First identification of the new SARS-CoV-2 Omicron variant (B.1.1.529) in Italy

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Abstract

We identified the first case in Italy of SARS-CoV-2 B.1.1.529 variant by whole genome sequencing in an Italian subject travelling from Mozambique. Specific mutation profiles deserve further investigations to clarify potential effects on vaccination efficacy.

This case highlights the crucial role of rapid and continuous surveillance of SARS-CoV-2 variant circulation.

Keywords: SARS-CoV-2; viral variants; Omicron; B.1.1.529; COVID-19.

Since the worldwide emergence of the COVID-19 outbreak, SARS-CoV-2 pandemic has been characterized by subsequent waves of viral propagation sustained by different viral strains with peculiar transmissibility, disease severity, risk of death, and potential escape from the immune response. The strong sequencing effort put in place by the international scientific community allowed to characterize numerous lineages differing in abundance and type of amino-acidic mutations. Particular attention was directed to the so-called Variants of Concern (VOCs), for which evidence of significant impact on epidemiological and clinical consequences are currently available. The lineages classified as VOCs until October 2021 were B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta) [1]. Starting from Summer 2021 Delta variant overcame the Alpha one establishing itself as the globally dominant strain with its rapid diversification into several sub-lineages (AY.X). The last identified VOC was Omicron (B.1.1.529), isolated in November in Botswana and South Africa, presenting 32 aminoacidic changes in the Surface (S) protein: to date, a total of 189 sequences were available on GISAID database [2], mainly from Africa (144/189), while information on possible alteration in immunity and natural infection course has still to be generated [3].

The arising concerns about Omicron variant are based on its unprecedentedly large mutation pattern and its rapid diffusion that have strongly stimulated containment measures and travel policy strategies worldwide to prevent global spread.

According to the Italian SARS-CoV-2 surveillance program indication for travellers of 2nd March 2021 (updated on 22nd October 2021) the countries are classified into 5 classes of risk and only inbound travellers from specific places are tested for SARS-CoV-2. In particular, the subjects coming from Africa are requested to fill in the Passenger Locator Form, to have a negative molecular or antigenic test for SARS-CoV-2 within 72 hours and to observe a 10-day isolation period or staying in Italy less than 120 hours [4].

Here we report the first case of SARS-CoV-2 B.1.1.529 identified in Italy, in an Italian subject travelling from Mozambique. After the Belgium case, this is the second patient harbouring Omicron variant in Europe according to GISAID. Moreover, other cases have been reported in Netherlands, United Kingdom, Germany and Austria.

A 48 year old man, who had undergone a full vaccination with heterologous CAZD1222 (ChAdOx1, AstraZeneca-Oxford) and BNT162b2 prime-boost vaccination (Comirnaty, Pfizer-BioNTech) in June 2021, resulted positive for SARS-CoV-2 RNA in November and he reported a recent business travel to Mozambique with two stopovers in Johannesburg and Doha.

Before leaving Mozambique, the subject had a negative SARS-CoV-2 RNA result (day 0) and two days later, upon arrival in Italy, he visited his family in Southern Italy. On day 5 he moved to Milan due to another scheduled journey to Africa: for this reason, a new nasopharyngeal swab (NPS) was collected which revealed the infection. Later on he started to experience fever (38°C), myalgia, fatigue, and headache. According to the rules of contact tracing his relatives were tested, revealing the positivity for SARS-CoV-2 RNA. No patient required hospitalization and at present nearly all of them are fully recovered.

Thanks to the National SARS-CoV-2 surveillance program indication for travellers, a NPS was collected and resulted positive using the SARS-CoV-2 ELITe MGB Kit on InGenius platform (EliTech Group, France) with a Cycle threshold (Ct) value of 21 for both RNA-dependent RNA polymerase and open reading frame 8 targets.

Being the Clinical Laboratory of Microbiology, Virology and Bioemergencies of L. Sacco University Hospital a regional reference centre for SARS-CoV-2 emergency and viral variant surveillance, we received an aliquot for genotyping analysis. According to the National Surveillance Program on variant circulation, the criteria for genotyping include arrival from area at risk of variants, cluster of outbreak, potential cases of reinfection, vaccinated subjects. As initial screening, a specific variant RT-PCR based test was performed by means of Allplex™ SARS-CoV-2 Variants I+II Assays kit (Seegene Inc., Republic of Korea), that detected N501Y and K417N mutations plus 69/70 deletions in S gene, a mutation pattern inconclusive for well-known α, β, γ, nor δ VOCs and suggestive of new variant of concern [5].

To assign the definitive lineage, whole genome sequencing (WGS) analysis was conducted on iSeq platform (Illumina) using CleanPlex® SARS-CoV-2 Panel kit (Paragon Genomics): FASTQ file was then processed on the Sophia bioinformatic platform (SOPHiA GENETICS, Switzerland) and the resulting FASTA was uploaded on the Italian web-based portal I-Co-Gen (https://irida.iss.it/) as coordinated by ISS. I-Co-Gen reported the presence first of all of an early warning due to the presence of amino acid substitution S371L specific for the new lineage B.1.1.529, and then finally the lineage was assigned by the I-Co-Gen platform. The sequence is also present on GISAID with code EPI_ISL_6777160. Lineage assignment was confirmed by Pangolin COVID-19 Lineage Assigner (https://pangolin.cog-uk.io/). This result permitted to share data with the Istituto Superiore di Sanità (ISS, National Institute of Health) and the network of laboratories participating in the genomic surveillance program. In addition to molecular analyses, viral isolation was performed on Vero E6 cells (VERO C1008 ATCC® CRL-1586). The availability of isolated virus would allow to evaluate the antibody response against the new variant by *in-vitro* serum neutralization test on COVID-19 convalescent

subjects as well as vaccinated ones [6]. Since mutations in S gene were associated with higher infectivity, escape to monoclonal antibodies and immune response, as well as to syncytium formation, it is crucial to identify aminoacidic alterations and test their effect on human antibodies activity [7-9]. Moreover, the comparison with the other VOCs can contribute to better estimate the level of concern of Omicron variant [10].The in vitro results could contribute to strengthen the third COVID-19 vaccine dose recommendation considering also the dangers of potential waning immunity [11].

The multistep approach based on specific variant RT-PCR assay and the subsequent WGS of samples with atypical pattern of mutations could be a powerful algorithm for a first step screening before the sequencing of the entire genome of SARS-CoV-2 in order to report the new SARS-CoV-2 variants to public health authorities.

A prompt and accurate surveillance of SARS-CoV-2 variant at national level could play a crucial role not only to quickly identify new variants but also for public health purposes.

Thanks to this rapid report, on 26th November 2021 health authorities have immediately released an urgent update to manage arrivals from Sub-Saharan Africa. In addition to previous rules, it's necessary to perform a molecular or antigenic test at the airport or harbor upon arrival and to undergo a 10-day isolation period with another molecular test thereafter.

In conclusion, the excellent integrated surveillance system implemented in Italy allowed us to rapidly reveal the new variant and to limit its circulation, even if in an interconnected world travel bans might slow but unfortunately will not eliminate global spread.

Notes

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