Brief Report

Time-of-Day Variation in SARS-CoV-2 RNA Levels during the Second Wave of COVID-19

Xiaodong Zhuang, Wei Wang, Helene Borrmann, Peter Balfe, Philippa C. Matthews, David W. Eyre, Elizabeth B. Klerman and Jane A. McKeating

1 Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK
2 Division of Sleep and Circadian Disorders, Brigham and Women’s Hospital, Division of Sleep Medicine, Harvard Medical School, Boston, MA 02115, USA
3 The Francis Crick Institute, London NW1 1AT, UK
4 Division of Infection and Immunity, University College London, London WC1E 6BT, UK
5 Department of Infection, University College London, Hospital NHS Foundation Trust, London WC1E 6BT, UK
6 Big Data Institute, Nuffield Department of Population Health, University of Oxford, Oxford OX3 7LF, UK
7 Division of Sleep Medicine, Harvard Medical School, Boston, MA 02115, USA
8 Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA
9 Chinese Academy of Medical Sciences Oxford Institute, University of Oxford, Oxford OX3 7FZ, UK

* Correspondence: jane.mckeating@ndm.ox.ac.uk
† These authors contributed equally to this work.

Abstract: Circadian rhythms influence and coordinate an organism’s response to its environment and to invading pathogens. We studied the diurnal variation in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in nasal/throat swabs collected in late 2020 to spring 2021 in a population immunologically naïve to SARS-CoV-2 and prior to widespread vaccination. SARS-CoV-2 diagnostic PCR data from 1698 participants showed a significantly higher viral load in samples obtained in the afternoon, in males, and in hospitalised patients when linear mixed modelling was applied. This study illustrates the importance of recording sample collection times when measuring viral replication parameters in clinical and research studies.

Keywords: circadian; SARS-CoV-2; RNA; diurnal; COVID-19

1. Introduction

COVID-19 caused by SARS-CoV-2 is one of the greatest health challenges we have faced in the 21st century. The concerted effort of academia, industry, government and regulatory bodies has resulted in effective vaccines and drug discovery programmes that limit SARS-CoV-2 transmission and disease severity [1]. At the time of writing, more than 5 million people have died, and the pandemic remains a global challenge. Understanding host pathways and associated factors that define susceptibility to SARS-CoV-2 infection and disease severity will inform future clinical management and public health measures to control this disease.

Circadian rhythms are endogenous daily oscillations that influence and coordinate an organism’s response to its environment. Many aspects of host immunity are regulated by the circadian clock and these immune rhythms are likely to have evolved to defend against diurnal peaks of pathogen encounters (reviewed in [2]). A recent report demonstrated significant time-of-day variation in multiple immune parameters, including lymphocyte and neutrophil counts in >300,000 participants in the UK Biobank, highlighting the rhythmicity in innate and adaptive immune responses [3]. In models of viral or bacterial infection, genetic disruption of the circadian clock increase disease severity [4–11]. Lung diseases frequently show time-of-day variation in respiratory function and severity of symptoms [12], with the key circadian component, BMAL1, regulating inflammation [13]. Influenza A virus infection of mice lacking BMAL1 showed a higher viral burden in the lung [14] and elevated
inflammatory responses [4,11]. There is an emerging picture of time-of-day dependency of virus replication (reviewed in [15]), suggesting that circadian regulation of infection is ubiquitous.

We recently identified a role for BMAL1 in regulating SARS-CoV-2 infection in vitro [16], suggesting that virus replication may vary during the day, and this could influence transmission. To explore the relevance of this observation, we performed a retrospective study to assess the relationship between SARS-CoV-2 RNA levels in nasal/throat swabs and time of sample collection in a cohort of 1698 adults tested by the Oxford University Hospitals, UK, from late 2020 to Spring 2021. This period covered the second wave of SARS-CoV-2 transmission in the UK involving the Alpha variant and was prior to widespread vaccination, providing an opportunity to study the daily variation in SARS-CoV-2 RNA levels in an immunologically naïve population.

2. Methods
2.1. Sample Collection

Samples were obtained from adults on hospital wards or admission units, classified as in-patients, or from out-patient centres. As health care workers were prioritized for SARS-CoV-2 vaccination in the UK from late December 2020, they were excluded from our analysis. We obtained anonymised SARS-CoV-2 PCR data from combined nasal/throat swabs collected from Nov 2020 to May 2021 from the Infections in Oxfordshire Research Database with Research Ethics Committee approvals (19/SC/0403 and ECC5-017(A)/2009). The following information was available: age, sex, time of sample request and time of receipt in diagnostic laboratory.

2.2. SARS-CoV-2 RNA Quantification

Viral RNA was measured using the Thermo Fisher TaqPath COVID-19 RT-PCR Kit that measures ORF1ab, Spike (S) and Nucleocapsid (N) gene transcripts. As an internal control for the quality of RNA isolation clinical samples were supplemented with MS2 phage RNA prior to extraction. S gene amplification will fail when the infecting variant has genetic mutations or deletions in S, and these samples are defined as S Gene Target Failures (SGTF). Sequencing of viruses showed that the Victoria (VIC) strain was circulating at this time and the SGTF samples represented infection with the Alpha variant (∆69/70 Spike) [13]. Viral loads (VLs) were estimated from the Ct values using standard curves for each amplicon, as previously reported [12,13]. The interval between sample request and laboratory receipt times allowed us to assess the effect of transport or storage delays on the VL estimates. The VL in samples with intervals of 0–3 or 3–6 h showed a median Log$_{10}$ VL of 4.2 and 3.9, respectively; however, samples with an interval >6 h had a reduced Log$_{10}$ VL of 3.7. To reduce variance in VL and to increase confidence in sample time for statistical evaluation, we selected samples with a <6 h interval and used the sample request time for all analysis.

2.3. Statistical Analysis

We selected to use a linear mixed-effects model that can account for multiple factors known to influence VL measurements. VLs were log$_{10}$-transformed and the sample request time (6:00–11:59 a.m. vs. 12:00–17:59 p.m.), age groups (18–39, 40–59, 60–79 and 80–104 years), sex (male vs. female), location (in-patients vs. out-patients) and virus strain (VIC vs. Alpha), together with their interactions, were included in the model as fixed effects. Participants were treated as random effects to account for inter- and intra-individual variability. Additional analyses were performed where the sample request time was classified into three intervals (morning 6:00–9:59 a.m., mid-day 10:00 a.m.–13:59 p.m. and afternoon 14:00–17:59 p.m.) or age was considered as a continuous variable. To assess the non-linear effects of age on VL, a B-spline fit of participant age was modelled [17], with residual plots used to check model assumptions and goodness of fit. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) with the significance level set at $\alpha = 0.05$. All tests were two-sided.
3. Results

The SARS-CoV-2 VIC and Alpha strains were co-circulating in Nov–Dec 2020, whereas by Apr–May 2021 the Alpha strain accounted for >90% of infections (Figure 1A). To compare VIC and Alpha RNA levels, we estimated the VL for VIC using either two (ORF1 and N) or three (ORF1, N and S) amplicons and observed an excellent agreement ($r^2 = 0.97$) (Figure 1B); we therefore used the ORF1 and N Ct values to estimate VL for all samples. In total, 85% of sample request times occurred during the working day (06:00–18:00) (Figure 1C), leading us to partition the data into morning (a.m.: 6:00–11:59) and afternoon (p.m.: 12:00–17:59). Participants were classified into four age groups (18–39, 40–59, 60–79 and 80–104 years) (Table 1).

![Figure 1](image-url)

**Figure 1.** (A) Monthly COVID-19 PCR samples detected in Oxford UK as Victoria (VIC, grey) or Alpha (blue) strain between Nov 2020 and May 2021. (B) Viral Load (VL) estimates for the VIC samples derived from either 2 (ORF1, N; x-axis) or 3 (ORF1, N and S; y-axis). Ct values agreed closely ($r^2 = 0.97$). (C) Sample request times from in-patient and out-patient groups listed by hour of the day and by VIC or Alpha strain. (D) Violin plots of $\log_{10}$ VL of the infecting virus strain (VIC/Alpha), partitioned by in- and out-patient groups (location) and by sample time (a.m., 06:00–11:59; p.m., 12:00–17:59). Median $\log_{10}$ VL is depicted with a solid red bar and the interquartile range with hashed red lines.
Table 1. Number of participants in each sex, age group, in-patient vs. out-patient, and AM/PM category.

| Age Group | Female In-Patients | Male In-Patients | Female Out-Patients | Male Out-Patients | Total |
|-----------|--------------------|------------------|---------------------|-------------------|-------|
| 18–39     | 31 AM | 39 PM | 71 AM | 84 PM | 23 AM | 28 PM | 50 AM | 77 PM | 403 |
| 40–59     | 44 AM | 67 PM | 56 AM | 56 PM | 88 AM | 84 PM | 62 AM | 62 PM | 519 |
| 60–79     | 90 AM | 89 PM | 70 AM | 66 PM | 159 AM | 164 PM | 67 AM | 62 PM | 767 |
| 80–104    | 83 AM | 87 PM | 40 AM | 50 PM | 56 AM | 91 PM | 50 AM | 46 PM | 503 |
| Total     | 248 | 282 | 237 | 256 | 326 | 367 | 229 | 247 | 2192 |

Several factors in this dataset suggest that simple univariate analyses are not appropriate: (i) in-patients (n = 1223) had a higher VL than out-patients (n = 969) (mean Log_{10} VL 3.87 vs. 3.61, p < 0.003) and (ii) in-patients were older (median of 66 vs. 54 years, p < 0.0001) and included a higher proportion of males (52% vs. 48%, p = 0.099). We therefore selected a linear mixed-effects modelling approach to assess the effect of sample request time, age, sex, in-patient vs. out-patient and virus strain (VIC vs. Alpha) as fixed effects on VL. Our analysis showed the VL associated with: (i) sample request time, with an estimated log_{10} VL of 3.56 in the morning and 3.75 in the afternoon (p = 0.044); (ii) sex, with an estimated log_{10} VL of 3.75 in males vs. 3.56 in females (p = 0.041); (iii) location, with an estimated log_{10} VL of 3.79 in the in-patients vs. 3.52 in the out-patients (p = 0.007) (Table 2). In summary, SARS-CoV-2 VL was significantly higher in samples requested in the afternoon, in males, and in in-patients.

Table 2. Linear mixed-effect modelling results (Type III tests of fixed effects).

| Main Effects: | Num DF | F-Value | p-Value |
|---------------|--------|---------|---------|
| Time (AM/PM)  | 1      | 4.09    | 0.044   |
| Age           | 3      | 0.14    | 0.935   |
| Sex           | 1      | 4.19    | 0.041   |
| In/Out patient| 1      | 7.28    | 0.007   |
| VIC/Alpha     | 1      | 2.94    | 0.087   |
| Interaction terms: | | | |
| Time × Age    | 3      | 1.69    | 0.169   |
| Time × Sex    | 1      | 0.48    | 0.487   |
| Time × In/Out patient | 1 | 8.45 | 0.004 |
| Time × VIC/Alpha | 1 | 4.17 | 0.042 |
| Age × Sex     | 3      | 0.93    | 0.426   |
| Age × In/Out patient | 3 | 7.93 | <0.0001 |
| Age × VIC/Alpha | 3 | 2.02 | 0.11 |
| Sex × In/Out patient | 1 | 2.76 | 0.097 |
| Sex × VIC/Alpha | 1 | 0.04 | 0.847 |
| In/Out patient × VIC/Alpha | 1 | 0.18 | 0.671 |

Bold values denote statistical significance at the p < 0.05 level.

It is noteworthy that out-patients infected with the VIC strain showed an 11-fold increase in VL between morning and afternoon, whereas the Alpha strain only showed a 2.4-fold increase (Figure 1D). We noted significant interactions between sample request time and location (p = 0.004), sample request time and SARS-CoV-2 strain (p = 0.042), and between age and location (p < 0.0001) (Table 2). For example, the largest difference between in-patients and out-patients was seen in the 80–104-year group (estimated Log_{10} VL = 4.08 vs. 3.28) and the smallest difference in the 40–59-year group (estimated Log_{10} VL = 3.69 vs. 3.53). Analysing the data using three time intervals for sample request time (6:00–9:59; 10:00–13:59 and 14:00–17:59) (Table 3), or treating age as a continuous variable and applying a B-spline analysis to account for non-linearity (GLIMMIX method [17]), produced similar results (Table 4).
Table 3. Analyses with three time groups.

| Main Effects:                  | Num DF | F-Value | p-Value |
|-------------------------------|--------|---------|---------|
| Time (AM/Mid-day/PM)          | 2      | 3.09    | 0.046   |
| Age                           | 3      | 0.44    | 0.725   |
| Sex                           | 1      | 1.92    | 0.166   |
| In/Out patient                | 1      | 11.68   | 0.0007  |
| VIC/Alpha                     | 1      | 4.2     | 0.041   |
| Interaction terms:            |        |         |         |
| Time × Age                    | 6      | 1.79    | 0.099   |
| Time × Sex                    | 2      | 1.4     | 0.248   |
| Time × In/Out patient         | 2      | 3.17    | 0.043   |
| Time × VIC/Alpha              | 2      | 3.8     | 0.023   |
| Age × Sex                     | 3      | 1.03    | 0.381   |
| Age × In/Out patient          | 3      | 6.98    | 0.0001  |
| Age × VIC/Alpha               | 3      | 2.08    | 0.102   |
| Sex × In/Out patient          | 1      | 1.22    | 0.27    |
| Sex × VIC/Alpha               | 1      | 0       | 0.97    |
| In/Out patient × VIC/Alpha    | 1      | 0.48    | 0.491   |

Bold values denote statistical significance at the $p < 0.05$ level.

Table 4. Analyses with age as a continuous variable.

| Main Effects:                  | Num DF | F-Value | p-Value |
|-------------------------------|--------|---------|---------|
| Time (AM/PM)                  | 1      | 6.71    | 0.0096  |
| Age (B-spline forms)          | 6      | 0.96    | 0.4482  |
| Sex                           | 1      | 3.2     | 0.0737  |
| In/Out patient                | 1      | 1.13    | 0.2882  |
| VIC/Alpha                     | 1      | 3.64    | 0.0567  |
| Interaction terms:            |        |         |         |
| Time × Age                    | 6      | 2.23    | 0.0382  |
| Time × Sex                    | 1      | 0.22    | 0.6354  |
| Time × In/Out patient         | 1      | 7.02    | 0.0061  |
| Time × VIC/Alpha              | 1      | 4.11    | 0.0427  |
| Age × Sex                     | 6      | 1.22    | 0.2949  |
| Age × In/Out patient          | 6      | 4.66    | 0.0001  |
| Age × VIC/Alpha               | 6      | 1.41    | 0.2054  |
| Sex × In/Out patient          | 1      | 2.81    | 0.094   |
| Sex × VIC/Alpha               | 1      | 0       | 0.9711  |
| In/Out patient × VIC/Alpha    | 1      | 0.45    | 0.5004  |

Bold values denote statistical significance at the $p < 0.05$ level.

4. Discussion

Our analysis showed a time-of-day influence on SARS-CoV-2 RNA levels in nasal/throat swabs with modest but significantly higher VL estimates in the afternoon ($\log_{10}$ VL 3.7 vs. 3.8 copies/sample, $p = 0.044$) after adjustment for multiple factors. Our observations are consistent with a study from McNaughton et al., who reported a diurnal variation in SARS-CoV-2 PCR test results from >80,000 nasopharyngeal samples, showing a 2-fold variation in test positivity with a peak of positive results at 2 p.m. [18]. In contrast, two studies assessing the time of sampling on SARS-CoV-2 diagnostic test results using repeated saliva collections reported a trend for higher VLs in samples collected earlier in the day [19,20]. However, the conclusions from the latter two studies are limited by the small cohort sizes ($n = 16$ and $n = 13$, respectively).

Cortisol levels are known to oscillate in a diurnal manner and a recent study showed this rhythmic pattern was reduced in COVID-19, with a more significant perturbation in hospitalized patients with more severe disease [21]. As circadian amplitude and timing can differ in hospitalized (compared with non-hospitalized) patients, resulting from multiple factors including disease severity, medication and changes in the environment (e.g., light-
ing) [22–24], it is important to compare the diurnal variation in SARS-CoV-2 VL separately between in- and out-patients. We noted a greater difference between the morning and afternoon VL in out-patients infected with VIC ($\log_{10}$ VL 2.68 in AM and 3.74 in PM) compared with Alpha ($\log_{10}$ VL 3.36 in AM and 3.74 in PM), that may relate to different replication rates or sites of infection between the variants [25].

Many respiratory infections follow a seasonal pattern, including COVID-19 [26,27]. Given the link between seasonality and circadian rhythms [28,29], it will be important to identify if SARS-CoV-2 variants evolve and adapt to seasonal effects, as this could influence our timing of booster vaccination programs.

Limitations of this study include the lack of information on actual sample collection time, medical or medication history, dietary information, sleep and shift-work patterns of the participants; and the heterogeneity of the cohort with subjects sampled in different stages of COVID-19—all of which could influence virus replication [30,31]. Our cohort does not include children or clinically vulnerable groups, such as immunocompromised patients. Finally, the clinical relevance of VL measurements and their association with COVID-19 severity is not known [32].

This study highlights the value of recording sample time in clinical and research studies and suggests time-of-day factors should be considered when designing clinical trials to evaluate antiviral drug efficacy where the most frequently measured end point is VL, and when designing epidemiological studies that track viral transmission. These time-of-day-dependent changes in VL could impact the interpretation of results from diagnostic assays, as discussed by McNaughton et al. [18], as well as disease severity and mortality [33]. It will be of interest to analyse the time-of-day dependency of other SARS-CoV-2 variants including Delta and Omicron and to assess the impact of vaccination on VL.

Author Contributions: X.Z. designed the study, analysed data and co-wrote the manuscript; W.W. designed the study, analysed data and co-wrote the manuscript; H.B. provided circadian expertise and co-wrote the manuscript; P.B. designed the study, analysed data and co-wrote the manuscript; P.C.M. provided access to patient data sets; D.W.E. provided access to patient data sets, analysed data and co-wrote the manuscript; E.B.K. designed the study, analysed data and co-wrote the manuscript; J.A.M. designed the study, analysed data and co-wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: J.A.M. is funded by a Wellcome Investigator Award 200838/Z/16/Z, UK Medical Research Council project grant MR/R022011/1 and Chinese Academy of Medical Sciences Innovation Fund for Medical Science, China (grant number: 2018-I2M-2-002). E.B.K. is funded by NIH K24-HL105664, P01-AI009975, R01-HL128538, and U54-AI062322, and the Leducq Trans-Atlantic Network of Excellence on Circadian Effects in Stroke. W.W. is funded by NCATS Harvard Clinical and Translational Science Center grant 5UL1TR002541-02. D.W.E. is a Robertson Foundation Fellow. P.C.M. is funded by a Wellcome intermediate fellowship grant Ref 110110/Z/15/Z, by the Francis Crick Institute and by UCL/UCLH NIHR BRC.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and anonymised data were obtained from the Infections in Oxfordshire Research Database with Research Ethics Committee approvals (19/SC/0403, ECC5-017 (A)/2009).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The authors declare that all data supporting the findings of this study are available in the article.

Acknowledgments: We thank all the people of Oxfordshire UK who contributed to the Infections in Oxfordshire Research Database. Research Database Team: L Butcher, H Boseley, C Crichton, O Freeman, J Gearing (community), R Harrington, M Landray, A Pal, TEA Peto, TP Quan, J Robinson (community), J Sellors, B Shine, AS Walker, D Waller. Patient and Public Panel: G Blower, C Mancey, PMcLoughlin and B Nichols.
Conflicts of Interest: X.Z. has no relevant disclosures. W.W. has a consultancy for the National Sleep Foundation. H.B. has no relevant disclosures. P.B. has no relevant disclosures. P.C.M. supervises a PhD studentship with GSK funding support. D.W.E. declares lecture fees from Gilead, outside the submitted work. E.B.K. reports travel support from Gordon Research Conference, Sleep Research Society, Santa Fe institute, DGS (German Sleep Society); consultancies for American Academy of Sleep Medicine Foundation, Circadian Therapeutics, National Sleep Foundation, Puerto Rico Science Technology Trust, Sanofi-Genzyme; E.B.K.’s partner owns Chronsulting; all are outside the scope of this work. J.A.M. has no relevant disclosures. The authors declare no conflict of interest.

References
1. Edwards, A.M.; Baric, R.S.; Saphire, E.O.; Ulmer, J.B. Stopping pandemics before they start: Lessons learned from SARS-CoV-2. Science 2022, 375, 1133–1139. [CrossRef] [PubMed]
2. Wang, C.; Lutes, L.K.; Barnoud, C.; Scheiermann, C. The circadian immune system. Sci. Immunol. 2020, 7, eabm2465. [CrossRef] [PubMed]
3. Wyse, C.; O’Malley, G.; Coogan, A.N.; McConkey, S.; Smith, D.J. Seasonal and daytime variation in multiple immune parameters in humans: Evidence from 329,261 participants of the UK Biobank cohort. iScience 2021, 24, 102255. [CrossRef]
4. Sengupta, S.; Tang, S.Y.; Devine, J.C.; Anderson, S.T.; Nayak, S.; Zhang, S.L.; Valenzuela, A.; Fisher, D.G.; Grant, G.R.; Lopez, C.B.; et al. Circadian control of lung inflammation in influenza infection. Nat. Commun. 2019, 10, 4107. [CrossRef]
5. Scheiermann, C.; Kunisaki, Y.; Lucas, D.; Chow, A.; Jang, J.E.; Zhang, D.; Hashimoto, D.; Merad, M.; Frenette, P.S. Adrenergic nerves govern circadian leukocyte recruitment to tissues. Immunity 2012, 37, 290–301. [CrossRef]
6. Sutton, C.E.; Finlay, C.M.; Raverdeau, M.; Early, J.O.; DeCourcey, J.; Zaslona, Z.; O’Neill, L.A.J.; Mills, K.H.G.; Curtis, A.M. Loss of the molecular clock in myeloid cells exacerbates T cell-mediated CNS autoimmune disease. Nat. Commun. 2017, 8, 1923. [CrossRef] [PubMed]
7. Zhang, Z.; Hunter, L.; Wu, G.; Maidstone, R.; Mizoro, Y.; Vonslow, R.; Fife, M.; Hopwood, T.; Begley, N.; Saer, B.; et al. Genome-wide effect of pulmonary airway epithelial cell-specific Bmal1 deletion. FASEB J. 2019, 33, 6226–6238. [CrossRef]
8. Gibbs, J.; Ince, L.; Matthews, L.; Mei, J.; Bell, T.; Yang, N.; Saer, B.; Begley, N.; Poolman, T.; Pariollaud, M.; et al. An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. Nat. Med. 2014, 20, 919–926. [CrossRef]
9. Pariollaud, M.; Gibbs, J.E.; Hopwood, T.W.; Brown, S.; Begley, N.; Vonslow, R.; Poolman, T.; Guo, B.; Saer, B.; Jones, D.H.; et al. Circadian clock component REV-ERBalpa controls homeostatic regulation of pulmonary inflammation. J. Clin. Investig. 2018, 128, 2281–2296. [CrossRef]
10. Early, J.O.; Menon, D.; Wyse, C.A.; Cervantes-Silva, M.P.; Zaslona, Z.; Carroll, R.G.; Palsson-McDermott, E.M.; Angiari, S.; Ryan, D.G.; Corcoran, S.E.; et al. Circadian clock protein BMAL1 regulates IL-1beta in macrophages via NRF2. Proc. Natl. Acad. Sci. USA 2018, 115, E8460–E8468. [CrossRef]
11. Ehlers, A.; Xie, W.; Agapov, E.; Brown, S.; Steinberg, D.; Tidwell, R.; Sajol, G.; Schulz, R.; Weaver, R.; Yu, H.; et al. BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes. Mucosal. Immunol. 2018, 11, 97–111. [CrossRef] [PubMed]
12. Scheiermann, C.; Gibbs, J.; Ince, L.; Loudon, A. Clocking in to immunity. Nat. Rev. Immunol. 2018, 18, 423–437. [CrossRef] [PubMed]
13. Ince, L.M.; Zhang, Z.; Beesley, S.; Vonslow, R.M.; Saer, B.R.; Matthews, L.C.; Begley, N.; Gibbs, J.E.; Ray, D.W.; Loudon, A.S.I. Circadian variation in pulmonary inflammatory responses is independent of rhythmic glucocorticoid signaling in airway epithelial cells. FASEB J. 2019, 33, 126–139. [CrossRef] [PubMed]
14. Edgar, R.S.; Stangherlin, A.; Nagy, A.D.; Nicoll, M.P.; Efstathiou, S.; O’Neill, J.S.; Reddy, A.B. Cell autonomous regulation of herpes and influenza virus infection by the circadian clock. Proc. Natl. Acad. Sci. USA 2016, 113, 10085–10090. [CrossRef]
15. Bormann, H.; McKeating, J.A.; Zhuang, X. The Circadian Clock and Viral Infections. J. Biol. Rhythm. 2021, 36, 9–22. [CrossRef]
16. Zhuang, X.; Tsukuda, S.; Wrensch, F.; Wing, P.A.C.; Schilling, M.; Harris, J.M.; Bormann, H.; Morgan, S.B.; Cane, J.L.; Mailly, L.; et al. The circadian clock component BMAL1 regulates SARS-CoV-2 entry and replication in lung epithelial cells. Science 2021, 24, 103144. [CrossRef]
17. Dean, C.B.; Nielsen, J.D. Generalized linear mixed models: A review and some extensions. Lifetime Data Anal. 2007, 13, 497–512. [CrossRef]
18. McNaughton, C.D.; Adams, N.M.; Hirschie Johnson, C.; Ward, M.J.; Schmitz, J.E.; Lasko, T.A. Diurnal Variation in SARS-CoV-2 PCR Test Results: Test Accuracy May Vary by Time of Day. J. Biol. Rhythm. 2021, 36, 595–601. [CrossRef]
19. Hung, D.L.; Li, X.; Chiu, K.H.; Yip, C.C.; To, K.K.; Chan, J.F.; Sridhar, S.; Chung, T.W.; Lung, K.C.; Liu, R.W.; et al. Early-Morning vs Spot Posterior Oropharyngeal Saliva for Diagnosis of SARS-CoV-2 Infection: Implication of Timing of Specimen Collection for Community-Wide Screening. Open Forum Infect. Dis. 2020, 7, ofaa210. [CrossRef]
20. Katayama, Y.; Murai, R.; Moriai, M.; Nirasawa, S.; Saeki, M.; Yakuwa, Y.; Sato, Y.; Asanuma, K.; Fujiyama, K.; Ito, M.; Kato, Y.; et al. Does the timing of saliva collection affect the diagnosis of SARS-CoV-2 infection? J. Infect. Chemother. 2022, 28, 1012–1014. [CrossRef]
21. Yavropoulou, M.P.; Filippa, M.G.; Mantzou, A.; Ntziora, F.; Mylona, M.; Tektonidou, M.G.; Vlachogiannis, N.I.; Paraskevis, D.; Kaltas, G.A.; Chrousovs, G.P.; et al. Alterations in cortisol and interleukin-6 secretion in patients with COVID-19 suggestive of neuroendocrine-immune adaptations. Endocrine 2022, 75, 317–327. [CrossRef] [PubMed]
22. Haspel, J.; Kim, M.; Zee, P.; Schwarzmeier, T.; Montagnese, S.; Panda, S.; Albani, A.; Merrow, M. A Timely Call to Arms: COVID-19, the Circadian Clock, and Critical Care. *J. Biol. Rhythm.* 2021, 36, 55–70. [CrossRef] [PubMed]
23. Telias, I.; Wilcox, M.E. Sleep and Circadian Rhythm in Critical Illness. *Crit. Care* 2019, 23, 82. [CrossRef] [PubMed]
24. Meira, E.C.M.; Miyazawa, M.; Gozal, D. Putative contributions of circadian clock and sleep in the context of SARS-CoV-2 infection. *Eur Respir J.* 2020, 55, 2001023. [CrossRef]
25. Lee, J.Y.; Wing, P.A.; Gala, D.S.; Noerenberg, M.; Jarvelin, A.I.; Titlow, J.; Zhuang, X.; Palmalux, N.; Iselin, L.; Thompson, M.K.; et al. Absolute quantitation of individual SARS-CoV-2 RNA molecules provides a new paradigm for infection dynamics and variant differences. *Elife* 2022, 11, e74153. [CrossRef]
26. Hawkes, M.T.; Lee, B.E.; Kanji, J.N.; Zelyas, N.; Wong, K.; Barton, M.; Mukhi, S.; Robinson, J.L. Seasonality of Respiratory Viruses at Northern Latitudes. *JAMA Netw. Open* 2021, 4, e2124650. [CrossRef]
27. Audi, A.; Allbrahim, M.; Kaddoura, M.; Hijazi, G.; Yassine, H.M.; Zaraket, H. Seasonality of Respiratory Viral Infections: Will COVID-19 Follow Suit? *Front. Public Health* 2020, 8, 567184. [CrossRef]
28. Wirz-Justice, A.; Wever, R.A.; Aschoff, J. Seasonality in freerunning circadian rhythms in man. *Naturwissenschaften* 1984, 71, 316–319. [CrossRef]
29. Hannay, K.M.; Forger, D.B.; Booth, V. Seasonality and light phase-resetting in the mammalian circadian rhythm. *Sci. Rep.* 2020, 10, 19506. [CrossRef]
30. Prather, A.A.; Pressman, S.D.; Miller, G.E.; Cohen, S. Temporal Links Between Self-Reported Sleep and Antibody Responses to the Influenza Vaccine. *Int. J. Behav. Med.* 2021, 28, 151–158. [CrossRef] [PubMed]
31. Lange, T.; Perras, B.; Fehm, H.L.; Born, J. Sleep enhances the human antibody response to hepatitis A vaccination. *Psychosom. Med.* 2003, 65, 831–835. [CrossRef] [PubMed]
32. Despres, H.W.; Mills, M.G.; Shirley, D.J.; Schmidt, M.M.; Huang, M.L.; Roychoudhury, P.; Jerome, K.R.; Greninger, A.L.; Bruce, E.A. Measuring infectious SARS-CoV-2 in clinical samples reveals a higher viral titer:RNA ratio for Delta and Epsilon vs. Alpha variants. *Proc. Natl. Acad. Sci. USA* 2022, 119, e2116518119. [CrossRef] [PubMed]
33. Fajnzylber, J.; Regan, J.; Coxen, K.; Corry, H.; Wong, C.; Rosenthal, A.; Worrall, D.; Giguël, F.; Piechocka-Trocha, A.; Attyeo, C.; et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat. Commun.* 2020, 11, 5493. [CrossRef] [PubMed]