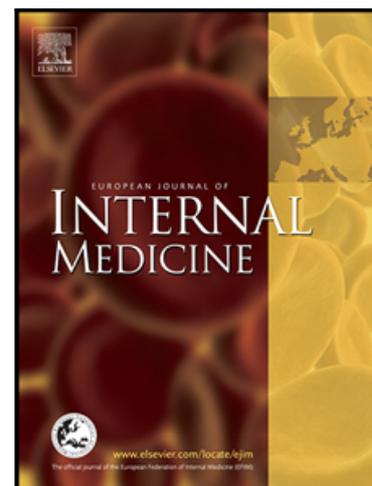


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SARS-CoV-2 infection among asymptomatic homebound subjects in Milan, Italy

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At the end of December 2019, the first cases of SARS-CoV-2 infection were identified in Wuhan, China. [1] In the following months, the outbreak of SARS-COV-2 infections spreads worldwide [1] As of May 1, 2020, more than 3 million of SARS-CoV-2 infections have been detected worldwide. These data were mainly collected from inpatients. On the other hand, asymptomatic or mildly symptomatic subjects are usually untested for SARS-CoV-2 although they are supposed to far outnumber the symptomatic patients. [2] Therefore, there is an urgent need to estimate as accurately as possible the real number of subjects who have been infected by the virus. The aim of this study was to assess the frequency of asymptomatic subjects with a SARS-CoV-2 positive nasal swab or presenting immunoglobulins against the virus in Milan (Italy).

This study is the first part of the UNICORN (“UNiversity against CORoNavirus”) project that is being conducted among the personnel of the University of Milan, the largest university in Lombardy (Italy). In this first part of the project, we aimed at investigating a sample of 200 asymptomatic subjects, enrolled during the lockdown period. Subjects with fever, any symptoms of flu-like infections or dyspnea at the moment of the recruitment or in the 14 previous days, subjects with close and prolonged contact with any person positive for SARS-CoV-2 or with signs or symptoms suggestive for infection in the previous 14 days could not participate. Each participant received an appointment at a fixed time to one of the two campuses fully dedicated to the study each for one day (March 30 and 31, 2020). Once at the campus, participants underwent a self-sampling nasal mid-turbinate swab (D.I.D. Diagnostic International, Milan, Italy) through a supervised onsite self-collection procedure according to the Centers for Diseases Control and Prevention Guidelines. [3] To detect SARS-CoV-2 RNA, a multiplex real time RT-PCR test (TaqPath™ Covid-19 CE-IVD RT-PCR kit, ThermoFisher Scientific) was applied. A 7.5 ml blood sample was also collected on mobile vehicles by

volunteers of the Italian Association of Blood Donors (AVIS Milano). Each blood sample was processed within 4 hours to obtain the plasma fraction. Total Antibodies (Total Ab), immunoglobulins M (IgM) and immunoglobulins G (IgG) against SARS-CoV-2 were tested using validated enzyme linked immunosorbent assay (ELISA) kits CE-IVD. [4, 5]. The Wantai SARS-CoV-2 Ab ELISA and the Anti-SARS-CoV-2 IgM ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China) were performed to measure Total Ab and IgM. These assays detect antibodies binding SARS-CoV-2 spike protein receptor binding domain (RBD) in human serum or plasma. The Anti-SARS-CoV-2 IgG ELISA (Euroimmun Medizinische Labordiagnostika, Lübeck, Germany) was used to detect IgG antibodies against SARS-CoV-2 spike protein subunit 1 (S1). After sampling procedures, participants were asked to fill-in an online structured questionnaire to collect data on: age, gender, education level, number of cohabitants (and the number of cohabitants aged 10 years or less), travels to Europe or other Continents from October 01, 2019, episodes of upper and lower respiratory infections from October 01, 2019, medical comorbidities and ongoing treatments. Finally, participants were asked if they were working at home or in university during the previous weeks. The characteristics of the subjects testing positive for nasal swab or at least one of the immunoglobulin tests were compared with the characteristics of those testing negative for all the tests by the Fisher exact test and Wilcoxon rank-sum test for not-normally distributed variables. The study was approved by the ethics committee of the University of Milan.

Among the 200 subjects enrolled in this study, 197 subjects (99%) completed the protocol, while 3 subjects revoked their participation before sample collection. A total of 31 subjects (16%) presented at least one positive test as given in Figure 1. In detail, the SARS-CoV-2 RNA was detected in the nasal swab of 21 subjects (11%). Twenty subjects (10%)

presented antibodies against SARS-CoV-2: total Ab were detected in 11 subjects (5 were positive and 6 were weakly positive), IgM in 12 subjects (5 were positive and 7 weakly positive) and IgG in 14 subjects (11 positive and 3 weakly positive). Ten out of these 21 subjects with a positive nasal swab were also positive for one or more of the antibody tests.

The subjects positive for at least one of the tests did not differ from the other participants as regards to age, gender, number of cohabiting family members (including children <10 years of age), travels from October 1, 2019, number of upper and lower respiratory infections in the previous months and the frequency of comorbidities. Among subjects with a positive nasal swab, 18 (86%) worked at home during the four weeks preceding the enrolment.

In this study conducted among a group of asymptomatic subjects in Milan, 11% presented a positive SARS-CoV-2 nasal swab and 10% had antibodies against SARS-CoV-2. The data on asymptomatic carriers of SARS-CoV-2 are of great interest, considering that the study was performed three weeks after the Italian Government had applied strict homebound measures to all citizens. The reasons underlying the infection of these subjects remain unknown. Milan is the capital of Lombardy, one of the regions with the highest SARS-CoV-2 local transmission in the world and it is possible that some subjects have been infected before the introduction of homebound measures. However, literature suggests that only a minority of asymptomatic cases are still positive for SARS-CoV-2 RNA in the nasal swab after 14 days. [6] Moreover, the large majority of positive subjects did not report any symptoms in the weeks preceding the enrolment, and some subjects never had symptoms in the previous 6 months.

Approximately 10% of subjects presented with antibodies against SARS-CoV-2. The ability of antibodies against SARS-CoV-2 infection is still under debate. However, these data

suggest that only a minority of asymptomatic subjects in Milan developed antibodies against this virus so far.

Some considerations about the usefulness of antibodies testing for large-scale screening should be acknowledged. Data collected among inpatients affected by SARS-CoV-2 have shown that the seroconversion occurs in almost all cases within 2-3 weeks after symptoms onset. [7,8] Testing for antibodies among asymptomatic subjects has been strongly emphasized as a promising strategy to guide societies to a gradual reopening, and many international authorities are addressing efforts to this goal. [9] The results of this study suggest that the use of antibodies for the general public should be cautious and new studies are needed to test their reliability in asymptomatic subjects.

Our study has some limitations. First, the self-sampling strategy could have increased the number of false negatives. Second, the presence of viral RNA does not necessarily mean that the virus is present in a replicative state. [10] Third, we did not test plasma samples for virus neutralization.

In conclusion, this study suggests that approximately 15% of homebound subjects in Milan has been infected by SARS-CoV-2. The reliability of antibodies tests this virus deserves further studies before its systematic use for screening in the general population.

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Authors' contributions

GM: Study design, literature search, data collection, data analysis, data interpretation, writing

EM: Data collection, data analysis, data interpretation

VB: Study design, figures, data collection, data analysis, data interpretation, writing

UNICORN CONSORTIUM: Subject's enrolment, Laboratory analysis, data interpretation.

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Competing interests

The authors have nothing to disclose.

CONFLICTS OF INTEREST

All authors declare no conflict of interest

Ethics committee approval

The study was approved by the ethics committee of the University of Milan (approval number 17/20, approval date March 6, 2020) and conducted in accordance with the Declaration of Helsinki.

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Figure legend

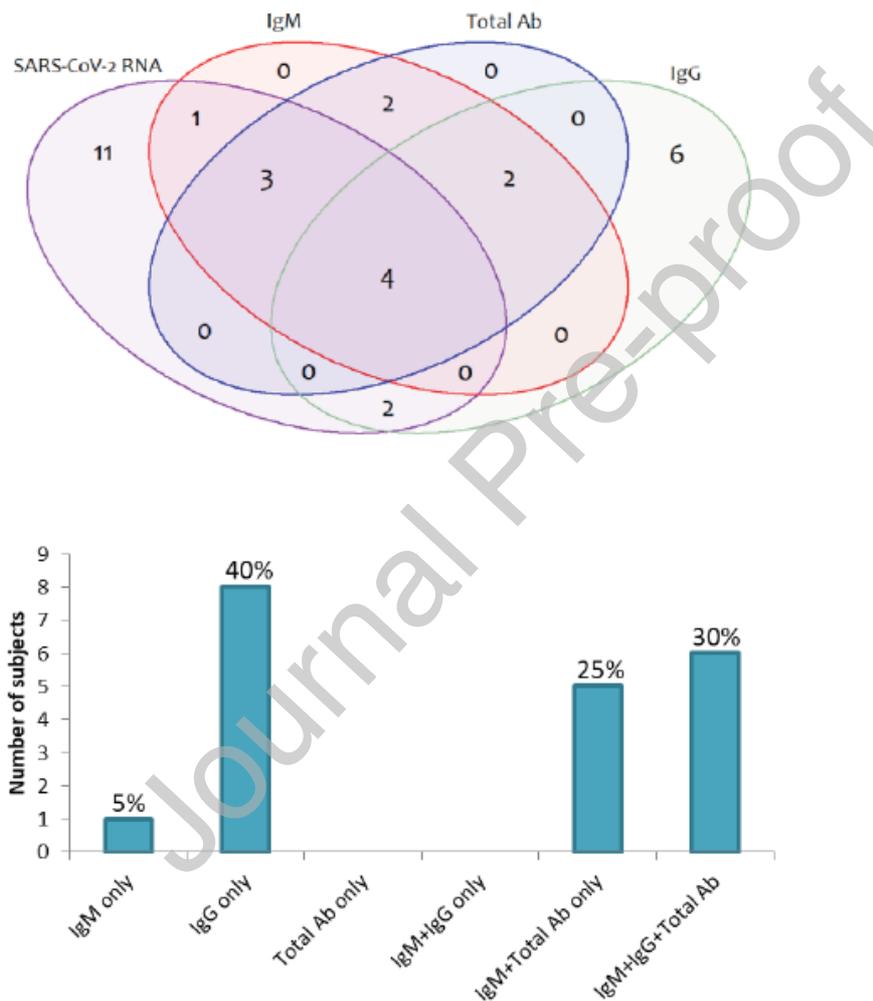


Fig 1, left panel. The figure describes the symptoms reported by subjects testing positive for one or more of the SARS-CoV-2 marker, including upper airways infections (red), lower airway infections (brown) and fever (blue). **Right panel.** The table specifies what marker resulted positive for each subject.