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BRIEF REPORT

Children account for a small proportion of diagnoses of SARS-CoV-2 infection and do not exhibit greater viral loads than adults

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Abstract

Previous reports have suggested that children are less affected than adults by SARS-CoV-2. We analyzed SARS-CoV-2 diagnoses between February 27, 2020, and March 14, 2020, and mortality among positive patients in Marseille university hospitals. Of 4050 tested individuals, 228 were positive. Deaths occurred in 2/99 documented cases (both > 85 year-old). Children were majorly asymptomatic. Incidence increased by 7.4-fold between 1–5 and 45–65 years then decreased. It was significantly lower among 0–1 year- (0%) and 1–5 (1.1%) and 5–10 (3.6%)-year-old children than among subjects > 18 years (6.5%). Viral loads did not differ between children and adults. Children may not contribute significantly to virus circulation.

Keywords SARS-CoV-2 · Covid-19 · Children · Transmission · Viral load

A new coronavirus, named SARS-CoV-2, has emerged in humans since December 2019 in the region of Wuhan in China [1]. In France, the first case was diagnosed on January 24, 2020, and on March 14, 2020, the number of confirmed cases was 3661, and the number of deaths was 79 [2]. As was the case previously in a dozen countries, the decision was made on March 12, 2020, to close schools, and universities, in order to limit the transmission of SARS-CoV-2 across the French population.

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Among parameters associated with infectivity of respiratory viral infections are duration of viral shedding and viral load that are positively correlated with the transmission risk. In the case of influenza virus infections, children are considered important drivers of transmission of the virus in the community and were described as more infectious than adults [3, 4]. In contrast, reports available from China have suggested that children are less affected than adults by the SARS-CoV-2 [5, 6]. No study has focused on SARS-CoV-2 among children in France. We describe here the number of infections and viral load in children comparatively to those in adults for cases tested in our French institution.

Viral RNA was extracted from nasopharyngeal secretions collected with Virocult swabs (Medical Wire and Equipment Company, Corsham, Wilts, England) using the EZ1 Virus Mini Kit v2.0 on the EZ1 instrument (Qiagen, Courtaboeuf, France) or the QIAamp Viral RNA Mini Kit (Qiagen, Courtaboeuf, France) on the QIAcube automated nucleic acid purifier (Qiagen). Then, SARS-CoV-2 RNA was assessed by a real-time reverse transcription (RT)-PCR system targeting the envelope protein (E)-encoding gene with the LightCycler Multiplex RNA Virus Master kit on a LightCycler 480 instrument (Roche Diagnostics, Mannheim, Germany), as previously described [7, 8]. Estimated detection threshold was 200 copies/mL. An internal control phage was used to ensure RNA extraction and PCR accuracies [7, 8]. Ten

swabs were weighed before and after collection of nasopharyngeal secretions to be able reporting the number of RNA copies/g of secretions. Mean weight was 220 ± 35 mg. Copy number was calculated using a positive control synthetic RNA corresponding to the PCR target region. Then, this number was multiplied by 23 as volume used for viral RNA extraction was 200 μL and total volume of swab fluid was 1 mL (factor = 5) and RNA copy number per swab was converted to that per g (factor = 4.5). We considered the time period starting from the first SARS-CoV-2-positive diagnosis, during which we had evidence that SARS-CoV-2 was present in our geographical region and may circulate. We analyzed the presence of clinical symptoms and the mortality rate among SARS-CoV-2-positive patients sampled in Marseille university hospitals for whom this information was available. Statistical analyses were performed with the OpenEpi online tool (https://www.openepi.com/Menu/OE_Menu.htm).

Between the 27th of February when we began to have positive tests until the 14th of March (17-day period), we tested 4766 respiratory samples from 4050 patients for the presence of SARS-CoV-2 RNA and found that 228 (5.6%) were positive. Tests were performed for people with a broad range of age as 15% of the 4050 subjects were < 10 years, 18% were < 15 years, and 18% were > 65 years (Fig. 1a). As a comparison, 18% of the inhabitants of Marseille city and Bouches-du-Rhône department counted in 2016 were < 15 years (https://www.insee.fr/fr/statistiques/2011101?geo=COM-13055; https://www.insee.fr/fr/statistiques/2011101?geo=DEP-13). Mean age (± standard deviation) of these 4050 patients was 40 ± 25 years. The proportion of children was significantly lower among SARS-CoV-2-positive than -negative subjects. Thus, 4% and 8% of positive subjects were < 10 and 15 years, respectively, whereas these proportions were 15% and 19%, respectively, among negative subjects [10 and 17/228 versus 578 and 735/3822 ($p < 10^{-5}$ for both age groups)] (Fig. 1b). In addition, the proportion of positive subjects was significantly lower or showed a tendency to be significantly lower among children whose age was comprised between 0 and 1 years (0/110; 0%), 1–5 years (3/285; 1.1%), and 5–10 years (7/194; 3.6%) than among subjects > 18 years (208/3205; 6.5%) ($p < 10^{-3}$, $p < 10^{-3}$, and $p = 0.074$, respectively) (Fig. 1c). Besides, among SARS-CoV-2-positive subjects, viral loads did not differ significantly between children or adolescents and adults. Indeed, mean cycle threshold (Ct) value was 24.8 ± 4.6 overall and 24.9 ± 4.3 in children < 10 years, 26.0 ± 4.9 among children and adolescents between 10 and 18 years, and 24.8 ± 4.6 among adults (Fig. 2a). We further considered particularly the Ct values < 19, < 23, and < 26 as we determined that they corresponded to viral loads > 10 billions, > 1 billion, and > 100 millions RNA copies/g of nasopharyngeal secretions. The proportion of Ct values < 19 was 0% (0/10), 0% (0/10) and 9% (19/208) for subjects < 10 years, between 10 and 18 years, and > 18 years, respectively (Fig. 2b). In addition, a tendency toward a significant difference was found between the proportions of Ct values < 19 among subjects < 18 years (0/20; 0%) and those between 45 and 55 years (6/43; 18%) ($p = 0.090$). Finally, the proportions of Ct values comprised between 19 and 26 did not differ significantly between children < 10 years (7/10; 70%), children and adolescents between 10 and 18 years (4/10, 40%), and adults (100/208; 48%).

We collected the presence of clinical symptoms among children and determined the mortality rate among the 99 SARS-CoV-2-positive patients sampled in Marseille university hospitals. Two (2.0%) of them died after being diagnosed with Covid-19. They were 87 and 89 year-old and were admitted with severe acute respiratory syndrome (SARS), and were out of 5 patients > 85 years. The three other patients > 85 years were symptomatic at admission: one presented SARS, one pneumonia, and one upper respiratory tract infection. Among 9 children or adolescents with clinical documentation, six were asymptomatic, one had cough and fever, one had upper respiratory tract infection, and one had isolated fever. No death was observed in subjects younger than 85 years in our series. Mean age ± standard deviation for the 129 remaining patients was 47 ± 21 years (range, 1.4–89).

We report in the present work for the first time in France based on the testing of 4050 patients and a series of 228 diagnosed SARS-CoV-2 infections that children and adolescents represented a low proportion of these infections, were majorly asymptomatic, and exhibited viral loads that did not differ significantly at the time of diagnosis with those among adults, and even tended to be lower. Regarding mortality, we observed that 2/5 patients older than 85 years, both admitted with SARS, died. Based on the first Chinese reports on the epidemiology of SARS-CoV-2 infections, it early appeared that these infections were uncommon in children [5, 6]. Thus, children < 10 years and aged of 10–19 years represented 1% each of 72,314 Covid-19 cases in a large study [5], and few pediatric cases have been reported overall [6, 9]. Consequently, it was questioned if children may be less susceptible to Covid-19 [6]. In addition, infections in children were found to be associated with milder clinical symptoms and with faster recovery compared to those in adults [10–12]. These epidemiological and clinical patterns are similar to those previously described for SARS-CoV and MERS-CoV infections [13–16]. Several series of childhood cases of SARS-CoV-2 infections have been reported in China, but overall, information are lacking on incidence relatively to that among adults, and on viral loads in clinical samples. In the largest study conducted to date, Dong et al. reported 731 laboratory-confirmed pediatric cases, among whom 94 (13%) were asymptomatic, 315 (43%) presented mild severity of illness, 300 (41%) moderate severity, 18 (2%) were severe cases, 3 (0.4%) needed intensive cares, and one (0.1%) 14-year-old patient died [12]. Lu et al. reported 171 cases who

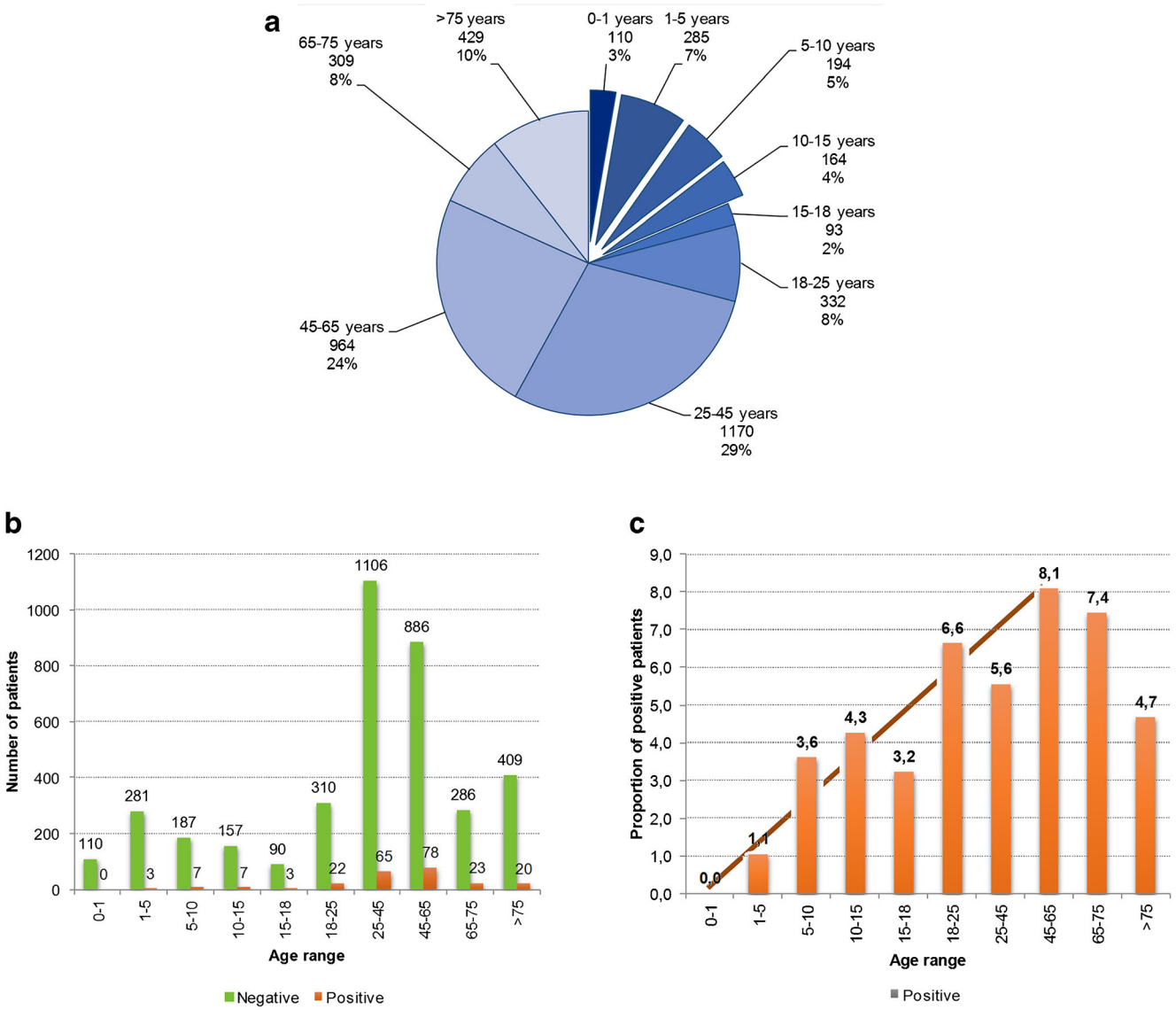

Fig. 1 Distribution of ages among people tested for SARS-CoV-2 infection between the 27th of February and the 14th of March, 2020 (**a**), distribution of ages among SARS-CoV-2-negative and positive subjects (**b**), and proportion of positive tests according to groups of age (**c**)

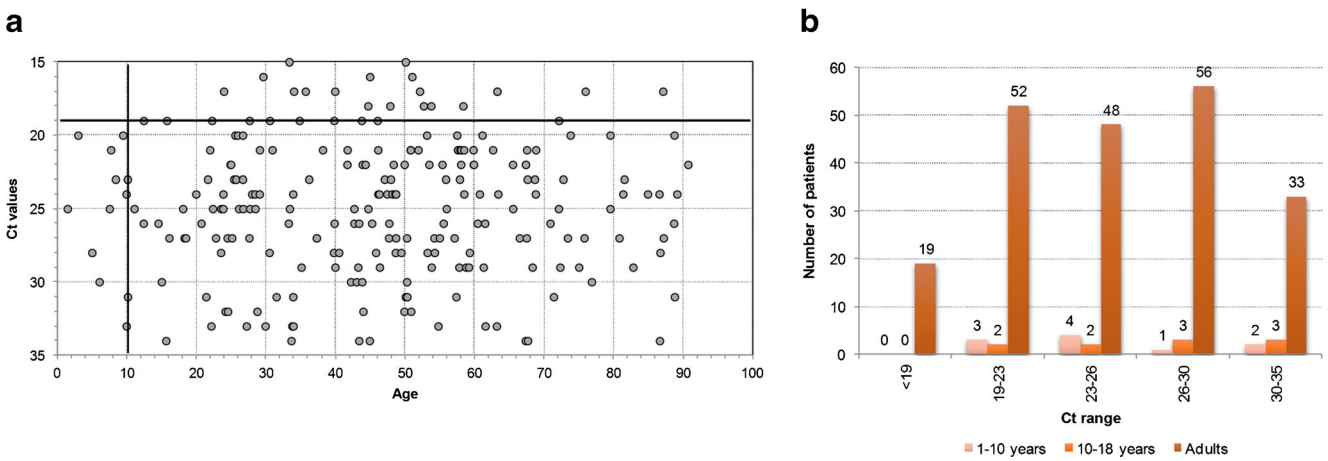

Fig. 2 Dot plot representation of the relationship between the age of SARS-CoV-2-positive subjects and the Ct values of PCR tests (**a**) and distribution of Ct values of PCR according to groups of age (**b**)

represented 12% of 1391 children with known contact with confirmed or suspected SARS-CoV-2 infections [17]. They described confirmed family members in 77% of the cases, and a milder clinical course in most children compared to adults; one 10-month-old child with intussusceptions died. In a study of 10 symptomatic pediatric cases, SARS-CoV-2 RNA was detected in nasopharyngeal/throat swabs for a mean duration of 12 days (range, 6–22 days) after illness onset [9]. Household exposure was found in seven cases. In another series that included 31 children whose age ranged between 1.5–17 years, 94% of the cases were in family clusters, and 39% were asymptomatic [18]. Liu et al. described that 1.6% ($n = 6$) of 366 hospitalized children with respiratory infections were SARS-CoV-2 positive [19]. The age of these cases ranged between 1 and 7 years. One of them was admitted to an intensive care unit but all recovered. Wang et al. reported a series of 37 SARS-CoV-2-positive children whose age ranged between 7 months and 18 years [20]. Family cluster transmission was suspected in 87% of these cases. Seven cases were asymptomatic and one was severe. Finally, 35% of 82 cases of a median age of 10 years from mainland China had an infected family member [21]. Hence, overall, a majority of childhood cases were part from familial clusters.

In summary, in contrast to flu, our findings confirm that children represent a small proportion of SARS-CoV-2 cases and do not have higher viral loads than adults, and may not be a major reservoir or vector of infections. This is a proof of concept that predictive models based on previously known respiratory viral diseases are vain.

Author contributions Conceived and designed the experiments: DR and PC. Contributed materials/analysis tools: all authors. Analyzed the data: PC, JCL, AM, DR. Wrote the paper: PC and DR.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Ethics All data have been generated as part of the routine work at Assistance Publique-Hôpitaux de Marseille (Marseille university hospitals), and this study results from routine standard clinical management. The study was a retrospective analysis of patients' biological and registry data issued from the hospital information system, which is an authorized healthcare database. Access to the registry was approved by the data protection committee of our institution (Assistance publique des hôpitaux de Marseille, APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73.

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