally, systems have been established and are being strengthened to detect ‘signals’ of potential VOIs or VOCs and assess these based on the risk posed to global public health” [9]. In South Africa, the Network for Genomics Surveillance in South Africa was formed to understand the spread of SARS-CoV-2 [18]. During the first COVID-19 epidemic wave in South Africa, 16 SARS-CoV-2 lineages specific to South Africa, 16 SARS-CoV-2 lineages specific to South Africa.
Africa were identified from 1365 high-quality whole genomes [18]. From these 16 lineages, 3 main clusters (B.1.1.54, B.1.1.56, and C.1 SARS-CoV-2 lineages) were identified to have caused approximately 42% of SARS-CoV-2 infections in South Africa [18]. Another sublineage specific to South Africa was the B.1.106 lineage that emerged in Kwa-Zulu Natal province in a nosocomial outbreak during the first COVID-19 epidemic wave [18]. The prevalence of this sublineage decreased as a result of control measures [18,19]. The C.1 lineage (first identified C lineage of SARS-CoV-2) was the most geographically spread lineage during the first COVID-19 epidemic wave in South Africa [18]. Before the resurgence of the second COVID-19 epidemic wave in South Africa, the beta (B.1.351, B.1.351.2, and B.1.351.3 lineages) SARS-CoV-2 VOC (formerly GR/501Y.V2) was identified in an analysis of 2704 South African SARS-CoV-2 genotypes (samples collected till December 14, 2020) from the GISAID database. The beta (B.1.351 lineage) SARS-CoV-2 VOC was detected in samples collected in October 2020 [20]. The beta SARS-CoV-2 lineage became the dominant lineage in South Africa’s second COVID-19 epidemic wave, rapidly replacing the 3 main clusters (B.1.1.54, B.1.1.56, and C.1 SARS-CoV-2 lineages) identified during the first COVID-19 epidemic wave [20]. During the resurgence of the third COVID-19 epidemic wave in South Africa, 4 SARS-CoV-2 variants were identified: alpha, beta, eta, and delta SARS-CoV-2 variants. Genomic data for South African samples identified 65% of 1147 whole genomes from May 2021 as the beta SARS-CoV-2 variant. The alpha, delta, and eta SARS-CoV-2 variants accounted for 6%, 16%, and 1% of those samples, respectively. In June 2021, with 2931 genetic sequences in that period, the delta SARS-CoV-2 variant had become the dominant variant in samples collected in South Africa at 66%, while the beta and alpha SARS-CoV-2 variants accounted for 16% and 4%, respectively [21]. By the end of South Africa’s third COVID-19 epidemic wave in September 2021, the delta SARS-CoV-2 variant accounted for 96% of the 186 whole-genome sampled in that period, while the C.1 SARS-CoV-2 lineage accounted for 1% of those samples [21]. The C.1 SARS-CoV-2 lineage, a new South Africa-specific SARS-CoV-2 lineage (evolved from the C.1 SARS-CoV-2 lineage), was identified in South African samples in May 2021. The C.1.2 lineage was detected across the majority of South African provinces and in 7 other countries [22]. On November 25, 2021, the National Institute for Communicable Diseases (NICD) in South Africa confirmed the detection of the omicron SARS-CoV-2 VOC (B.1.1.529 lineage) in SARS-CoV-2 genomes of 22 laboratory-confirmed cases of COVID-19 [23]. The investigation into the initially identified cases of the B.1.1.529 SARS-CoV-2 lineage in South Africa was triggered by the absence of the S gene (S-gene dropout or-gene target failure) in a specific PCR assay because of the 69-70del deletion [24]. A similar observation was made during the early identification of the alpha SARS-CoV-2 VOC (B.1.1.7 lineage). The omicron SARS-CoV-2 VOC (B.1.1.529 lineage) was the dominant SARS-CoV-2 lineage in the fourth COVID-19 wave in South Africa, accounting for 82% of SARS-CoV-2 infections in November 2021 and 98.5% of SARS-CoV-2 infections in South Africa by January and February 2022 [25]. By the time of the resurgence of the fifth COVID-19 epidemic wave in South Africa in April 2022, the omicron SARS-CoV-2 VOC had evolved into sublineages. A total of 2459 whole genomes from South African samples with confirmed SARS-CoV-2 infection in April 2022 identified the omicron SARS-CoV-2 sublineages (BA.4, BA.2, and BA.5) in 54%, 25%, and 19% of the respective samples, respectively [26]. By the end of the fifth COVID-19 epidemic wave in South Africa in July 2022, 339 whole genomes from South African samples with confirmed SARS-CoV-2 infection in that period were identified to be largely omicron SARS-CoV-2 sublineages (BA.5 and BA.4), accounting for 96% of those samples [27].

COVID-19 Vaccination in South Africa

The immunity against COVID-19 in humans is thought to be both innate and adaptive. Most patients with COVID-19 who recovered developed antibodies against SARS-CoV-2 within 1 to 3 weeks [28]. The SARS-CoV-2 seroprevalence in South Africa’s first COVID-19 epidemic wave was estimated to be between 31% and 46% [29], while in the second COVID-19 epidemic wave, it was estimated to be 35.8% [30-32]. COVID-19 vaccination in South Africa commenced during South Africa’s second COVID-19 epidemic wave and was limited to frontline workers, such as health care workers, owing to the limited access to vaccines at the time [33]. COVID-19 vaccines were then largely administered to the rest of the South African population during the third and fourth COVID-19 epidemic waves. According to the WHO, there are currently 52 COVID-19 candidate vaccines in the clinical evaluation stages, of which 13 are in phase 3. Some vaccines have been reported to have an efficacy greater than 90% (BioNTech or Fosun Pharma or Pfizer, AstraZeneca, Sinovac, and Sputnik V vaccines) [28]. South Africa’s COVID-19 vaccination run through COVID-19 vaccination programs and clinical trials have largely administered the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson or Janssen doses to the South African population. By August 26, 2022, a total of 37.4 million people had been vaccinated against COVID-19 in South Africa, mainly with the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson or Janssen COVID-19 vaccines [34]. The Johnson & Johnson or Janssen COVID-19 vaccine is a viral vector vaccine, whereas the Pfizer-BioNTech (Comirnaty) COVID-19 vaccine is an mRNA vaccine. Both vaccines are dependent on the encoded SARS-CoV-2 spike (S) proteins to induce an immune response after vaccination. Thus, SARS-CoV-2 VOCs and VOIs with mutations on the spike (S) proteins emerging after the development of these vaccines have hampered their efficacy, requiring booster doses [35].

The evolution of SARS-CoV-2 has played a major role in the resurgence of the COVID-19 epidemic waves in South Africa and across the globe. South Africa has a unique observation of the evolution of SARS-CoV-2, with distinct SARS-CoV-2 lineages dominating certain epidemic periods. This unique observation allows for an investigation of the impact of the detected SARS-CoV-2 lineages on COVID-19 transmissibility and severity through the analysis of epidemiological data. In this study, a descriptive and inferential statistical analysis was conducted on South African COVID-19 epidemiological data to describe and investigate the impact of SARS-CoV-2 lineages.

Methods

The general methodology in this study involved the collation and stratification of South African COVID-19 epidemiological data, covariance, regression analysis of epidemiological data, normalization, and comparative inferential statistical analysis through null hypothesis testing (paired 2-tailed t tests).

Collation of South African COVID-19 Epidemiological Data

South African COVID-19 reported case data (cumulative and daily COVID-19 cases, recovered, and deaths) for the reporting period from January 22, 2020, to August 18, 2022, were obtained from the Johns Hopkins University Center for Systems Science and Engineering COVID-19 Database [2]. The South African COVID-19 testing data (cumulative and daily COVID-19 tests) were obtained from the Our World In Data project [36] for the reporting period from February 14, 2020, to June 22, 2022. South African COVID-19 hospitalization data were obtained from the NICD DATCOV surveillance system [37] for the period May 24, 2020, to August 18, 2022. The NICD DATCOV surveillance system in South Africa only started publishing reports on the reporting date of May 24, 2020; thus, data from March 5, 2020, to May 23, 2020, in the first COVID-19 epidemic wave period are missing. Data from October 9, 2020, to October 26, 2020, in the second COVID-19 epidemic wave period were also missing. The number of hospitals reporting to the NICD DATCOV surveillance system during South Africa's first COVID-19 epidemic wave period was initially 204 facilities and the number of facilities increased to 666 by the end of the fifth COVID-19 epidemic wave period. The South African COVID-19 hospitalization data obtained in this study were composed of the number of facilities reporting, admission status data (daily COVID-19 hospital admission cases, hospitalized in high care, intensive care unit [ICU], isolation ward, on oxygen, and ventilator), cumulative COVID-19 admission age profile, cumulative COVID-19 hospital deaths age profile, and cumulative COVID-19 patients discharged alive. The COVID-19 hospital daily discharge rate (DR) and case fatality rate (CFR) were then calculated based on the methodology described in the study by Mabuka et al [38]. Weekly South African natural and excess (natural) deaths were obtained from the South African Medical Research Council [39] for the reporting period of December 29, 2019, to August 20, 2022. The weekly unreported excess deaths (natural) to COVID-19 death ratio (ECDR) was then calculated based on the methodology in the study by Mabuka et al [38].

South African COVID-19 vaccination data (cumulative number of people who received at least 1 dose of a COVID-19 vaccine per population age group) for the reporting period of February 17, 2021, to August 18, 2022, were obtained from the Department of Health Republic of South Africa COVID-19 Public Dashboard [34]. The data in the respective dashboards only contained COVID-19 vaccination records captured on the live Electronic Vaccination Data System and excluded vaccination records captured on paper [34]. South African community mobility data, which includes the change from baseline in movement in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential locations, were obtained from the Google Community Mobility Reports [40] with data from the period of February 15, 2020, to August 18, 2022. The reports are created using aggregated data from users who have turned on their location history in their Google accounts. The baseline in these reports was the median values of movement in the respective locations from January 3 to February 6, 2020.

Stratification of South African COVID-19 Epidemiological Data

Study analysis data sets from the collated South African COVID-19 epidemiological data were produced using SAS Base 9.4 software. To draw inferential comparisons regarding the impact of the evolution of SARS-CoV-2 and COVID-19 vaccinations in South African COVID-19 epidemiology, the collated South African COVID-19 epidemiological data were stratified based on the observed COVID-19 epidemic wave periods 1, 2, 3, 4, and 5 in South Africa. The South African COVID-19 epidemic wave periods 1, 2, 3, 4, and 5 were classified as collated data from March 5 to September 30, 2020; October 1, 2020, to April 26, 2021; April 27 to November 14, 2021; November 15, 2021, to April 1, 2022; and April 2 to July 31, 2022, respectively. The labels of stratified variables were given a suffix reference of “1,” “2,” “3,” “4,” and “5” for the 5 COVID-19 epidemic periods, respectively. For cumulative epidemiological data (South African cumulative COVID-19 admission age profile, cumulative COVID-19 hospital deaths age profile, and cumulative COVID-19 patients discharged alive), the data were adjusted using equation 1 to remove the cumulative data from the previous COVID-19 epidemic period:

Cumulative epidemic variable adj (n), i = cumulative epidemic variable (n), i − cumulative epidemic variable (n), j (1)

where n is the number of patients, i is the reported date, and j is the last reported date of the previous COVID-19 epidemic period. The stratification of data in this study was done by splitting the data using the epidemic period variable in SPSS (version 28; IBM Corp).

On the basis of a review of the literature [18,20,21,25-27,41], Table S1 in Multimedia Appendix 1 summarizes the South African SARS-CoV-2 lineage clusters observed in the South African genomic data during the respective observed COVID-19 epidemic wave periods. On the basis of this stratification, the cluster of lineages identified in Table S1 in Multimedia Appendix 1 was assumed to be the SARS-CoV-2 lineages resulting in the respective COVID-19 epidemic waves in South Africa.

Covariance and Regression of South African Epidemiological Data

In this study, covariance between the following epidemiological data was investigated: (1) COVID-19 daily tests and cases, (2) the number of reporting hospitals to NICD DATCOV and COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily COVID-19 hospitalized cases.
COVID-19 cases, hospital-to-active cases (HA), CFR, DR, admission status, and admitted death status with daily cumulative COVID-19 vaccinated people. The covariance was investigated by applying Bivariate Analysis using 2-tailed Pearson and Spearman tests in SPSS. The 2 respective parametric and nonparametric methods were applied, considering the distribution of data and linearity. The assumed linearity was based on 2-tailed Pearson correlations with Pearson correlations above 0.850 or below 0.850, with \( P < .001 \) considered as having a high degree of linearity. For data with assumed linearity, further analysis was conducted by applying an ANOVA using the Univariate General Linear Model.

Normalization and Paired \( t \) tests on South African Epidemiological Data

In this study, COVID-19 transmissibility was measured through the magnitude of mean and variance of the percentage of daily COVID-19 positive tests, considering the linear positive correlation between daily COVID-19 tests and cases. COVID-19 severity was measured through the magnitude of the mean and variance of the COVID-19 hospital admission cases, admission status, admission age profile, death age profile, CFR, DR, and ECDR. Considering the linear positive correlation between COVID-19 active and hospital admission cases, a normalized parameter (COVID-19 HA) was used to normalize the variance of active cases in hospital admission cases.

For the comparative inferential statistical analysis conducted to understand the impact of SARS-CoV-2 evolution on COVID-19 transmissibility and severity in South Africa, descriptive statistics and paired samples \( t \) test at 95% CIs were conducted on the normalized parameters in SPSS. The paired \( t \) test was conducted between the COVID-19 epidemic wave periods based on the following \( t \) test pairings: normalized parameter 1 with normalized parameter 2 (pair 1), normalized parameter 1 with normalized parameter 3 (pair 2), normalized parameter 1 with normalized parameter 4 (pair 3), normalized parameter 1 with normalized parameter 5 (pair 4), normalized parameter 2 with normalized parameter 3 (pair 5), normalized parameter 3 with normalized parameter 4 (pair 6), and normalized parameter 4 with normalized parameter 5 (pair 7) were the suffix number representing the COVID-19 epidemic wave period.

Data Handling and Limitations

The propagated error due to data capturing or data reliability from data sources were not accounted for. Each descriptive and inferential statistical analysis test was applied independently to the analysis data sets, and there was no codependent or propagated error in the results of the applied statistical tests. The time variance of data was accounted for by reporting values to 95% CIs. Missing data values were not included in the analysis. The Pearson and Spearman correlation coefficients are limited to determining the strength of the association between ≥2 or more variables; however, they do not determine the causality of this relationship. In this study, COVID-19 seroprevalence was not investigated as a confounder because of the limitations of these data.

Ethics Approval

An internal ethical assessment was conducted within the Afrikan Research Initiative at the start of the ARI COVID-19 Research Project, and no regional ethics approval was requested for this study. Data used in this study were obtained from public sources with an Open Data Licence. Patient data were obtained from the public source and anonymized following the local regulations of the Protection of Personal Information Act in South Africa.

Results

Covariance and Regression of South African COVID-19 Epidemiological Data

Table 1 shows that the mean COVID-19 daily tests in the first, second, third, fourth, and fifth South African COVID-19 epidemic wave periods were 20,575 (SD 14,062), 31,046 (SD 14,115), 41,315 (SD 16,108), 35,226 (SD 17,078), and 23,419 (SD 7229), respectively.
The Pearson (coefficient of determination) correlation coefficients between COVID-19 daily tests (independent variable) and cases (dependent variable) in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa were determined to be 0.910 (0.828), 0.877 (0.769), 0.893 (0.797), 0.859 (0.737), and 0.749 (0.562), respectively (shown in Table S2 in Multimedia Appendix 1). The F test values between the mean square regression and residual for the daily COVID-19 tests and cases linear regression in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa are shown in Table S3 in Multimedia Appendix 1, and the unstandardized and standardized coefficients are shown in Table S4 in Multimedia Appendix 1. The Pearson (coefficient of determination) correlation coefficients between facilities reporting to the NICD DATCoV (independent variable) and COVID-19 hospital admission cases (dependent variable) in the first, second, third, and fourth COVID-19 epidemic waves in South Africa were determined to be 0.932 (0.869), 0.819 (0.671), 0.967 (0.936), 0.919 (0.845), and 0.863 (0.745), respectively (Table S5 in Multimedia Appendix 1). The Pearson (coefficient of determination) correlation coefficients between COVID-19 active (independent variable) and hospital admission cases (dependent variable) in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa were determined to be 0.936 (0.113), 0.212 (0.045), 0.385 (0.148), and −0.249 (−0.062), respectively (Table S5 in Multimedia Appendix 1). The Pearson (coefficient of determination) correlation coefficients between daily COVID-19 tests and cases in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa are presented in Table S6 in Multimedia Appendix 1.

South Africa’s COVID-19 NPI health policy response to the COVID-19 epidemic waves in South Africa was implemented in the form of National Lockdown Alert Level policies. The National Lockdown Alert Level policies were largely entry and exit screening at borders, limitations of movements and gatherings, closure and limitations of institutions and business activities, ban and limiting of alcohol and tobacco industries, isolation, quarantine of potentially infected persons, contact-tracing protocols, use of personal protective equipment, and hygienic protocols [38,42]. The adjustment in the alert levels resulted in eased movement restrictions compared with their predecessors. Table S7 in Multimedia Appendix 1 shows the summary of COVID-19 NPI policies implemented in South Africa during the first, second, third, fourth, and fifth COVID-19 epidemic waves.

The mean change in movement from baseline in the retail and recreation, grocery and pharmacy, parks, transit stations, workspaces, and residential locations in South Africa during the implementation of the no national lockdown; National Lockdown Level 5, 4, 3, and 2; and National Lockdown Adjusted Level 4, 3, 2, and 1 are shown in Multimedia Appendix 2. In general, COVID-19 NPI policies in South Africa resulted in a negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workplaces. Residential locations in South Africa had a positive mean change in movement from baseline. A decrease in the national lockdown alert levels resulted in a decrease in the modulus mean change in movement from the baseline in locations in South Africa. The national lockdown adjusted alert levels resulted in a lower modulus mean change in movement from the baseline in locations in South Africa than the lowest unadjusted Alert Level (National Lockdown Alert Level 2). Concerning movement, the most affected locations in South Africa by the COVID-19 NPI policies implemented were the retail and recreation, transit stations, workplaces, and residential locations. The National Lockdown Alert Level 5 had the largest impact on the movement in South African communities, whereas the National Lockdown Adjusted Alert Level 1 had the least impact on movement in South African communities. Figure 1 shows the mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workplaces.

Table 1: Statistical sample number (n), range, mean (SD), and coefficient of skewness of daily COVID-19 tests and cases in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n⁴</th>
<th>Values, mean (SD; range)</th>
<th>Skewness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily COVID-19 cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>237</td>
<td>2845 (3794; 0-13,944)</td>
<td>1.53</td>
</tr>
<tr>
<td>2</td>
<td>208</td>
<td>4336 (5034; 437-21,980)</td>
<td>1.79</td>
</tr>
<tr>
<td>3</td>
<td>197</td>
<td>6672 (6252; 0-26,485)</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>137</td>
<td>5705 (6816; 0-37,875)</td>
<td>1.95</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>3293 (2791; 0-13,613)</td>
<td>1.31</td>
</tr>
<tr>
<td>Daily COVID-19 tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>203</td>
<td>20,575 (14,062; 4-56,663)</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>201</td>
<td>31,046 (14,115; 10,402-77,167)</td>
<td>1.16</td>
</tr>
<tr>
<td>3</td>
<td>191</td>
<td>41,315 (16,108; 13,507-96,896)</td>
<td>0.62</td>
</tr>
<tr>
<td>4</td>
<td>133</td>
<td>35,226 (17,078; 12,942-104,831)</td>
<td>1.40</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>23,419 (7299; 9149-39,613)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

⁴Values, n represents the number of observations or records pooled for the mean sample size calculations. All other values represent absolute descriptive statistical values.
and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential locations in South Africa during the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa. Figure 1 shows that the negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workplaces gradually decreased during each consecutive COVID-19 epidemic wave. The mean change in movement from the baseline in residential locations was positive for the 5 COVID-19 epidemic waves. Figure 1 also shows that the mean change in movement from baseline in South African locations was positive during the fourth and fifth COVID-19 epidemic waves.

The cumulative (maximum) number of people receiving at least 1 dose of the COVID-19 vaccine relative to the total population per age group in the first, second, third, fourth, and fifth COVID-19 epidemic waves is shown in Multimedia Appendix 3. By August 30, 2022, a total of 37,456,345 doses of COVID-19 vaccines had been administered in South Africa. Of the 37,456,345 nationally administered doses, 9,190,172 (24.5%) doses of the COVID-19 vaccine administered were the Johnson & Johnson COVID-19 vaccine, with 1,385,476 (3.7%) doses being the Johnson & Johnson COVID-19 vaccine booster dose; 28,266,173 (75.5%) of the nationally administered amount was the Pfizer COVID-19 vaccine, with 14,452,185 (38.6%), 11,515,875 (30.7%), and 2,298,113 (6.14%) being the Pfizer COVID-19 vaccine first, second, and third booster doses, respectively [34]. Figure 2 shows the mean daily number of COVID-19 vaccinated people per age group during the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa. Figure 2 shows that the mean daily COVID-19 vaccination rate per age group was lowest in the second and fourth COVID-19 epidemic waves and highest in the third COVID-19 epidemic wave for all age groups. There were no reported administered COVID-19 vaccinations in South Africa during the first COVID-19 epidemic wave. Figure 2 shows that there was a decrease in the mean daily COVID-19 vaccination rate in all age groups after the third COVID-19 epidemic wave.

The Spearman correlation coefficients and $P$ values among daily COVID-19 cases, change in daily COVID-19 cases, HA, CFR, DR, admission status, and admitted death status with daily cumulative COVID-19 vaccinated people are shown in Table S8 in Multimedia Appendix 1. The Spearman correlation coefficients and $P$ values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth, and fifth COVID-19 epidemic waves in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916), and 0.811 (95% CI 0.673-0.895), respectively, with $P<.001$. The Spearman correlation coefficients and $P$ values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 ($P=.79$; 95% CI $−0.207$ to 0.266), $−0.014$ ($P=.93$; 95% CI $−0.341$ to 0.316), and $−0.077$ ($P=.62$; 95% CI $−0.374$ to 0.233), respectively. These results show a significant strong positive monotonic correlation ($P<.001$) between daily COVID-19 cases and daily cumulative COVID-19 vaccinations and a weak monotonic correlation between the change in daily COVID-19 cases and daily cumulative COVID-19 vaccinations. The Spearman correlation coefficients and $P$ values between the daily cumulative COVID-19 vaccinated people and the daily HA were $−0.983$ (95% CI $−0.989$ to $−0.972$), $−0.852$ ($P=.93$; 95% CI $−0.921$ to $−0.731$), and $−0.917$ ($P=.62$; 95% CI $−0.955$ to $−0.850$), respectively, with $P<.001$. These results show a significantly strong negative monotonic correlation ($P<.001$) between daily COVID-19 HA and daily cumulative COVID-19 vaccinated individuals. The Spearman correlation coefficients and $P$ values between the daily cumulative COVID-19 vaccinated people and the CFR were 0.380 ($P<.001$; 95% CI 0.160-0.565), 0.192 ($P=.25$; 95% CI $−0.150$ to 0.494), and 0.264 ($P=.09$; 95% CI $−0.049$ to 0.529), respectively. These results show a moderate positive monotonic correlation between hospital CFR and daily cumulative COVID-19 vaccinated people. The Spearman correlation coefficients between daily cumulative COVID-19 vaccinated people and the percentage hospitalized age groups of 18 to 34 years showed a significantly strong positive monotonic correlation ($P<.001$), while the age groups of 50 to above 60 years showed a significantly strong negative monotonic correlation ($P<.001$).
Figure 1. Mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residences during the South African first, second, third, fourth, and fifth COVID-19 epidemic waves.

Figure 2. Mean daily number of COVID-19 vaccinated people per age group during the first, second, third, fourth, and fifth COVID-19 epidemic wave in South Africa.

Descriptive Statistics and Paired Samples t tests of South African COVID-19 Epidemiological Data

COVID-19 Detection in South Africa

Table 2 shows the descriptive statistics for COVID-19 active and daily positive COVID-19 tests for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa.

The daily positive COVID-19 tests indicate the transmissibility of COVID-19 based on the detection rate of COVID-19 and account for covariance in the testing rate for statistical comparison between the epidemic wave periods. The mean daily positive COVID-19 tests in South Africa’s first and second COVID-19 epidemic waves were not statistically different ($P=.97$). The mean daily positive COVID-19 tests in South Africa’s third, fourth, and fifth COVID-19 epidemic waves were 15.7%, 18.4%, and 24.3% more than those of the first and second COVID-19 epidemic wave periods, respectively. The difference among the mean daily positive COVID-19 tests in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods can also be observed in Figure 3. A paired t test of the daily positive COVID-19 tests between the first and second COVID-19 epidemic wave periods (pair 1)
showed no significant difference at a 95% CI between these COVID-19 epidemic periods with $P = .97$ (Table 3). The paired $t$ test of the daily positive COVID-19 tests between test pairs 2 to pair 7 showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods (Table 3).

**Table 2.** Statistical sample number (n), range, and mean (SD) of COVID-19 active cases and daily positive COVID-19 tests in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n$^a$</th>
<th>Values, mean (SD; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 active cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>237</td>
<td>45,851 (53,975; 0-173,590)</td>
</tr>
<tr>
<td>2</td>
<td>208</td>
<td>66,178 (53,878; 19,809-239,799)</td>
</tr>
<tr>
<td>3</td>
<td>197</td>
<td>89,171 (63,613; 16,243-211,052)</td>
</tr>
<tr>
<td>4</td>
<td>137</td>
<td>75,527 (65,996; 10,849-216,947)</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>35,833 (22,323; 9644-81,174)</td>
</tr>
<tr>
<td>Daily positive COVID-19 tests (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>203</td>
<td>11.5 (8.58; 0-34.0)</td>
</tr>
<tr>
<td>2</td>
<td>201</td>
<td>11.5 (8.45; 2.7-33.7)</td>
</tr>
<tr>
<td>3</td>
<td>191</td>
<td>13.3 (9.72; 0-31.6)</td>
</tr>
<tr>
<td>4</td>
<td>133</td>
<td>13.3 (9.91; 0-60.7)</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>13.3 (8.49; 0-41.6)</td>
</tr>
</tbody>
</table>

$^a$Values, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.

**Figure 3.** The mean daily positive COVID-19 tests (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.
Table 3. Mean paired differences, SD of paired differences, SE of mean (SE mean), 95% CI of the upper and lower difference, t value, df, and P value (significance, 2-tailed) for the daily positive COVID-19 tests in pair 1 to pair 7 (paired samples t test).

<table>
<thead>
<tr>
<th>Sample t test pairing</th>
<th>Paired variables</th>
<th>Paired differences, mean (SD)</th>
<th>t test (df)</th>
<th>Significance (2-tailed), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>Daily positive COVID Test1—daily positive COVID Test2</td>
<td>−0.03 (13.26)</td>
<td>−0.04 (198)</td>
<td>.97</td>
</tr>
<tr>
<td>Pair 2</td>
<td>Daily positive COVID Test1—daily positive COVID Test3</td>
<td>−2.78 (14.42)</td>
<td>−2.65 (187)</td>
<td>.009</td>
</tr>
<tr>
<td>Pair 3</td>
<td>Daily positive COVID Test1—daily positive COVID Test4</td>
<td>−5.00 (15.10)</td>
<td>−3.66 (121)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 4</td>
<td>Daily positive COVID Test1—daily positive COVID Test5</td>
<td>−10.8 (10.67)</td>
<td>−7.39 (52)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 5</td>
<td>Daily positive COVID Test2—daily positive COVID Test3</td>
<td>−1.20 (5.95)</td>
<td>−2.80 (191)</td>
<td>.006</td>
</tr>
<tr>
<td>Pair 6</td>
<td>Daily positive COVID Test3—daily positive COVID Test4</td>
<td>5.22 (14.87)</td>
<td>3.96 (126)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 7</td>
<td>Daily positive COVID Test4—daily positive COVID Test5</td>
<td>2.71 (8.85)</td>
<td>2.37 (59)</td>
<td>.02</td>
</tr>
</tbody>
</table>

COVID-19 Hospital Admissions in South Africa

Table 4 shows the descriptive statistics for the COVID-19 HA for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. The second COVID-19 epidemic wave period in South Africa had the highest number of COVID-19 HA, followed by the third COVID-19 epidemic wave period. The mean paired difference of the COVID-19 HA in pairs 1, 2, 3, 4, 5, 6, and 7 was −14.0%, −10.6%, −4.62%, −7.72%, 2.31%, 4.02%, and −2.34%, respectively (Table 5). The difference between the mean number of COVID-19 HA in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods can also be observed in Figure 4. Paired t tests of the COVID-19 HA among pairs 1, 2, 3, 4, 5, 6, and 7 showed a significant difference at a 95% CI between the respective COVID-19 epidemic periods with P < .001 (Table 5).

Multimedia Appendix 4, Multimedia Appendix 5, and Multimedia Appendix 6 show the COVID-19 HA and vaccinated people in the first half period of the third, fourth, and fifth COVID-19 epidemic wave in South Africa, respectively. The first half period of the COVID-19 epidemic wave is the period from the first case to the peak of the epidemic wave. Multimedia Appendices 4-6 show that the number of COVID-19 HA decreased while the number of COVID-19 vaccinated people increased in the first half period of the third, fourth, and fifth COVID-19 epidemic wave in South Africa.

Table 4. Statistical sample number (N), minimum, maximum, and mean (SD) of COVID-19 hospital-to-active cases in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, N</th>
<th>Values, mean (SD; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 hospital-to-active cases (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>6.80 (1.82; 4.02-12.7)</td>
</tr>
<tr>
<td>2</td>
<td>189</td>
<td>18.7 (7.08; 0-32.4)</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>16.5 (5.56; 9.28-29.0)</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>9.84 (4.48; 3.65-24.2)</td>
</tr>
<tr>
<td>5</td>
<td>113</td>
<td>4.48 (3.32; 3.73-21.7)</td>
</tr>
</tbody>
</table>

*aValues, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.*
Table 5. Mean paired differences, SD of paired differences, SE of mean (SE mean), t value, df, and P value (significance, 2-tailed) for the COVID-19 hospital-to-active (HA) cases in pair 1 to pair 7 (paired samples t test).

<table>
<thead>
<tr>
<th>Sample t test pairing</th>
<th>Paired variables</th>
<th>Paired differences, mean (SD)</th>
<th>t test (df)</th>
<th>Significance (2-tailed), P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>HA1—HA2</td>
<td>−14.0 (8.59)</td>
<td>−18.3 (126)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 2</td>
<td>HA1—HA3</td>
<td>−10.6 (5.99)</td>
<td>−19.7 (122)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 3</td>
<td>HA1—HA4</td>
<td>−4.62 (4.41)</td>
<td>−7.48 (50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 4</td>
<td>HA1—HA5</td>
<td>−7.72 (4.01)</td>
<td>−11.2 (33)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 5</td>
<td>HA2—HA3</td>
<td>2.31 (7.34)</td>
<td>4.33 (189)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 6</td>
<td>HA3—HA4</td>
<td>4.02 (5.21)</td>
<td>9.07 (137)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pair 7</td>
<td>HA4—HA5</td>
<td>−2.34 (3.04)</td>
<td>−8.42 (119)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Figure 4. The mean COVID-19 hospitalized-to-active cases (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.

COVID-19 Hospital Admission Status in South Africa

Table 6 shows the descriptive statistics for the COVID-19 hospital admission status for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 5 shows the COVID-19 hospital admission status profiles in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 5 shows that most COVID-19 hospitalized cases in South Africa were hospitalized in the general ward (60.0%-79.1%). Figure 5 also shows that the COVID-19 patients on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the ICU (8.07%-16.7%). The number of hospitalized COVID-19 cases admitted to the general ward was highest in the fourth and fifth COVID-19 epidemic wave periods, followed by the first COVID-19 epidemic wave period. The number of South African patients with COVID-19 admitted in high care, in the ICU, and on ventilators was highest in the first COVID-19 epidemic wave. The number of patients with COVID-19 on oxygen was highest in the second COVID-19 epidemic wave. The general trend showed a decrease in patients admitted on oxygen, in the ICU, and on a ventilator in the fourth and fifth COVID-19 epidemic waves compared with previous COVID-19 epidemic waves. Patients in high care have remained relatively in the mean range of 5.65% (SD 0.80%) to 7.93% (SD 1.03%) throughout the COVID-19 epidemic in South Africa. Paired t tests of the mean COVID-19 hospital admission status in pairs 1, 2, 3, 4, 5, 6, and 7 showed a significant difference at a 95% CI between the respective COVID-19 epidemic periods with $P < .001$ (Table S9 in Multimedia Appendix 1). Except for the COVID-19 hospitalized cases admitted in the general ward, in high care, on oxygen, and on ventilator between the fourth and fifth COVID-19 epidemic waves (pair 7), whose difference was found not to be statistically significant (Table S9 in Multimedia Appendix 1).
Table 6. Statistical sample number (n), range, and mean (SD) of COVID-19 hospitalized cases in the general ward, in high care, in the intensive care unit (ICU), on oxygen, and on ventilator in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Values, mean (SD; range)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COVID-19 hospitalized general ward (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>72.8 (2.62; 68.7-78.1)</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>61.7 (9.61; 52.6-86.2)</td>
<td>92.4</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>60 (4.10; 50.9-70.7)</td>
<td>16.8</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>79.1 (5.45; 63.8-84.4)</td>
<td>29.7</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>79.1 (3.71; 56.9-84.3)</td>
<td>13.8</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized high care (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>7.93 (1.03; 5.07-10.5)</td>
<td>1.07</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>5.65 (0.80; 4.09-7.81)</td>
<td>0.647</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>6.09 (0.81; 4.51-7.80)</td>
<td>0.651</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>6.96 (1.33; 4.66-11.0)</td>
<td>1.76</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>7.81 (3.22; 4.29-30.0)</td>
<td>10.34</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized ICU (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>16.7 (2.17; 11.9-20.6)</td>
<td>4.69</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>10.9 (1.49; 7.96-14.1)</td>
<td>2.21</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>12.4 (1.12; 9.65-15.1)</td>
<td>1.25</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>8.07 (0.89; 6.33-10.1)</td>
<td>0.789</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>9.74 (1.28; 6.97-11.8)</td>
<td>1.646</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized on oxygen (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>16.8 (5.76; 10.6-30.8)</td>
<td>33.2</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>21.4 (3.12; 14.7-27.7)</td>
<td>9.72</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>15.1 (3.64; 8.43-23.9)</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>11.4 (2.02; 7.66-16.3)</td>
<td>4.07</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>11.2 (2.18; 8.81-26.3)</td>
<td>4.75</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized on ventilator (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>7.81 (1.79; 4.87-10.8)</td>
<td>3.20</td>
</tr>
<tr>
<td>2</td>
<td>187</td>
<td>5.34 (1.17; 3.28-6.93)</td>
<td>1.38</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>6.49 (0.68; 3.86-8.08)</td>
<td>0.458</td>
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<tr>
<td>4</td>
<td>138</td>
<td>3.29 (0.45; 2.47-4.50)</td>
<td>0.205</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>3.41 (1.74; 2.33-16.3)</td>
<td>3.013</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.
COVID-19 Hospital Admission Age Profile in South Africa

Table 7 shows the descriptive statistics for the COVID-19 hospital admission age profiles for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa.

Figure 6 shows the COVID-19 hospital admission age profile in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 6 shows that most COVID-19 hospitalized cases in South Africa’s first, second, third, and fourth COVID-19 epidemic wave periods were in the ages of 40 to 49 years (16.8%-20.4%) and 50 to 59 years (19.8%-25.3%), respectively. Patients admitted owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%) and highest in the fourth and fifth COVID-19 epidemic wave periods. Figure 6 shows that the mean age profile for COVID-19 hospital admissions for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods had a similar relative normal distribution within the admitted age groups. In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, while those for age groups between 30 and 79 years decreased.

Paired $t$ tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for all age groups showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table S10 in Multimedia Appendix 1), except for the following:

- Age groups between 20 and 29 years, 50 and 59 and 60 and 69 years between COVID-19 epidemic waves 2 and 3 ($P=.12$, $P=.08$, and $P=.68$, respectively).

Figure 5. The mean COVID-19 hospitalized admission status (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.
Table 7. Statistical sample number (n), range, mean, and SD of COVID-19 hospitalized cases in the ages of 0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and 80 to 89 years in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Values, mean (SD; range)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 hospitalized admitted (age 0-9 years; %)</td>
<td>1</td>
<td>110</td>
<td>2.32 (0.58; 1.68-3.96)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>187</td>
<td>1.98 (0.25; 1.81-3.66)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>202</td>
<td>2.47 (0.19; 1.14-3.48)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>138</td>
<td>3.63 (0.40; 2.92-4.14)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>4.59 (0.21; 4.21-4.83)</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 10-19 years; %)</td>
<td>1</td>
<td>108</td>
<td>1.75 (0.22; 1.23-2.62)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>179</td>
<td>2.23 (0.18; 1.98-2.56)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>202</td>
<td>2.62 (0.20; 1.19-3.68)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>138</td>
<td>3.43 (0.18; 3.09-3.66)</td>
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<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>3.78 (0.03; 3.71-3.81)</td>
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<tr>
<td>COVID-19 hospitalized admitted (age 20-29 years; %)</td>
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<td>8.04 (2.11; 0.04-14.6)</td>
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<td>2</td>
<td>187</td>
<td>7.39 (1.19; 0.63-13.8)</td>
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<td>3</td>
<td>202</td>
<td>7.78 (2.83; 3.30-47.3)</td>
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<td>4</td>
<td>138</td>
<td>9.03 (0.34; 8.29-9.32)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>9.42 (0.01; 9.40-9.43)</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 30-39 years; %)</td>
<td>1</td>
<td>126</td>
<td>18.1 (3.27; 11.4-29.8)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>187</td>
<td>15.3 (2.49; 13.0-30.5)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>202</td>
<td>14.8 (0.98; 6.36-18.9)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>138</td>
<td>15.9 (0.16; 15.5-16.0)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>16.0 (0.05; 16.0-16.1)</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 40-49 years; %)</td>
<td>1</td>
<td>75</td>
<td>20.4 (2.64; 18.8-27.4)</td>
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<tr>
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<td>2</td>
<td>181</td>
<td>18.17 (1.52; 17.2-36.5)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>201</td>
<td>17.73 (1.11; 7.70-22.6)</td>
</tr>
<tr>
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<td>4</td>
<td>138</td>
<td>17.3 (0.40; 17.0-18.6)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>16.8 (0.13; 16.7-17.0)</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 50-59 years; %)</td>
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<td>25.3 (4.07; 3.54-38.4)</td>
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<tr>
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<td>2</td>
<td>186</td>
<td>22.5 (2.57; 21.0-39.1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>201</td>
<td>22.1 (1.21; 9.76-27.2)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>138</td>
<td>20.6 (0.64; 19.4-21.8)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126</td>
<td>19.8 (0.19; 19.6-20.1)</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 60-69 years; %)</td>
<td>1</td>
<td>125</td>
<td>17.46 (2.73; 10.5-24.3)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>186</td>
<td>17.32 (1.91; 14.9-28.1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>202</td>
<td>17.11 (1.03; 7.65-22.5)</td>
</tr>
<tr>
<td>Parameter and COVID-19 epidemic wave</td>
<td>Values, n&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Values, mean (SD; range)</td>
<td>Variance</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>---------------------</td>
<td>------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>15.66 (0.29; 15.3-16.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>15.13 (0.07; 15.1-15.3)</td>
<td>0.01</td>
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<tr>
<td>COVID-19 hospitalized admitted (age 70-79 years; %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>10.17 (1.98; 4.56-16.3)</td>
<td>3.91</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>9.90 (1.16; 8.08-13.9)</td>
<td>1.33</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>10.6 (3.61; 5.44-60.5)</td>
<td>13.04</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>9.63 (0.08; 9.47-9.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>9.55 (0.07; 9.45-9.63)</td>
<td>0.00</td>
</tr>
<tr>
<td>COVID-19 hospitalized admitted (age 80-89 years; %)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125</td>
<td>5.498 (1.54; 0.88-9.43)</td>
<td>2.38</td>
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<tr>
<td>2</td>
<td>183</td>
<td>4.77 (0.45; 3.74-7.16)</td>
<td>0.20</td>
</tr>
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<td>202</td>
<td>5.01 (0.57; 2.41-7.16)</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>4.74 (0.19; 4.44-4.92)</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>4.87 (0.12; 4.69-5.01)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.

**Figure 6.** The mean COVID-19 hospitalized admission age profile (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.

**COVID-19 Hospital Deaths Age Profile in South Africa**

Table 8 shows the descriptive statistics for the COVID-19 hospital death age profiles for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Table 8 also shows the cumulative COVID-19 death age risk ratio, with age groups of 0 to 9 years as the reference.

Figure 7 shows the mean COVID-19 hospital death age profiles in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 7 shows that most COVID-19 hospitalized deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%). COVID-19 hospitalized deaths in the age groups of 0 to 29 years were relatively low (0.227%-4.89%). Figure 7 also shows that the COVID-19 hospitalized death age profiles for the first, second, and third COVID-19 epidemic wave periods were similar in distribution, while the distributions in the fourth and fifth COVID-19 epidemic waves were also similar. The mean COVID-19 hospitalized deaths in the fourth and fifth COVID-19 epidemic waves for the age groups of 0 to 49 years and 80 to
89 years were significantly higher than those observed in the first, second, and third COVID-19 epidemic waves, while the age groups of 50 to 69 years were significantly lower than the respective COVID-19 epidemic waves. General trends show an increase in COVID-19 hospitalized deaths in the age groups of 0 to 49 years and 80 to 89 years after each consecutive COVID-19 epidemic wave and a decrease in the age groups of 50 to 79 years. The cumulative risk of death in COVID-19 hospitalized deaths increased with increasing age groups. The cumulative risk of death in COVID-19 hospitalized deaths in the age groups above 40 years was significantly lower in the fourth and fifth COVID-19 epidemic waves when compared with prior COVID-19 epidemic waves.

Paired t tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for the age groups of 10 to 39 years and 50 to 89 years showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with \( P < 0.01 \) (Table S11 in Multimedia Appendix 1). Paired t tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for the age groups of 40 to 49 years showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with \( P < 0.01 \), except for the paired t tests among the first, fourth, and fifth COVID-19 epidemic waves. The differences were statistically insignificant with \( P = 0.34 \) and \( P = 0.32 \) (Table S11 in Multimedia Appendix 1).
Table 8. Statistical sample number (n), range, mean, SD of COVID-19 hospitalized deaths in the ages of 0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and 80 to 89 years in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n</th>
<th>Values, mean (SD; range)</th>
<th>Variance</th>
<th>CFARR&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COVID-19 hospitalized deaths (age 0-9 years; %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>0.227 (0.154; 0-1.15)</td>
<td>0.024</td>
<td>Reference 1</td>
</tr>
<tr>
<td>2</td>
<td>174</td>
<td>0.23 (0.082; 0-0.56)</td>
<td>0.007</td>
<td>Reference 2</td>
</tr>
<tr>
<td>3</td>
<td>198</td>
<td>0.837 (5.3; 0.12-75)</td>
<td>28.1</td>
<td>Reference 3</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>1.88 (1.043; 0.16-7.85)</td>
<td>1.087</td>
<td>Reference 4</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>1.23 (1.193; 0.08-7.69)</td>
<td>1.424</td>
<td>Reference 5</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized deaths (age 10-19 years; %)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>0.298 (0.17; 0-1.70)</td>
<td>0.029</td>
<td>1.31</td>
</tr>
<tr>
<td>2</td>
<td>182</td>
<td>0.309 (0.109; 0.23-1.45)</td>
<td>0.012</td>
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</tr>
<tr>
<td>3</td>
<td>201</td>
<td>0.273 (0.076; 0-0.36)</td>
<td>0.006</td>
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</tr>
<tr>
<td>4</td>
<td>134</td>
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<td>0.54</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>0.94 (0.711; 0.03-5.56)</td>
<td>0.506</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized deaths (age 20-29 years; %)</strong></td>
<td></td>
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<tr>
<td>1</td>
<td>126</td>
<td>1.57 (0.65; 0-7.04)</td>
<td>0.422</td>
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<tr>
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<td>183</td>
<td>1.58 (0.442; 1.14-4.93)</td>
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<tr>
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<td>200</td>
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<td>1.45</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>4.68 (2.31; 0.94-21.45)</td>
<td>5.34</td>
<td>2.49</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>4.89 (4.21; 0.34-22.22)</td>
<td>17.73</td>
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<tr>
<td><strong>COVID-19 hospitalized deaths (age 30-39 years; %)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>6.02 (2.01; 1.03-26.82)</td>
<td>4.02</td>
<td>26.5</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>4.51 (0.828; 2.93-8.75)</td>
<td>0.686</td>
<td>19.6</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>5.08 (1.51; 3.07-25)</td>
<td>2.27</td>
<td>6.06</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>11.9 (6.29; 4.05-51.78)</td>
<td>39.52</td>
<td>6.33</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>10.6 (3.23; 0.92-25.93)</td>
<td>10.45</td>
<td>8.65</td>
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<tr>
<td><strong>COVID-19 hospitalized deaths (age 40-49 years; %)</strong></td>
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<td>126</td>
<td>13.7 (4.8; 7.22-63.29)</td>
<td>23.04</td>
<td>60.2</td>
</tr>
<tr>
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<td>8.78 (1.87; 4.19-13.75)</td>
<td>3.50</td>
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</tr>
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<td>11.0</td>
</tr>
<tr>
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<td>138</td>
<td>14.2 (6.54; 11.17-59.95)</td>
<td>42.82</td>
<td>7.57</td>
</tr>
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<td>5</td>
<td>125</td>
<td>13.2 (3.26; 1.58-18.73)</td>
<td>10.64</td>
<td>10.7</td>
</tr>
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<td><strong>COVID-19 hospitalized deaths (age 50-59 years; %)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>76</td>
<td>24.8 (2.68; 14.78-37.50)</td>
<td>7.21</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>179</td>
<td>17.1 (3.15; 9.29-19.43)</td>
<td>9.93</td>
<td>74.6</td>
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<tr>
<td>3</td>
<td>202</td>
<td>20.3 (1.7; 17.14-25)</td>
<td>2.88</td>
<td>24.2</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>15.8 (7.21; 12.03-70.04)</td>
<td>52.0</td>
<td>8.42</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>16.1 (4.38; 1.90-25.78)</td>
<td>19.2</td>
<td>13.1</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized deaths (age 60-69 years; %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>125</td>
<td>29.5 (4.11; 23.80-39.86)</td>
<td>16.9</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>23.5 (4.62; 13.13-36.67)</td>
<td>21.32</td>
<td>103</td>
</tr>
<tr>
<td>3</td>
<td>201</td>
<td>26.9 (2.6; 24.81-36.97)</td>
<td>6.78</td>
<td>32.1</td>
</tr>
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</table>
COVID-19 CFR, DR, and ECDR in South Africa

Table 9 shows the descriptive statistics for the COVID-19 hospital CFR, hospital DR, natural deaths, excess natural deaths, weekly reported COVID-19 deaths, and weekly unreported ECDR for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Paired \( t \) tests of the CFR between the COVID-19 epidemic waves showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with \( P < .001 \) (Table S12 in Multimedia Appendix 1). Except for the paired \( t \) tests among the third, fourth, and fifth COVID-19 epidemic waves. Paired \( t \) tests of the DR between the COVID-19 epidemic waves showed no statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with lowest \( P = .27 \) (Table S12 in Multimedia Appendix 1). Except for the

### Parameter and COVID-19 epidemic wave

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n(^a)</th>
<th>Values, mean (SD; range)</th>
<th>Variance</th>
<th>CFARR(^b,c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>134</td>
<td>18.5 (2.05; 0-24.09)</td>
<td>4.22</td>
<td>9.82</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>15.9 (3.61; 1.49-22.22)</td>
<td>13.01</td>
<td>12.9</td>
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<tr>
<td><strong>COVID-19 hospitalized deaths (age 70-79 years; %)</strong></td>
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<td></td>
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<tr>
<td>1</td>
<td>125</td>
<td>20.7 (3.33; 11.75-25.45)</td>
<td>11.11</td>
<td>91.3</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>16.6 (3.7; 8.28-22.28)</td>
<td>13.7</td>
<td>72.4</td>
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<tr>
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<td>202</td>
<td>22.6 (2.18; 15.13-50)</td>
<td>4.74</td>
<td>27.0</td>
</tr>
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<td>138</td>
<td>20.1 (10.5; 6.67-102)</td>
<td>110</td>
<td>10.7</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>19.1 (7.1; 1.56-91.57)</td>
<td>50</td>
<td>15.6</td>
</tr>
<tr>
<td><strong>COVID-19 hospitalized deaths (age 80-89 years; %)</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125</td>
<td>13.5 (3.44; 2.16-18.18)</td>
<td>11.8</td>
<td>59.6</td>
</tr>
<tr>
<td>2</td>
<td>183</td>
<td>8.77 (2.22; 4.95-27.54)</td>
<td>4.93</td>
<td>38.2</td>
</tr>
<tr>
<td>3</td>
<td>202</td>
<td>14.3 (1.03; 11.74-25)</td>
<td>1.06</td>
<td>17.1</td>
</tr>
<tr>
<td>4</td>
<td>136</td>
<td>17.7 (8.8; 5.13-87.65)</td>
<td>77.5</td>
<td>9.39</td>
</tr>
<tr>
<td>5</td>
<td>126</td>
<td>18.2 (10.38; 1.11-92.06)</td>
<td>107.8</td>
<td>14.8</td>
</tr>
</tbody>
</table>

\(^a\)Values, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.

\(^b\)CFARR: cumulative COVID-19 death age risk ratio.

\(^c\)CFARR with COVID-19 epidemic wave references, reference 1, reference 2, reference 3, reference 4, and reference 5 as the mean COVID-19 hospitalized deaths (age 0 to 9 years; %) for the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa, respectively.

Figure 7. The mean COVID-19 hospitalized death age profile (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.
Paired $t$ tests between the third and fourth COVID-19 epidemic periods. Paired $t$ tests of the ECDR between the COVID-19 epidemic waves showed no statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with lowest $P=.69$ (Table S12 in Multimedia Appendix 1).

Paired $t$ tests of the weekly COVID-19 deaths and excess deaths between the COVID-19 epidemic waves showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table S12 in Multimedia Appendix 1).
Table 9. Statistical sample number (n), range, mean, SD of COVID-19 hospital case fatality rate (CFR), hospital discharge rate (DR), weekly natural deaths, excess natural deaths, reported COVID-19 deaths, and the weekly unreported excess deaths (natural) to COVID-19 death ratio (ECDR) in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

<table>
<thead>
<tr>
<th>Parameter and COVID-19 epidemic wave</th>
<th>Values, n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Values, mean (SD; range)</th>
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<td><strong>CFR</strong></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>121</td>
<td>2.06 (1.10; 0 to 6.9)</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>2.33 (1.59; 0 to 12.9)</td>
</tr>
<tr>
<td>3</td>
<td>199</td>
<td>1.76 (1.18; 0.07 to 10.1)</td>
</tr>
<tr>
<td>4</td>
<td>129</td>
<td>1.63 (7.57; 0 to 86.3)</td>
</tr>
<tr>
<td>5</td>
<td>117</td>
<td>0.99 (1.72; 0 to 18.1)</td>
</tr>
<tr>
<td><strong>DR</strong></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>121</td>
<td>8.40 (4.89; 0 to 42.5)</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>8.03 (8.62; 0 to 77.6)</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>6.11 (2.90; 0.19 to 16.0)</td>
</tr>
<tr>
<td>4</td>
<td>137</td>
<td>9.30 (7.70; 0 to 63.2)</td>
</tr>
<tr>
<td>5</td>
<td>118</td>
<td>10.1 (10.7; 0 to 111.9)</td>
</tr>
<tr>
<td><strong>ECDR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>1.88 (2.34; −0.37 to 6.43)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>1.99 (1.38; 0.30 to 4.25)</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>2.78 (1.92; 0.53 to 8.96)</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>4.81 (5.58; −0.43 to 17.64)</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>13.3 (8.48; 5.34 to 30.30)</td>
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<tr>
<td><strong>Weekly excess deaths</strong></td>
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<td></td>
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<tr>
<td>1</td>
<td>22</td>
<td>2134 (2155; 35 to 6676)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>3822 (4277; 752 to 16,123)</td>
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<tr>
<td>3</td>
<td>29</td>
<td>3856 (2837; 834 to 10,339)</td>
</tr>
<tr>
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<td>19</td>
<td>1655 (954; 832 to 3571)</td>
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<td>20</td>
<td>1197 (492; 383 to 2055)</td>
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<td><strong>Weekly natural deaths</strong></td>
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<td>36</td>
<td>9975 (2387; 7819 to 15,865)</td>
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<td>2</td>
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<td>11,941 (4341; 9041 to 24,215)</td>
</tr>
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<td>3</td>
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<td>12,751 (3262; 8863 to 19,959)</td>
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<tr>
<td>4</td>
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<td>9558 (1168; 8495 to 11,891)</td>
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<td>5</td>
<td>20</td>
<td>10,070 (624; 8945 to 11,197)</td>
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<td><strong>Weekly COVID-19 deaths</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
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</tr>
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<td>30</td>
<td>1258 (1123; 324 to 3942)</td>
</tr>
<tr>
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<td>29</td>
<td>1228 (902; 155 to 2916)</td>
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<td>4</td>
<td>18</td>
<td>577 (456; 85 to 1674)</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>101 (71; 0 to 232)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.
Discussion

Principal Findings

The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the 5 COVID-19 epidemic waves in South Africa. The \( F \) test values and standardized coefficients obtained between the respective parameters using ANOVA and the Univariate General Linear Model indicated that the residual error between the linear predicted values and the actual values was relatively small, showing a high degree of linearity. On the basis of the Probability Theory, an increase in testing results in an increased probability of the outcome of detection of a positive COVID-19 test. In this study, COVID-19 transmissibility was measured based on the magnitude of the mean and variance of daily COVID-19 positive tests (COVID-19 detection rate). Considering the linear positive association between daily COVID-19 tests and cases, the COVID-19 detection rate normalized the variance between the respective parameters. The mean daily positive COVID-19 tests in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 11.5% (SD 8.58%), 11.5% (SD 8.45%), 13.3% (SD 9.72%), 13.1% (SD 9.91%), and 14.3% (SD 8.49%), respectively. The mean daily positive COVID-19 test results in South Africa’s first and second COVID-19 epidemic waves showed no significant difference at a 95% CI with \( P = .97 \). The mean daily positive COVID-19 tests in South Africa’s third, fourth, and fifth COVID-19 epidemic waves were 15.7%, 18.4%, and 24.3% higher than those of the first and second COVID-19 epidemic wave periods, respectively, with statistically significant differences at a 95% CI. These results suggest that the COVID-19 transmission rates in the first and second COVID-19 epidemic waves in South Africa were similar, while the COVID-19 transmission rate was higher in the third, fourth, and fifth COVID-19 epidemic waves than in the first and second waves.

The negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workplaces gradually decreased during each consecutive COVID-19 epidemic wave as NPIs in South Africa were relaxed. The mean change in movement from baseline in residential locations was positive for the 5 COVID-19 epidemic waves. By the fourth and fifth COVID-19 epidemic waves, the mean change in movement from the baseline in all South African locations was positive, indicating greater movement than that observed before the COVID-19 pandemic (baseline, median values of movement in the respective locations from January 3 to February 6, 2020). The daily effective contact rate is the average number of adequate contacts per infective per day; it is directly proportional to the reproductive number [43]. Mabuka et al [38] showed through stochastic COVID-19 modeling that adjusting the NPIs by 1 Alert Level in South Africa translated into a reduction in the effective SARS-CoV-2 daily contact number by 4.13% to 14.6%. Thus, the relaxation of NPIs in South Africa after each consecutive COVID-19 epidemic wave could have possibly contributed to the increase in COVID-19 transmissibility in the COVID-19 epidemic waves.

The emerging dominant SARS-CoV-2 lineages in the South African SARS-CoV-2 genotypes collected during the first, second, third, fourth, and fifth COVID-19 epidemic waves were alpha (B.1.1.54, B.1.1.56, and C.1), beta (B.1.351), delta (B.1.617.2), and omicron B.1.1.529 and omicron BA.4, BA.2*, BA.5, BA.4, and BA.5.* SARS-CoV-2 variants or lineages, respectively. The beta, delta, and omicron SARS-CoV-2 lineages had major mutations in the spike protein. The beta (B.1.351) SARS-CoV-2 variant had 8 of its 17 mutations in the spike protein at Δ69-70 deletion, Δ144 deletion, N501Y, Δ570D, P681H, T716L, S982A, and Δ1118H [44-46]. The delta (B.1.617.2) SARS-CoV-2 variant had 10 mutations in the spike protein at T19R (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, and Δ950N [47]. The omicron SARS-CoV-2 variant (B.1.1.529 lineage) had at least 34 mutations (30 amino acid substitutions, 3 small deletions, and 1 small insertion) in its genome. In total, 15 of the 30 amino acid substitutions in the omicron SARS-CoV-2 variant were in the receptor-binding domain. The key amino acid substitutions in the spike (S) protein were at Δ67V, Δ679-70, Δ951, Δ144-144, Y145D, del211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F [48]. SARS-CoV-2 during infection binds to human angiotensin-converting enzyme (ACE) 2 receptors through the SARS-CoV-2 spike protein (S1). Some of the mutations observed in these lineages showed increased affinity by the spike protein in SARS-CoV-2 to the human ACE 2 receptors [49,50]. Thus, the increased COVID-19 transmission rate in the third, fourth, and fifth COVID-19 epidemic waves in South Africa could have been also a result of the mutations in the detected SARS-CoV-2 lineages in the respective COVID-19 epidemic waves.

The values of the Pearson, standardized coefficients, and coefficient of determination obtained between the number of COVID-19 active and hospital admission cases in this study indicated a strong linear positive association between COVID-19 active and hospital admission cases in the 5 COVID-19 epidemic waves in South Africa. This correlation was also well demonstrated by stochastic COVID-19 epidemiological models [38,51,52]. Considering this linear positive association between COVID-19 active and hospital admission cases, COVID-19 HA was used to understand the impact of SARS-CoV-2 lineages and COVID-19 vaccination on COVID-19 hospitalization in South Africa. The mean COVID-19 daily HA in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 6.80% (SD 1.82%), 18.7% (SD 7.08%), 16.5% (SD 5.56%), 9.84% (SD 4.48%), and 11.6% (SD 11.6%), respectively. The second COVID-19 epidemic wave period in South Africa had the highest number of COVID-19 HA, followed by the third COVID-19 epidemic wave period. The COVID-19 HA decreased during the first half period of the third, fourth, and fifth COVID-19 epidemic waves (Multimedia Appendices 4-6). The first half period of the COVID-19 epidemic wave is the period from the first case to the peak of the epidemic wave.
Most COVID-19 hospitalized cases in South Africa were hospitalized in the general ward (60.0%-79.1%). Patients with COVID-19 on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the ICU (8.07%-16.7%). The general trend showed a decrease in patients admitted on oxygen, in the ICU, and on a ventilator in the fourth and fifth COVID-19 epidemic waves compared with previous COVID-19 epidemic waves. The patients in high care remained relatively similar, with a mean range of 5.65% to 7.93% throughout the COVID-19 epidemic in South Africa. Most COVID-19 hospitalized cases in South Africa’s first, second, third, and fourth COVID-19 epidemic wave periods were in the ages of 40 to 49 years (16.8%-20.4%) and 50 to 59 years (19.8%-25.3%). Patients admitted owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%) and highest in the fourth and fifth COVID-19 epidemic wave periods. In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, whereas for age groups between 30 and 79 years, they decreased. Most COVID-19 hospitalized deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%). COVID-19 hospitalized deaths in the age groups of 0 to 29 years were relatively low (0.227%-4.89%). The worldwide incidence of COVID-19 deaths in children has been reported to be low [8]. The cumulative risk of death in COVID-19 hospitalized deaths increased with increasing age groups. The cumulative risk of death in hospitalized COVID-19 deaths in the age groups above 40 years was significantly lower in the fourth and fifth COVID-19 epidemic waves when compared with prior COVID-19 epidemic waves. General trends show an increase in COVID-19 hospitalized deaths in the age groups between 0 and 49 years and 80 and 89 years after each consecutive COVID-19 epidemic wave and a decrease in the age groups between 50 and 79 years.

By the end of the fifth COVID-19 epidemic wave, 20,323,729 COVID-19 vaccine doses had been administered in South Africa. The COVID-19 vaccines administered in South Africa were Pfizer-BioNTech (Comirnaty) and Johnson & Johnson or Janssen COVID-19 vaccines. The percentage of the population per age group in South Africa who had received at least 1 dose of the COVID-19 vaccine by the end of the fifth COVID-19 epidemic wave was 32.8%, 52.9%, 66%, and 71.6% of the total population in the age groups of 18 to 34 years, 35 to 49 years, 50 to 59 years, and above 60 years, respectively. The COVID-19 vaccination rate in South Africa had dropped drastically in all age groups since the third COVID-19 epidemic wave. The values of the Spearman correlation coefficients obtained between the daily cumulative COVID-19 vaccinated people and COVID-19 HA in the half period of the third, fourth, and fifth COVID-19 epidemic waves in this study indicated a strong negative monotonic association between the cumulative COVID-19 vaccinated people and COVID-19 HA. The Spearman correlation coefficients between daily cumulative COVID-19 vaccinated people and the percentage of hospitalized age groups of 18 to 34 years showed a significantly strong positive monotonic association, while the age groups of 50 years to above 60 years showed a significantly strong negative monotonic association. The values of the Spearman correlation coefficients obtained between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases in the half period of the third, fourth, and fifth COVID-19 epidemic waves in this study indicate a weak monotonic association between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases. These results suggest that COVID-19 vaccination had an association with the reduction in COVID-19 hospital admission. Pfizer-BioNTech (Comirnaty) and Johnson & Johnson or Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 76.7%, respectively [53,54].

The mean COVID-19 hospital CFR in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 2.06% (SD 1.10%), 2.33% (SD 1.59%), 1.76% (SD 1.18%), 1.63% (SD 7.57%), and 0.99% (SD 1.72%), respectively. The mean COVID-19 hospital DR in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 8.40% (SD 4.89%), 8.03% (SD 8.62%), 6.11% (SD 2.90%), 9.30% (SD 7.70%), and 10.05% (SD 10.73%), respectively. The CFRs in the third, fourth, and fifth COVID-19 epidemic waves in South Africa were lower than those observed in the first and second COVID-19 epidemic waves. The mean ECDR in South Africa’s first, second, third, fourth, and fifth COVID-19 epidemic wave periods was 1.88% (SD 2.34%), 1.99% (SD 1.38%), 2.78% (SD 1.92%), 4.81% (SD 5.58%) and 13.3% (SD 8.48%), respectively. The ECDR values obtained in this study suggest that there was a relatively high number of deaths related to the COVID-19 pandemic which occurred outside South African hospitals and were unreported. Paired t tests of the ECDR between the COVID-19 epidemic waves showed statistically significant indifferences at 95% CI, indicating that the COVID-19 death rate occurring outside South African hospitals was similar in the 5 COVID-19 epidemic waves. According to the NICD, most hospitalized cases in the third, fourth, and fifth COVID-19 epidemic waves were mostly patients unvaccinated against COVID-19 (66.4%) [55,56].

Conclusions
The relaxation of COVID-19 NPI health policies in South Africa and the evolution of SARS-CoV-2 were associated with increased COVID-19 transmission and severity in the South African population. COVID-19 vaccination in South Africa was strongly associated with a decrease in COVID-19 hospitalizations and severity in South Africa.

Acknowledgments
The Afrikan Research Initiative (ARI) would like to thank the members of the ARI African Disease Demographic Research Group and the African COVID-19 Modelling Research Group for their voluntary commitment to working on the ARI COVID-19
Research Project. The authors acknowledge the work of the National Institute for Communicable Diseases, Western Cape Department of Health Provincial Health Data Centre, South African Medical Research Council, and the Network for Genomics Surveillance in South Africa in which the ARI COVID-19 Project draws a lot of its data. Finally, the authors want to salute the scientific community, governments, health care workers, and essential personnel in their response to the pandemic, and the authors pay homage to those who have lost their lives due to the COVID-19 pandemic.

This study is part of the ARI COVID-19 Research Project, which is currently not funded. Data used in this study were obtained from public sources.

**Conflicts of Interest**
None declared.

Multimedia Appendix 1
SARS-CoV-2 lineages identified in genome samples and COVID-19 nonpharmacetical intervention policies implemented in the South African COVID-19 epidemic waves; Pearson and Spearman correlation coefficients, ANOVA, univariate general linear model coefficients, and paired samples t tests for South African COVID-19 epidemiological data.

[DOCX File, 75 KB - xmed_v4i1e34598_app1.docx ]

Multimedia Appendix 2
Mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residences during the implementation of South African COVID-19 nonpharmaceutical intervention policies.

[PNG File, 125 KB - xmed_v4i1e34598_app2.png ]

Multimedia Appendix 3
Maximum number of people with at least 1 dose of COVID-19 vaccine administered per total population age group during the first, second, third, fourth, and fifth COVID-19 epidemic wave in South Africa.

[PNG File, 74 KB - xmed_v4i1e34598_app3.png ]

Multimedia Appendix 4
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the third COVID-19 epidemic wave.

[PNG File, 102 KB - xmed_v4i1e34598_app4.png ]

Multimedia Appendix 5
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the fourth COVID-19 epidemic wave.

[PNG File, 87 KB - xmed_v4i1e34598_app5.png ]

Multimedia Appendix 6
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the fifth COVID-19 epidemic wave.

[PNG File, 94 KB - xmed_v4i1e34598_app6.png ]

**References**


Abbreviations

ACE: angiotensin-converting enzyme
CFR: case fatality rate
DR: discharge rate
ECDR: excess deaths (natural) to COVID-19 death ratio
HA: hospital-to-active cases
ICU: intensive care unit
NICD: National Institute for Communicable Diseases
NPI: nonpharmaceutical intervention
VOC: variant of concern
VOI: variant of interest
WHO: World Health Organization

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Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years

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Abstract

The renin angiotensin system is composed of several enzymes and substrates on which angiotensin converting enzyme (ACE) I and renin act to produce angiotensin II. ACE1 and its substrates control blood pressure, affect cardiovascular and renal function, hematoepoiesis, reproduction, and immunity. The increased expression of ACE1 has been observed in human monocytes during congestive heart failure and abdominal aortic aneurysm. Moreover, T lymphocytes from individuals with hypertension presented increased expression of ACE1 after in vitro stimulation with angiotensin II (ATII) with the highest ACE1 expression observed in individuals with hypertension with low-grade inflammation. Our group and others have shown that aging is associated with comorbidities, chronic inflammation, and immunosenescence, but there is a lack of data about ACE1 expression on immune cells during the aging process. Therefore, our aim was to evaluate the levels of ACE1 expression in nonlymphoid cells compared to lymphoid that in cells in association with the immunosenescence profile in adults older than 60 years. Cryopreserved peripheral blood mononuclear cells obtained from blood samples were used. Cells were stained with monoclonal antibodies and evaluated via flow cytometry. We found that ACE1 was expressed in 56.9% of nonlymphocytes and in more than 90% of lymphocytes (all phenotypes). All donors exhibited characteristics of immunosenescence, as evaluated by low frequencies of naïve CD4+ and CD8+ T cells, high frequencies of effector memory re-expressing CD45RA CD8+ T cells, and double-negative memory B cells. These findings, in addition to the increased C-reactive protein levels, are intriguing questions for the study of ACE1, inflammaging, immunosenescence, and perspectives for drug development or repurposing (Reviewed by the Plan P #PeerRef Community).

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KEYWORDS

aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging
**Introduction**

Angiotensin converting enzyme (ACE1, also known as CD143) and renin are components of the renin angiotensin system (RAS) acting to produce angiotensin II. In a simplistic definition, RAS is composed of a vasoconstrictor, proinflammatory ACE1/angiotensin II (ATII)/ATII receptor type 1 (AGTR1) axis, and a vasodilating anti-inflammatory ACE2/angiotensin-(1-7) [Ang-(1-7)]/Mas receptor axis (Figure 1). In addition to blood pressure control, ACE1 and its peptide substrates affect cardiovascular and renal function, hematopoiesis, reproduction, and the immunity [1,2]. Thus, it seems crucial that the RAS presents an inflammatory axis and an anti-inflammatory axis for adequate regulation of the immune response. ACE1 expression has been not only observed in tissues, but also its soluble form has been found in urine, serum, seminal fluid, amniotic fluid, and cerebrospinal fluid [3].

*Figure 1.* The renin angiotensin system. ACE: angiotensin converting enzyme; ACE1: angiotensin converting enzyme 1; AGTR1: angiotensin II type 1 receptor; AGTR2: angiotensin II type 2 receptor.

The expression of ACE1 in cells from the immune system has been reported in health and disease. Costerousse et al [4] observed, via reverse transcriptase–polymerase chain reaction and southern blot analysis, the expression of ACE1 in monocytes, macrophages, and T cells but not in B cells in healthy adult donors. In addition, ACE1 activity was very low in monocytes, whereas it was high in macrophages (monocytes driven to differentiation). T cells presented intermediary ACE1 activity and B cells expressed no activity [4]. In patients with type 1 diabetes (median age 29 years, normotension), higher ACE1 and lower ACE2 expression were observed when compared to healthy controls (median age 32 years, normotension) [5]. Coppo et al [6] found that T cells in culture had increased mRNA expression of ACE1 and AGTR1 in individuals with obesity with low-grade inflammation (high-sensitivity C-reactive protein [CRP] level of >3 mg/dL). ACE1 activity was also increased in the supernatant of a T cell culture in individuals with obesity with a high-sensitivity CRP level of >3 mg/dL. Moreover, expression of RAS genes in T cells and levels of inflammatory cytokines in the serum were oppositely associated with serum levels of insulin [6,7]. Ulrich et al [8] have shown that the increased expression of ACE1 in monocytes was associated with kidney and cardiovascular disease progression, suggesting that circulating leukocytes can modulate local immune responses via their own RAS components [8-10].

Considering that aging has been associated with comorbidities, low-grade chronic inflammation, and altered frequency or function of immune cells [11-14], it seems reasonable to suggest that ACE1 play an important role in the aging process. ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. ACE1 polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between ACE1 polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20]. In hematopoietic stem/progenitor cells isolated from peripheral blood, Joshi et al [21] showed that aging is associated with decreased ACE2 and increased ACE1 protein expression. This imbalance suggests a bias to the detrimental proinflammatory axis of the local RAS. Considering the scarce information about ACE1 expression in the phenotypes of T and B cells, we aimed to investigate ACE1 expression in cells from the immune system and parameters of immunosenescence in adults older than 60 years. Results herein show different levels of expression of ACE1 in nonlymphoid versus lymphoid cells, with expression being higher in lymphoid cells.
Methods

Overview

Blood was collected from adults (n=6, four females and 2 males) aged 64-67 years in 2015. Peripheral blood mononuclear cells were isolated using a Ficoll–Hypaque density gradient (Amersham Biosciences) and centrifugation. Viable cells were counted, adjusted to $2 \times 10^6/100 \mu$L in 80% fetal bovine serum and 20% dimethylsulfoxide (Sigma), and frozen stored until the phenotyping. In 2021, cells were thawed, checked for viability, and stained with monoclonal antibodies to the T cell phenotypes CD4 PerCP Cy5.5, CD8 APC Cy7, CD27 APC, CD45RA PE; B cell phenotypes CD19 PE, CD27 APC, IgD PE Cy5.5 (eBioscience), and ACE CD143 fluorescein isothiocyanate (R&D Systems). After 30 minutes of incubation in the dark at 4°C, the cells were washed with phosphate-buffered saline and centrifuged. Living cells (based on forward and side scatter) were acquired in the BD FACSCanto II flow cytometry system using the DIVA software (Becton Dickinson).

For assessing metabolic parameters, the serum of studied individuals was previously isolated through centrifugation and frozen stored until use. Measurement of metabolic parameters was performed in the Laboratório Central–Hospital São Paulo, Federal University of São Paulo.

Statistical Analysis

Data are presented as mean (SD) values. To test the normality of data, we used the Shapiro-Wilk test. We considered $P$ values for interindividual differences in each variable, since individuals were aged differently (biological aging) and thus, physiological parameters could be affected by genetics, lifestyle, nutrition, and comorbidities. A $P$ value less than .05 was considered significant.

Ethics Approval

The Ethics Committee of the Federal University of São Paulo approved all procedures (protocol 10904).

Results

Table 1 shows that older adults are heterogeneous for some physiological parameters such as glucose, urea, glycated hemoglobin, and CRP.

Table 2 and Figures 2-4 show that CD143 (ACE1) is expressed in almost 100% of lymphocytes, whereas it is expressed in 56.9% (SD 20.6%) of nonlymphocytes. CD8$^+$ T cells presented the highest expression (98.4%), followed by CD19$^+$ B cells (93.7%, SD 3.4%) and CD4$^+$ T cells (90.7%, SD 8.7%). In T cells, ACE1 is expressed in all phenotypes (naïve, central memory, effector memory, and effector memory re-expressing CD45RA [EMRA]). In B cells, ACE1 was expressed in naïve, unswitched memory, switched memory, and double-negative (DN) cells.

Table 3 shows that characteristics of senescent T cells were observed in both males and females, such as low expression in naïve CD4$^+$ and CD8$^+$ T cells and high expression in EMRA CD8$^+$ T cells.

Table 4 shows that aging adults with lower percentages of naïve B cells also presented a higher percentage of DN memory B cells.

Table 1. Physiological parameters observed in older adults.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Unit (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td></td>
<td>(mg/dL)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td></td>
<td>(mg/dL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Individual participants' values</th>
<th>Cholesterol</th>
<th>High-density lipoprotein</th>
<th>Triglycerides</th>
<th>Glucose</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Albumin</th>
<th>Glycated hemoglobin</th>
<th>C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>207, 253, 181, 223</td>
<td>137, 176, 96, 150, 186</td>
<td>152, 152, 130, 149</td>
<td>163, and 130</td>
<td>80, 86, 137, 83, 89, and 165</td>
<td>30, 40, 28, 28, 29, and 28</td>
<td>0.86, 0.73, 0.84, 0.68</td>
<td>0.79, and 1.01</td>
<td>3.4</td>
</tr>
<tr>
<td>Overall, mean (SD)</td>
<td>217.3 (27.2)</td>
<td>145.0 (30.4)</td>
<td>146.0 (12.1)</td>
<td>106.7 (32.5)</td>
<td>30.5 (4.3)</td>
<td>0.82 (0.1)</td>
<td>3.8 (0.4)</td>
<td>6.2 (0.8)</td>
<td>7.6 (7.2)</td>
</tr>
</tbody>
</table>

$^a P > .10$.  
$^b P = .047$.  
$^c P = .02$.  
$^d P = .03$.  

https://med.jmirx.org/2023/1/e45220
Table 2. CD143 (ACE1) expression in lymphocytes and nonlymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>Lymphocytes (%)</th>
<th>Nonlymphocytes&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD&lt;sup&gt;4+&lt;/sup&gt;CD143&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;CD143&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Individual participants’ values</td>
<td>84.8, 77.6, 96.9, 98.8, 87.8, and 98.3</td>
<td>97.1, 96.7, 99.0, 99.6, 98.5, and 99.6</td>
</tr>
<tr>
<td>Overall, mean (SD)</td>
<td>90.7 (8.7)</td>
<td>98.4 (1.3)</td>
</tr>
<tr>
<td></td>
<td>74.6, 35.4, 47.7, 75.0, 32.9, and 75.9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>P = .08.
<sup>b</sup>P > .15.

Figure 2. Flow cytometry gating strategy for B cell phenotypes and CD143 expression. (A) All cells and gates for lymphocyte (green) based on forward scatter (FSC-A) and side scatter (SSC-A); (B) exclusion of doublets (from the lymphocyte gate); (C) CD19<sup>+</sup> B cells (from the doublets exclusion gate); (D) CD143<sup>+</sup>ACE1 cells (from the CD19<sup>+</sup> B cells’ gate); and (E) B cell phenotypes and CD143+IgM+IgD+CD27<sup>-</sup> (naïve), IgMlowIgD+CD27<sup>+</sup> (memory-unswitched), IgM-IgD-CD27<sup>+</sup> (memory-switched), and IgM+IgD-CD27<sup>-</sup> (memory double-negative). DN: double-negative; FSC: forward scatter; Mem: memory; SSC: side scatter.
Figure 3. Flow cytometry gating strategy for T cell phenotypes and CD143 expression. (A) All cells and gates for lymphocyte (green) based on forward scatter (FSC-A) and side scatter (SSC-A); (B) exclusion of doublets (from the lymphocyte gate); (C) CD4$^+$ and CD8$^+$ T cells (from the doublets exclusion gate); (D) CD143$^+$ACE1 cells (from the CD4$^+$ and CD8$^+$ T cells’ gate); (E) T cell phenotypes and CD143$^+$, CD45RA$^+$CD27$^-$ (naïve), CD45RA$^-$CD27$^+$ (central memory), CD45RA$^-$CD27$^-$ (effector memory), and CD45RA$^+$CD27$^-$ (effector memory re-expressing CD45RA) cells. FSC: forward scatter; SSC: side scatter.

Figure 4. Flow cytometry gating strategy for nonlymphocytes and CD143 expression. (A) All cells and gates for lymphocytes (P1) and nonlymphocytes based on forward scatter (FSC-A) and side scatter (SSC-A) and (B) CD143$^+$ACE1 cells (from the nonlymphocyte gate). FSC: forward scatter; SSC: side scatter.

Table 3. Phenotypes of CD4$^+$ and CD8$^+$ T cells.

<table>
<thead>
<tr>
<th>CD4$^+$ T cells (%)</th>
<th>CD8$^+$ T cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naïve$^a$</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Individual participants’ values</td>
<td>27.6, 43.3, 13.4, 24.8, 24.8, and 32.6</td>
</tr>
<tr>
<td>Overall, mean (SD)</td>
<td>25.7 (11.7)</td>
</tr>
</tbody>
</table>

$^a$P > .10.  
$^b$P = .047.
Table 4. Phenotypes of CD19+ cells.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Naive (%)</th>
<th>Unswitched memory (%)</th>
<th>Switched memory (%)</th>
<th>Double-negative memory (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, mean (SD)</td>
<td>53.2 (17.9)</td>
<td>6.5 (2.4)</td>
<td>15.3 (10.6)</td>
<td>25.1 (9.8)</td>
</tr>
</tbody>
</table>

*P > 0.10.

Discussion

Our results show that for the studied population, chronological aging and biological aging are asynchronous. Even among individuals with a small chronological difference (64 to 67 years), there is heterogeneity in physiological parameters such as glucose, urea, glycated hemoglobin, and CRP. Changes in the same functional parameters have been reported by Carlsson et al [22] and Helmerson-Karlqvist [23] in healthy older adults. Carlsson’s [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24].

The link between the RAS and inflammation has been suggested but its role is not completely clear under physiological and pathological conditions [25,26]. In addition, the association between altered ACE1 expression in tissues (brain, muscle, heart, and vessels) and the development and progression of age-related conditions such as Alzheimer disease, sarcopenia, and cardiovascular disease has been suggested, but results are controversial [17,27-30].

There are few studies showing the association between ACE1 expression in cells from the immune system (monocytes and T cells) and the progression of kidney and cardiovascular disease [8,9,31,32]. Therefore, considering the lack of information on this issue, we questioned whether ACE1 (CD143) was highly expressed in cells from the immune system during the aging process. We found that ACE1 was expressed in almost 100% of T (CD4+ and CD8+) and B lymphocytes and in all phenotypes of these cells. In nonlymphoid cells, mean ACE1 expression was 56.9% (SD 20.6%). In agreement with our findings, independent studies showed that T cells from healthy donors and monocytes from patients with congestive heart failure expressed ACE1, but there has been no investigation on cell phenotypes [25,26]. Our study is the first to show that either inexperienced (naive) or fully activated (memory) cells express ACE1. Our findings suggest that the expression of ACE1 in lymphoid and nonlymphoid cells reflects health status, since our studied population presented changes in physiological parameters and high levels of ACE1 expression in immune cells. Previous independent studies showed that patients with unstable angina [32] or acute myocardial infarction [33] presented higher expression of ACE1 in T cells and dendritic cells than control subjects. In addition, markers of cell (lymphoid and nonlymphoid) functional status, such as inflammatory or growth factor production, could be modulated by ACE inhibitors (ACEi). Accordingly, mononuclear leukocytes from healthy subjects incubated with an endotoxin exhibited high levels of tissue factor activity, which was reduced in the presence of captopril in a dose-dependent pattern. This result could be related to the antithrombotic effect of ACEi [34]. In patients with congestive heart failure, immune cells cultured with lipopolysaccharide secreted high levels of the proinflammatory tumor necrosis factor α, and these levels were significantly reduced in the presence of captopril [35].

It may be proposed that mechanistically, ATII is produced by mononuclear cells or lymphocytes and, at the same time, ATII induces immunologic activation in these cells. Therefore, the inflammatory axis ACE1/ATII/AGTR1 and the counterregulator ACE2/Ang-(1-7)/Mas receptor axis [36,37] could play a role in chronic diseases, inflamming, and immunosenescence observed in older adults. Our studied population presented changes in some physiological parameters and increased levels of CRP. This inflammatory profile [24], in addition to more than 90% of T and B cells expressing ACE1 in our population of older adults, suggest a correlation among aging, inflamming, and ACE1 expression. Independent of chronological age, inflammation (even if related to subclinical diseases) may be a contributor to disease progression when the balance with anti-inflammation is shifted [38]. In this context, the regulation of ACE1/ACE2 expression could be explored as a target for the balance of exacerbated inflammatory reactions. Considering that the equilibrium between ACE1 and ACE2 expression could play an important role in healthy aging, our subsequent studies will be focused on ACE1 and ACE2 expression in cells from the immune system.

The phenotype of T and B lymphocytes has been used to identify senescence in immune cells. CD4+ T cells present changes during the aging process with a decrease in naive phenotypes and an increase in effector memory phenotypes, whereas CD8+ T cells show a decrease in the naive phenotype and an increase in the effector memory and EMRA phenotypes [12,39,40]. It has been shown that the reduction in naïve B cells is accompanied by no change in memory-unswitched and memory-switched B cells but an increase in the percentage of double-negative B cells [41-44]. Using these phenotypes, we found a similar senescent phenotype in some of the studied aging adults. The reduction in naive lymphocytes has been related to impaired antigen responsiveness, and for B cells, a decrease in the production of antibodies has been observed [45,46]. The increased percentage of DN memory B cells has been linked to autoimmune diseases [47,48]. We observed ACE1 expression in more than 90% of T cells and B cells and in all phenotypes. ACE1 was expressed in nonlymphocytes in a range of 32.9% to 75.9%. Our findings suggest that ACE1 could play

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a role in several processes linked to aging, including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE1 suppress the autoimmune process in a number of autoimmune diseases such as experimental autoimmune encephalomyelitis, arthritis, autoimmune myocarditis [49].

This study is the first to compare the expression of the protein ACE1 between different cell types, both lymphoid cells (CD4+ and CD8+ T cells and B cells) and nonlymphocytes in older adults. It was also observed that even though the study participants were in the early stage of chronological aging (64 to 67 years), they presented heterogeneity in physiological parameters, signs of inflaming (increased CRP levels), and immunosenescence, including low expression in naïve T and B cells in addition to the accumulation of terminally differentiated CD8+ T cells and DN B cells. This study has limitations such as the small sample size and the lack of young adults for comparison. As an example, the subject presenting the highest CRP and albumin levels also exhibited a high percentage of ACE1 expression in T cells (CD4+ and CD8+), B cells, and nonlymphoid cells, in addition to the lowest percentage of CD4+ naïve cells, and the highest percentage of CD8+ terminally differentiated (EMRA) and DN B cells. However, due to the small sample size, it was not possible to associate the high expression of ACE1 in immune cells with inflaming and immunosenescence. Correlation of physiological parameters and health status with ACE1 expression and investigating whether age and associated chronic diseases could lead to increased ACE1 expression would yield important information. Moreover, we only determined CRP as a marker of inflaming, and interleukin 6 and tumor necrosis factor α would be desirable to complete our panel. Functional analyses are needed to clarify the impact of ACE1 expression on immune cells and whether ACE1 and angiotensin receptor blockers administered to patients with hypertension somehow affect immunity. Recently, it was shown that membrane-bound ACE2 acts as a receptor for SARS-CoV-2, but the possible effects on RAS components [AT1, Ang-(1-7), ACE1, ACE2, AT1, and Mas] and whether ACE1 and angiotensin receptor blockers interfere with the mitigation of COVID-19 require further investigation [50-54]. Therefore, it is important to emphasize the negative impact of chronic diseases on the outcomes of older adults during a viral infection and how ACE1 or ACE2 expression in immune cells could provide information regarding diagnosis and treatment.

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Conflicts of Interest

None declared.

Editorial Notice

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References


Abbreviations

ACE1: angiotensin converting enzyme
ACEi: angiotensin converting enzyme inhibitors
AGTR1: angiotensin II receptor type I
Ang-(1-7): angiotensin-(1-7)
ATII: angiotensin II
dN: double-negative
EMRA: effector memory re-expressing CD45RA
RAS: renin angiotensin system

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Protocol

Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review

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Abstract

Background: The postacute sequelae of COVID-19 (PASC) is a syndrome characterized by persistent COVID-19 symptoms or the onset of new symptoms following recovery from the initial or acute phase of the illness. Such symptoms often occur 4 or more weeks after being diagnosed with COVID-19. Although a lot of work has gone into understanding the long-term mental health effects of PASC, many questions related to the etiology and risk of this condition remain.

Objective: This protocol is for a systematic review assessing the association between PASC and adverse psychiatric outcomes and whether people with PASC are at greater risk of developing an adverse psychiatric outcome than those without PASC.

Methods: Various medical literature databases (e.g., PubMed and EMBASE) will be searched for eligible articles, using predefined search criteria. Gray literature will also be explored. Epidemiological observational studies and secondary analyses of randomized controlled trials that report a quantitative relationship between PASC and at least one adverse psychiatric outcome will be included. The Population, Exposure of interest, Comparator, and Outcome framework will be used as a standardized framework for the inclusion criteria. The Joanna Briggs Institute critical appraisal tools will be used to assess methodological quality and critically appraise the risk of bias in included studies. A random-effects meta-analysis will be conducted if possible. A formal narrative synthesis will be performed if a meta-analysis is impossible due to substantial heterogeneity across studies. The Grading of Recommendations Assessment, Development and Evaluation approach will be used to rate the cumulative certainty of the evidence for all outcomes. Ethical approval is not required. The study results will be published in a peer-reviewed journal.

Results: This study documents and addresses etiology, risk factors, and long-term symptoms of COVID-19 among people with PASC. It focuses on a key priority area for new evidence syntheses on the clinical management of COVID-19 and pandemic-related conditions. It will include evidence on nonhospitalized and hospitalized patients with a history of PASC.

Conclusions: Substantial heterogeneity across studies may limit the ability to perform a meta-analysis. Findings will inform disease prevention, decision-making, health care policy, and clinical research (Reviewed by the Plan P #PeerRef Community).

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KEYWORDS
COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

Introduction

Overview
COVID-19 is a contagious illness caused by SARS-CoV-2. Persistent and long-lasting (>4 weeks) symptoms following infection with acute COVID-19 have given rise to a syndrome known as post-acute sequelae of COVID-19 (PASC) or post–COVID-19 condition [1-3]. Incidence and prevalence estimates for people with COVID-19 presenting with or reporting persistent psychiatric symptoms after months of initial infection range from 0.8% to 49% [1-5]. Among 44,759 people with no recorded history of psychiatric illness, the estimated overall probability of being diagnosed with new-onset psychiatric illness in the 90 days following a confirmed diagnosis of COVID-19 was 5.8% in a retrospective cohort study [6].

Similarly, clinical anxiety and depression, as well as other psychiatric sequelae, have been reported following diagnosis with COVID-19 in other studies [6-8]. Although sex and age are considered to be sociodemographic risk factors for PASC, there is no consensus on other baseline clinical features that act as independent predictors of PASC [9,10]. The prevalence of PASC symptoms is higher in women than in men [10].

Among people aged 35-49 years, the prevalence of PASC is 26.8% compared with 26.1% and 18% among people aged 50-69 years and 70 years or older, respectively [10]. Persistent symptoms occur weeks and months after infection irrespective of initial disease severity (mild, moderate, severe, and critical) [11,12]. Mendez et al [13] reported in their cross-sectional study that 2 months after discharge, neurocognitive impairment, psychiatric morbidity, and poor quality of life were markedly prevalent among 179 COVID-19 survivors who had been hospitalized [13]. Nevertheless, Vannorsdall and Oh [14] posit that current research on the postacute phase following hospitalization has been conflicting due to the absence of a detailed, standardized neuropsychological evaluation of patients with COVID-19 after hospitalization [14]. In addition, they stated that literature on PASC and adverse mental health outcomes are mostly limited to studies that cannot establish causal relationships or lack generalizability (eg, case reports, case series, and data obtained from cognitive screening instruments) [14]. Thus, more high-quality studies are warranted [15].

In a study where the short-term and long-term sequelae of COVID-19 were systematically evaluated, PASC was categorized as short-term (1 month), intermediate-term (2-5 months), and long-term (>6 months) following COVID-19 diagnosis [13]. Clinical manifestations of PASC were classified into organ systems, including cardiovascular, dermatologic, digestive, ear, nose, and throat; mental health, neurologic, and respiratory; constitutional symptoms; and functional mobility [13]. The mechanisms leading to the postacute and chronic neuropsychiatric manifestations of COVID-19 may be due to the direct effect of the viral infection and the indirect effect on mental health due to social isolation, posttraumatic stress, and job loss. Specifically, correlations have been observed between COVID-19 posttraumatic stress scores, general distress, and sleep disruption [13,14]. Despite those correlations, Khubchandani et al [16] stated that the causal pathways and etiology of adverse mental health outcomes in people who were infected with COVID-19 are multidimensional and complex [16,17].

To clarify whether COVID-19 is a risk factor for psychiatric disorders and vice versa, an electronic health record network cohort study of 69 million people consisting of 62,354 people with a COVID-19 diagnosis compared the rates of psychiatric sequelae of health in the initial 4 months of the pandemic (January to April 2020) and subsequently (after April 2020) [8]. The study found that the rate of all diagnoses of psychiatric disorders and relapses was greater following COVID-19 infection than after control health events (eg, influenza infection, skin infection, other respiratory tract infections, and fracture) [8].

Likewise, a diagnosis of psychiatric disorder in the 12 months preceding the COVID-19 pandemic was associated with a 65% increased risk of COVID-19 (relative risk [RR] 1.65, 95% CI 1.59-1.71; P<.001) compared with a matched cohort of people with specific physical risk factors for COVID-19 without a psychiatric diagnosis [18]. Whereas these associations were partly attributed to illness severity and pandemic-related contextual factors (eg, social isolation, overwhelmed health care systems, and stigma), they do not adequately account for observed differences in psychiatric sequelae [18]. Moreover, the inability to conclusively determine why there were between 2- and 3-fold increases in the risk of neuropsychiatric complications following a COVID-19 infection, in this and other studies, calls for further examination of the association between COVID-19 and risk factors for psychiatric morbidity [8,16,18].

Rationale

With many long-term adverse mental health outcomes linked to COVID-19, effective interventions that optimize recovery and minimize relapse are needed. Such interventions may serve as appropriate tools to evaluate risk factors that may cause maladaptive psychiatric responses [19]. Furthermore, they may aid with the management of anxiety, fear, frustration, stigma, and paranoia by mitigating psychopathological symptoms and reducing contextual stress [19,20]. Interventions that have been assessed in patients with COVID-19 include web-based and physical psychotherapeutic approaches; for example, cognitive behavioral therapy, emotional freedom techniques, and ultrabrief psychological interventions; combined psychiatric and psychological interventions; technology and media; complementary and alternative therapies; self-care; spirituality and religion; and pharmacotherapies [21-23].
Evidence on the effectiveness of these interventions is mixed and not thoroughly synthesized, with quality inadequately assessed in earlier studies, and may vary depending on COVID-19 duration and severity. In a randomized controlled clinical trial of 51 people with COVID-19 consisting of an experimental group receiving progressive muscle relaxation technology for 30 minutes each day for 5 consecutive days and a control group receiving only usual care and treatment, participants in the experimental group reported lower depressive symptoms, lower anxiety levels, and better sleep quality than those in the control group [24]. Another randomized controlled trial of 30 hospitalized patients with COVID-19 assigned to an experimental or control group reported an improvement in all outcome measures among intervention group subjects compared to controls [24]. In that study, a short 4-session crisis intervention package tailored to cover COVID-19–specific guidance was delivered by clinical psychologists [24]. Topics covered included tension reduction, relaxation, adjustment, responsibility skills enhancement, and promoting resilience [24]. Outcome measures in the study were derived from the Depression, Anxiety, and Stress Scale, Symptom Checklist 25, and the abbreviated version of the World Health Organization Quality of Life assessment [24]. Lack of cultural specificity, methodological issues, small sample sizes, lack of follow-up, unadjusted confounding factors, and brief time spans in both studies limit their generalizability [23,24].

During the COVID-19 pandemic, digital interventions to deliver health care have gained widespread acceptance [25]. Remote care coordination and provision have been adopted to help reduce the risk of disease transmission [25]. Mobile apps have also been used for contact tracing and information dissemination [25]. Although an evidence synthesis of digital interventions to attenuate the adverse effects of the COVID-19 pandemic on the mental health of the public highlighted their importance in mental disorder prevention and mental health promotion; it noted that evidence on their cost-effectiveness, process quality, and long-term outcomes is sparse [26]. Furthermore, the negative impact and risks of the COVID-19 pandemic are sometimes more significant in vulnerable and clinically extremely vulnerable populations (eg, people older than 70 years, pediatric patients with cystic fibrosis, or people with developmental disabilities) who may be digitally disadvantaged [26-29].

Presently, it is unclear what duration of PASC, etiologies, and risk factors are most associated with the manifestation or persistence of adverse psychiatric outcomes (eg, depression, anxiety, substance use disorder, posttraumatic stress disorder, psychosis, dementia, nonsuicidal self-injury [self-harm], or suicide) compared with other health events. A prospective cohort study of patient-reported outcome measures 3 months after initial COVID-19 symptom onset noted impairment with self-care and anxiety or depression as being present in 13% and 22% of its 78 subjects, respectively, with at least 1 Charlson comorbidity at baseline compared to subjects without any Charlson comorbidities (4% and 9% respectively). Among subjects without any Charlson comorbidities, 70% reported an abnormal PROM, and 33% had at least 1 moderate issue in at least 1 EQ-5D assessment [30]. In addition, questions remain about the long-term (≥6 months) outcomes of COVID-19 [30].

Although some studies indicate that most people who acquire COVID-19 are at risk of psychiatric sequelae and their symptoms tend to improve over time, others suggest that symptoms may worsen over time or point to a different disease trajectory [29,30]. Research and any future recommendations about PASC and mental health should be guided by the best available evidence.

Few epidemiological studies have investigated the short- and long-term impact of COVID-19 and PASC on mental health. Thus, this study will examine the causes of adverse psychiatric outcomes and risk factors in people with PASC. Furthermore, prior studies on this and related topics report internal validity and generalizability (external validity) limitations due to evidence derived solely from electronic health records, single networks, or claims data. Because data on the psychiatric sequelae of PASC are conflicting and sparse, it is imperative to systematically summarize the evidence and combine the results of various scientific studies.

This study aims to generate a new hypothesis on causality and provide a more precise estimate of the risk factors underlying PASC and adverse psychiatric outcomes. An initial search of peer-reviewed and gray literature found no systematic reviews and meta-analyses on the topic. This protocol is for a systematic review that assesses the literature on PASC duration and risk factors that act as determinants (etiologies) of adverse psychiatric outcomes.

Objectives

The primary objective of this systematic review is to determine whether people with PASC are at greater risk of developing an adverse psychiatric outcome (depression, anxiety, substance use disorder, posttraumatic stress disorder, psychosis, dementia, nonsuicidal self-injury [self-harm], or suicide) than those without PASC.

Secondary review questions include the following:

1. Does the association between PASC and an adverse psychiatric outcome vary with age, sex, severity of COVID-19 (mild, moderate, severe, and critical), and duration of PASC (short-term [1 month], intermediate-term [2-5 months], and long-term [≥6 months] following COVID-19 diagnosis or hospital discharge)?

2. Is PASC an independent risk factor for an adverse psychiatric outcome?

Methods

This protocol has been drafted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance for protocols (PRISMA-P) [31]. The systematic review will explicitly report any amendments and modifications made to this protocol.
Eligibility Criteria

Study Design and Characteristics
The review will include observational studies, namely, retrospective studies, and prospective longitudinal cohort studies, case-control studies, cross-sectional studies, case series, and case reports. Secondary analyses of randomized controlled trials will also be included. Effect measures of risk factors, including the incidence rate ratio, risk difference, relative risk, odds ratio (OR), and hazard ratio central to the primary outcome, will be included. Risk factors predispose people with PASC to an adverse psychiatric outcome [32,33]. Such risk factors are associated with an increased probability of people with PASC having a negative mental health outcome [32,33]. Information on the relationship between risk factors and incidence of primary and secondary outcome measures will be included. Studies that do not report a quantitative relationship between PASC and at least one adverse psychiatric outcome will be excluded.

COVID-19 diagnosis must have been confirmed on the basis of clinical suspicion or with a positive nucleic acid amplification test such as reverse transcriptase–polymerase chain reaction, an antigen test, or a serologic test (eg, a rapid serology test or enzyme-linked immunosorbent assay) [34]. Studies will be included if subjects were longitudinally observed since the initial diagnosis of COVID-19; that is, during the acute phase or since the time of PASC onset (postacute or chronic phase) [34]. A follow-up time of at least 1 month since COVID-19 diagnosis is required [35]. Primary and secondary outcomes will encompass etiology, risk factors, symptom and illness severity, duration of PASC, and adverse events [34,35].

Participants
Studies with adult participants (aged 18 years or older) will be included. Pediatric and animal studies will not be included. There will be no sex, ethnicity, or race limitations. The search dates will range from December 2019 (date of the first confirmed case of COVID-19) until March 2023 (the anticipated completion date of the review). COVID-19 filters will be used, if necessary, to limit search results to COVID-19– and PASC-related articles.

Exposure

Primary Measure
PASC, for this review, is defined as a continuing symptomatic illness or the emergence of new symptomatic illness in people with a confirmed history of COVID-19 after recovery from the acute phase of the illness. PASC will be categorized as short-term (1 month), intermediate-term (2-5 months), and long-term (26 months) following a COVID-19 diagnosis or hospital discharge.

Secondary Measure
The severity of COVID-19 (mild [including asymptomatic], moderate, severe, or critical) will be considered.

Comparators (Controls)

Primary Measure
People with a confirmed history of COVID-19 without PASC will be considered.

Secondary Measure
The severity of COVID-19 (mild [including asymptomatic], moderate, severe, or critical) will be considered.

Outcomes

Primary Outcome Variable
Primary outcome variables will include adverse psychiatric outcomes such as depression, anxiety, substance use disorder, posttraumatic stress disorder, and psychosis.

Secondary Outcome Variables
Secondary outcome variables will include nonsuicidal self-injury (self-harm) and suicide.

Information (Evidence) Sources and Search Strategies
Information, including titles and abstracts extracted from evidence sources, will be initially screened against the review questions. Information deemed eligible for inclusion will undergo more comprehensive screening. Once an article, study, or review is considered suitable for inclusion, it will be placed in the list of included studies. The steps above will be carried out for each information source, after which duplicates will be removed. The study selection process will be described in a PRISMA flow diagram and reported in the systematic review.

Author AE will develop the search strategy in consultation with a medical research librarian. The following databases and evidence sources will be searched: PubMed, Ovid MEDLINE, Embase, JBI EBP Database, CINAHL Plus, UpToDate, APA PsycInfo, Google Scholar, ProQuest Dissertations & Theses Global, Scopus, Web of Science, the University of Toronto COVID-19 Data & Statistical Sources, Centre for Addiction and Mental Health (CAMH) COVID-19 National Survey Dashboard reports, and COVID-END. Gray literature will also be considered where appropriate. Search strategies will be comprehensive and adapted for each information source. See Appendix 1 for a sample of the Embase search strategy.

The Covidence or JBI SUMARI software will be used during the systematic review process for screening, appraisal of evidence sources, data extraction, synthesis, and study completion.

Ethics and Dissemination
Ethical approval is not required for this study. The study findings will be disseminated via preprints, peer-reviewed publications, conference abstracts, posters, plain-language summaries, presentations, and infographics.

Patient and Public Involvement
Input on the review questions and outcomes was informally sought from patients and people who had been previously diagnosed with COVID-19 and PASC.
Results

Study Selection
Information, including titles and abstracts, extracted from information sources will be initially screened by AE and a second reviewer against the research questions. Information deemed eligible for inclusion will undergo more comprehensive screening. Once an article, study, or review is considered suitable for inclusion, it will be placed in the list of included studies. The steps above will be carried out for each information source, after which, duplicates will be removed. Disagreements on inclusion will be resolved through discussion or arbitration. The study selection process will be described in a PRISMA flow diagram and reported in the systematic review.

Data Extraction and Management
Data will be extracted on primary and secondary outcome measures following the PRISMA guideline for systematic reviews [36]. Outcome and effect size measures (eg, adjusted and ORs, risk ratios [RRs], hazard ratios, and SEs), P values, associated 95% credibility intervals, and associated 95% CIs. RRs for subgroups (eg, age, sex, duration of PASC, and COVID-19 severity) will be extracted if reported. The following data will also be extracted: authorship, publication year, journal name, study design, study location, sample size, baseline characteristics of the study participants, demographics (age, sex, ethnicity, or race of subjects), study population characteristics (eg, general population, prisoners, and health care workers), the definition of PASC, duration of PASC, comorbidities, other risk factors, duration of follow-up, list of adjusted and unadjusted confounders (eg, hospitalization, occupation, and symptom recognition) and a list of adjusted and unadjusted confounders (eg, age, sex, nature of exposure, and type of intervention), and propensity methods.

Two reviewers will conduct the data extraction. Discrepancies in data extraction will be resolved through discussion or arbitration.

Risk of Bias in Individual Studies
The JBI critical appraisal checklist will be used to determine the methodological quality and to critically appraise the risk of bias for included studies. Assessment will be done at the study and outcome level. Information related to a variable (exposure, outcome, or covariate), misclassification, confounders, participant selection, reverse causation, missing data, study power, and generalizability will be appraised. Two reviewers will initially pilot the checklist to enhance consistency, mitigate potential issues with mechanistic scoring, and mitigate performance bias in the overall risk-of-bias assessment. Studies that do not adequately report on statistical analyses or address confounders, biases (selection, performance, detection, or attrition) and other biases will be deemed lower-quality studies—that is, when they consistently have “no,” “unclear,” and “not applicable” ratings’ across relevant items.

Discussion
Summary treatment effects estimated as continuous outcomes will be converted to ORs, RRs, incidence rate ratios, risk differences, and number needed to treat with a 95% CI or 95% credibility interval (along with the baseline risk) for easier interpretation where possible. A random-effects meta-analysis will be conducted if possible. Statistical heterogeneity across studies will be explored using the Higgins $I^2$ and Cochran Q statistics. A Cochran Q test based on a chi-square statistic with a P value of <.05, and greater than the df, will indicate heterogeneity. The $I^2$ statistic will be interpreted as follows: 0%-40%, minimal heterogeneity; 30%-60%, moderate heterogeneity; 50%-90%, substantial heterogeneity; and 75%-100%, considerable heterogeneity. If there is substantial heterogeneity, subgroup analysis (based on the duration of PASC or COVID-19 severity) will be conducted. Subgroup effect sizes (Cohen d or Hedges g) and correlations will be assessed and compared with unadjusted values to interpret for meaningful effects. Observed effects will be considered robust if the effect estimates of the primary outcome remain consistent or there are no large differences in the magnitude of effect across subgroups. Subgroup analyses will not be performed if there is minimal or moderate heterogeneity. A formal narrative synthesis will be performed if meta-analysis is not possible. The reasons for not pooling data (eg, high statistical, methodological, and clinical heterogeneity) will be reported in the review.

A methodological quality-based sensitivity analysis presented as a summary table will be used to assess the robustness of the findings. Authors of included studies with missing information will be contacted for clarification. The Grading of Recommendations, Assessment, Development, and Evaluations approach will be used to rate the overall certainty of the evidence obtained from the study.

Authors’ Contributions
AE conceived, designed, and drafted the study protocol in its entirety.

Conflicts of Interest
None declared.

Editorial Notice
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References

https://med.jmirx.org/2023/1/e43880


34. Caliendo AM, Hanson KE. COVID-19: Diagnosis. UpToDate. 2022. URL: https://tinyurl.com/53h6me78 [accessed 2023-01-12]


Abbreviations

OR: odds ratio
PASC: postacute sequelae of COVID-19
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR: risk ratio