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Letter to the Editor

Seroprevalence of SARS-CoV-2 significantly varies with age: Preliminary results from a mass population screening

Dear Editor,

We read with interest the article by C. Dimeglio and colleagues, in which the authors apply a mathematical model to conclude that a SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) seroprevalence of at least 50% is required to avoid an infection rebound after removal of containment measures. In our study we found that SARS-CoV-2 seroprevalence was dramatically lower than this threshold, even in an area of initially unrestricted viral circulation.

Italy was the first European country that suffered a widespread spread of Coronavirus Disease 2019 (COVID-19), caused by a novel betacoronavirus which was first identified in China and denominated SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), which caused hundreds of thousands of cases. During the epidemic, testing was restricted to severely symptomatic cases. Consequently, the true extent of the SARS-CoV-2 infection remains unknown.

We estimated SARS-CoV-2 seroprevalence in the municipality of Castiglione d’Adda, a rural town of about 4550 inhabitants located South-East of Milan, which has been heavily affected by SARS-CoV-2 infection since the earliest stages of the epidemic. As of June 21, 2020, 184 confirmed cases of COVID-19 were reported, the large majority of which requiring hospitalization, accounting for about 4% of the total population. At the same time, 47 deaths were officially attributed to COVID-19.

In this study, the entire population of Castiglione D’Adda was invited to perform a lateral-flow immunocromatographic tests on capillary blood (Prima Lab, Switzerland) from the 18th of May to the 7th of June. News about the mass screening was disseminated by the town municipality. A random sample of 562 subjects (stratified per sex and age) was invited to undergo confirmatory tests by chemiluminescent method on venipuncture drawn blood (CLIA, IgG anti-SARS-CoV-2, Abbott, USA) and SARS-CoV-2 PCR on NPS, regardless of RICT results. More detailed information about the randomization procedure and the study design are available on the complete protocol, published on medRxiv pre-print server.

The analysis of IgG prevalence in the different age groups was performed by logistic regression models with response variable equal to 1 for positive IgG results, and 0 for negative IgG results. Age and gender were included as independent variables. Results were reported in terms of estimated probabilities of being positive to IgG test as a function of age, with respective 95% confidence intervals.

Results presented in this paper are based on 509 people selected in the random sample who agreed to undergo venipuncture to perform CLIA serologies. Characteristics of the selected population are reported in Table 1.

The overall seroprevalence found in the tested sample was 22.6% (95% confidence interval 17.2%- 29.1%). Interestingly, seroprevalence increases with increasing age (as shown in Table 1). In multivariate analyses, a significant effect of age was found (p=0.0001) while no significant association between IgG positivity and gender emerged (p=0.2560). The possible existence of a non-linear effect of age was tested by including spline polynomials, without significant results (p=0.9078). Furthermore, an age/gender interaction effect did not result significant (p=0.5199). Estimates of probabilities of being positive to IgG test, from a

Table 1
Characteristics of 509 subjects in the random sample.

| Gender (Female) | IgG negative (n = 394) | IgG positive (n = 115) |
|-----------------|-----------------------|------------------------|
| Age (years; median, SD) | 46±0, 20±6 | 55±4, 19±5 |
| Age groups: | | |
| 0–19 | 56 (91±8%) | 5 (9±2%) |
| 20–39 | 92 (82±9%) | 19 (17±1%) |
| 40–59 | 142 (78±0%) | 40 (22±0%) |
| ≥ 60 | 104 (67±13) | 51 (32±9%) |
| Contact with verified case | | |
| Smoker | 92 (23±6%) | 61 (53±0%) |
| Cardiovascular diseases | | |
| CAD/MI | 10 (2±5%) | 3 (4±3%) |
| Arthritism | 14 (3±6%) | 5 (4±3%) |
| Hypertension | 68 (17±3%) | 32 (27±8%) |
| Other | 14 (3±6%) | 14 (12±2%) |
| At least one of the above: | | |
| Asthma | 84 (21±3%) | 47 (40±9%) |
| Rheumatic diseases | 19 (4±8%) | 11 (9±6%) |
| Diabetes mellitus | 12 (3±0%) | 6 (6±2%) |
| Chronic Lung diseases | | |
| Asthma | 20 (5±1%) | 2 (1±7%) |
| COPD | 1 (0±3%) | 1 (0±9%) |
| Other | 9 (2±3%) | 4 (3±5%) |
| At least one of the above: | | |
| Onco logical pathologies | 29 (7±4%) | 7 (6±1%) |
| Breast tumours | 20 (5±1%) | 6 (5±2%) |
| Ovarian neoplasms | 2 (0±5%) | 2 (1±7%) |
| At least one of the above: | | |
| Symptomatic | 22 (5±6%) | 8 (7±0%) |

Numerical variables are presented as means.

CAD: Coronary Artery Disease; MI: Myocardial Infarction; COPD: Chronic Obstructive Pulmonary Disease.

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model including only age as independent variable, are reported in Fig. 1.

Since the early phases of the pandemic, advanced age was identified as an independent predictor for severe disease and worse outcomes6. Beside this, it remains unclear if the limited number of cases reported in children7 is due to a milder course of disease, with a larger percentage of asymptomatic cases, or to a lower susceptibility to infection, as our results seem to suggest.

Different ACE2 expression according to age have been postulated to explain clinical expression and susceptibility to the infection. In particular, a higher expression of ACE2 in lung tissues in advanced age groups had been speculated8, 9. Moreover, a variable susceptibility to other coronavirus such as HCoV-NL63, which also use ACE2 as cell receptor in humans, in different age groups, has been also reported in different age groups8.

Another possible explanation may be that an asymptomatic/pauci-symptomatic infection, more common in younger subjects, could elicit a less marked, or transient, antibody response, as already found in the closely related Middle East Respiratory Syndrome Coronavirus (MERS-CoV)10.

A possible confounding factor in our findings could be related to social distancing measures: schools of any grade were among the first institutions to be closed in Italy, starting from the 5th of March. This could have led to a lower exposure to the infection in children in pre-schooler and scholar age groups.

In conclusion, our findings suggest that SARS-CoV-2 IgG seroprevalence increases with increasing age and these data suggest a lower susceptibility to infection in the lower age groups. These findings have important implications in epidemiology and public health, particularly in designing future population screenings, and could be an important contribution in the re-opening process, especially considered that more than three-fourths of the population could be still susceptible to SARS-CoV-2 infection, even in an area of initially unrestricted viral circulation.

Declaration of Competing Interests

The authors declare no conflicts of interest. All authors have seen and approved the final manuscript.

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MG, GP, FC, DB, AG, RR and EB defined the study protocol. RR, AP, FC, DB and GP cooperated in the practical execution of the study and data gathering. GP drafted a first version of the manuscript, which was then revised and integrated by MG, EB, AG, CEG and SC. CO was responsible of serologies and NPSs execution. EB, PB and GM analyzed the data. All authors approved the final version of the manuscript.

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Ethical approval

The study was approved by University of Milan’s Ethical Committee.

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Gabriele Pagani*, Federico Conti, Andrea Giacomelli,
Dario Bernacchia
Università degli Studi di Milano, Italy
Infectious Diseases department, III Division, ASST FBF-Sacco,
Milano, Italy
Rossana Rondonin, Andrea Prina
Medispa s.r.l, Italy
Vittore Scolari
Sorbonne Université, Paris, France
Cecilia Eugenia Gandolfi
Università degli Studi di Milano, Italy
Silvana Castaldi
Università degli Studi di Milano, Italy
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico di Milano, Italy
Giuseppe Marano
Università degli Studi di Milano, Italy
Cosimo Ottomano
Synlab s.p.a, Belgium
