

## **Patient management**

During the study period, COVID-19 patients who required hospitalization could be admitted either to the Lannion hospital, a 550 acute care beds hospital in an agglomeration of 50,000 inhabitants, or the Saint-Brieuc hospital, a 750 acute care beds hospital in an agglomeration of 150,000 inhabitants. Patients who required endotracheal intubation were transferred to the intensive care unit (ICU) of the Saint-Brieuc hospital.

All patients with COVID-19 confirmation or suspicion, based on clinical and/or radiological findings, were isolated with droplet transmission precautions for at least 10 days, including 48 h after clinical improvement and apyrexia. Patients who required ICU management were isolated during 14 days, and immunocompromised patients were isolated during 24 days, including 48 h after clinical improvement and apyrexia. Infection control measures included admission to a single room, wearing gown and gloves, protection glasses, surgical mask (if treated with O<sub>2</sub> flow <6 L/min) or FFP2 mask (if treated with O<sub>2</sub> flow ≥6 L/min) and reinforced hand hygiene. All COVID-19 patients received prophylactic therapy with heparin, according to national guidelines, and those who required treatment with O<sub>2</sub> received dexamethasone, 6 mg daily for 10 days or until they no longer required O<sub>2</sub>. Tocilizumab could be prescribed at the discretion of the physicians in charge.

## **Virological methods**

Different commercially available RT-PCR assay were used at Lannion and Saint-Brieuc hospitals: Xpert® Xpress SARS-CoV-2, GeneXpert Cepheid®, BD SARS-CoV-2 reagents for BD Max, BD®, Viasure SARS-CoV-2 Real Time PCR Detection, Certest®. Screening for VOCs was performed on positive RT-PCR samples using ID SARS-CoV-2/UK/SA Variant Triplex, ID Solutions®. When B.1.616-related COVID-19 was suspected, samples were sent to the National Reference Center (Institut Pasteur, Paris) for WGS.

SARS-CoV-2 serology (IgM/IgG) of suspected COVID-19 cases was performed using AdviseDx SARS-CoV-2 Alinity Abbott®.

## **Whole Genome Sequencing**

RNA extracted from clinical samples was used for SARS-CoV-2 WGS using a highly multiplexed PCR amplicon approach[1] with the ARTIC Network multiplex PCR primers set v3 (<https://artic.network/ncov-2019>). Synthesized cDNA was used as template and amplicons were generated using two pooled primer mixtures for 35 rounds of amplification. Libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina) and sequenced on an Illumina NextSeq500 (2x150 cycles) on the Mutualized Platform for Microbiology (P2M) at Institut Pasteur.

## **Genome assembly and phylogenetic analysis**

Illumina adaptors, low quality reads, primer sequences were trimmed with Trimmomatic v0.36 [2] and reads were assembled with Megahit [3] as well as mapped onto reference genome Wuhan/Hu1/2019 (NCBI Nucleotide – NC\_045512, GenBank – MN908947) using the CLC Genomics Suite v5.1.0 (QIAGEN). SAMtools v1.3 [4] was used to sort the aligned bam files and generate alignment statistics. Aligned reads were manually inspected using Geneious prime v2020.1.2 (2020) (<https://www.geneious.com/>), and consensus sequences were generated (> 5X read-depth coverage for a base call). SARS-CoV-2 sequences generated during this study (n=6) were combined to a global dataset of sequences available on GISAID to produce a subsampled phylogenetic tree (n=3238) with augur and auspice as implemented in the Nextstrain pipeline version (April, 2021) (<https://github.com/nextstrain/ncov>).

### **Sensitive analysis**

Since Healthcare-associated infection has been described with various definitions, we performed a sensitivity analysis in which the definition of nosocomial infection would be restricted to patients who developed symptoms > 8 days after hospital admission (<https://www.ecdc.europa.eu/en/covid-19/surveillance/surveillance-definitions>). This new definition conducted to reclassification of 41 cases, previously considered as nosocomial, who would be classified as community-acquired and reclassification of 3 B.1.616 cases as being unknown variant cases, instead of B.1.616 probable cases. Therefore, the B.1.616 group would be constituted of 31 patients, 14 with WGS confirmed B.1.616 infection and 17 with SARS-CoV-2 ~~SARS-COV-2~~ infection and close contact with a confirmed case at least 8 days before symptoms onset. In multivariable analysis, results remain in line with those reported in our first analysis and B.1.616 infection remains independently associated with a higher risk of severe disease (aHR = 3.77, 95%CI [1.43 – 9.88]; p=0.007).

### **Supplementary Figure 1: Flow Chart**

Note. VOC, variant of concern

### **Supplementary Figure 2: Epidemiological curve of 86 inpatients living in the B.1.616 epidemic area**

Note. VOC, variant of concern. WGS : Whole Genome Sequencing. RT-PCR: Reverse-transcriptase Polymerase Chain Reaction. CT: Computed tomography

### **Supplementary Figure 3: Flow Chart of B.1.616 cases according to diagnosis criteria.**

Note. VOC, variant of concern. WGS : Whole Genome Sequencing. RT-PCR: Reverse-transcriptase Polymerase Chain Reaction.

## REFERENCES

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