



HAL
open science

A new SARS-CoV-2 variant poorly detected by RT-PCR on nasopharyngeal samples, with high lethality: an observational study

Pierre Fillâtre, Marie-José Dufour, Sylvie Behillil, Remi Vatan, Pascale Reusse, Alice Gabellec, Nicolas Velmans, Catherine Montagne, Sophie Geffroy
Du Coudret, Edith Droumaguet, et al.

► To cite this version:

Pierre Fillâtre, Marie-José Dufour, Sylvie Behillil, Remi Vatan, Pascale Reusse, et al.. A new SARS-CoV-2 variant poorly detected by RT-PCR on nasopharyngeal samples, with high lethality: an observational study. *Clinical Microbiology and Infection*, 2022, 28 (2), pp.298.e9-298.e15. 10.1016/j.cmi.2021.09.035 . hal-03373018

HAL Id: hal-03373018

<https://hal.science/hal-03373018v1>

Submitted on 26 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

A new SARS-CoV-2 variant poorly detected by RT-PCR on nasopharyngeal samples, with high lethality: an observational study

Pierre Fillâtre, M.D.¹, Marie-José Dufour, M.D.², Sylvie Behillil P.D.³, Remi Vatan, M.D.^{4, 5}, Pascale Reusse, M.D.⁶, Alice Gabellec, M.D.⁶, Nicolas Velmans, M.D.⁴, Catherine Montagne, M.D.⁷, Sophie Geffroy Du Coudret, M.D.⁸, Edith Droumaguet, M.D.⁹, Véronique Merour, M.D.¹⁰, Vincent Enouf, Ph.D.^{3, 11}, Rodolphe Buzele, M.D.⁵, Marion Valence, M.D.⁵, Elena Guillotel M.D.¹², Bertrand Gagniere, M.D.¹³, Artem Baidaliuk, Ph.D.¹⁴, Anna Zhukova, Ph.D.¹⁵, Mathieu Tourdjman, M.D.¹⁶, Vincent Thibault M.D., Ph.D.¹⁷, Claire Grolhier M.D.¹⁷, Charlotte Pronier M.D.¹⁷, François-Xavier Lescure M.D., Ph.D.¹⁸, Etienne Simon-Loriere, Ph.D.¹⁴, Dominique Costagliola, Ph.D.¹⁹, Sylvie Van Der Werf M.D., Ph.D.^{3*}, Pierre Tattevin, M.D., Ph.D.^{20*}, Nicolas Massart M.D.^{1*}

¹ Service de Réanimation polyvalente, CH de Saint-Brieuc, 10, rue Marcel Proust 22000 Saint-Brieuc, France.

² Service de Biologie médicale, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

³ National Reference Center for Respiratory Viruses, Molecular Genetics of RNA Viruses, CNRS-UMR 3569, University of Paris, Institut Pasteur, Paris, France

⁴ Service de Médecine Interne, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

⁵ Service de Maladies Infectieuses, CH de Saint-Brieuc, 10 rue Marcel Proust 22000 Saint-Brieuc, France.

⁶ Service de Gériatrie, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

⁷ Service d'Imagerie Médicale, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

⁸ Service de Pneumologie, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

⁹ Département d'informatique médicale, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

¹⁰ Plateforme de surveillance continue, CH de Lannion, Centre Hospitalier Pierre-le-Damany, 22303 Lannion, France.

¹¹ Mutualized Platform of Microbiology, Pasteur International Bioresources Network, Institut Pasteur, Paris, France

¹² Service de Biologie médicale, CH de Saint-Brieuc, 10, rue Marcel Proust 22000 Saint-Brieuc, France.¹³ Santé publique France, French national public health agency, Cellule Bretagne, Rennes, France

¹⁴ Evolutionary Genomics of RNA Viruses, Institut Pasteur, Paris, France

¹⁵ Unité Bioinformatique Evolutive & Hub de Bioinformatique et Biostatistique - Département Biologie computationnelle, USR3756 (C3BI/DBC), Institut Pasteur, Paris, France

¹⁶ Santé publique France, the French public health agency, Saint-Maurice, France

¹⁷ Service de Virologie, CHU de Rennes, 2, rue Henri le Guilloux 35000 Rennes, France

¹⁸ Department of Infectious and Tropical Diseases, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France; Infections Antimicrobials Modelling Evolution (IAME) UMR 1137, University of Paris, Paris, France

¹⁹ Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique (IPLESP), Paris, France

²⁰ Service de maladie infectieuse et de réanimation médicale, CHU de Rennes, 2, rue Henri le Guilloux 35000 Rennes, France.

* These authors equally contributed to this manuscript.

Keywords : COVID-19; SARS-CoV-2 variants; Severity of illness index; Coronavirus Infections / epidemiology; Coronavirus Infections / virology

Corresponding author Pierre FILLÂTRE, Service de Réanimation, CH de Saint-Brieuc, 10, rue Marcel Proust 22000 Saint-Brieuc, France, pierre.fillatre@armorsante.bzh, +33296017060

Alternate corresponding author : Pierre TATTEVIN, Service de maladie infectieuse et de réanimation médicale, CHU de Rennes, 2, rue Henri le Guilloux 35000 Rennes, France. pierre.tattevin@chu-rennes.fr, +33299284321

ABSTRACT

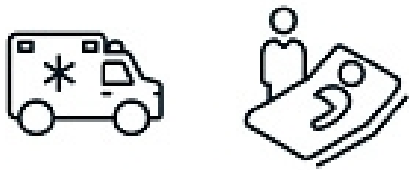
Objectives: In early January 2021, an outbreak of nosocomial cases of COVID-19 emerged in Western France, with RT-PCR tests repeatedly negative on nasopharyngeal samples but positive on lower respiratory tract samples. Whole genome sequencing (WGS) revealed a new variant, currently defining a novel SARS-CoV-2 lineage: B.1.616. In March, WHO classified this variant as ‘under investigation’ (VUI). We analyzed the characteristics and outcomes of COVID-19 cases related to this new variant.

Methods: Clinical, virological, and radiological data were retrospectively collected from medical charts in the two hospitals involved. We enrolled inpatients with either: i) positive SARS-CoV-2 RT-PCR on a respiratory sample; ii) seroconversion with anti-SARS-CoV-2 IgG/IgM; iii) suggestive symptoms and typical features of COVID-19 on a chest CT scan. Cases were categorized as either: i) B.1.616; ii) variant of concern (VOC); iii) unknown.

Results: From January 1st to March 24th, 2021, 114 patients fulfilled inclusion criteria: B.1.616 (n=39), VOC (n=32), and unknown (n=43). B.1.616-related cases were older than VOC-related cases (81 years interquartile range [IQR] [73-88], vs 73 years IQR [67-82], $P<0.05$) and their first RT-PCR tests were rarely positive (6/39, 15% vs 31/32, 97%, $P<0.05$). B.1.616 variant was independently associated with severe disease (multivariable Cox model HR 4.0 95% CI [1.5-10.9]), and increased lethality: 28-day mortality 18/39 (46%) for B.1.616, vs. 5/32 (16%) for VOC, $P=0.006$.

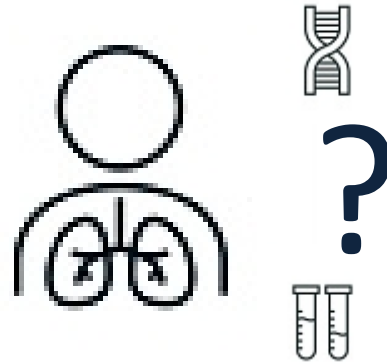
Conclusion: We report a nosocomial outbreak of COVID-19 cases related to a new variant, B.1.616, poorly detected by RT-PCR on nasopharyngeal samples, with high lethality.

B.1.616 variant COVID-19 outbreak poorly detected but highly lethal



SARS-COV 2 nosocomial outbreak
in western France
114 infected patients

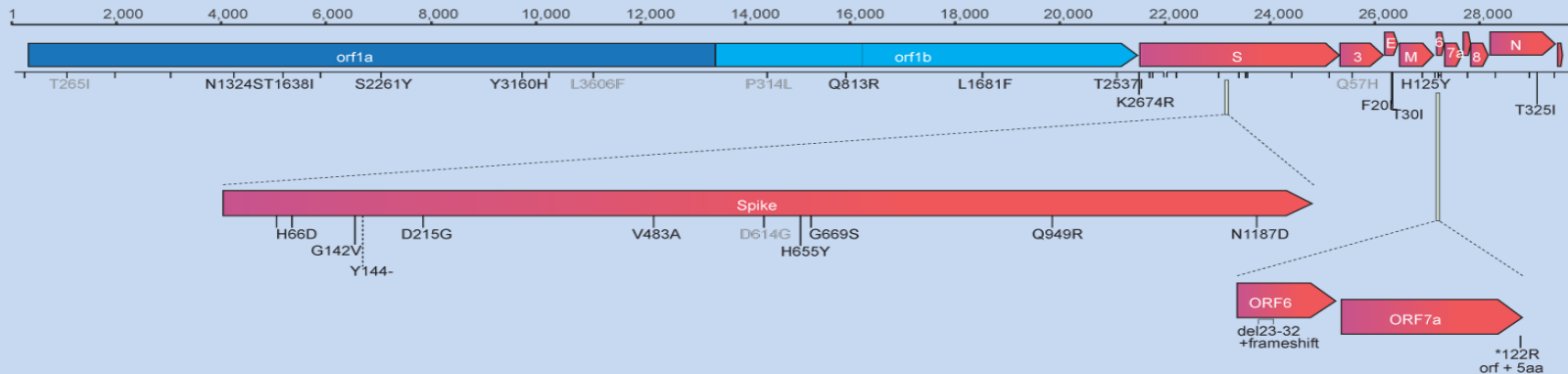
Low rate of positive
nasopharyngeal RT-PCR



Only **15%** positive at first test



65% of severe disease
28 day mortality : 46%



MANUSCRIPT

INTRODUCTION

At the end of 2020, novel concerns were raised with the detection of rapidly spreading variants of concern (VOCs) associated with increased transmissibility, increased severity and/or immune escape properties [1–5]. In January 2021, an outbreak of cases highly suggestive of COVID-19 despite negative RT-PCR tests on repeated nasopharyngeal samples was reported at the Lannion hospital in Western France. Of note, when applied on lower respiratory tract samples, the performance of RT-PCR tests was preserved, suggesting that failure to detect this variant on nasopharyngeal samples was due to a viral load below the limit of detection in the upper respiratory tract, rather than to genomic mismatches between routine RT-PCR targets and this variant, which was confirmed by genomic data. Whole-genome sequencing (WGS) from lower respiratory tract samples of several cases identified a previously unknown variant of SARS-CoV-2 belonging to clade GH/20C (GISAID/Nextstrain nomenclatures) carrying several amino acid substitutions or deletions in the Spike protein, which received the B.1.616 Pango lineage designation. In March, the French public health agency, the National Reference Center for respiratory viruses, and the WHO classified B.1.616 as variant under investigation (VUI) [5,6]. We aimed to characterize this variant in terms of virological features, clinical presentation, and outcomes.

METHODS

Setting and Patients

We conducted a retrospective cohort study of all patients who were diagnosed with SARS-CoV-2 infection at the Lannion and Saint-Brieuc hospitals from January 1st to March 24th, 2021. SARS-CoV-2 infection was defined by at least one of the following: i) positive SARS-CoV-2 RT-PCR on a respiratory sample; ii) seroconversion based on paired sera tested for anti-SARS-CoV-2 IgG/IgM; iii) suggestive symptoms and typical features of COVID-19 on chest CT scan [7].

COVID-19 cases were categorized in one of the following groups: i) B.1.616; ii) VOC; iii) unknown. The B.1.616 group included patients with B.1.616 infection documented by WGS (confirmed B.1.616 case), and patients for whom the SARS-CoV-2 isolate could not be characterized, but who were close contacts of at least one patient with documented B.1.616 infection (probable B.1.616 case). Close contact was defined as household, occupational, or nosocomial (hospitalization in the same ward). Infection was considered as related to this contact when patients developed symptoms

or first positive test at least 48 hours after the first contact. Since all cases of B.1.616 infection confirmed by WGS lived in the Lannion district, this place was considered as the epidemic area.

The VOC group included all cases due to VOCs B.1.1.7, B.1.351 and P.1. Routine screening for these VOCs has been performed by RT-PCR targeting N501Y mutation (common to B.1.1.7, B.1.351 and P.1) and 69-70 deletion (B.1.1.7 specific) in all patients with positive RT-PCR since February 10th, 2021. COVID-19 patients not fulfilling the criteria for the first two groups were categorized in the ‘unknown’ group. These patients were included in the study for clinical, biological, and radiological description. However, since it was not possible to assign them to either the B.1.616 group, or the VOC group, they were not included in the primary analysis. Cases were categorized as nosocomial if symptoms appeared at least 2 days after hospital admission [8]. Patients management, virological methods and statistical analysis are reported on supplementary data.

Outcomes

Primary outcome was severity defined as a score >5 in the WHO clinical progression scale, which is achieved when patients require non-invasive ventilation or high-flow oxygen [9]. Secondary outcomes were ICU admission and 28 day-mortality.

Ethics

Patients or closest relatives were informed of the retrospective collection of data and could refuse to participate. The French infectious diseases society ethics committee (CER-MIT) approved the study (N° COVID 2021-06). Written informed consent was waived.

RESULTS

Genomic characteristics of B.1.616

Phylogenetic analysis revealed an original variant carrying a unique constellation of mutations, which received the B.1.616 Pango lineage designation [1] (Figure 1). It is characterized by 9 amino acid changes and one deletion in the S protein in comparison with the original Wuhan strain (H66D, G142V, Y144del, D215G, V483A, D614G, H655Y, G669S, Q949R, N1187D), several unique amino-acid changes in the E, M, and N proteins, in ORF1ab and ORF3, as well as by a deletion and frameshift in ORF6 and replacement of the stop codon of ORF7a resulting in a 5 amino acids extension at its C-terminus (Supplementary Table 1). Interestingly, some mutations (Y144- and H655Y) in the S protein

have been observed in VOCs B.1.1.7 and P.1, respectively. The V483A is located in the receptor binding motif next to residue 484, for which the E484K change found in several VOCs has been associated with reduced neutralization by post-infection and post-vaccination antibodies [10–12]. ORF6 and ORF7a are two proteins that antagonize various steps of type I interferon production and signaling [13–15].

Population

From January 1st to March 24th, 2021, 268 patients were hospitalized with SARS-CoV-2 infection in Lannion or Saint Briec hospitals, of whom 86 lived in the B.1.616 epidemic area (Supplementary Figure 1). CT-scan was typical of COVID-19 in 58/86 (67%) and serology was positive in 31/86 (36%). SARS-CoV-2 was detected by RT-PCR in 50/86 (58%) patients, including 14 with all B.1.616 genomic characteristic features (confirmed B.1.616). In addition, 25 patients developed COVID-19 not related to a VOC at least 48 hours after close contact with a confirmed B.1.616 case (probable B.1.616 cases). These 39 patients (14 confirmed and 25 probable) constituted the B.1.616 group (supplementary Figure 1).

During the study period, 108 patients were hospitalized in a ward where at least one case of B.1.616 COVID-19 was admitted, for a total of 780 patient-days at risk: 37/108 (34%) developed COVID-19 symptoms after they were admitted for another reason and were categorized as nosocomial B.1.616 cases (12 confirmed, and 25 probable). Therefore, the B.1.616 incidence rate in these wards was estimated at 47/1000 patient-days at risk. In addition, 47/86 patients living in the epidemic area were hospitalized because of COVID-19, in the absence of any contact with a B.1.616 case, of whom 4 were infected by a VOC (B.1.1.7, n=3; B.1.351, n=1), and 43 were assigned to the unknown group. Epidemiological curve and infection control measures are reported in supplementary Figure 2. The two B.1.616 cases not healthcare-associated were the wife of a confirmed case who visited him just before he was diagnosed with COVID-19, and a physician who developed infection after taking care of a WGS-confirmed case.

Finally, among the 182 COVID-19 inpatients living outside of the B.1.616 epidemic area, 39 were screened for VOCs, of whom 28 (72%) were infected with the B.1.1.7 (n=27) or the B.1.351 (n=1) variants. These 28 patients, combined with the 4 patients from the B.1.616 epidemic area infected with a VOC, were assigned to the VOC group.

Baseline characteristics are displayed on Table 1. Briefly, COVID-19 cases in the B.1.616 and unknown groups were older than in the VOC group (respectively, 81 years [73-88] vs 80 years [68-87] vs 73 years [67-82], $P=0.022$). B.1.616 cases were less likely to be documented at first RT-PCR (15% vs 23% for unknown variant vs 97% for VOC, $P<0.001$), even though time from symptoms onset to first RT-PCR test was shorter in B.1.616 group (0 [0-2] vs 0 [0-5] vs 3 [1-5];

$p=0.004$), Table 1. Although B.1.616 cases had more RT-PCR tests ($P<0.001$), and more lower respiratory tract samples ($P<0.001$), only 26 (66%) had at least one positive RT-PCR. Among the 13 patients classified as B.1.616 in the absence of any positive RT-PCR, 13 had typical radiological findings on CT-scan, and 4 had SARS-CoV-2 seroconversion, with a median time from symptoms onset to positive serology of 7 days [5-8] (Supplementary Table 2). In addition, the median cycle threshold (CT) in nasopharyngeal samples was higher in the B.1.616 and the unknown variant group, at 29 [27-36] and 35 [30-40], vs 19 [14-22] in the VOC group, $P<0.001$. Supplementary Fig 3 illustrate diagnosis criterion in VOC and B.1.616 groups.

Outcomes

As planned, we compared outcomes of B.1.616 and VOC cases. Patients with B.1.616 COVID-19 had worse clinical outcomes: 27 (65%) reached a WHO score >5 within 28 days from onset, 14 (36%) were admitted in the ICU, and 18 (46%) died within 28 days, Table 2. Variables independently associated with time to poor outcome in Cox proportional hazard regression (after adjusting for B.1.616, age, chronic respiratory disease, immunodepression, hypertension, healthcare-associated COVID-19), were B.1.616 (HR 4.0 [1.5-10.9]), and chronic respiratory diseases (Table 3). Lethality was higher for B.1.616, at 18/39 (46%) vs. 5/32 (16%) for VOC, $P=0.011$, Figure 2, although this was not significant after adjustment for age and healthcare-associated infection (aHR 2.4 95% CI [0.76-7.44]).

We performed a sensitivity analysis in which the definition of nosocomial infection was restricted to patients who developed symptoms >8 days after admission (<https://www.ecdc.europa.eu/en/covid-19/surveillance/surveillance-definitions>); B.1.616 remains independently associated with severe disease (aHR 3.77, 95% CI [1.43-9.88]) (supplementary data).

DISCUSSION

The low rate of SARS-CoV-2 detection by RT-PCR tests on nasopharyngeal samples within a large cluster of COVID-19 cases led to the identification of a novel variant, B.1.616. Although COVID-19 cases due to this variant had clinical, biological and radiological findings in line with classical features of COVID-19, B.1.616 was associated with more severe disease. A clinical trial reported 39% of severe patients who required high-flow oxygen or more invasive support at day 28, as compared to 65% for B.1.616 cases in our study, but that study was conducted prior to the emergence of VOCs, patients enrolled were younger (mean, 60 years), and had less comorbidities (81% with ≥ 1 comorbidity) [16]. In-hospital mortality ranged from 21% to 36% in previous studies (21), with higher mortality in nosocomial COVID-19 (22), or in elderly patients [17–19]. The high mortality rate among B.1.616 cases in our study must be interpreted with caution, as

patients enrolled combined both pejorative factors, age (median, 81 years [73-88]), and healthcare-acquisition (35/39, 90%). The B.1.616 group included both WGS-confirmed B.1.616 cases, and COVID-19 cases following close contact with a confirmed case (probable B.1.616), in the absence of WGS confirmation. This conservative bias implies that comparisons with controls may misjudge the actual differences, with the 25 probable cases being frailties, therefore increasing morbidity. Other variants have been associated with an increase or a decrease in COVID-19 severity [4,20]. By the time of this writing, four variants are classified by the WHO as VOC, based on ‘evidence of an increase in transmissibility, more severe disease, significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures’ [5].

The second most salient feature of COVID-19 cases related to B.1.616 was the high number of negative or weakly positive RT-PCR tests on nasopharyngeal samples. Previous studies estimated that 10-16% of patients with COVID-19 have negative RT-PCR tests on nasopharyngeal samples [21,22]. A meta-analysis concluded that 1.8 to 33% of RT-PCR tests on first nasopharyngeal samples in COVID-19 patients are found negative [23]. In our study, 20/26 (77%) of B.1.616 cases with at least one positive RT-PCR were tested negative for RT-PCR on their first nasopharyngeal sample. Failure to detect B.1.616-related COVID-19 with the gold-standard diagnostic test most likely contributed to the emergence of several clusters, since implementation of specific infection control measures mostly relied on virological confirmation during the study period. Retrospective review of medical charts found that, in several cases, the diagnosis was suspected early in the COVID-19 course, but specific infection control measures were interrupted once RT-PCR tests returned negative. Failure to detect B.1.616 on nasopharyngeal samples is even more problematic for the screening of contacts. Indeed, the incidence of nosocomial COVID-19 was much higher during the early phase of the outbreak, before we realized the low yield of nasopharyngeal samples for this variant. Of note, although ‘diagnostic detection failures’ is one of the criteria to define VOC, none of the four major VOCs nor any of the current VUI meet this classification criteria. As RT-PCR assays used in France target at least two different viral genomic regions, it is unlikely that the B.1.616 strain would not be detected due to specific mismatches. Identification of this new lineage in lower respiratory tract samples confirmed the correct detection of the viral genome by commercially available assays used during the study period. Repeated failures to detect B.1.616 on nasopharyngeal samples were therefore most likely due to SARS-CoV-2 viral loads below detection threshold in this site, rather than sub-optimal sensitivity of routine RT-PCR tests. Of note, RT-PCR was positive on nasopharyngeal samples for only 32% of patients with pneumonia due to SARS-CoV [24], and the Middle East respiratory syndrome-related coronavirus (MERS-CoV) is mostly detected in lower respiratory tract specimens, with high viral load, whereas nasopharyngeal specimens are poorly contributive [25].

In our study, among patients with a positive RT-PCR assay in the B.1.616 group, the sensitivity of one, two, three, and four tests on nasopharyngeal samples were, respectively, 6/39 (15%), 15/39 (38%), 16/39 (41%), and 21/39 (54%). RT-PCR tests on sputum, or broncho-alveolar lavage (BAL), were positive in 8/39 (20%) B.1.616 cases with previous negative nasopharyngeal RT-PCR tests. As samples from the lower respiratory tract are more difficult to obtain in frail patients, the real extent of the B.1.616-related COVID-19 outbreak in our institution has probably been underestimated. A large surveillance study, with sequencing of a representative sample of 15% of all RT-PCR-positive COVID-19 cases during the study period found no community-acquired B.1.616-related COVID-19 (Flash study#5, SpF, Paris, France, unpublished data), but the low detection in standard sampling may have contributed to this result.

Our study has limitations. First, the small sample size and the retrospective design both limit the statistical power. However, due to the fast pace of the pandemic, early communication on the characteristics of new variants is warranted, and this would be the first case series of B.1.616-related COVID-19 cases. Second, B.1.616 confirmed cases were those for whom a deep respiratory sample was obtained, mostly motivated by disease severity, hence constituting a selection bias. Finally, the selection of controls may be an additional limitation: VOC-related cases were selected as controls mostly because these cases could be reliably classified as ‘non-B.1.616’. Since VOC screening was not performed until February 10th, misclassification may have occurred, with inclusion of VOC cases among the 25 probable B.1.616 cases. However, the proportion of VOC in this area was very low on January 7th, estimated at less than 1% [26]. Moreover, inclusion of VOC patients in the B.1.616 group would lead to underestimation of differences between groups. In addition, all consecutive cases with available VOC screening were enrolled in the control group, which limits selection bias.

We report a nosocomial outbreak of COVID-19 cases related to a new variant, B.1.616, characterized by poor detection with RT-PCR tests on nasopharyngeal samples despite typical clinical, radiological, and biological features of COVID-19. The novel variant reported here adds to the diversity of emerging SARS-CoV-2 variants with impact on early diagnosis and control. This work also highlights the difficulties to manage nosocomial cases when the gold-standard test fails to confirm the diagnosis. With constantly emerging new variants, one should remain attentive to any unusual clinical situation that could be linked to such emergence.

Funding for the study: None

SW lab is funded by Institut Pasteur, CNRS, Université de Paris, Santé publique France, Labex IBEID (ANR-10-LABX-62-IBEID), REACTing (Research & Action Emerging Infectious Diseases), and by the H2020 project 101003589 (RECOVER). ESL acknowledges funding from the INCEPTION programme (Investissements d’Avenir grant ANR-16-CONV-0005).

Conflict of interest:

All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years. All authors have no other relationships or activities that could appear to have influenced the submitted work.

SW has patents issued and pending for SARS-associated coronavirus diagnostics

Funding: No external funding was received for this study

Acknowledgments: We thank Olivia DA CONCEICAO and Cylia IMEKHLAF for their help in collecting data from patients charts. We would like to thank all the healthcare workers, public health employees, and scientists involved in the COVID-19 response and this outbreak.

We acknowledge the authors, originating and submitting laboratories of the sequences from GISAID (Table S2). We avoided any direct analysis of genomic data not submitted as part of this paper and used this genomic data only as background. This work used the computational and storage services (Maestro cluster) provided by the IT department at Institut Pasteur, Paris.

Author contribution

Conception and design: PF, SB, FXL, DC, SVDW, PT, NM

Acquisition of the data PF, MJD, RV, PR, AG, NV, CM, SGDC, ED, VM, RB, MV, EG, VT, CG, CP, SVDW, NM

Analysis and interpretation PF, SB, VE, BG, AB, AZ, MT, ESL, DC, SVDW, PT, NM

Drafting or revising the article PF, SB, VE, BG, AB, AZ, MT, ESL, DC, SVDW, PT, NM

All authors approved the final version

REFERENCES

- [1] Rambaut A, Holmes EC, O’Toole Á, Hill V, McCrone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol* 2020;5:1403–7. <https://doi.org/10.1038/s41564-020-0770-5>.
- [2] Cele S, Gazy I, Jackson L, Hwa S-H, Tegally H, Lustig G, et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature* 2021. <https://doi.org/10.1038/s41586-021-03471-w>.
- [3] Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* 2021. <https://doi.org/10.1038/s41586-021-03398-2>.
- [4] Davies NG, Jarvis CI, CMMID COVID-19 Working Group, Edmunds WJ, Jewell NP, Diaz-Ordaz K, et al. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature* 2021. <https://doi.org/10.1038/s41586-021-03426-1>.
- [5] Weekly epidemiological update on COVID-19 - 27 April 2021 n.d. <https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---27-april-2021> (accessed May 2, 2021).
- [6] Nouveau variant détecté et sous surveillance en Bretagne n.d. /presse/2021/nouveau-variant-detecete-et-sous-surveillance-en-bretagne (accessed April 26, 2021).
- [7] Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. *Eur Radiol* 2020;30:4381–9. <https://doi.org/10.1007/s00330-020-06801-0>.
- [8] Rhee C, Baker M, Vaidya V, Tucker R, Resnick A, Morris CA, et al. Incidence of Nosocomial COVID-19 in Patients Hospitalized at a Large US Academic Medical Center. *JAMA Netw Open* 2020;3. <https://doi.org/10.1001/jamanetworkopen.2020.20498>.
- [9] WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020;20:e192–7. [https://doi.org/10.1016/S1473-3099\(20\)30483-7](https://doi.org/10.1016/S1473-3099(20)30483-7).
- [10] Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. *Cell Host Microbe* 2021;29:463-476.e6. <https://doi.org/10.1016/j.chom.2021.02.003>.
- [11] Liu Z, VanBlargan LA, Bloyet L-M, Rothlauf PW, Chen RE, Stumpf S, et al. Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. *Cell Host Microbe* 2021;29:477-488.e4. <https://doi.org/10.1016/j.chom.2021.01.014>.
- [12] Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature* 2021;592:616–22. <https://doi.org/10.1038/s41586-021-03324-6>.
- [13] SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists - PubMed n.d. <https://pubmed.ncbi.nlm.nih.gov/32529952/> (accessed May 2, 2021).
- [14] Li J-Y, Liao C-H, Wang Q, Tan Y-J, Luo R, Qiu Y, et al. The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Res* 2020;286:198074. <https://doi.org/10.1016/j.virusres.2020.198074>.
- [15] Xia H, Cao Z, Xie X, Zhang X, Chen JY-C, Wang H, et al. Evasion of Type I Interferon by SARS-CoV-2. *Cell Rep* 2020;33:108234. <https://doi.org/10.1016/j.celrep.2020.108234>.
- [16] Rosas IO, Bräu N, Waters M, Go RC, Hunter BD, Bhagani S, et al. Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. *N Engl J Med* 2021;384:1503–16. <https://doi.org/10.1056/NEJMoa2028700>.
- [17] Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020;323:2052–9. <https://doi.org/10.1001/jama.2020.6775>.
- [18] Rickman HM, Rampling T, Shaw K, Martinez-Garcia G, Hail L, Coen P, et al. Nosocomial Transmission of Coronavirus Disease 2019: A Retrospective Study of 66 Hospital-acquired Cases in a London Teaching Hospital. *Clin Infect Dis* 2021;72:690–3. <https://doi.org/10.1093/cid/ciaa816>.
- [19] Mendes A, Serratrice C, Herrmann FR, Genton L, Périvier S, Scheffler M, et al. Predictors of In-Hospital Mortality in Older Patients With COVID-19: The COVIDAge Study. *J Am Med Dir Assoc* 2020;21:1546-1554.e3. <https://doi.org/10.1016/j.jamda.2020.09.014>.
- [20] Young BE, Fong S-W, Chan Y-H, Mak T-M, Ang LW, Anderson DE, et al. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *Lancet* 2020;396:603–11. [https://doi.org/10.1016/S0140-6736\(20\)31757-8](https://doi.org/10.1016/S0140-6736(20)31757-8).
- [21] REMAP-CAP Investigators, Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. *N Engl J Med* 2021. <https://doi.org/10.1056/NEJMoa2100433>.

- [22] RECOVERY Collaborative Group, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* 2021;384:693–704. <https://doi.org/10.1056/NEJMoa2021436>.
- [23] Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, Del Campo R, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: A systematic review. *PLoS One* 2020;15:e0242958. <https://doi.org/10.1371/journal.pone.0242958>.
- [24] Peiris JSM, Chu CM, Cheng VCC, Chan KS, Hung IFN, Poon LLM, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 2003;361:1767–72. [https://doi.org/10.1016/s0140-6736\(03\)13412-5](https://doi.org/10.1016/s0140-6736(03)13412-5).
- [25] Guery B, Poissy J, el Mansouf L, Séjourné C, Ettahar N, Lemaire X, et al. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. *Lancet* 2013;381:2265–72. [https://doi.org/10.1016/S0140-6736\(13\)60982-4](https://doi.org/10.1016/S0140-6736(13)60982-4).
- [26] Gaymard A, Bosetti P, Feri A, Destras G, Enouf V, Andronico A, et al. Early assessment of diffusion and possible expansion of SARS-CoV-2 Lineage 20I/501Y.V1 (B.1.1.7, variant of concern 202012/01) in France, January to March 2021. *Euro Surveill* 2021;26. <https://doi.org/10.2807/1560-7917.ES.2021.26.9.2100133>.

Table 1: Baseline characteristics of in-patients with SARS-COV 2 infection according with variants responsible for infection.

Variables	B.1.616	Undetermined	VOC	p-value
	n=39	variant n=43	n=32	
Age, year [IQR]	81 [73 – 88]	80 [68 – 87]	73 [67 – 82]	0.022
Male, n (%)	21 (54%)	24 (56%)	15 (47%)	0.732
Body mass index, kg/m ² [IQR]	25 [22 – 29]	26 [23 – 28]	31 [27 – 33]	0.012
<i>Comorbidities</i>				
No comorbidity, n (%)	6 (18%)	9 (19%)	4 (13%)	0.812
Cardiovascular disease, n (%)	22 (56%)	20 (47%)	11 (34%)	0.560
Chronic respiratory disease, n (%)	8 (21%)	9 (21%)	5 (16%)	0.824
Chronic kidney failure, n (%)	12 (31%)	7 (16%)	5 (16%)	0.185
Cirrhosis, n (%)	4 (10%)	4 (9%)	1 (3%)	0.518
Neurological disease, n (%)	11 (28%)	5 (12%)	10 (31%)	0.082
Cancer, n (%)	12 (31%)	11 (26%)	10 (31%)	0.826
Immunodepression, n (%)	4 (10%)	1 (2%)	5 (16%)	0.120
Diabetes, n (%)	9 (23%)	11 (26%)	6 (19%)	0.783
Hypertension, n (%)	22 (56%)	19 (44%)	22 (69%)	0.105
Health-care associated COVID-19, n (%)	37 (95%)	17 (40%)	10 (31%)	<0.001
<i>Clinical findings</i>				
Fever, n (%)	24 (61%)	27 (63%)	21 (66%)	0.397
Temperature, °C	38.2 [37.0 – 38.8]	38 [37.7 – 38.5]	37.8 [37.0 – 38.8]	0.732
Dyspnoea, n (%)	26 (67%)	27 (63%)	14 (44%)	0.118
Oxygen saturation, %	91 [90 – 94]	93 [92 – 96]	94 [89 – 96]	0.171
Cough, n (%)	14 (36%)	15 (35%)	14 (44%)	0.705
Headache, n (%)	2 (5%)	3 (7%)	1 (3%)	0.876
Delirium, n (%)	6 (15%)	5 (12%)	2 (6%)	0.534
Fatigue, n (%)	9 (23%)	14 (33%)	12 (38%)	0.400
Anosmia, n (%)	1 (3%)	2 (5%)	0	0.777
Digestive symptoms, n (%)	4 (10%)	5 (12%)	1 (3%)	0.417
Rhinorrhoea, n (%)	0	1 (2%)	2 (6%)	0.279
No symptoms – no. (%)	0	4 (9%)	4 (12%)	0.053
<i>Biological findings</i>				
Neutrophils, x 10 ⁹ /L	5140 [3245 – 7775]	4050 [3150 – 8110]	4130 [1440 – 6700]	0.166
Lymphocytes, x 10 ⁹ /L	650 [425 – 1055]	730 [580 – 1050]	780 [610 – 1060]	0.526
Serum C-reactive protein, mg/mL	79 [43 – 122]	51 [16 – 142]	49 [12 – 92]	0.163
D-dimeres, µg/L	1330 [589 – 1714]	722 [521 – 1609]	1528 [930 – 1942]	0.476

<i>Thorax Computed Tomography (CT)</i>				
No CT scan, n	9 (23%)	18 (42%)	12 (37%)	
<25%, n (%) *	9(23%)	9 (21%)	5 (16%)	0.953
25-50%, n (%) *	12 (35%)	11 (26%)	9 (28%)	
50-75%, n (%) *	8 (21%)	5 (12%)	5 (16%)	
>75%, n (%) *	1 (3%)	0	1 (3%)	
Pulmonary embolism, n (%)	3 (8%)	1 (2%)	2 (6%)	0.583
Time from symptoms onset to first RT-PCR test, days [IQR]	0 [0 – 2]	0 [0 – 5]	3 [1 – 5]	0.004
Time from symptoms onset to first SARS-CoV-2 detection, days [IQR]	3 [2 – 9]	4 [1 – 7]	1 [0 – 4]	0.059
First RT-PCR positive for SARS-CoV-2, n (%)	6 (15%)	10 (23%)	31 (97%)	<0.001
At least one RT-PCR positive for SARS-CoV-2, n (%)	26 (67%)	24 (56%)	32 (100%)	<0.001
<i>Number of RT-PCR Test per patient</i>				<0.001
1	6 (15%)	13 (30%)	31 (97%)	
2	12 (31%)	17 (40%)	1 (3%)	
>3	21 (54%)	13 (30%)	0	
<i>Site of sample (First positive RT-PCR only), n</i>				<0.001
Endotracheal aspirate	0	2 (5%)	0	
Expectoration	2 (5%)	0	0	
Broncho-alveolar lavage	5 (13%)	0	0	
Nasopharyngeal swab	13 (33%)	22 (451%)	32 (100%)	
Stool	0	1 (2%)	0	
<i>CT value (Positive RT-PCR only)</i>				
Nasopharyngeal sample	29 [27 – 36]	35[30 – 40]	19 [14 – 22]	<0.001
Lower respiratory tract sample	33 [33 – 35]	45 [45 – 45]	26	0.035

Note. VOC, variant of concern, *

Table 2: 28 days clinical outcomes of study patients according with variants responsible for infection.

Variables	B.1.616 n=39	Undetermined variant n=43	VOC n=32	p-value
Intensive care unit admission, n (%)	14 (36%)	5 (12%)	10 (31%)	0.028
WHO score >5 within 28 days from onset, n (%)	27 (65%)	14 (40%)	10 (31%)	<0.001
28 day-mortality, n (%)	18 (46%)	10 (23%)	5 (16%)	0.011

Note. WHO, world health organization; VOC, variant of concern

Table 3: Risk factors for poor outcome (WHO>5) among the 39 B.1.616-related cases and the 32 VOC-related COVID-19 cases (cox proportional hazard regression)

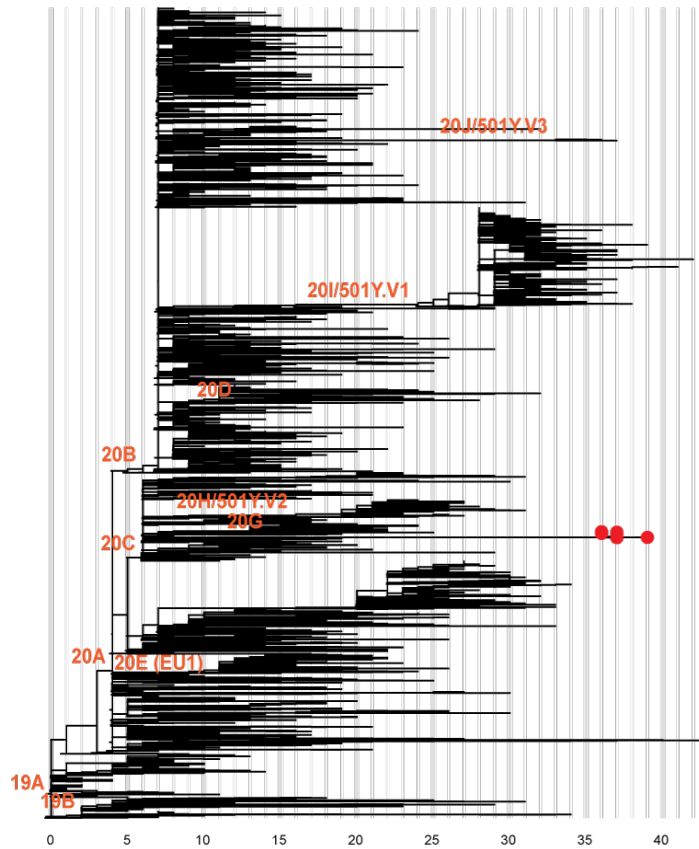
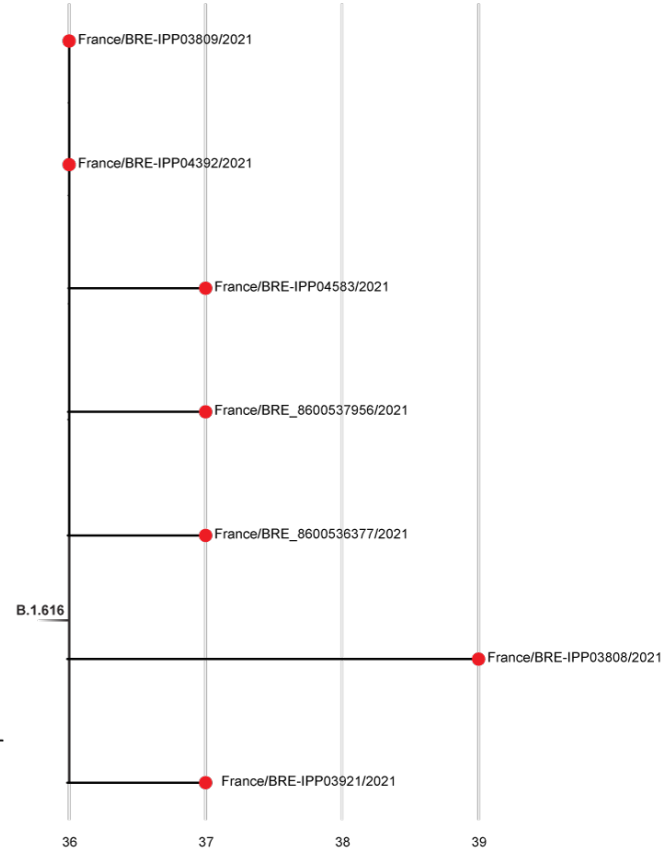
Variable	Univariate analysis		Multivariable analysis	
	HR	IC	HR	IC
B.1.616 (vs VOC)	3.0	[1.4 – 6.1]	4.0	[1.5 – 10.9]
Age per supplementary 10 years	1.0	[0.8 – 1.2]	0.9	[0.6 – 1.2]
Male	1.3	[0.7 – 2.6]		
Cardiovascular disease	1.0	[0.5– 1.9]		
Chronic respiratory disease	2.5	[1.2 – 5.2]	2.6	[1.2 – 5.6]
Chronic kidney failure	1.1	[0.5 – 2.3]		
Cirrhosis	1.4	[0.4 – 4.6]		
Neurological issue	0.7	[0.3 – 1.6]		
Cancer	0.7	[0.3 – 1.4]		
Immunodepression	0.3	[0.1 – 1.4]	0.6	[0.1 – 2.9]
Diabetes	1.0	[0.4 – 2.1]		
Hypertension	0.5	[0.3 – 1.1]	0.7	[0.3 – 1.6]
Healthcare associated COVID	1.4	[0.7 – 2.7]	0.9	[0.3 – 2.1]

Note. VOC, variant of concern

Figure 1: Phylogenetic analysis of the B.1.616 lineage and characteristic non-synonymous substitutions. A. Subsampled global phylogenetic maximum likelihood tree of SARS-CoV-2 with annotated Nextstrain clades next to the corresponding nodes and tips highlighted only for sequences from the Pangolin B.1.616 lineage. B. Detailed view of the B.1.616 lineage. A-B. Branch lengths correspond to the number of nucleotide substitutions (shown below the tree) from the reference Wuhan-Hu-1 strain (NC_045512). C. Nucleotide and amino acid substitutions from the reference Wuhan-Hu-1 strain shared among the sequences from Lannion, France are represented as ticks along the SARS-CoV-2 genome and are annotated with text if non-synonymous. Light grey text annotated amino acid substitutions are not unique to the Lannion (B.1.616) lineage.

Figure 2: Survival curves

Note. VOC, variant of concern

A**B****C**