

THE EVOLUTION OF SARS-COV-2 INFECTION IN THE PEDIATRIC WARD AT ST. ANDREW'S COUNTY EMERGENCY CLINICAL HOSPITAL CONSTANTA

Ramona-Anca Sterian¹, Sergiu Chirila², Lavinia Daba²

¹ County Clinical Emergency Hospital of Constanta

² Faculty of Medicine, University "Ovidius" of Constanta

Ramona-Anca Sterian

email: anca.rizea93@yahoo.com

ABSTRACT

In the period April 24, 2020 – December 31, 2021 at Saint Andrew's County Emergency Clinical Hospital's Molecular Biology Laboratory were performed 2856 RT-PCR tests on childrens. This method consists of two steps: extraction and amplification. RT-PCR is the golden standard to diagnose infection with Covid-19.

The tests were performed on patients of both genders and under 18 years old. Statistics show that both male and female patients were affected by Sars-Cov-2 in relatively equal proportions: in the first year (male 46% and female 54%), followed by the next year (male 48% and female 52%).

The results concluding, that during the study, in 2020, 6.98% of the total number of tests came out positive, 92.32% came out negative, 0.70% inconclusive. In 2021, 4.21% of the total number of tests came out positive, 95.56% came out negative, 0.23% inconclusive.

This study highlights the situation of Covid-19 cases encountered at childrens from the Pediatric section diagnosed in Constanta, Romania.

Keywords: Covid-19, pediatric, children, DNA

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID-19 is a serious threat to healthcare system all over the world. Most of the countries have declared restrictive measures, such as lockdown or stay-at-home orders, in order to contain the pandemic at the local level by reducing individual contact. At this moment, the world has already exiting the lockdown, including Romania (1).

Based on the WHO, there have been 548,990,094 confirmed cases worldwide, including 6,341,637 deaths. COVID-19 cases with little to no symptoms that never got tested

or confirmed positive are not included (2).

SARS-CoV-2 is an enveloped, non-segmented, positive sense RNA virus that is included in the sarbecovirus, ortho corona virinae subfamily which is broadly distributed in humans and other mammals (3). Structurally, SARS-CoV-2 has four main structural proteins including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also several accessory proteins (4).

Diagnosis of SARS-CoV-2 infection is currently based on real-time reverse transcriptase-polymerase chain reaction (RT-PCR) performed on either nasopharyngeal swabs (NPS) or

oropharyngeal swabs (OPS). RT-PCR represents the first-line diagnostic modality to test patients and otherwise asymptomatic population for COVID-19 (5).

Low incidence of the disease among the pediatric population it is a topic of great interest for current and future research. The literature mentions several theories.

It's been shown that angiotensin-converting enzyme 2 receptors would be the gateway to human cells for some coronaviruses, including SARS-CoV-2. Also, the low expression of these receptors in the respiratory tract of children could be the reason why they are less vulnerable to SARS-CoV-2. Another hypothesis is the impairment of endothelial that occurred before the infectious aggression, which facilitates the spread of infection and the inflammatory process.

Because in the pediatric population there is a low incidence of diseases recognized as causing endothelial damage (for example cardiovascular diseases, diabetes) it is estimated that the spread of the inflammatory process would limit the conditions of a normal endothelium.

Many viral infections that childrens have in the first upcoming years of life, as well as the administration of vaccines stimulates their immune response. The innate immune system plays an important factor in protection against SARS-CoV-2 (6).

In light of these events, this study aims to highlight:

- Number of cases at children diagnosed with the help of St. Andrew's County Emergency Clinical Hospital's Molecular Biology Laboratory, Romania, Constanta
- Prevalence of Covid-19 children's gender-wise
- Average age of infection in pediatric section.

Materials and Methods

Reported COVID-19 cases among children between April 24, 2020 – December 31, 2021 were diagnosed and some of them hospitalized in the pediatric section at St. Andrew's County Emergency Clinical Hospital Constanta.

Inclusion criteria:

- Specific symptoms like: high temperature,

cough, lost of taste or smell, shortness of breath, a headache, sore throat, diarrhea, etc.

- Non-specific symptoms: wheeze, coryza, respiratory distress, dehydration, conjunctivitis, and rash.
- Under age patients <18

The study took place between April 24, 2020 – December 31, 2021 in the Pediatric Section at St. Andrew's County Emergency Clinical Hospital Constanta on a total number of 2856 patients tested.

At early signs or symptoms of COVID-19, the children along side with a parent or a guardian tutor, suspects of COVID-19, were hospitalized at Pediatric section of St. Andrew's County Emergency Clinical Hospital Constanta, in order to diagnose with the method RT-PCR (Real-Time Polymerase Chain Reaction) and following a specialized treatment. In other cases, which are not severe, they go home and take a specialized treatment.

The study uses the following markers, such as: age, gender, probable causes of infections.

PCR (polymerase chain reaction) is a technique used for amplification of DNA strands. Is typically considered the gold standard in the analysis of nucleic acid. When working with RNA as a starting material, however, RT-PCR (reverse transcription-PCR) is used to obtain DNA from the RNA template. This is done by converting RNA into cDNA (complementary DNA) using RT-PCR, and then amplifying the cDNA into DNA via standard PCR (7).

This method follows two steps:

- Extraction of viral RNA
- Amplification of viral RNA

Principle of the procedure: A sample is collected from the parts of the body where the COVID-19 virus gathers, such as a person's nose or throat.

Nucleic acid extraction was accomplished by the work of four automatic devices available at the St. Andrew's County Emergency Clinical Hospital's Molecular Biology Laboratory Constanta.

Extractor (for example Bioneer- Fig.1) is a fully automatic nucleic acid extraction device created for quickly extraction of DNA or RNA in a short period of time. It can work up to 48 samples

at the same time. It has an automatic function of pipetting and perforating the cartridges, which reduces the errors from human manipulation. The estimate time for completing the process is approximately 60 minutes.

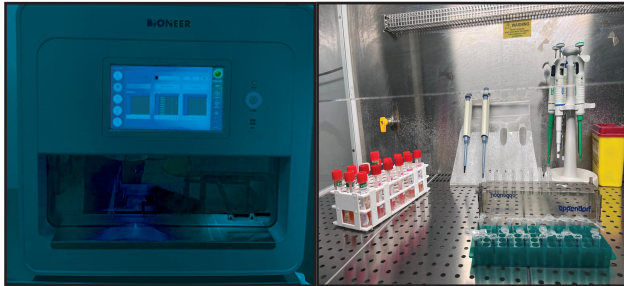


Figure 1 a. Extractor Bioneer b. Extraction

The extraction process is consisted: preparation of the sample, the selection of the extraction process, pipetting the sample - mixture of the patient's respiratory specimen with chemical reagents and last, but not least, operating and introducing the sample tubes in the device. The extracted RNA is loaded automatically in elution tubes. The elution tubes are kept in the freezer at -20 degrees Celsius, until the amplification process starts.

Molecular Biology Laboratory Constanta has two devices in his inventory for detection and identification of SARS-CoV-2.

Reagent mixture, extracted nucleic acid, negative and positive control) is pipetted.

Reagents and the extracted nucleic acids must be defrosted for at least 10 minutes to room temperature. It uses at least one negative control (water) and one positive control for one complete test. Centrifuge all reagents, controls, samples before use. The mixture of reaction is being prepared (for example Master Mix, Enzyme, depends on kit), and then, shake and centrifuge for 10 seconds. The mixture is pipetted in each well from the reagent mixture, then from the extracted nucleic acid assigned to each patient's well, in the final negative and positive control in the corresponding wells. They are sealed with a special foil, and they are inserted in the amplifier. This process multiplies DNA into hundreds of thousands of copies so that the doctor can confirm or infirm the diagnosis of Covid-19 (depending of the curves- Fig. 6, the presence/absence of the genes N, E, RdRp).

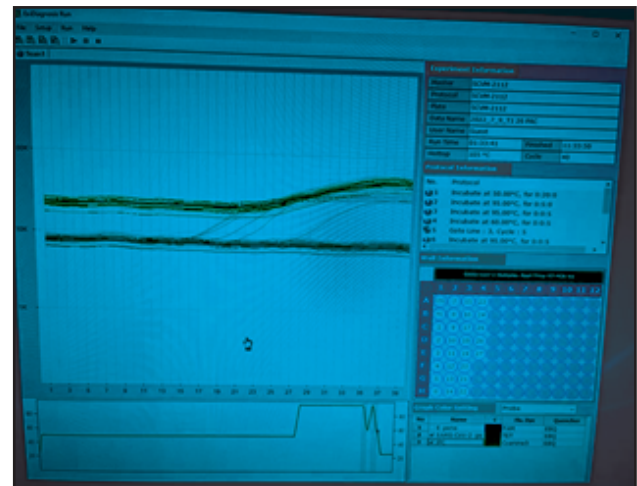


Figure 2 Amplification heat cycles

Results

During the period of the study (between April 24, 2020- December 31, 2021) at St. Andrew's County Emergency Clinical Hospital's Molecular Biology Laboratory Constanta, were tested a total of 2856 patients (childrens). In 2020 (April 24, 2020- December 31, 2020), 716 tests were performed, of which: 50 children came out positive (6.98%), inconclusive 5 patients (0.70%), and the rest of the tests came out negative 661 patients (92.32%).

In 2021 (January 1st, 2021- December 31, 2021) 2140 tests were performed, of which: 90 tests were positive (4.21%), 5 inconclusive tests (0.23%), the rest of the tests came out negative 2045 (95.56%).

The tests that came out positive, meant that they had two or three genes present (gene E, RdRp, N), the inconclusive ones meant that they had only one gene present (any of the three), and they had to be repeated. The inconclusive tests came out like this, due to variable factors, or due to a poor swab technique, when taking the patient's respiratory specimen, defective VTM (viral transmission medium). The negative tests, when interpreted, had no gene present.

Comparing the year 2020 with 2021, we can observe that in 2021 more tests were performed on children, that in the previous year, but the number of positive tests decreased from 6.98% to 4.21%, due to the implementation of sanitary precautions.

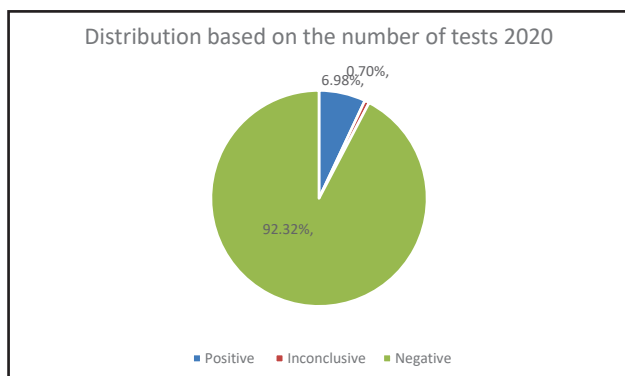


Figure 3 Distribution based on the number of tests 2020

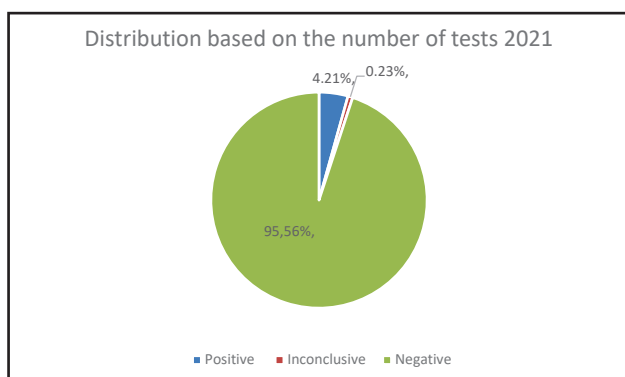


Figure 4 Distribution based on the number of tests 2021

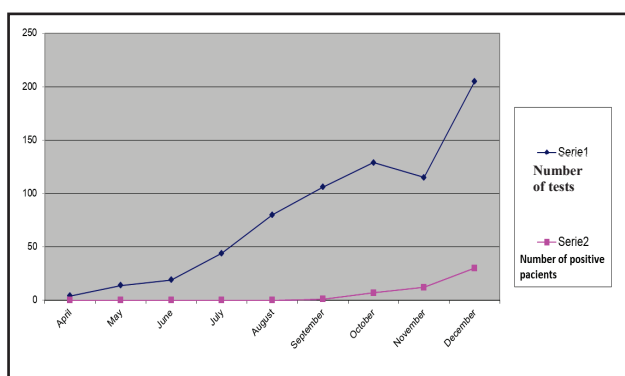


Figure 5 Distribution of total number of tests performed vs positive tests depending on the months of 2020

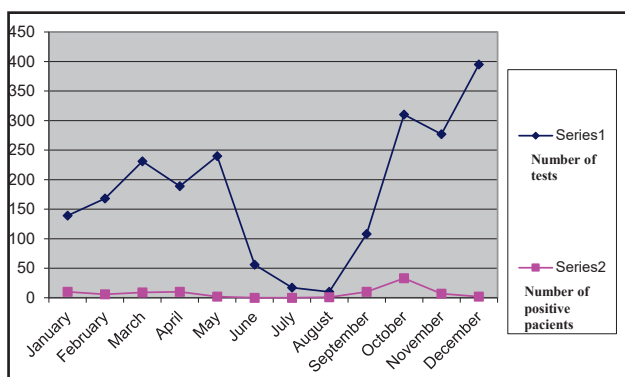


Figure 6 Distribution of total number of tests performed vs positive tests depending on the months of 2021

Also, in 2020 much alike in 2021, most of the tests were performed in December, during the winter holidays. As can be seen, the difference is that in 2020, the month with the most patients diagnosed with Covid-19 was December (30 cases), while in 2021 the peak of infections took place in October (33 cases), after school started they required presence in the classroom. For a period of time, the courses took place online.

During the summer time, there were made the least amount of tests, due to the summer vacation of children and the preference not to test at the first symptom.

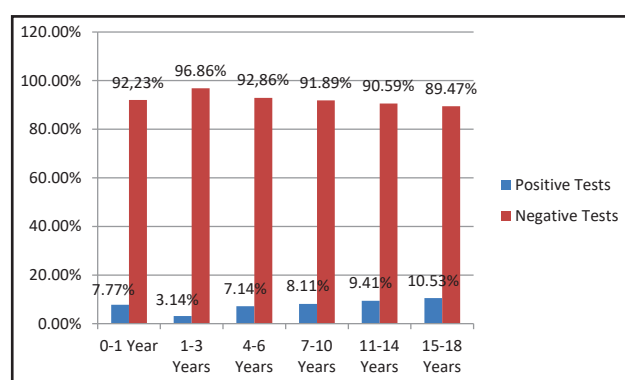


Figure 7 Distribution of negative and positive tests according to age – 2020

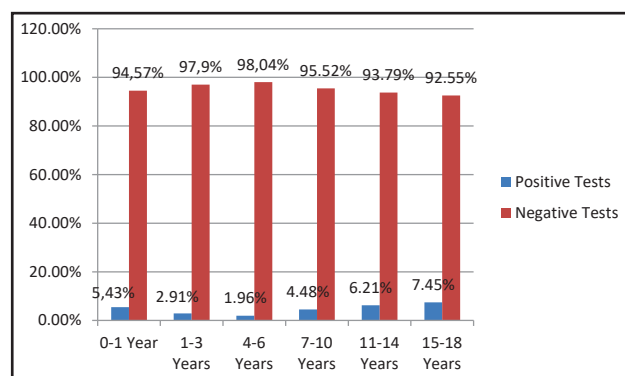


Figure 8 Distribution of negative and positive tests according to age - 2021

In the first pandemic year, the rate of positive tests was higher, due to the fact that fewer tests were performed than in 2021. Children, of all ages, from the youngest 1 day old to 17 years and 11 months were affected by Covid-19. The majority of them, developed an asymptomatic, mild or an average form, and the rest, in a small proportion, suffered a severe form.

Most positive tests in the first two years of the pandemic were found in the 15-18 group

age, followed by the 11-14 group age. The least affected were 1-3 years group in 2020, and in 2021 were 4-6 years group.

At Constanta's St. Andrew's Hospital's Molecular Biology Laboratory, the average age affected by the disease at children is around 7 years.

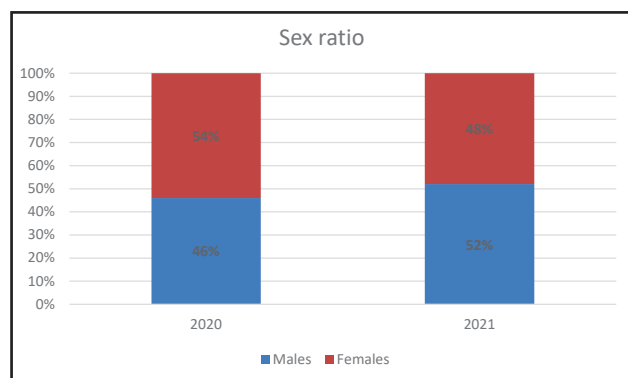


Figure 9 Sex Ratio

As for gender, Covid-19 affected both girls and boys in a slightly equal number.

Discussions

Although the average tests of positive patients at children in the first two years of the pandemic is 5,6%, does not reflect the real situation, because some refused to be tested, some parents refused to hospitalize their children with symptoms, others were ignorant of the action of self-isolation and/or self-quarantine or indication for appropriate treatment.

The rate of positive cases classified by gender of male patients under 18 years of age in Constanta, Romania is 49%. According to a case study in Ontario, Canada done on a sample of 70,187 positive cases of patients under 18 years of age, shows that an average of 51,4% are male patients totaling 36,062 cases.

Female patients under 18 years have the rate of positive cases in Constanta, Romania is 51%, compared to the results of the study in Ontario, Canada, where the percentage is 48,6% (8).

Children, of all ages, were affected by Covid-19 infection with an average age of 7 years. In Constanta, Romania, at St. Andrew's County Emergency Clinical Hospital in 2020-2021, the most affected group age was 15-18 years old,

followed by 11-14 years old. According to a case study in United States of America, were made on children under 18 years, the most affected ages groups by Covid-19 infection were: 5-11 years (38.3%), followed by the age group 12-15 years (27%) (9).

The majority of children's tests were performed in the autumn and winter season when parents were alarmed by the symptoms. Most positive cases were in December 2020, during the winter holidays, and in October 2021, when children returned to school, they entered in the community, which led to an increase in the transmission of the disease. The same thing happened in the United States of America in October 2021, when children returned to school, the number of positive cases increased by 10.6%, according to the study (10).

In the summer season, the number of tests decreased, when either children went on vacation, or simply, parents refused to test them.

The Covid-19 vaccine can prevent the children from getting the infection and spread at home or at school, to not develop a severe form, to avoid the hospitalization, and possible death (in rare cases, at those with comorbidities). Examples of diseases who can lead into a severe form: asthma or cronic lung disease, diabetes, obesity, sickle cell disease, etc. The children or teenagers can develop complications, such as Multisystem Inflammatory Syndrome (11).

In the early of 2020, when Sars-Cov-2 pandemic started, there were no vaccine available. They appeared in the begining of 2021. At the begining, vaccination rate was high, but after a period of time, the percentage decreased because people got skeptical. Vaccines are available to children by age group: from 6 months to 5 years (requires three shots), ages 6 through 11 years (involves two shots), ages 12 through 17 (this vaccine involves two shots aswell) (12).

During the pandemic, a lot of countries have imposed travel restrictions, and because of that reason adults, along side with childrens, the number of tests and vaccinations has increased. But, a portion of population remains distrustful towards vaccines due to fear and the side effects it can cause in the body.

Conclusions

The risk of SARS-CoV-2 is lower on children than at adults. In general, children have asymptomatic or mild forms, rarely severe forms, especially those with comorbidities.

At a certain point, the implemented restrictions helped contain the spread of infection in community. Vaccination is important at those with high risk of severe forms.

At the beginning, there were fewer people tested, and because of the fact that number of cases increased, also the number of positive tests increased, as well.

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