

## Case report

# SARS-CoV-2 detection and sequencing in heart tissue associated with myocarditis and persistent arrhythmia: A case report



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## ABSTRACT

**Background:** SARS-CoV-2 uses the human cell receptor angiotensin-converting enzyme (ACE2). ACE2 is widely present in the cardiovascular system including the myocardium and the conduction system. COVID-19 patients that present severe symptoms have been reported to have complications involving myocardial injuries caused by the virus. Here we report the detection of SARS-CoV-2 by whole genome sequencing in the endocardium of a patient with severe bradycardia.

**Case presentation:** We report a case of a 34-year-old male patient with COVID-19 tested by PCR, he started with gastrointestinal symptoms, however, he quickly deteriorated his hemodynamic state by means of myocarditis and bradycardia. After performing an endocardium biopsy, it was possible to identify the presence of SARS-CoV-2 in the heart tissue and to sequence its whole genome using the ARTIC-Network protocol and a modified tissue RNA extraction method. The patient's outcome was improved after a permanent pacemaker was implanted.

**Conclusions:** It was possible to identify a SARS-CoV-2 clade 20A in the endocardium of the reported patient.

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## Introduction

SARS-CoV-2, as well as SARS-CoV, uses the human cell receptor angiotensin-converting enzyme (ACE2), which is a transmembrane carboxypeptidase that removes carboxy-terminal amino acids from peptide substrates [1]. Single-cell sequencing analysis of ACE2 expression has identified lung, heart, esophagus, kidney, bladder, and ileum as organs at risk for SARS-CoV-2 infection [2]. ACE2 is considered vital in the cardiovascular system as it is involved in normal heart function and the development of blood hypertension [3]. COVID-19 patients that present severe symptoms

have been reported to have complications involving myocardial injuries caused by the virus [3,4]. The mechanisms that could lead to these myocardial damages are thought to involve ACE2 related signaling pathways, cytokines produced by T helper cells, and hypoxemia [3]. Chronic cardiovascular damage may also be a consequence of COVID-19, this type of damage in the cardiovascular system has already been reported in SARS-CoV positive patients. Furthermore, patients with previous cardiovascular diseases have shown to be more susceptible to infection and are more likely to present severe symptoms [3].

The presence of SARS-CoV-2 in cardiac tissue has been previously reported in a child with Multisystem inflammatory syndrome in children (MIS-C), it was also detected in the heart by RT-PCR on a post-mortem sample [4]. We report a case of COVID-19 in a young adult that presented myocardial injuries damaging the heart conduction system, leading to the requirement of a permanent pacemaker. The virus was confirmed in heart biopsy

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specimens by sequencing the SARS-CoV-2 genome using the MinION Oxford Nanopore technology. Assessing the impacts that SARS-CoV-2 can cause in the cardiovascular system remains important for the effective treatment of patients in this ongoing pandemic.

### Case presentation and methods

A 34-year-old police male patient with a previous history of treated genital herpes, who worked in close contact with confirmed COVID-19 patients, went to the emergency room at Hospital Quito N° 1 de la Policía Nacional after 1 week of fever, odynophagia, abdominal pain accompanied by vomiting and anorexia. The patient was evaluated for a preliminary diagnosis of gastroenteritis with a primary diagnosis of bacterial gastrointestinal infection and prescribed amoxicillin + clavulanic acid, acetylsalicylic acid, and azithromycin for 5 days ambulatory. After one week, he is reassessed despite partial improvement and admitted to the hospital with the diagnosis of uncomplicated cholelithiasis. At admission, a nasopharyngeal swab was taken for a control RT-qPCR SARS-CoV-2 test which came back with a positive result and symptomatic treatment was started. The next morning, he suddenly presented hypotension associated with bradycardia of up to 20 bpm and was transferred to the intensive care unit (ICU). In the ICU, he maintained the low heart rate until positive intravenous chronotropic drugs and was performed immediate orotracheal intubation. The echocardiogram detected poor contractility and severe systolic dysfunction with LVEF 35 % associated with low output bradyarrhythmia suggested myocarditis. The electrocardiogram showed non-sinus rhythm, sinus arrest with idioventricular leakage of 35 bpm with QRS around 150 ms with the morphology of right bundle branch block with extreme right axis deviation (Fig. 1). Despite the chronotropic drugs and intubation for 2 days, he persisted in bradycardia and hypotension and it was decided to implant a temporary pacemaker with an active fixation lead. Based on chronotropic improvement, the

patient progressively increased his hemodynamic status and achieved vasoactive drugs removal and orotracheal extubation, he remained in the ICU for 2 more days and then was transferred to hospitalization. During hospitalization, he had a favorable clinical evolution with a normal functioning temporary pacemaker. After 15 days, a new SARS-CoV-2 RT-qPCR test was performed for control with a negative result, for which it was released from respiratory isolation. A control echocardiogram showed ventricles of normal size, LVEF 48 %, and discrete biventricular global hypokinesia. TAPSE 11 mm, cardiac output: 2.5 L/min, for which beta-blockers and ACE inhibitors were administered. Due to the heart failure and the high suspicion of viral myocarditis, a contrasted cardiac magnetic resonance and Holter scan of arrhythmias were performed. Both tests confirmed the signs of myopericarditis. Additionally, the patient, since admission to ICU and after removal of the urethral catheter, presents persistent obstructive urinary symptoms and scrotal edema (the patient had antecedents of untreated genital herpes). It was diagnosed with urethral meatus stenosis and scheduled weekly dilations (3 in total are performed). A culture of urethral secretion reported carbapenemase-producing *Klebsiella* sp. and *Enterococcus faecalis*, which is why broad-spectrum antibiotic therapy was started with meropenem and ampicillin plus a seminal fluid culture with negative growth. The 14 days of meropenem and 21 days of ampicillin ended, without complications or serious adverse effects.

The contractile mechanical improvement without any amelioration of the rhythm and heart rate plus the need to stratify the severity of the conduction system is not clearly explained by the cardiac MRI findings (Fig. 2). To define the prognosis and to decide to implant a permanent pacemaker were the reasons to perform an endocardium biopsy and an electrophysiological test. Six anatomic pathological samples were taken from the interventricular septum near the AV and parahisian junction. Three samples were sent for histopathology analysis and three for viral RNA whole genome sequencing to confirm bacterial endocarditis caused by *Klebsiella* and/or *Enterococcus* or viral myocarditis due to SARS-

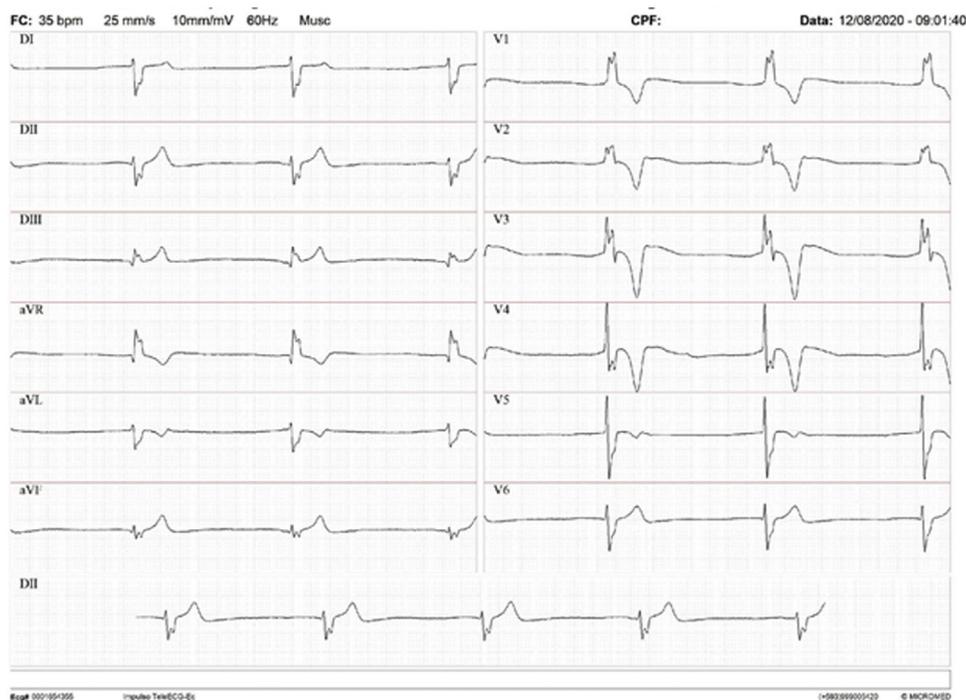
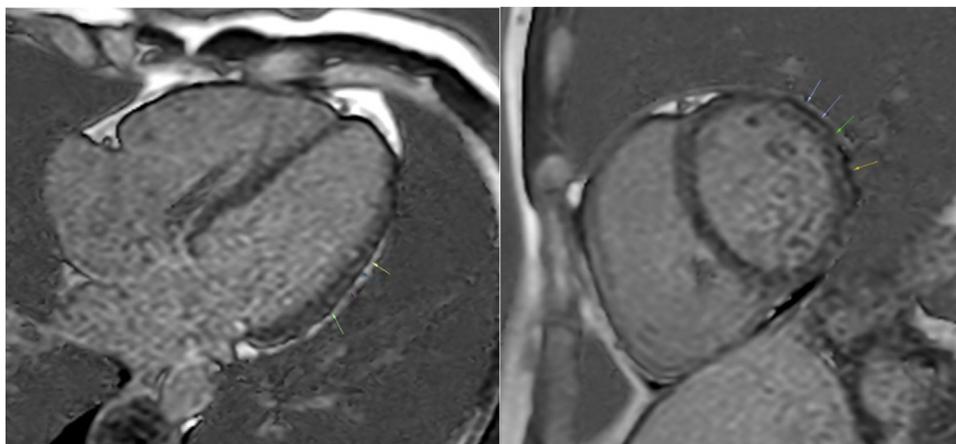


Fig. 1. Basal electrocardiogram made at the patient's admission. Electrocardiogram showing absence of atrial electrical activity, idioventricular rhythm with 30 beats per minute heart rate.



**Fig. 2. Cardiac MRI during acute myocarditis.** Semiquantitative post gadolinium analysis during T1 sequence. Mesoepicardial myocardial fibrosis with non-coronary pattern in the left ventricle middle portion free lateral wall. Estimated fibrosis burden: 2%.

CoV-2, respectively. According to histopathological analysis, the sample was not compatible with acute inflammation and no residual fibrosis was found (supplementary Fig. 1).

The electrophysiological study found significant deterioration of the conduction system manifested by atrial silence and absence of command in pacing indicating that the intrinsic sinus function was abolished. The atrioventricular conduction was not assessable due to lack of atrial capture at different points, absence of His potential in electrogram and idioventricular escape of 35 beats per minute, ventricular stability to the pacing test with three basic cycles of 600, 500 and 430 ms and three extra-stimuli until refractory right ventricular period. These findings determined a permanent dual chamber pacemaker placement. The patient was discharged after the procedure, hemodynamically stable, afebrile, with blood pressure 90/60 mm Hg, heart rate 60 per minute, and without signs of respiratory or cardiac failure, being followed up by urology and cardiology in an outpatient clinic.

The SARS-CoV-2 whole genome sequencing was performed on the endomyocardial biopsy samples. Briefly, three biopsy samples (about 2 mm each one) were pooled and viral RNA extraction was performed using the Quick RNA Viral Kit (Zymo, USA). Retro-transcription of RNA to cDNA was performed using the ARTIC protocol [5]. Primer Scheme V3 from the ARTIC network protocol for nCoV-2019 was used for viral whole-genome sequencing [5,6].

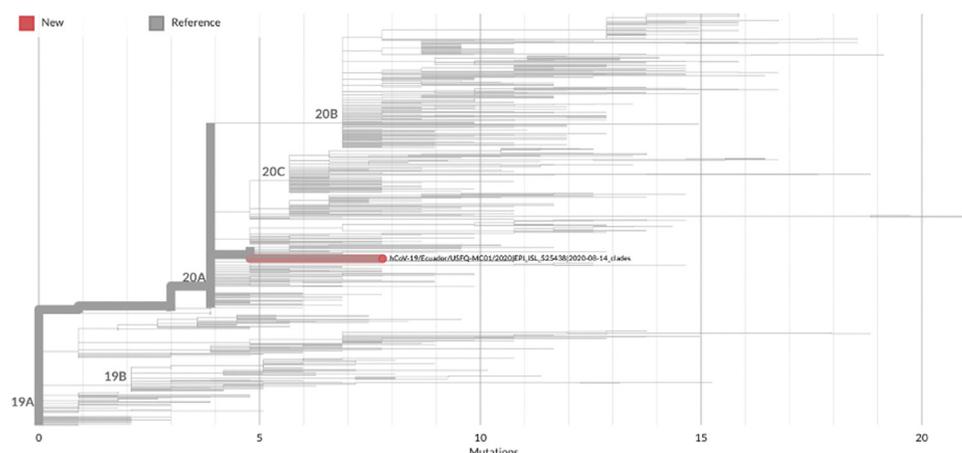
Library preparation was performed using the Barcoding kit (SQK-RBK004 - Oxford Nanopore Technologies), and then the library was loaded into a MinION Flowcell (FLO-MIN 106). nCoV-2019 novel coronavirus bioinformatics protocol from the ARTIC Network pipeline (<https://github.com/artic-network/rampart>) was used to monitor sequencing in real-time and Guppy (version 3.4.5) [7] was used for Basecalling of FAST5 files. Porechop (version 0.2.4) (<https://github.com/rrwick/Porechop>) was used to perform demultiplexing and adapter removal, and Nanoplot was used to determine sequence quality [8]. Finally, the online tools NextClade (v0.4.0) [9] and CoV-GLUE [10] were used to determine the sequence clade and to perform lineage classification and mutation resolution.

**Results**

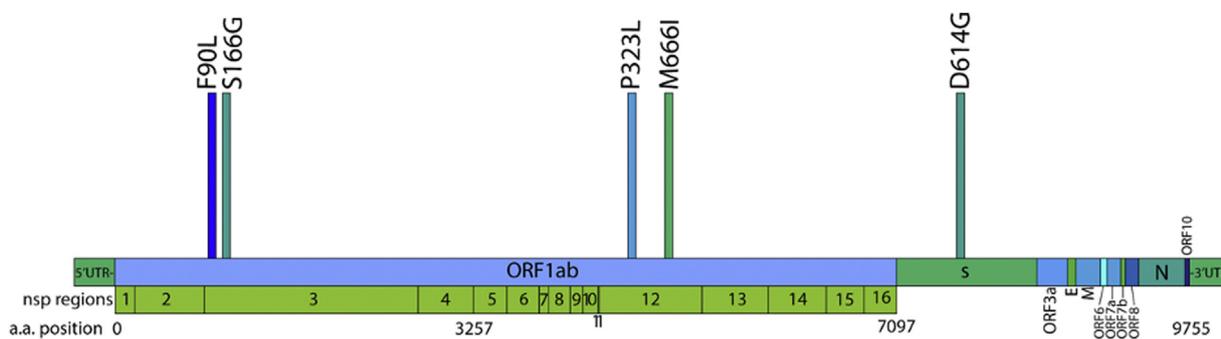
*Whole genome sequencing*

The sequence obtained had a total of 29,849 bp, it was uploaded to GISAID under the accession number EPI\_ISL\_525438. Phylogenetic analysis placed the strain in the 20A clade according to NextClade (Fig. 3) and in the B.1.5.6 lineage according to CoV-GLUE.

When compared with the Wuhan-Hu-1 reference genome, seven SNPs were identified: T2987C, C3037 T, A3215 G, C14408 T,



**Fig. 3. Phylogenetic placement of the sequence recovered from the heart tissue (highlighted in red).** The sequence was aligned with a representation of the global SARS-CoV-2 genetic diversity. The tree was acquired from NextClade v0.4.0, which uses a banded Smith-Waterman algorithm with an affine gap-penalty [12].



**Fig. 4.** Genome annotation and amino acid changes identified in the SARS-CoV-2 sequence of the cardiac tissue, compared to the Wuhan-Hu-1 reference genome (GenBank accession number MN908947).

**Table 1**  
Mutations identified in the sequence after comparison with the Wuhan-Hu-1 reference genome.

| Mutation | Localization | Type         | a.a. replacement |
|----------|--------------|--------------|------------------|
| T2987C   | nsp3/ORF1ab  | transition   | F90L             |
| C3037T   | nsp3/ORF1ab  | transition   | ---              |
| A3215G   | nsp3/ORF1ab  | transition   | S166G            |
| C14408T  | nsp12/ORF1ab | transition   | P323L            |
| G15438T  | nsp12/ORF1ab | transversion | M666I            |
| A20268G  | nsp15/ORF1ab | transition   | ---              |
| A23403G  | S            | transition   | D614G            |

G15438 T, A20268 G, and A23403 G, which determine five amino acid replacements: F90 L, S166 G (in nsp3/ORF1ab), P323 L, M666I (in nsp12/ORF1ab) and D614 G (in S) (Table 1 and Fig. 4).

**Discussion and conclusions**

In the present case report, SARS-CoV-2 was detected on cardiac tissue using Oxford Nanopore technology. For this approach, the ARTIC protocol was followed. We obtain a total of 29,849 pb. The sequence was compared with the reference strain Wuhan-1 (GenBank accession number MN908947), and a phylogenetic tree was obtained with Nextclade. The sample analyzed belongs to the clade 20A. This clade is the most prevalent in Europe, America, Asia, USA, and India (Nextstrain, 2020). The most common mutations reported in this clade are P323 L in RdRP, F942 F in NSP3, and D614 G in the spike protein [11]. In Addition to P323 L and D614 G, we found three additional substitutions F90 L, M666I, and S166 G. D614 G is increasingly common and it has been reported in 70.46 % of the reported SARS-CoV-2 genomes [12,13].

Mutation P323 L was also found in our study and has been described in 71 countries considered as one of the most prevalent [14]. It is suggested that this mutation has co-evolved with D614 G. This coexistence is probably contributing to viral replication and infectivity [15]. Regarding mutation M666I, it was found with a prevalence of 0.3 % in Wales, Scotland, and England. S166 G has been previously reported in the USA, Singapore, Denmark, Austria, and Scotland [14]. Interestingly, substitution F90 L has not been reported yet.

Even though SARS-CoV-2 presents a clear tropism for the respiratory tract, ACE2 receptor has been found in different cells, cardiovascular tissue damage caused by COVID19 has been increasingly reported and viral transcription on cardiomyocytes has been confirmed [3,16]. The mechanisms in which the virus produces these myocardial injuries are not fully understood [3]. The virus has been only detected in heart tissue through RT-qPCR. To our knowledge, this is the first report of a sequenced SARS-CoV-2 genome in heart. Since SARS-CoV produces chronic disease on the heart, assessment of how SARS-CoV-2 produces damage in

heart tissues could be valuable to fully understand the disease and its possible treatment.

**Availability of data and methods**

The sequence obtained from the SARS-CoV-2 genome is publicly hosted by GISAID under the accession number EPI\_ISL\_525438. Detailed methods used for SARS-CoV-2 whole genome sequencing and cardiac tissue RNA extraction are available from the corresponding author on reasonable request.

**Ethical approval and consent to participate**

The study protocol was approved by the Institutional Review Board of the Universidad San Francisco de Quito P2020-022IN (CEISH No. 1234) and by the Ecuadorian Ministry of Public Health MSP-CGDES-2020-0121-O. The patient provided written informed consent for sample analysis and publication.

**Author contribution**

Diego Egas was involved in patient treatment and clinical data gathering, Juan José Guadalupe performed lab work, manuscript writing and data analysis, Belén Prado-Vivar performed lab work and data analysis, Mónica Becerra-Wong performed lab work, manuscript writing and data analysis, Sully Márquez performed lab work and data analysis, Stalin Castillo was involved in patient treatment and clinical data gathering, Johanna Latta was involved in patient treatment and clinical data gathering, Francisco Rodriguez was involved in patient treatment and clinical data gathering, Giovanni Escorza was involved in patient treatment and clinical data gathering, Gabriel Trueba was involved in study design, data analysis and manuscript writing, Michelle Grunauer was involved in study design, data analysis and manuscript writing, Verónica Barragán was involved in study design, data analysis and manuscript writing, Patricio Rojas-Silva was involved in study design, data analysis and manuscript writing, Paúl Cárdenas was involved in study design, data analysis and manuscript writing. All authors have reviewed the data and approved the final manuscript.

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**Declaration of Competing Interest**

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.idcr.2021.e01187>.

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