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Short Communication

Infection sustained by lineage B.1.1.7 of SARS-CoV-2 is characterised by longer persistence and higher viral RNA loads in nasopharyngeal swabs

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ABSTRACT

- Following the announcement on December 2020 about the emergence of a new variant (VOC 202012/01, B.1.1.7 *lineage*) in the United Kingdom, a targeted surveillance was put in place in the Abruzzo region (Italy), which allowed detection of 313 persons affected by *lineage* B.1.1.7, up to the 20th of February 2021. We investigated the results of RT-PCR on nasopharyngeal swabs tested from December 2020 to February 2021 to verify any difference on the viral load and persistence between people infected by *lineage* B.1.1.7 and others. Statistically significant lower values of C_T associated with the detection of the N protein encoding gene (C_T N) were observed in persons with *lineage* B.1.1.7 infection (median C_T N = 15.8) in comparison to those infected by other lineages (median C_T N = 16.9). A significantly longer duration of the persistence of SARS-CoV-2 RNA in nasopharyngeal swabs was observed in persons with lineage B.1.1.7 infection (16 days) in comparison to those infected by other lineages (14 days).

Introduction

Starting from March 2020, nasopharyngeal swabs collected in three provinces (Chieti, L'Aquila and Teramo) of Abruzzo, a central Region of Italy, were tested daily for the presence of SARS-CoV-2 RNA at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM) (Danzetta et al., 2020).

Several SARS-CoV-2 variants are now circulating globally and some of them have raised international concern. One of these is certainly represented by the Variant of Concern (VOC) 202012/01 which belongs to the lineage B.1.1.7 (Rambaut et al., 2020). VOC 202012/01 is considered to have higher transmission capabilities (Davies et al., 2021), and although mechanisms underlying VOC 202012/01 spread are largely unknown, VOC 202012/01 seems to be associated with higher viral loads and prolonged viral persistence in infected patients (Kissler et al., 2021). Furthermore, a six nucleotide deletion in the S protein encoding gene of VOC 202012/01is responsible for the S-gene drop out of a commonly used SARS-CoV-2 RNA real time-based detection kit (Thermoscentific, Waltham-MA, USA), which simultaneously detects two additional regions of the SARS-CoV-2 genome, namely ORF1ab and N protein encoding genes.

In order to unravel the spread of VOC 202012/01 in Abruzzo, a surveillance plan was established by the IZSAM. A two-step strategy was adopted (Bal et al., 2021). The first included a random selection of swabs resulting positive for SARS-CoV-2 RNA in a time period ranging from the beginning of December 2020 to February 20th 2021 but showing a readout pattern characterized by the S-gene drop-out. The second included whole genome sequence analysis of the S-negative swab samples with Threshold cycle (C_T) values $\leq$20. Overall, at least 10% of all positive samples were sequenced.

In the period of observation, 1724 samples tested positive for SARS-CoV-2 RNA with the S-negative readout pattern. Of these,

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Table 1
C_T N gene values of the first positive nasopharyngeal swab and persistence of positivity in patients tested from December 2020 to February 2021.

	C_T N values			Duration of positivity in nasopharyngeal swabs (days)		
	Patients with lineage B.1.1.7 (n = 313)	Others (n = 2344)		Patients with lineage B.1.1.7 (n = 136)	Others (n = 965)	
Mean	15.4	16.4	P < 0.0001	17.4	17.1	P < 0.0317
Median	15.8	16.9		16.0	14.0	
2.5 percentile	9.6	10.4		7.0	10.0	
97.5 percentile	19.6	19.9		39.0	30.6	

655 were sequenced. VOC 202012/01 was detected in 313 individuals, mostly originating from the province of Chieti (n = 258, 82.4%), which experienced an upsurge of COVID-19 cases in the first two months of the year 2021.

Hence, we deeply investigated the molecular results of nasopharyngeal swabs tested from December 2020 to February 2021 to verify whether VOC 202012/01 was associated with higher RNA loads and prolonged persistence in the respiratory tract with respect to those of other SARS-CoV-2 variants.

Methods

The workflow for SARS-CoV-2 RNA detection and sequencing have been previously described by our group (Danzetta et al., 2020). Sequences, once produced, are regularly shared with the GISAID database. For the estimation of the viral load in tested swabs, the C_T values associated with the detection of N protein encoding gene (C_T N) were chosen, since this protein is less affected by the mutation mainly targeting the S gene (Wu and Brian, 2010). The C_T N gene values of the first positive nasopharyngeal swab of patients tested from December 2020 to February 2021 were analysed by comparing the median C_T N values observed in the 313 VOC 202012/01-infected individuals with homologous values in individuals with S-positive results (n = 2344). To obtain two comparable sub-populations only those individuals with C_T values $\leq$20 were considered.

To verify any difference in the duration of the positivity at the molecular test, a further subset of the two previously mentioned groups was defined. Reasonably, only those individuals with a final negative result, thus allowing definition of the end of the positivity period, were included in the analysis. The difference (in days) between the date of the first and last positive nasopharyngeal swab was considered for each infected individual.

The statistical analysis was performed using StatTools© (Palisade Corporation, Ithaca, NY, USA). A Mann–Whitney test was used to assess the statistical significance of differences among the C_T N median values, whereas Chi-square and Fisher exact tests were used for comparing the percentages of people in the two groups who had clinical symptoms and those who died. Level of statistical significance was set at 0.05.

Results and discussion

A statistically significant difference (Mann–Whitney Test, P < 0.0001) was observed between the median values of C_T N observed in VOC 202012/01-infected individuals (median C_T N = 15.8) in comparison to S-positive infected individuals (median C_T N = 16.9) (Table 1). Furthermore, a statistically significant difference (Mann–Whitney Test, P = 0.0317) was observed between the median values of the duration of RNA positivity at the molecular test in VOC 202012/01-infected individuals (n = 136; median value = 16 days) in comparison to those of S-positive infected individuals (n = 965; median value = 14 days) (Table 1).

Viral load kinetics and the duration of viral shedding are important determinants for disease transmission (Cevik et al., 2021). In this regard also our analysis, performed in a given time period, suggests that VOC 202012/01 persists longer in the respiratory tract of infected individuals reaching higher RNA loads with respect to those of other SARS-CoV-2 variants. Although not a good predictor for viral load at individual level (Dahdouh et al., 2020), C_T values may give an indirect indication of the viral load in the population, as already seen in other studies (Veronesi et al., 2020; Hay et al., 2021).

One limitation of our study is the lack of information on the clinical status of all persons of two groups, which could be linked to different levels of C_T values and duration of the disease. However, the information about the presence or absence of clinical signs and the exitus of the disease was available for 140 VOC 202012/01-infected individuals and 961 S-positive infected individuals. In particular, 83.6% (C.I. 95%: 76.5%–88.8%) and 81.2% (C.I. 95%: 78.6%–83.5%) of individuals showed COVID-19 clinical signs in VOC 202012/01-infected and S-positive infected persons, respectively. The difference of the two percentages is not statistically significant (Chi-square: 0.4686, P = 0.4936). Similarly, the difference between the percentages of deaths in the two groups, 2.1% (C.I. 95%: 0.8%–6.1%) for VOC 202012/01-infected and 4.1% (C.I. 95%: 3.0%–5.5%), was not significant (exact Fisher value = 0.3493). These findings, although limited to a sub-sample of the study population, suggest a similar clinical picture and gravity in the two populations.

Further analyses are reasonably warranted in order to establish the correlation between C_T values originating from infections with different variants and the presence of infectious (then transmissible) virus, the evolution of the spread of VOC 202012/01 in a given area and the impact on hospitalization and access to intensive care unit.

Conflict of interest

None.

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Ethical approval

The results analysed in the present study derive from the official control activities performed by the Public Health Local Authority and no ethical approval is specifically requested.

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