Viral load dynamics in healthcare workers with COVID-19 during Delta and Omicron era

Brian M.J.W. van der Veer (brian.vander.veer@mumc.nl)
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands
https://orcid.org/0000-0001-8837-8283

Jozef Dingemans
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands
https://orcid.org/0000-0001-8079-3087

Leonie E.A. Bank
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands
https://orcid.org/0000-0001-9599-0315

Christian J.H. von Wintersdorff
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands
https://orcid.org/0000-0003-4409-4611

Inge H.M. van Loo
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands
https://orcid.org/0000-0002-5960-4357

Paul H.M. Savelkoul
Department of Medical Microbiology, Maastricht University Medical Center+, Maastricht, Netherlands

Research Article

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Abstract

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, several variants of concern (VOC) have emerged and became the dominant strain. These are considered VOC because of traits like increased transmissibility, increased severity, and immune-evasion. Understanding their viral load dynamics, with the use of longitudinal follow-up data, can help to understand transmission and could inform policy makers on for example infection prevention guidelines. However, longitudinal follow-up studies are scarce. In this study, we were able to monitor the viral load dynamics of the Delta, Omicron BA.1 and BA.2 variants via an unique dataset that was obtained as a consequence of the implemented infection prevention guidelines for healthcare workers at our institution, based on longitudinal follow-up of viral load. We found that the dynamics are different between Delta, Omicron BA.1, and Omicron BA.2 variants, the latter having the highest viral load on day 5 (5.7 $\log_{10}$ copies/mL compared to 4.3 $\log_{10}$ copies/mL for Delta and 4.8 $\log_{10}$ copies/mL for BA.1) and day 7 (4.4 $\log_{10}$ copies/mL compared to 3.3 $\log_{10}$ copies/mL for Delta and 3.8 $\log_{10}$ copies/mL for BA.1). However, the infection duration does not appear to be different between these variants. Still, considerable viral loads even after the suggested quarantine period were observed, in particular for the Omicron BA.2 sub variant. This highlights the need for a tailored approach per variant as results of previously circulating variants do not always match. This is in particular important for healthcare workers as they can transmit SARS-CoV-2 to vulnerable patients.

Introduction

Since 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of people [1,2]. In 2021, different vaccines were released and today many people have been vaccinated and even received a booster vaccine. However, as SARS-CoV-2 mutates, variants emerge that are able to evade the current vaccines which are based on the original Wuhan variant [3]. Some variants have been considered a variant of concern (VOC) because of traits like increased transmissibility, increased severity, and immune-evasion. Delta, Omicron BA.1, and Omicron BA.2 harbor these traits, with Delta causing more severe disease while Omicron shows enhanced immune escape properties and appears to be more infectious [4]. Transmission and dominance of these variants are multifactorial in which viral load dynamics is one of the hallmarks for potential transmission. Only limited studies have focused on viral load dynamics due to lack of longitudinal data [5]. In our institution we implemented infection prevention guidelines for healthcare workers based on longitudinal follow-up of viral load. These data are unique since long term follow-up studies are scarce. Longitudinal follow-up data could aid in understanding transmission and how variants become dominant. Also, it could inform policy makers on for example quarantine duration. In this study we focused on SARS-CoV-2 load dynamics in our population of healthcare workers that are mostly vaccinated and received a booster vaccine, thereby comparing dynamics in viral load of Delta, BA.1, and BA.2 variants.

Methods

Follow-up procedure

Healthcare workers (n=1501) were tested for SARS-CoV-2 because of COVID-19 related symptoms, contact tracing, or outbreak investigation. When tested positive for SARS-CoV-2, healthcare workers were retested until a negative test or a Ct-value of ≥35 to as part of infection prevention guidelines of Maastricht UMC+. The time in days until Ct-value of ≥35 or a negative test was considered as infection duration. All nasopharyngeal swabs were uniformly collected by trained personnel and deposited in 2 mL of a mixture (1:1 ratio) of GLY viral transport medium (Mediaproducts BV) and Lysis Buffer (Perkin Elmer) and stored under the same conditions prior to testing. RNA extraction and diagnostic RT-PCR was performed as previously described by von Wintersdorff et al 2021 [6].

Variant determination

Of the 1501 healthcare workers tested between 01 November 2021 and 18 February 2022, 511 were genotyped. One sample with a sufficiently high load for sequencing (Ct- value ≤30) was selected per healthcare worker to determine the SARS-CoV-2 genotype. Whole genome sequencing was performed in 230/511 cases as described by von Wintersdorff et al. 2021 with an updated primer scheme [6]. The remaining 280 were genotyped using a RT-qPCR that discriminates between Delta, BA.1, and BA.2 variant on a Quantstudio 5 system (Thermofisher). Concentrations for the multiplex qPCR were 400nM primer and 200nM probe for both Spike targets S24-26 and S69-70 (see primer and probe sequences in the supplemental table). The assay consisted of 5µl primer/probe mix, 5 µl TaqPath 1-Step RT-qPCR Master Mix (Thermofisher), and 10µl RNA eluate. PCR protocol was 2 min at 25°C, 15 min at 50°C, 2 min at 95°C followed by 42 cycles of 3s at 95°C and 30s at 60°C. A positive signal of both targets is the Delta variant, S24-26 only is BA.1, and S69-70 only is the BA.2 variant.

Data analysis

Descriptive statistics were used to describe the median age, sex, and days of infection duration between healthcare workers with a known variant versus unknown (e.g. variant was not determined) or per variant. Ct-values were converted to viral loads using a standard curve as described in von Wintersdorff et al. 2021 but negative samples, were set to 0.1 viral copies/mL to be included in the load dynamics models [6]. The viral load values were $\log_{10}$ transformed for analysis. Using locally estimated scatterplot smoothing (LOESS), viral load dynamics were compared between Delta, BA.1, and BA.2 variants. The average load values of the first 10 days since the initial PCR test were compared between the variants. The sum of the difference in load values between two variants was divided by the days to calculate the average load difference between the two variants. All analyses were performed using R statistical software (version 3.6.2) and the figure was made with ggplot2 (version 3.3.2).

Competing interests

None to report

Results

There were 1501 healthcare worker cases from 01 November 2021 till 18 February 2022. Of these the variant was genotyped in 511/1501 cases. The healthcare workers with a known variant had similar age and sex distribution (table 1). Furthermore, the duration of infection was similar in typed healthcare worker cases as compared to non-typed cases (table 1).

Table 1 Characteristics of the study population by typing results

	Typed (n = 511)	Not typed (n = 991)	p-value
Age (median [IQR])	37 [27 – 46]	38 [27 – 49]	0.15
Sex			
Women (% [n])	76 [390]	72 [718]	
Men (% [n])	24 [120]	28 [273]	0.11
Duration of infection (days) (median [IQR])	12 [10 – 15]	12 [7 – 16]	0.15

Genotyping results (n=511) revealed that 62/511 healthcare workers were infected with the Delta variant, 412/511 with BA.1, and 37/511 with BA.2. The population characteristics and duration of infection were comparable between the three variants (table 2). However, viral load dynamics was different between variants (figure 1). Delta had on average mostly the lowest viral load in the first 10 days of infection, BA.1 had a slightly higher average load (approximately 2-fold), and BA.2 had the highest average load of approximately 7-fold and 3-fold compared to Delta and BA.1 respectively (figure 1 and table 3).

Table 2 Characteristics of the study population with a genotyping result per variant

	Delta (n = 62)	BA.1 (n = 412)	BA.2 (n = 37)	p-value
Age (median [IQR])	40 [26 – 49]	37 [27 – 46]	36 [27 – 45]	0.71
Sex				
Women (% [n])	82 [51]	76 [314]	78 [25]	
Men (% [n])	18 [11]	24 [97]	32 [12]	0.25
Duration of infection (days) (median [IQR])	12 [10 – 17]	12 [10 – 15]	11 [10 – 13]	0.30

Table 3 Average viral load values per day since initial positive test per variant.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Delta log_{10} copies/mL	6.3	5.9	5.6	5.2	4.8	4.3	3.9	3.3	2.9*	2.5	2.2
BA.1 log_{10} copies/mL	5.9	5.7	5.7	5.6	5.3	4.8	4.3	3.8	3.4	3.0*	2.6
BA.2 log_{10} copies/mL	6.1	6.4	6.5	6.4	6.1	5.7	5.1	4.4	3.7	3.0*	2.6

* first viral load value surpassing Ct-value 30 (log_{10} 3.2 copies / mL)

Discussion

In this short communication we report viral load dynamics in healthcare workers. The dynamics are different between Delta, BA.1, and BA.2 variants, the latter having the highest viral load in the first 10 days since initial PCR (7-fold and 3-fold respectively). However, the infection duration does not appear to be significantly different between variants.

The viral load dynamics of BA.2 in particular could have implications for transmission resulting in adaptation of the quarantine rules and advises compared to the rules and advises for the previous variants like Delta. For example in the Netherlands, the current quarantine advise is shortened to 5 days, and 7 days for healthcare workers, with the requirement that one has to be asymptomatic for at least one day. Based on the results of our study this would mean that 5 days after initial PCR the viral load of a BA.2 infected healthcare worker is significantly higher (5.7 log_{10} copies/mL [Ct-value 21]) compared to BA.1 (4.8 log_{10} copies/mL [Ct-value 24]), which is higher than for Delta (4.3 log_{10} copies/mL [Ct-value 26]) (table 3). After 7 days the viral loads are lower but for BA.2 a load of 4.4 log_{10} copies/mL (Ct-value 26) was detected, which is as high as Delta at day 5. Our result also show that at day 5 one still has a considerable load that is well above 3.2 log_{10} copies/mL (Ct-value 30).

The detected loads are most probably representing intact viral particles as samples with viral loads as low as 4.5 log_{10} copies/mL can be cultured (approximately 20%) of both vaccinated and unvaccinated individuals [7]. Shamier et al. 2021 also demonstrated that on average the culture success is lower in sample from vaccinated individuals, likely because of presence of antibodies. By carefully extrapolating the data from vaccinated individuals this would mean that at one week since initial positive test about 25% of the healthcare workers, infected with BA.2 variant, would still have intact SARS-CoV-2 viral particles. Five days after initial positive test up to 45% of the BA.2 infected HCW and up to 25% of the BA.1 infected HCW would have intact viral particles. However, the culture results of Shamier et al. 2021 were based on variants that were dominant before the Omicron era [7]. Since we know that in particular the Omicron variant is able to evade the immune system by escaping antibody responses, the presented interpreted percentages could even be higher. However, cellular tropism is shifted in the Omicron variant as it has impaired cell entry in lung and gut cells compared to Delta [8].

Apart from intact viral particles, the BA.2 variant might again lead to hospitalization as it is more infectious than the BA.1 variant [9]. In addition, a previous BA.1 exposure does not appear to provide protection for a BA.2 infection [10]. In January 2022 the BA.2 variant became the dominant SARS-CoV-2 variant in Denmark and together with high numbers of infections and lifted infection control measures, there was a surge of hospitalization. Therefore, the higher viral load dynamics of BA.2 compared to Delta and BA.1 should be considered in quarantine rules or advises, especially for healthcare workers who work with vulnerable patients.

One of the strengths of this study is the unique infection prevention strategy that was followed in our institution, allowing us to closely monitor infection progression per individual healthcare worker. Unfortunately we do not have information regarding vaccination status of the healthcare workers. However, we can assume most are vaccinated and received their booster based on anonymized data of our hospital. Also, we do not have data on symptoms per time point, thus association between viral load to symptoms is not possible nor the given advise to about length of quarantine. In addition, the number of cases with BA.2 is relatively small, but the viral load dynamics clearly differ, and the 95% confidence interval of the regression model is barely overlapping with Delta and BA.1 variants (figure 1).

In conclusion, viral load dynamics are different between the Delta, BA.1, and BA.2 variants with the latter having the highest viral loads, contributing to the higher transmission potential. This results in considerable viral loads even after the suggested quarantine period. As especially BA.2 has high loads after the advised quarantine period, that were shortened in the Netherlands, this could facilitate transmission of the virus. This highlights the need for a tailored approach per variant when it becomes dominant as results of previous variants do not always match. This tailored approach is especially important for healthcare workers as they can transmit SARS-CoV-2 to vulnerable patients in for example hematology or oncology wards.

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Declarations

Ethical statement In the Netherlands, research is required to be reviewed by a Medical Research Ethics Committee if it is subject to the Dutch Medical Research Involving Humans Subjects Act (WMO). Your research: Is it subject to the WMO or not? https://english.ccmo.nl/investigators/legal-framework-for-medical-scientific-research/your-research-is-it-subject-to-the-wmo-or-not. Accessed 19 April 2022 Retrospective research that is carried out on existing patient files or material is exempt from the WMO according to the Dutch Central Committee on Research Involving Human Subjects (CCMO). The data presented in this study were retrospectively retrieved from infectious disease control activities of the Maastricht University Medical Centre+ (MUMC+). Therefore, this study does not fall under the scope of the WMO and is exempt from medical ethical approval. No additional administrative permissions were required as the data is owned by the MUMC+.

Figures

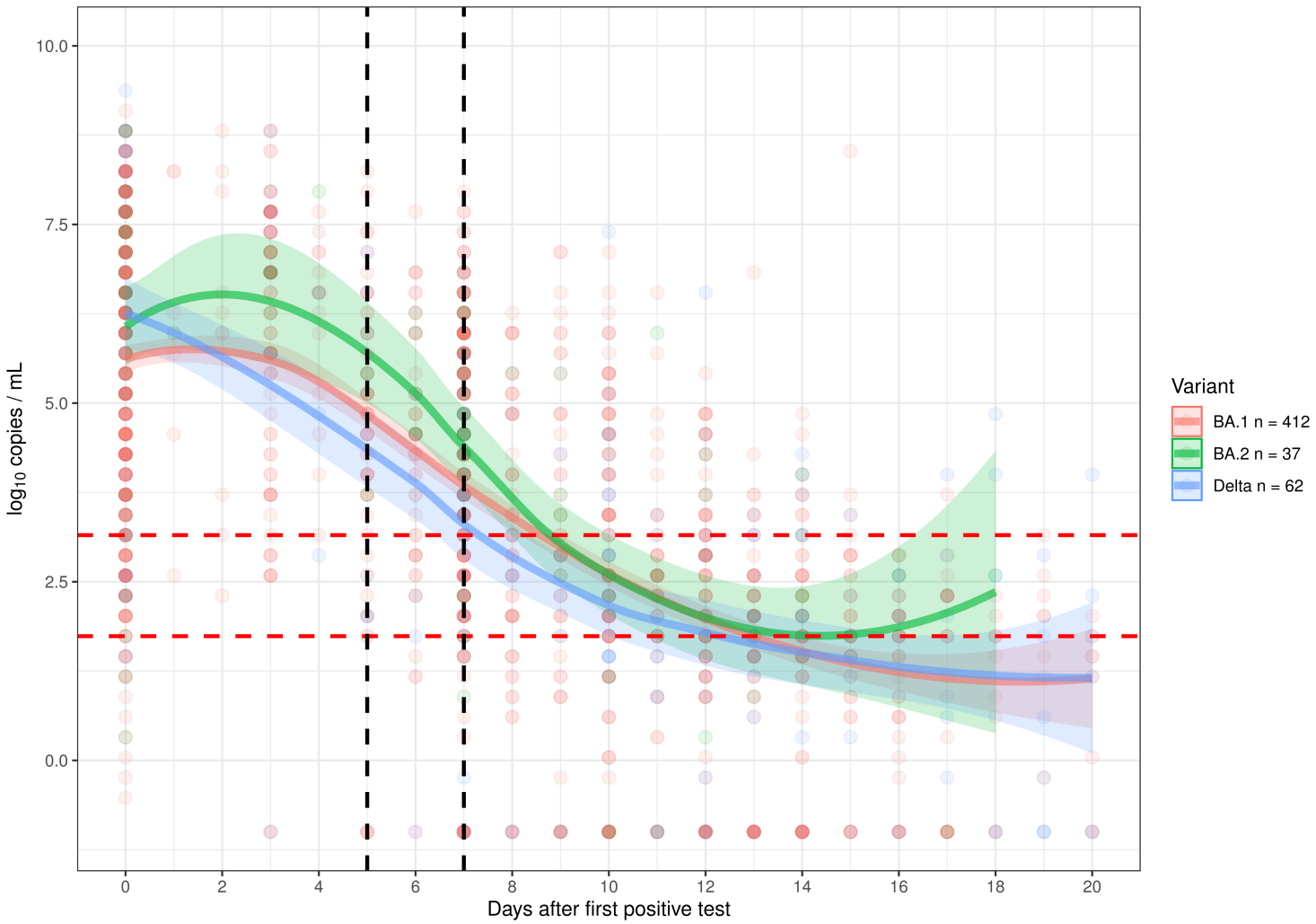

Figure 1

Viral load dynamics per variant. SARS-CoV-2 negative samples are -1 $\log_{10}$. The horizontal red lines represent Ct-values 30 (3.2 $\log_{10}$ copies/mL) and 35 (1.7 $\log_{10}$ copies/mL). Vertical black lines represent day 5 and 7 post initial positive test.