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neuroimaging, with traumatic cause considered the most likely aetiology.

Although prone positioning ventilation is a strategy that reduces mortality in patients with ARDS,<sup>7,8</sup> it is not without risk and, while it cannot be confirmed unequivocally, in our case in the context of mechanical ventilation and prone positioning, traumatic aetiology was postulated as the most likely. Incorrect use of the laryngoscope and prolonged pressure on the trachea due to excessive inflation of the endotracheal tube cuff have been suggested as the most common mechanisms of injury in ventilated patients.<sup>9</sup>

The treatment of this syndrome involves establishing a speech therapy and swallowing rehabilitation programme. In this case, the patient experienced complete clinical recovery of swallowing and speech capacity after six months of rehabilitation, with complete mobility of the vocal cords confirmed via control fiberoptic laryngoscopy. This clinical course points to mild neuropraxia or axonotmesis as the mechanism of injury.

Although the scientific evidence is limited, the majority of diagnosed cases are neuropraxia-type injuries, which, by definition, is a reversible process.<sup>10</sup> The definitive diagnosis is established by electromyography. However, it is not performed routinely due to difficult access to the muscles of the larynx and because it is an uncomfortable technique. As such, a favourable clinical course would confirm such injury. The therapy aims to reduce recovery time and achieve early functionality.

Finally, the neurological repercussions that orotracheal intubation can cause related to prone positioning changes must be stressed. Although most cases of this syndrome are mild, irreversible damage could occur, requiring the use of a nasogastric tube or gastrostomy to avoid the risk of bronchial aspiration.

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## De novo emergence of the mutation E484K in a SARS-CoV-2 B.1.1.7 lineage variant<sup>☆</sup>



### Aparición de novo de la mutación E484K en una variante del linaje B.1.1.7 de SARS-CoV-2

Since its first description in December 2020<sup>1</sup>, the SARS-CoV-2 variant of concern VOC-202112/01 (also known as lineage B.1.1.7, 20I/501Y.V1 or, recently, according to the WHO, simply alpha<sup>2</sup>) has been spreading all over the world. In our geographical area, it became predominant from the beginning of March 2021, accounting for more than 90% of new infections in June 2021<sup>3</sup>. This is mainly due to the mutations that this variant accumulates in the gene that encodes the spike (S gene), especially in the receptor-binding domain (RBD). These mutations, especially the N501Y mutation that it shares with the beta (B.1.351 or 20H/501Y.V2) and gamma (P.1 20J/501Y.V3) variants of concern, among others, are related to an increased binding affinity of the spike with angiotensin-converting enzyme II and an increase in transmissibility<sup>4</sup>. These

last two variants also share the E484K mutation, also in the RBD, which could be related to a certain degree of escape from the action of the vaccines<sup>5</sup>. Therefore, when the first sequences of the B.1.1.7 lineage with the E484K mutation emerged, the British authorities declared them variants of concern VOC-202102/02<sup>6</sup>. However, the cluster in which these variants were framed does not seem to have been as successful and represents only 0.225% of the sequences of the B.1.1.7 lineage included in the GISAID (Global Initiative on Sharing All Influenza Data).

In accordance with our centre's variant screening protocol, all SARS-CoV-2 positive samples with Ct <32 are tested using the Allplex™ SARS-CoV-2 Variants I Assay kit (Seegene, Korea), which simultaneously detects H69/V70, E484K and N501Y mutations. At the end of April, we identified a sample which was positive for all three targets studied. In addition to the sample from the patient who had all three mutations, samples from the other three positive cases from the family cluster of which the patient had been a close contact were sequenced, presenting a profile compatible with the B.1.1.7 lineage but without the E484K mutation. For the sequencing of the viral genome, the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific, USA) was used<sup>7</sup>. Libraries were prepared following the manufacturer's instructions and loaded onto a 540 chip and the Ion GeneStudio™ S5 (Thermo Fisher Scientific, USA)

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**Table 1**  
Coverage of the mutations present in the family cluster.

	Test sample	Relative 1	Relative 2	Relative 3
C241T	2935	7356	4892	3110
C913T	5499	10533	7634	5110
C3037T	6899	10817	8752	8597
C3267T	1870	6997	4489	3645
C4464T	7318	13962	8950	5797
A5041G	3407	6980	4543	2567
C5388A	4309	8448	5309	3474
G5763A	7673	12951	9983	10079
C5986T	2074	3458	1213	449
T6954C	2822	7202	3136	933
11288-11297	6911	11107	8345	7157
C11668T	5339	7078	3973	2795
C12439T	6903	11349	7622	6920
C14407T	4004	6209	3489	2521
C14408T	4006	6230	3496	2525
C14676T	5486	8911	6115	4265
T15096C	3185	5267	3462	3199
C15279T	1811	6655	4096	2838
T16176C	6050	11721	8117	9837
C18647T	2354	6501	4085	3055
21766-21772	5587	6261	5807	5072
21994-21997	7153	7985	6569	5075
G23012A	8771			
A23063T	2137	5422	4300	1691
C23271A	4375	7485	6071	5999
A23403G	13281	16004	13387	13398
C23604A	9736	12985	11754	14791
C23709T	7735	11037	9594	7600
T24506G	14234	17157	15581	13242
G24914C	9398	14695	11772	7804
C27972T	25717	28812	39283	27683
G28048T	25829	28835	39267	27589
A28095T	12218	16000	19763	6728
A28111G	12192	15990	19727	6687
28274	9517	11135	18433	8315
G28280C	10624	12112	19790	8916
A28281T	10686	12175	19865	8933
T28282A	10689	12181	19875	8936
C28320T	10853	12294	20071	9036
G28881A	6831	10521	12661	5721
8882A	6840	10531	12673	5728
G28883C	6841	10531	12673	5728
C28977T	6715	10284	12336	5378

platform. The genome was assembled using the IRMA plugin<sup>8</sup> and its consistency was checked using the Integrative Genomics Viewer (IGV) program<sup>9</sup>. The Nextstrain webApp<sup>10</sup> was also used for both clade assignment and visualisation of mutations. The sequences obtained by next-generation sequencing confirmed the results of the PCR variant screening techniques. The test sample presented the G23012A mutation that conditions the amino acid change in the E484K S gene. None of the other three samples had that mutation in the assembly, and the A readings at position 23012 accounted for less than 1% of the readings in each of the samples. Except for the G23012A mutation, the four strains of the family cluster were identical and had both the characteristic mutations of the B.1.1.7 lineage and some of their own (see Table 1).

Despite belonging to the B.1.1.7 lineage and also exhibiting the E484K mutation, the sequence of our sample did not share the rest of the characteristic mutations of VOC-202102/02. The appearance of this mutation was therefore probably independent to those found in February 2021 in the United Kingdom, similar to other synchronous appearances of this mutation. In this case, the epidemiological chain of transmission is quite clear, since the infection of the patient with the E484K mutation appeared later: one week after the rest, and when the patient had been in isolation for six days for being a close contact of a confirmed case, so this mutation probably emerged *de novo*, either in the patient himself or in any of the other three members of the cluster after taking their respective

samples. Fortunately, the patient had no subsequent close contacts and no new variants belonging to the B.1.1.7 lineage with the E484K mutation have been observed in the daily screenings carried out in our health organisation.

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## Could the stethoscope be a SARS-CoV-2 vector?



### ¿Podría ser el fonendoscopio un vector del SARS-CoV-2?

Dear Editor,

SARS-CoV-2 is a viral disease that is transmitted by different mechanisms, among which are aerosols and fomites. The stethoscope is a medical device that is used for different patients, which is known for its ability to transmit other infectious diseases between patients and healthcare workers.<sup>1,2</sup> Usually, the stethoscope is placed on the front and back of the chest, while the patient breathes or even coughs on it. Despite the exponential growth of knowledge about the infection by SARS-CoV-2, to date, no study has been published that analyzes the possibility that the stethoscope acts as a fomite in the transmission of SARS-CoV-2. We conducted the present study to assess the ability of transmitting SARS-CoV-2 through the stethoscope.

In our hospital, a clean stethoscope was placed in each isolation room with symptomatic patients with pneumonia due to SARS-CoV-2. During the months of January and February 2021, we studied the presence of SARS-CoV-2 in 100 stethoscopes from specific SARS-CoV-2 rooms. Two hours after conducting the respiratory assessment, samples for PCR detection of SARS-CoV-2 RNA were taken using a swab with a synthetic tip and a plastic shaft rubbing the diaphragm for 10 s. A real-time Seegene PCR that detected 3 specific genes (RdRP, E and N) was used. The stethoscopes were not disinfected since the first day of admission of the patients. Fifty-four of them were in single rooms, and the remaining in double rooms. The patients admitted to these rooms had a median hospital stay prior to inclusion in the study of 7 days (3–12). The presence of SARS-CoV-2 was confirmed with nasopharyngeal swabs on the day of admission. PCR was used in 75 of the cases, with a mean cycle threshold (Ct) of  $26 \pm 5.1$ . The remaining 71 were confirmed by antigen detection by chemiluminescence, which could be a limitation of the study. SARS-CoV-2 RNA was not detected in any of the samples obtained from the stethoscopes.

Despite the importance of standard precautions, such as environmental cleaning and hand hygiene, which prevent the transmission of other microorganisms, the demonstration that a single route of transmission is capable of transmitting SARS-CoV-2 in real situations is very complex. The most studied and known SARS-CoV-2 transmission mechanism is produced by drops, caused by direct, indirect or close contact with infected people through the contaminated secretions expelled during speech (5–10  $\mu\text{m}$ ).

Airborne transmission caused by the suspension of aerosols in the air for long periods, especially in closed environments with poor ventilation (<5  $\mu\text{m}$ ), has also been established.<sup>3</sup> The last studied mechanism, transmission by fomites, is caused by respiratory secretions deposited on different surfaces and objects, which can be maintained for long periods (from hours to days), depending on the type of surface, especially in hospital environments. This fact has motivated the performance of various studies that consider the possibility of this route of transmission plausible, especially in rooms of patients infected by SARS-CoV-2. The virus is more stable in plastic and steel (stethoscope materials) than in copper and cardboard, and viable virus remains can be detected up to 72 h later, with stability kinetics like SARS-CoV-1.<sup>4</sup> Environmental contamination has been described in rooms with symptomatic patients with SARS-CoV-2 infection, being more frequent on the floor and bed rail, associated with a lower cycle threshold and during the first week of admission. This is probably due to direct contamination by either the patient or by healthcare workers after contacting with infected respiratory fluids.<sup>5</sup> There are controversial studies that describe the presence of SARS-CoV-2 RNA in the hospital environment, but none of them has shown it as the cause of an outbreak.<sup>6,7</sup> Our study revealed that, despite including symptomatic patients with low Ct, the presence of SARS-CoV-2 on stethoscopes was not found.

In conclusion, the stethoscope as a medical tool that is in contact with the patient is not a fomite capable of transmitting SARS-CoV-2 but this fact does not mean that systematic cleaning should not be performed.

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## Conflicts of interest

None to declare.

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