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ABSTRACT

Objectives

SARS-CoV-2 has emerged among humans in China since December 2019 and has now spread outside this country. Chinese reports have suggested that children are less affected than adults, but scarce data have been reported so far and no data are available for France.

Methods

We analyzed the number of SARS-CoV-2 RNA tests of respiratory samples sent to our laboratory between end of February and mid-March 2020. Clinical symptoms and mortality rate were analyzed among SARS-CoV-2-positive patients sampled in Marseille university hospitals.

Results

Between February, 27th and March 14th, 2020 we performed SARS-CoV-2 RNA testing on respiratory samples from 4,050 individuals and diagnosed 228 cases. Among 99 documented cases, 2 (both >85 year-old and admitted with acute respiratory distress) died (2.0%), while children in our series were majoritarily asymptomatic. We observed an increasing incidence (7.4-fold rise) of positive tests between 1-5 year and 45-65 years, then a decrease >65 years. The proportion of positive subjects was significantly lower among children whose age was 0-1 year (0%), 1-5 years (1.1%) and 5-10 years (3.6%) than among subjects >18 years (6.5%). In addition, SARS-CoV-2-positive children exhibited viral loads that do not differ significantly compared to those of adults, proportion of high viral loads (Ct<19) being 0%, 0% and 9% for subjects <10 years, between 10-18 years and >18 years, respectively.

Conclusion

Thus, children and adolescents accounted for a low proportion of SARS-CoV-2 infections and did not exhibit higher viral loads than adults, and they may not contribute significantly to the virus circulation.

Key words: SARS-CoV-2; Covid-19; children; transmission; viral load

TEXT

INTRODUCTION

A new coronavirus, named SARS-CoV-2, has emerged in humans since December 2019 in the region of Wuhan in China [1]. It is a new causative agent of respiratory disease, named Covid-19, which can include pneumonia and be life-threatening. This virus adds to the 6 coronaviruses previously detected in humans, including human coronaviruses (HCoV) 229E, OC43, HKU1, and NL63, which circulate worldwide and are common [2,3], as well as the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) that circulated temporarily and mainly in Asia in 2003 [4] and the Middle-East respiratory syndrome-associated coronavirus (MERS-CoV) that remained located in the Middle East [5]. SARS-CoV-2 has most often generated great fears in Western countries, first even before the detection of the first cases in early February 2020, then from the time when the number of cases increased significantly and the first deaths were observed. In France, the first case was diagnosed on January 24, 2020 and on March 14, 2020 the number of confirmed cases was 3,661 and the number of deaths was 79 [6]. 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.

Among the parameters associated with infectivity of respiratory viral infections are the duration of viral shedding and the 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 [7,8]. In contrast, reports available from China have suggested that children are less affected than adults by the SARS-CoV-2 [9,10]. No study has focused on SARS-CoV-2 among children in France. We describe here the number of infections and the viral load

measured in children comparatively to those in adults for cases tested in our French institution.

MATERIALS AND METHODS

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 [11,12]. We assessed the weight of nasopharyngeal secretions collected on swabs by measuring the weight of 10 swabs before and after collection of nasopharyngeal secretions, to be able to report the number of copies per g of secretions. Mean weight was 220±35 mg. The number of copies was calculated using a synthetic RNA corresponding to the PCR system target region. Then, this number was multiplied by 23, taking into account that the volume used for viral RNA extraction was 200 µL compared to the total volume of Virocult swab fluid of 1 mL (factor of 5) and converting the number of RNA copies per swab to that per g (factor of 4.5). To compare the proportion of positive tests in the differents age groups we considered the period starting from the first diagnosis of SARS-CoV-2 infection, 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 determined the mortality rate among SARS-CoV-2-positive patients sampled in Marseille university hospitals since we had for them information on clinical presentation and follow-up. Statistical analyses were performed with the OpenEpi online tool

(https://www.openepi.com/Menu/OE_Menu.htm) using the Chi-square or Fischer tests for the comparisons of proportions and the Anova test for the comparison of mean values. A p value of 0.05 was considered as the significance threshold.

RESULTS

We implemented the testing by real-time RT-PCR of SARS-CoV-2 RNA in respiratory samples at the clinical diagnosis laboratory of our institute since the 29th of January, 2020. This laboratory is opened 7/7 and 24/24 and is the single one that diagnoses infectious agents for the four university hospitals of Marseille, Southeastern France. In the setting of the emergence of SARS-CoV-2 in China that further reached countries outside the Asian continent, it has been the first laboratory to diagnose SARS-CoV-2 infections in the Southeastern region, Provence-Alpes-Côte d'Azur, which accounts for approximately 7% of the French population. Between the 29th of January, 2020 and the 14th of March, 2020, we tested 6,721 respiratory samples from 5,645 patients for the presence of SARS-CoV-2 RNA. Regarding the 17-day period from the 27th of February when we began to have positive tests until the 14th of March, we tested 4,766 respiratory samples from 4,050 patients for the presence of SARS-CoV-2 RNA and found that 228 (5.6%) were positive.

During this period of time, tests were performed for people with a broad range of age as 15% of the 4,050 subjects were younger than 10 years and 18% were older than 65 years (**Figure 1a**). Mean age (± standard deviation) of these patients was 40±25 years. The proportion of children was significantly lower among SARS-CoV-2-positive subjects than among those SARS-CoV-2-negative. Thus, 4% and 8% of positive subjects were children younger than 10 and 15 years, respectively, whereas these proportions were 15% and 19%, respectively, among negative subjects [10 and 17 out of 228 versus 578 and 735 out of 3,822 ($p<10^{-5}$ for both groups of age)] (**Figure 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 0-1 years (0/110; 0%), 1-5 years (3/285; 1.1%) and 5-10 years (7/194; 3.6%) than among subjects older than 18 years (208/3,205; 6.5%) ($p<10^{-3}$, $p<10^{-3}$ and p=0.074, respectively) (**Figure 1c**).

Besides, among SARS-CoV-2-positive subjects, viral loads did not differ significantly between children or adolescents and adults. Indeed, the mean cycle threshold (Ct) value was 24.8±4.6 overall while it was 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 (**Figure 2a**). We further considered particularly the Ct values lower than 19, 23 and 26 as we determined that they corresponded to viral loads greater than 10 billions, 1 billion and 100 millions RNA copies per g of nasopharyngeal secretions. The proportion of Ct values lower than 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 (**Figure 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 younger than 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 older than 85 years. The three other patients older than 85 years in our series 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.

DISCUSSION

We report in the present work for the first time in France based on the testing of 4,050 patients and a series of 228 diagnosed SARS-CoV-2 infections that children and adolescents represented a low proportion of these infections, were majoritarily asymptomatic, and exhibited viral loads that did not differ significantly with those among adults, and even tended to be lower. Regarding mortality, we observed that 2 of 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 [9,10]. Thus, children <10 years and aged of 10-19 years represented 1% each of 72,314 Covid-19 cases in a large study [9], and few pediatric cases have been reported overall [10,13]. Consequently, it was questioned if children may be less susceptible to Covid-19 [10]. In addition, infections in children were found to be associated with milder clinical symptoms and with faster recovery compared to those in adults [14–16]. These epidemiological and clinical patterns are similar to those previously described for SARS-CoV and MERS-CoV infections [17–20]. 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 [16]. Lu et al. reported 171 cases who represented 12% of 1,391 children with known contact with confirmed or suspected SARS-CoV-2 infections [21]. 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 [13]. Household exposure was found in seven cases. In another series that included 31 children whose age ranged between 1.5 and 17 years, 94% of the cases were in family clusters, and 39% were asymptomatic [22]. Liu et al. described that 1.6% (n=6) of 366 hospitalized children with respiratory infections were SARS-CoV-2 positive [23]. 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 [24]. 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 [25]. 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.

Acknowledgments

None.

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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Conflicts of interest

The authors have no conflicts of interest to declare. 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.

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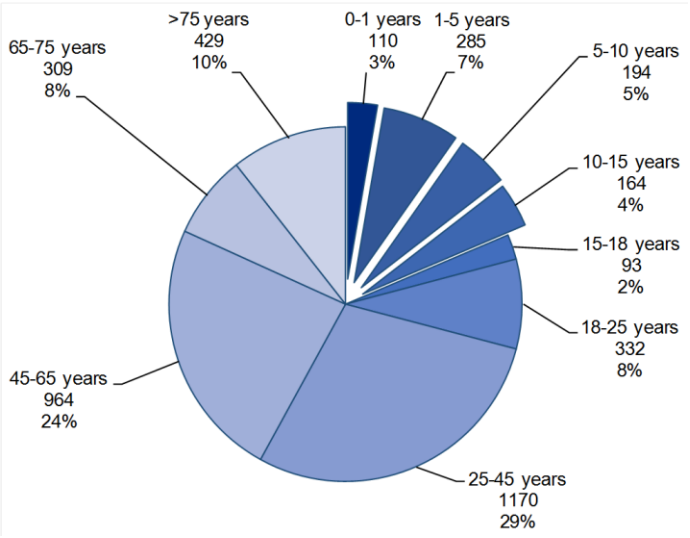
FIGURE LEGEND

Figure 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).

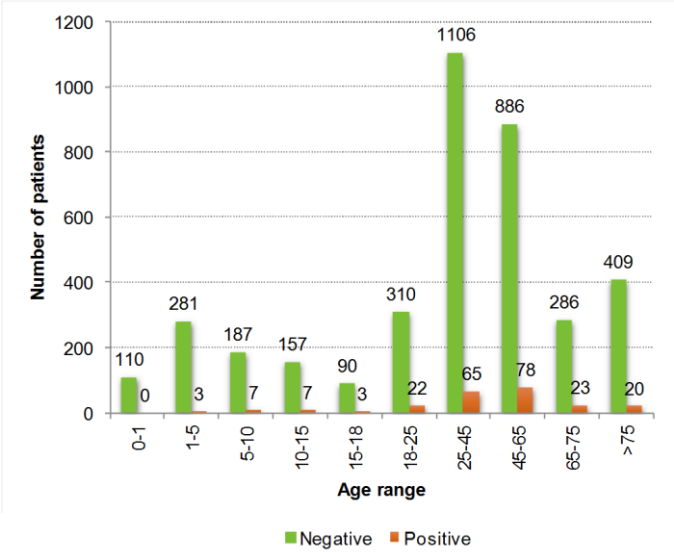
Figure 2. Dot plot representation of the relationship between the age of SARS-CoV-2-positive subjects and the Ct values of PCR tests (**a**) and distribution of Ct values of PCR according to groups of age (**b**).

Fig. 1

a.


0-1 years
110
3%
1-5 years
285
7%
5-10 years
194
5%
10-15 years
164
4%
15-18 years
93
2%
18-25 years
332
8%
25-45 years
1170
29%
45-65 years
964
24%
65-75 years
309
8%
>75 years
429
10%

b.


Number of patients
1200
1000
800
600
400
200
0
110
0
281
3
187
7
157
7
90
3
310
22
1106
65
886
78
286
23
409
20
0-1
1-5
5-10
10-15
15-18
18-25
25-45
45-65
65-75
>75
Age range
Negative
Positive

c.

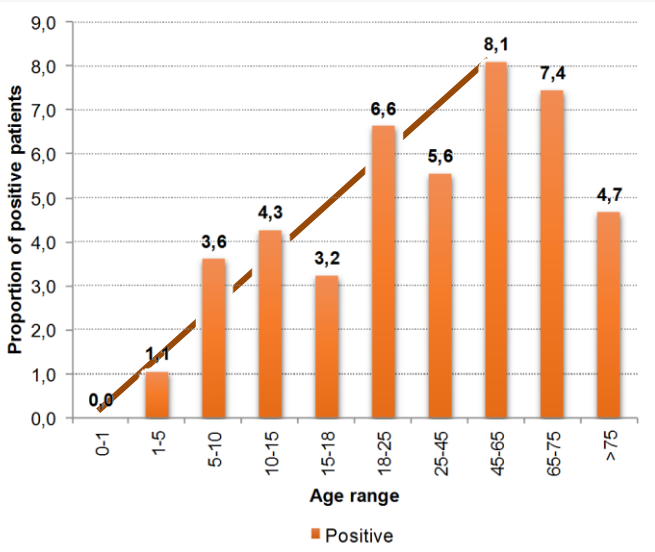
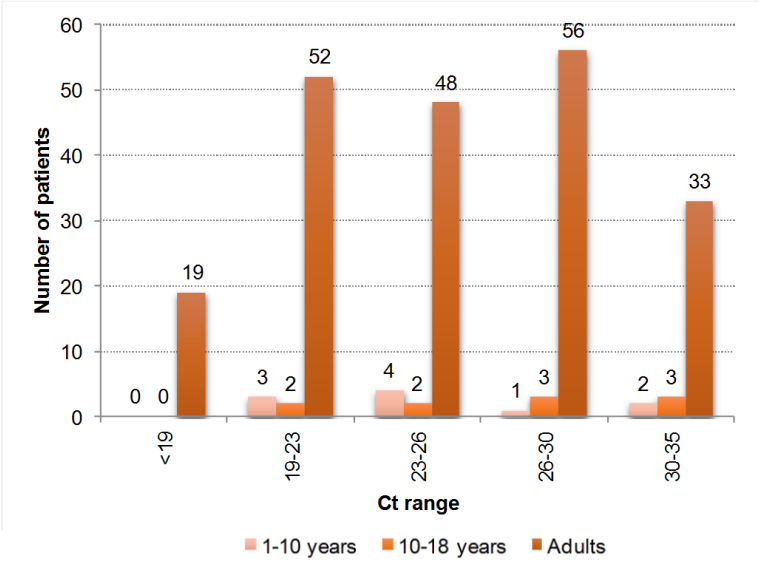

Proportion of positive patients
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
0,0
1,1
3,6
4,3
3,2
6,6
5,6
8,1
7,4
4,7
0-1
1-5
5-10
10-15
15-18
18-25
25-45
45-65
65-75
>75
Age range
Positive

Fig. 2

a.


Ct values
Age
15
20
25
30
35
0
10
20
30
40
50
60
70
80
90
100

b.


Number of patients
Ct range
60
50
40
30
20
10
0
0 0
19
3 2
52
4 2
48
1 3
56
2 3
33
<19
19-23
23-26
26-30
30-35
1-10 years
10-18 years
Adults