Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection 82 (2021) 186–230

Contents lists available at ScienceDirect

Journal of Infection

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

Letters to the Editor

Detection of group B streptococcus colonisation in pregnant women: Comparison of two different culture methods and study of antimicrobial resistance patterns

Dear Editor,

We read with interest the recent article by Collins et al.[1] regarding under-identification of hospital clusters of Group B Streptococcus (GBS) disease outbreaks.

The first step in preventing GBS disease in infants is to correctly identify women carrying GBS at 35–37 weeks of pregnancy. These women should then be offered the opportunity to receive intrapartum antibiotic prophylaxis (IAP) to prevent neonatal GBS disease. Approximately 11 to 35% of pregnant women are colonised with GBS in the rectum and/or vagina. In the absence of IAP the rate of transmission of GBS from mother to newborn is approximately 50% and it is estimated that 1 to 2% of newborns will develop invasive GBS disease within the first week of life[2]. It is therefore crucial that the identification of GBS from swabs uses the most sensitive method available.

The recommendations of Public Health England (PHE) for the isolation of GBS from vaginal and rectal swabs offer different options: the use of an enrichment culture medium (ECM, Todd-Hewitt broth with gentamicin or colistin and nalidixic acid or LIM broth), followed by subculture on blood agar, selective agar or chromogenic agar. Most UK microbiology laboratories use direct plating onto chromogenic agar as time-to-result is reduced from 30–72 h to 18–24 h. Few studies have compared the classical culture method using ECM plus non-selective agar with direct plating onto a chromogenic medium showing a similar sensitivity[3], and to our knowledge, there are no studies comparing the latter method with a double selective culture. PHE current guidelines also recommend the latex agglutination test (LAT) for serotyping of GBS.

We compared the sensitivity of two different GBS culture methods and two serotyping techniques in 597 women from 35 weeks of gestation onwards who were swabbed with a double head recto-vaginal swab at two hospitals in London from 2nd July 2018 to 31st December 2018. We compared direct plating onto chromogenic agar with ECM followed by plating onto chromogenic agar. We used both LAT and multiplex PCR to assess serotypes. Further we analysed the antimicrobial resistance of any clinical GBS isolate from all the vaginal and rectovaginal swabs obtained during the study period.

The overall colonisation rate was 20% (120/597), 19% based on ECM and chromogenic agar versus 15% based on chromogenic agar alone. The positivity rate of the ECM-chromogenic agar method was 97% versus 75% by direct plating ($p < 0.0001$) (Table 1).

We serotyped 116 positive isolates by LAT and 113 by a multiplex PCR (3 isolates were not serotyped by PCR because of the low colony growth). The serotype distribution is shown in Fig. 1. Six samples were non-typable by LAT but identified through PCR (serotypes Ia, Ib, II, IV and two V). For 30% of serotypes (32/107) LAT yielded a different serotype than that identified by PCR. In addition, PCR identified a small proportion of serotypes IV and VI, not detected by LAT.

A total of 509 GBS positive isolates, including those of our study, were tested for antibiotic resistance. 100% of the isolates were sensitive to penicillin but 26.9% (95%CI 23.1–30.8%) were found to be resistant to clindamycin. Teicoplanin resistance was low at 5.1% (95%CI 3.4–7.4%).

Our results suggest that unless the double selective culture method is used some women might be misclassified as GBS negative and not receive antibiotics during labour, potentially resulting in neonatal GBS disease. This has implications for laboratories across the world as few use double selective cultures, and double selective testing is not mandated in any guidelines.

The serotype distribution of GBS isolates identified in our cohort is similar to that described globally, with serotypes III, II and Ia being the most common[4]. Knowledge of serotypes will inform vaccine design and subsequent monitoring of serotype replacement. LAT is the most commonly used phenotypic method for GBS serotype identification. However, due to poor capsular expression, capsule operon mutations or rearrangements, LAT can fail to assign the correct serotype. Although some studies have shown a higher concordance between molecular and phenotypic methods[5], our study shows that results obtained using LAT only match those using PCR in 70% of cases, similar to the results from the Oslo GBS-study, which showed an agreement of 71.1%[6]. Genomic determination of capsular serotype has also been shown to be superior to LAT with discordance attributed to the subjectivity of the LAT methodology or laboratory transcription errors[7].

With regard to resistance rates for antibiotics used in GBS colonised women allergic to penicillin, a resistance rate higher than expected was found for clindamycin (26.9%) compared to the rates described in the literature for the UK in recent years[8]. However, resistance to clindamycin reported by PHE in GBS isolates from bacteraemia is similar to our results in colonised women, 29% in 2018[9], as well as in the US, up to 33%[10]. These results support the recent change of the empirical antibiotic of choice, from clindamycin to vancomycin, in penicillin allergic women in UK guidelines.

In conclusion, our data confirm the use of ECM to ensure that GBS is correctly identified antenatally and advocate for the use of molecular serotyping methods to ensure accurate serotype information is available nationally in preparation for vaccine licensure.

https://doi.org/10.1016/j.jinf.2021.01.001

Table 1
Number of positive GBS isolates, positivity rate (relative sensitivity) and colonisation rate detected by a double selective culture using enrichment culture media (ECM) and direct plating onto chromogenic agar.

	Number of positivity	Positivity rate (%)	Colonisation rate (%)
ECM	116	97 (116/120)	19 (116/597)
Direct plating	90	75 (90/120)	15 (90/597)

Description: The positivity rate of ECM was 97% versus 75% by direct plating ($p < 0.0001$).

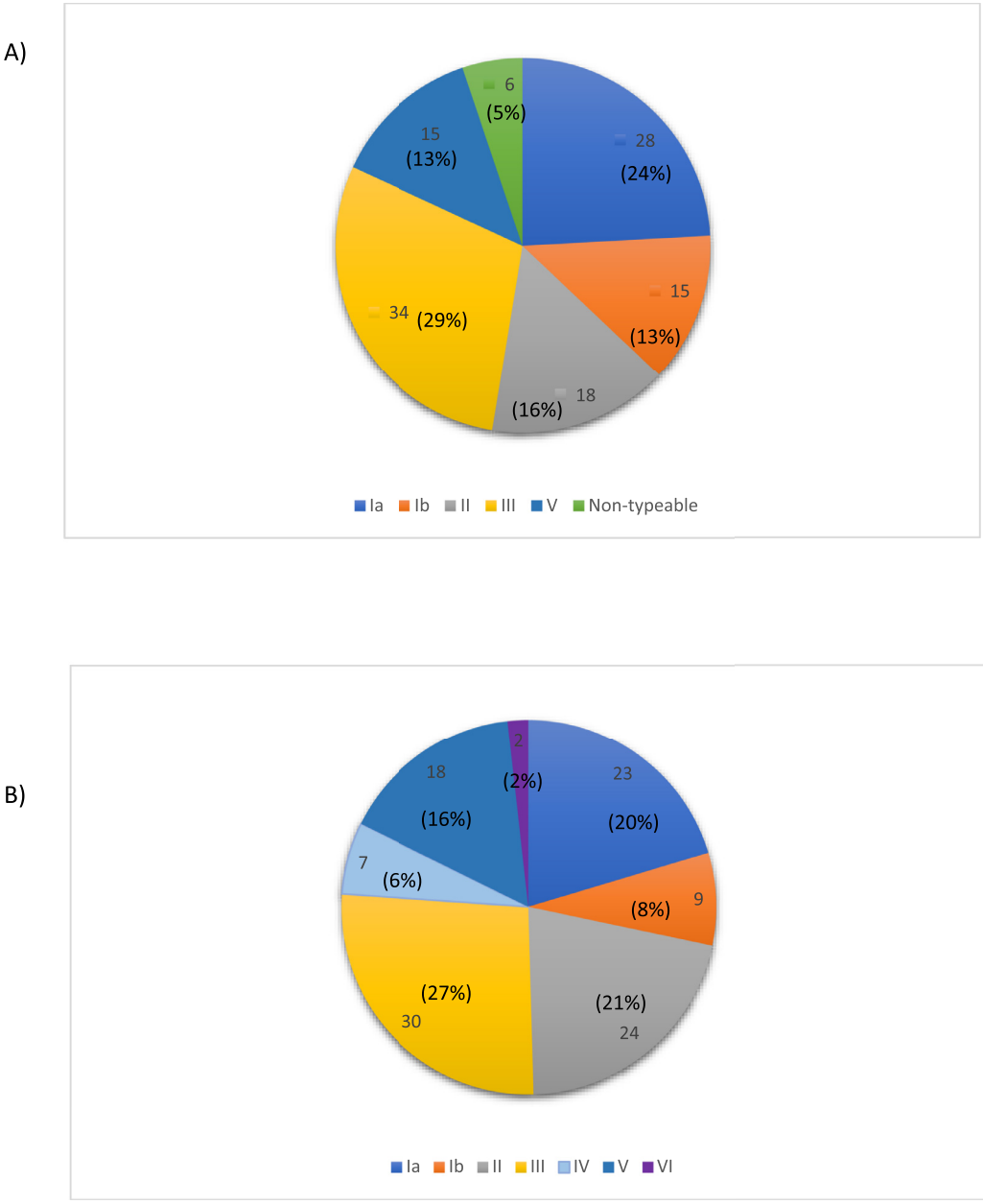

Fig. 1. GBS serotype distribution using a latex agglutination test (LAT) and a multiplex PCR
Description: A) GBS serotype distribution using a latex agglutination test (LAT) B) GBS serotype distribution using a multiplex PCR. Total numbers of serotyped isolates are expressed in the figure and their percentages in brackets.

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Accepted 5 January 2021
Available online 8 January 2021

https://doi.org/10.1016/j.jinf.2021.01.001

Analytical and clinical performance of the panbio COVID-19 antigen-detecting rapid diagnostic test

Dear Editor,

Recent articles in this Journal have suggested the potential of antigen-based rapid diagnostic tests (Ag-RDT) as low-cost and ease-of-use tools for massive screening and epidemiological surveillance of SARS-CoV-2 spread.[1,2] Based on a pre-screening of four Ag-RDT on 40 frozen specimens from nasopharyngeal swabs with known PCR results (Table S1, Appendix), we selected the Panbio COVID-19 Ag Test (Abbott) for investigating its analytical and clinical performance.

The analysis of serial dilutions of a SARS-CoV-2 isolate, propagated in Vero E6 cells, yielded a limit of detection (LoD) of 6.5×10^5 genome copies/reaction (Table S2). According to this value, the test would not detect SARS-CoV-2 infection in respiratory specimens with very low viral load. Still, the LoD was one logarithmic unit below the 10^6 copies/mL threshold necessary for successful virus isolate from respiratory samples.[3]

The clinical performance was analysed on frozen swabs from 1406 individuals (mean age 40.4 years; SD 24.5) with an RT-qPCR result available: 951 (67.6%) positive and 455 (32.4%) negative. Overall, 446 (31.7%) and 473 (33.6%) samples were nasopharyngeal swabs from symptomatic individuals and contacts exposed to symptomatic cases, respectively, and 487 (34.6%) were nasal mid-turbinate swabs from asymptomatic individuals collected in screening campaigns. The cycle threshold (Ct) of PCR-positive samples was <20, 20–24, 25–29, and >30 in 258 (17.1%), 305 (32.1%), 285 (30.0%), and 103 (10.8%), respectively (median Ct 23.6; interquartile range 19.7–27.3).

Overall, the Ag-RDT identified the presence of SARS-CoV-2 in 872 of 951 PCR-positive samples (sensitivity 91.7%; 95% CI 89.8–93.4) and ruled out its presence in 450 of 455 PCR-negative samples (specificity 98.9%; 97.5–99.6)(Table S3). In line with previous reports of clinical performance of this test,[2] sensitivity increased with lower Ct values (Ct <25, 98.2%; Ct<30, 94.9%) and was higher among samples collected in the setting of case identification (92.6%) and contact tracing (94.2%) than asymptomatic screening (79.5%). The increasing trend of sensitivity with lower Ct values was maintained in the setting of asymptomatic screening: sensitivity of samples with Ct <25 and <30 were 100% and 98.6%, respectively (Fig. 1). The high sensitivity of the Ag-RDT in samples with low Ct values, which was consistent with the LoD, has important implications for using this test as an epidemiological surveillance tool. A growing body of evidence indicates that

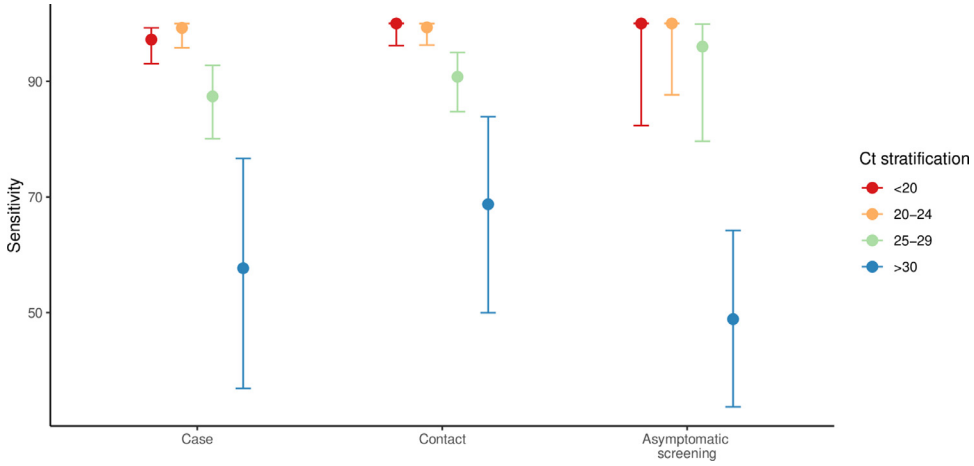

Fig. 1. SARS-CoV-2 detection using the Ag-RDT Panbio COVID-19 Ag-Test on PCR-positive samples according to rt-qPCR Ct value. Sensitivity (95CI) of the Ag-RDT according to the disease status and RT-qPCR Ct value.

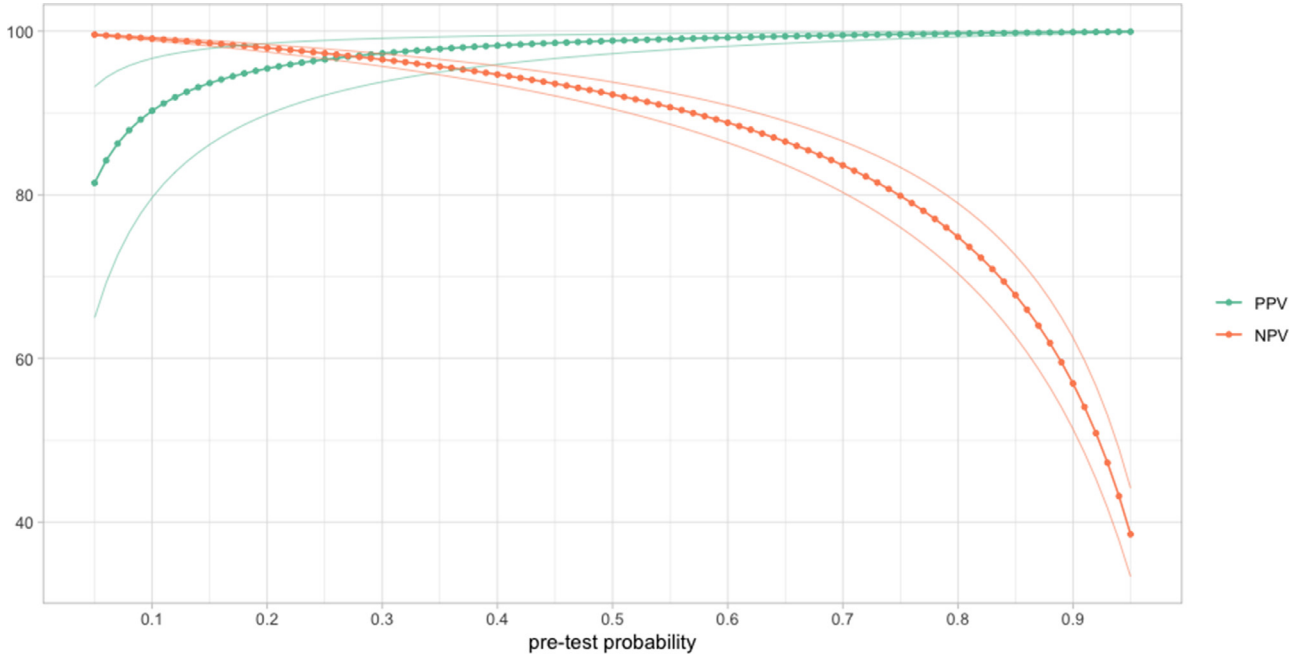

Fig. 2. Modelling of positive predictive value (PPV) and negative predictive value (NPV) assuming different pre-test probabilities. Dots represent the PPV and NPV at sequential increment of 0.01; lines are the 95% confidence interval . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PCR-positive respiratory specimens with low viral load (i.e., Ct >25 or $<10^6$ copies/mL) have a limited capacity for effective transmission.[3,4] Also, studies based on contact tracing strategies showed that the secondary attack rate significantly increases among index cases with Ct values <25.[5] The higher sensitivity of the Ag-RDT in samples with low Ct, irrespective of the presence of symptoms, indicates that the test is particularly suitable for identifying individuals who are contagious.

Like most studies investigating the clinical performance of RDTs,[6] our assessment was retrospectively on frozen samples from three different settings. Our internal validation showed no relevant differences between tests performed on fresh samples using the Abbot test Kit buffer and 1:3 dilutions of the Kit buffer and frozen specimens stored on transport media. Although we encourage internal validation before using this approach, our experience suggests the suitability of parallel sampling for Ag-RDT and PCR tests. Another consequence of not sampling in the intended setting was the impossibility of direct estimates of the positive predictive value (PPV) and negative predictive value (NPV). Alternatively, we modelized the PPV and NPV assuming a wide range of prevalence (i.e., pre-test probability) in the target population (Fig. 2). At a pre-test probability of 5%, consistent with the prevalence observed in asymptomatic screening campaigns in high-risk settings,[7] the NPV was 99.6% (99.5–99.7) (Table S4) and increased as the pre-test probability dropped. Correspondingly, the PPV at 5% pre-test probability was 81.5% (65.0–93.2) and decreased as pre-test probability decreased. At 5% pre-test probability, the estimated number of false-negative and false-positive values per thousand tests were 4 (3–5) and 12 (4–27), respectively (TableS5).

Our analytical and clinical performance findings suggest that the Ag-RDT cannot replace nucleic acid amplification tests (NAAT) as a tool to confirm or rule out the presence of SARS-CoV-2. However, the high sensitivity of NAAT has raised questions as to the clinical and epidemiological meaning of being positive for

SARS-CoV-2 infection. In some patients, low levels of viral RNA can remain detectable by RT-qPCR for months, with doubtful transmission capacity. The Ag-RDT reliably identifies people with high viral loads, and therefore it could be useful for screening strategies to identify and isolate asymptomatic COVID-19 people while they are still infectious. Although the kinetics of SARS-CoV-2 have not been well established, current evidence suggests that this time window may begin approximately four days after exposure and last for nearly ten days.[8] Of note, during the exponential phase, viral RNA may rise from undetectable levels (i.e., Ct >40) to millions of RNA copies/mL (i.e., Ct < 25) in the order of a day,[9] thus limiting the temporal validity of a negative result.

Currently, the WHO recommends using Ag-RDTs to support the diagnosis of cases and contacts during outbreak investigations and monitor trends in disease incidence, particularly in remote settings or closed groups (e.g., schools, care homes, or prisons), but not to screen asymptomatic populations.[10] However, our findings suggest that Ag-RDT might be useful for screening asymptomatic individuals, particularly in communities with high prevalence. Furthermore, the high sensitivity for detecting infected individuals with transmission capacity makes the test suitable for creating safe environments in time-limited social activities with high-risk of transmission, including–but not limited to–visiting relatives at nursing homes, playing sports, going to a crowded place like movie theatres, music concerts, and airports.

Contributors

OM, AA, BB, CGB, IB, JV designed the study. AA, BB, MU, MCM, JR, LR performed the laboratory procedures, and organized the data. DO did statistical analysis. OM wrote the first draft with revisions and input from JR, JS, CE, GF, QB, BC, JA, MVM, CGB. All authors approved the final version.

Declaration of Competing Interest

We declare no conflicts of interest.

Acknowledgements

The authors would like to thank Gerard Carot-Sans (PhD) for providing professional medical writing support during the preparation of the manuscript. We thank Andrea Tiburcio Lara and Elisabeth Bascuñana Prieto for technical support

Bárbara Baró is a Beatriu de Pinós postdoctoral fellow granted by the Government of Catalonia's Secretariat for Universities and Research, and by Marie Sklodowska-Curie Actions COFUND Programme (BP3, 801370).

Funding

Blueberry diagnostics, Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, and #YoMeCorono.org crowfunding campaing.

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Accepted 24 December 2020
Available online 7 January 2021

https://doi.org/10.1016/j.jinf.2020.12.033

Revisiting the county/city-level event risk assessment during the COVID-19 pandemic

Dear Editor,

Risk communication is vital in medicine and public health.[1,2] Risk-related information can differentially affect people's attitude and behaviors depending on how the information is presented.[3] Tools that properly assess and communicate health-related risks are urgently needed by health departments and governments to inform their decision-making. A recent paper in *Journal of Infection*[4] (and others[5,6]) responded to such an urgent need and aimed to make the "invisible" risk of COVID-19 at mass-gatherings visible and available to stakeholders and the general public. This letter attempts to provide feedback to improve both the utility of the tool and the likelihood for its successful implementation.

The author introduced a series of formulas and a R shiny app (https://yukifuruse.shinyapps.io/covid_eventrisk_en/), which inputs the daily number of newly reported cases of a region, the population size of the region, and expected attendees at an index event held in the region and outputs the probability of the event containing at least one infectious individual. The formulas could tell users the level of their "risk" in sharing the same environment (event) with one or more infectious individuals. This is analogous to a weather forecast, which informs users of the "risk" of rain. The formulas and the app can reflect continuous updates in the SARS-CoV-2-related evidence (e.g. asymptomatic ratio) and input region-specific parameter values (e.g. the fraction of the reported cases by testing among the actual infected individuals).

The app has garnered growing media attention in Japan and other countries (e.g. covered by *TV Tokyo, YouTube* [https://www.youtube.com/watch?v=r86YO7FZWxs], and *Wall Street Journal* [https://www.wsj.com/livecoverage/covid-2020–12–03/card/VleP5zyCw8feYTIybuQf]), where self-restraint requests made by governments are not legally enforced; as a result, holding/suspending or attending/not attending an event largely depends on the hosts' and attendees' discretion. Without the app, people would have difficulty knowing the level of the local risk involved in their events and going-out decisions. Therefore, the app could improve people's risk perception and enhance the scientific communication on SARS-CoV-2.

A potential pitfall in the app use relates to the limitations drawn by the lack of the micro-level network data and the assumptions of the formulas. The formulas assume that infectious individuals are randomly located in the social networks within the index region (of the event) and that the attendees of the event are randomly selected from the region. To illustrate this importance, we calculate the probability that there will be at least one infectious individual at an event of 50 attendees in a region of 100,000 individuals with 20 new cases per day using the app's default setting on these parameters as of Dec/28/2020, which is 35.2%. *What is the origin of the 20 daily new cases there?* They are typically secondary cases arising within the region. When that is the case, the infections do not occur at random locations, but rather spread like a snowball in the region's social networks.[7,8] Here, let's divide the region into two "communities": the first community contains a majority of the cases (e.g. 15 cases in 10,000 population), and the second community contains the rest (5 cases in 90,000 population). The divide of a region's singular social network may relate to geography, age, risk preference, occupation, or others. Most of the individuals in the first community have a smaller "degree of separation" from the infectious individuals compared to those in the second community.

Then, let's recalculate the probability using the same formulas per community. The probability of the event of 50 attendees to contain at least one infectious individual in the first community is 96.5%. If the event is planned for the first community, the app's estimation of 35.2% could lead people to dramatically underestimate their true risk of attending the event (35.2 compared to 96.5). This underestimation may make people overconfident in the first community (more widespread communities) and contribute to further infection spread there.

The recalculated risk in the second community is 11.3%. If the event is planned for the second community, the app's estimated risk of 35.2% could lead people to over-estimate their risk and avoid the event due to misperception of risk (35.2 compared to 11.3). This overestimation may make people in the second community (less widespread communities) less confident and result in unnecessarily sacrificing social and economic activities there, which may cause "quarantine fatigue".[7,9]

In sum, as with any new tool or program, this app and its effects should be studied to better understand its potential implications and implementation-related issues, including people's willingness to attend events, and health departments' response to event risks. The issue presented in this letter does not appear to stem from the formulas itself, but from the lack of data availability on COVID-19 case data at the municipality level due to privacy protection in most countries. This issue, which is shared with comparable event risk assessment tools[5,6] (e.g. in ref,[5] the event risk is provided at the county level in the US, UK, and other European countries), might be resolved if the microdata of communities (where we can assume random mixing) are made available.

Declaration of Competing Interest

AN is a consultant to Urbanic & Associates. HT is the Founder and CEO, Corporate Health, Inc. in Japan. YT is the CEO of Decades Inc. in Japan, and an employee of SoftBank Corp. SDY is a consul-

tant and advisor for digital health startups, and receives royalties from HarperCollins Publishers for the book, Stick with It.

Funding

Support for this research was provided by a grant from the UCLA Fielding School of Public Health High-Impact Data Initiative (AN). Additional support for AN and SDY was provided by grant P30DA027828 from the National Institute on Drug Abuse, awarded to C. Hendricks Brown. SDY received support from the National Institute of Allergy and Infectious Diseases (NIAID) Young: 7R01AI132030; and National Institute of Mental Health (NIMH) 5R01MH106415. The opinions expressed herein are the views of the authors and do not necessarily reflect the official policy or position of the National Institute on Drug Abuse, or any other part of the US Department of Health and Human Services.

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Accepted 31 December 2020
Available online 3 January 2021

https://doi.org/10.1016/j.jinf.2020.12.031

Regional and temporal disparities of excess all-cause mortality for Germany in 2020: Is there more than just COVID-19?

Dear Editor,

An excess all-cause mortality has been described during the first peak of the COVID-19 pandemic in spring 2020, with countries with high case numbers and corresponding case-fatality rates being particularly affected.[1,2] In this light, Stang and colleagues presented a corresponding analysis in this journal investigating weekly death counts for Germany up to early June.[3] At the same time, reduced hospitalization rates for various acute medical conditions as well as reduced performance of urgent procedures were reported in this period worldwide and for Germany.[4–6] Whether the latter observations influence long-term mortality irrespective of infection rates is unclear since current studies only investigated death rates until mid-2020. With this letter, we want to extend all-cause mortality statistics for Germany up to late October.

Data on daily deaths were accessible from the Federal Bureau of Statistics.[7] We analyzed death rates from 01/01/2020–10/25/2020 and compared them to averaged death rates from 2016 to 2019. Numbers of COVID-19 cases and deaths with proven SARS-CoV-2-infection were reported from the Robert-Koch-Institute.[8] According to COVID-19 case numbers per 100,000 inhabitants within a federal state we calculated tertiles defining COVID-19 case volume with corresponding low, intermediate and high infection rates. Based on nonoverlapping 95% confidence intervals (CI) of LOESS curves, three time periods were defined (winter: 01/17–03/16; spring surge: 03/23–05/06; summer surge: 07/30–10/15; Fig. 1). Inferential statistics were based on generalized linear mixed models (GLMM) and Poisson GLMMs. Effects were estimated with the lme4 package (version 1.1–21) in the R environment for statistical computing (version 3.6.1). We report incidence rate ratios (IRRs, calculated by exponentiation of the regression coefficients) plus 95% CIs and p values.

Compared to the expected number of deaths based on 2016–2019, data showed a reduced all-cause mortality during the winter period (IRR 0.941; 95%CI 0.935–0.947; $p < 0.01$) and an excess all-cause mortality both during spring surge (IRR 1.078; 95%CI 1.070–1.087; $p < 0.01$) and summer surge (IRR 1.050; 95%CI 1.043–1.056; $p < 0.01$). Overall, an excess mortality irrespective of subdivision of time intervals resulted in 2020 compared to previous years (IRR 1.012; 95%CI 1.009–1.016; $p < 0.01$). Higher age was associated with increased all-cause mortality counts in the total observational period, spring and summer surge intervals but without such interaction during winter period. All-cause mortality was higher in men

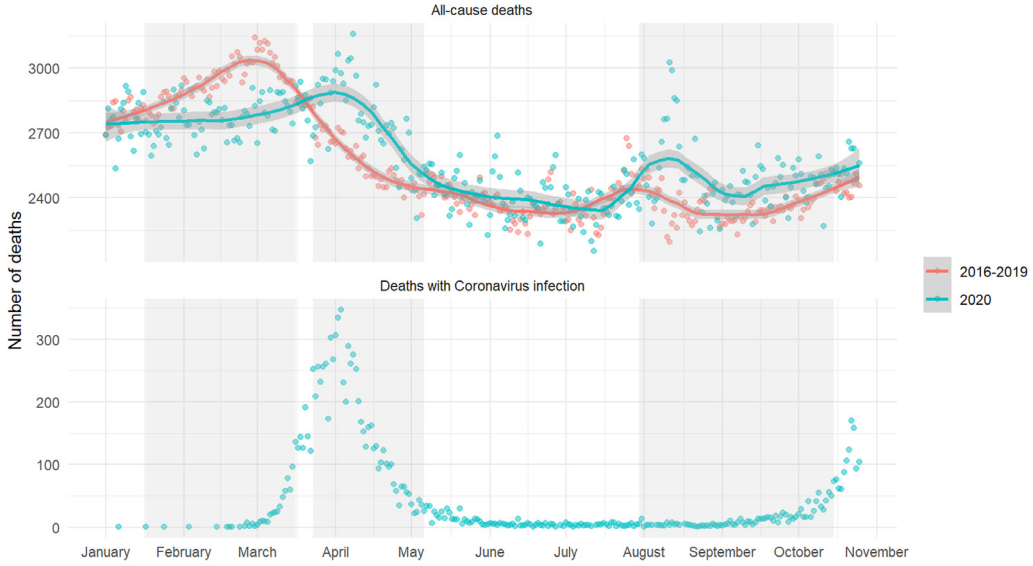

Fig. 1. Daily all-cause mortality in 2020 compared to previous years including illustrated intervals of significant differences and COVID-19-associated deaths (All dates in figures represent the 2020 data. Due to the leap year in 2020, there is no one-to-one match for daily data between 2016 and 2019 and 2020).

Table 1
All-cause deaths and interactions with age, gender and COVID-19 case volume in different observational periods.

time intervals	influencing factor	death counts (2016–2019)*	death counts (2020)*	IRR (95%CI)	*p* value	interactions IRR (95%CI)	*p* value
Total	overall	2551.0	2582.8	1.012 (1.009–1.016)	<0.01	/	/
	age [years]						
	<65	2648.9	2605.5	0.984 (0.976–0.992)	<0.01	1.029 (1.017–1.041)	<0.01
	65–74	2671.9	2703.2	1.012 (0.1.003–1.020)	<0.01	1.037 (1.027–1.046)	<0.01
	>75	12,528.2	12,774.6	1.020 (1.016–1.024)	<0.01		
	gender						
	female	9058.7	9046.6	0.999 (0.994–1.003)	0.55	1.029 (1.023–1.036)	<0.01
	male	8790.3	9036.6	1.028 (1.023–1.033)	<0.01		
	COVID-case volume						
	low	558.7	562.2	1.006 (0.999–1.013)	0.08	1.002 (0.993–1.011)	0.65
	intermediate	651.2	656.5	1.008 (1.002–1.015)	<0.01	1.011 (1.003–1.019)	<0.01
	high	1341.1	1364.0	1.017 (1.013–1.022)	<0.01		
Winter	overall	2943.3	2769.8	0.941 (0.935−0.947)	< 0.01	/	/
	age [years]						
	<65	2861.7	2728.7	0.953 (0.938–0.969)	<0.01	1.008 (0.985–1.032)	0.50
	65–74	2981.5	2865.5	0.961 (0.946–0.977)	<0.01	0.992 (0.974–1.010)	0.37
	>75	14,620.7	13,824.7	0.946 (0.939–0.953)	<0.01		
	gender						
	female	10.523.5	9759.9	0.927 (0.919–0.936)	<0.01	1.048 (1.035–1.061)	<0.01
	male	9940.5	9659.0	0.972 (0.963–0.980)	<0.01		
	COVID-case volume						
	low	646.0	602.7	0.933 (0.920–0.947)	<0.01	1.005 (0.986–1.026)	0.63
	intermediate	750.6	703.8	0.938 (0.925–0.950)	<0.01	1.012 (0.995–1.029)	0.11
	high	1546.7	1463.3	0.946 (0.937–0.955)	<0.01		
Spring surge	overall	2556.8	2757.2	1.078 (1.070−1.087)	< 0.01	/	/
	age [years]						
	<65	2646.2	2667.7	1.008 (0.988–1.029)	0.43	1.046 (1.017–1.077)	<0.01
	65–74	2668.4	2814.7	1.055 (1.034–1.076)	<0.01	1.088 (1.064–1.113)	<0.01
	>75	12,464.7	13,678.1	1.097 (1.087–1.107)	<0.01		
	gender						
	female	8999.7	9538.7	1.060 (1.048–1.071)	<0.01	1.034 (1.018–1.050)	<0.01
	male	8779.6	9621.9	1.096 (1.084–1.108)	<0.01		
	COVID-case volume						
	low	564.0	579.4	1.027 (1.010–1.045)	<0.01	1.029 (1.005–1.053)	0.02
	intermediate	653.8	690.8	1.057 (1.040–1.074)	<0.01	1.081 (1.059–1.104)	<0.01
	high	1339.0	1487.0	1.111 (1.098–1.123)	<0.01		
Summer surge	overall	2368.7	2486.0	1.050 (1.043−1.056)	< 0.01	/	/
	age [years]						
	<65	2529.2	2509.2	0.992 (0.976–1.008)	0.33	1.050 (1.026–1.073)	<0.01
	65–74	2527.8	2632.1	1.041 (1.025–1.058)	<0.01	1.066 (1.048–1.085)	<0.01
	>75	11,582.3	12,253.5	1.058 (1.050–1.066)	<0.01		
	gender						
	female	8416.2.	8737.2	1.038 (1.029–1.047)	<0.01	1.008 (0.992–1.025)	0.02
	male	8223.1	8657.6	1.053 (1.044–1.062)	<0.01		
	COVID-case volume						
	low	517.9	541.0	1.044 (1.030–1.059)	<0.01	1.008 (0.989–1.026)	0.42
	intermediate	605.5	637.2	1.052 (1.039–1.066)	<0.01	1.006 (0.989–1.022)	0.50
	high	1245.2	1307.8	1.050 (1.041–1.060)	<0.01		

* Death counts are given on a daily basis for the overall comparison of periods / COVID-19 case volume and on a weekly basis for age / genderIRR: incidence rate ratio.

than in women in all studied periods. COVID-19 case volume interacted with death counts overall and during the spring surge, but not in the other intervals. Results of interaction analysis including CIs and p-values are shown in Table 1.

Our study showed temporal evolution of all-cause death rates during the course of the ongoing COVID-19 pandemic with the longest observational period so far. Findings from Stang and colleagues were confirmed with an excess mortality during spring most likely attributable to COVID-19-associated deaths as indicated by the timely correlation and the pronounced effect in areas with high infection rates.[3] Higher excess death rates in men, groups of higher age and in areas with high COVID-19 case numbers are in accordance to previous studies.[2,9] Of note, an overall excess mortality in 2020 resulted due to another increase in death rates in August/September, which cannot be explained by COVID-19-attributed. The Federal Bureau of Statistics suspects a connection to the extraordinary summer heat wave which might be a possible explanation. However, being aware of the substantial changes in care pathways even of patients without COVID-19, this could also be a consequence of postponed treatments and patients' avoidance of entering the health care system.[6] This assumption is supported by findings of higher out-of-hospital cardiac arrest rates and proportional increases in deaths due to heart diseases.[10] Whether there is an additional effect of late COVID-19 sequelae is unclear. Further research is needed to confirm our findings and identify causes of this alarming trend.

Declaration of Competing Interest

Nothing to declare.

Funding

There has been no funding in connection with this study.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Accepted 19 December 2020
Available online 23 December 2020

https://doi.org/10.1016/j.jinf.2020.12.018

Evaluation of the rapid antigen test Panbio COVID-19 in saliva and nasal swabs in a population-based point-of-care study

Dear Editor,

Recent articles in this Journal have described the usefulness of saliva to detect SARS-CoV-2 infection through real-time reverse transcription polymerase chain reaction (rRT-PCR) [1,2]. We analyzed the performance of antigen-detecting rapid diagnostic tests (Ag-RDT) in saliva and nasal samples.

Ag-RDT directly identify SARS-CoV-2 proteins produced by replicating virus in respiratory secretions [3]. In contrast to the reference nucleic acid amplification tests (NAAT), such as rRT-PCR, the Ag-RDT are relatively inexpensive, simple to perform, do not require infrastructure, and enable obtaining point-of-care results within a few minutes [4]. Accordingly, despite being less sensitive than NAAT, Ag-RDT are more advantageous for guiding patient management at point-of-care, repeat testing, and timely large-scale public health decisions to prevent transmission [5,6]. Panbio COVID-19 Ag-RTD (Abbott Rapid Diagnostic Jena GmbH, Jena, Germany) is a recent generation, highly sensitive and specific antigen test for the qualitative detection of SARS-CoV-2 antigen in human nasopharyngeal swab (NPS) specimens. Because obtaining a NPS requires trained healthcare professionals and a personal protective equipment (PPE), availability of a simpler and accurate alternative sample that could even be self-collected, like nasal swab (NS) or saliva, would further ease the procedure and allow large-scale testing. We evaluated the performance of Panbio COVID-19 Ag-RDT in NS and saliva specimens compared with rRT-PCR in

Table 1
Performance of the Panbio COVID-19 antigen Rapid Test Device.

Overall	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
NP sample	76	1	519	56	57.3% (48.3–65.8)	99.8% (98.8–100)
Nasal sample	59	0	527	73	44.7% (36.1–53.6)	100% (99.1–100)
Saliva sample	28	0	489	93	23.1% (16.2–31.9)	100% (99–100)
Nasal+saliva	60	0	489	61	49.6% (40.4–58.8)	100% (99–100)
Nasal sample						
Symptomatic	51	0	297	46	52.6% (42.2–62.7)	100% (98.4–100)
Ct ≤ 25	43	0	0	12	78% (65–88)	
Ct ≤ 30	48	0	0	25	66% (54–76)	
Ct ≤ 35	51	0	0	43	54% (44–64)	
Asymptomatic	8	0	230	27	22.9% (11–40.6)	100% (98–100)
Ct ≤ 25	8	0	0	5	62% (32–85)	
Ct ≤ 30	8	0	0	15	35% (17–57)	
Ct ≤ 35	8	0	0	24	25% (0–69)	
Saliva sample						
Symptomatic	25	0	277	64	28.1% (19.3–38.8)	100% (98.3–100)
Ct ≤ 25	21	0	0	30	41% (28–56)	
Ct ≤ 30	25	0	0	42	37% (26–50)	
Ct ≤ 35	25	0	0	61	29% (20–40)	
Asymptomatic	3	0	212	29	9.4% (2.5–2.6)	100% (97.8–100)
Ct ≤ 25	3	0	0	9	25% (7–57)	
Ct ≤ 30	3	0	0	17	15% (4–39)	
Ct ≤ 35	3	0	0	26	10% (3–28)	
Nasal+saliva						
Symptomatic	55	0	277	37	58.4% (47.5–68.6)	100% (98.3–100)
Ct ≤ 25	45	0	0	8	85% (72–93)	
Ct ≤ 30	52	0	0	18	74% (62–84)	
Ct ≤ 35	55	0	0	34	62% (51–72)	
Asymptomatic	9	0	212	24	27.3% (13.9–45.8)	100% (97.8–100)
Ct ≤ 25	9	0	0	4	69% (39–90)	
Ct ≤ 30	9	0	0	12	43% (23–66)	
Ct ≤ 35	9	0	0	21	30% (0–69)	

Unless specified, all analyses have been performed in nasopharyngeal samples. TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPA, positive percent agreement; NPA, negative percent agreement; NP, nasopharyngeal; Ct, cycle threshold of RT-PCR.

NPS in a large prospective study conducted in three primary care centers between 15th September and 29th October 2020. Consecutive adults and children, either with COVID-19 signs/symptoms or asymptomatic contacts, were included. Informed consent was obtained from all the patients, and the study was approved by the Hospital General Universitario de Elche COVID-19 Institutional Advisory Board. Patients were asked to fill a questionnaire about symptoms and to collect saliva into a 100 ml sterile empty container. Then, a NS and two consecutive NPS were obtained by a qualified nurse according to the recommended standard procedure. The antigen kit was used according to the manufacturer's instructions. rRT-PCR testing was performed according to the manufacturer's guidelines on Cobas z 480 Analyzer (Roche, Basilea, Suiza). Positive and negative percent agreement (PPA, NPA) were calculated for Panbio antigen test in the NPS, NS and saliva samples compared to the rRT-PCR test in NPS. The study included 659 patients with NS samples, of whom 610 (92.6%) had a saliva sample. 265 (40.2%) patients were asymptomatic and 394 (59.8%) had symptoms, with median (Q1-Q3) duration of 3 (2–5) days. Median (Q1-Q3) age was 38 (21–49.8) years, 76 (11.5%) had ≤14 years, 45 (7.6%) >65 years, 372 (56.4%) were women, and 157 (23.8%) had a comorbid condition, the most frequent hypertension in 46 (7%), dyslipidemia in 39 (5.9%), obesity in 29 (3.2%) and diabetes in 21 (4.4%) patients. rRT-PCR was positive in NPS in 132 (20%) patients, with median (Q1-Q3) cycle threshold (Ct) of rRT-PCR of 24 (17.6–31). Table 1 shows the performance of Ag-RDT in NS, saliva and NS/saliva. Ag-RDT was positive in 76 (11.7%), 59 (9%), 28 (4.6%) and 60 (9.1%) NPS, NS, saliva and any of NS or saliva (NS/saliva) samples, respectively. Median (Q1-Q3) Ct value in NPS of antigen-positive NS samples was 17 (14–21.5) and of antigen-negative NS samples 29.5 (25.6–33); and 17.9 (15.8–19.3) and 28 (19.6–32) in antigen-positive and antigen-negative saliva samples, respectively. The PPA (95% CI) was 57.3% (48.3–65.8) in NPS, 44.7% (36.1–53.6) in NS, 23.1% (16.2–31.9) in saliva, and 49.6% (40.4–58.8) in NS/saliva. In all cases, NPA was 100%. Ag-RDT performance was dependent on the Ct values and the presence of symptoms (Fig. 1A-C). For symptomatic patients with Ct<25, the PPA (95% CI) was 78% (65–88) in NS, 41% (28–56) in saliva and 85% (72–93) in NS/saliva samples. Ag-RDT performed better with duration of symptoms <7 days (Fig. 1D). The best test performance was observed for NS/saliva in symptomatic patients with <7 days and Ct≤25, with PPA (95% CI) of 92% (78–98), and 85.1% (71.1–93.3) for Ct ≤ 30. In NS, PPA was 87.8% (72.9–95.4) and 79.6% (65.2–89.3) for Ct ≤ 25 and Ct ≤ 30, respectively, and <7 days with symptoms. Symptoms associated with higher sensitivity of the Ag-RDT in NS/saliva samples were sore throat, with PPA (95% CI) of 69% (49–84), and ageusia with 66% (12.5–98.2). Results from this large study show that the overall sensitivity of Panbio Ag-RDT was lower in NS and saliva than in NPS, particularly in asymptomatic patients, although the specificity was 100% in all samples. The same as with Ag-RDT in NPS, sensitivity was highly dependent on the Ct values and the presence and duration of symptoms [7]. In NS samples, the sensitivity in symptomatic patients with Ct ≤ 30 and duration of symptoms <7 days met the minimum test performance requirements to be adequate for the diagnosis of SARS-CoV-2 infection [5], although the greatest performance was observed with the combination of NS and saliva samples. Therefore, although the saliva alone did not show a satisfactory performance, it added sensitivity to the NS for SARS-CoV-2 diagnosis. Infectivity risk has been associated with Ct values and duration of symptoms, with no viral growth observed in samples with PCR Ct values >25–30 [8,9], and symptom duration >8 days [9,10]. Consequently, the contagious risk of symptomatic patients not detected by the Ag-RDT in NS/saliva samples may be low. In addition to self-collection, NS and saliva samples allow performing the test without safe isolation conditions requirement to avoid propagation, thereby widening the

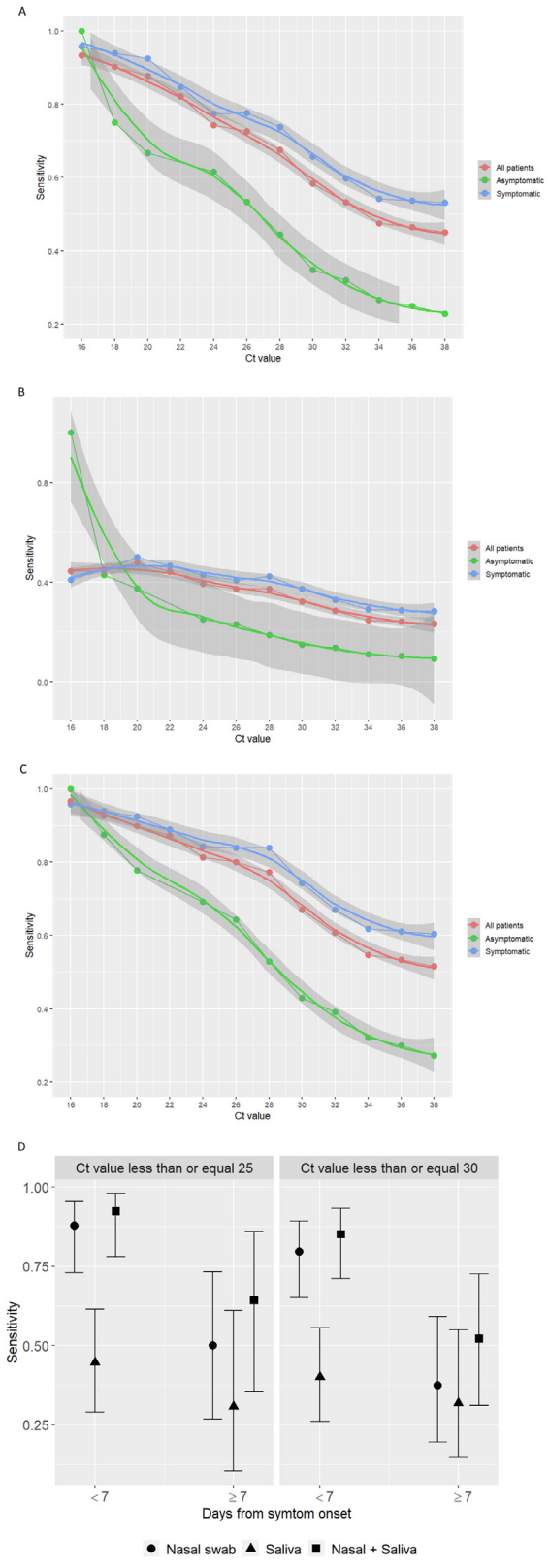

Fig. 1. Performance of Panbio COVID-19 antigen Rapid Test Device in nasal, saliva and nasal + saliva samples according to the presence of symptoms and cycle threshold values. A: Performance in nasal samples. B: Performance in saliva samples. C: Performance in nasal + saliva samples. D: Performance in symptomatic patients according to cycle threshold values and days from symptom onset.

settings where the test can be performed, and facilitating the procedure in children since it causes much less discomfort. Moreover, the same diagnostic kit could even be used to analyze both samples, through insertion of the NS in the saliva specimen. In conclusion, because of the low performance observed in asymptomatic patients, NS and saliva samples are not good options for screening or surveillance of SARS-CoV-2 with Ag-RDT. However, in settings with no availability of PPE or trained personnel, or with no safe conditions for the Ag-RDT procedure, the combination of saliva and nasal samples could be a suitable alternative to the NPS for the point-of-care diagnosis of symptomatic patients with SARS-CoV-2 infection.

Declaration of Competing Interest

None.

Acknowledgements

Members of the COVID19-Elx-Rapid Diagnostic Tests Study Group

Félix Gutiérrez, Mar Masiá, Sergio Padilla, Guillermo Telenti, Lucia Guillen, Cristina Bas, María Andreo, Fernando Lidón, Vladimir Ospino, José López, Marta Fernández, Vanesa Agulló, Gabriel Estañ, Javier García, Cristina Martínez, Leticia Alonso, Joan Sanchís, Ángela Botella, Paula Mascarell, María Selene Falcón, Sandra Ruiz, José Carlos Asenjo, Carolina Ding, Mar Carvajal, Inmaculada Candela, Jorge Guijarro, Cristina la Moneda, Cristina Jara, Raquel Mora, Juan Manuel Quinto, Sergio Ros, Daniel Canal, Pascual Pérez, Montserrat Ruiz, Alba de la Rica, Carolina Garrido, Manuel Sánchez, Jaime Sastre, Carlos de Gregorio, Francisco Carrasco, Juan Navarro, Andrés Navarro, Nieves Gonzalo, Clara Pérez, Adoración Alcalá, José Luis Rincón, Juan Antonio Gutiérrez.

Funding

This work was supported by the RD16/0025/0038 project as a part of the Plan Nacional Research + Development + Innovation ($R+D+I$) and cofinanced by Instituto de Salud Carlos III - Subdirección General de Evaluación y Fondo Europeo de Desarrollo Regional; Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias [grant number PI16/01740; PI18/01861; CM 19/00160, COV20–00005]).

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Accepted 7 December 2020
Available online 9 December 2020

https://doi.org/10.1016/j.jinf.2020.12.007

Myxovirus resistance protein A in peripheral blood predicts supplemental oxygen need in COVID-19

Dear Editor,

We read with great interest the report on the usefulness of FebriDx (Lumos diagnostics, Sarasota, Florida, US) in the diagnosis of COVID-19 published in October in this journal[1]. FebriDx, which detects Myxovirus resistance protein A (MxA) and C reactive protein (CRP), is not available in Japan, but we also have considered that MxA would be useful for Coronavirus disease 2019 (COVID-19) practice. Therefore, we would like to introduce our study.

MxA induced exclusively by type I and III interferons (IFNs) as a specific response to viral infections, has activity against a wide range of viruses[2]. Although few studies have clarified the relationship between coronavirus and MxA, there is a report that symptomatic patients have higher MxA levels than asymptomatic patients in respiratory viral infections[3]. Therefore, we hypothesized that severely ill patients with COVID-19 had high blood MxA levels and that there was a difference in MxA levels between patients who would need oxygen and those would not. Since the need for supplemental oxygen is a major factor in determining whether a patient requires hospitalization, we examined the relationship between MxA levels and the need for supplemental oxygen and clarified whether MxA is a useful predictor of oxygen demand.

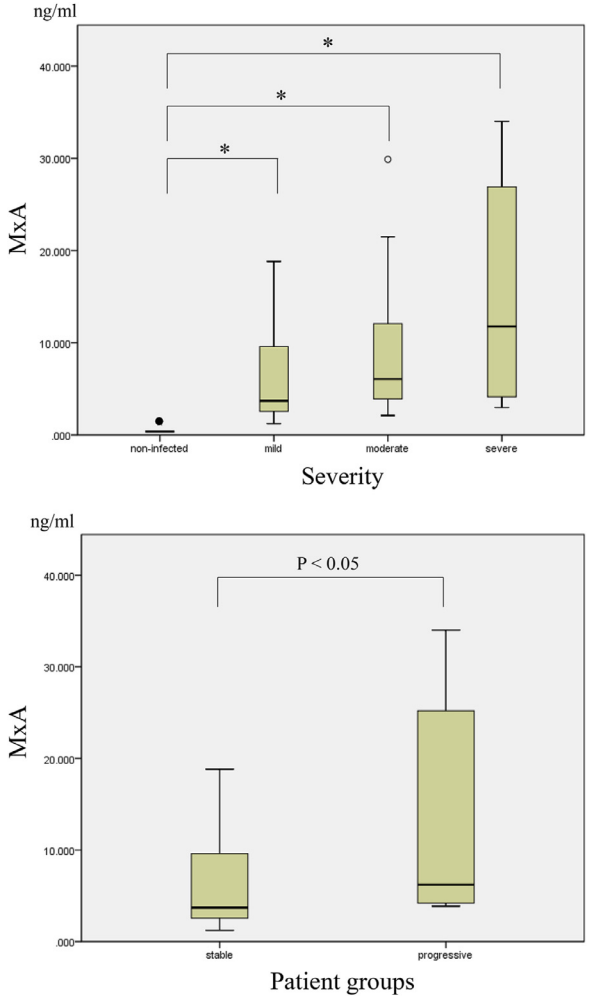

Fig. 1. Relationship between COVID-19 severity and MxA levels. Blood MxA levels in COVID-19 patients. Boxes show the median (line) and interquartile range (IQR) Whiskers show the highest and lowest values that are no greater than 1.5 times the IQR. A) Comparison of COVID-19 patients with non-infected individuals. Patients were divided according to severity as follows: mild, patients who did not require oxygen supplementation; moderate, patients who required oxygen by conventional nasal cannula; severe, patients who required oxygen by high-flow nasal cannula or ventilator. The days after onset when sample was taken were as follows: mild, 5 days [4–6]; moderate, 7 days [5–8.5]; severe, 7.5 days [5.25–8], respectively). The clear circle indicates an outlier case with values 1.5–3.0 times the IQR. The black circle indicates an extreme case with values >3 times the IQR. *$p < 0.001$ by Kruskal–Wallis test. B) MxA levels in patients who did not require supplemental oxygen at admission. Patients who did not require oxygen supplementation at the time of admission were divided stable patients who did not require oxygen supplementation throughout hospitalization (stable) and progressive patients who did (progressive). The blood samples of two groups were taken at approximately the same days after onset (5 days [4–6] and 5 days [4–7], respectively). There was a significant difference between the two groups ($p < 0.05$ by Mann–Whitney U test).

This study investigated 48 patients with COVID-19 who were admitted to Japan Self-Defense Forces Central Hospital in Tokyo, Japan. All patients were SARS-Cov-2 positive Japanese adults who were confirmed by real-time polymerase chain reaction (PCR) using a nasopharyngeal swab specimen and confirmed to have pneumonia by chest computed tomography. We also included 14 non-infected outpatients with metabolic syndrome. We excluded participants had lung disease or had used antivirals or steroids before admission. The whole blood MxA levels were quantified by sand-

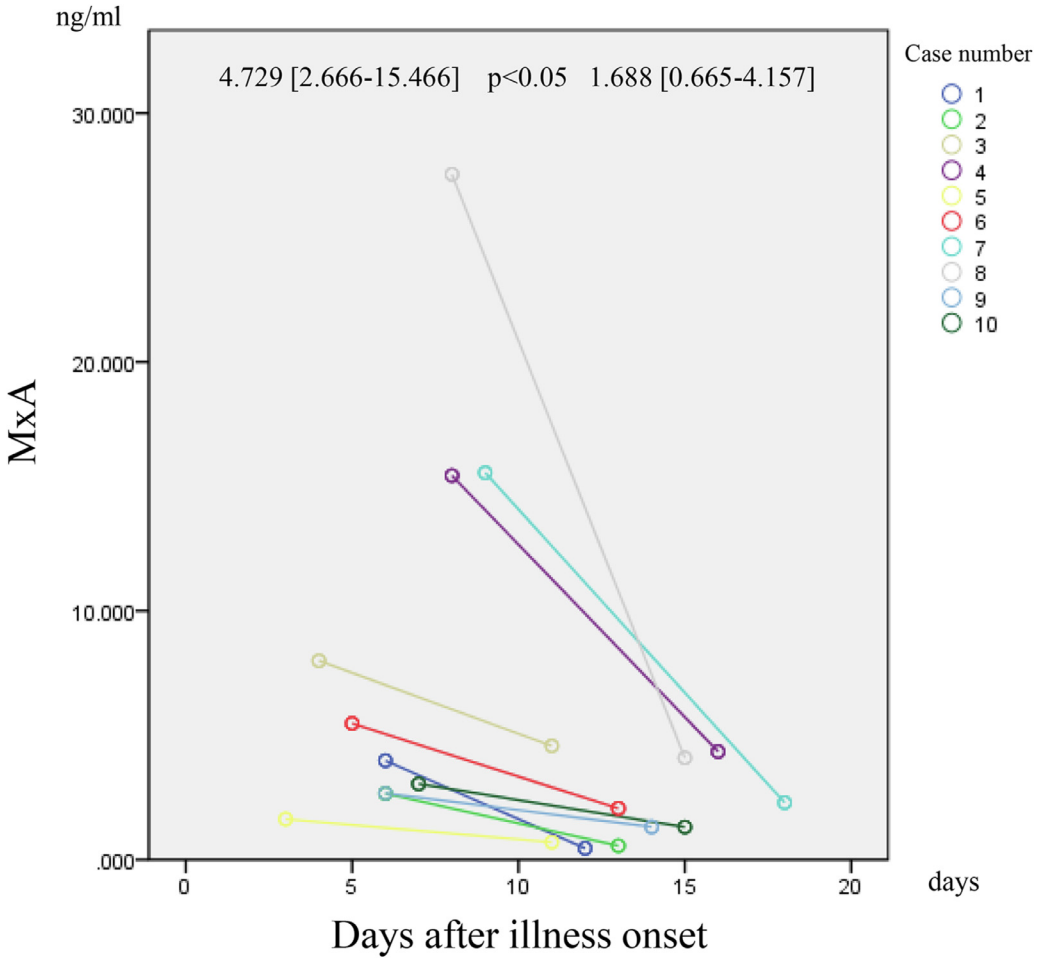

Fig. 2. Dynamics of MxA. Blood MxA levels in the same individuals at admission and several days thereafter. The median and interquartile range are shown above the groups; p < 0.05 by Wilcoxon signed-rank test.

wich enzyme-linked immunosorbent assay (ELISA) using a MxA Protein Human ELISA kit (BioVendor GmbH, Heidelberg, Germany). The obtained values are shown by as median [interquartile range, IQR]. Statistical analyses were performed using SPSS v19 software for Windows (IBM Corp., Armonk, NY).This study was reviewed and approved by Japan Self-Defense Forces Central Hospital (approval number 02–025).

We compared the patient oxygen demand during hospitalization, and categorized the patients into four groups as follows: non-infected individuals (non-infected, $n = 14$), patients who did not require oxygen (mild, $n = 23$), patients who required oxygen by conventional nasal cannula (moderate, $n = 15$), and patients who required oxygen by high-flow nasal cannula or ventilator (severe; $n = 10$). The three groups of patients with COVID-19 had significantly higher MxA levels than the non-infected group (0.386 [0.371–0.395]; $p < 0.001$). Severely ill patients showed the highest MxA levels (mild, 3.715 [2.560–9.600]; moderate, 6.079 [3.922–12.084]; severe, 11.777 [5.216–25.183]) (Fig. 1A). This result highlights the efficacy of MxA in the diagnosis of COVID-19, and agrees with the report by Clark et al[1]. Furthermore, MxA was also associated with the severity of COVID-19.

Next, we divided the 35 patients who did not require oxygen at the time of admission into two groups: those who did not require oxygen supplementation during hospitalization (stable patients, $n = 23$) and those that did (progressive patients, $n = 12$). The progressive patients had significantly higher MxA levels than the stable patients (6.203 [4.237–23.350] vs 3.715 [2.560–9.600]; $p < 0.05$) (Fig. 1B). This result shows that MxA predicts the need for supplemental oxygen in COVID-19.

Additionally, we investigated the dynamics of MxA levels in 10 patients using blood specimens drawn at admission and several days thereafter. MxA levels decreased after admission in all cases (4.729 [2.666–15.466] vs 1.688 [0.665–4.157]; $p < 0.05$), suggesting that they peak before onset or in the early stage of disease (Fig. 2).

There were reported that the impaired response of IFN type I and III in patients with COVID-19 wasassociated with a persistent blood viral load and an exacerbated inflammatory response, leading to severe illness[4–7]. Based on these reports and taking into consideration that MxA expression is strictly controlled by type I and III IFNs[2,8], MxA expression should be suppressed in COVID-19 patients, especially those who are critically ill. In contrast, all patients in our study, including those critically ill on mechanical ventilation, had significantly higher levels of MxA than the non-infected individuals. Additionally, we found that MxA levels were higher in critically ill patients and patients requiring oxygen supplementation (Fig. 1). Impaired IFNs response can be due to either reduced or delayed IFNs production. Our results support the delayed IFNs production theory[6,7] rather than that of reduced IFNs production[4,5]. Delayed IFNs may be associated with a high blood viral load, which increase IFNs production and leads high MxA production.

Our finding that MxA declines from the early stage of onset (Fig. 2) is similar to the levels of type I IFN over time in patients with COVID-19[4,9]. When taking into consideration that MxA has

fast induction time, up to 10-fold higher MxA protein levels were observed at 24–48 h after IFN induction in vitro[10], we think that MxA has a peak in the early stage of infection. It suggests may be useful as an auxiliary test instead of real-time PCR, which is insufficiently sensitive to diagnose SARS-CoV-2 infection, not only in the early stage of onset but also in the incubation period. When applied as a screening test for immigration, it may significantly reduce the number of people who need to be quarantined for 14 days.

However, the rapid change in MxA over time also means that care must be taken when interpreting MxA level. In other words, when comparing data, samples with the same days after onset should be compared. Detailed data on changes over time will be needed for clinical application.

In conclusion, we revealed that MxA in peripheral blood predicts the need for supplemental oxygen in patients with COVID-19. We consider that our results are beneficial from a clinical viewpoint. We hope that research on the relationship between MxA and COVID-19 will progress and subsequently contribute to clinical practice.

Declaration of Competing Interest

The authors declare no conflicts of interest associated with this manuscript.

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Accepted 6 December 2020
Available online 8 December 2020

https://doi.org/10.1016/j.jinf.2020.12.006

Clinical efficacy and safety of favipiravir in the treatment of COVID-19 patients

Dear Editor,

Recently, Saito et al.[1] showed the first two COVID-19 waves in Japan and found the difference between first and second waves in terms of the burden of medical system, patients' clinical characteristics and outcomes. In addition, we also noted that 1806 (68.3%) patients had received favipiravir for at least once during their hospitalization in this series.[1] However, the efficacy of favipiravir for treating COVID-19 remained unclear and was not assessed in this study. In fact, several clinical studies[2-6] investigating the usefulness of favipiravir for COVID-19 patients have been published and provides us the opportunity to re- assess the clinical efficacy and safety of favipiravir. Therefore, we did a meta-analysis of these clinical studies to provide an update data to clarify this issue –the clinical efficacy and safety of favipiravir in the treatment of COVID-19 patients.

Only clinical studies that compared the clinical efficacy of favipiravir and other alternative agents or placebo in the treatment of COVID-19 patients were identified. The results of clinical improvement were extracted for the analysis of primary outcome. In addition, the rate of viral clearance and the risk of adverse events (AEs) were collected as secondary outcomes. All statistical analyses were performed using Review Manager (RevMan) version 5.3.

Overall, five clinical studies[2-6] fulfilled the inclusion criteria and were included in this meta-analysis. A total of 552 patients were enrolled in this study, including 252 and 300 patients received favipiravir and comparator, respectively. Except Cai et al's study was before-after controlled trial,[3] all the other were randomized controlled trials.[2,4-6] Three studies[3-5] was conducted in a single center and two were multicenter studies.[2,6] Three studies were conducted in China[3,4,6] and one each in Russia[2] and Oman.[5] The

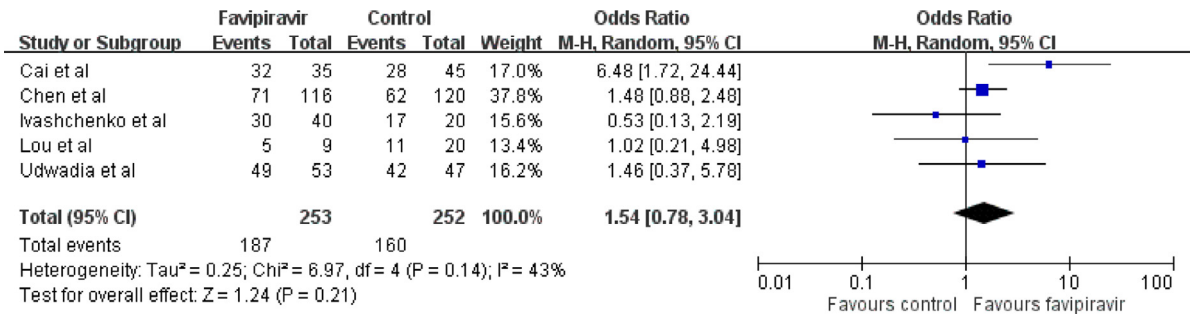

Fig. 1. Forest plot of clinical improvement rate between favipiravir and comparators.

comparator varied, including lopinavir/ritonavir,[3] umifenovir,[6] hydroxychloroquine,[5] baloxavir[4] and standard of care.[2,4]

First, the pooled analysis of five studies[2-6] showed that favipiravir was associated with a higher clinical improvement rate than control group, but the difference did not reach statistical significance (odds ratio [OR], 1.54; 95% CI, 0.78–3.04; $I^2 = 43\%$, Fig. 1). Second, no significant difference was observed between favipiravir and comparator in terms of viral clearance rate at day 4–5 (OR, 2.70; 95% CI, 0.65–11.28; $I^2 = 85\%$), at day 7–8 (OR, 1.08; 95% CI, 0.58–1.98; $I^2 = 0\%$), at day 10–12 (OR, 2.69 95% CI, 0.98–7.35; $I^2 = 57\%$) and at day 14–16 (OR, 2.32; 95% CI, 0.81–6.66; $I^2 = 36\%$). Finally, favipiravir group was associated with a similar risk of adverse event as control group (OR, 0.571.05; 95% CI, 0.15–7.13; $I^2 = 93\%$) in the pooled analysis of 3 studies.[3,5,6]

In this study, we cannot find the better clinical improvement and higher viral clearance rate of COVID-19 patients receiving favipiravir than those receiving comparators or standard of care. In addition, we also did not find that favipiravir was associated with a higher risk of adverse events than comparators. However, our findings should be interpreted cautiously due to several limitation. First, the study design and the comparators in each clinical study varied. Second, the numbers of study and patients were limited. Third, most findings were based on the analysis of data with high heterogeneity. Therefore, further large scale randomized controlled trial is warranted to investigate the role of favipiravir in the treatment of COVID-19 patients.

In conclusion, based on low-quality evidence, there is no conclusive evidence that favipiravir would provide any additional benefit to COVID-19 patients. Therefore, further recommendation of favipiravir for COVID-19 patients should be halted until high-quality evidence from further ongoing randomized controlled trials.

Funding

No.

Declaration of Competing Interest

None to declare.

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Accepted 4 December 2020
Available online 7 December 2020

https://doi.org/10.1016/j.jinf.2020.12.005

High rate of infections during ICU admission of patients with severe SARS-CoV-2 pneumonia: A matter of time?

Dear Editor,

A recent meta-analysis published in this journal showed that the prevalence of bacterial co-infections in critically ill COVID-19 patients in Intensive Care Units (ICUs) hover around 14% (95% CI 5–26%).[1] In contrast, other authors, applying molecular screening for co-infection, described co-infection rates as high as 28% and 41% in two ICUs.[2,3] In other meta-analysis,[4] secondary bacterial infection during admission on hospitalized adult patients was identified in 14.3% of COVID-19 patients (95% CI 9.6–18.9%), although this prevalence might not reflect the rates in ICUs. In this work, we want to report our findings in a Spanish ICU with high incidence of both co-infection and secondary infections during the first wave of the pandemic.

Table 1
Patient characteristics of infected and non infected patients.

Characteristic	Patients	No infection vs infection
Gender (male/female)	61/31 = 92	
Age (mean ± SD)	64 ± 12	
Days at ICU (median and IQR)	12 (5–25)	6 (2–11) vs 21 (12–37), $p < 0.01$
Days of ICU in patients with MV	17 (10–28)	9 (4–13) vs 22 (16–40), $p < 0.01$
Days of MV (median and IQR)	13 (8–26)	8 (4–11) vs 18 (12–18), $p < 0.01$
Scores II (mean ± SD):		
APACHE	16 ± 5	
SOFA	7 ± 3	
Barthel	98 ± 7	
Comorbidities (%)		
Hypertension	40%	
Obesity	40%	
Smoking	37%	
Lung disease	34%	
Organ failure (%)		
Hemodynamic	50%	
Acute renal failure	41%	
Multiple organ dysfunction syndrome	42%	
Coagulopathy	24%	
Liver failure	10%	
Life support (%)		
Vasopressors	72%	62% vs 83%, $p < 0.05$
Mechanical ventilation (MV)	75%	61% vs 85%, $p < 0.01$
Prone position	56%	42% vs 70%, $p < 0.01$
CRRT	8%	
ECMO	2%	
Death (%)	33%	
Treatment (%)		
Lopinavir/ritonavir	91%	
Hydroxychloroquine	91%	
Interferon	46%	
Tocilizumab	26%	
Antibiotics	90%	82% vs 96%, $p < 0.03$
Ceftriaxone	64%	
Azithromycin	47%	
Levofloxacin	37%	
Piperacillin-tazobactam	32%	16% vs 43%, $p < 0.01$
Linezolid	27%	11% vs 39%, $p < p < 0.04$
Meropenem	20%	8% vs 28%, $p < 0.03$
Ceftazidime	11%	5% vs 33%, $p < 0.01$
Antifungal	12%	0% vs 20%, $p < 0.01$
Methylprednisolone	51%	
Dexamethasone	17%	

We prospectively included all consecutive patients who were admitted to the ICU at the Araba University Hospital in Vitoria-Gasteiz (Spain) with the diagnosis of severe pneumonia caused by SARS-COV-2 between March 4th and June 2nd, 2020 (first wave). Outcomes of the first 48 patients were reported previoulsy.[5] The study protocol was approved by the hospital Ethics and Clinical Research Committee, and informed consent was waived. All patients had a positive SARS-CoV-2 test using real-time reverse transcriptase PCR (Cobas Roche Diagnostics SLU) either from nasopharyngeal swabs or lower respiratory tract aspirates. Data were expressed as median and interquartile range or percentages as appropriate.

Ninety-two patients were admitted to the ICU during this period. Table 1 shows the demographic and clinical data of the patients. In 63 patients, broncho-alveolar lavage (BAL)/trach aspirates were collected for microbiologic culture, and in 33 of them (52%), an automated multiplex PCR test targeting 27 pathogens and 7 antimicrobial resistance genes was performed (analysis time of about 67 min; Film BIOFIRE® FILMARRAY® Respiratory 2.1 plus Panel, FA-RP). None of the 33 FA-RP tests (14 performed on admission) identified other respiratory viruses.

At admission or in the first 48 h of stay in the ICU, 32 microbial isolates were found in 24 patients (26%, 24/92). In these patients, concordant results between the FA-RP ($\geq 10^4$ DNA copies/ml) and cultures (BAL cut-off of 10^4 CFU/ml) were obtained in 11 of 14 patients (overall agreement = 78%, kappa = 0.59 [95% CI 0.21–0.96]). Discordant results were obtained in 3 samples (*Moraxella catarrhalis, Proteus* spp and *Streptococcus agalactiae*). Table 2 shows the microbial isolates obtained from microbiologic cultures. The most frequently isolated microorganisms in respiratory samples were *Staphylococcus aureus, Streptococcus pneumoniae* and *Haemophilus influenzae*, microorganisms that commonly colonise the nasopharynx. Other identified isolates were *Pseudomonas aeruginosa, Serratia marcescens* and *Enterococcus faecium* (mostly in respiratory samples), microorganisms that are frequently nosocomial. These data contrast with the low rates reported by other authors in Spain[6,7] and focus on the peculiarity of critical patients also in this scenario. Likewise, it should be noted that the hospital stay before admission to the ICU of the patients with *P. aeruginosa* infection was markedly longer (median 9 days) than that of the general group (median 3 days).

On the other hand, 125 microbial isolates were found in 43 patients (47%, 43/92) during their stay in the ICU. Most samples were respiratory (52%), followed by urinary (22%), blood (18%) and catheter tips (8%). The most common isolated microorganisms were *P. aeruginosa, E. faecium* and *Enterobacterales,* which represent half of the isolates in all secondary infections. *Candida* spp isolated from respiratory samples and coagulase-negative staphylococci in blood cultures and urine cultures may be considered as normal microbiota or contaminants, respectively. The contam-

Table 2
Clinical isolates from microbiologic cultures distributed by infectious site.

Isolates	RC		BC		UC		Catheter		Others		Total	
S. pneumoniae	5	0	0	0	0	0	0	0	0	0	5	0
H. Influenzae	5	1	0	0	0	0	0	0	0	0	5	1
S aureus	5	6	1	0	1	0	0	0	0	0	7	6
CNS	0	2	0	5	0	9	0	3	0	0	0	19
E. faecalis	1	6	0	2	0	5	0	1	0	0	1	14
E. faecium	1	4	0	4	1	9	0	1	0	1	2	19
P. aeruginosa	4	14	0	3	0	3	0	1	0	1	4	22
M. catarrhalis	2	0	0	0	0	0	0	0	0	0	2	0
S. marcescens	3	1	1	0	0	0	0	0	0	0	4	1
Stenotrophomonas	1	5	1	0	0	0	0	0	0	0	2	5
E. coli	0	8	0	0	0	3	0	0	0	0	0	11
Candida species	0	7	0	1	0	6	0	0	0	1	0	15
Aspergillus	0	2	0	0	0	0	0	0	0	0	0	2
Klebsiella	0	2	0	1	0	0	0	0	0	1	0	4
Enterobacter spp	0	4	0	1	0	0	0	1	0	0	0	6
Total	27	62	3	17	2	35	0	7	0	4	32	125

RC: respiratory cultures (sputum or tracheal aspiration), BC: blood cultures, UC: urine cultures.
First column: Coinfection Second column; Secondary infection

inant isolates may be explained by the use of personal protective equipment and the unfamiliarity of protocols by healthcare workers who do not usually work in the ICU. During admission, concordant results between the FA-RP and cultures were obtained in 12 out 19 patients (overall agreement = 63%, kappa = 0.31 [95% CI -0.05–0.67]). Discordant results were obtained in 6 samples, *E. faecalis* (2), *Aspergillus fumigatus* (2), *E. faecium* (1) and *Candida albicans* (1), targets not included in the panel. Out the two patients with culture-positive of *A. fumigatus*, one was considered to be colonized, and the other one, which had a positive galactomannan serum antigen, was considered as possible case of COVID-19 associated pulmonary aspergillosis (CAPA), and treated accordingly. Even though the AspICU algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients requires a positive respiratory culture to identify *Aspergillus*, the real prevalence remains elusive because of the current absence of a standardized definition for non-proven disease in non-neutropenic critically ill patients.[8,9] Nine multidrug-resistant strains were isolated, which represented 6% of microbial isolates: extended-spectrum beta-lactamase *E. coli* (4); multi-resistant *P. aeruginosa* (2), and methicillin-resistant *S. aureus* (3).

Regarding the consumption of antimicrobials, 90% of the patients received at least one antibiotic for a median of 6 days (IQR 2–10). Twelve percent of patients received antifungal treatment. Regarding the analytical data, the maximum levels of procalcitonin (Abbott Alinity I BRAHMS PCT) were significantly lower in patients who did not present infection (median 0.4 ng/mL (IQR 0.1–1.4) vs median 1.2 ng/mL (IQR 0.3–2.6). Although it is known that procalcitonin is a non-sensitive marker,[10] it can be useful, framed in the appropriate clinical context, to suspect the presence of an associated infection.

Infected patients upon admission to the ICU presented significantly longer ICU stay (median 21 vs 6 days), required mechanical ventilation (83% vs 62%) and/or prone position (70% vs 40%) more frequently, and were the patients who received more antibiotics to treat nosocomial infections (see table 1). However, we cannot determine whether longer stays is a cause or a consequence of the development of infection. Finally, the overall mortality was 33%. without difference regardless the presence of an infection, in contrast to what it was observed by other authors.[6,10]

In conclusion, based on our experience, the incidence of infections during SARS-Cov-2 infection, both at the beginning and during admission, is higher than that reported by other authors, which leads to greater morbidity, longer stay, higher antimicrobial use, and potential selection for resistant microorganisms. We must optimize the antimicrobial stewardship concept to tackle the challenge we face.

Acknowledgment

The authors would like to thank all the staff of the ICU and the microbiology laboratory of the Araba University Hospital for the enormous effort made during these months to guarantee clinical care and, in addition, to be able to carry out this research.

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Accepted 2 December 2020
Available online 5 December 2020

https://doi.org/10.1016/j.jinf.2020.12.001

Role of endothelial dysfunction in the thrombotic complications of COVID-19 patients

Dear Editor,

We note with interest the review of Kunutsor SK. et al.[1] on cardiovascular implications of coronavirus disease 2019 (COVID-19). Indeed, Sars-Cov-2 infection begins in lungs but moves rapidly to the vascular system with platelet alterations and blood clotting abnormalities, and associates with a high incidence of cardiovascular events and venous thromboembolism (VTE), especially in critically ill patients (10–34%).[2]

Based on autopsy findings, endothelial injury has been hypothesized to play a crucial role in the Sars-Cov-2 associated procoagulant condition.[3] Very few studies, however, have assessed circulating biomarkers of endothelial damage in COVID-19 patients. Among these particularly interesting are circulating endothelial cells (CECs), circulating endothelial progenitor cells (EPCs), endothelial extracellular vesicles (EEVs) and soluble forms of endothelial adhesive proteins (CAM) which are known to be altered in conditions associated with enhanced cardiovascular risk and to be predictive of vascular complications in various conditions, including infectious diseases.[4] For these parameters no data are available, to our knowledge, in Sars-Cov-2 infection. Aim of our study was to assess the role of cellular and soluble circulating endothelial derangement parameters as markers of endothelial damage in COVID-19 patients and to unravel if they may identify patients developing VTE or adverse outcome.

Fifty-six COVID-19 patients and 36 healthy, age- and sex-matched controls were enrolled in a multicenter study in the Umbria Region, Italy. Peripheral blood was collected for EEVs, CECs and EPCs by flow cytometry and for sVCAM and sICAM by ELISA.[5] Four of the enrolled patients were reassessed after disease recovery confirmed by 2 negative nasopharyngeal swabs (mean 72.7 days, 95% CI 32.8–112.6, after the second).

Statistical analyses were performed using GraphPad Prism 8.4 for Windows software. Data not normally distributed were analyzed with the Mann Whitney test; otherwise with the two-tailed unpaired Student's *t*-test. $P<0.05$ was considered statistically significant.

The study was approved by the local Ethics Committees (CEAS Umbria n. 3656/20, University of Perugia Bioethics Committee n. 2020–36,346).

Demographics, clinical and main laboratory features of the study population are summarized in Table 1. All patients were hospitalized (median hospitalization 26 days) and were studied on average 4.2 ± 0.5 days (95%CI 3.2–5.2) from the last positive nasopharyngeal swab. Sixteen patients (28.5%) were admitted into the intensive care unit (ICU) while the others into non-ICU COVID-19 wards. Five ICU and 3 non-ICU patients died during hospitalization. Of the 56 patients, 19 had a partial pressure of oxygen/fraction of inspiration oxygen ratio (PaO_2/FiO_2) lower than 300 and 31 required mechanical ventilation (15 invasive and 16 non invasive). Ten (17.8%) developed 11 thrombotic events (one suffered two thrombotic events) during or immediately after hospitalization (median 9.5 days) confirmed by computed tomography pulmonary angiography or compression ultrasonography (6 pulmonary embolism, 4 deep vein thrombosis, 1 cava vein thrombosis): of these, six were under prophylactic low molecular weight heparin (LMWH) ($n=3$ standard-dose, $n=3$ intermediate-dose) and four under therapeutic-dose LMWH (one for atrial fibrillation and one for a previous pulmonary embolism).

COVID-19 patients had significantly higher CECs and EEVs in comparison with healthy subjects (21.5 ± 2.2 vs $8.1 \pm 1.4/\mu$l, $p<0.01$ and 286.5 ± 38 vs $127.6 \pm 21/\mu$l, $p<0.05$ respectively). CECs correlated with C-reactive protein levels ($r=0.49$, $p<0.05$), neutrophil-to-lymphocyte ratio ($r=0.40$, $p<0.01$) and D-Dimer ($r=0.45$, $p<0.05$), biomarkers of inflammation and hypercoagulability, but did not differ between patients who developed a thrombotic event and those who did not.

Three distinct populations of circulating EPCs (CD34+ and CD309+) were detected based on their CD45 expression. CD45 negative ($CD45^{neg}$), which express the regenerative potential of EPCs against vascular damage, were significantly lower in COVID-19 patients compared to controls (Fig. 1A), while a significant increase of CD45 positive intermediate ($CD45^{+int}$) (Fig. 1B) and CD45 positive high ($CD45^{+high}$) was observed, suggesting that these EPCs with high phagocytic capability may represent a reactive mechanism to limit viral proliferation.[6]

COVID-19 patients also had higher plasma levels of soluble markers of EC disturbance, sVCAM-1 (3122 ± 324 vs 1135 ± 82 ng/ml, $p<0.001$) and sICAM-1 (Fig. 1C) and VWF:Ag and VWF:RCo (Table 1), as compared with controls. Notably, sICAM-1 was significantly more elevated in COVID-19 patients admitted into ICU compared to those not in ICU (Fig. 1D) and in patients with reduced PaO_2/FiO_2 ratio compared to those with normal PaO_2/FiO_2 (Fig. 1E), suggesting that severe respiratory syndrome and hypoxemia are associated with endothelial damage. A significant correlation was also found between sICAM-1 and the SOFA score ($r=0.65$, $p<0.01$), suggesting that elevated sICAM-1 may represent a marker of severe disease evolution in Sars-Cov-2 infection.

D-dimer, VWF:Ag (not shown) and sICAM-1 (Fig. 1F) were significantly higher in patients who developed VTE than in pa-

Table 1
Demographic and clinical characteristics of the study population.

	COVID-19 patients $n=56$	Healthy subjects $n=36$	p value
Age (years)	72.1 ± 1.8	68.0 ± 3.0	ns
Sex (% M)	57.1%	40.1%	ns
Leukocytes (x $10^3/\mu L$)	7.0 ± 0.7	5.7 ± 0.8	ns
Neutrophil-to-lymphocyte ratio (NLR)	8.0 ± 0.9	2.0 ± 0.2	<0.005
Platelets (x $10^3/\mu L$)	211.1 ± 17.0	208 ± 17.2	ns
D-dimer (ng/ml)	1663 ± 299.0	180.6 ± 21.7	<0.0001
Fibrinogen (mg/dL)	403.1 ± 27.0	323.2 ± 26	ns
VWF: Ag (%)	273.8 ± 26.4	104.0 ± 7.0	<0.0001
VWF: RCo (%)	298.0 ± 28.0	90.0 ± 4.9	<0.0001
Procalcitonin (ng/ml)	1.2 ± 0.5	N.A.	
CRP (mg/dL)	4.8 ± 1.5	N.A.	
LDH (U/L)	247.2 ± 33.3	N.A.	
PaO_2/FiO_2	241.3 ± 27.1	N.A.	
SOFA score (total)	6.0 ± 0.4	N.A.	
Days from positive swab	4.2 ± 0.5	N.A.	
Thrombotic events (n)	11	N.A.	
Comorbidities			
Hypertension (n)	32	3	<0.01
Type 2 Diabetes Mellitus (n)	11	1	<0.05
Obesity (n)	12	1	<0.05
Smoker (n)	6	3	ns
Atrial Fibrillation (n)	7	0	ns
Cirrhosis (n)	1	0	ns
Kidney failure (n)	7	0	ns
Stroke (n)	4	0	ns
Peripheral artery disease (n)	7	0	ns
COPD (n)	4	0	ns
Drugs			
Antihypertensive agents (n)	11	1	<0.05
Statins (n)	11	0	<0.05
Antiplatelet treatments:			
Aspirin (n)	9	0	<0.05
Anti $P2Y_{12}$ (n)	3	0	ns
Anticoagulant treatments:			
LMWH (n)	45	0	<0.0001
-standard	*32*		
-incremented	*8*		
-therapeutic	*5*		
Apixaban (n)	6	0	ns
COVID-19 Treatments			
Hydroxycloroquine (n)	5	N.A.	
Darunavir/Cobicistat (n)	2	N.A.	
Tolicizumab (n)	1	N.A.	

Results are reported as mean±SEM if not differently indicated. N.A. not applicable; SOFA: sequential organ failure assessment.

tients who did not. ROC curve analysis showed that sICAM-1 >519.06 ng/ml discriminates COVID-19 patients with VTE from those without with moderate accuracy (AUC= 0.83, $p<0.01$) (Suppl. Fig. 1).

Most patients were under standard- ($n=32$) or incremented-dose ($n=8$) prophylactic LMWH (40/56, 71%) but no differences between treated and untreated patients were found for any of the circulating endothelial dysfunction markers assessed.

In patients who had recovered from COVID-19, CECs, EMPs, EPCs, VWF:Ag, VWF:RCo, sICAM-1 and sVCAM-1 returned to levels close to those of healthy controls, suggesting that endothelial damage is strictly dependent on active COVID-19 infection (Suppl. Fig. 2).

Our results show that COVID-19 patients have increased circulating CECs, EMPs and phagocytic EPCs and increased plasma levels of sICAM-1, sVCAM-1, VWF:Ag and VWF:RCo, with concomitant decrease of angiogenic EPCs, proving that circulating parameters of endothelial derangement are strongly altered in COVID-19 patients. In particular, plasma levels of sICAM-1 and of sVCAM-1 were more than threefold increased probably reflecting the enhanced adhesiveness of microvascular endothelium mediating the strong leukocyte extravasation in tissue, in particular in lungs. Moreover, the endothelial activation triggered by SARS-CoV-2 probably contributes to the strong in vivo platelet activation found in COVID-19 patients and to platelet adhesion to lung endothelium leading to lung injury.

Elevated sICAM-1 predicts cardiovascular events in apparently healthy men and in patients with cardiovascular disease and is associated with recurrent VTE,[7] and in our study strongly associated with VTE incidence and disease severity, therefore this marker warrants more extensive investigation for prognostic prediction in COVID-19 patients.

In our study prophylactic-dose LMWH did not affect biomarkers of endothelial dysfunction, in agreement with low clinical efficacy in preventing VTE in COVID-19 patients.[8] A recent study evaluated the impact of therapeutic-dose anticoagulation given to COVID-19 patients prior to hospitalization on endothelial damage, measured by CECs, suggesting that early treatment may prevent COVID-19-associated endothelial lesion.[9] Thus sICAM-1 might be used as an indicator to switch to therapeutic dose heparin in high-risk patients.

Finally, our data, strongly confirming that COVID-19 is an endothelial disease, provide the rationale for the search of novel therapeutic strategies targeting inflammatory mediators and/or promoting endothelial protection/repair to prevent the thrombotic and systemic complications of COVID-19.

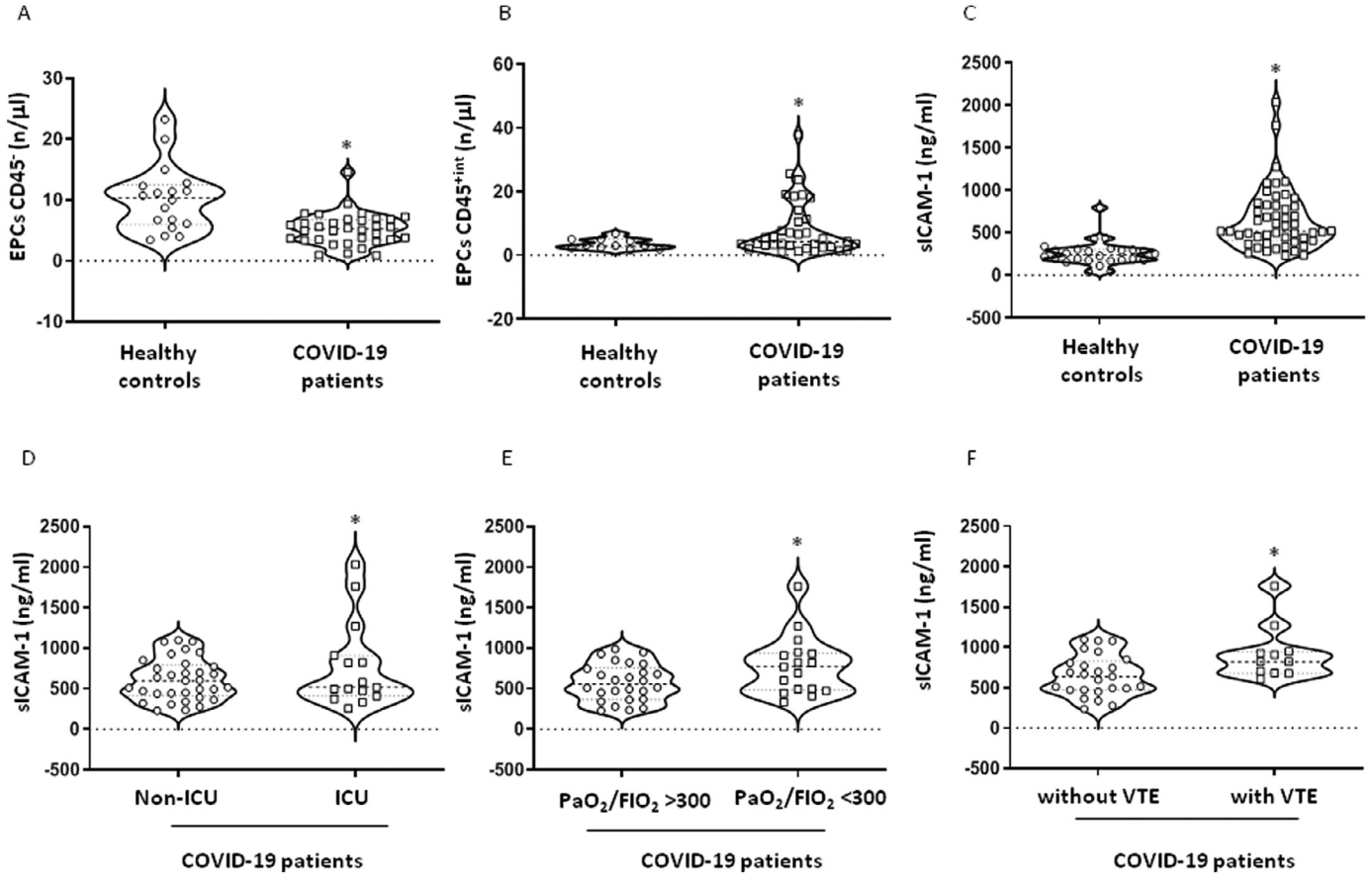

Fig. 1. *Cellular and soluble markers of endothelial dysfunction in COVID-19 samples*. Subpopulations of circulating EPCs based on CD45 expression: angioblast EPCs $CD45^{neg}$ (A) and hematopoietic EPCs $CD45^{+int}$ (B) in COVID-19 patients and in healthy controls. Results are expressed as absolute number/μl. *=$p<0.05$ vs healthy controls. sICAM-1 (C) levels are increased in COVID-19 patients compared to healthy controls ($p<0.05$) and in plasma of COVID-19 patients admitted into intensive care unit (ICU) or in non-ICU COVID-19 wards ($p<0.05$) (D), with normal or abnormal PaO_2/FIO_2 ratio ($p<0.05$) (E) and patients with or without thromboembolic event ($p<0.05$) (F). (*=$p<0.05$).

Declaration of Competing Interest

The authors declare no conflict of interest.

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Acknowledgments

The contribution of Luisa Golia (Section of Anesthesia, Intensive Care, and Pain Medicine, Azienda Ospedaliera-Universitaria Santa Maria della Misericordia, Perugia, Italy), Valentina Bubba (Division of Internal Medicine, ASL 1 Umbria, Città di Castello, Italy), Pierluigi Piergentili (Section of Anesthesia and Intensive Care, Presidio Alto Chiascio, USL Umbria 1, Gubbio, Italy) with patient enrollment is kindly acknowledged.

Stago s.r.l. (Italy) kindly gave some of the reagents used for the study.

Funding Statement

This work was supported by a fellowship from Fondazione Umberto Veronesi to L. Bury and E. Falcinelli.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.11.041.

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Accepted 28 November 2020
Available online 2 December 2020

https://doi.org/10.1016/j.jinf.2020.11.041

SARS-CoV-2 has been circulating in northeastern Brazil since February 2020: evidence for antibody detection in asymptomatic patients

Dear Editor,

Grall et al.[1] reports that asymptomatic patients with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) contaminate their masks and surroundings objects (mobiles, doorbell and bed table) with potential to play a role in transmission. Very few surveys focus on the prevalence of asymptomatic infections and although seroprevalence surveys provide insights of the proportion of the population infected ([2,3]) and the intensity of infection, they are unable to distinguished whether a significant proportion of infections are asymptomatic. Our country, Brazil, has the third highest global burden of Coronavirus Disease-19 (COVID-19), after the USA and India,[4] but does not systematically monitor the proportion of the population with SAR-CoV-2 antibodies. Moreover, the Ministry of Health recommends confirming the presence of the virus only in symptomatic individuals and the total number of infections is likely to be substantially higher.[5]

The first case of COVID-19 in Brazil was reported the 26th February 2020[4]. However, the virus was identified in sewage samples in Santa Catarina State, in the South of Brazil, as early as the 27th November, suggesting the virus had been circulating several months earlier, and, possibly, among asymptomatic infections.[7] This situation is similar to Italy, where the virus was discovered in sewage samples in Milan, in December 2019, even though the first COVID-19 case was reported two months later, the 21st February 2020.[8]

We report here evidence that SARS-CoV-2 may have circulated in the Northeast of Brazil before the first COVID-19 cases were reported in the region. In Aracaju, Sergipe State, in Northeast Brazil, the first case of COVID-19 was documented the 14th March 2020[4], but we report here that patients undergoing routine blood examinations for causes unrelated to COVID-19 had SARS-CoV-2 Immunoglobulins (IgM and IgG) before clinical cases were reported in the State

We obtained 987 anonymized serum samples collected from January 2020 to April 2020. Samples had been collected by a private laboratory from patients undergoing laboratory tests for routine examinations and health checks for causes unrelated to COVID-19. Most of the tests had been paid by health insurance companies, which usually indicate the patients were of upper socioeconomic status. All samples were kept frozen in the laboratory blood bank at −80 °C and tested for the presence of anti-SARS-CoV-2 IgM and IgG using two in vitro diagnostic tests. The first assay was a lateral flow immunochromatography (Nantong Egens Biotechnology CO, Ltd., China), reported by the manufacturer to have 96.9% sensitivity and 100% specificity. The second assay was a lateral flow sandwich detection immunofluorescence technology (iChroma2™ COVID-19 Antibody), which was used with the Ichroma™ II Reader (Bodytech Med Inc., South Korea). The assay is reported to have 95.8% sensitivity and 97.0% specificity.[3] All samples were thawed the day of testing using a 37 °C thermal bath. Samples were considered positive if they were positive by both assays. Assays with only one positive test were repeated. Only samples with two positive assays were considered positive. The study protocol was approved by the research ethics committee Federal University of Sergipe and individual consent was waived (CAAE: 36.401.320.0.0000.5546).

Fig 1, Table 1,[6]

The mean (SD) age of the 987 participants was 38.9 (22.2) years, ranging from birth to 90 years and 683 (69.2%) were women (Table). Sixteen (1.6%) samples tested positive for both assays, 968 (98.1%) were negative and three (0.3%) were indeterminate. Thirteen of the participants with positive samples were women and three men. Seven (43.8%) samples were IgM positive, three (18.8%) IgG positive and six (37.5%) were positive for both (Table). These sero-positive individuals had similar age, gender and residence than individuals reported within the first 30 days after the first COVID-19 case in the State[3]. These included 46 RT-PCR positive cases, of which 17 were men (37%) and 29 women (63%); of which 34 (74%) were adults aged between 20 and 60 years and 37 (80.4%) lived within in Aracaju City.

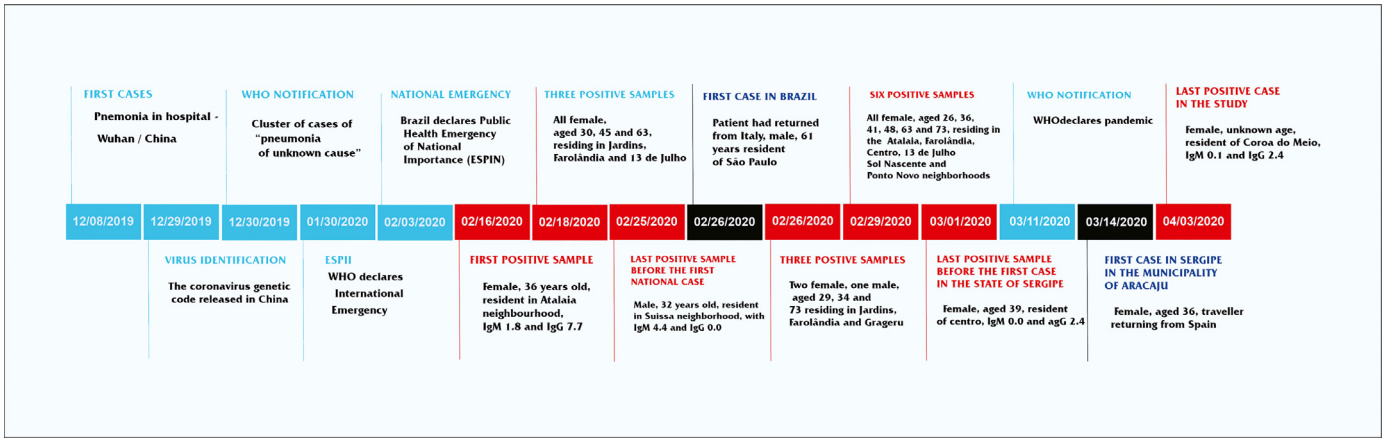

Fig. 1. Analysis of the timeline of events, with the evolution of SARS-CoV-2 in the world, Brazil and Sergipe
Legend: Red: our results; Blue: world events; Black: official notification.

Table 1
Positive tests participants characteristics´(Age, Sex, and district of residence) and of IgM and IgG analysis for SARS-CoV-2, Aracaju/SE, Brazil.

Sample	Sample date	IgM	IgG	Sex	Age	District	Economic range*
49	16/02/2020	1.8	7.7	F	36	Atalaia	A
121	18/02/2020	2.4	3.3	F	30	Jardins	A
123	18/02/2020	7.9	14.8	F	63	Treze de Julho	A
124	18/02/2020	3.4	6.1	F	45	Farolândia	B
271	25/02/2020	4.4	0.0	M	32	Suissa	B
309	26/02/2020	3.9	0.0	F	34	Farolândia	B
305	26/02/2020	3.0	8.7	M	73	Grageru	B
306	26/02/2020	1.7	7.4	F	29	Jardins	A
468	29/02/2020	4.4	0.0	F	41	Centro	C
483	29/02/2020	0.2	2.5	F	36	Farolândia	B
477	29/02/2020	4.4	0.0	F	26	Atalaia	A
475	29/02/2020	1.8	0.0	M	48	Treze de Julho	A
472	29/02/2020	1.7	0.0	F	63	Jabotiana	B
454	29/02/2020	1.7	0.0	F	73	Ponto Novo	B
486	01/03/2020	0.0	2.4	F	39	Centro	C
938	03/04/2020	0.1	2.4	F	47	Coroa do Meio	B

Legends: * A- Neighborhoods with residents with salaries above $ 2000; B- Neighborhoods with residents with salaries $ 300 to $ 1900; C- neighborhoods with residents with salaries below $ 300.

Aracaju has reported the highest number of COVID-19 cases in Sergipe[5]. Initial COVID-19 cases occurred in the Southern districts of the city, which are inhabited by high income populations who frequently travel to the South and Southeast regions of the country, where the pandemic was first reported. Our study has the limitations that participants were not screened for the presence of SAR-CoV-2 antigens and that the assays may have had false positive results resulting from cross-reactions with endemic infections. However, we used assays based on different technologies, and it is likely their combination may have provided higher specificity than the individual assays.[9]

Our data suggest that SARS-CoV-2 may have circulated in Northeast Brazil before the first COVID-19 case reported. As samples were collected for routine screening of pre-operative procedures or to monitor other morbidities, it is possible that most of these infections were asymptomatic at the time of testing. It is possible that large popular events occurring in February in Brazil, such as the traditional Carnival (21^{st}–25^{th}), may have accelerated the spread of the virus throughout the country. Our findings are in agreement with sewage-based studies in Santa Catarina[7] and Italy[8], suggesting that clinical cases were preceded by asymptomatic infections several weeks earlier.

Acknowledgments

We thank the Santa Helena Laboratory who donated the blood samples. We also thank the staff at the Department of Medicine and Pharmacy (LaBiC) of UFS, and acknowledge financial support received from the Ministério Público do Trabalho, Ministério Público Federal and Ministério Público Estadual.

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Accepted 28 November 2020
Available online 1 December 2020

https://doi.org/10.1016/j.jinf.2020.11.037

Test on stool samples improves the diagnosis of hospitalized patients: Detection of SARS-CoV-2 genomic and subgenomic RNA

Dear Editor,

As reported in this journal, SARS-CoV-2 has a prolonged detection rate in stool samples, although the clinical significance of this remains unclear.[1] Other studies have shown that the virus is detected longer in stool, but its infectivity or replication status is not yet well-established. However, some association between the detection of SARS-CoV-2 subgenomic RNA (sgRNA) and virus isolation in cell culture has been observed.[2–5] On the other hand, virus detection in upper respiratory tract (URT) samples lasts, in general, up to the eighth day after symptom onset, with a decrease in detection beyond the fifth day.[6] Many hospitalized patients suspected of having COVID-19 arrive late or when it is no longer possible to detect viral RNA in URT samples, thus imposing a problem on their clinical management given the pandemic. In order to better understand whether virus detection in stool could improve the diagnosis of COVID-19, we evaluated the detection of viral genomic RNA (gRNA) in 74 hospitalized patients admitted to the São Paulo university hospital with negative results in samples obtained from naso- or oropharyngeal swabs, including 3 patients with SARS-CoV-2-positive nasopharyngeal and stool samples as a control group. We also attempted to detect at least two different sgRNAs as possible markers of viral replication.

For RNA preparation from stool samples, ~2.0 ng of stool was homogenized in 2.0 mL of sterile lactated Ringer's solution and centrifuged at 9,300 x g for one minute. 150 uL of supernatant was used for RNA extraction with the Quick-RNA Virus kit (Zymo Research, USA) according to the manufacturer's instructions.

Molecular detection was performed by real-time RT-PCR. SARS-CoV-2 gRNA amplification was aimed at the Envelope (E) gene,[6] and positive stool samples were used for sgRNAs aimed at the E[6] and Nucleocapsid (N) messenger RNAs (mRNA). N mRNA was detected using the same forward primer as E sgRNA, with reverse primer and probe from the CDC USA N1 target protocol (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html), as shown in Fig. 1.

The reactions were carried out as described elsewhere.[7] The cycle threshold (Ct) values were used as a semi-quantitative parameter, meaning that the higher the RNA sample concentration, the lower the Ct value. The variation in Ct values for each sample between gRNA, their sgRNA counterparts, and between sgRNAs, were calculated as ΔCt.

Viral RNA was detected in 23.0% (17/74) of the stool samples, with a mean Ct value of 27.4 ± 6.0 (mean ± SD). In those positive samples, the N and E sgRNAs were detected in 94.11% (16/17) and 58.82% (10/17), respectively.

The ΔCt values were on average 7.22 ± 1.42 and 2.93 ± 1.83 higher for E and N sgRNAs, respectively (Table 1), corresponding to a lower sensibility in the order of 2 and 1 Log10, in relation to gRNA detection. The mean ΔCt value between E and N sgRNAs was 4.43 ± 0,61.

The number of days after the onset of symptoms in patients with SARS-CoV-2 detected in stool varied from 2 to 37 days (mean of 13.3 ± 11), with a mean hospitalization time of 24.7 ± 16.7 days.

The presence of SARS-CoV-2 in stool samples could be related to the swallowing of respiratory secretions from the URT or residues of infected antigen-presenting immune cells,[8] or, more likely, due to virus replication in gastrointestinal epithelial cells.[4] However, the detection of viral RNA in stool was not related to gastrointestinal symptoms or COVID-19 severity.[5,9]

We were able to detect the SARS-CoV-2 sgRNA of the E and N genes in positive stool samples. Interestingly, the authors who developed the protocol for the detection of E sgRNA did not report any detection in the stool of hospitalized patients.[6]

When comparing the ΔCt between gRNA and their corresponding sgRNAs in each sample, we observed a difference of 1 to 2 log10 of sensitivity for mRNAs of N and E, respectively. Those differences could be explained by the fact that N sgRNA is the most abundantly expressed transcript during viral replication, followed by E sgRNA, in an amount of approximately 1.5 Log10 lower transcripts.[10] We also roughly observed this difference between N and

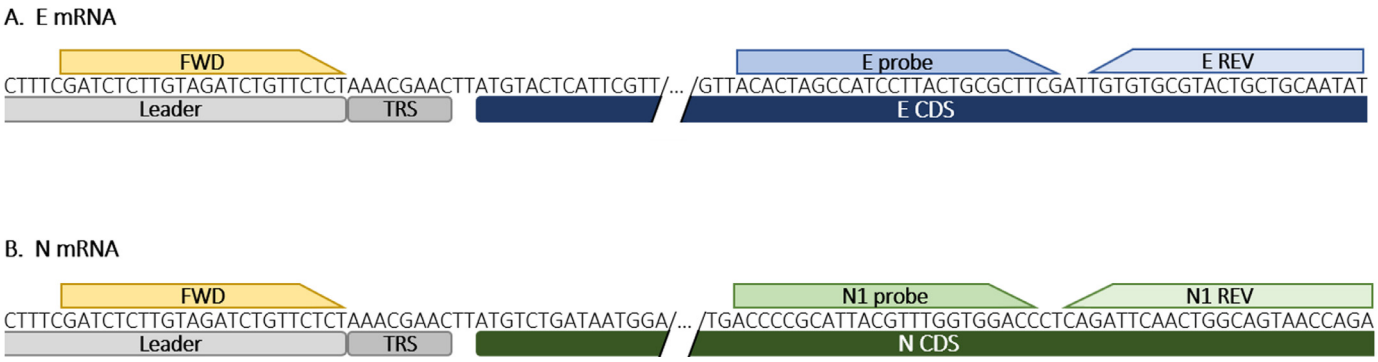

Fig. 1. Real-time RT-PCR oligonucleotide binding sites for amplification of E and N mRNAs. A) E mRNA. B) N mRNA. The leader sequence and transcription-regulating sequence (TRS) are shown in grey. The common forward primer is shown in yellow. The coding sequence (CDS), probe, and reverse primers for E and N are shown in blue and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Ct values of gRNA and sgRNA in stool samples, and ΔCt.

Patient	Threshold cycle values					
	gRNA	sgRNA (E)	sgRNA (N)	ΔCt (sgE-gE)	ΔCt (sgN-gE)	ΔCt (sgE-sgN)
2920	15.93	21.10	16.93	5.17	1.00	4.17
2089*	19.56	26.75	21.89	7.19	2.33	4.86
3201	21.95	30.93	26.22	8.98	4.27	4.71
2615	23.81	31.68	26.86	7.87	3.05	4.82
2449	23.92	32.47	27.00	8.55	3.08	5.47
3859	24.14	29.41	26.00	5.27	1.86	3.41
2555	26.59	33.07	29.00	6.48	2.41	4.07
4604	26.97	35.95	31.95	8.98	4.98	4.00
1724*	27.16	33.32	29.00	6.16	1.84	4.32
3973	29.78	37.34	38.00	7.56	8.22	−0.66†
4478	30.04	N.D.	33.88	–	3.84	–
1450*	33.68	N.D.	37.00	–	3.32	–
5153	33.84	N.D.	36.51	–	2.67	–
4485	34.10	N.D.	N.D.	–	–	–
4914	34.37	N.D.	36.38	–	2.01	–
2980	34.44	N.D.	36.00	–	1.56	–
4598	35.51	N.D.	36.02	–	0.51	–
Mean ± SD	*27.99 ± 5.89*	*31.20 ± 4.66*	*30.54 ± 6.17*	*7.22 ± 1.42*	*2.93 ± 1.83*	*4.43 ± 0.61*

*Positive controls. † non-computed value. Ct, threshold cycle. gRNA, genomic RNA. sgRNA, subgenomic RNA. E, envelope protein. N, nucleocapsid protein. ΔCt, the difference between Ct values of sgRNAs and gRNA. N.D., not detected.

E sgRNA detection with a $\Delta Ct = 4.43 \pm 0.61$. In spite of these findings, the detection of sgRNAs in clinical samples per se does not necessarily imply infectivity, which is more related to days post symptoms and viral load.[2,3,6] However, the detection of more than one sgRNA could be used as a marker of viral replication, although further studies are needed to confirm this hypothesis.

In conclusion, viral detection in stool improves the diagnosis of COVID-19, especially in patients who are suspected of being infected but with negative results in URT samples.

Declaration of Competing Interests

None.

Acknowledgements

L.V.L.M, L.K.S.L, G.R.B., A.P.C.C., and J.M.A.C. are fellows of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil. D.D.C. is a fellow of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

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Accepted 28 November 2020
Available online 1 December 2020

https://doi.org/10.1016/j.jinf.2020.11.034

Venous thrombosis prevention & COVID-19: Increased anticoagulation reduces proximal deep vein thrombosis in mechanically ventilated COVID-19 patients

Dear Editor,

Coronavirus disease-2019 (COVID-19) has been associated with life-threatening thromboembolic complications due to increased inflammation, marked hypercoagulability and endothelial activation.[1–3] Preventing deep vein thrombosis (DVT) and pulmonary embolism is important, since ~10% of COVID-19-related deaths are caused by pulmonary embolism complicating DVT.[4] Recently, enhanced prophylactic or therapeutic anticoagulation dose regimens have been recommended by experts and adopted in some centres.[5,6] However, direct comparative studies of the different anticoagulation regimens are lacking.

In our intensive care unit (ICU), we established a DVT prevalence of 46% in mechanically ventilated COVID-19 patients on standard prophylactic anticoagulation[7] and subsequently, we increased anticoagulation to reduce thromboembolic complications. We designed this before-after observational exploratory study to evaluate the risk/benefit ratio of increased (IA) *versus* standard prophylactic anticoagulation (SPA) in mechanically ventilated COVID-19 patients. The study was part of the COVID-ICU and French COVID-19 cohort registries and received approval from the ethics committee of our institution (N°, IDRCB, 2020-A00256-33; CPP, 11-20.20.02.04.68737).

We included all consecutive patients admitted for COVID-19-related pneumonia requiring tracheal intubation. We excluded patients on long-term therapeutic anticoagulation before ICU admission. To diagnose DVT, an initial ultrasound was routinely performed during the first week after intubation, and in DVT-free patients, a second ultrasound was performed ~1week later by certified sonographers (SV/PB) according to guidelines.[8] The study was composed of two periods, defined according to the type of anticoagulation received from intubation to the first ultrasound examination. In both groups, if DVT was diagnosed, therapeutic anticoagulation was initiated.

Patients admitted from 2020/03/11 to 2020/04/01 (SPA group) received prophylactic anticoagulation with subcutaneous enoxaparin 40 mg once daily or unfractionated heparin 15000IU/day if creatinine clearance <15 mL/min. Patients admitted from 2020/04/02 to 2020/10/12 (IA group) received either prophylactic double-dose enoxaparin 40 mg twice daily or therapeutic anticoagulation with either enoxaparin 1 mg/kg twice daily or unfractionated heparin to reach plasma anti-Xa activity of 0.3–0.6IU/mL. Supportive care included optimized mechanical ventilation, vasopressors, sedation and muscular paralysis according to guidelines. Dexamethasone, antiviral and other immunomodulatory drugs were administered according to the physicians in charge.

The efficacy endpoint was the prevalence of femoral/popliteal DVT, known to be strongly associated with pulmonary embolism.[9] The efficacy endpoint was also compared between patients treated with double-dose prophylactic enoxaparin (0.4 mg twice daily) and patients treated with standard enoxaparin prophylaxis (0.4 mg once daily). The safety endpoint was the number of patients with at least one major bleeding (MB) defined according to guidelines,[10] *i.e.* bleedings causing death, decreasing hemoglobin by ≥2 g/dL, requiring transfusion of ≥2 blood units or occurring in a critical organ.

Quantitative variables are expressed as medians [25th–75th percentiles] and categorical variables as percentages. Parameters were compared between SPA and IA patients using Mann-Whitney and Fisher's exact tests as appropriate. An exploratory generalized multilinear regression model was built to adjust for parameters significantly different between groups. *P*-values ≤0.05 were considered significant. Based on the 26% prevalence of femoral/popliteal DVT in the SPA group and a presumed reduction to <5% in the IA group, 42 patients/group were required for 95% confidence interval and 80% statistical power.

Ninety-three patients were included, 50 in the SPA and 43 in the IA group. Baseline characteristics did not differ significantly between the groups (Table 1). The initial ultrasound was performed 2 days [1–4] post-intubation. Time from ICU admission to the first ultrasound was 4days [2–6] in the SPA *versus* 5days [3–8] in the IA group, $P=0.03$. In 37 of the femoral/popliteal DVT-free patients, a second ultrasound was performed 8days [7–10] post-intubation. Anticoagulant treatment is presented in Table 1. At the time of the initial ultrasound C-reactive protein, fibrinogen and D-dimer were remarkably elevated at 223 mg/L [132–307], 7.6 g/L [6.2–8.6] and 3180 ng/mL [1495–6808], respectively. Twenty-nine patients (31%) required renal replacement therapy (RRT) while 12 (13%) were treated with extracorporeal membrane oxygenation (ECMO), 3/50 (6%) in the SPA and 9/43 (21%) in the IA group ($P=0.06$).

Prevalence of femoral/popliteal DVT was significantly reduced in the IA in comparison with the SPA group (two (5%) *versus* 13 (26%), $P=0.01$; Fig. 1). The two DVT in the IA group and one DVT in the SPA group were associated with femoral central venous catheters. After adjustment for parameters significantly different between groups, anticoagulant treatment was the only factor associated with DVT ($P=0.02$). Prevalence of femoral/popliteal DVT was decreased, *i.e.* 1/25 patients treated with enoxaparin 0.4 mg twice/day (2% of the IA group) *versus* 11/42 patients treated with enoxaparin 0.4 mg/day (22% of the SPA group), $P=0.02$.

MB occurred 10 days [8–13] post-intubation in 11 patients (26%) in the IA group *versus* seven patients (14%) in the SPA group ($P=0.19$). MB occurred while on ECMO and/or RRT in 15/18 cases (83%). One patient died of intracranial hemorrhage. In patients

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease-2019; DVT, deep vein thrombosis; ECMO, extracorporeal membrane oxygenation; IA, increased anticoagulation; ICU, intensive care unit; MB, major bleeding; SARS-CoV-2, Severe acute respiratory syndrome coronavirus-2; SOFA score, Sequential Organ Failure Assessment score; SPA, standard prophylactic anticoagulation.

Table 1
Main characteristics, biological parameters, anticoagulant treatment and outcome in 93 mechanically ventilated COVID-19 patients.

Parameters	All patients($N=93$)	Standard prophylaxis($N=50$)	Increased anticoagulation($N=43$)	P
Patient characteristics				
Male gender, N (%)	64 (69)	36 (72)	28 (65)	0.51
Age (years)	63 [56–71]	62 [54–69]	65 [58–73]	0.14
Body mass index (kg/m^2)	29 [25–32]	28 [25–31]	30 [25–34]	0.18
Past hypertension, N (%)	49 (53)	23 (46)	26 (60)	0.21
Diabetes, N (%)	36 (39)	22 (44)	14 (33)	0.29
Ischemic heart disease, N (%)	11 (12)	9 (18)	2 (5)	0.58
SOFA score on admission	6 [3–8]	6 [4–9]	5 [3–8]	0.14
Main biological parameters				
PaO_2/FiO_2 (mmHg)	151 [113–240]	179 [117–258)]	141 [110–188]	0.15
PT (ratio of normal)	1.18 [1.12–1.27]	1.17 [1.11–1.25]	1.19 [1.14–1.31]	0.17
APTT (ratio of normal)	1.23 [1.12–1.50]	1.21 [1.10–1.43]	1.29 [1.20–1.65]	0.04
Plasma fibrinogen (g/L)	7.6 [6.2–8.6]	8.1 [6.7–8.8]	7.2 [6.1–8.1]	0.07
Plasma D-dimer (ng/mL)	3180 [1495–5808]	3500 [2000–7760]	2710 [1465–4135]	0.10
White blood cells (G/L)	10.4 [7.8–14.0]	10.5 [8.0–13.8]	9.8 [7.1–14.3]	0.57
Lymphocytes (G/L)	0.72 [0.42–1.20]	0.74 [0.45–1.14]	1.0 [0.43–1.24]	0.74
Platelets (G/L)	274 [197–367]	271 [200–365]	274 [194–372]	0.99
CRP (mg/L)	223 [132–307]	246 [180–304]	180 [117–307]	0.18
Serum creatinine (µmol/L)	98 [67–154]	94 [70–150]	99 [63–173]	0.87
Serum ALT (IU/L)	33 [23–50]	32 [21–48]	34 [25–54]	0.46
Anti-COVID-19 and supportive treatments				
Lopinavir/ritonavir combination, N (%)	12 (13)	12 (24)	0 (0)	0.003
Azithromycin, N (%)	42 (45)	16 (32)	26 (61)	0.01
Hydroxychloroquine, N (%)	25 (27)	14 (28)	11 (26)	0.82
Dexamethasone, N (%)	41 (44)	13 (26)	28 (65)	0.0002
Vasopressor treatment, N (%)	45 (49)	27 (54)	18 (42)	0.21
Renal replacement therapy, N (%)	29 (31)	14 (28)	15 (35)	0.51
ECMO, N (%)	12 (13)	3 (6)	9 (21)	0.06
Anticoagulation regimen				
Standard prophylaxis before initial ultrasound, N (%)	50 (54)	50 (100)	0 (0)	<0.0001
Standard prophylactic enoxaparin, N (%)	42 (45)	42 (84)	0 (0)	<0.0001
Standard prophylactic unfractionated heparin, N (%)	8 (9)	8 (16)	0 (0)	0.05
Double-dose prophylactic enoxaparin, N (%)	25 (27)	0 (0)	25 (58)	<0.0001
Therapeutic anticoagulation before initial ultrasound, N (%)	18 (19)	0 (0)	18 (54)	<0.0001
Therapeutic enoxaparin before initial ultrasound, N (%)	6 (6)	0 (0)	6 (14)	0.01
Therapeutic unfractionated heparin before ultrasound, N (%)	12 (13)	0 (0)	12 (28)	<0.0001
Endpoints				
Femoral/popliteal DVT, N (%)	15 (16)	13 (26)	2 (5)	0.01
Femoral/popliteal DVT, on enoxaparin prophylaxis, N (%)	13 (14)	11 (22)	1 (2)	0.02
DVT below the popliteal level, N (%)	23 (25)	12 (24)	11 (26)	1.0
Major bleeding, N (%)	18 (19)	7 (14)	11 (26)	0.19
Therapeutic anticoagulation at major bleeding, N (%)	16 (17)	9 (18)	8 (19)	1.0
Death, N (%)	38(44)	18 (34)	20 (56)	0.08

DVT, deep vein thrombosis; SOFA score, Sepsis-related Organ Failure Assessment score; PaO2/FiO2, oxygen arterial partial pressure/fraction of inspired oxygen ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; CRP, C-reactive protein; ALT, alanine-aminotransferase; ECMO, extracorporeal membrane oxygenation.

treated with 0.4 mg/day enoxaparin in the SPA group, one MB occurred *versus* none in patients treated with enoxaparin 0.4 mg twice/day in the IA group. In the SPA group, 17/50 (34%) died as compared to 19/43 (44%) in the IA group ($P=0.08$), while 7/43 (16%) are still hospitalized.

Our most important finding is that IA is associated with decreased femoral/popliteal DVT prevalence in comparison with SPA in mechanically ventilated COVID-19 patients. The second important finding is that enoxaparin 0.4 mg twice daily regimen[5] seems effective for DVT prophylaxis compared with standard enoxaparin prophylaxis.

To our knowledge, this is the first study comparing SPA with IA strategies and showing a significant reduction in femoral/popliteal DVT using systematic ultrasound screening. Our data suggests that double-dose enoxaparin prophylaxis (40 mg twice daily) may have a favorable risk/benefit ratio, worth exploring in further studies. DVT below the popliteal level were not reduced in the IA group, suggesting that at this level, venous stasis and/or endothelial lesion may play a more important role than hypercoagulation.

Our study strength is that ultrasound was performed in all patients, avoiding biases related to the absence of systematic screening. Limitations include absence of randomization and small sample size precluding assessment of the effect on mortality.

In conclusion, using systematic ultrasound screening, we observed a decrease in femoral/popliteal DVT prevalence while increasing anticoagulation compared to standard prophylaxis in mechanically ventilated COVID-19 patients. The favorable risk/benefit ratio of prophylactic double-dose enoxaparin 40 mg twice-daily regimen is worth exploring in future studies.

Declaration of Competing Interest

The authors declare no competing interests.

Consent for publication

All the authors agree to publish.

Funding

The study, analysis, and manuscript preparation were completed as part of official duties at the university hospital. There was no additional funding.

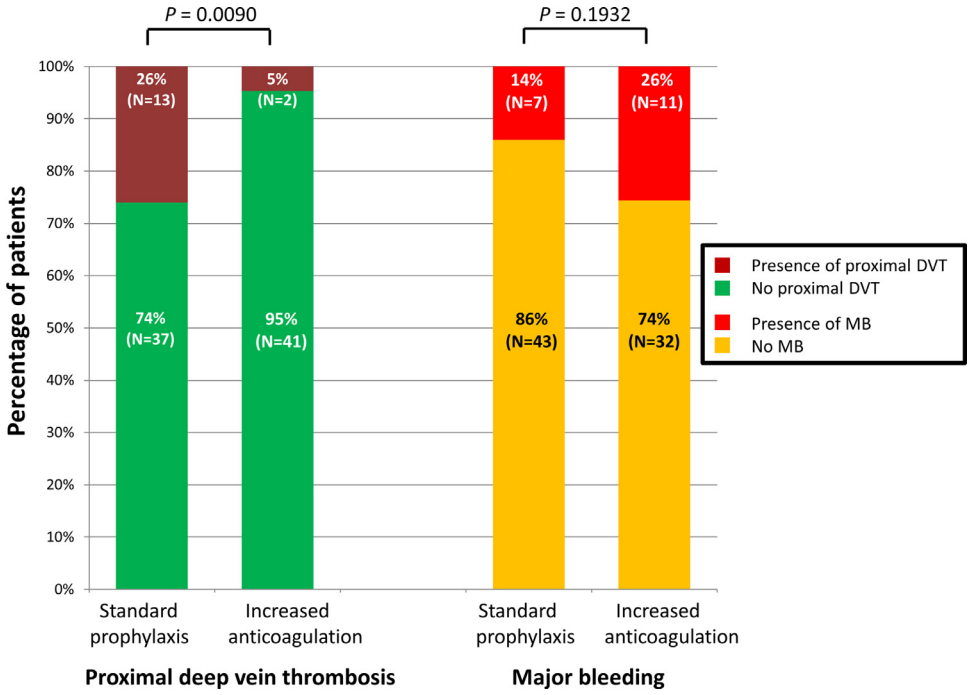

Fig. 1. Before-after comparison of proximal deep vein thrombosis (DVT) and major bleeding (MB) prevalence in 93 mechanically ventilated COVID-19 patients.

Ethics approval and consent to participate

The study was part of the COVID-ICU and French COVID-19 cohort registries and approved by our institutional ethics committee (N°, IDRCB, 2020-A00256-33; CPP, 11-20-20.02.04.68737).

Availability of data and materials

Drs. Voicu, Mebazaa and Mégarbane contributed to the study concept and design. Drs. Voicu, Choustermann, Deye, Malissin, Le Gall, Barthélémy, Sutterlin, Naïm, Mrad, Pépin-Lehalleur, Le Dorze, de Roquetaillade, Ekhérian, Gayat, Sidéris, Mebazaa, and Mégarbane contributed to the patient management. Drs. Voicu and Bonin performed lower limbs systematic duplex ultrasound. All authors contributed to the acquisition, analysis, or interpretation of data. Drs. Voicu and Mégarbane contributed to drafting the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. Dr. Mégarbane has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

The authors would like to thank Drs. Virginie Siguret and Alain Stépanian for performing the coagulation tests and Mrs. Alison Good (Scotland, UK) for her helpful review of the manuscript.

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Accepted 10 November 2020
Available online 20 November 2020

https://doi.org/10.1016/j.jinf.2020.11.019

Early clinical experience with imatinib in COVID-19: Searching for a dual effect

Dear Editor,

We have read with interest the recent articles by Chen et al.[1] and Chan and colleagues,[2] in which the antiviral action of favipiravir and sofosbuvir/daclatasvir against SARS-CoV-2 is assessed. Data reported by the authors are certainly instructive, but, in our opinion, finding drugs not only with antiviral effects, but also with immunomodulatory properties, would be more advantageous, since a heightened immune response has been observed in severe COVID-19.[3] In this regard, tyrosine kinase inhibitor (TKI) imatinib, originally designed to treat chronic myeloid leukaemia (CML), has been also proposed as a possible therapy for COVID-19[4] given its antiviral and immunomodulatory effects observed in preclinical models, as well as the lower incidence of SARS-CoV-2 infection reported among CML patients who were being treated with TKIs.[5]

In March 2020, due to lack of robust data favouring any specific therapy for COVID-19, and after reviewing the theoretical framework about the potential utility and safety of imatinib, it was considered as a possible off-label treatment in some of our patients in order to perform a preliminary analysis of both its clinical effects and safety profile that could subsequently support the design of the COVINIB Study Group (CSG) clinical trial (NCT04346147), which is currently ongoing.

In this letter, we would like to briefly describe the characteristics of twenty consecutive COVID-19 patients admitted to our hospital from March to May 2020 who received imatinib as part of their treatment for SARS-CoV-2 infection. As in other studies, our series involved the off-label administration of a drug for a transmissible disease which had become pandemic even before any therapy was found effective. In such scenarios, WHO offers ethical guidance. In order to meet its recommendations, previous evidence regarding potential effectiveness and safety of imatinib in COVID-19 was carefully reviewed, leading to the creation of the CSG. Informed consent was obtained from each patient before imatinib was started, and the study was approved by the Hospital Ethics Committee. A dosage of 400 mg daily was chosen since it is a usual initial dose in hematological disorders. Treatment duration, however, depended on the clinical course, the appearance of possible adverse reactions and the criteria of the attending physician. Data regarding previous medical history and clinical, radiological and laboratory information were collected.

Table 1 summarises the main characteristics of patients. The median age was 73 years, and 45% of patients were male. The prevalence of hypertension, hyperlipidaemia, diabetes mellitus and previous cardiac and pulmonary disease was 65%, 45%, 30%, 20% and 20%, respectively; four subjects were ex-smokers, and only one was a current smoker. All subjects but one (number 7) had pneumonia, which was bilateral in eighteen patients. Polymerase chain reaction test for SARS-CoV-2 in nasopharyngeal swab was positive in all cases except one (number 13), in whom the diagnosis of COVID-19 was made on the basis of clinical, radiological and laboratory features, according to WHO case definitions at that time.

Hydroxychloroquine (HCQ) and lopinavir/ritonavir (LPV/r), were the most common concomitant therapies. On the basis of the latest evidence, these two drugs seem to have had slight impact, if any, on patients' outcomes. On the contrary, corticosteroids have been found useful in COVID-19;[6] however, according to our protocol at that time, they were indicated in patients whose clinical course deteriorated despite other treatments. Half of the cases were considered to have a favourable clinical course, and subsequently did not receive corticosteroids.

Six patients reported mild gastrointestinal symptoms (which are among the most common side effects of imatinib): all of them had nausea, and one had also diarrhea. No other adverse events were recorded, and this TKI was not discontinued in any patient because of side effects. These data seem to be in line with the safety profile of imatinib observed in CML patients: adverse reactions are infrequent and usually related to long-term therapy. Furthermore, digestive complaints are also associated with other treatments (such as HCQ or LPV/r), and could even be part of the clinical spectrum of COVID-19.

Three patients died in our series. All of them were male, over the age of 70 years and had previous comorbidities. In addition, they all needed supplemental oxygen and had bilateral pneumonia and poor prognostic laboratory features at imatinib onset. All these factors might also explain their fatal outcome, which could not be directly linked to imatinib administration.

Six patients received imatinib within the first week from the beginning of the symptoms. In this scenario, the drug might display antiviral properties (observed in in vitro studies against coronaviruses closely related to SARS-CoV-2).[7] However, there are conflicting data regarding the real antiviral effect of imatinib against SARS-CoV-2.[8,9]

Table 1
Characteristics of COVID-19 patients treated with imatinib. Subjects are arranged chronologically according to imatinib start date.

Case	Age (yr)	Sex	Days of symptoms* (Md: 8; IQR: 6–9.3)	Oxygen therapy†	C-reactive protein (Highest, mg/dL) (Md: 14.8; IQR: 11–23.5)	Lymphocytes (Lowest, 10^9/L) (Md: 0.74; IQR: 0.5–1.18)	D-dimer (Highest, ng/mL) (Md: 1230.5; IQR: 523–1922.5)	Days of imatinib (Md: 9.5; IQR: 7–11.8)	Days of hospitalization (Md: 10; IQR: 7–18)	Other therapies		Final vital status
										Before imatinib	After/with imatinib	
1‡	38	F	12	LFNC	21.2	0.84	1129	5	11	HCQ, LPV/r	–	Recovered
2	60	F	9	Mask	16.7	0.88	5650	15	27	HCQ, LPV/r	C	Recovered
3	87	M	5	Mask	30.7	0.36	1501	4	13	HCQ, LPV/r, AZM	C	Dead
4	76	M	8	Mask	18.1	0.31	20,539	5	10	HCQ, LPV/r	C	Dead
5	74	M	9	Mask	30.8	0.52	359	7	10	HCQ, LPV/r	C	Dead
6	58	M	9	LFNC	11.5	1.20	488	13	7	HCQ, LPV/r	–	Recovered
7	75	F	6	LFNC	0.9	1.45	392	8	6	–	HCQ	Recovered
8	40	M	7	LFNC	26.3	1.41	628	15	7	LPV/r	HCQ	Recovered
9	76	F	6	IMV	24.1	0.29	22,787	3	28	HCQ, LPV/r	C, TCZ	Recovered
10	67	F	8	LFNC	1.5	0.77	1234	10	8	HCQ, LPV/r	C	Recovered
11	83	M	6	LFNC	11.0	0.66	281	13	12	HCQ, LPV/r	–	Recovered
12	76	M	13	No need	12.5	0.71	2388	9	3	HCQ, LPV/r	–	Recovered
13	71	F	8	Mask	21.6	0.50	1837	11	22	HCQ, LPV/r, AZM, C, TCZ	–	Recovered
14	74	M	11	Mask	30.2	0.45	1927	11	21	HCQ, LPV/r, AZM, C, TCZ	–	Recovered
15	72	M	8	LFNC	5.3	1.20	1227	10	4	HCQ, LPV/r	–	Recovered
16	65	F	10	Mask	17.9	1.11	669	8	10	HCQ, LPV/r	C, TCZ	Recovered
17	52	F	8	LFNC	11.0	0.52	292	12	10	HCQ, LPV/r	–	Recovered
18	83	F	2	Mask	12.9	0.55	1909	11	17	HCQ, AZM, C	–	Recovered
19	79	F	8	LFNC	12.5	1.01	1666	7	18	–	HCQ	Recovered
20	71	F	8	No need	1.8	1.38	720	7	11	–	HCQ	Recovered

Abbreviations: yr = years; Md = median value; IQR = interquartile range; *F* = female; *M* = male; LFNC = low-flow nasal cannula; Mask = Venturi mask or nonrebreather face mask; IMV = invasive mechanical ventilation; HCQ = hydroxychloroquine; LPV/*r* = lopinavir/ritonavir; AZM = azithromycin; *C* = corticosteroids; TCZ = tocilizumab. Reference range for C-reactive protein: <0.5 mg/dL. Reference range for D-dimer: <500 ng/mL.
*Days from symptom onset to treatment with imatinib.
†Highest oxygen support needed during admission.
‡This case has been previously published (Morales-Ortega et al.)[4].

Another rationale for the potential beneficial role of imatinib in COVID-19 is its immunomodulatory effect, which would probably become more valuable from the second week after symptom onset; 70% of our patients received the drug at that time. Evidence from animal and human-cell studies suggests that imatinib can attenuate inflammatory cytokine release, including interleukin-6 and tumor necrosis factor-alpha, probably by inhibiting NF-κB signaling pathway,[10] which seems to play a prominent role in the immune-mediated lung injury observed in severe COVID-19.[3] Moreover, imatinib has also been found to prevent pulmonary damage by reducing tissue edema and maintaining endothelial barrier integrity in murine models of acute inflammatory lung injury.[10]

This preliminary study, which is limited by its observational design and a low number of cases, did not find differences when comparing patients who received imatinib before or after day 7 from the onset of symptoms in terms of age, sex, duration of imatinib therapy, concomitant treatments, C-reactive protein and D-dimer levels, lymphocyte count, days of hospitalization and death.

To our knowledge, this is the first case series of COVID-19 patients in whom imatinib was used as a treatment for this condition. This report cannot provide enough evidence for the effectiveness of this drug against SARS-CoV-2 infection, but the safety of a short-term treatment with imatinib, as well as its potential antiviral and immunomodulatory properties, suggests that it could be an acceptable option to explore in controlled clinical trials.

Funding

None

Ethical statement

This study was approved by the Ethical Committee of Hospital Universitario de Fuenlabrada (IRB protocol 20/42).

Author contributions

Conception and design: AMO, BFP, FB, MGG, JVSL and DBB. Data analysis and acquisition: AMO, LRP, BFP, and DBB. Interpretation of the data: AMO, BJB and DBB. Drafting or revision of the manuscript: AMO, BJB, AIFS, FB and DBB. Final approval of the manuscript: all authors.

Declaration of Competing Interests

David Bernal-Bello is the principal investigator of a non-sponsored randomised trial investigating the therapeutic role of imatinib and baricitinib in COVID-19 patients (NCT04346147). The rest of the authors are sub-investigators in this project. All authors declare no other competing interests.

Acknowledgments

The authors would like to thank Dr. Jaime García de Tena (Department of Medicine, Universidad de Alcalá, Madrid, Spain) and Dr. Miguel Ángel Canales Albendea (Department of Haematology, Hospital Universitario La Paz, Madrid, Spain) for their helpful comments on previous drafts of this manuscript.

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Accepted 1 February 2021
Available online 5 February 2021

https://doi.org/10.1016/j.jinf.2021.02.002

Protecting healthcare workers in conflict zones during the COVID-19 pandemic: Northwest Syria

Dear Editor,

We read with interest Jones et al's correspondence on SARS-CoV-2 seroprevalence among health workers and share experiences of COVID-19 cases among health-workers in Northwest Syria (NWS).[1] This geographical area includes parts of Aleppo and Idleb governorates and shelters 4.17 million civilians (of whom 2.6 million are internally displaced and 1.4 million reside in camps). It shares closed borders with Turkey on one side and closed lines with areas under government control on the other side. (See Fig. 1) Most people in NWS live in overcrowded, unhygienic conditions with poor access to water, sanitation and hygiene (WASH) and healthcare.[2] This area has seen protracted conflict with recurrent attacks on WASH infrastructure, healthcare facilities and healthcare workers in contravention of International Humanitarian Law.[3] Healthcare workers face numerous challenges relating to the effects of conflict on the health system, inadequate personal protective equipment (PPE,) poor infection prevention and control (IPC) practices, insufficient resources and severe under-staffing. Our aim is to identify the trajectory of COVID-19 cases and the proportion of infected healthcare workers in Northwest Syria.

Methods

We retrospectively reviewed data collected by the Early Warning, Alert and Response Network (EWARN) in NWS. At present, there are two parallel surveillance systems for infectious diseases in Syria with the Early Warning, Alert and Response System (EWARS) covering areas under government control since 2012, and EWARN predominantly operating in areas outside of government control since 2013.[4] In March 2020, the NWS COVID-19 Task Force was established with the aim of addressing lab testing, contact tracing, establishing isolation centres and COVID-19 treatment centres, as well as measures to interrupt transmission (lockdown, self-isolation, quarantine, and public health education) at the community level.[2] Each suspected case which met the case definition (adapted from WHO's global surveillance guidelines) was investigated and data were collected via Excel forms then by using the Go.Data application (https://www.who.int/godata). A nasopharyngeal swab was collected for rt-PCR testing in Idleb city. Although EWARN was established in mid-2013 and aggregated data for acute respiratory illnesses has been collected since then, PCR testing only became available at the start of 2020, and testing for SARS-CoV-2 as of March 2020.

Results

The first case of COVID-19 in NWS was confirmed in a doctor working in Bab Al Hawa (a border-located hospital) on 9th July 2020; after this, a cluster was noted among Bab Al Hawa staff before several community clusters became evident towards the end of July 2020, suggesting community transmission. Since then, there has been a steady increase in cases across the region, reaching (as of 16th January 2021) 20,822 cases among 80,326 tests (26% positivity.) (See Fig. 2) Of these, more cases were detected among males (12,993, 62%) than females (7829, 38%), with 16,324 (78%) of cases aged between 15 and 50 years. After the initial phase where most cases were reported from Al Bab city in Aleppo governorate (1505 reported cases), the trend shifted towards Idleb city with 4160 reported cases and an increase in the cases detected from districts where camps were concentrated. These were mainly in Dana in Idleb governorate with 3111 confirmed cases and A'zaz in Aleppo governorate with 1465 cases. Camps accounted for 10% of cases (2176.) 5% (1041) of the total cases had moderate manifestations in addition to 142 severe cases. There have been 376 (1.8%) attributed fatalities to date out of 423 deaths among cases with confirmed SARS-CoV-2.

The percentage of healthcare providers amongst the overall cases dropped from almost 25% during the early stages of community transmission to just under 16% (a total of 1837 cases among healthcare workers) as of 28th December 2020 and down to 13% (2692 cases) as of 16th January 2021. As of 19th January 2021, there have been 1076 cases among nurses and 390 among physicians. 6 healthcare workers (5 physicians and 1 nurse) have died.

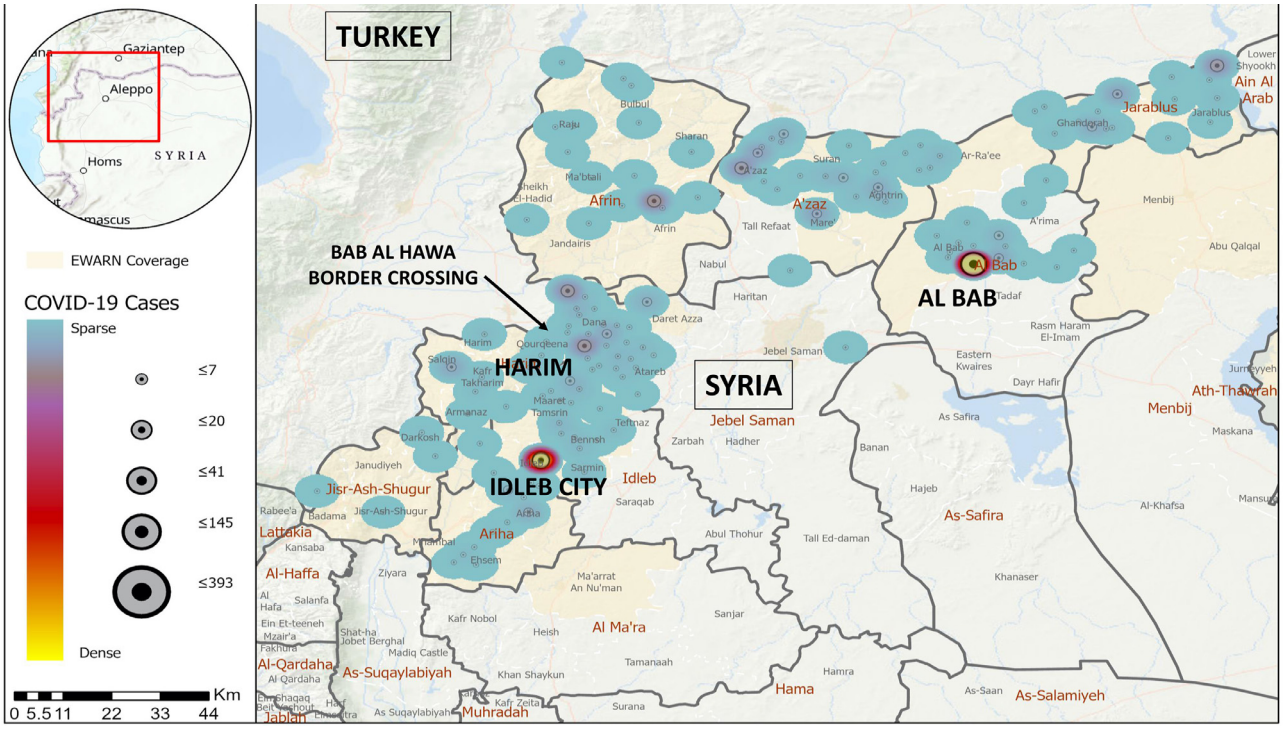

Fig. 1. This shows a map of northwest Syria showing Bab Al-Hawa border crossing and the density of COVID-19 cases across districts covered by the Early Warning and Response Network. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

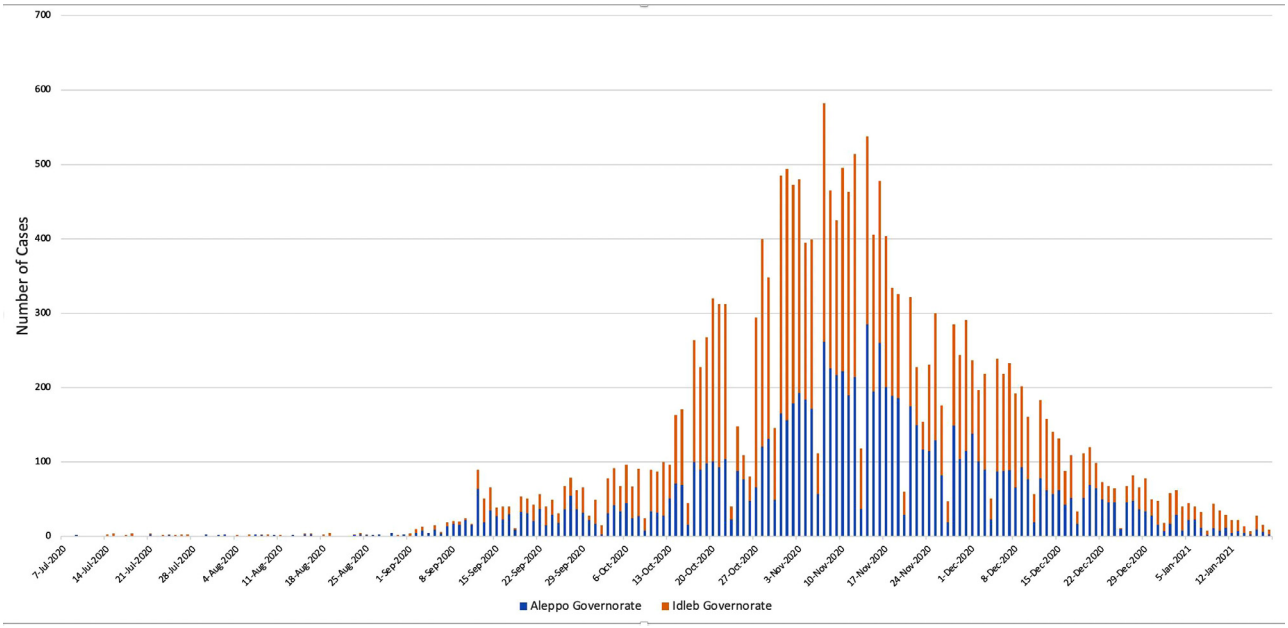

Fig. 2. This bar chart shows the number of cases in Idlib and Aleppo governorates over time.

As of January 2021, there are 9 hospitals (8 active), with 615 available beds, 142 available intensive care beds and 55 ventilators available for COVID-19 patients. In addition to 36 community treatment centres (22 active), including 960 beds.

Discussion

The increase in the numbers of cases of COVID-19 in NWS was initially slow however as of mid-September 2020, cases increased rapidly and have overwhelmed hospital and staff capacity though the rate of new cases appears to be slowing. We highlight a number of findings. As elsewhere, the impact on healthcare workers both personally and professionally has been grave with a large proportion affected; however, healthcare workers in this area work in an already exhausted and under-resourced health system after almost a decade of conflict and face ongoing attacks.[5] The effects on the health workforce in areas under government control have been even more stark with suggestions that at least 165 doctors have lost their lives, however, official confirmation has been suppressed.[6] Seroprevalence studies among healthcare workers have not been performed so the true extent of exposure remains unknown. The high representation of healthcare workers among positive cases is likely due to increased exposure as in other contexts but also access to testing, particularly early in the outbreak. Secondly, early in the outbreak, there were fewer cases than expected in the camps however, as of the end of October 2020, cases are increasing; the slow increase is likely a result of poor access to testing, poor trust in local healthcare providers and restricted movement. This is being addressed and a number of activities including more sampling locations, camp screening around the first confirmed cases in some camps, and more community engagement activities were conducted targeting the camps area. Lastly, the declared number of cases (around 0.5% of the population) likely represents an underestimate of cases due to under-testing and underdiagnosis due to weakened health system capacity after almost a decade of protracted conflict. Conclusions: The high prevalence of COVID-19 among healthcare workers in NWS is of major concern. Measures to protect healthcare workers from increased rates of infection are urgently required; these include prioritization for vaccination, improved access to PPE, and refining IPC precautions in health facilities.

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Accepted 30 January 2021
Available online 3 February 2021

https://doi.org/10.1016/j.jinf.2021.01.027

High-risk exposure without personal protective equipment and infection with SARS-CoV-2 in-hospital workers - The CoV-CONTACT cohort

Dear Editor,

Two recent studies published in this journal focused on SARS-CoV-2 infection among hospital workers (HWs), the first one reported the prevalence of SARS-CoV-2 carriage among HWs and the second, the clinical presentation of symptomatic HWs in order to identify new cases as early as possible and to stop nosocomial transmission[1,2]. The objective of the present study was to estimate within the hospital, the risk of in-hospital HWs infection following a high-risk exposure to SARS-CoV-2-infected subject without personal protective equipment.

We conducted the CoV-CONTACT study, a prospective cohort which included HWs, hereafter referred to as "contacts" with an high risk exposure to an SARS-CoV-2-infected person (either a patient or a colleague) hereafter referred to as "index", in the 1000 bed Bichat Claude Bernard University Hospital (Paris, France) between March, 3rd 2020 and April, 27th 2020[3]. Exposure was considered to be at high-risk of SARS-CoV-2 transmission if it occurred i) face-to-face, within one meter and without protective surgical or FFP2/N95 mask, and ii) during a discussion or while the index had an episode of coughing or sneezing, and iii) in the 72 h prior to, or following the virological diagnosis, or during the symptomatic period of the index.

Following exposure and upon written informed consent, daily symptoms were self-reported for 30 days; nasopharyngeal swabs for SARS-CoV-2 RT-PCR were performed at inclusion and at days 3, 5, 7 and 12; SARS-CoV-2 IgG serology (LuLISA N and EuroIMMUN[4,5]) was assessed at inclusion and at day 30. Confirmed infection was defined by positive RT-PCR or seroconversion, and possible infection by one general and one specific symptom for two consecutive days. SARS-CoV-2 seroconversion was defined as the apparition of a positive SARS-CoV-2 serology at the D30 visit, or as an at least two-fold increase of the LuLISA signal or EuroIMMUN ratio between inclusion and day 30. The primary endpoint was confirmed or possible SARS-CoV-2 infection, hereafter referred to as "SARS-CoV-2 infection".

The 146 analysed contacts were exposed to 42 COVID-19 index. No contacts worked in a front-line COVID-19 unit (Table 1). Exposure to patient decreased from 67.4% (56/83) before March, 18th (the date of the widespread use of masks in the hospital) to 15.9% (10/63) after March, 18th.

Overall, 24 /146 contact subjects (16.4%, 95%CI [11.0%–23.7%]) had at least one SARS-CoV-2-positive nasopharyngeal swab; 16/146 contact subjects (10.9%) had positive serology at inclusion which did not respond to the seroconversion definition, revealing a pre-existing infection and 31 additional contact subjects (21.2%, 95%CI [15.1%–28.9%]) exhibited a seroconversion at D30. Based on self-administered questionnaires, 59/146 contact subjects (40.4%, 95%CI [32.5%–48.9%]) met the definition of a clinical infection Fig. 1. Seven out of 24 subjects with positive SARS-CoV-2 nasopharyngeal RT-PCR had a positive RT-PCR before the symptoms onset; the first positive nasopharyngeal RT-PCR was observed as early as six days before symptoms onset. At day 30, 63/146 contacts (43.2%, 95%CI [35.1%–51.6%]) had SARS-CoV-2 infection (confirmed in 35 (23.9%, 95%CI [17.5%; 31.9%]), and possible in 28 (19.2%, 95%CI [13.3%; 26.7%])). In the multivariable analysis, the variables associated with SARS-CoV-2 infection were being a non-caregiver HW (aOR = 4.1, 95%CI [1.4; 12.2], $p = 0.010$) and being exposed to a SARS-CoV-2-infected patient (aOR = 2.6, 95%CI [1.2; 5.7], $p = 0.013$) rather to an infected colleague (Table 1).

Following universal masking for HWs on March, 18th in our hospital, high-risk exposure to SARS-CoV-2-positive patients dropped by 4 and high-risk exposure to SARS-CoV-2-positive colleagues became predominant, making colleagues-to-colleagues transmission a potentially major route of infection[6]. Of note, none of the exposures between a HW and a SARS-CoV-2 infected patient occurred in the front-line services where the mask was worn by all caregivers from the beginning of the epidemic. These exposures occurred, prior to universal masking, in second-line services in which patients had not been previously identified as COVID-19. The profession of the contact subjects was associated with infection, but we did not find any association with the type of activities of the HWs.

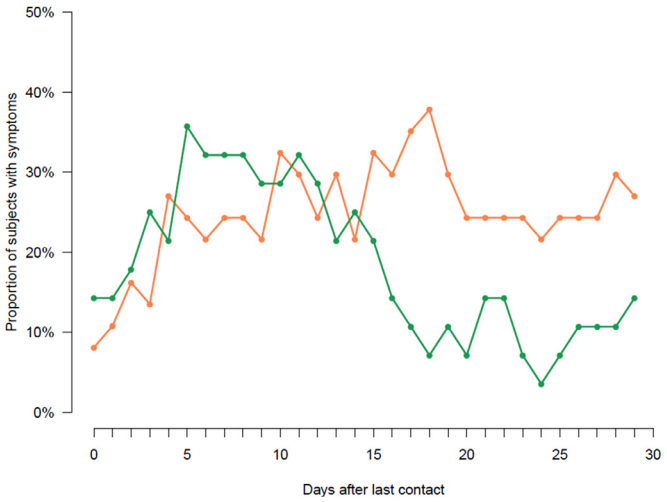

Fig. 1. Proportions of symptomatic contact subjects among the 146 contacts of the CoV-CONTACT cohort. The orange curve corresponds to contacts subjects with confirmed SARS-CoV-2 infection (*i.e.*, virologically- or immunologically-proven, $n = 35$). The green curve corresponds to contacts subjects with possible SARS-CoV-2 infection (*i.e.*, clinically-suspected without viro-immunological confirmation, $n = 28$).

The 10.9% rate of HWs with SARS-Cov-2 antibodies at inclusion revealing a pre-existing infection while they were not working in front-line services, is close to the seroprevalence of 8.8% reported in the Paris area in the general population during this period[7,8]. In addition to these HWs already infected at inclusion, 31 others (21.2% of the total population) seroconverted at day 30.

We cannot state with certainty that contacts meeting the definition of confirmed infection acquired their infection as a result of the exposure leading to their inclusion in the study. There are several arguments in favor of the link between exposure and infection: the RT-PCR positivity within 12 days after contact, the chronology of symptom onset after contact, and the seroconversion rate observed within the 30 days following the exposure, which is much higher than that observed in the community between March and May 2020[7, 8]. In addition, the subjects included were counseled to strictly adhere to protective measures to avoid any chain of transmission during the D0-D30 period, limiting the risk of further exposure.

All together, the rate of transmission observed in HWs after high-risk exposure, which could be as large as 43%, and close to a recent report[9], strengthens the conclusion that universal masking of HW, both during contacts with patients and colleagues, and at all times, as soon as the epidemic has been identified, is essential to prevent HWs infection and maintain hospital capacities during outbreaks[10].

Acknowledgments

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Principal investigator: Duval Xavier

Table 1
Characteristics of the 146 contacts with high-risk exposure to SARS-CoV-2 included in the CoV-CONTACT cohort, according to the infection status at D30.

Variable	All contacts ($N = 146$)	Contacts with SARS-CoV-2 infection ($N = 63$)	Contacts with no SARS-CoV-2 infection ($N = 83$)	OR [95%CI]	*p*-value	aOR [95%CI]	*p*-value
Contact characteristics							
Age (year)	35 [29;46] ($N = 146$)	35 [28.5;45.5] ($N = 63$)	35 [30;47] ($N = 83$)	0.99 [0.96;1.02]	0.46		
Male gender	35/146 (24%)	11/63 (17.5%)	24/83 (28.9%)	0.52 [0.23;1.14]	0.11		
HW functions							
Medical doctor / Resident / Midwife	49/146 (33.6%)	14/63 (22.2%)	35/83 (42.2%)	1 (ref)	–	1 (ref)	–
Registered nurse / Certified nurse assistant /Physiotherapists / Hospital Students	74/146 (50.7%)	36/63 (57.1%)	38/83 (45.8%)	2.37 [1.11;5.22]	0.028	1.76 [0.78;4.03]	0.18
Non-caregiver HWs	23/146 (15.8%)	13/63 (20.6%)	10/83 (12%)	3.25 [1.17;9.36]	0.025	4.06 [1.42;12.18]	0.010
Coexisting conditions							
Obesity (BMI > 30 Kg/m²)	27/146 (18.5%)	13/63 (20.6%)	14/83 (16.9%)	1.28 [0.55;2.98]	0.56		
Tobacco use	36/146 (24.7%)	17/63 (27%)	19/83 (22.9%)	1.24 [0.58;2.66]	0.57		
Cardiopathy	8/146 (5.5%)	5/63 (7.9%)	3/83 (3.6%)	2.3 [0.54;11.57]	0.27		
Chronic respiratory disease	21/146 (14.4%)	7/63 (11.1%)	14/83 (16.9%)	0.62 [0.22;1.59]	0.33		
Chronic kidney disease	2/146 (1.4%)	2/63 (3.2%)	0/83 (0%)	NE	0.99		
Diabete	1/146 (0.7%)	0/63 (0%)	1/83 (1.2%)	NE	0.99		
Immusuppressive therapy	7/146 (4.8%)	4/63 (6.3%)	3/83 (3.6%)	1.81 [0.38;9.47]	0.45		
Current pregnancy	1/111 (0.9%)	0/52 (0%)	1/59 (1.7%)	NE	0.99		
Type of exposition							
Contact with > 1 index	26/146 (17.8%)	13/63 (20.6%)	13/83 (15.7%)	1.4 [0.59 ;3.3]	0.44		
Types of index subject							
Contacts with infected HW(s) only	80/146 (54.8%)	27/63 (42.9%)	53/83 (63.9%)	1 (ref)	–	1 (ref)	–
Contacts with infected patient	66/146 (45.2%)	36/63 (57.1%)	30/83 (36.1%)	2.36 [1.21;4.65]	0.01	2.62 [1.24;5.71]	0.013
Maximal SARS-CoV-2 viral load in the index subject	9.3 [7.5;10.8] ($N = 145$)	10 [7.6;10.8] ($N = 62$)	8.7 [7.5;10.8] ($N = 83$)	1.1 [0.93;1.31]	0.25		
Cumulated length of exposure > 30 min	98/143 (68.5%)	38/61 (62.3%)	60/82 (73.2%)	0.61 [0.3;1.23]	0.17		
Exposure to infected patient ($N = 66$)							
Care during an aerosol-generating procedure	6/66 (9.1%)	3/36 (8.3%)	3/30 (10%)	0.82 [0.14;4.73]	0.81		
Care without aerosol-generating procedure	55/66 (83.3%)	30/36 (83.3%)	25/30 (83.3%)	1 [0.26;3.7]	1		
Presence in the patient's room during an aerosol-generating procedure	22/66 (33.3%)	13/36 (36.1%)	9/30 (30%)	1.32 [0.47;3.8]	0.6		
Other type of contact	12/66 (18.2%)	10/36 (27.8%)	2/30 (6.7%)	5.38 [1.27;37.23]	0.04		
Exposure to a SARS-CoV-2-infected HCW ($N = 92$)							
Face-to-Face discussion	86/92 (93.5%)	31/34 (91.2%)	55/58 (94.8%)	0.56 [0.1;3.2]	0.5		
Participation in a joint meeting	25/92 (27.2%)	9/34 (26.5%)	16/58 (27.6%)	0.95 [0.35;2.43]	0.91		
Lunch sharing	20/92 (21.7%)	6/34 (17.6%)	14/58 (24.1%)	0.67 [0.22;1.89]	0.47		
Other type of contact	9/92 (9.8%)	3/34 (8.8%)	6/58 (10.3%)	0.84 [0.17;3.42]	0.81		

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Sponsor: Inserm

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Gov identification number: NCT0425989

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Accepted 30 January 2021
Available online 3 February 2021

https://doi.org/10.1016/j.jinf.2021.01.026

Side effect of a 6 p.m curfew for preventing the spread of SARS-CoV-2: A modeling study from Toulouse, France

Dear Editor,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged in Wuhan, China in December 2019 spreads mainly by sustained human-to-human transmission[1]. This spread has been so rapid that the WHO declared the resulting disease a pandemic[2]. After a first lockdown in March 2020, SARS-CoV-2 resumed its rampage in Europe, including France, at the end of the summer. We have used data from the measures to limit virus transmission, mask wearing, restricted access to public spaces and curfews, taken by several large cities to quantify their impact on virus proliferation[3]. The French authorities declared a new lockdown from October 29 to November 28, followed by a gradual release with a 8 p.m curfew from December 15, 2020. This curfew has shown its effectiveness in restricting the spread of the virus in France[3]. A recent study published in this journal assessed the impact of community-wide mask-wearing on the spread of SARS-CoV-2 in the Hong Kong population during the first phase of the epidemic, March 2020[4]. The efficacy of these public health measures has been widely questioned despite the fact that of they have all helped to restrict the spread of the virus[3,5]. We have examined the impact of the 6 p.m. curfew imposed by the French government from January 16, 2021 on the resumed proliferation of the virus after the New Year celebrations using data for the city of Toulouse, France.

Our model is a discretized version of a susceptible infectious and recovered (SIR)-type model[6]. These compartmental models are well suited to studies of the spread of SARS-CoV-2 in different populations[7,8]. Our model[3,5,9] includes a diffusion/transmission coefficient R_0 that varies with the likelihood of contagion, and a reduction coefficient $\widehat{c}$ that accounts for the impact of public health measures on virus transmission in the French city of Toulouse. The model predicts how the SARS-CoV-2 virus would have evolved and projects the daily percentage of new positive cases. We estimated $\hat{c}$ by correcting the values predicted by the model with observed data so that predictions and observations coincide over a given period. This model was then used to measure the influence of each individual public health measure on the dynamics of the SARS-CoV-2 infection. We focused on two periods: January 1–January 15, 2021, when an 8 p.m curfew was in force immediately after the New Year, and January 20–January 24, 2021, when the curfew was lowered to 6 p.m.

The January 1–January 15, 2021 period makes it possible to assess adherence to the curfew during the end-of-year holidays. The circulation of the virus among Toulouse inhabitants was reduced by 38% by the 8 pm curfew[5]. There should have been a 7–8% increase in positive RT-PCR tests between January 10 and 15 if the curfew had been strict adhered to. Instead, it was closer to 8.5–9%, which corresponds to less constraint of 37%. Using these data, the percentage of new positive cases per day would increase to 15.4% at the end of May 2021 and only then decrease to 10% of positive

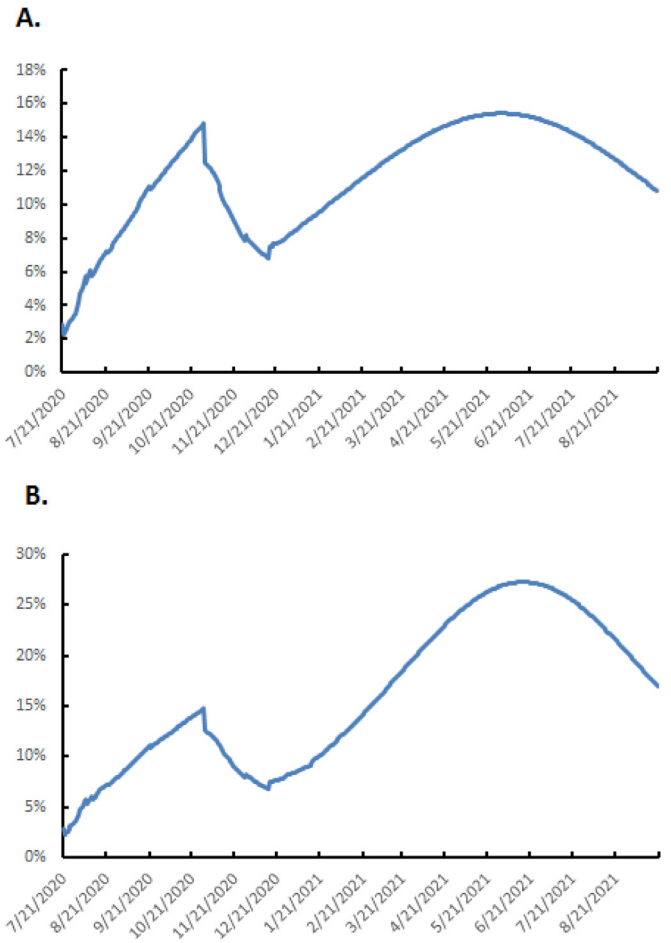

Fig. 1. SARS-CoV-2 infections per day, July 21, 2020 - September 20, 2021 according to the protective measures adopted. A: closure of some public spaces, compulsory masks and 8 pm curfew from December 15, 2020. B: closure of some public spaces, compulsory masks and 6 pm curfew from January 15, 2021.

tests in early February 2021 (Fig. 1A). The nation-wide 6 p.m curfew stating on January 16, 2021 provided the second data set for Toulouse (January 20–24, 2021). The real increase in positive PCR tests was above 10%, which was even greater than that predicted by the model after an 8 p.m curfew. The corresponding constraint was therefore 35% and the spread of virus would continue to increase, reaching 27.3% on June 15, 2021, before starting to decrease (Fig. 1B).

The 6 p.m curfew was intended to keep the circulation of SARS-CoV-2 under control after the Christmas/New Year period but it had exactly the opposite effect in the Toulouse urban area; it reduced the stress on virus spread by 2%. This could be because the more restrictive evening curfew results in larger groups of people in shops and supermarkets before they all hurried to get home.

This study shows that certain health measures can be ill-suited to local epidemiological situations and that their implementation must be accompanied by analysis of the local situation to avoid triggering an undesirable opposite effect.

Acknowledgements

The English text was edited by Dr Owen Parkes.

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Accepted 29 January 2021
Available online 31 January 2021

https://doi.org/10.1016/j.jinf.2021.01.021

Increased inflammatory markers reflecting fibrogenesis are independently associated with cardiac involvement in hospitalized COVID-19 patients

Dear Editor,

Dear Editor, a high proportion of COVID-19 infected patients have cardiac involvement [1], and elevated surrogate markers of myocardial injury and stress, such as troponins [2] and N-terminal pro-B-type natriuretic peptide (NT-proBNP) , are found in patients with poor outcome. Beyond direct angiotensin-converting enzyme 2 (ACE2) related mechanisms, overwhelming inflammatory responses could activate regulatory fibrotic pathways, induce tissue damage and be harmful for the host, and such “hyperinflammatory” mechanisms may be involved in COVID-19 associated car-

Table 1
Characterization of the study group.

	Controls	All patients	Cardiovascular endpoint	
	($n=16$)	($n=39$)	No ($n=17$)	Yes ($n=22$)
Women, n (%)	7 (44)	10 (25)	6 (35.3)	4 (18.2)
Age, years	66 ± 7	60 ± 15	58 ± 13	63 ± 16
Time from symptoms, days	–	9.6±3.6	9.7 ± 4.2	9.6 ± 3.2
Caucasian, n (%)	16 (100)	28 (70)	10 (59)	17 (77)
Current smoker, n (%)	3 (19)	8 (20)	2 (12)	7 (32)
P/F ratio	–	41 ± 15	44 ± 18	38 ± 12
Comorbidities, n (%)				
Cardiovascular	0 (0)	9 (23)	2 (12)	7 (32)
Pulmonary	0 (0)	1 (3)	0 (0)	1 (3)
Asthma	0 (0)	8 (20)	4 (24)	4 (18)
Renal	0 (0)	4 (10)	0 (0)	4 (18)
Liver	0 (0)	0 (0)	0 (0)	0 (0)
Neurological	0 (0)	1 (3)	0 (0)	1 (5)
cancer	0 (0)	1 (3)	1 (6)	0 (0)
hematological	0 (0)	1 (3)	1 (6)	0 (0)
Obesity	0 (0)	5 (13)	2 (12)	3 (14)
Diabetes	0 (0)	3 (8)	1 (6)	2 (9)
Rheumatic	0 (0)	4 (10)	1 (5)	3 (14)
Biochemistry				
Hemoglobin, g/dL	14.4±0.9	13.3±1.7**	12.7±1.6	13.7±1.7
Leukocytes, $x10^9$/L	5.6±1.9	6.6±3.2	5.3 ± 2.0	7.7 ± 3.6*
Lymphocytes, $x10^9$/L	1.68 ± 0.66	1.07 ± 0.45**	1.22 ± 0.45	0.95 ± 0.42
Monocyte, $x10^9$/L	0.54 ± 0.18	0.44 ± 0.2	0.48 ± 0.22	0.41 ± 0.17
Neutrophils, $x10^9$/L	3.24 ± 0.71	5.09 ± 3.2*	3.5 ± 1.9	6.3 ± 3.6*
Platelets, $x10^9$/L	254 ± 70	202 ± 59**	212±52*	194±66
ALT, U/L	29 ± 13	43 ± 40	58±55	32±19
AST, U/L	32 ± 9	49 ± 38	58±47	36±10
eGFR, mL/min/1.73m^2	82 ± 12	82 ± 30	88 ± 20	77 ± 37
CRP, mg/L†	1.6 [0.8. 3.9]	53 [31, 153]***	31 [15,41]	144 [53,191]***

Continuous data are given as mean±standard deviation. Cut-offs for NT-proBNP, cTni and cTnT are give in the methods section. *$p < 0.05$, **$p < 0.01$ vs. patients with no ICU/Death. †median [25th, 75th percentile].

diac involvement [3]. However, systemic inflammatory responses have been linked to both pulmonary and myocardial injury during COVID-19 disease [4], and it is unclear if enhanced cardiac stress is due to respiratory failure, rather than direct cardiac involvement. Underlying cardiovascular disease (CVD) promotes poor prognosis in COVID-19 disease and could further enhance the inflammatory burden.

We examined a range of inflammatory and fibrotic markers during COVID-19 hospitalization in relation to elevation of troponins and NT-proBNP. As cardiac markers and acute phase responses are influenced by kidney function, we focused on identifying inflammatory and fibrotic markers that displayed an association with elevated cardiac markers, beyond that explained by hyperinflammation (CRP), kidney- (estimated glomerular filtration rate, eGFR) and respiratory-function (P/F ratio) and co-morbid CVD.

Thirty-nine adult patients (≥18 years old) with confirmed COVID-19 were consecutively recruited between March 6 and April 14 to a clinical cohort study (Norwegian SARS-CoV-2 study; ClinicalTrials.gov, number NCT04381819). Clinical information and routine laboratory samples were collected at the earliest time-point after hospitalization. 1–3 plasma samples were collected at day 0–2 (within 48 h of admission), day 3–5 and day 7–10. Informed consents were obtained from all patients or next-of-kin if patients were incapacitated of giving consent. For reference, inflammatory markers were also analyzed in plasma from 16 healthy controls (Table 1). The study was approved by the South-Eastern Norway Regional Health Authority (reference number: 106,624).

The CV endpoint was defined prior to analysis as cardiac markers above reference values at any time during hospitalization (Fig. 1A/B): NT-proBNP (women: <50 years (y) ≥170 ng/L; 50–69 $y \geq 300$ ng/L; ≥70 $y \geq 760$ ng/L, men: <50y≥85 ng/L; 50–69 $y \geq 250$ ng/L; ≥70 $y \geq 500$ ng/L) or cardiac (c) Tnt (≥14 ng/mL), cTni (women ≥15 ng/mL, men ≥30 ng/mL). Cut-off references as provided by local laboratories based on product information from Roche (NT-proBNP and TnT) and Abbot (TnI).

A list of the various markers in relation to tissues and functions is given in Fig. 1A. Plasma markers were measured in duplicate by enzyme immunoassays using commercially available antibodies (R&D Systems, Minneapolis, MN) in a 384 format with intra-assay coefficient of variation <5%.

Patient characteristics were compared using student's *t*-test or chi-square for continuous and categorical variables, respectively (Table 1). Associations between the temporal profile of the inflammatory and fibrotic markers and CV-endpoint were evaluated in a generalized linear mixed model with patient number as random factor and time as fixed and cumulatively including CRP, eGFR, P/F ratio and comorbid CVD as covariates. These are reported with the F-statistic (Fig. 1A). Due to high number of markers, limited patient population with varying follow-up samples and multiple covariates we did not perform post-hoc testing. Markers of interest were visualized (Fig. 1B) and scatterplots (Pearson) with NT-proBNP and cardiac troponins assessed at each time-point (Fig. 1C). P-values are two-sided and considered significant when <0.05.

Of 39 COVID-19 patients, 18 and 10 patients had levels of NT-proBNP and troponins above age- and sex- adjusted reference levels, respectively, during hospitalization. Combined, 22 patients had cardiac markers above reference limits, defined as the CV endpoint in the study. These patients were characterized by high neutrophil counts and markedly higher CRP levels (Table 1).

The linear mixed model revealed multiple markers that were associated the CV endpoint in unadjusted analysis (Fig. 1A). However, after full adjustment for CRP, eGFR, P/F ratio and comorbid CVD, only the fibrotic markers GDF-15, POSN, TIMP1 and YKL-40 remained associated with the CV endpoint (Fig. 1A). All markers revealed a stable temporal profile and for GDF15, TIMP1 and YKL40, levels remained higher compared to patients without the CV endpoint and healthy controls (Fig. 1B). Of note, Spd, NGAL and in particular the vascular markers PTX3 and sTNFR1 were associated with the CV endpoint adjusting for CRP and eGFR but not following adjustment for pulmonary function.

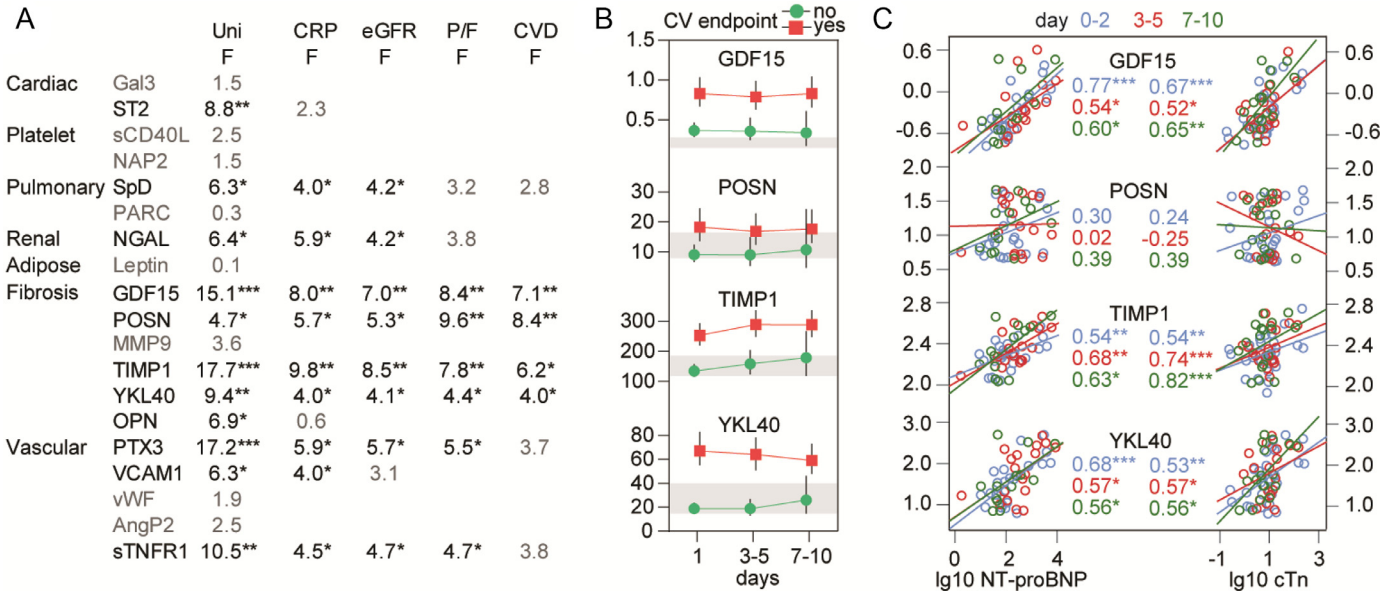

Fig. 1. Inflammatory markers and cardiac involvement during COVID-19 disease. A) Circulating markers measured in the study reflecting inflammation in relevant tissues or cells (pulmonary, adipose, cardiac, renal, platelets) or related to function (fibrogenesis, vascular inflammation). The table shows the F statistic from the generalized linear mixed model evaluated the impact of the temporal course of plasma markers on the CV endpoint. In adjusted analysis, C-reactive protein (CRP), estimated glomerular filtration rate (eGFR), P/F ratio and presence of cardiovascular comorbidity (CVD) were cumulatively added as covariates. $^{*}p < 0.01$, $^{**}p < 0.01$, $^{***}p < 0.001$. B) Temporal course of GDF15, POSN, TIMP-1 and YKL-40 (all ng/mL) during COVID-19 infection according to the CV-endpoint. Data are presented as back-transformed estimated marginal means with 95% confidence intervals from the mixed model analysis (see statistical methods). The gray area represents the estimated marginal mean (line) and 95% confidence interval (gray area) of healthy controls ($n = 16$). Available samples at the different time-points was 0–2 days: $n = 31$, 3–5 days: $n = 22$, 7–10 days: $n = 19$. C) Pearson correlation between NT-proBNP and cardiac troponins (cTn) and the selected markers (log transformed) at different time-points (day 0–2 blue, day 3–5 red, day 7–10 green) during the course of the study. SpD, surfactant protein D; PARC/CCL18, pulmonary and activation-regulated chemokine; ST-2, suppression of tumorigenesis-2; Gal-3, Galectin-3 ; sCD40L, soluble CD40 ligand; NAP2/CXCL7, neutrophil activating peptide; NGAL, neutrophil gelatinase-associated lipocalin; vWF, von Willebrand factor; AngP2, angiopoietin 2; PTX-3, pentraxin 3; sTNFR1, soluble tumor necrosis factor receptor type 1; CXCL16, C-X-C Motif Chemokine Ligand 16; VCAM1, vascular cell adhesion molecule 1; GDF-15, growth differentiation factor; POSN, periostin; OPN, osteopontin; MMP-9, matrix metallopeptidase 9; TIMP1, tissue inhibitor of matrix metalloproteinase; YKL-40 also known as chitinase-3-like protein 1. . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 1C shows the correlation analysis with continuous measures of NT-proBNP (left side) and troponins (right side) during the course of the study. As shown, GDF-15, TIMP-1 and YKL-40 were strongly positively associated with NT-proBNP and troponins and these associations were consistent at all time-points.

In the present study, over half of the hospitalized COVID-19 patients reached the CV endpoint as reflected by elevated levels of NT-proBNP and cardiac troponins supporting frequent cardiac involvement in these patients. Enhanced fibrosis has been related to respiratory failure in COVID-19 patients, but the fibrotic markers GDF-15, POSN, TIMP-1 and YKL-40 remained elevated in patients with cardiac involvement following adjustment with the P/F ratio obtained at the same time of sampling. Previous experimental and clinical studies have identified a role for TIMP-1, GDF-15 and YKL-40 [5–7] in promoting cardiac fibrosis and we suggest that the strong correlation with cardiac markers reflects a more direct role in cardiac fibrosis in COVID-19 patients.

Cardiac involvement in COVID-19 disease has been speculated to involve downregulation of the ACE2, and of relevance, downregulation of ACE2 in experimental models enhances cardiac remodeling and fibrosis involving upregulation of TIMP-1 [8] and POSN [9]. Furthermore, GDF-15 correlated with poor outcome in hospitalized COVID19 patients [10]. Thus, activation of these inflammatory pathways involved in fibrogenesis and ECM remodeling may represent novel targets for intervention in COVID-19 patients.

In conclusion, our study shows that fibrosis and ECM remodeling may play an important role in the cardiac involvement during COVID-19 infection

Declaration of Competing Interest

None

Author contributions

Conception and design: TU and PA. Data analysis and acquisition:

TU, AMDR, JCH, ARH, FM, SGD, KT, LH. Interpretation of the data: TU and PA. Drafting or revision of the manuscript: TU and PA. Final approval of the manuscript: all authors.

Sources of support

This study received funding from the Research Council of Norway grant no 312780 and has received private donation from Vivaldi Invest A/S owned by Jon

Stephenson von Tetzchner.

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Accepted 25 January 2021
Available online 28 January 2021

https://doi.org/10.1016/j.jinf.2021.01.017

Host-cell recognition through GRP78 is enhanced in the new UK variant of SARS-CoV-2, *in silico*

Dear Editor,

In this Journal we previously reported the predicted SARS-CoV-2 spike-host cell receptor GRP78 binding site (1). New SARS-CoV-2 variant VUI 202,012/01 started in the UK and currently spreading in Europe and Australia during the last few days. The new variant bears about nine mutations in the spike protein (Δ69–70, Δ145, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H). The N501Y lies in the receptor-binding domain (RBD) of the spike and interacts with the host-cell receptor ACE2 responsible for viral recognition and entry. We tried to simulate the system of ACE2-SARS-CoV-2 spike RBD in the wildtype and mutated isoform of the RBD (N501Y). Additionally, the GRP78 association with the ACE2-SARS-CoV-2 spike RBD is modeled at the presence of this mutant variant of the viral spike.

Based on our previous study, the Heat Shock Protein A5 (HSPA5), also called, Glucose Regulated Protein 78 (GRP78) or Bip, is predicted to bind to the receptor-binding domain (RBD) of the SARS-CoV-2 Spike.[1] The GRP78 is predicted to bind the Spike protein alongside the putative host-cell receptor, the Angiotensin-Converting Enzyme 2 (ACE2).[2,3] The binding of GRP78 to the spike/ACE2 complex is predicted using HADDOCK 2.4 webserver[4] (Fig. 1A). PyMOL V2.2.2 was utilized to do a point mutation (N501Y) to resemble the RBD mutation found in the new variant of COVID-19.[5] We docked GRP78 with both wild type SARS-CoV-2 Spike RBD-ACE2 complex (WT ACE2-RBD), and N501Y mutant SARS-CoV-2 Spike RBD-ACE2 complex (Mut ACE2-RBD). GRP78 and SARS-CoV-2 Spike RBD's active sites were T428, V429, V432, T434, F451, S452, V457 & I489 and C480-C488, respectively, and the rest of HADDOCK options were kept as default. The carbohydrate moieties (NAG) attached to the proteins were held in the structure.

The HADDOCK score values for the GRP78 against WT ACE2-RBD and Mut ACE2-RBD are -74.3 ± 0.9 and -95.6 ± 1.0, respectively. This indicates better binding for the GRP78 against Mut ACE2-RBD than the WT ACE2-RBD complexes. There is a 28.7% increase in the HADDOCK score of GRP78 to the Mut ACE2-RBD form compared to the WT ACE2-RBD. The interactions between GRP78 and the two complexes are presented in Table 1. GRP78 is tightly bound to the mutated complex with three H-bonds and five hydrophobic contacts instead of two H-bonds and three hydrophobic contacts in the case of WT ACE2-RBD, respectively. On the other hand, the docking scores and the interactions established upon docking of the ACE2 into SARS-CoV-2 spike RBD in wildtype and N501Y mutant isoforms are shown in Table 1.

As shown in the table, the HADDOCK score of ACE2 to both WT RBD and Mut RBD of SARS-CoV-2 spike protein is almost the same. The interactions are established through a dozen H-bonds, about eight hydrophobic contacts, and a salt bridge. The Y501 in the spike's mutant variant engaged in H-bond with K353 and formed π-stacking interaction with Y41 of the ACE2.

The best result from the two docking experiments (GRP78 against WT ACE2-RBD and Mut ACE2-RBD) was selected for Molecular Dynamic Simulation (MDS) using Nanoscale molecular dynamics software (NAMD) version 2.13.[6] The necessary files for MDS were generated using the CHARMM-GUI webserver. The system's temperature and salt concentration were set to be 310 K and 0.154 M NaCl to resemble the physiological conditions. The system was minimized for 20,000 steps in a constant number of atoms, constant volume, constant temperature (NVT) ensemble using a conjugate gradient algorithm. The system was then equilibrated in a constant number of atoms, constant pressure, and constant temperature (NPT) ensemble for one nanosecond period. The pressure was controlled by the Nose-Hoover Langevin piston set to at-

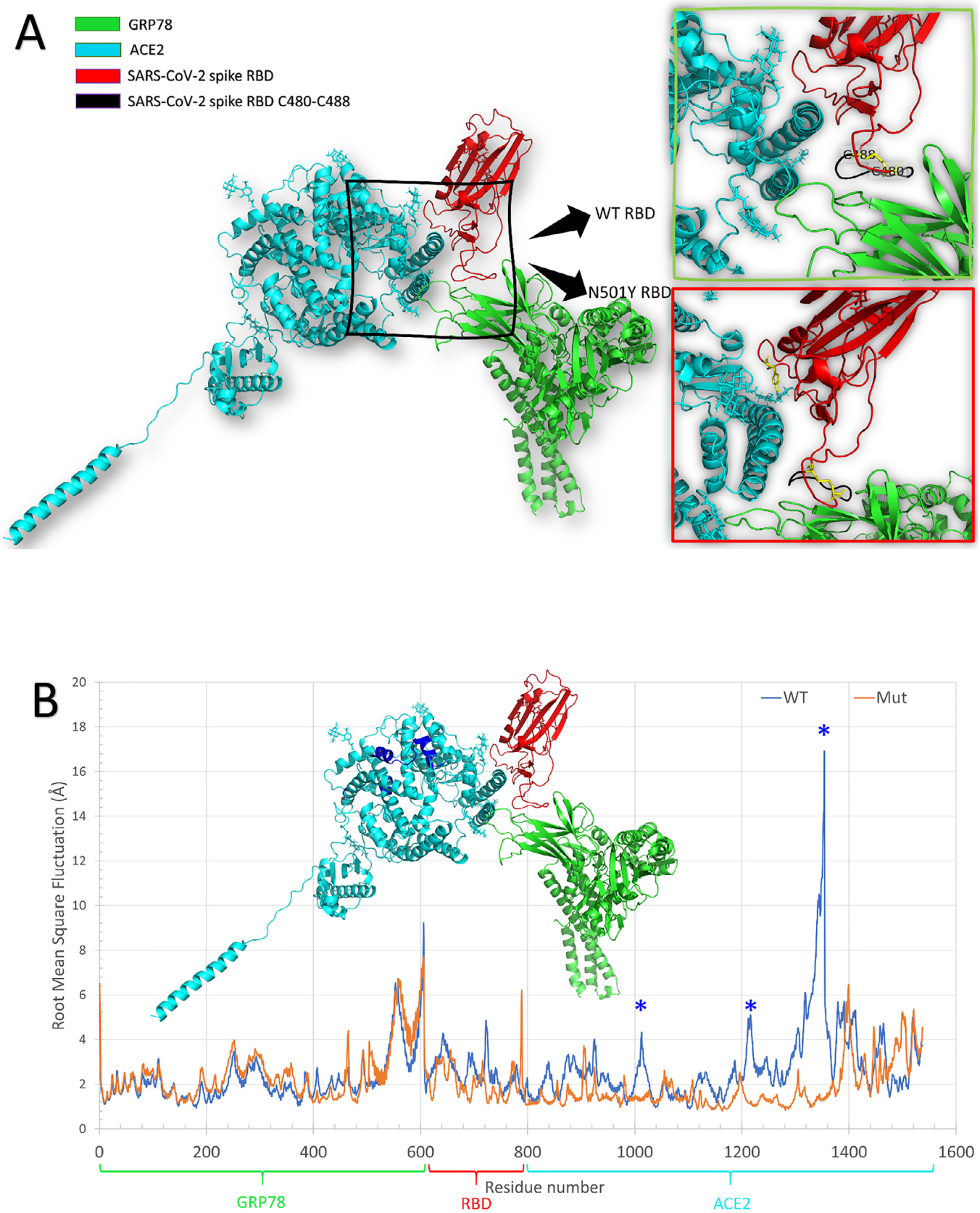

Fig. 1. (A) The docking of GRP78 (5E84) to ACE2-SARS-CoV-2 Spike RBD complex (6M17). The enlarged panels on the right side show the docking pose in WT RBD (up) and the N501Y mutant variant (down). The C480-C488 of the RBD that was reported to bind GRP78 is shown in black cartoons. (B) The per -residue RMSF calculated for the 25 ns period MDS on the GRP78-RBD-ACE2 where both WT and N501Y mutant RBD is used. The proteins are represented with the same coloring scheme as Fig. 1. Blue asterisks denote the blue regions in the ACE2.

Table 1
The interaction patterns after docking GRP78 into the WT ACE2-RBD and Mut ACE2-RBD and ACE2 into the WT RBD and Mut RBD (N501Y). Bold residues are the interacting residues found in both WT and Mut ACE2-RBD complexes, while blue residues form Pi-stacking interactions.

		H-bonding			Hydrophobic interaction			Salt Bridge		
complex	**HADDOCK score**	**No.**	**Amino acids involved from RBD**	**Amino acids involved from GRP78**	**No.**	**Amino acids involved from RBD**	**Amino acids involved from GRP78**			
WT ACE2-RBD-GRP78	**−74.3 ± 0.9**	2	N481 and **F486**	E427 and **G454**	3	T478, **P479**, and **V483**	**V453 (2)** and **V457**			
Mut ACE2-RBD-GRP78	**−95.6 ± 1.0**	3	E471, T478, and **F486**	G430, S452, and **G454**	5	**P479**, N481, **V483 (2)**, and F486	T428, **V453 (2), V457**, and V490			
complex	**HADDOCK score**	**No.**	**Amino acids involved from RBD**	**Amino acids involved from ACE2**	**No.**	**Amino acids involved from RBD**	**Amino acids involved from ACE2**	**No.**	**Amino acids involved from RBD**	**Amino acids involved from ACE2**
WT ACE2-RBD	**−126.1** ± 3.3	**12**	**K417, Y449**, Y473, **N487, Y489, Q493**, S494, **T500(3)**, G502, and V503	E23, **D30, H34(2)**, D38, Y41, **Y83(2)**, T324, **K353**, D355, and **R357**	**9**	**F456(2)**, Y473, A475, **F486(2)**, Y489, and **T500(2)**	Q24, **T27(3)**, D30, **Y41, M82, Y83**, and D355	**1**	**E484**	**K31**
Mut ACE2-RBD	**−120.8** ± 1.7	**13**	**K417**, G446, **Y449**, Y453, **N487(3), Y489**, F490, **Q493**, Q498(2), **T500**, and Y501	Q24, T27, **D30**, K31(2), **H34**, Q42(4), **Y83(2), K353**, and **R357**	**8**	**F456(2), F486(3), F500**, Y501, and Y505	**T27(2)**, K31, **Y41, M82, Y83(2)**, and K353	**1**	**E484**	**K31**

mospheric pressure (1.01325 bar), while Langevin dynamics control the temperature. Finally, a production run of 25 ns was initialized in the NVT ensemble. The force field used was CHARMM36 force field parameters. TIP3P water model is used in the system simulation using NAMD 2.13 software.[7] Different in-house scripts and the visualizing molecular dynamics (VMD) software tools are used to analyze data.[8,9]

Fig. 1B shows the superposition of the per-residue Root Mean Square Fluctuations in Å (calculated during 25 ns MDS) in the case of WT ACE2-RBD-GRP78 (blue line) and Mut ACE2-RBD-GRP78 (orange line). The WT complex show three highly flexible regions (blue asterisks) in the ACE2 (blue cartoon), while the other proteins show no significant differences. The systems need more in-depth analysis and calculations that require more time.

In this letter, we propose to shed light on the effect of the new variant mutation N501Y of the RBD on the viral recognition by the host cell-surface GRP78. This recognition can be targeted by peptides, antibodies, and phytochemicals (10).

Declaration of Competing Interest

All the authors declare no competing interest in this work.

Author contribution

A.E. own the research idea, wrote the manuscript, and draw figures, while I.I. performed the calculations and make the tables. All the authors approve the final version of the manuscript.

Acknowledgment

Cairo University supported this work through the COVID-19 grant.

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Accepted 19 January 2021
Available online 22 January 2021

https://doi.org/10.1016/j.jinf.2021.01.015

Quality of life after COVID-19 without hospitalisation: Good overall, but reduced in some dimensions

Dear Editor,

We have with interest read the paper by Garrigues et al.[1], showing that symptoms may persist several months after hospitalization for COVID-19, as also shown elsewhere.[2] Garrigues et al. also showed that there was little difference in health-related qual-

Table 1
EQ-5D-5 L and RAND-36 scores and z-scores for comparison with Norwegian general population norms* ($N = 458$).

		Scale score	Z-score		
Instrument	n	Mean (SD)	Mean (SD)	P**	<5th percentile, n (%)****
EQ-5D index ***	456	0.82 (0.17)	−0.07 (1.00)	0.13	26 (6)
RAND-36 (range 0 to100)					
Physical functioning	457	86.1 (18.2)	−0.04 (1.10)	0.39	42 (9)
Role limitations-physical	457	70.5 (40.1)	−0.17 (1.16)	0.002	73 (16)
Bodily pain	458	75.6 (24.2)	−0.01 (0.96)	0.79	34 (7)
General health	451	65.6 (19.3)	−0.35 (0.95)	<0.001	49 (11)
Energy/fatigue	458	56.8 (23.9)	−0.20 (1.14)	<0.001	58 (13)
Social functioning	458	79.6 (23.9)	−0.32 (1.17)	<0.001	65 (14)
Role limitations-emotional	457	80.2 (35.2)	−0.15 (1.18)	0.008	68 (15)
Emotional well-being	458	78.2 817.6)	−0.16 (1.13)	0.003	49 (11)

* adjusted for age and sex.
** z-score=0.
*** range −0.654 to 1.00.
**** lower score than the 5th percentile in the norm population ($z<-0.1645$).

ity of life (HRQoL) between ward and ICU patients.[1] There is less evidence for the non-hospitalised, but breathlessness and fatigue is common after several months.[3, 4]

The majority of those diagnosed with COVID-19 are not hospitalised, and yet there is little evidence relating to their HRQoL, though a recent study reported on quality of life in non-hospitalised subjects recruited from a Facebook support group for patients with persistent complaints after confirmed/suspected COVID-19.[5]

The present study assessed HRQoL with the widely used EQ-5D and RAND-36 instruments in a population-based cohort of non-hospitalised subjects in Norway, on average 4 months after their COVID-19. Scores were compared with general population norms.

This was a cross-sectional survey of a geographical cohort in the catchment areas of Akershus University (Ahus) and Østfold Hospitals, covering about 900,000 inhabitants in 2020, or 17% of the population of Norway.[4] Prior to 1 June 2020, 1029 PCR SARS-CoV-2-positive subjects ≥18 years were identified, of whom, 938 were eligible for the survey (Supplement, Fig.1).

At the end of June 2020, subjects received a postal invitation asking them to sign a consent form on-line via their personal identification number and national electronic identification system, and thereafter received an online web-questionnaire. Alternatively, they could complete and return paper versions. Non-respondents received a postal reminder after 5 weeks.

The questionnaire included the EQ-5D-5 L and RAND-36 (SF-36), and the modified Medical Research Council (mMRC) dyspnoea scale,[6] other health-related information and background characteristics. The EQ-5D-5 L assesses five items or dimensions of health — mobility, self-care, usual activities, pain/discomfort, anxiety/depression — with five response levels from no problems to extreme problems/unable to do. Responses to all five dimensions represent a health state with a value attached, an index score, anchored at 0=dead and 1=full health, with values <0 indicating states worse than dead. Scoring was based on the UK EQ-5D-3 L algorithm together with a mapping algorithm (7). The RAND-36 assesses eight dimensions of health with two to five levels. Items sum to give eight scores from 0 to 100 (best health possible).[8]

The distributions of crude EQ-5D dimension scores were compared with Norwegian general population norms[9] using Fisher's exact test. Respondents' EQ-5D index scores and RAND-36 scores were compared with general population norms[9,10] after matching in age- and sex- specific strata. Z-scores were used, representing the difference from the mean of the norm population reported in number of SDs. We used the paired *t*-test to test for statistical significance, using a 5% significance level. We also present the percentages scoring below the 5th percentile of the norms ($z<-1.645$). The Regional Committees for Medical and Health Research Ethics, Health Region South East (approval no. 2020/ 149,384) and the Data Protection Officer at Akershus University Hospital approved the study.

The questionnaire was completed by 458 (49%) subjects at a median of 117.5 days after COVID-19 onset. Their mean age was 49.5 (SD 15.3) and 256 (56%) were women (Supplement, Table 1). In total, 289 (65%) reported no dyspnoea, 110 (25%) grade 1 and 48 (11%) grade 2–4.

Similar response distributions to the general population were found for the five EQ-5D dimensions (Fig. 1). However, COVID-19 subjects had higher proportion responses to the level 2, indicative of slight problems, for mobility and usual activities dimensions. For these two dimensions, the distribution of scores differed from the general population norms.

EQ-5D index scores were not different from those for the general population (Table 1). The mean z-scores for the eight dimensions of the RAND-36 were negative, indicating poorer health, for the COVID-19 subjects. The largest mean z-scores, were found for general health followed by social functioning and energy/fatigue. Compared to general population norms, differences ($p<0.01$) were found for 6 of 8 RAND-36 dimension scores, the exceptions being physical functioning and pain (Table 1).

This is one of the first studies to assess HRQoL in non-hospitalised COVID-19 subjects at follow-up, using the two most widely used patient-reported outcome measures, EQ-5D and RAND-36. Lower scores than the norm population were found across important aspects of health 1.5 to 6 months following COVID-19 symptom onset. Participants had lower mobility scores on the EQ-5D, corresponding to the finding of a high proportion of subjects with persistent dyspnoea on the mMRC scale.

The mean EQ-5D index of 0.82 was similar to that reported for hospitalised patients on average 111 days after admission,[1] however >1 SD higher than the 0.62 reported for Facebook-recruited subjects.[5]

There is some evidence for prolonged fatigue for non-hospitalised patients in the months after being diagnosed with COVID-19.[3] The current study not only shows that other aspects of health are also affected in these subjects, but compared to the general population, social functioning and general health were the most affected, as shown by z-scores. Moreover, differences from RAND-36 population norms were most apparent for dimensions associated with aspects of mental health, which in addition to social functioning, included role-limitations due to emotional problems and emotional well-being.

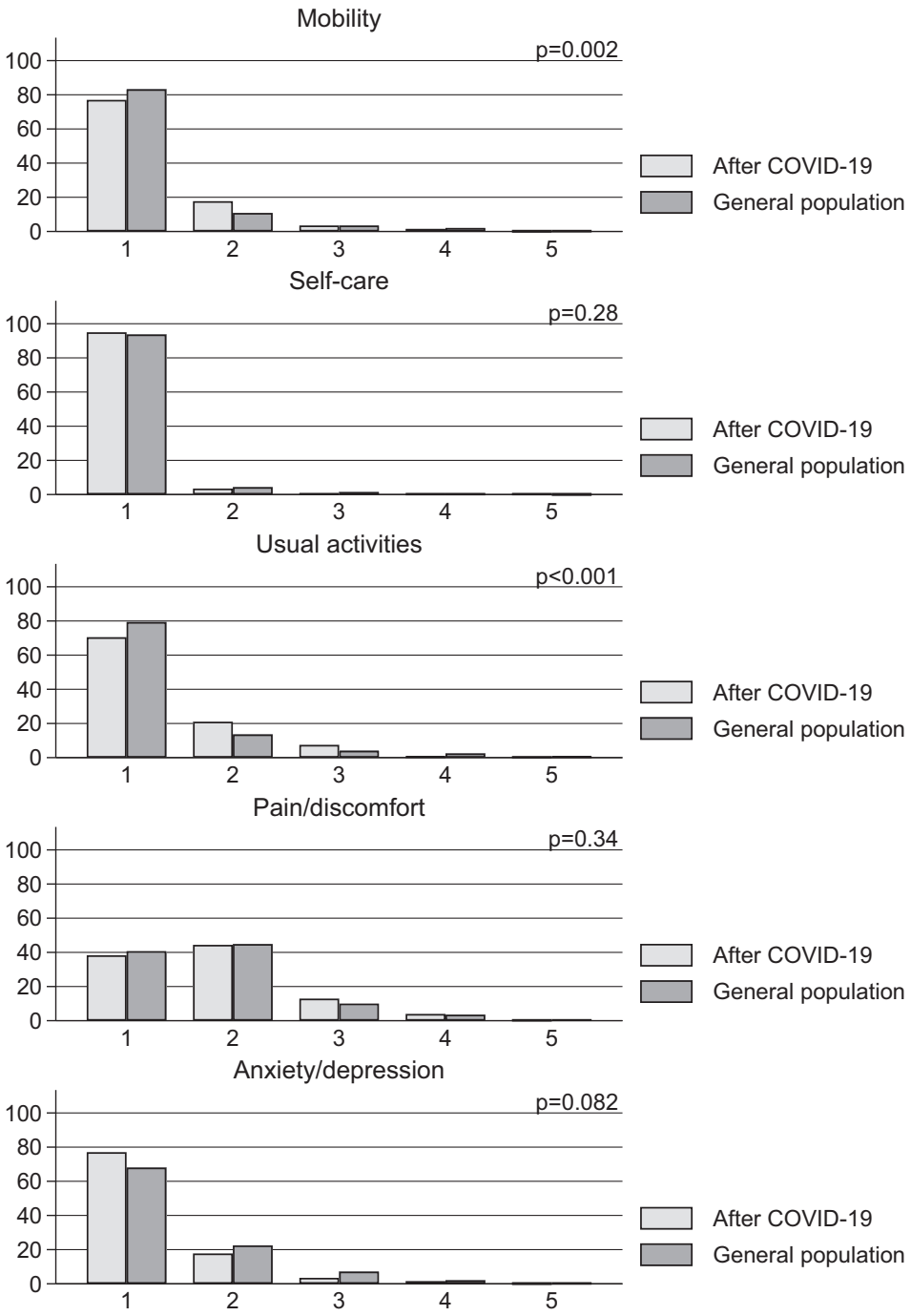

Fig. 1. EQ-5D dimension scores 1.5–6 months after start of COVID-19 and for Norwegian general population norms. P-values are for comparison of the distributions using Fisher's exact test.

This population-based study had a response rate of 49%, with low response in three districts having a high proportion of immigrants. Moreover, the responses were somewhat biased towards females and subjects >50 years of age, which is common in epidemiological surveys.

There is accumulating evidence that COVID-19 has a long-term impact on both hospitalised and non-hospitalised patients, which includes not just symptoms, but a broader impact on aspects of quality of life including mental health. The longer-term follow-up of both groups is recommended, and the use of widely used instruments such as the EQ-5D and RAND-36, will help understand how their health is affected over time compared to the general population.

In conclusion, in this study of non-hospitalised subjects, EQ-5D index scores did not differ from the general population norms. However, several important dimensions of HRQoL, including aspects of mental health, were lower than general population norms 1.5–6 months after COVID-19 onset.

Author contributions

All authors have made substantial contributions to this work and have reviewed and approved the final version. Concept and design: all authors. Acquisition of data: KS, GE, WG. Statistical analysis: KS. Interpretation of data: KS, AMG. Writing original draft: AMG, KS. Writing review and editing: all authors.

Declaration of Competing Interest

None of the authors declared any competing interest relative to the present study

Financial Support

None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.01.002.

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Accepted 8 January 2021
Available online 9 January 2021

https://doi.org/10.1016/j.jinf.2021.01.002