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Abstract

Purpose: Clinical data suggest that during the current COVID-19 pandemic children are less prone than adults to SARS-CoV-2 infection. Our purpose was to determine the frequency of SARS-CoV-2 in children *vs.* adults during the 2020 pandemic in Warsaw, Poland and to investigate whether RSV and/or influenza A/B infections were associated with SARS-CoV-2 infections.

Methods: We present results of RT-PCR tests for SARS-CoV-2 performed in Warsaw, Poland. Some of the pediatric subjects were also PCR-tested for RSV, and A and B influenza. We compared the test results from the four groups of symptomatic and asymptomatic subjects: 459 symptomatic pediatric patients (children 0-18 years old), 1774 symptomatic adults, 445 asymptomatic children, and 239 asymptomatic adults.

Results: 3.26% (15/459) of symptomatic pediatric patients were positive for SARS-CoV-2 in contrast to 5.58% (99/1774) of symptomatic adults (p=0.0448). There were no SARS-CoV-2 positive cases in the group of asymptomatic children (0/445) and two positive cases in the group of asymptomatic adults (2/239), i.e. 0.83%. In the group of symptomatic pediatric patients 17.14% (6/35) (p=0.0002) were positive for RSV, 8.16% (4/49) were positive for influenza A, and 2.04% (1/49), thus 10.20% (5/49) (p=0.0176) for influenza A/B.

Conclusion: Children were less prone to SARS-CoV-2 infection than the adults during the COVID-19 pandemic in Warsaw. Higher percentage of symptomatic children was infected with RSV or influenza A/B than with SARS-CoV-2. This suggests a necessity for the testing for all these viruses for an early identification and isolation of SARS-CoV-2-positive patients for an ensuing 2020 autumn return of COVID-19.

Key words: COVID-19, SARS-CoV-2, 2020 pancemic, pediatric patients

Introduction

Severe Acute Respiratory Syndrome coronavirus-2 or SARS-CoV-2 emerged in Wuhan in Hubei province in China in December 2019 and rapidly had propagated all over the world causing the COVID-19 pandemic, declared by WHO on March 11 (WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020; https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020). The pandemic and number of cases are still unfolding in many countries while we report this data. In Poland, the first case was reported on March 4th, 2020. During the first two weeks of the epidemic, the whole Masovia area, including the Polish capital, Warsaw, became one of the major infected areas in Poland, with 3581 cases by 1st June 2020.

There is a growing body of evidence from different countries with COVID-19 pandemic that children are less frequently infected with SARS-CoV-2. Children are mostly asymptomatic or experience much milder symptoms than the adults (Balasubramanian et al., 2020; Cristiani et al., 2020; Dong et al., 2020; Jiatong et al., 2020; Lee et al., 2020; Marraro and Spada, 2020 Morand et al., 2020; Zimmermann and Curtis, 2020), although a low percentage of the

infected children developed the pediatric multi-system inflammatory syndrome (Viner and Whittaker, 2020). Severe (2-3%) or critical illness (0.61%) have been reported in pediatric patients analyzed in two systematic reviews and meta-analysis of data from several countries (Liguoro et al., 2020; Dhir et al., 2020). The purpose of our study was to establish if a lower frequency of infection, and lesser severity in children were also true for the 2020 COVID-19 pandemic in Warsaw, Poland.

Material and Methods

Patients

Analyses were performed on two main groups of patients: children – from 1 month to 18 years old, and adults – over 18 years old. Patients from two Warsaw hospitals were enrolled: the Pediatric Teaching Clinical Hospital of Medical University of Warsaw and the Hospital for Infectious Diseases (Warsaw, Poland). Each of these two groups was divided into two subgroups: symptomatic (separately children and adults) and asymptomatic (separately children and adults). Patients presenting with fever, respiratory tract infection syndromes, gastrointestinal symptoms, dyspnea, cough, chest pain, and seizures were included in the symptomatic groups. Two asymptomatic groups were: 1. asymptomatic children who underwent screening for SARS-CoV-2 at the admission for planned hospitalisation unrelated to COVID-19, 2. asymptomatic legal guardians of the asymptomatic children and medical staff involved in contact tracing.

SARS-CoV-2 analysis

Throat or nasopharynx swabs were obtained from the symptomatic and asymptomatic subjects. Immediately after collection, the swabs were placed in viral preservation medium, transported to laboratory in temperature 2-8°C and stored in such conditions until processing. Total RNA was extracted from 140 µl specimen with manual centrifuge column isolation kit (Viral RNA Isolation Kit, ZJ Bio-Tech C, China) according to manufacturer's instruction. Extraction volume of 60 µl was obtained for each sample. A 5 µl aliquot of RNA was used for real-time RT-PCR, which targeted simultaneously 3 genes specific for SARS-CoV-2: gene E, N, and ORF1ab (Liferiver Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit, Shanghai ZJ Bio-Tech C, Shanghai, China). Real time PCR was performed upon CFX96 ™ Real-Time PCR Detection System (Bio-Rad, USA). The specificity of the test is 98.1 %, analytical sensitivity $1x10^3$ copies/ml. The protocol was performed according manufacturer's instruction. Real-time RT-PCR was performed under the following conditions: 45°C for 10 min and 95°C for 15 min, followed by 45 cycles of amplification at 95°C for 15 sec and 60°C for 1 min. Criteria for judging results: CT value < 43 positive; 43 ≤ CT value ≤ 45 suspicious positive and ≥ 45 negative. The positive should meet for all 3 genes tested. Internal control was added into extraction mixture on isolation stage to monitor the whole process. Positive and negative control was included in each run of amplification.

RSV and Influenza A/B analysis

Selected samples of pediatric symptomatic patients were also tested for the presence of RSV and Influenza A/B virus with qualitative real-time PCR test Xpert® Xpress Flu/RSV (Cepheid, USA). 35 patients were tested for RSV and 49 patients for influenza A and B.

Nasopharyngeal specimen were collected from selected paediatric symptomatic patients. After collection the swab were placed in universal transport medium (UTM, Copan Diagnostics). The samples were tested immediately after collection with qualitative real-time PCR test Xpert® Xpress Flu/RSV (Cepheid, USA) according manufacturer's instruction on GeneXpert I apparatus. The volume of 300 µl was transferred to the cartridge. Isolation and amplification stage was done automatically by the instrument. Each test was equipped in internal control which monitored whole process. External positive and negative controls were tested with each new applied lot of Xpert Xpress Flu/RSV. The RT-PCR test targets few genes of examined viruses: genes encoding matrix protein, PB2 and PA for Flu A, genes encoding matrix protein and non-structural protein for fly B, genes encoding nucleocapsid of RSV A and RSV B. Sensitivity of the test is estimated on 99%, specificity on 98%. Results were presented as the percentage of all patients in the given group.

Statistical analysis

The data from the adults and children, as well as RSV positive, and influenza A and B positive cases within the selected samples in the symptomatic children group, were analyzed by Chi-square test. The P-value <0.05 was considered as statistically significant. We also analyzed data using the Fisher test.

Results

SARS-CoV-2 RT-PCR tests were performed in two centers in Warsaw, Poland, during 3 months between March and May 2020, which corresponded to the first three months of COVID-19 epidemics in Poland. Patients were divided into four groups.

1. symptomatic pediatric patients (n = 459),

2. symptomatic adult patients (n = 1774),

3. asymptomatic children (n = 445),

4. asymptomatic adults (n = 239).

The age strata in each group are showed in Suppl. Materials 1 section.

The tests showed 3.26% (15/459) positive cases among the symptomatic pediatric patients, 5.58% (99/1774) among the symptomatic adults, none in the the asymptomatic children (0/445), and 0.83% (2/239) in the asymptomatic adults.

The statistical analysis performed with the chi-square test has shown significantly (p=0.0448) lower incidence of SARS-CoV-2 infection in the symptomatic pediatric population than in the symptomatic adults. This trend was also observed in the asymptomatic groups, however the result was not stiatistically significant (p=0.0533) (Fig. 1). The Fisher test analysis gave similar results (Fig. 1 Suppl. Materials).

The age of 15 positive paediatric symptomatic patients, uniformly covering the whole period between 0.6 and 14.1 years of age, was as follows: 0.6; 06; 0.8; 1.0; 1.8; 4.2; 5.0; 7.3; 9.8; 10.0; 11.0; 11.3; 13.0; 14.0 and 14.1 years.

The analysis of the symptomatic non-SARS-CoV-2 patients showed that 17.14% (6/35) (p=0.0002) of children were positive for RSV, 8.16% (4/49) for influenza A, and 2.04%

(1/49) for influenza B; thus 10.20% (5/49) (p=0.0176) were positive for A and B influenza together (the same 49 patients were tested for influenza A and B). These data were also analyzed using two statistical tests: chi-square (Fig. 2) and the Fisher test (Fig. 2 Suppl. Materials). In other words, 17.14% of pediatric patients negative for SARS-CoV-2 were positive for RSV, and 10.20% for influenza A/B. Thus, statistically significantly higher number of pediatric symptomatic patients were positive for either RSV or influenza A/B than for SARS-CoV-2. There was also a single pediatric case of pertussis (whooping cough) diagnosed based on the clinical symptoms. These data show that during the first three months (March-May 2020) of the COVID-19 pandemic in Warsaw, Poland, significantly less pediatric patients were infected with SARS-CoV-2 than RSV and influenza together. Thus, children were more likely to be infected with RSV or influenza A/B than with SARS-CoV-2.

Disussion

Our study showed that children presenting symptoms suggestive of COVID-19 and requiring hospitalisation or an emergency assessment, had significantly lower SARS-CoV-2 infection than hospitalised adults. This data are consistent with the previous studies from different countries during the 2020 COVID-19 pandemic. Asymptomatic, mild, and moderate symptoms were observed in more than 90% of all COVID-19 positive children, severe and critical cases were observed in 5.9% of children in comparison to 18.5% in adults (Dong et al., 2020). Cristiani et al. (2020) report that among the total number of 44,672 positive cases, the Chinese Centre of Disease Control and Prevention report showed only 416 pediatric cases in 0–9 years age group (0.9%) with no fatalities and 549 cases in 10–19 years age group (1.2%) with 1 fatality (0.2%). Brodin (2020) reports Korean data showing that 6.3% of all positive cases were children under 19 years of age. (reviewed also by Balasubramanian et al., 2020; Jiatong et al., 2020; Lee et al., 2020; Marraro and Spada, 2020; Morand et al., 2020; Zimmermann and Curtis, 2020).

Interestingly, SARS-CoV-1 infection rate during 2002-2003 epidemic had shown similar characteristics regarding children *vs*. adults in China, and other countries (Cao et al., 2020; Dong et al., 2020; Sørensen et al., 2006; Stockman et al., 2007; Zhao et al., 2006). Therefore, it seems that children are more resistant to both SARS-CoV-1 and SARS-CoV-2 infection.

There are several factors that might explain this phenomenon. First of all, children, because the milder symtomps of SARS-CoV-2 infection are tested less frequently than the adults. There may be also an input from the environmental/social factors. Children may be better protected and sheltered against the virus during the pandemic than the adults, and the rate of children infection may be influenced by the quality of the parental care (Zimmermann and Curtis, 2020).

Alternatively, some endogenous factors may play a role. They may include a different age-dependent expression and/or distribution of ACE2 receptor for SARS-CoV-1 and SARS-CoV-2 (suggested by Li et l., 2003; Lovren et al., 2008; Tai et al., 2020; Tipnis et al., 2000; Walls et al., 2020; Wysocki et al., 2010; Brodin, 2020; Miri et al., 2020; Xu et al., 2020). Indeed, a lower level of ACE2 expression in the nasal epithelial cells of children, especially the youngest children, was confirmed by a recent study of Bunyavanich (2020) suggesting

that this can be a reason for a lower infectability of children with SARS viruses. Nevertheless, the data about the impact of ACE2 expression pattern are still fragmentary.

One of the reasons for the severity of the COVID-19 is the over-reactivity of the immune response in the lungs, where the alveolar macrophages induce the cytokine storm (Merad and Martin 2020). In children, the immune system is immature (Kloc et al., 2020), thus aggressive cytokine response is rarely seen (Simon et al., 2015) resulting in milder COVID-19 symptoms. The severity of COVID-19 in children was recently reviewed by two meta-anlyses (Liguoro et al., 2020; Dhir et al., 2020). Even though the reviews had large cohorts (7480, 4857 patients), the severity of illness was described only in 20-34% of all patients (1475 and 1666 respectively). In the review by Liguro et al. (2020), 12% of neonates were severely ill. In the second review, neonates were not included in the study. The estimated reported mortality in these two studies was 0.08% and 0.1% of analyzed patients. Higher morbidity and mortality ratios were described by the ptbnet COVID-19 study Group who collected data from 25 European countries during the peak of the pandemic in Europe in April 2020 (Gotzinger et al., 2020). Out of 582 patients, 8% required ICU admission, and 4% required mechanical ventilation. The case fatality rate was 0.69%, and age younger than 1 month, male sex, signs or symptoms of lower respiratory tract infections at presentation, and the presence of pre-existing medical conditions were associated with ICU admission.

Another plausible explanation might be that the children who attend day-care, kindergarten, and school and are frequently exposed to the respiratory viruses, develop some basal level of the antibodies, which give cross protection against SARS-CoV-2 (Schuez-Havupalo et al., 2020).

In the case of newborns, and very young babies, a part of explanation can be that they are protected either by antibodies transferred during pregnancy through the placenta from mother (Jackson and Nazar, 2006; Palmeira et al., 2011, Brodin, 2020). or the compounds, such as the Lactoferrin, a globular glycoprotein present in the breast milk that have the antimicrobial and immunomodulatory roles in protecting the newborn from the various infections (Telang 2018), including the necrotizing enterocolitis (Sherman, 2013) and SARS (Lang et al., 2011).

A plausible hypothesis for a lower number of positive cases among children is that a high percentage of them might have been protected by the cross-immunity conferred by infections with other viruses including human coronaviruses (e.g. Brodin, 2020). Also, the vaccination against other infectious disesases may stimulate the cross-immunity. The young patients were probably better protected because of freshly acquired immune memory (Miri et al., 2020). Of special interest can be the so-called trained immunity caused by vaccinations with live viruses, such as OPV, anti-measles vaccine, or mycobacteria, including BCG (Netea et al., 2020; Chumakov et al., 2020). Moreover, the authors also hypothesized that if SARS-CoV-2 will mutate, and the specific vaccine will be weak or ineffective, the OPV may be the only real solution in the future (Chumakov et al., 2020). In this case, again, recent vaccination might give a better protective effect.

Interestingly, the PCR tests of children included in our study showed that, within the first three months of the COVID-19 pandemic in Warsaw, the children had significantly higher risk for RSV or influenza A/B than for SARS-CoV-2 infection. If this bias toward RSVand influenza A/B infection persists for the next seasonal peak of flu, and respiratory infections, the physicans in Masovia area should keep in mind that the common flu/respiratory infections

are much more frequent than COVID-19. However taking into account the risk of spreading COVID-19, authorities should perform a routine broad testing for all these viruses. The data from many countries show that an early detection of SARS-CoV-2 infection is one of the most effective strategies of pandemic control.

In addition, our study did not show any case of the co-infection between SARS-CoV-2 and other viruses tested, or between RSV and Influenza A/B. Reina and Dueñas (2019) showed that the RSV and influenza co-infection occurred in 4.8% of the total studied RSV cases. It is possible that the limited number of pediatric patients, which were tested for RSV and Influenza A/B in our study is responsible for this discrepancy. In contrast, some studies have reported the presence of coinfections with SARS-CoV-2 and other respiratory tract pathogens, both in children and adults. In most cases studied, the researchers have reported coinfection with others than SARS-CoV-2 coronaviruses (HKU1, NL63, 229E, OC43), influenza, and parainfluenza viruses, RSV, rhinoviruses-enteroviruses, human methapneumoviruses, adenoviruses, and *Mycoplasma pneumoniae*. Nevertheless, the coinfection rates were low and ranged between 0.1% - 3%, and the studies did not clearly assess whether they influenced the course of infection with SARS-CoV-2 or not. In general, the clinical course was mild, ending with full recovery (Nowak et al., 2020; Wee et al., 2020; Blasco et al., 2019).

We want to emphasize some limitations of our study. First, the number of examined patients was not very high. We wanted to present our results as quickly as possible to spread the information included in this article before the onset of the expected autumn/winter wave of the pandemic, and for this reason, we have chosen to describe the results of the first 3 months of the pandemic. It allows to draw conclusions, but further studies should be performed to verify whether the observed perecentage of children vs. adults infections are similar during the whole period of the pandemic period without the anti-SARS-CoV-2 vaccination.

Conclusions

The results of our study are consistent with the previous data showing that SARS-CoV-2 infection in children occurs less often than in the adults. Routine testing for SARS-CoV-2 is crucial for the control of the pandemic, and discerning between COVID-19 and common seasonal respiratory infections.

Declarations:

Funding

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Availability of data and material

All data and material is freely available.

Ethical Approval

No ethical approval was obtained because this study did not involve a clinical evaluation, did not involve laboratory animals and invasive procedures.

Authors` contributions

EK, AL, SL, MK and JZK designed the study. AZ, MW, DK performed tests, MK and JZK wrote the manuscript, EK, AZ, MW, DK, EP, KB, AL, SL, CK, AH and JZK analysed results, AL and SL performed the statistical analysis, EK, AZ, MW, DK, EP, AL, SL, CK, AH, MK and JZK edited the manuscript.

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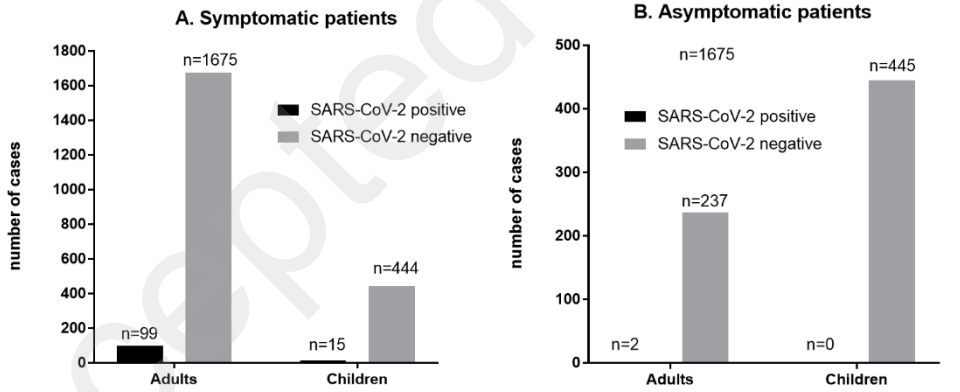
Visualistaion of figures and their legends



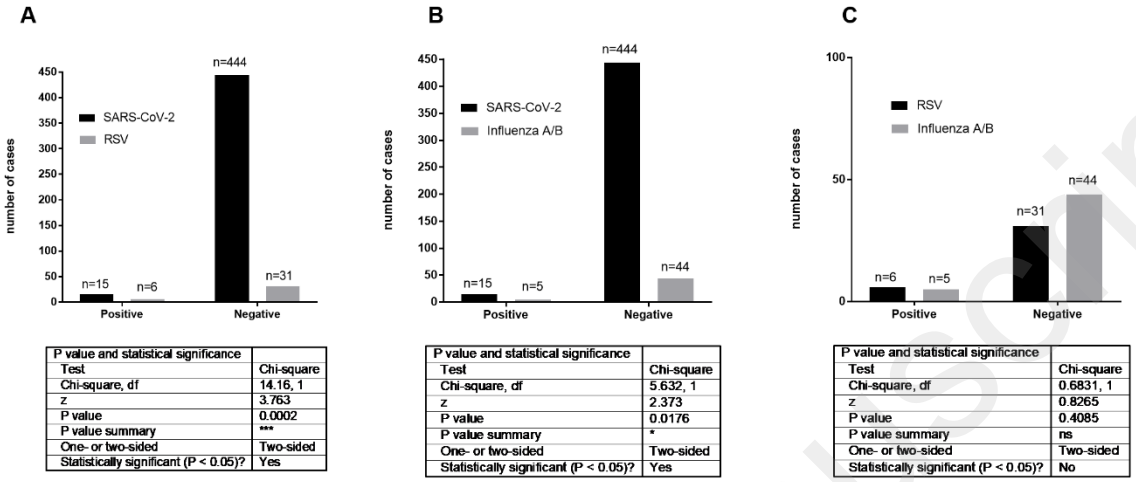
P value and statistical significance	
Test	Chi-square
Chi-square, df	4.026, 1
z	2.006
P value	0.0448
P value summary	*
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes

P value and statistical significance	
Test	Chi-square
Chi-square, df	3.735, 1
z	1.933
P value	0.0533
P value summary	ns
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	No

Fig. 1

The statistical analysis by chi-square test of the results obtained in: A – symptomatic patients, B – asymtomaptic patients.

Fig. 2



P value and statistical significance	
Test	Chi-square
Chi-square, df	14.16, 1
z	3.763
P value	0.0002
P value summary	***
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes

P value and statistical significance	
Test	Chi-square
Chi-square, df	5.632, 1
z	2.373
P value	0.0176
P value summary	*
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes

P value and statistical significance	
Test	Chi-square
Chi-square, df	0.6831, 1
z	0.8265
P value	0.4085
P value summary	ns
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	No

The statistical analysis by chi-square test of the results obtained in: A – SARS-CoV-2 vs. RSV tests; B – SARS-CoV-2 vs. influenza A/B; C- RSV vs. influenza A/B.

Supplementary Materials 1

Age strata in the four groups of patients studied:

1. symptomatic pediatric patients (n = 459): 0-5 years: 37.6%; 5-10 years: 29.1%; 10-15 years: 29.1%; 15-18 years: 4.2%

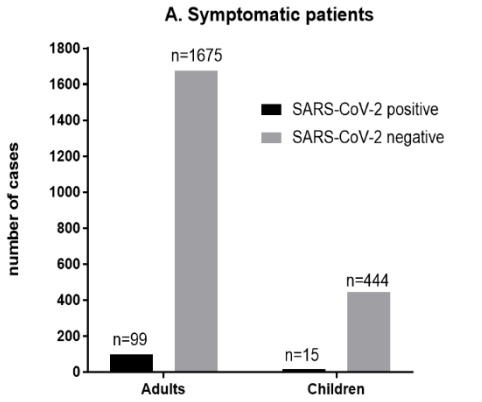
2. symptomatic adult patients (n = 1774): 18-30 years: 26.0%; 30-40 years: 21.0%; 40-50 years: 27.5%; 50-60 years: 7.5%; 60-70 years: 10.5%; 80 and more years: 7.5%.

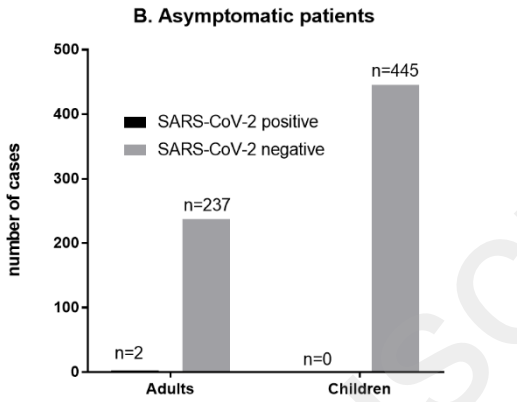
3. asymptomatic children (n = 445): 0-5 years: 45.0%; 5-10 years: 25.0%; 10-15 years: 17.5%; 15-18 years: 12.5%

4. asymptomatic adults (n = 239): 18-30 years: 24.0%; 30-40 years: 15.0%; 40-50 years: 26.5%; 50-60 years: 17.5%; 60-70 years: 8.5%; 80 and more years: 8.5%.

Supplementary Materials 2

Fig. 1 Suppl. Materials



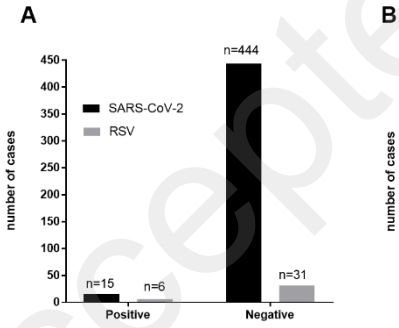


P value and statistical significance	
Test	Fisher's exact test
P value	0.0435
P value summary	*
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes

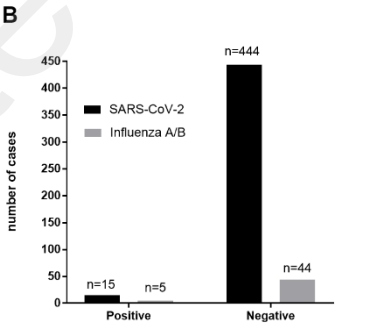
P value and statistical significance	
Test	Fisher's exact test
P value	0.1218
P value summary	ns
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	No

The statistical analysis by Fisher test of the results obtained in: A – symptomatic patients, B – asymtomaptic patients.

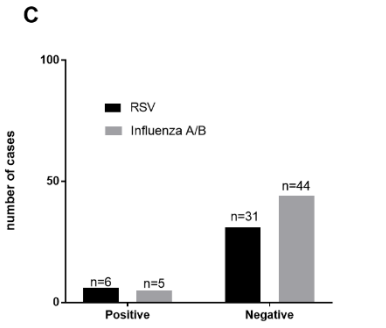
Fig. 2 Suppl. Materials



P value and statistical significance	
Test	Fisher's exact test
P value	0.0027
P value summary	**
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes



P value and statistical significance	
Test	Fisher's exact test
P value	0.0346
P value summary	*
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	Yes



P value and statistical significance	
Test	Fisher's exact test
P value	0.5189
P value summary	ns
One- or two-sided	Two-sided
Statistically significant (P < 0.05)?	No

The statistical analysis by Fisher test of the results obtained in: A – SARS-CoV-2 vs. RSV tests; B – SARS-CoV-2 vs. influenza A/B; C- RSV vs. influenza A/B.