Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

European Journal of Internal Medicine 78 (2020) 161–163

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Letter to the Editor

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

ARTICLE INFO

Keywords:
UNICORN
COVID-19
SARS-CoV-2
Immunoglobulins
Viral RNA
Asymptomatic carriers
Nasal swab

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 Fig. 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

https://doi.org/10.1016/j.ejim.2020.06.010
Received 30 May 2020; Accepted 6 June 2020
Available online 09 June 2020

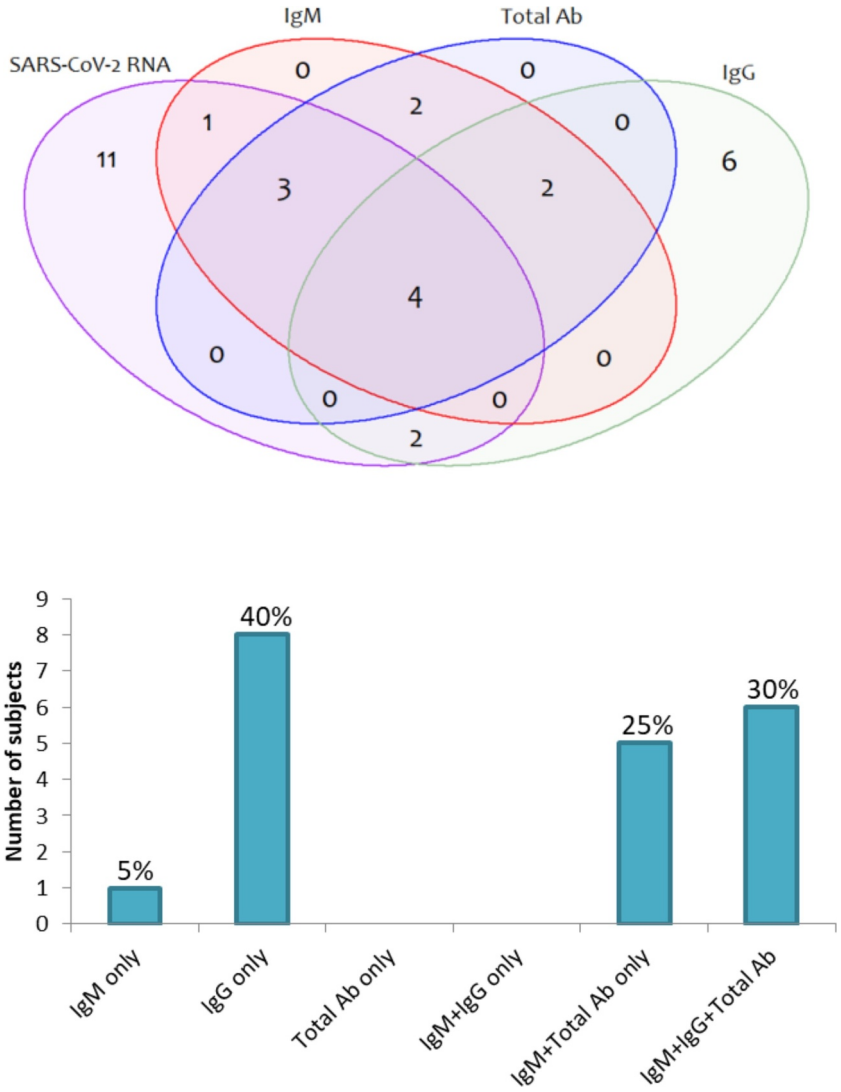

Fig. 1. Upper panel. Venn diagram showing the number of asymptomatic subjects testing positive for SARS-CoV-2 for RNA in the nasal mid-turbinate (violet circle), for circulating IgM (red circle), IgG (green circle), and Total Ab (blue circle). **Lower panel**. Histogram showing the frequency and percentage of subjects testing positive for IgM, IgG and Total antibodies (Total Ab). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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.

Funding

Action “Ricerche Emergenza coronavirus”, University of Milan, 2020.

Financial disclosure statement

GPM and VB received a grant from “Ricerche Emergenza coronavirus”, University of Milan, 2020 to support the study (https://lastatalenews.unimi.it/statale-individuati-sette-progetti-ricerca-ad-alta-priorita-contro-covid-19). Funds have been used for purchasing reagents.

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.

CRediT authorship contribution statement

Gregorio Paolo Milani: Conceptualization, Investigation, Methodology, Writing - original draft. **Emanuele Montomoli:** Data curation, Formal analysis, Writing - review & editing. **Valentina Bollati:** Conceptualization, Investigation, Methodology, Project administration, Writing - original draft. **Benedetta Albetti:** . **Claudio Bandi:** . **Tommaso Bellini:** . **Matteo Bonzini:** . **Marco Buscaglia:** . **Carlo Cantarella:** . **Laura Cantone:** . **Michele Carugno:** . **Sergio Casartelli:** . **Guido Cavaletti:** . **Sarah D'Alessandro:** . **Francesca De Chiara:** . **Serena Delbue:** . **Laura Dioni:** . **Ivano Eberini:** . **Chiara Favero:** . **Luca Ferrari:** . **Monica Ferraroni:** . **Laura Galastri:** . **Cristina Galli:** . **Mirjam Hoxha:** . **Simona Iodice:** . **Carlo La Vecchia:** . **Chiara Macchi:** . **Ilaria Manini:** . **Serena Marchi:** . **Jacopo Mariani:** . **Elena Pariani:** . **Angela Cecilia Pesatori:** . **Federica Rota:** . **Massimiliano Ruscica:** . **Tommaso Schioppo:** . **Letizia Tarantini:** . **Claudia Maria Trombetta:** . **Maria Grazia Valsecchi:** . **Marco Vicenzi:** . **Giuliano Zanchetta:** .

Declaration of Competing Interests

None.

Acknowledgments

We thank all the AVIS-Milano volunteers for their support with blood collection, Patrizia Angiolillo for electronic questionnaire preparation, Nicla Diomede for informatics security, Elena del Giorgio for support during subject's enrolment, Angelo Casertano and all the University of Milan staff for their precious help.

References

[1] World Health Organization (WHO). WHO director-general's opening remarks at the media briefing on COVID-19: 11 March 2020. Published March 11, 2020. Accessed March 11, 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020.

[2] Day M. Covid-19: four fifths of cases are asymptomatic, China figures indicate. BMJ 2020;369:m1375.

[3] Centers for diseases control and prevention guideline for “collecting, handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19)”, (2020). cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.

[4] GeurtsvanKessel CH, OKBA NMA, Igloi Z, Embregts CWE, Laksono BM, et al. (2020) Towards the next phase: evaluation of serological assays for diagnostics and exposure assessment. medRxiv: 2020.2004.2023.20077156.

[5] Lassaunière R, Frische A, Harboe ZB, Nielsen AC, Fomsgaard A, et al. (2020) Evaluation of nine commercial SARS-CoV-2 immunoassays. medRxiv: 2020.2004.2009.20056325.

[6] Wu J, Liang J, Zhou H, Peng F, Wang B, et al. Clinical features and outcomes of asymptomatic cases of SARS-CoV-2 infection. J Infect 2020. S0163-4453(20)30238-3.

[7] Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020. https://doi.org/10.1038/s41591-020-0897-1.

[8] To KK, Tsang OT, Leung WS, Tam AR, Wu TC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20(5):565-574.

[9] Abbasi J. The promise and peril of antibody testing for COVID-19. JAMA 2020. https://doi.org/10.1001/jama.2020.6170.

[10] Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol 2009;7:439–50.

Gregorio Paolo Milani[a,b], Emanuele Montomoli[c], on behalf of the UNICORN Consortium investigators, Valentina Bollati[d,*], Benedetta Albetti[e], Claudio Bandi[f], Tommaso Bellini[g], Matteo Bonzini[e,h], Marco Buscaglia[g], Carlo Cantarella[e], Laura Cantone[e], Michele Carugno[e,h], Sergio Casartelli[i], Guido Cavaletti[j], Sarah D'Alessandro[k], Francesca De Chiara[i], Serena Delbue[k], Laura Dioni[e], Ivano Eberini[l], Chiara Favero[e], Luca Ferrari[e], Monica Ferraroni[m], Laura Galastri[i], Cristina Galli[n], Mirjam Hoxha[e], Simona Iodice[e], Carlo La Vecchia[m], Chiara Macchi[l], Ilaria Manini[c], Serena Marchi[c], Jacopo Mariani[e], Elena Pariani[n], Angela Cecilia Pesatori[e,h], Federica Rota[d], Massimiliano Ruscica[l], Tommaso Schioppo[o], Letizia Tarantini[e], Claudia Maria Trombetta[c], Maria Grazia Valsecchi[p], Marco Vicenzi[q,r], Giuliano Zanchetta[g]

[a] *Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy*

[b] *Pediatric Unit, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy*

[c] *Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy*

[d] *EPIGET Lab, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy*

[e] *Fondazione IRCCS Ca’ Granda-Ospedale Maggiore Policlinico, Occupational Health Unit, Milan, Italy*

[f] *Department of Biosciences and Pediatric Clinical Research Center "Romeo and Enrica Invernizzi", University of Milan, Milan, Italy*

[g] *Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, 20129, Italy*

[h] *Occupational Health Unit, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy*

[i] *AVIS (Associazione Volontari Italiani Sangue) Milano, Milan, Italy*

[j] *Experimental Neurology Unit, School of Medicine and Surgery; NeuroMI (Milan Center for Neuroscience), University of Milano-Bicocca, Monza, MB, Italy*

[k] *Department of Biomedical, Surgical and Dental Sciences, Laboratory of Translational Research, Via Carlo Pascal 36, 20133 Milano, Italy*

[l] *Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*

[m] *Branch of Medical Statistics, Biometry, and Epidemiology "G. A. Maccacaro", Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy*

[n] *Department of Biomedical Sciences for Health, University of Milan, Milan, Italy*

[o] *Division of Rheumatology, ASST Pini-CTO, Milan, Italy*

[p] *Center of Biostatistics for Clinical Epidemiology, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

[q] *Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Cardiovascular Disease Unit, Internal Medicine Department, Milan, Italy*

[r] *Dyspnea Lab, Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy*

E-mail address: valentina.bollati@unimi.it (V. Bollati).

[*] Corresponding author.