Using proxy markers from routine diagnostic PCR testing to assess the disease severity of new SARS-CoV-2 variants

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March 2023

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Declaration

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Hannah Hussey

10 April 2023

Abstract

Background

With the emergence of new SARS-CoV-2 variants, understanding the clinical implications of these variants in our South African setting is critical. The Delta variant (B.1.617.2) has been associated with more severe disease, but most of this data is from high income countries. And while the Omicron variant (B.1.1.529, sub-lineages BA.1 and BA.2) has been associated with a reduced risk of severe disease, it is uncertain whether this is caused by a decrease in variant virulence or by higher levels of population immunity.

Methods

We used a novel proxy marker, RNA-dependent RNA polymerase (RdRp) target delay in the Seegene Allplex™ 2019-nCoV polymerase chain reaction assay, to identify suspected Delta variant infection in routine laboratory data. All cases diagnosed on this assay in the public sector in the Western Cape, South Africa, from 1 April to 31 July 2021 (for analysis of clinical severity of Delta vs previously circulating [mainly Beta] variant analysis) and from 1 November to 14 December 2021 (for analysis of clinical severity of Omicron vs Delta variant analysis), were included in the dataset provided by the Western Cape Provincial Health Data Centre (PHDC). We calculated odds/hazard ratios for the association between the proxy marker and death/hospitalization, adjusted for socio-demographic factors, comorbidities, prior diagnosed infection and vaccination status.

Results

For the analysis of the clinical severity of Delta vs previously circulating (mainly Beta) variants, we included 11,355 cases with 700 deaths. RdRp target delay (RTD - i.e., suspected Delta infection) was associated with higher mortality (adjusted odds ratio [aOR] 1.45; 95% confidence interval [CI]: 1.13-1.86), compared to presumptive Beta infection (absence of RTD). Prior diagnosed infection during the previous COVID-19

wave, which was driven by the Beta variant, was protective (aOR 0.32; 95%CI: 0.11-0.92) as was vaccination (aOR [95%CI] 0.15 [0.03-0.62] for complete vaccination [≥28 days post a single dose of Ad26.COV2.S or ≥14 days post second BNT162b2 dose]).

For the Omicron (BA.1/BA.2) vs Delta variant analysis, we included 150 cases with RTD and 1486 cases without RTD. Cases without RTD (i.e., suspected Omicron cases) had a lower hazard of admission (adjusted hazard ratio [aHR], 0.56; 95% CI 0.34-0.91). Complete vaccination was protective against admission, with an aHR of 0.45 (95% CI, 0.26-0.77).

Conclusion

RdRp target delay, a proxy for infection with the Delta variant, is associated with an increased risk of mortality amongst those with laboratory-diagnosed COVID-19 in our setting. Omicron has resulted in a lower risk of hospital admission compared with contemporaneous Delta infection, when using the proxy marker of RTD. Under-ascertainment of reinfections with an immune escape variant remains a challenge to accurately assessing variant virulence.

This study also resulted in two publications, that could be disseminated, initially on MedRxiv, and shared with policy makers in the Department of Health and other scientific colleagues in time to influence the pandemic response.

Acknowledgements

While the epidemiological analyses presented here are my own work, they were only possible because of the groundwork laid by others. Prof Marvin Hsiao, Dr Ziyaad Valley-Omar and the team of virologists at the Western Cape National Health Laboratory Service (NHLS) discovered the proxy marker RdRp target delay that could be used to identify the SARS-CoV-2 Delta variant. Prof Andrew Boulle, along with other data scientists, including Dr Alexa Heekes, established the Provincial Health Data Centre (PHDC) which collates all available electronic health data in the Western Cape Department of Health, including all SARS-CoV-2 laboratory data.

In 2021 I was fortunate to be working in the Western Cape Department of Health's Epidemiology and Surveillance Unit, under Prof Mary-Ann Davies, where I became involved in research looking at SARS-CoV-2 variants. And it was thanks to the wisdom, generosity, and kindness of Prof Davies as a supervisor that I was able to complete this dissertation, that linked the proxy marker of RdRp target delay to clinical outcome data at the PHDC.

Out of this dissertation two manuscripts were submitted for publication. Professors Davies, Hsiao and Boulle provided invaluable support for me in the analysis and writing up of these manuscripts, and we were furthermore assisted by the virology team at NHLS, clinicians working in COVID-19 at Groote Schuur and Tygerberg Hospitals, as well as colleagues from the National Institute of Communicable Diseases. The full author lists are available in the Appendices.

And lastly, I would like to thank my family, and especially my husband, Christian, for their love and patience over the last few years. This dissertation is dedicated to my daughter, Josie, who chose to be born at the start of a pandemic.

Author's note

Please note that this MMed thesis is made up of two publications that were started in June and December 2021, as the new SARS-CoV-2 variants of Delta and Omicron, respectively, emerged and there was limited understanding of variant disease severity.

At that time, I was rotating through the Western Cape Epidemiology and Surveillance Unit, under the supervision of Prof Mary-Ann Davies, who was my line manager in the Provincial Department of Health, as well as my MMed supervisor. Ethics approval for the studies fell under the overall HREC approval that Prof Davies and the Provincial Department of Health had to conduct analyses of routine health data to understand factors associated with COVID-19 outcomes in the Western Cape. The analyses were written up and placed in the public domain (the electronic preprint server MedRxiv) as rapidly as possible in order to provide evidence in a timely manner for health and other policymakers, both in South Africa and internationally, to best plan for and manage the infection surges associated with the emergence of the different variants.

At the time of the Omicron study (November to December 2021), the various Omicron sub-lineages had not been fully appreciated, but the dominant sub-lineages of that time were BA.1 and BA.2. Therefore, when the protocol or published manuscript refer to the Omicron variant, they are in fact referring to the Omicron sub-lineages of BA.1 and BA.2 only.

List of abbreviations

aHR	Adjusted hazard ratio
aOR	Adjusted odds ratio
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease of 2019
Ct	Cycle threshold
EVDS	Electronic Vaccination Data System
HIV	Human immunodeficiency virus
NGS-SA	Network for Genomic Surveillance in South Africa
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
PCR	Polymerase chain reaction
PHDC	Provincial Health Data Centre
RdRP	RNA-dependent RNA-polymerase
RNA	Ribonucleic acid
RTD	RNA-dependent RNA-polymerase (RdRP) target delay
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SGTF	S-gene target failure
TB	Tuberculosis
VOC	Variant of concern

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Chapter 1: Literature review and protocol

Title: Using proxy markers from routine PCR diagnostic testing to assess the disease severity of new SARS-CoV-2 variants

Purpose of the study

With the emergence of new SARS-CoV-2 variants like Delta (B.1.617.2) and Omicron (B.1.1.529), it is crucial to understand the disease severity associated with them in our setting, the Western Cape, South Africa. As we only have limited resources for genomic sequencing, however, novel methods such as RNA-dependent RNA-polymerase (RdRp) target delay, a proxy marker on routine diagnostic PCR testing, can be used to assess variant disease severity. We therefore aim to use this methodology in two separate studies to assess the disease severity of the Delta and Omicron variants.

Background and literature review

COVID-19 in the Western Cape

Since the emergence of SARS-CoV-2 through to March 2022, the Western Cape has experienced four major waves of infection. These waves peaked in June 2020, and January 2021, August 2021 and December 2021, and were caused by the ancestral strain, then almost completely by the Beta (B.1.351), Delta (B.1.617.2) and Omicron (B.1.1.529, BA.1/BA.2 sub-lineages) variants, respectively (Figure 1) (1). Data from the Network of Genomic Surveillance in South Africa describe how >90% of cases in each wave were caused by the dominant variant in each wave (Figure 2a and 2b) (1).

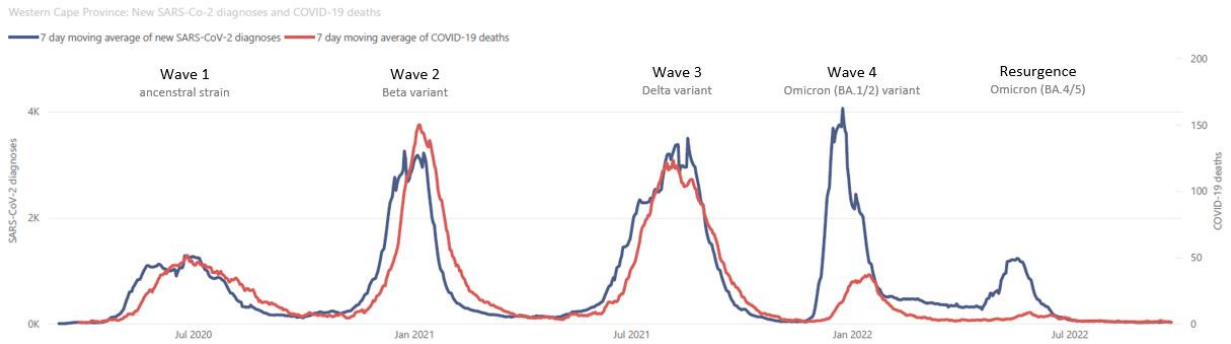

Figure 1: 7-day moving average of new SARS-CoV-2 cases (blue line) and 7-day moving average for COVID-19 related deaths for the Western Cape, South Africa, March 2020 - August 2022.

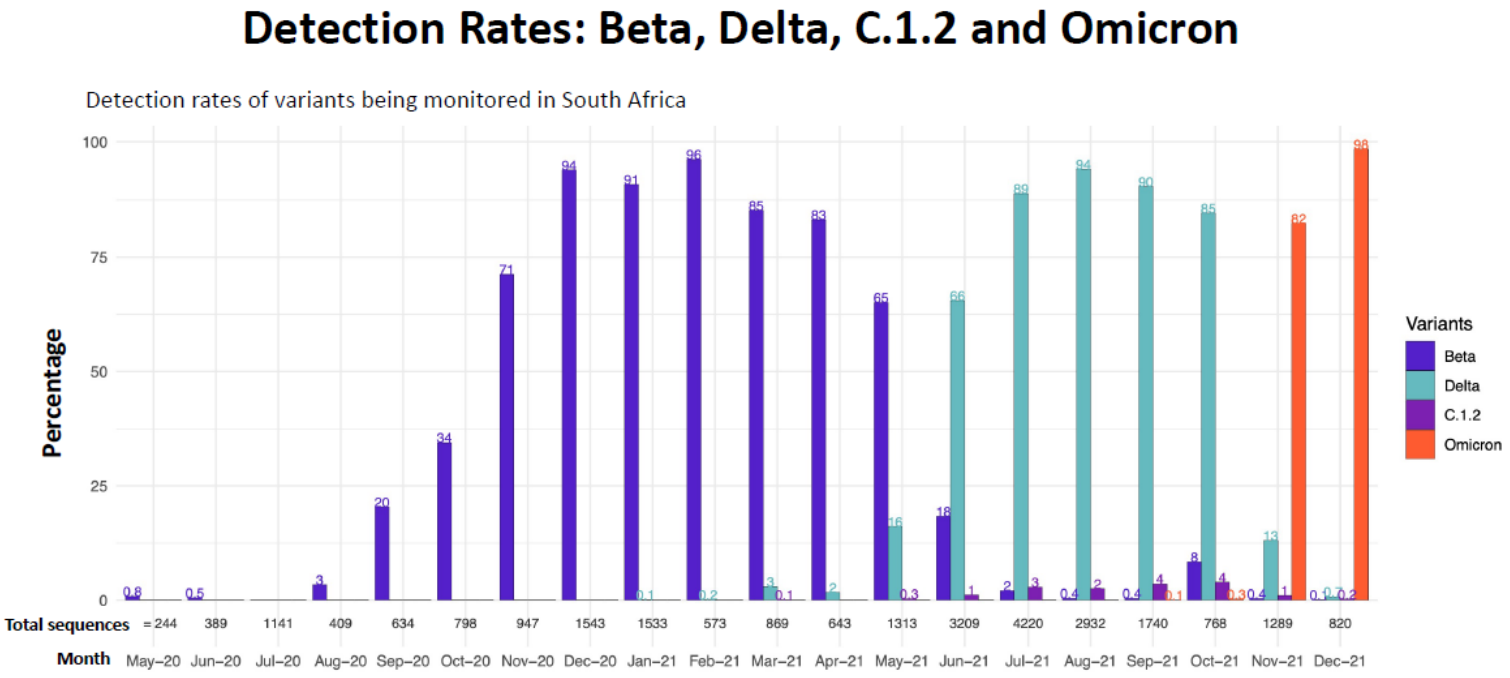

Figure 2a: Detection rates of variants being monitored in South Africa. Graphic taken from NGS-SA SARS-CoV-2 Sequencing Update 31 December 2021 (1).

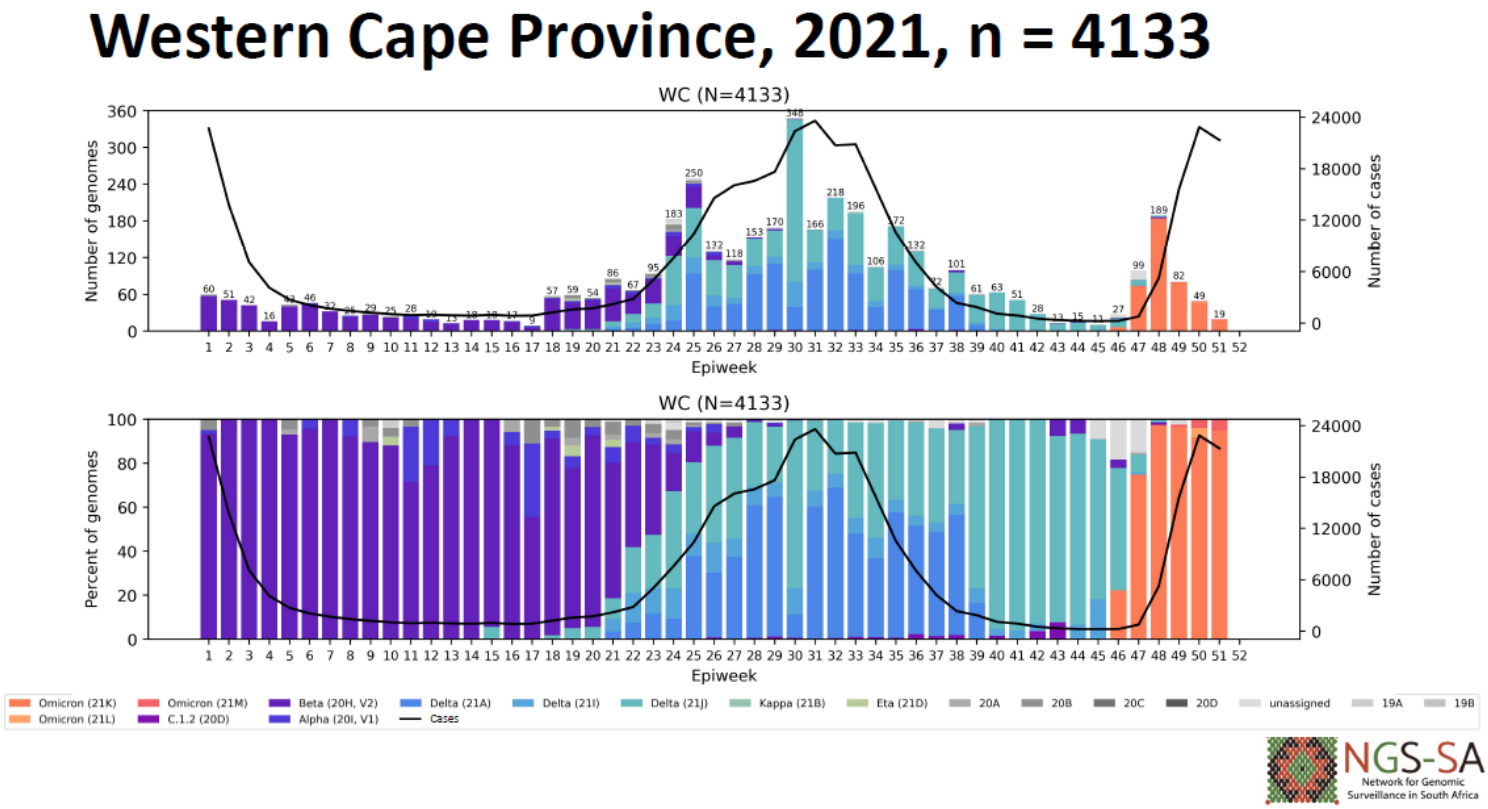

Figure 2b: Number and proportion of SARS-CoV-2 cases in the Western Cape caused by variants. Graphic taken from NGS-SA SARS-CoV-2 Sequencing Update 31 December 2021 (1).

This data on circulating variants is determined by genomic sequencing, where, following routine diagnostic PCR testing, a small subset of specimens is selected and sent on to laboratories working within the Network for Genomic Surveillance of South Africa (NGS-SA) (1). As only a small number of specimens are selected for sequencing and sequencing data can take weeks to months to become available, it is challenging to use this data to assess the clinical outcomes of a new variant.

Initial analyses of variant disease severity have come from high-income countries, which have the resources and ability for large volumes of genetic sequencing (2–7). These studies often assessed morbidity and not mortality, and their findings are not always generalizable a sub-Saharan African setting, as healthcare system resources and capacities differ, as do the patient populations. In the Western Cape,

not only do we have high background SARS-CoV-2 seroprevalence rates, but high levels of comorbid infectious diseases such as HIV and tuberculosis, and access to vaccination was delayed until late in the Delta wave, except for older persons and health care workers (8).

Alternatives to genomic sequencing to assess variant disease severity

A recent study by Davies et al assessed mortality in the Western Cape but used the start of the COVID-19 wave time periods themselves as proxies for the variant. In that study, which used the mortality during the third wave ("Delta": 26 May - 23 June 2021) as the reference period, both the second wave ("Beta": 25 October – 21 November 2020) and fourth wave ("Omicron": 14 November – 11 December 2021) had lower mortality with aHR of 0.60 (95%CI 0.48-0.74) and 0.41 (95%CI 0.29-0.59) respectively (9).

The challenge with using the wave time periods as proxies for the variant, is that the health system might respond differently at different time periods, e.g., with better treatment algorithms like the introduction of steroid treatments, or by making more high flow oxygen beds available. In addition, if a variant was highly infectious and resulted in a high number of SARS-CoV-2 cases, but was not actually very virulent, it would still result in a high number of deaths, as the high admission pressure itself would contribute to mortality (10). And while this study did adjust for vaccination status and documented prior infection, it is unknown whether the individuals infected in the earlier waves are comparable to those infected in the later waves, and whether some other factor that contributed to their differential infection risk might also play a role in determining their disease severity.

For all these reasons, using the wave themselves as proxies for the different variants can be challenging, and our study, therefore, aims to compare the outcomes of contemporaneous cases instead.

RdRp target delay (RTD)

The Seegene Allplex™ 2019-nCoV PCR assay (Seegene Inc, USA) is widely used on the National Health Laboratory Services platform, accounting for 28% of SARS-CoV-2 tests performed nationally (11). This assay measures three gene targets: E, N and RdRp (RNA-dependent RNA polymerase). Because of the G15451A mutation found in the Delta variant, there is a RdRp primer mismatch, i.e., the RdRp probe is slow to pick up positive specimens and the RdRp cycle threshold (Ct) value is higher than expected (11). Thus, a proxy marker for the Delta variant has been suggested, called RdRp target delay (RTD) which is calculated as follows: Ct value of RdRP – Ct value of E > 3.5 (11). When compared to genomic sequencing data, this method has a sensitivity of 93.6% and specificity of 89.7% in detecting the Delta variant (11).

The failure of one probe in the face of different SARS-CoV-2 variant is not uncommon, with S-gene target failure (SGTF) on the Thermo Fisher Taqpath™ PCR assay with the Alpha and Omicron variants being well documented and being used to assess variant disease severity (12–15). In SGTF the failure of the probe is complete, but in RTD the failure is incomplete and only relative to the other probes.

Unfortunately, very little routine Taqpath™ PCR assay testing is done in the Western Cape NHLS, limiting our ability to do SGTF analyses. But as both the Beta and the Omicron variants lack RTD, RTD provides us with an alternative proxy marker with which to assess variant disease severity. Figure 3 illustrates how the proportion of Seegene Allplex™ cases in the Western Cape with RTD increased dramatically with the emergence of the Delta variant, and then quickly decreased with the emergence of the Omicron variant. Since Omicron rapidly replaced Delta, with minimal other variants co-circulating (1), we can use the absence of RTD to identify suspected Omicron cases.

The fourth wave in South Africa was driven by the BA.1 and BA.2 sub-lineages of Omicron, but since then BA.4 and BA.5 have become dominant (1). As the proxy marker of RTD only identifies Omicron by the absence of Delta, we are not able to distinguish the Omicron sub-lineages from one another, and this

study therefore will focus on the earlier sub-lineages of BA.1 and BA.2. However, this is not necessarily a limitation, as the clinical disease with BA.4 and BA.5 has been found to be similar to that of BA.1 (16).

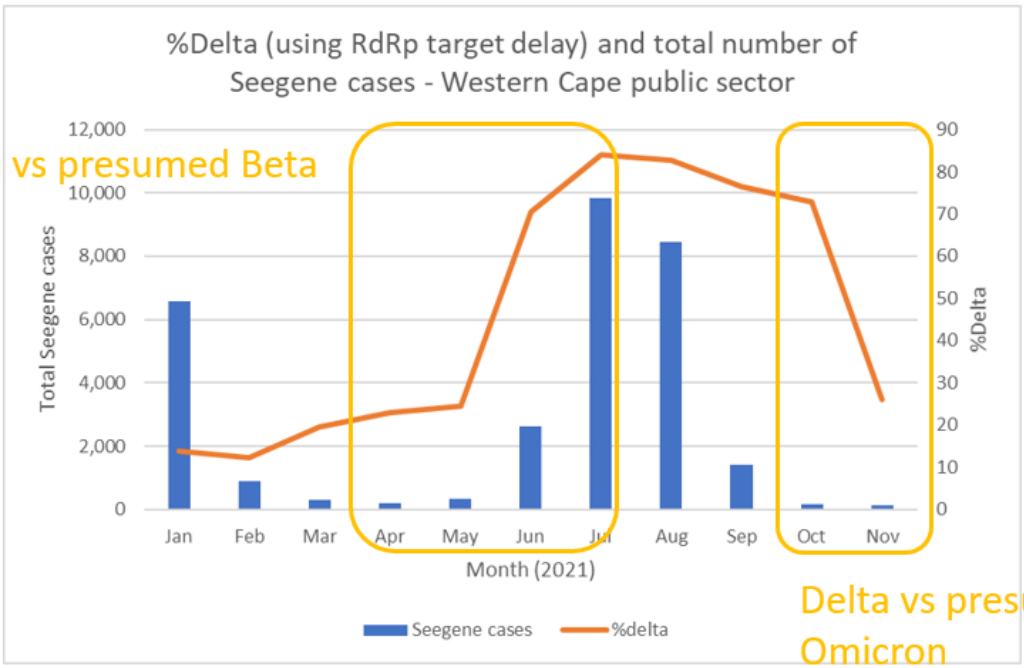

Figure 3: Total number of Seegene Allplex™ SARS-CoV-2 PCR positive cases in the Western Cape public sector (blue bar graphs), with the proportion of RdRp target delay (RTD) (red line graph). The two study time periods are highlighted in yellow.

Delta variant

Before the emergence of Omicron, Delta was the dominant variant globally and increased clinical severity has been noted in high-income countries (2–7). An increased risk of hospital admission, when compared to Alpha, was seen in England and Denmark with an aHR 2.26 (95%CI 1.32-3.89) and RR 2.83 (95%CI 2.02-3.98), respectively (2,3). For a composite outcome that combined high-flow oxygen, ICU admission and/or death Delta was associated with aOR 4.9 [95%CI 1.43-30.78], when compared to the ancestral strain in Singapore and an aOR of 3.61 (1.65-7.91), when compared to Beta in Qatar (4,7).

By the time the Delta variant emerged in these countries, their COVID-19 vaccination programmes were already well established (17). Since most of the severe disease and death in these studies occurred in those who were unvaccinated, the concern is that differences in vaccination status, which are often related to socioeconomic inequalities (18), might contribute to the clinical severity observed and confound the association between variant and clinical outcome. This is in contrast to the Western Cape, where only 5% of all adults, mostly in older age groups, were fully vaccinated by 31 July 2021 (17).

Even though the Delta variant no longer dominates globally, it continues to circulate at low levels and understanding the disease severity associated with it and its spectrum of mutations remains important, as illustrated by the recent emergence of an Omicron-Delta recombinant strain (BA.1 x AY.4) (19). Furthermore, disease severity of new variants is often described relative to that of the previously dominant variant (20). The Omicron variant, for instance, has been reported to cause less severe disease than the Delta variant, but this must be understood in the context of the Delta variant already being associated more severe disease than the Alpha or Beta variants (21).

Omicron variant

Figure 1 demonstrates that in the first three waves COVID-19 mortality tracked consistently with new SARS-CoV-2 cases. But in the fourth wave, even though there was a large spike in cases, it was not accompanied by a correspondingly large increase in mortality, suggesting that the variant of the fourth wave, Omicron, was less virulent. Even amongst those few deaths in people with diagnosed SARS-CoV-2 infection that did occur in the fourth wave, there was an increase in the proportion of deaths due to other causes, with the SARS-CoV-2 infection being incidental, highlighting further the decreased disease severity associated with Omicron (22).

Initial studies from South Africa, which used the wave time periods as proxies for the variant, suggest that Omicron is associated with less severe disease (23–25). This has been corroborated by similar findings in the United Kingdom (26,27), as well as an SGTF analysis by the National Institute of Communicable Diseases (NICD) in South Africa (28). The latter study found an aOR 0.3 (95%CI 0.2–0.5) for severe disease in cases with SGTF (presumed Omicron cases), compared to those without SGTF (presumed Delta), however vaccination status was based on self-report and likely not fully adjusted for. (28).

The challenge with Omicron, however, is that, as an immune escape variant, infection in individuals who have previously been infected or been vaccinated is more likely than with other variants, and this prior exposure is highly protective (9,20,28). Given that by the time Omicron emerged, the South African population had already undergone three large waves of SARS-CoV-2 infections with a resultant high background seroprevalence (73.1% [72.0-74.1%] of all participants had antibodies positive SARS-CoV-2 in a survey conducted 22 October – 9 December 2021), and that the vaccination programme had begun in earnest, disentangling these factors from the apparent decreased variant virulence of Omicron is particularly challenging (29,30).

Conclusion

While there is literature on variant disease severity available, it is often from high-income countries, or uses the wave period as a proxy for the variant and compares cases at different time periods. Our study, therefore, aims to look at variant disease severity, by using RTD to allow us to compare contemporaneous cases, without having to sequence a large number of specimens.

For the Delta variant analysis, we will focus on mortality with Delta, when compared to the Beta variant specifically, taking into account vaccination and comorbidity data, as that is a gap in the literature. And

for the Omicron variant analysis, we intend to perform a study similar to the NICD SGTF analysis, but with complete vaccination data and using more robust comorbidity data than the NICD had available.

Aims and objectives

Aim: To assess the disease severity of new variants, namely Delta and Omicron, in adults in the Western Cape public sector using contemporaneous cases and RTD, a proxy marker in routine diagnostic PCR testing

Objectives:

1. Assess the association of COVID-19 hospital admission and/or death with the "new variant", compared to the previously circulating dominant variant by using RTD as follows:
    a. Delta (RTD present) vs Beta (RTD absent)
    b. Omicron (RTD absent) vs Delta (RTD present)

2. Assess risk factors associated with worse clinical outcomes, including co-morbidities, vaccination status and recorded re-infections

Methodology

Setting

In the Western Cape the Provincial Health Data Centre (PHDC) collates all electronic health data for patients in the public sector (31). Since March 2020, the PHDC has also been collating all available COVID-19 data, including laboratory tests, hospital admissions and deaths. SARS-CoV-2 vaccination data from the national Electronic Vaccination Data System (EVDS) is also obtained at the PHDC and linked through the national identifier.

PCR test results performed in the Western Cape National Health Laboratory Service (NHLS) are sent to the PHDC, along with details on the type of PCR assay used and the cycle threshold values for the different PCR targets. This data is used to calculate RTD in test samples done with the Seegene Allplex^{TM} assay, and then linked to other clinical data at the PHDC.

Study design

Two separate survival analyses using retrospective cohorts will be performed, with identical methodologies, except the time periods will be different, and the main exposure of interest will change depending on the variant of interest:

1. Delta variant, identified by the presence of RTD, conducted 1 April – 31 July 2021
2. Omicron variant, identified by the absence of RTD, conducted 15 October – 14 December 2021

Study population

All positive Seegene Allplex^{TM} PCR tests conducted on adults (≥18 years) in the Western Cape NHLS in the time period when both the old and new variants were co-circulating will be included. If insufficient cases

are available during the Omicron study time period (i.e., if the study is underpowered), we will expand the population to all those aged 15 years and above, as the clinical disease seen in this population is similar to that of young adults (32).

Data analysis

Cox regression will be done to calculate hazard ratios, taking into account time from diagnosis to time of outcome. Cox regression is preferred to logistic regression as with time the new variant replaces the old variant, and outcomes from SARS-CoV-2 can take weeks to occur. If the dataset is extracted too soon after the emergence of the new variant, the new variant will appear less severe, as insufficient time has occurred for the outcomes to occur, regardless of whether it actually is less severe or not. However, if sufficient time has passed, logistic regression is a reasonable alternative option.

The outcomes assessed will be:

- COVID-19 related hospital admission, defined as a date of admission within 21 days of the COVID-19 diagnosis (excluding specialized psychiatry and orthopedic rehabilitation hospitals, where the COVID-19 diagnosis would likely be incidental)
- ICU admission during a COVID-19 admission
- COVID-19 related death, defined as a death within 28 days of a COVID-19 diagnosis, or within 14 days of discharge from COVID-19 admission
    - Includes out of hospital deaths; excludes deaths flagged as incidental by COVID-19 local case manager
- Composite outcome: COVID-19 hospital admission and/or COVID-19 death

As outcomes are expected to be worse with the Delta variant, and there is limited data on Delta variant associated mortality available, in the first study we will focus on the outcome of mortality. However, as the fourth wave has resulted in very few deaths, and incidental deaths are increasingly an issue (22), we might not be able to assess mortality in the Omicron study. If that is the case, the Omicron study will then rather focus on COVID-19 related hospitalization, with and without ICU admission.

The primary analysis will be done on all included specimens. Secondary analyses will also be performed:

- Stratifying by age (<50 years and ≥ 50 years)
- Restricting to admitted cases only

These secondary analyses will be performed to try to take into account the issue of limited testing capacity, where those with or at risk of severe disease were more likely to get tested for SARS-CoV-2.

All models will be adjusted for the following variables:

- Age (in 10-year age groups), sex, sub-district of residence
- Known PHDC comorbidities: HIV, diabetes, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD) /asthma, hypertension, tuberculosis (ever), pregnancy (current)
- Recorded reinfection status, defined as a positive SARS-CoV-2 test > 90 days after a previous test. The "current" infection will be diagnosed with a Seegene Allplex^{TM} PCR test, but the test for the first infection can be an antigen test or a PCR test using any assay type
- Vaccination status, grouped into
    - Unvaccinated: no documented SARS-CoV-2 vaccination data on EVDS

- Fully vaccinated: ≥28 days post vaccination with a single dose of Janssen/Johnson & Johnson (Ad26.COV2.S), or ≥14 days post second dose of Pfizer–BioNTech (BNT162b2)
- Partially vaccinated: >1 day after (first) vaccination dose until meeting criteria for full vaccination above

Booster vaccinations will not be considered as booster vaccine availability was very limited by the time Omicron emerged, and so there were too few individuals to include.

- Admission pressure, defined as weekly COVID-19 admissions in the week of diagnosis, as it is a known independent driver of mortality (10)

Limitations and feasibility

There are two main limitations of this study design. Firstly, although RTD is a reliable proxy marker for the Delta variant it is not as accurate as whole genome sequencing, and misclassification may dilute the effect seen. Secondly, as we are dependent on using routine electronic health data, and not all COVID-19 cases are tested in our setting, this might also introduce bias, as particularly those with mild or asymptomatic disease are less likely to present for and access testing. In a seroprevalence study after the first wave of infections, case ascertainment was noted to be as low as 4% of all infections that had occurred (8).

While testing capacity did increase somewhat in the later waves, particularly after the introduction of antigen tests in October 2020 (34), low case ascertainment poses two related issues. Firstly, less cases are available to be included in the analysis, and the cases that are available are more likely to have severe disease. Secondly, low case ascertainment in previous waves means that there might be low ascertainment of reinfections for current cases. Unrecorded previous infections could provide protection for a new variant, making it appear less virulent. This is particularly a challenge when trying to assess an immune escape variant, like Omicron (30). As prevalence of prior infection differed by sub-district of

residence, we will adjust for that, which should somewhat mitigate differential ascertainment of prior infection.

Other limitations are that RTD can only be calculated for the Seegene Allplex™ assay, and therefore a large number patients who had other PCR assays or antigen tests could not be included, decreasing the sample size available markedly. This is a particular concern in the Omicron study as there was a rapid takeover of the new variant from a baseline of very low diagnosed cases (see Figure 3). In addition, there will probably be very low numbers of severe outcomes or deaths in the Omicron wave, and so uncertainty remains as to whether our study will be sufficiently powered.

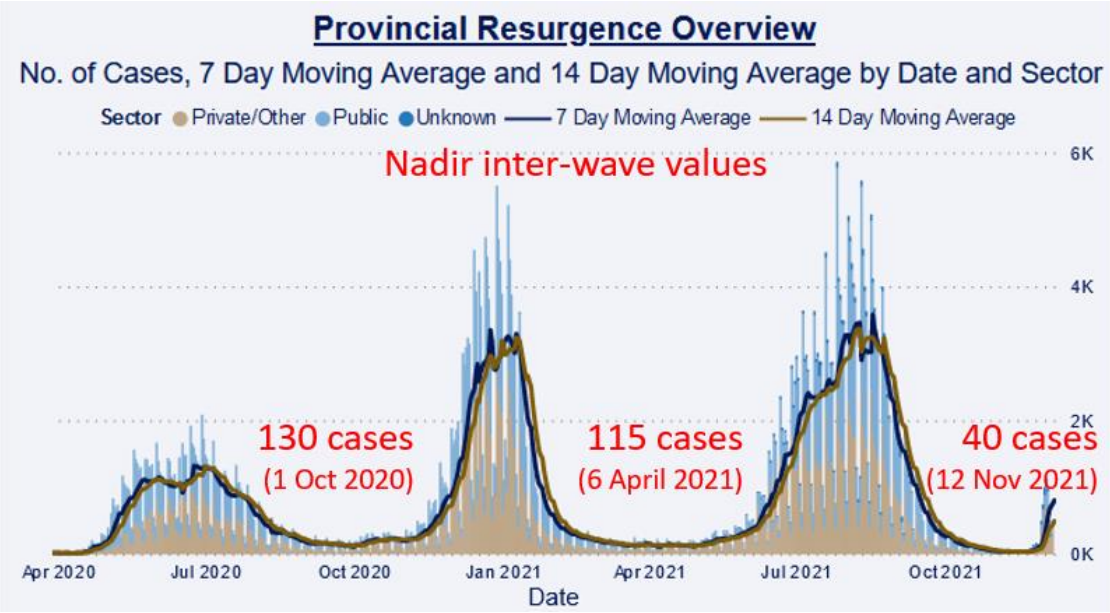

Figure 4: Nadir inter-wave values using the 7-day moving average of new SARS-CoV-2 cases (source Western Cape Department of Health: Internal COVID-19 dashboard)

In addition, different laboratories servicing different geographic areas tend to use different PCR assays, and therefore we do need to confirm that the demographic and clinical features of SARS-CoV-2 cases

diagnosed with and without the Seegene Allplex™ assay are similar, and that our findings are generalizable to all COVID-19 cases in the province.

As we are using routine electronic health data from the public sector in the Western Cape, we need to be cautious of any other biases that exist in this observational data. For instance, the individuals that have "good" health seeking behavior, might be more likely to present for/access SARS-CoV-2 vaccination, and might be the same individuals that would be more likely to present for/access SARS-CoV-2 diagnostic testing, and thus be diagnosed with COVID-19 infection.

Ethical considerations

As a retrospective study, there are no major risks to the participants, apart from a minor theoretical risk of breach of confidentiality, which we will actively mitigate. De-identified clinical data with an anonymous study identifier will be downloaded from the PHDC and stored in a secure password protected cloud storage database for use only by the study researchers.

We are also asking for waiver of informed consent, as we are using data that has already been collected from patients as part of routine COVID-19 surveillance and clinical management.

This study will have limited individual benefit to the study participants, but significant population level benefits, including implications for health services planning, risk stratification of patients, and informing necessity for lockdown type measures, both locally and internationally.

Also, please note that while this protocol pertains to my MMed, Prof Mary-Ann Davies and the PHDC have HREC approval to conduct analyses to describe the COVID-19 outbreak and factors associated with clinically severe outcomes (HREC Ref 460/2020). As I was working in Epidemiology and Surveillance with

Prof Mary-Ann Davies in 2021, I have started working on this topic of disease severity using RTD, using the initial broader PHDC ethics approval.

Dissemination of results

A report and presentation on the study will be shared with all relevant stakeholders, including the Western Cape Department of Health, the NHLS, the National Institute of Communicable Diseases and the Network for Genomics Surveillance in South Africa (NGS-SA). Manuscripts will be drafted for submission to peer-reviewed journals and posted to MedRxiv while awaiting peer-review.

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Chapter 2: Publication ready manuscripts

The analyses were conducted in real time with the emergence of the variants - first Delta, and then Omicron - and so even though the methodologies used are essentially the same, they are presented separately here. The two variants also have different characteristics, especially with regards to immune escape, creating markedly different challenges in the observational data, which also support them being addressed separately.

Please note that while the Omicron analysis has been published in a peer reviewed journal (International Journal of Infectious Diseases – see Appendix 3), the Delta analysis is still undergoing peer review but is available online as a preprint (Gates Open – see Appendix 2). The difference in publication status is most likely due to more timely submission to a journal for the Omicron analysis, when the variant was still topical, as there is no substantive difference between the two analyses, beyond, of course, the variant being analyzed.

2a) Delta analysis: "Higher mortality associated with the SARS-CoV-2 Delta variant in the Western Cape, South Africa, using RdRp target delay as a proxy: a cross-sectional study"

Abstract

Background: The SARS-CoV-2 Delta variant (B.1.617.2) has been associated with more severe disease, particularly when compared to the Alpha variant. Most of this data, however, is from high income countries and less is understood about the variant's disease severity in other settings, particularly in an African context, and when compared to the Beta variant.

Methods: A novel proxy marker, RNA-dependent RNA polymerase (RdRp) target delay in the Seegene Allplex™ 2019-nCoV (polymerase chain reaction) PCR assay, was used to identify suspected Delta variant infection in routine laboratory data. All cases diagnosed on this assay in the public sector in the Western Cape, South Africa, from 1 April to 31 July 2021, were included in the dataset provided by the Western Cape Provincial Health Data Centre (PHDC). The PHDC collates information on all COVID-19 related laboratory tests, hospital admissions and deaths for the province. Odds ratios for the association between the proxy marker and death were calculated, adjusted for prior diagnosed infection and vaccination status.

Results: A total of 11,355 cases with 700 deaths were included in this study. RdRp target delay (suspected Delta variant) was associated with higher mortality (adjusted odds ratio [aOR] 1.45; 95% confidence interval [CI]: 1.13-1.86), compared to presumptive Beta infection. Prior diagnosed infection during the previous COVID-19 wave, which was driven by the Beta variant, was protective (aOR 0.32; 95%CI: 0.11-

0.92) as was vaccination (aOR [95%CI] 0.15 [0.03-0.62] for complete vaccination [≥28 days post a single dose of Ad26.COV2.S or ≥14 days post second BNT162b2 dose]).

Conclusion: RdRp target delay, a proxy for infection with the Delta variant, is associated with an increased risk of mortality amongst those who were tested for COVID-19 in our setting.

Introduction

To date, the Western Cape Province of South Africa, has experienced four large waves of SARS-CoV-2 infections. The first wave caused by early ancestral clades of SARS-CoV-2 peaked in June 2020, the second wave with the Beta variant (B.1.351) peaked in December 2020, the third wave caused by the Delta variant (B.1.617.2) peaked in August 2021 and the fourth most recent wave was caused by the Omicron variant peaked in December 2021 [1].

Prior to the arrival of Omicron, Delta had been the dominant variant globally, its increased clinical severity particularly when compared to Alpha is well documented [2–6]. Most of the severe disease in these studies occurred in those who were unvaccinated. The concern is that in these settings with well-established vaccination programmes, there are differences between the vaccinated and unvaccinated population, largely driven by socioeconomic disparities [7], that might contribute to the clinical severity seen and confound the association between variant and disease severity. In addition, there is only limited data on Delta disease severity within a sub-Saharan African setting.

The start of the third wave of COVID-19 in the Western Cape Province was characterized by a rapid transition from the previously dominant Beta variant to Delta [8]. Using a novel proxy marker for Delta, namely RNA-dependent RNA polymerase (RdRp) target delay (RTD) in (polymerase chain reaction) PCR

positive samples, our objective was to assess mortality associated with Delta, compared to Beta, in our population which had relatively low levels of vaccine coverage as well as a high prevalence of comorbidities, including HIV and tuberculosis.

Methods

RdRP target delay

RTD, defined as a difference in cycle threshold (Ct) value of >3.5 in the RdRp relative to E gene target in the Seegene Allplex™ 2019-nCoV PCR assay (Seegene Inc, USA), has recently been described [9]. This phenomenon is due to the G15451A mutation found in Delta, resulting in a RdRp primer mismatch [9]. When evaluated against genomic data, this method had a sensitivity of 93.6% and specificity of 89.7% in detecting Delta [9]. RTD has been used to assess variant disease severity in our setting during the fourth wave [10].

Population and statistical analysis

We included all COVID-19 cases diagnosed on the Seegene Allplex™ 2019-nCoV diagnostic PCR assay in the Western Cape public sector from 1 April to 31 July 2021, a period when both Beta and Delta were co-circulating (Appendix Figure 1) All available cases were included without sampling. Alpha and non-variant of concern lineages accounted for a negligible number of infections at this time [8]. Follow-up ended on 31 August 2021, at which point most of the expected COVID-19 related outcomes would have occurred. Approximately 70% of the Western Cape population uses the public sector for health services and the Western Cape Provincial Health Data Centre (PHDC) collates all available electronic health data on these patients [11]. The PHDC combines laboratory data on COVID-19 tests with hospital admission and death

data, as well as information on known comorbidities and where available, vaccination status. Use of de-identified linked data provided by the PHDC in COVID-19 analyses has been previously described [12]. COVID-19 deaths were defined analytically as deaths within 28 days of diagnosis of COVID-19 or 14 days after discharge from hospital (where hospital admission occurred within 21 days of the COVID-19 diagnosis), without a non-COVID-19 cause of death recorded by the health facility or COVID-19 case managers. Out-of-facility deaths were determined by civil identifier linkage of patients with COVID-19 diagnoses to the national population register.

We undertook logistic regression using Stata 13.1 to determine the association between RTD and mortality, adjusted for age, sex, known comorbidities, prior diagnosed infection, vaccination status at time of diagnosis, sub-district of residence, month of diagnosis and hospital admission pressure (number of public sector admissions in the calendar week of diagnosis, categorized into four [weekly admission of ≤350, ≤700, ≤1000 and >1000]). All variables were included as binary or categorical variables. Prior diagnosed infections were defined as a positive COVID-19 test more than 90 days prior to the current test and classified into those with their first infection in the first wave (1 March – 30 September 2020) or second wave (1 October 2020 – 31 March 2021). Vaccination status at time of COVID-19 diagnosis was determined by the PHDC prior to de-identification by linking COVID-19 cases with the national Electronic Vaccination Data System (EVDS) through national civil identifiers in both the PHDC and EVDS databases. EVDS, a national clinical data system that is not publicly accessible, is one of the data sources integrated into the PHDC [11]. For the purposes of this study, we defined complete vaccination analytically as ≥28 days post-vaccination with Janssen/Johnson & Johnson (Ad26.COV2.S), or ≥14 days post second dose of Pfizer–BioNTech (BNT162b2). Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination.

A secondary analysis was done, using the same methodology as above, but stratifying the cases into those aged less than 50 years and those aged 50 years and above.

Ethical approval

The study was approved by the University of Cape Town Research Ethics Committee (HREC 460/2020).

Results

We included 11,355 cases tested using the Seegene Allplex TM assay, which were 22% of all positive test results in the province in that time period (Appendix Table 1). The median age was 43 years (interquartile range [IQR] 32-55) and 44% were male. RTD was present in 9106 (80%) of the cases included (Table 1). Patient characteristics were similar in those with and without RTD. There was, however, a difference in Ct values (average of the E and N gene targets); those with RTD had lower median Ct values (26.1, IQR 22.2-30.9, vs 32.7 IQR 25.6-37.6).

Amongst the Seegene Allplex TM cases in our study, there were 700 deaths meeting the above definition for COVID-19-associatedness, with a case fatality rate of 6.3% in those with RTD, compared to 5.8% in those without. After adjusting for all covariates, we found that RTD was associated with death among cases (aOR 1.45 [95% CI 1.13-1.86]) and among those admitted to hospital (aOR 1.39 [95% CI 1.03-1.88]) (Table 2).

Prior diagnosed infection with a first infection in the Beta dominated second wave (vs no prior diagnosed infection), was protective against death (aOR 0.32 [95% CI 0.11-0.92]), whereas prior diagnosed infection with a first infection in the first wave was not (aOR: 1.56 [95% CI 0.50-4.92]). Vaccination was protective

against death. The aORs (95% CI) for partial and full vaccination were 0.59 (0.45-0.77) and 0.15 (0.03-0.62) respectively.

In a stratified analysis the association between RTD and death was similar in both those aged <50 and ≥50 years (aOR [95%CI] of 1.32 [0.77-2.26] and 1.44 [1.09-1.91] respectively).

Discussion

RTD, a proxy marker for the Delta variant, was associated with an increased risk for death compared to presumptive Beta variant infection, while prior diagnosed infection (with first infection in the second wave) and vaccination were strongly protective. These findings are corroborated by a recent study from the Western Cape, which is the only other study to assess mortality with the Delta variant to compared to Beta. In that analysis, the wave itself was used as a proxy for variant, and the third wave ("Delta": 26 May - 23 June 2021) had a higher aOR for mortality when compared to the second wave ("Beta": 25 October - 21 November 2020) of 1.79 (95%CI 1.41-2.27) [13], which is slightly higher than this analysis. Although the confidence intervals do overlap, the possible difference might be explained by the inclusion of cases after testing restrictions were in place in this analysis.

The increased transmissibility of Delta is well established and may be due to its higher viral load [6]. In our analysis, specimens with RTD had lower Ct values than those without, suggesting a higher viral load. This, along with immune evasion by Delta, could contribute to the increased disease severity seen [14]. Nonetheless, our results need to be interpreted in the context of our setting where not all COVID-19 cases would have accessed testing. Delta often results in mild symptoms and during wave surges, as was the case from 15 June 2021 until the end of the analysis period, in the public sector only those aged ≥45 years

or with comorbidities or requiring hospital admission would be eligible for all SARS-CoV-2 testing [15]. The interpretation of our results is therefore, that among those who access a PCR test, the Delta variant is associated with worse outcomes. While it is unclear if this finding can be extrapolated to all those with COVID-19 in our setting, similar findings from countries with more widespread testing support the increased clinical severity of Delta [2–6].

Hospital admission was more likely with Delta compared to Alpha in England (aHR 2.26 [95%CI 1.32-3.89]) [2] and Denmark (RR 2.83 [95%CI 2.02-3.98]) [3]. In Singapore, infection with Delta and Beta vs. wild-type SARS-CoV-2 were both associated with a composite outcome of oxygen use, intensive care admission and death (aOR 4.9 [95%CI 1.43-30.78] and 1.69 [95%CI 0.19-14.69] respectively) [4]. Similarly, a recent study from Qatar found that Delta compared to Beta had an aOR of 3.61 (1.65-7.91) for severe-critical disease, which was defined as intensive care unit admission, use of high-flow oxygen, mechanical ventilation, or death [16]. Delta (vs wild-type) was associated with mortality in Canada (aOR 2.32 [95% CI 1.47-3.30]) as were N501Y-positive variants (Alpha, Beta, Gamma) (aOR 1.51 [95%CI 1.30-1.74]) [5].

However, most of these countries had relatively high COVID-19 vaccination coverage rates by the time the Delta variant became dominant there [17]. Since most severe COVID-19 cases would be in unvaccinated people, differences between the vaccinated and the unvaccinated population might confound associations with variant infection and disease severity. By contrast in the Western Cape by 31 July 2021, only 5.0% of all adults, mostly in older age groups, were fully vaccinated [17]. In addition, most of these studies compared the outcomes of Delta to Alpha. The Alpha variant has been shown to have worse clinical outcomes compared to wild-type, with an aHR for death of 1.73 (95%CI 1.41 - 2.13) [18].

Despite Delta no longer being the dominant variant, it continues to circulate globally and understanding and quantifying the disease severity associated with it and its spectrum of mutations remains critical. A

Delta resurgence cannot be excluded, particularly with the recent emergence of the Omicron-Delta recombinant strain (BA.1 x AY.4) that the World Health Organisation has declared to be a variant under monitoring [19]. In addition, disease severity of dominant circulating variants is often calculated relative to the previously dominant variants [20]. The Omicron variant, for instance, has been found to cause less disease than the Delta variant, but this has to be understood in the context of the Delta variant already being associated with more severe disease compared to Alpha or Beta, hence quantifying the clinical severity of different variants is important for such comparisons [10,13].

This study has several limitations. First, we could only include cases tested on the Seegene Allplex^{TM} 2019-nCoV assay, excluding cases diagnosed by other PCR methods or antigen testing. However, the included cases are mostly representative of all diagnosed PCR cases in the Western Cape (Appendix Table 1). Patients tested on this assay were similar in age, sex, known comorbidities, prior diagnosed infection and vaccination status to those who tested positive using other PCR assays. As different laboratories service different geographical regions there was a difference, however, in the district of residence. Patients who tested positive on antigen tests tended to be younger, have fewer comorbidities and fewer were admitted to hospital. This is in accordance with the Western Cape's testing guidelines where PCR was preferred for hospital patients, while antigen testing was promoted at primary health care facilities[15].

Second, while RTD is a reliable proxy marker for Delta, it is not as accurate as whole genome sequencing, and misclassification may have diluted the effect of Delta. Third, we could only assess the effect of prior laboratory-confirmed COVID-19 infection and seroprevalence studies suggest considerably higher numbers were infected, even after the first wave, and that prior infection prevalence differed by sub-district of residence [21]. While we did adjust for sub-district, the absence of a protective effect of prior infection in the first wave may be due to insufficient numbers of those infections being diagnosed. Fourth,

the inclusion of cases after testing restrictions were introduced is likely to result in an underestimate of Delta disease severity. However, in the stratified analysis, those younger than 50 years, who would be more likely to be affected by the testing restrictions, still had a similar findings to those >50 years. Fifth, although we adjusted for COVID-19 hospital admissions to account for escalating service pressure during the wave surge, we could not adjust for non-COVID-19 admissions that might have added to pressure on facilities and contributed to mortality.

And finally, there are innate limitations in using observational data from surveillance and routine health records to assess variant disease severity, particularly as potential biases around testing patterns cannot always be fully adjusted for [20].

Conclusion

In this study we found that RTD, a useful proxy for infection with the Delta variant, is associated with an increased risk of mortality amongst those who were tested for COVID-19 in our setting. This suggests that the Delta variant is associated with an increased risk of mortality when compared with other variants, and the Beta variant in particular.

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Tables and Figures

Table 1: Characteristics of all COVID-19 cases (Seegene Allplex™ 2019-nCoV assay positive) in the Western Cape, April - July 2021, by presence of RNA-dependent RNA polymerase target delay (RTD).

		All cases (n=11,355; 700 deaths)		RdRP Target Delay (n=9,106; 570 deaths)		No RdRP Target (n=2,249; 130 deaths)	
		n	%	n	%	n	%
RTD	**absent**	2249	19.8				
	present	9106	80.2				
Sex	**female**	6411	56.5	5143	56.5	1268	56.4
	male	4944	43.5	3963	43.5	981	43.6
Age category	**20-29yrs**	2243	19.8	1810	19.9	433	19.3
	30-39yrs	2623	23.1	2149	23.6	474	21.1
	40-49yrs	2427	21.4	1956	21.5	471	20.9
	50-59yrs	2113	18.6	1701	18.7	412	18.3
	60-69yrs	1177	10.4	899	9.9	278	12.4
	≥70yrs	772	6.8	591	6.5	181	8.1
Comorbidities	**HIV positive**	726	6.4	560	6.2	166	7.4
	Diabetes	1546	13.6	1218	13.4	328	14.6
	Current tuberculosis	94	0.8	66	0.7	28	1.2
	Hypertension	2475	21.8	1963	21.6	512	22.8
	Chronic Kidney Disease	374	3.3	299	3.3	75	3.3
	Chronic Obstructive Pulmonary Disease	876	7.7	685	7.5	191	8.5
Prior diagnosed infection	**None**	11107	97.8	8914	97.9	2193	97.5
	1st infection in 1st wave	64	0.6	46	0.5	18	0.8
	1st infection in 2nd wave	184	1.6	146	1.6	38	1.7
Vaccination status at time of diagnosis¶	**Unvaccinated**	10422	91.8	8358	91.8	2064	91.8
	Partially vaccinated	694	6.1	544	6.0	150	6.7
	Completely vaccinated	239	2.1	204	2.2	35	1.6

¶ Fully vaccinated was defined as ≥28 days post-vaccination with Janssen/Johnson & Johnson or ≥14 days post second dose of Pfizer–BioNTech. Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination.

Table 2: Logistic regression for outcome of death in a) all positive Seegene Allplex™ cases; b) restricted to those admitted to hospital only; c) stratified by age into those aged under 50 and over 50 years.

		Adjusted§ OR (95%CI) for outcome of death			
		All cases (n=11 355; 700 deaths)	Only admitted cases* (n=1 856; 612 deaths)	All cases < 50 years (n=7 293; 113 deaths)	All cases ≥ 50 years (n=4 062; 587 deaths)
RTD	absent	Ref	Ref	Ref	Ref
	present	1.45 (1.13-1.86)	1.39 (1.03-1.88)	1.32 (0.77-2.26)	1.44 (1.09-1.91)
Sex	female	Ref	Ref	Ref	Ref
	male	1.50 (1.25-1.80)	1.19 (0.95-1.47)	1.16 (0.78-1.72)	1.63 (1.33-2.00)
Age category	20-29yrs	Ref	Ref	Ref	
	30-39yrs	2.75 (1.19-6.31)	2.19 (0.81-5.93)	2.49 (1.07-5.78)	
	40-49yrs	6.89 (3.15-15.05)	5.91 (2.27-15.54)	6.48 (2.92-14.37)	
	50-59yrs	11.49 (5.31 - 24.88)	6.86 (2.66-17.67)		Ref
	60-69yrs	24.64 (11.36-53.42)	11.75 (4.56-30.28)		2.16 (1.68-2.79)
	≥70yrs	72.81 (33.54-158.06)	25.93 (10.03-67.01)		6.43 (4.96-8.33)
Comorbidities†	HIV positive	1.66 (1.11-2.47)	1.14 (0.69-1.88)	2.21 (1.27-3.85)	0.99 (0.53-1.85)
	Diabetes	2.60 (2.14-3.16)	1.19 (0.95-1.49)	2.58 (1.55-4.32)	2.61 (2.11-3.22)
	Current tuberculosis	3.62 (1.76-7.42)	1.57 (0.68-3.61)	4.90 (1.90-12.65)	2.38 (0.78-7.20)
	Hypertension	1.13 (0.93-1.38)	0.95 (0.76-1.20)	1.07 (0.63-1.82)	1.16 (0.93-1.43)
	Chronic Kidney Disease	2.17 (1.65-2.86)	1.76 (1.29-2.42)	9.33 (4.20-20.72)	1.87 (1.41-2.50)
	Chronic Obstructive Pulmonary Disease	1.27 (1.00-1.62)	0.91 (0.69-1.19)	1.95 (1.08-3.53)	1.17 (0.90-1.52)
Prior diagnosed infection	None	Ref	Ref	Ref	Ref
	1st infection in 1st wav	1.56 (0.50-4.92)	2.63 (0.51-13.64)	1.11 (0.14-78.87)	2.12 (0.49-9.17)
	1st infection in 2nd wa	0.32 (0.11-0.92)	0.34 (0.10-1.09)	0.68 (0.15-3.19)	0.14 (0.03-0.67)
Vaccination status at time of diagnosis¶	Unvaccinated	Ref	Ref	Ref	Ref
	Partially vaccinated	0.59 (0.45-0.77)	0.79 (0.57-1.09)	1.47 (0.32-6.66)	0.57 (0.44-0.75)
	Completely vaccinated	0.15 (0.03-0.62)	0.14 (0.02-1.20)	(omitted)	0.22 (0.05-0.96)

§ Adjusted for all variables in the model, as well as month of diagnosis, sub-district of residence and number of COVID-19 admissions in week of diagnosis.

* Date of hospital admission within 21 days of COVID-19 date of diagnosis.

† The reference group here is the absence of that specific comorbidity.

¶ Fully vaccinated was defined as ≥28 days post-vaccination with Janssen/Johnson & Johnson or ≥14 days post second dose of Pfizer–BioNTech. Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination.

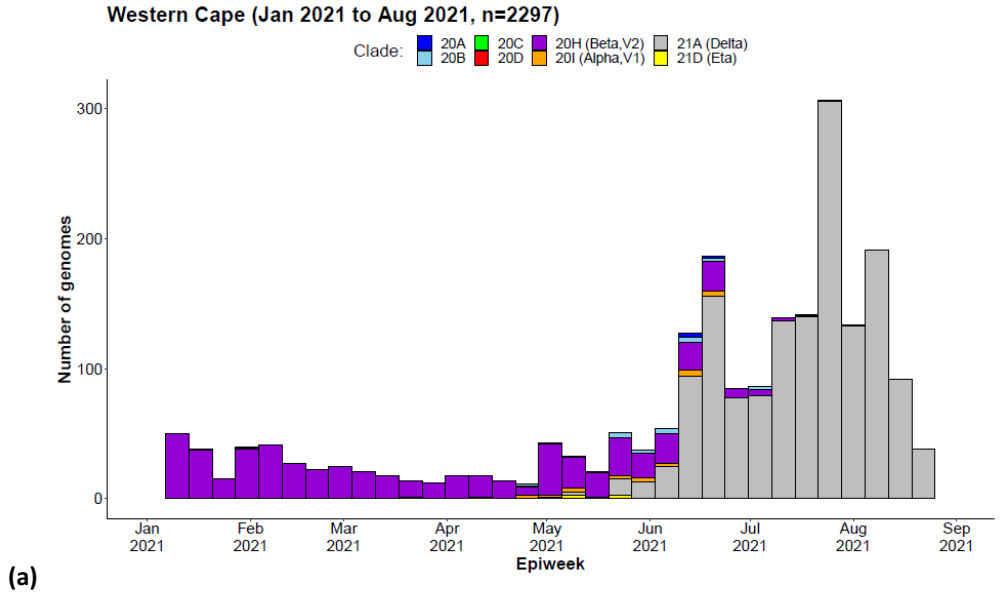
Appendix Table 1: All positive SARS-CoV-2 tests in the Western Cape public sector, April to July 2021

		Total positive tests (n=50 768)		Seegene AllplexTM PCR (n=11,355; 22.4%)		Other PCR tests (n=25 042; 49.3%)		Antigen tests (n=14 371; 28%)	
		n	%	n	%	n	%	n	%
Month of diagnosis	**April**	165	1.5	900	3.6	106	0.7		
	May	302	2.7	1260	5.0	637	4.4		
	June	2161	19.0	5223	20.9	2836	19.7		
	July	8727	76.9	17659	70.5	10792	75.1		
District of residence	**Cape Winelands**	508	4.5	2449	9.8	3125	21.8		
	Central Karoo	246	2.2	431	1.7	302	2.1		
	City of Cape Town	4975	43.8	12039	48.1	8954	62.3		
	Garden Route	4642	40.9	4063	16.2	961	6.7		
	Overberg	426	3.8	2448	9.8	522	3.6		
	West Coast	557	4.9	3503	14.0	486	3.4		
	Unallocated	1	0.0	109	0.4	21	0.2		
Sex	**Male**	4944	43.5	10602	42.3	6427	44.7		
Age category	**20-29yrs**	2243	19.8	4805	19.2	2711	18.9		
	30-39yrs	2623	23.1	5497	22.0	3410	23.7		
	40-49yrs	2427	21.4	5200	20.8	3025	21.1		
	50-59yrs	2113	18.6	5104	20.4	3187	22.2		
	60-69yrs	1177	10.4	2660	10.6	1241	8.6		
	≥70yrs	772	6.8	1776	7.1	797	5.6		
Comorbidities	**HIV positive**	726	6.4	1586	6.3	405	2.8		
	Diabetes	1546	13.6	3433	13.7	1488	10.4		
	Current tuberculosis	94	0.8	284	1.1	60	0.4		
	Hypertension	2475	21.8	5357	21.4	2272	15.8		
	Chronic Kidney Disease	374	3.3	702	2.8	270	1.9		
	Chronic Obstructive Pulmonary Disease	876	7.7	2115	8.5	878	6.1		
Prior diagnosed infection	**None**	11107	97.8	24339	97.2	14245	99.1		
	1st infection in 1st wave	64	0.6	202	0.8	43	0.3		
	1st infection in 2nd wave	184	1.6	501	2.0	83	0.6		
Vaccination status at time of diagnosis*	**Unvaccinated**	10422	91.8	22930	91.6	13223	92.0		
	Partially vaccinated	694	6.1	1457	5.8	904	6.3		
	Completely vaccinated	239	2.1	655	2.6	244	1.7		
Admitted§	**Case admitted to hospital**	1856	16.4	4964	19.8	1649	11.5		

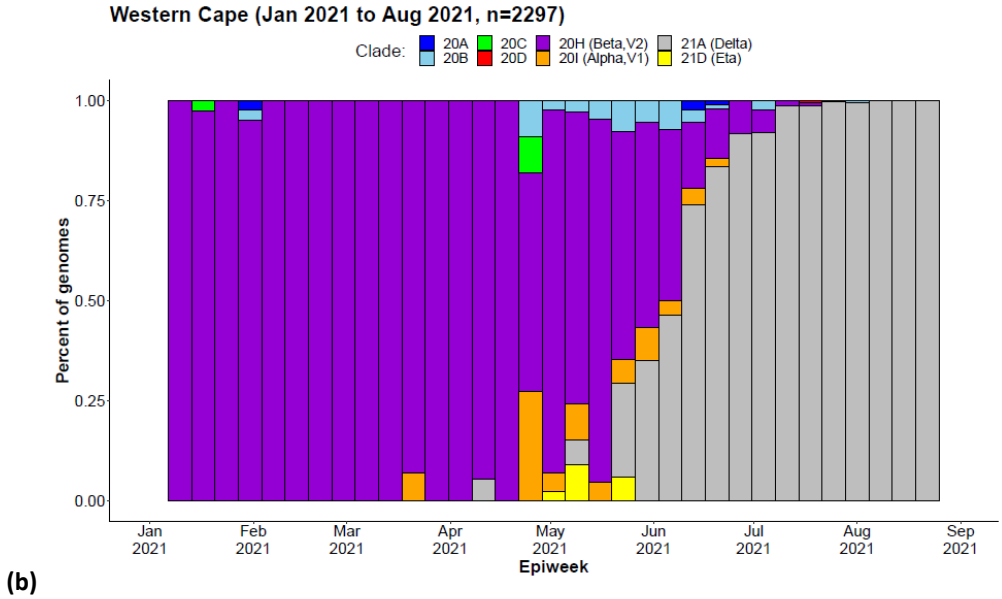
* Fully vaccinated was defined as ≥28 days post-vaccination with Janssen/Johnson & Johnson or ≥14 days post second dose of Pfizer–BioNTech. Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination.

§ Admission with 21 days before or after COVID-19 diagnosis date

Appendix Figure 1



(a)



(b)

Appendix Figure 1: Weekly frequency and distribution of SARS-CoV-2 variants circulating in the Western Cape Province, South Africa, 1 January to 31 August 2021: a) absolute count of genomes sequenced, b) proportion of genomes sequenced. (2).

2b) Omicron analysis: "Assessing the clinical severity of the Omicron variant in the Western Cape Province, South Africa, using the diagnostic PCR proxy marker of RdRp target delay to distinguish between Omicron and Delta infections – a survival analysis"

Abstract

Background: The extent to which the reduced risk of severe disease seen with SARS-CoV-2 Omicron is due to a decrease in variant virulence, or higher levels of population immunity, is currently not clear.

Methods: RdRp target delay (RTD) in the Seegene Allplex™ 2019-nCoV PCR assay is a proxy marker for the Delta variant. The absence of this proxy marker in the transition period was used to identify suspected Omicron infections.

Cox regression was performed for the outcome of hospital admission in those who tested positive for SARS-CoV-2 on the Seegene Allplex™ assay from 1 November to 14 December 2021 in the Western Cape Province, South Africa, public sector. Vaccination status and prior diagnosed infection, were adjusted for.

Results: 150 cases with RTD and 1486 cases without RTD were included. Cases without RTD had a lower hazard of admission (adjusted Hazard Ratio [aHR] of 0.56, 95%CI 0.34-0.91). Complete vaccination was protective of admission with an aHR of 0.45 (95%CI 0.26-0.77).

Conclusion: Omicron has resulted in a lower risk of hospital admission, compared to contemporaneous Delta infection, when using the proxy marker of RTD. Under-ascertainment of reinfections with an immune escape variant remains a challenge to accurately assessing variant virulence.

Background

With the rapid global spread of the Omicron (B.1.1.529) SARS-CoV-2 variant of concern (VOC), understanding the clinical implications of this new VOC is critical (Viana et al., 2022). Emerging data from both South Africa and the United Kingdom suggest that it is associated with a reduced risk of severe disease (Abdullah et al., 2021; Ferguson et al., n.d.; Jassat et al., 2021; Maslo et al., 2021; Sheikh et al., 2021; Wolter et al., 2021). The extent to which this reflects a difference in the inherent virulence of Omicron, or just higher levels of population immunity, due to previous infection and/or vaccination, is currently not clear.

In November 2021 in the Western Cape Province, South Africa, following a period of very low incidence, Omicron rapidly replaced Delta (B.1.617.2) as the dominant variant and drove the fourth wave of infections. Omicron is known to result in S-gene target failure (SGTF) on the Thermo Fisher Taqpath™ PCR assay (Scott et al., 2021). Unfortunately, too little routine diagnostic testing is done using this assay in the Western Cape National Health Laboratory Service (NHLS) to power an SGTF analysis.

RdRp target delay (RTD) is a proxy marker for the Delta variant on routine diagnostic testing with the Seegene Allplex™ 2019-nCoV PCR assay, similar in concept to S-gene target failure. RTD has a 93.6% sensitivity and 89.7% specificity in detecting the Delta variant when compared to genomic sequencing

(Valley-Omar et al., 2022). This proxy marker has previously successfully been used to assess the association of the Delta variant and mortality in the third wave (Hussey et al., 2021).

As the Seegene Allplex™ assay is widely used by the Western Cape NHLS, and as Omicron rapidly displaced the Delta variant with minimal other variants detected, the absence of RTD in Seegene Allplex™ cases can be used to identify likely Omicron infections during the replacement period. The absence of RTD tracks well with SGTF in the province (Figure 1), as well as genomic sequenced data (Network for Genomic Surveillance in South Africa (NGS-SA), 2021; Viana et al., 2022). We used this proxy marker to assess the clinical severity associated with Omicron infection.

Methods

RTD is defined as a difference in cycle threshold value of RdRp gene target - E gene target > 3.5 in the Seegene Allplex™ 2019-nCoV PCR assay (Valley-Omar et al., 2022). Cases diagnosed using this assay have previously been found to be representative of cases diagnosed by any PCR assay in the Western Cape Province public sector (Hussey et al., 2021).

We included all COVID-19 cases, aged 15 years and older, diagnosed on the Seegene Allplex TM 2019-nCoV diagnostic PCR assay in the Western Cape Province public sector from 1 November to 14 December 2021, a period when both Delta and Omicron were co-circulating and other lineages were negligible (Network for Genomic Surveillance in South Africa (NGS-SA), 2021; Viana et al., 2022). Follow-up ended 6 January 2022. Children were excluded from this analysis as their testing and admission patterns are different compared to adults.

The Western Cape Provincial Health Data Centre (PHDC) collates all available electronic health data on public sector patients, including COVID-19 test results, comorbidities and hospital admissions. If an

individual tested positive for SARS-CoV-2 14 days before admission, or 7 days after the date of admission, and was not admitted to a specialized psychiatric and rehabilitation facilities, it was defined as COVID-19 related hospital admission.

We undertook a survival analysis using Cox regression, assessing time from date of diagnosis to date of hospital admission, which was our outcome of interest. Those whose date of admission was on or before the date of diagnosis were assigned one day of follow-up. Intensive Care Unit (ICU) admission or mortality were not assessed as too few of these severe outcomes had occurred. We adjusted for age, sex, known comorbidities, prior diagnosed infection (two positive COVID-19 tests more than 90 days apart) and vaccination status at time of diagnosis. Vaccination status was determined by linking COVID-19 cases with the national Electronic Vaccination Data System through national identifiers in the PHDC. Complete vaccination was defined ≥28 days post-vaccination with Janssen/Johnson & Johnson (Ad26.COV2.S), or ≥14 days post second dose of Pfizer–BioNTech (BNT162b2). Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination. Analyses were conducted using Stata 13.1.

Results

1 636 cases were tested on the Seegene Allplex TM assay during the study period and were included in this analysis, representing 14% of all cases diagnosed in the public sector at that time. We included 150 cases were with RTD (proxy for Delta) and 1486 cases were without RTD (proxy for Omicron/non-Delta). Patient demographic characteristics and comorbidities were similar in both groups (Table 1). The median age in both groups was 33 years (IQR 25-44 in those with RTD; IQR 26-44 in those without). The proportion

of cases that were fully vaccinated in both groups was also similar, although more infections following partial vaccination were seen in those without RTD.

The proportion of cases with a documented reinfection was 11% for both those with and without RTD. By using a stricter definition of RTD (i.e., requiring a larger difference in Ct values), a similar proportion of RTD cases that had documented reinfections were found (Supplementary Table 1).

21 cases (14%) were admitted to hospital in those with RTD, while 101 (6.8%) were admitted in those without RTD. Amongst those not admitted, 189 (12.5%) had a documented previous infection, while amongst those admitted, none had a documented previous infection and so we could not calculate the extent of protection from prior diagnosed infection against admission, although there appears to be benefit. The cases without RTD (i.e. suspected Omicron cases) had a lower hazard of admission (adjusted Hazard Ratio [aHR] of 0.56, 95% confidence interval [CI] 0.34-0.91) (Table 1), after adjusting for age, sex, prior diagnosed infection, vaccination status at time of diagnosis and known comorbidities, when compared to suspected Delta cases. Complete vaccination was protective of admission with an aHR of 0.45 (95%CI 0.26-0.77).

The proportional hazards assumption was tested and found to have held for each variable and the model as a whole (see Supplementary Table 2).

Discussion

Using the proxy marker of RTD absence, suspected Omicron cases were associated with a lower risk of hospital admission when compared to contemporaneous suspected Delta cases.

A study from the South African National Institute of Communicable Diseases found that using SGTF suspected Omicron cases had a lower odds of being admitted to hospital compared to non-SGTF infections (adjusted odds ratio [aOR] 0.2, 95%CI 0.1-0.3) (Wolter et al., 2021). However, this analysis was not able to adjust for vaccination status at time of diagnosis, potentially explaining the greater reduction in observed disease severity compared to our study. Similar contemporaneous SGTF studies from the UK have also found that SGTF was associated with a lower risk of hospitalisation, with an adjusted observed/expected ratio of 0.32 (95% CI 0.19, 0.52) (Sheikh et al., 2021) and a 40-45% reduction in risk of admission, which is similar to our result (Ferguson et al., n.d.).

Several studies have also found less severe disease in the fourth Omicron-driven wave compared to the third wave caused by Delta in South Africa (Abdullah et al., 2021; Jassat et al., 2021; Maslo et al., 2021), but as they are comparing non-contemporaneous cases it is difficult to account for the effect of the vaccination programme, which started too late to provide significant protection for the third wave (Mathieu et al., 2021), as well as the fact that more individuals were previously infected, and thus had some immunity at the start of the fourth wave compared to the third.

Anecdotally, this fourth wave has resulted in a relatively large proportion of admissions where COVID-19 was incidentally diagnosed (Abdullah et al., 2021). It is unfortunately not possible to tease out the primary indication for admission from our routine data and we were unable to assess severe outcomes due to the very small numbers of cases tested on the Seegene Allplex^{TM} assay who were recorded as having steroids prescribed electronically, being admitted to ICU or dying. The fact that full vaccination still provided substantial protection against admission, even with the contamination of incidental admissions, suggests that vaccination provides very strong protection against admission in the face of the Omicron variant, and that some of the admissions were likely due to COVID-19 disease. Vaccination itself might also be a proxy

marker for higher socio-economic status, access to health care or good health seeking behaviour, such as adherence to chronic medications, and as such might confer some protection against hospital admissions, irrespective of COVID-19.

Omicron is associated with an increased risk of reinfection (Pulliam et al., 2021). Previous infection in itself is protective against severe disease (Milne et al., 2021). In this analysis, no cases with a documented reinfection required hospital admission. But seroprevalence studies indicate only a small fraction of total COVID-19 cases in the Western Cape Province are laboratory confirmed (Hsiao et al., 2020), particularly when there have been testing restrictions during epidemic surges. Under-ascertainment of prior diagnosed infection is therefore likely, and this could considerably bias measures of disease severity for an immune escape variant compared to a variant that is not associated with reinfection. The extent of this residual confounding i.e., the contribution of this under-ascertainment of reinfections to the milder disease severity seen with Omicron, is thus still uncertain in our context of very high rates of unconfirmed prior infection. At the same time, if we are diagnosing a smaller proportion of Omicron cases by missing the more mild or asymptomatic infections, or if the Omicron variant results in more incidental hospital admissions and outcome misclassification, this could bias our findings in the opposite direction, and falsely elevate the disease severity seen with this variant.

And additional limitations to this study are the low numbers of SARS-CoV-2 infections during the Delta to Omicron transition period, particularly in the public sector, where this analysis was restricted to, as well as the increasing use of SARS-CoV-2 rapid antigen tests from which the variant proxy markers cannot be calculated. We also used a proxy marker for Omicron and not whole genome sequencing, as only limited sequencing is feasible in our setting. A proxy marker on routine diagnostic testing is likely to result in some

variant misclassification, which is evidenced by the high rate of reinfections seen in RTD cases. Attempts to narrow the definition of RTD were not able to resolve this. In addition, this analysis like many others, only compares disease severity between Omicron and Delta. Little is known of the severity of Omicron when compared to the ancestral strain and other non-Delta VOCs.

Conclusion

Omicron has resulted in a lower risk of hospital admission, when compared to contemporaneous Delta cases using the proxy marker of RTD in the Western Cape Province. Vaccination and documented previous infection are highly protective of hospital admission. Under-ascertainment of reinfections with an immune escape variant like Omicron remains a challenge to accurately assessing variant virulence.

Acknowledgements

We would like to acknowledge all patients in the Western Cape and to thank the Western Cape Department of Health Provincial Health Data Centre, the Western Cape Department of Health COVID-19 Outbreak Response Team, the Western Cape Communicable Disease Control sub-directorate and Western Cape health care workers involved in the COVID-19 response for their contributions to this report. In addition, we would like to thank the South African National Department of Health for assisting us with vaccination data from the Electronic Vaccination Data System (EVDS).

Ethics

The study was approved by the University of Cape Town Research Ethics Committee (HREC 460/2020).

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Tables and Figures

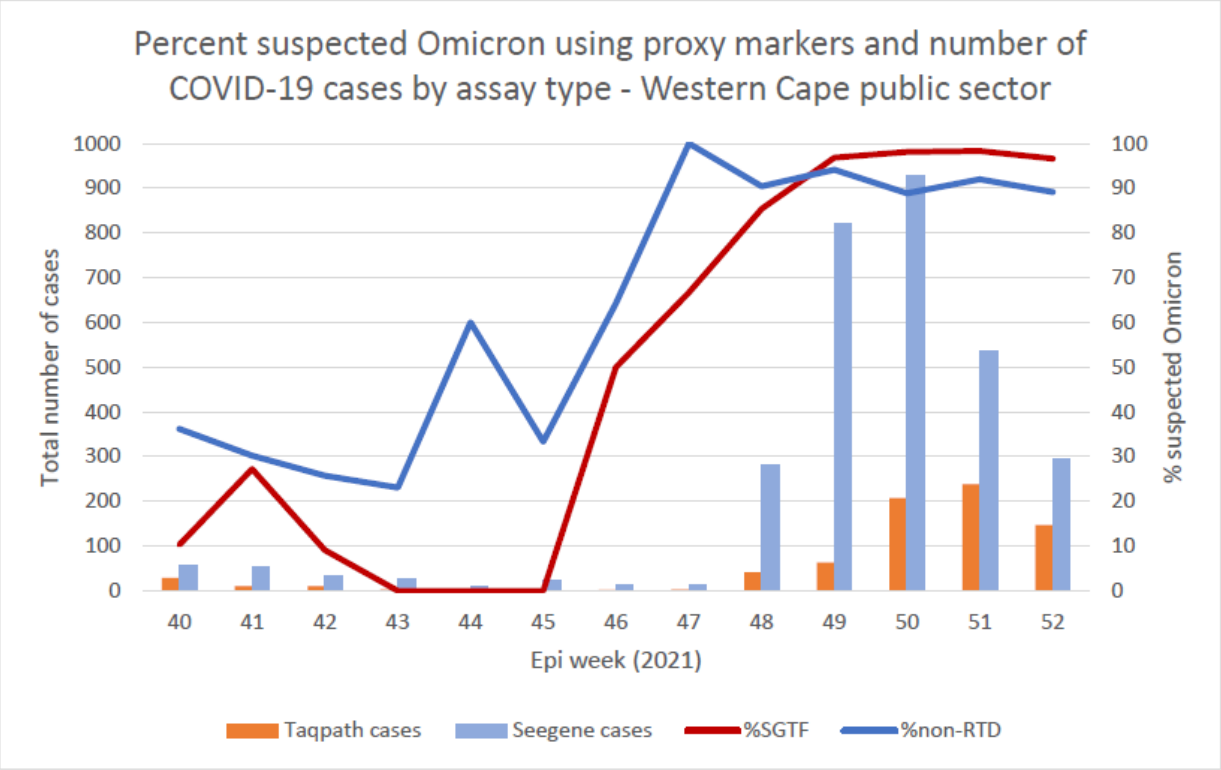


Figure 1: Percent suspected Omicron using the proxy markers of S-gene target failure in Thermo Fisher Taqpath™ cases and non-RdRp target delay in Seegene Allplex™ assay cases, in the Western Cape Province public sector adults.

Table 1: Characteristics of included cases, according to presence or absence of RdRp target delay, as well as adjusted hazard ratios (aHR) for the outcome of admission in adults in the Western Cape Province public sector, 1 November to 14 December 2021.

		RTD present	RTD absent	Cox regression for outcome of admission	
	n	150	1486	1636 (122 admissions)	
		n (%)	n (%)	adjusted HR	95% CI
RTD	**Present ("Delta")**			**Ref**	
	Absent ("Omicron")			**0.56**	0.34; 0.91
Sex	**Female**	80 (53.3%)	860 (57.9%)	**Ref**	
	Male	70 (46.7%)	626 (42.1%)	**1.03**	0.71; 1.48
Age category	**15-29 years**	57 (38%)	559 (37.6%)	**Ref**	
	30-39 years	43 (28.7%)	447 (30.1%)	**0.92**	0.56; 1.50
	40-49 years	21 (14%)	216 (14.5%)	**1.12**	0.62; 2.02
	50-59 years	15 (10%)	166 (11.2%)	**0.91**	0.46; 1.80
	60-69 years	6 (4%)	60 (4%)	**1.60**	0.73; 3.49
	≥70 years	8 (5.3%)	38 (2.6%)	**1.27**	0.56; 2.89
Comorbidity§	**HIV positive**	21 (14%)	142 (9.6%)	**1.29**	0.72; 2.30
	Diabetes	7 (4.7%)	77 (5.2%)	**1.30**	0.68; 2.46
	Tuberculosis (current)	4 (2.7%)	17 (1.1%)	**5.15**	2.44; 8.89
	Hypertension	19 (12.7%)	149 (10%)	**1.78**	0.99; 3.19
	Chronic Kideny Disease	4 (2.7%)	23 (1.6%)	**4.46**	2.24; 8.89
	COPD / Asthma	6 (4%)	79 (5.3%)	**2.26**	1.29; 3.96
Prior documented infection	**None**	134 (89.3%)	1313 (88.4%)	**Ref**	
	Yes	16 (10.7%)	173 (11.6%)	**0.00**	
Vaccination †	**None**	114 (76%)	1081 (72.7%)	**Ref**	
	Partial	3 (2%)	93 (6.3%)	**1.22**	0.63; 2.36
	Complete	33 (22%)	312 (21%)	**0.45**	0.26; 0.77

§ The reference group for the aHR here is the absence of that specific comorbidity.

† Fully vaccinated was defined as ≥28 days post-vaccination with Janssen/Johnson & Johnson or ≥14 days post second dose of Pfizer–BioNTech. Patients were deemed partially vaccinated from the day after their (first) vaccine dose until meeting criteria for complete vaccination.

Supplementary 1

Supplementary Table 1: Adjusting the difference in Ct values (RdRp – E), and the resultant adjusted Hazard Ratios for suspected Omicron cases being admitted, using the same survival analysis model as the main analysis

Seegene Allplex cases for the time period 1 November - 14 December 2021

Difference in Ct values (RdRp - E)	>3.5	>4	>4.5	>5
Number of cases with RTD	150	89	59	41
% documented reinfection in RTD cases	10.67	10.11	11.86	7.32
aHR (95%CI) for non-RTD cases for admission	0.56 (0.34-0.91)	0.49 (0.28-0.87)	0.52 (0.26-1.03)	0.56 (0.26-1.20)

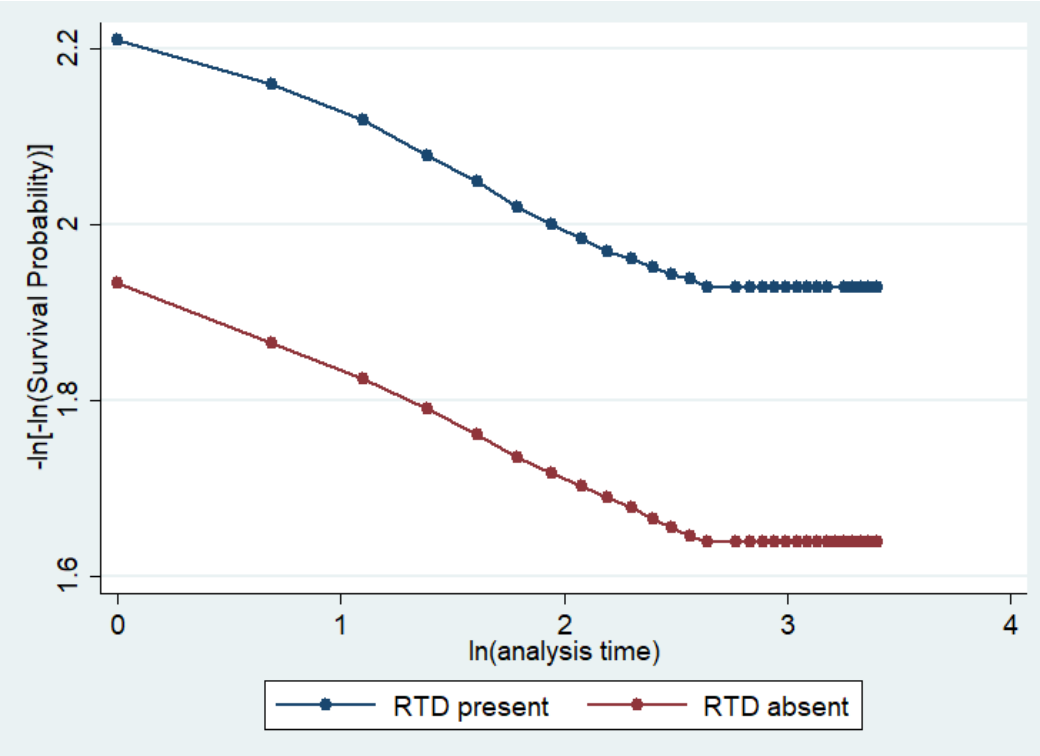
Using the original definition of RTD (>3.5), 16 RTD cases with reinfections (10.67%) were included in the main analysis. The proportion of RTD cases that were reinfections was 2.6, 2.1 and 0.8% in the months of August, September and October 2021 respectively. By narrowing the definition of RTD to require a larger difference in Ct values, less "Delta" cases were classified as such, but the aHR for suspected Omicron cases and admission remained similar, although the confidence intervals did widen.

The stricter definition of RTD, however, was not able decrease the proportion of cases with reinfection. In November, when Delta was still dominant, there were very low case numbers. After Omicron arrived, there was a massive increase in case numbers. A small proportion of misclassification of these higher numbers of Omicron cases is therefore able to markedly change the proportion of reinfection seen in the RTD cases.

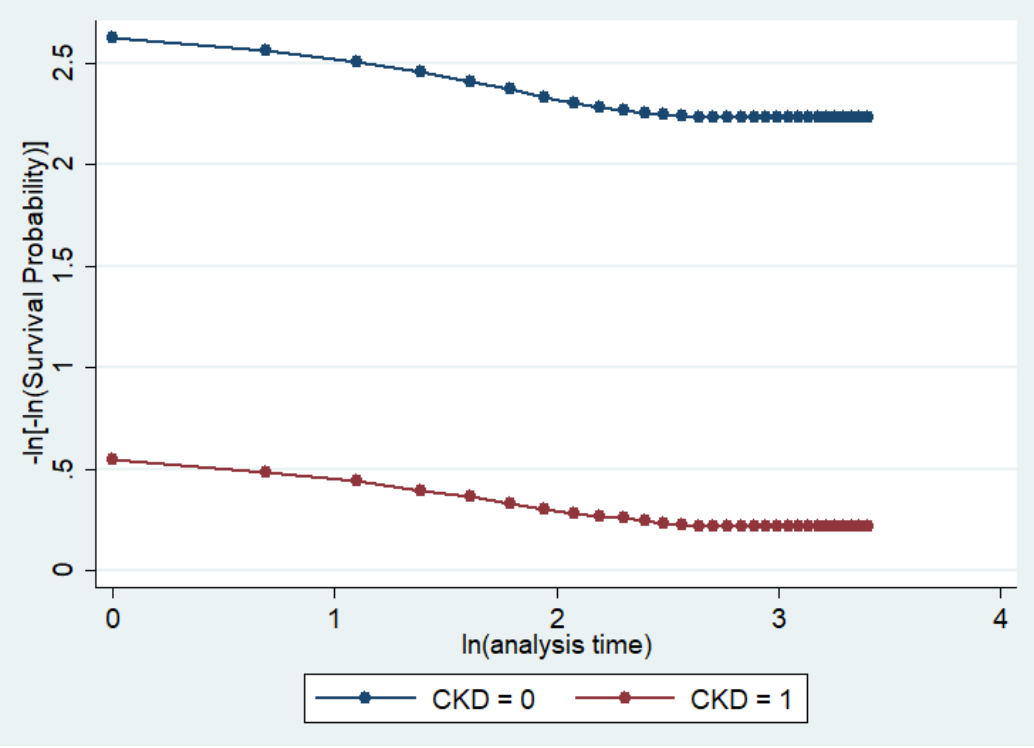
Supplementary Table 2: Assessing the proportional hazards assumption on the basis of Schoenfeld residuals, for each variable and the model as whole. Although the p values for CKD and complete vaccination were low, the log-log plots (Supplementary Figure 1) showed no gross violation.

		rho	chi2	df	Prob>chi2
Suspected Omicron		0.1213	1.97	1	0.1607
Male sex		-0.01886	0.05	1	0.8285
Age category	30-39 years	-0.06309	0.49	1	0.4818
	40-49 years	0.11636	1.72	1	0.1895
	50-59 years	-0.04749	0.27	1	0.6053
	60-69 years	-0.01227	0.02	1	0.8864
	≥70 years	0.06897	0.65	1	0.4216
Comorbidities	HIV positive	-0.0443	0.26	1	0.608
	Diabetes	-0.03791	0.23	1	0.6311
	TB (current)	0.0296	0.13	1	0.722
	HPT	-0.05891	0.49	1	0.4824
	CKD	0.1688	4.6	1	0.0319
	COPD	-0.07967	0.87	1	0.3522
Reinfection		0.08018	0	1	1
Vaccination	Partial	-0.03976	0.19	1	0.6656
	Complete	0.18966	4.41	1	0.0358
Global test			19.51	16	0.2433

Supplementary Figure 1:

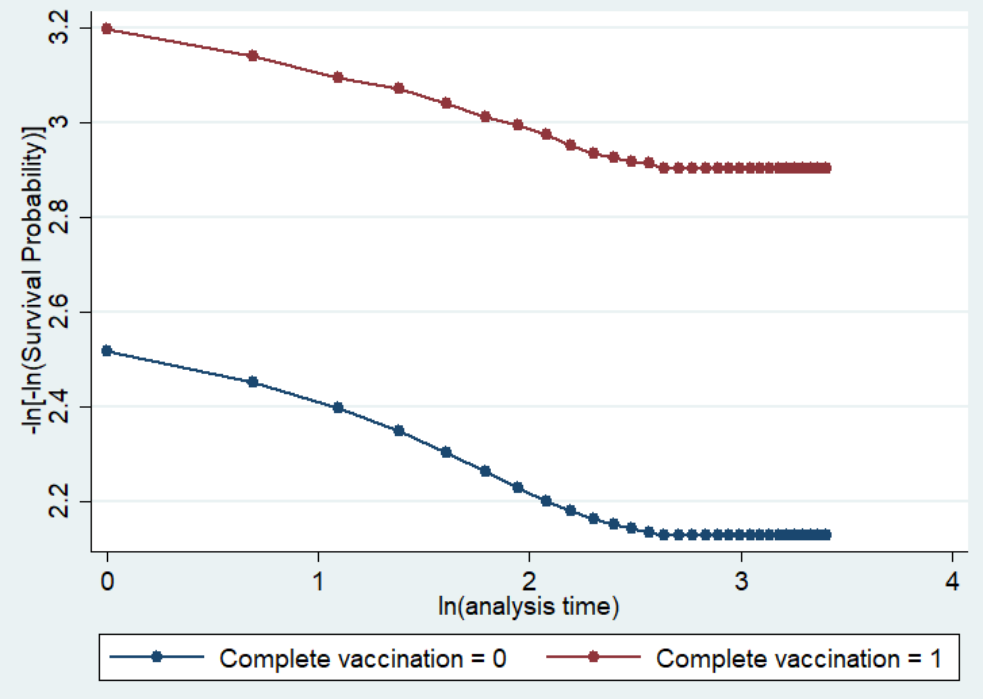


(a)



(b)

(c)



Supplementary Figure 1: Log-log plots for the covariates of (a) RTD, (b) CKD and (c) complete vaccination.

Appendices

Appendix 1 – UCT HREC approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

20 February 2023

HREC REF: 075/2023

Prof M Davies
School of Public Health & Family Medicine
Falmouth FHS
Email: mary-ann.davies@uct.ac.za
Student: Hannah.hussey@westerncape.gov.za

Dear Prof Davies

PROJECT TITLE: USING PROXY MARKERS FROM ROUTINE DIAGNOSTIC PCR TESTING TO ASSESS THE DISEASE SEVERITY OF NEW SARS-COV-2 VARIANTS-SUB-STUDY LINKED TO 460/2020-
(MMED CANDIDATE-DR HANNAH HUSSEY)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2024.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Hannah Hussey will also be involved in this study.

Please quote the HREC REF 075/2023 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Appendix 2 – Delta analysis – still under peer review

Currently still awaiting the second reviewer's feedback at Gates Open Research, but available online there as a preprint (https://gatesopenresearch.org/articles/6-117).

Gates Open Research

Gates Open Research 2022, 6:117 Last updated: 01 SEP 2022

Check for updates

RESEARCH ARTICLE

Higher mortality associated with the SARS-CoV-2 Delta variant in the Western Cape, South Africa, using RdRp target delay as a proxy: a cross-sectional study. [version 1; peer review: awaiting peer review]

Hannah Hussey[1,2], Mary-Ann Davies[1,3], Alexa Heekes[1,2], Carolyn Williamson[4-6], Ziyaad Valley-Omar[4,5], Diana Hardie[4,5], Stephen Korsman[4,5], Deelan Doolabh[4,5], Wofgang Preiser[5,7], Tongai Maponga[5,7], Arash Iranzadeh[4,5], Susan Engelbrecht[5], Sean Wasserman[6,8], Neshaad Schrueder[9], Linda Boloko[6,10], Greg Symons[10], Peter Raubenheimer[10], Abraham Viljoen[9], Arifa Parker[9], Cheryl Cohen[11,12], Waasila Jasat[11], Richard Lessells[13], Robert J Wilkinson[6,14,15], Andrew Boulle[1,3], Marvin Hsiao[4-6]

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[15]Department of Infectious Diseases, Imperial College London, London, UK

v1 **First published:** 31 Aug 2022, 6:117
https://doi.org/10.12688/gatesopenres.13654.1
Latest published: 31 Aug 2022, 6:117
https://doi.org/10.12688/gatesopenres.13654.1

Abstract

Background: The SARS-CoV-2 Delta variant (B.1.617.2) has been associated with more severe disease, particularly when compared to the Alpha variant. Most of this data, however, is from high income countries and less is understood about the variant's disease severity in other settings, particularly in an African context, and when compared

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

Appendix 3 – Published Omicron analysis

Published in the International Journal of Infectious Diseases in February 2022 (https://www.ijidonline.com/article/S1201-9712(22)00129-1/fulltext).

International Journal of Infectious Diseases 118 (2022) 150–154

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Assessing the clinical severity of the Omicron variant in the Western Cape Province, South Africa, using the diagnostic PCR proxy marker of RdRp target delay to distinguish between Omicron and Delta infections – a survival analysis

Hannah Hussey[1,2,*], Mary-Ann Davies[1,3], Alexa Heekes[1,2], Carolyn Williamson[4,5,6], Ziyaad Valley-Omar[4,5], Diana Hardie[4,5], Stephen Korsman[4,5], Deelan Doolabh[4,5], Wolfgang Preiser[5,7], Tongai Maponga[5,7], Arash Iranzadeh[4,5], Sean Wasserman[6,8], Linda Boloko[6,9], Greg Symons[9], Peter Raubenheimer[9], Arifa Parker[10], Neshaad Schrueder[10], Wesley Solomon[11], Petro Rousseau[11], Nicole Wolter[12,13], Waasila Jassat[12], Cheryl Cohen[12,14], Richard Lessells[15], Robert J Wilkinson[6,16,17], Andrew Boulle[1,3], Nei-yuan Hsiao[4,5,6]

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ARTICLE INFO

Article history:
Received 18 January 2022
Revised 8 February 2022
Accepted 23 February 2022

Keywords:
SARS-CoV-2
Omicron variant
clinical severity
South Africa
RdRp target delay

ABSTRACT

Background: At present, it is unclear whether the extent of reduced risk of severe disease seen with SARS-Cov-2 Omicron variant infection is caused by a decrease in variant virulence or by higher levels of population immunity.

Methods: RdRp target delay (RTD) in the Seegene Allplex™ 2019-nCoV PCR assay is a proxy marker for the Delta variant. The absence of this proxy marker in the transition period was used to identify suspected Omicron infections.

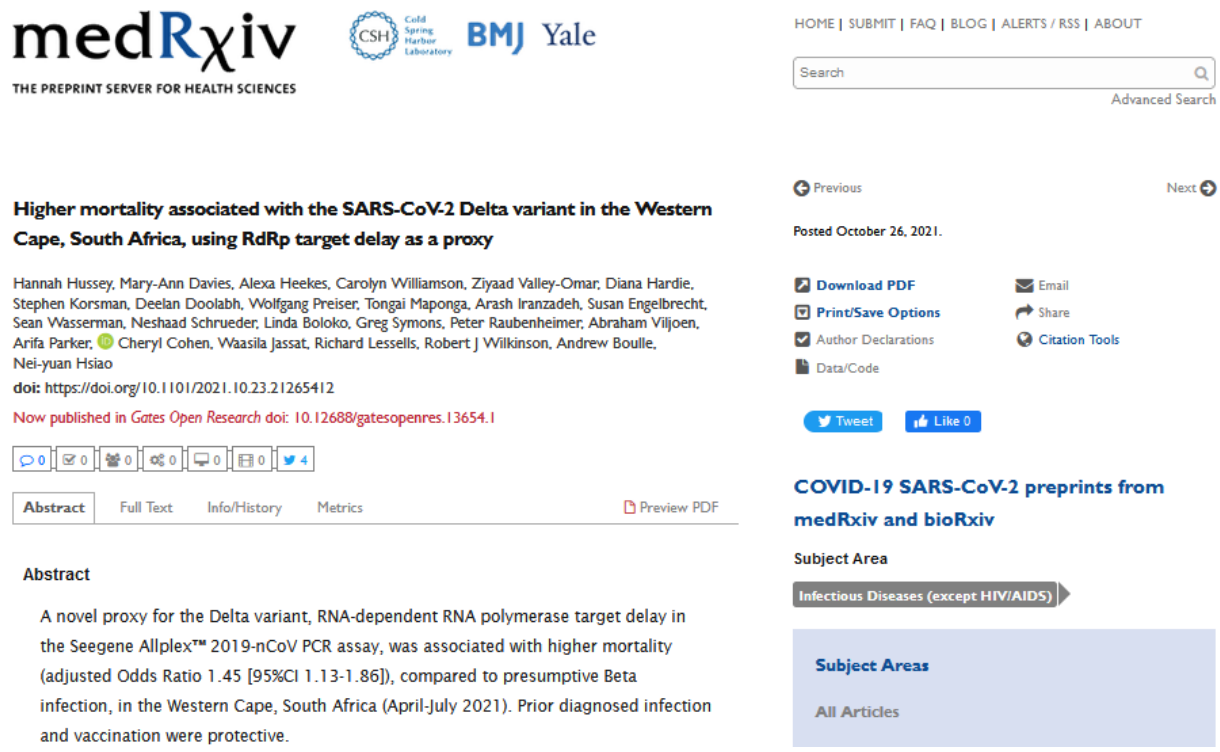
Cox regression was performed for the outcome of hospital admission in those who tested positive for SARS-CoV-2 on the Seegene Allplex™ assay from November 1 to December 14, 2021 in the Western Cape Province, South Africa, in the public sector. Adjustments were made for vaccination status and prior diagnosis of infection.

Results: A total of 150 cases with RTD and 1486 cases without RTD were included. Cases without RTD had a lower hazard of admission (adjusted hazard ratio [aHR], 0.56; 95% confidence interval [CI], 0.34-0.91). Complete vaccination was protective against admission, with an aHR of 0.45 (95% CI, 0.26-0.77).

Conclusion: Omicron has resulted in a lower risk of hospital admission compared with contemporaneous Delta infection, when using the proxy marker of RTD. Under-ascertainment of reinfections with an immune escape variant remains a challenge to accurately assessing variant virulence.

Appendix 4 – Preprints on MedRxiv

Delta analysis preprint available at https://www.medrxiv.org/content/10.1101/2021.10.23.21265412v1.



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Higher mortality associated with the SARS-CoV-2 Delta variant in the Western Cape, South Africa, using RdRp target delay as a proxy

Hannah Hussey, Mary-Ann Davies, Alexa Heekes, Carolyn Williamson, Ziyaad Valley-Omar, Diana Hardie, Stephen Korsman, Deelan Doolabh, Wolfgang Preiser, Tongai Maponga, Arash Iranzadeh, Susan Engelbrecht, Sean Wasserman, Neshaad Schrueder, Linda Boloko, Greg Symons, Peter Raubenheimer, Abraham Viljoen, Arifa Parker, Cheryl Cohen, Waasila Jassat, Richard Lessells, Robert J Wilkinson, Andrew Boulle, Nei-yuan Hsiao

doi: https://doi.org/10.1101/2021.10.23.21265412

Now published in *Gates Open Research* doi: 10.12688/gatesopenres.13654.1

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Abstract

A novel proxy for the Delta variant, RNA-dependent RNA polymerase target delay in the Seegene Allplex™ 2019-nCoV PCR assay, was associated with higher mortality (adjusted Odds Ratio 1.45 [95%CI 1.13-1.86]), compared to presumptive Beta infection, in the Western Cape, South Africa (April-July 2021). Prior diagnosed infection and vaccination were protective.

COVID-19 SARS-CoV-2 preprints from medRxiv and bioRxiv

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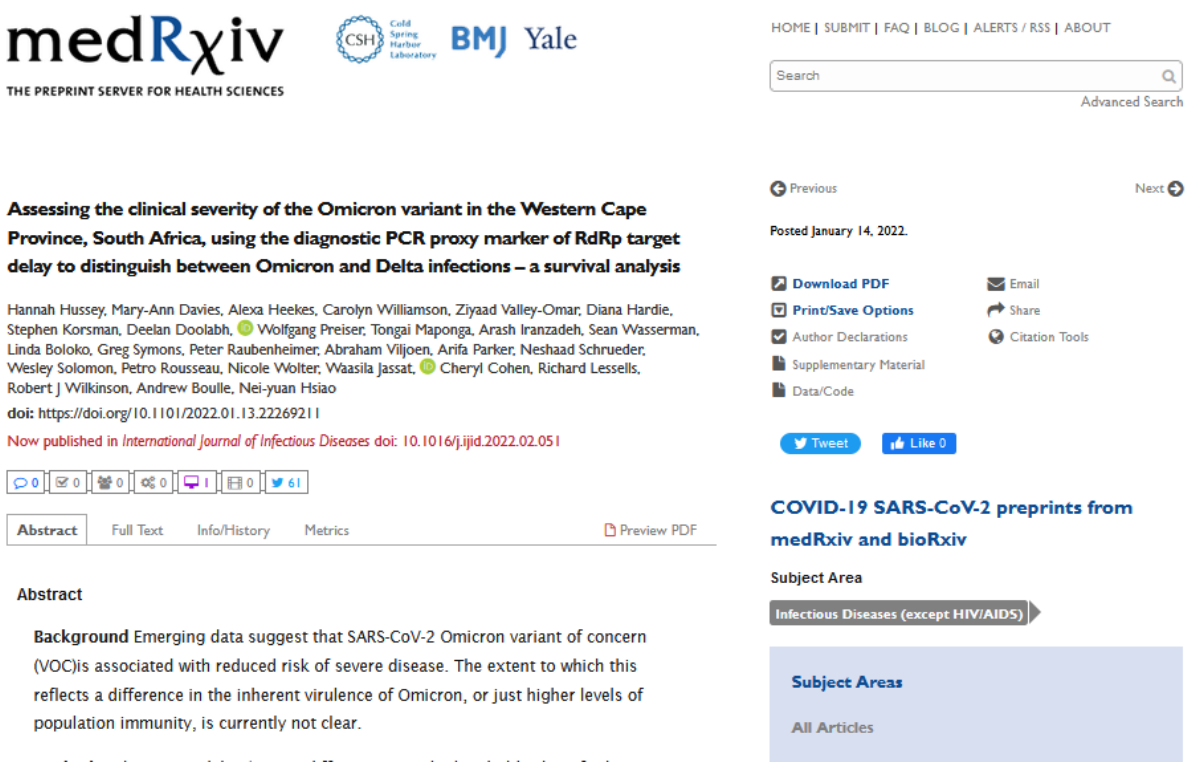
Subject Areas

All Articles

Omicron analysis preprint available at
https://www.medrxiv.org/content/10.1101/2022.01.13.22269211v1.



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doi: https://doi.org/10.1101/2022.01.13.22269211

Now published in *International Journal of Infectious Diseases* doi: 10.1016/j.ijid.2022.02.051

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COVID-19 SARS-CoV-2 preprints from medRxiv and bioRxiv

Subject Area

Infectious Diseases (except HIV/AIDS)

Abstract

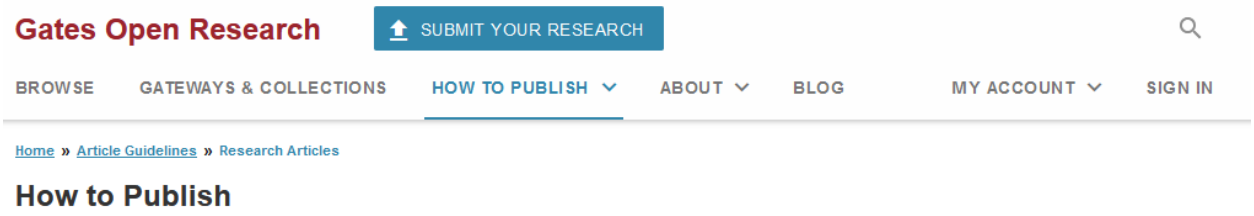
Background Emerging data suggest that SARS-CoV-2 Omicron variant of concern (VOC)is associated with reduced risk of severe disease. The extent to which this reflects a difference in the inherent virulence of Omicron, or just higher levels of population immunity, is currently not clear.

Subject Areas

All Articles

Appendix 5 – Author guidelines: Gates Open Research

Available from https://gatesopenresearch.org/for-authors/article-guidelines/research-articles



Guidelines for Article Preparation for Submission

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Details of each author's contribution must be listed in the Author contributions section.

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The format of the main body of the article is flexible: it should be concise, making it easy to read and review, and presented in a format that is appropriate for the type of study presented. A Research Article should be no more than 20,000 words.

For most Research Articles, the following standard format will be the most appropriate:

Introduction

Methods

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Articles in some areas of research, e.g. education, social sciences or economics, may benefit from a different structure, in which case a more flexible format is possible as long as the authors ensure that they describe their methods and sources in sufficient detail for others to be able to repeat the research.

Standards of reporting: Standards of reporting guidelines help authors to ensure that they have provided a comprehensive description of their research, making it easier for others to assess and reproduce the work; for more detail and a comprehensive overview, see the FAIRSharing initiative. Available reporting guidelines for biological research can be found using the MIBBI Foundry filter on the FAIRSharing website; the EQUATOR network provides a comprehensive list of reporting guidelines for health research.

Articles in Gates Open Research that report clinical trials must adhere to the CONSORT reporting guidelines. For reporting of the intervention methodology itself, Gates Open Research endorses the TIDieR checklist, an extension of the CONSORT statement. We ask authors to include a copy of the original trial protocol and a completed CONSORT checklist and flow diagram as supporting files, which will be published alongside the article. The trial registration number and registration date must be included in the Methods section. Any deviation from the original trial protocol must be explained in the article.

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All articles reporting *in vivo* experiments must conform with the ARRIVE Essential 10 checklist, and we encourage authors to ensure they report in line with the full ARRIVE 2.0 checklist. Authors should include a completed ARRIVE checklist with their article and this will be included in the Reporting

Guidelines section of the article when published. As the online version of your article will not have page numbers please use section names rather than page numbers when completing the checklist.

The NC3Rs provide detailed explanations of each item of the guidelines with examples of good reporting from the published literature here.

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When antibodies are used, the species in which the antibody was raised, the manufacturing company or source laboratory, the catalogue or reference number, and whether it is a polyclonal or monoclonal antibody should be included. In addition, if the antibody has been previously validated, a reference to the validation study should be included. If the antibody has not been validated, full details of the dilution and use of the antibody should be given in the Methods section.

Where proprietary software is used for analysis, we require that details of an open-access alternative that can perform an equivalent function are provided. Where authors have written their own code in the course of their analysis, we require this to be written in (or compatible with) an open-source programming language.

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If the study involves the use of a questionnaire that has been validated by a previous study, this should be cited and a URL link provided to the validated questionnaire. If the authors have created a novel questionnaire (or performed a translation), the article must state if the questionnaire has been validated, and provide the following information:

Initial ace validity testing

Preliminary pilot testing

Reliability testing (internal consistency, test-retest, inter-rater)

Any changes implemented resulting from preliminary testing

The novel questionnaire should be provided as extended data.

Ethics policies: All research must have been conducted within an appropriate ethical framework. For studies involving humans or animals, details of approval by the authors' institution or an ethics committee must be provided in the Methods section. Please refer to the detailed 'Ethics' section in our editorial policies for more information.

Clinical trials: If the data associated with your article relate to a clinical trial then the Trial Registration details must be provided: name of registry, registry number, registration date and URL of the trial in the registry database. We support the public disclosure of all clinical trial results (as mandated in the US FDA Amendments Act, 2007), for example on a public website, such as clinicaltrials.gov. The disclosure of results on such sites does not preclude the publication of articles reporting and/or analyzing the same datasets in Gates Open Research. For further details about trial registration, see our editorial policies.

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Authors should ensure that the terms *sex* and *gender* are used correctly throughout the article.

Title/abstract: it should be clear if the results can only be applied to one sex or gender.

Introduction: if sex and gender differences are expected in the results, these should be stated.

Methods:

if sex and gender differences were taken into consideration for the design of the study these should be stated. If they were not taken into consideration, the rationale should be given.

explanation of how sex of participants was defined should be stated, either based on self-report, assigned following external or internal examination of body characteristics, or through genetic testing or other means.

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6. Data (and Software) Availability

Underlying data

All articles must include a Data Availability statement, even where there is no data associated with the article - see our data guidelines and policies for more information.

The Data Availability statement should provide full details of how, where, and under what conditions the data underlying the results can be accessed; for practical guidance please see Add a Data Availability statement to your manuscript. See also Prepare your Data and Select a Repository for further guidance on data presentation, formatting and deposition.

If you have deposited your datasets or used data that are already available in a repository, please include the name of the repository, the DOI or accession number, and license. This should be done in the style of, for example:

Repository: Confounding factors considered by studies of vaping as a possible gateway to smoking. https://doi.org/10.5256/repository.4591.d34639.

This project contains the following underlying data:

Data file 1. (Description of data.)

Data file 2. (Description of data.)

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Where data are held in a structured, subject-specific repository, the following example would be appropriate:

NCBI Gene: Ihe1 intestinal helminth expulsion 1 [Mus musculus (house mouse)]. Accession number 107537.

If you are describing new software, please make the source code available on a Version Control System (VCS) such as GitHub, BitBucket or SourceForge, and provide details of the repository and the license under which the software can be used in the article.

For other scenarios, such as where data cannot be shared, please see Add a Data Availability statement to your manuscript for details of what must be indicated in your Data Availability statement.

Extended data

There are no figure or table limits for articles in Gates Open Research. Additional materials that support the key claims in the paper but are not absolutely required to follow the study design and analysis of the results, e.g. questionnaires, supporting images or tables, can be included as extended data; descriptions of the materials and methods should be in the main article. Extended data should be in a format that supports reuse under a CC0 license. Care should be taken to ensure that the publication of extended data in this instance does not preclude primary publication elsewhere.

If you have any extended data, please deposit these materials in an approved repository and include the title, the name of the repository, the DOI or accession number, and license in the manuscript under the subheading 'Extended data'. Please also include citations to extended data in the main body of the article. For practical guidance please see Add a Data Availability statement to your manuscript. See also Prepare your Data and Select a Repository for further guidance on data presentation, formatting and deposition.

Please note, information which can be used to directly identify participants should not be included in underlying and extended datasets, unless they have provided explicit permission to share their details. Please see our data guidelines for further information.

7. Reporting Guidelines

Articles in Gates Open Research must comply with consensus-based minimum reporting guidelines for research. Comprehensive lists of available reporting guidelines can be found on the EQUATOR network website for health research.

Checklists are available for a number of reporting guidelines, including:

Randomized controlled trials (CONSORT) and protocols (SPIRIT)

Systematic reviews and meta-analyses (PRISMA) and protocols (PRISMA-P)

Observational studies (STROBE)

Case reports (CARE)

Qualitative research (COREQ; SRQR)

In vivo animal studies (ARRIVE)

Please deposit completed reporting checklists and flow charts in an approved general repository; include the guideline type, name of the repository, the DOI, and license in the manuscript's Data availability statement in the style of, for example:

Repository: CONSORT checklist and flow chart for 'Title of paper'.
https://doi.org/10.5256/repository.4591.d34639.

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

8. Consent

For articles involving patient/participant data or information (e.g. personal genomics articles, case reports, clinical trials, questionnaires, observations), authors must ensure that they have written informed consent from all the subjects involved (or their legal guardian for a minor, or next of kin if the subject is deceased). For details, see our editorial policies.

If applicable, please include a section entitled "Consent" and state 'Written informed consent for publication of the participants/patients' details and/or their images was obtained from the participants/patients/parents/guardian/relative of the participant/patient.'

Any clinical photographs must be accompanied by written consent to publish from the patient(s) involved. Any distinguishing features, medical record numbers or codes that could be used to identify the patient concerned must be removed from clinical images.

9. Author Contributions

We are using the CRediT Taxonomy to capture author contributions as we believe that having more detail of who did what brings transparency, enables recognition for researchers, and provides greater accountability for all involved. For more information click here.

You do not need to include an Author Contributions section in your manuscript: on submission, you will be asked for the contributions made by each author, to be selected from the list below. Anyone who has

contributed but does not meet the criteria for authorship should be listed in the Acknowledgments section.

Contributor Role	Role Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims.
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse.
Formal Analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.
Funding Acquisition	Acquisition of the financial support for the project leading to this publication.
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.
Methodology	Development or design of methodology; creation of models.
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Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
Validation	Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.

Contributor Role	Role Definition
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If you do not have any competing interests, add the text 'No competing interests were disclosed'.

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Please provide details of the Gates Foundation grant(s) that supported the work presented in your article in the following example format:

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If applicable, please also list any other funders or employers who funded the work. For each funder, please state the funder's name, the grant number where applicable and known, and the individual to whom the grant was assigned.

Please do not list funding that you have that is not relevant to this specific piece of research.

12. Acknowledgments

This section should acknowledge anyone who contributed to the research or the writing of the article but who does not qualify as an author; please clearly state how they contributed. Authors should obtain permission to include the name and affiliation, from all those mentioned in the Acknowledgments section. Please note that grant funding should not be listed here.

13. Supplementary Material

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should be deposited in an approved repository and listed as part of the data availability statement. For more information, please see extended data.

14. References

References can be listed in any standard referencing style as long as it is consistent between references within a given article. However, basic requirements include:

Journal abbreviations should follow the Index Medicus/MEDLINE abbreviation approach.

Preprints can be cited and listed in the reference list.

Only articles, books and book chapters, datasets and abstracts that have been published or are in press, or are available through public e-print/preprint servers/data repositories, may be cited. Unpublished abstracts, papers that have been submitted to a journal but not yet accepted, and personal communications should instead be included in the text; they should be referred to as 'personal communications' or 'unpublished reports' and the researchers involved should be named. Authors are responsible for getting permission to quote any personal communications from the cited individuals.

Web links, URLs, and links to the authors' own websites should be included as hyperlinks within the main body of the article, and not as references.

References to trials on a clinical trial database should be as follows: [Authors/name of group], [title of the trial], In: ClinicalTrials.gov [cited year month date], Available from [URL of the link from ClinicalTrials.gov].

Example: Kovacs Foundation, The Effect of Ozone Therapy for Lumbar Herniated Disc. In: ClinicalTrials.gov [cited 2012 Aug 30], Available from http://clinicaltrials.gov/ct2/show/NCT00566007.

Datasets published or deposited elsewhere (for example, in figshare, Dryad, etc.) should be listed in the "References" section and the citation to the dataset should follow one of these examples.

15. Figures and Tables

All figures and tables should be cited and discussed in the article text. There is no limit to the number of figures and tables you can have. Figure legends and tables should be added at the end of the manuscript. Tables should be formatted using the 'insert table' function in Word, or provided as an Excel file. For larger tables or spreadsheets of data, please see our data guidelines. Files for figures are usually best uploaded as separate files through the submission system (see below for information on formats).

Any photographs must be accompanied by written consent to publish from the individuals involved. Any distinguishing features, including medical record numbers or codes in the case of clinical images that could be used to identify the patient or participant concerned must be removed from the images.

Titles and legends: Each figure or table should have a concise title of no more than 15 words. A legend for each figure and table should also be provided that briefly describes the key points and explains any symbols and abbreviations used. The legend should be sufficiently detailed so that the figure or table can stand alone from the main text.

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include a note in the legend to state that: 'This figure/table has been reproduced with permission from [*include original publication citation*]'.

Figure formats: For all figures, the color mode should be RGB or grayscale.

Line art: Examples of line art include graphs, diagrams, flow charts and phylogenetic trees. Please make sure that text is at least 8pt, the lines are thick enough to be clearly seen at the size the image will likely be displayed (between 75-150 mm width, which converts to one or two columns width, respectively), and that the font size and type is consistent between images. Figures should be created using a white background to ensure that they display correctly online.

If you submit a graph, please export the graph as an EPS file using the program you used to create the graph (e.g. SPSS). If this is not possible, please send us the original file in which the graph was created (e.g. if you created the graph in Excel, send us the Excel file with the embedded graph).

If you submit other forms of line art such as flow charts, diagrams or text to be displayed as an image, please export the image as an EPS file (e.g. if creating phylogenetic trees with specialized programs), or send us the original file that was used to create the image (e.g. EPS or AI files if Adobe Illustrator was used, or a DOC, DOCX, PPT, PPTX or equivalent file if Word or PowerPoint was used).

If none of the above options is possible then we also accept uncompressed TIFFs with a resolution of at least 600dpi at the size they are likely to be displayed at (see above).

16. Images (if applicable)

Photographs and microscopy images: Photographs and microscopy images should be submitted as uncompressed TIFFs with a resolution of at least 300dpi at the size they are likely to be displayed (see above).

Mixed images: Images that are a mix of half-tone images and line art (e.g. annotated gels or images with scale bars) should be submitted as TIFF files at a resolution of 500dpi or vector files (e.g. EPS or Adobe Illustrator files). Please ensure that the text size is at least 8pt and lines are thick enough to be clearly visible at the size the image will be displayed.

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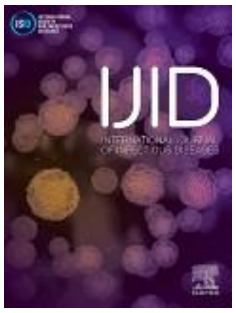
Electronic manipulation of images: The clarity of figures may be improved using image-editing software, but this must be done transparently and without misrepresenting the data (and the original, unaltered source data must be provided with the article). Brightness, contrasts or color balance may be used to enhance electronic images, but such changes must be applied to the whole image; any non-linear adjustments must be made explicit in the figure legend. Specific features within an image must not be added or changed (e.g. amplified, removed or obscured); and if figures are composed from images that have come from different sources, such as different gels, or from different parts of the same source, this must be made clear on the figure (e.g. by adding dividing lines). Authors are required to include details of all modifications made to images published as figures or uploaded as data in the Methods section of an article, including the name of the software (with version number) used to make these modifications. Please see our Policies on Image Manipulation for more information.

Appendix 6 – Author guidelines: International Journal of Infectious Diseases

INTERNATIONAL JOURNAL OF INFECTIOUS DISEASES

Official Publication of the International Society for Infectious Diseases

AUTHOR INFORMATION PACK



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ISSN: 1201-9712

DESCRIPTION

The International Journal of Infectious Diseases (IJID) is published monthly by the International Society for Infectious Diseases. *IJID* is a peer-reviewed, open access journal and publishes original clinical and laboratory-based research, together with reports of clinical trials, reviews, and some case reports dealing with the epidemiology, clinical diagnosis, treatment, and control of infectious diseases with particular emphasis placed on those diseases that are most common in under-resourced countries.

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The *International Journal of Infectious Diseases(IJID)* is published monthly by the International Society for Infectious Diseases.

IJID is a peer-reviewed, open access journal and publishes position papers, original clinical and laboratory-based research, together with reports of clinical trials, reviews, exceptional case reports. The interest areas of the *IJID* are epidemiology, clinical diagnosis, treatment, and control of infectious diseases with particular emphasis placed on under-resourced countries. The *IJID* does not publish veterinary studies and studies based on animal models alone.

Please visit our Open Access page for more information on article publishing charge. Editorials and Letters to Editor are not subject to this publication fee.

Manuscript types

Original articles cover research on infectious disease topics of broad interest. We particularly welcome papers that discuss epidemiological aspects of international health, clinical reports, clinical trials, systematic reviews and meta-analyses, and reports of clinical laboratory investigations. Original articles should not exceed 3500 words in length, with up to 5 figures/tables and no more than 30 references. The word count is from the introduction through to the end of the conclusion/discussion and does not include abstract, tables, figures, acknowledgements or reference list.

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Word count for a Review Article from introduction to conclusion/discussion: 2500 to max of 4000 words. There can be one or two figures/tables, an abstract, an introduction, a conclusion, and no more than 30 references.

Perspectives are papers that advance a hypothesis or represent an opinion based on scientific evidence relating to a topic of current interest or importance. They should be fully referenced (not more than 20), with one figure or table, and should not exceed 2000 words in length.

Correspondence (Letter to the Editor) is reserved for comments on papers recently published (within 3 months by volume) in the Journal. Maximum length 400 words, one table or figure and a maximum of 10 references. If you are reporting original data, please ensure that Article type is set to Original Article or Short Communication.

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Short Communications brief reports of unusual or preliminary findings. Maximum length 1200 words, two tables or figures and a maximum of 10 references.

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Contact details

If you have any problem submitting your paper online please contact Natalia Clarke at IJID@elsevier.com

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Please also ensure the font size is as required (12 pt).

Please include all the three statements at the end of your text, just before the reference list:

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BEFORE YOU BEGIN

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Please see our information on Ethics in publishing.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

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All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/ registrations, and grants or other funding. Authors should complete the declaration of competing interest statement using this template and upload to the submission system at the Attach/Upload Files step. Note: Please do not convert the .docx template to another file type. Author signatures are not required. If there are no interests to declare, please choose the first option in the template. More information.

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Where authors use generative artificial intelligence (AI) and AI-assisted technologies in the writing process, authors should only use these technologies to improve readability and language. Applying the technology should be done with human oversight and control, and authors should carefully review and edit the result, as AI can generate authoritative-sounding output that can be incorrect, incomplete or biased. AI and AI-assisted technologies should not be listed as an author or co-author, or be cited as an author. Authorship implies responsibilities and tasks that can only be attributed to and performed by humans, as outlined in Elsevier's AI policy for authors.

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Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyrightholder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

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Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/ sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

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Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the randomization procedure. The CONSORT checklist and template flow diagram are available online.

Registration of clinical trials

Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with International Committee of Medical Journal Editors recommendations. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects of health outcomes. Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example drugs, surgical procedures, devices, behavioural treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) will not require registration.

Registration of Systematic Reviews

Prospective registration of systematic reviews is required as from 6 September 2022 for any submitted manuscript reporting study results to be considered for publication in the International Journal of Infectious Diseases. Authors should register their protocols in any relevant open access online registry with searchable databases e.g. PROSPERO. The registration number should be included in the Methodology of the manuscript and the Abstract.

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