

Retrospective Analysis of SARS CoV-2 PCR Results Obtained between 2020-2022 at “St. Sofia” Medical Center and Hospital

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Abstract

In response to the global health emergency declared by the World Health Organization (WHO) due to the COVID-19 pandemic between 2020 and 2022, RT-PCR testing for SARS-CoV-2 was conducted at the PCR Laboratory of the “St. Sofia” Medical Center and General Hospital in Sofia, Bulgaria. Patients of various age groups and both sexes were tested. During the study period (2020–2022), the highest number of RT-PCR-confirmed SARS-CoV-2 cases was recorded in 2021. The results indicated an approximately equal male-to-female ratio, with higher positivity rates observed among older age groups. These findings are consistent with previously published data for the Sofia city region during the same period. No significant differences were observed between vaccine types in terms of protection, as the proportion of SARS-CoV-2-positive individuals was similar across vaccination groups.

Keywords: SARS-CoV-2, COVID-19, RT PCR, vaccine

Резюме

Във връзка с обявената от СЗО глобална извънредна ситуация с COVID-19 в PCR лабораторията при ДКЦ и МБАЛ „Св. София“ в град София, България бяха провеждани изследвания с RT PCR за SARS-CoV-2 на пациенти от различни възрастови групи от двата пола. За периода 2020г- 2022г, най-голям брой положителни за SARS-CoV-2 резултати от RT PCR са установени през 2021 година. Съотношението при тях между мъже и жени е еквивалентно, като се наблюдава по-голям брой положителни резултати при по-големите възрастови групи. Установените данни кореспондират на публикувани данни за региона на град София. Не се установи кой от типове COVID ваксини дава по-добра защита на пациентите, тъй като % на положителни за SARS-CoV-2 ваксинирани пациенти беше сходен.

Introduction

On the 30th of January 2020, the WHO Emergency Committee declared a global health emergency based on the rising number of reported cases of COVID-19 in China and other international locations (World Health Organization, 2023). Sources of SARS-CoV-2 infection include infected animal hosts and humans, with bats accepted as the most likely primary source of the virus. Both symptomatic and asymptomatic individuals can transmit the infection, mainly through droplets produced by coughing and sneezing, with contact-absorption transmission also playing a significant role in the spread of SARS-CoV-2 (Shi *et al.*, 2020). A single cough can release more than 3000 droplets, which may reach other individuals or contaminate surrounding surfaces; a small proportion of these

droplets may remain suspended in the air (Hafeez *et al.*, 2020).

SARS-CoV-2 is a β -coronavirus, and the amino acid sequences of its seven conserved domains within the genomic open reading frame 1ab (ORF1ab) are 79.6% identical to those of the original SARS-CoV. At the whole-genome level, SARS-CoV-2 shows 96% identity with a bat coronavirus (*Rhinolophus affinis*) found in Yunnan Province, China (Zhou *et al.*, 2020).

The SARS-CoV-2 virion has a diameter of 60–140 nm and contains a single-stranded positive-sense RNA genome of 29,891 base pairs (Zhou *et al.*, 2020). The coronavirus virion is typically spherical, although variations in shape can occur. It features petal-shaped surface projections composed

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of trimeric Spike (S) proteins, which are characteristic of coronaviruses. The S protein mediates viral attachment and membrane fusion during infection. Specifically, the S protein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of type II human lung pneumocytes, facilitating viral entry (Shi *et al.*, 2020).

In addition to the S protein, coronavirus genomes encode three other structural proteins: the Membrane (M) protein, the Envelope (E) protein, and the Nucleocapsid (N) protein. The M protein consists of 218–263 amino acids, possesses an N-terminus modified by O- or N-linked glycans, and a hydrophilic C-terminal tail. The E protein, composed of 74–109 amino acids, may contribute to increased virulence, and approximately 20 copies are typically present per virion. The N protein, containing 349–470 amino acids, is a phosphorylated RNA-binding protein that assists in the proper folding of the genomic RNA within the nucleocapsid (Shi *et al.*, 2020).

The initial clinical sign of SARS-CoV-2-associated disease (COVID-19) that enabled case detection was pneumonia. Gastrointestinal symptoms and asymptomatic infections have also been reported, particularly among young children. Current observations indicate an incubation period ranging from 0 to 24 days, with an average of approximately 3 days and a median of 5–7 days. In symptomatic patients, clinical manifestations typically appear within the first week after exposure and include fever, cough, nasal congestion, fatigue, and other signs of upper respiratory tract infection. Loss of taste and smell has also been noted in some cases. In approximately 75% of patients, the infection may progress to severe disease characterized by dyspnoea and radiological evidence of pneumonia on hospital admission (Velavan and Meyer, 2020).

Numerous studies have shown that COVID-19 mortality increases with age. Among individuals younger than 50 years, the mortality rate is approximately 0.40%; between 50–59 years, 1.3%; between 60–69 years, 3.60%; between 70–79 years, 8.00%; and in those aged 80 years and older, about 14.80% of infected individuals die (Hafeez *et al.*, 2020).

Highly transmissible variants of SARS-CoV-2, including Delta (B.1.617.2) and Omicron (B.1.1.529), emerged as “variants of concern” (VOCs) during the COVID-19 pandemic. The Delta variant is associated with high viral load and increased virulence, whereas the Omicron variant has demonstrated partial vaccine escape and high

transmissibility, but a significantly lower risk of hospitalization and mortality compared with the Delta variant (Gotseva *et al.*, 2024). A key strategy to prevent viral spread and reduce morbidity and mortality is the development of effective vaccines. One proposed vaccine approach involves the design of a polypeptide that mimics the binding motif of the Spike (S) protein. The objective is to block viral entry by saturating angiotensin-converting enzyme 2 (ACE2) receptors with antibodies or peptides before the virus can bind. This peptide would stimulate the immune system to produce antibodies against the virus, as both the S protein and ACE2 remain structurally stable across viral mutations (Mayer, 2020).

Two main types of vaccines - mRNA and vector-based - were the most widely administered during the COVID-19 pandemic. In the case of mRNA vaccines, such as those produced by BioNTech/Pfizer and Moderna, the whole virus is not introduced into the body. Instead, a segment of the coronavirus genetic material, namely messenger RNA (mRNA), is delivered directly. This mRNA encodes the viral Spike (S) protein. After vaccination, some human cells translate the mRNA and temporarily synthesize the S protein.

The bivalent Original/Omicron BA.4–5 vaccines contain mRNAs encoding the S proteins of both the original SARS-CoV-2 strain and the Omicron BA.4–5 sub variants, whose spike sequences are identical. The immune system subsequently recognizes this S protein as foreign and mounts a response by producing specific antibodies and activating T cells. The mRNA from the vaccine does not persist in the body; it is rapidly degraded after administration. In contrast, vector-based vaccines use a harmless carrier virus—most commonly an adenovirus—to deliver coronavirus genetic material into human cells, where it is expressed and triggers an immune response (Bulgarian Medicines Agency, 2023). Examples of COVID-19 vector vaccines include those developed by AstraZeneca and Janssen (Johnson & Johnson).

The COVID-19 pandemic has raised the need for different types of diagnostics, comparative validation of new tests, rapid approval by state and federal agencies, and rapid production of test kits to meet increased global demand. An essential part of COVID-19 diagnostics is the detection of SARS-CoV-2 using NAT (nucleic acid tests), in particular RT PCR (real-time reverse transcription polymerase chain reaction) in materials from symptomatic patients. Here, viral RNA is reverse transcribed

into DNA, which is amplified by PCR (Weissleder *et al.*, 2020).

Materials and Methods

Different degrees of SARS-CoV-2 detectability have been observed in various clinical materials. The RT-PCR detection rate of SARS-CoV-2 in patients with COVID-19 was highest in bronchoalveolar lavage fluid (93%), followed by sputum (72%) and nasopharyngeal swabs (63%), while lower detection rates were reported for pharyngeal swabs (32%) and stool samples (29%) (Vandenberg *et al.*, 2021). For this reason, at the “St. Sophia” Medical Center and Hospital, nasopharyngeal samples were selected for RT-PCR examination. These swab samples were collected using the transport and preservation medium provided by Biocomma Limited.

In RT-PCR testing for SARS-CoV-2, viral RNA is reverse transcribed into complementary DNA (cDNA), which is subsequently amplified by polymerase chain reaction. The assay employs a multiplex real-time reverse transcription PCR system using specific primers and fluorescent probes targeting the ORF1ab, E, and N genes of SARS-CoV-2, provided by Maccura Biotechnology Co., Ltd. Detection of viral nucleic acid is achieved through real-time monitoring of fluorescence intensity.

SARS-CoV-2 RNA isolated and purified from nasopharyngeal samples using the Auto-Pure 32A automated extraction system (Allsheng) was reverse transcribed into cDNA and then amplified with the Quant Gene 9600 system (BIOER). An internal control was included in each reaction to monitor the presence of PCR inhibitors in the samples and to prevent false-negative results, using the SARS-CoV-2 Fluorescent PCR provided by Maccura Biotechnology Co., Ltd. The internal control contains “bulletproof” RNA incorporating a fragment of the internal control gene. The positive control contains “armored” RNA with a fragment of the target gene, while the negative control consists of water treated with diethylpyrocarbonate.

RNA extraction, enrichment, and purification were performed using the Mag-Bind RNA Extraction Kit, which applies magnetic nanoparticle technology. The extraction reagent used was proteinase K. Under buffered conditions, nucleic acids in the samples were bound to magnetic nanoparticles, which were subsequently collected, transferred, and dispersed using external magnetic fields to achieve efficient extraction and separation of nucleic acids.

The quantitative PCR reaction mixture

contained primers, probes, deoxyribonucleotide triphosphates (dNTPs), Mg^{2+} , and buffer. The quantitative PCR enzyme mixture included Taq polymerase and uracil-DNA glycosylase, provided by Maccura Biotechnology Co., Ltd. A defined ratio of the two mixtures was combined to prepare the PCR mastermix, to which the extracted RNA samples were added.

PCR amplification was carried out in the thermal cycling system Quant Gene 9600 (BIOER). The cycling protocol, pre-programmed in the instrument, comprised the following sequential steps under specific temperature conditions: (1) reverse transcription; (2) activation of Taq polymerase and pre-denaturation; (3) denaturation, annealing, elongation, and fluorescence detection; and (4) cooling of the apparatus.

In this process, the probe hybridizes to a specific target sequence located between the forward and reverse primers. During the elongation phase of the PCR cycle, the 5'-nuclease activity of Taq polymerase degrades the probe, separating the reporter dye from the quencher dye and thereby generating a fluorescent signal. With each successive cycle, additional reporter molecules are released from their respective probes, resulting in a progressive increase in fluorescence intensity. The fluorescence is monitored at every PCR cycle by the accompanying software. Detection of viral RNA not only facilitates disease diagnosis but also provides valuable epidemiological and surveillance information (CDC, 2020).

The cycle threshold (Ct) value for each fluorescence channel and its corresponding amplification curve were reported using the SARS-CoV-2 Fluorescent PCR kit provided by Maccura Biotechnology Co., Ltd. The reference (threshold) Ct values were 38 for the ORF1ab and N genes, and 37 for the E gene. Samples with Ct values less than or equal to the reference were reported as positive for SARS-CoV-2, whereas samples with Ct values exceeding the reference were considered negative.

Results and Discussion

RT-PCR testing for SARS-CoV-2 was conducted in accordance with the manufacturers' instructions for the respective extraction and amplification kits. Inconclusive results, characterized by amplification of only a subset of the target genes in the SARS-CoV-2 RT-PCR assay, were most often observed in patients recovering from COVID-19 infection. However, such inconclusive findings may also result from mutations in primer or probe binding sites within the target gene, reagent insta-

bility, or cross-contamination of samples or amplification products (Jeon *et al.*, 2023).

RT-PCR data for SARS-CoV-2 obtained at St. Sofia Medical Center and General Hospital for the period from September 2020 to December 2022 are summarized in Table 1, which presents the total number of tests performed each year. The data are also expressed as percentages by sex relative to the total number of tests, along with the percentage of positive results for each sex relative to the total tested within that group.

The largest number of RT-PCR tests for SARS-CoV-2 during the study period was recorded in 2021, when 3712 samples were analyzed. This represents an almost threefold increase compared to the previous year and is consistent with the official data reported by the Regional Health Inspectorate (RHI) to the National Center for Public Health and Analysis (NCPHA), which documented a two-fold rise in confirmed cases and annual incidence in 2021 compared with 2020 (Marinova *et al.*, 2023).

Analysis of the 2021 dataset revealed an approximately equal gender distribution among tested individuals, with males comprising 50.2% and females 49.8%. In contrast, the proportion of females tested was higher in 2020 (53.17% vs. 46.8% males) and in 2022 (58% vs. 42.05% males). In 2020, the percentage of positive RT-PCR results for SARS-CoV-2 was identical between males and females, at 38.8% relative to the total number of tests performed for each sex. A trend of slightly higher positivity among females was observed in both 2021 and 2022.

A study conducted for the Sofia region similarly reported equal infection rates between sexes,

with a male-to-female ratio of 1:1 (83,488/94,659) (Marinova *et al.*, 2023). By comparison, an analysis of data from the 20 most severely affected European countries, as well as from the USA and Canada, indicated that male patients accounted for less than half of confirmed cases in all countries except Austria.

Table 2 presents the distribution of positive results among males and females across different age groups, expressed as a percentage of the total RT-PCR tests performed for SARS-CoV-2 in each respective year.

The lowest percentage of SARS-CoV-2-positive RT-PCR results was observed among children aged 1–9 years, with 0.29% in 2020, 0.32% in 2021, and 1.45% in 2022. This trend is consistent with global observations, where COVID-19 is infrequently reported in young children and, when present, typically manifests as a mild or asymptomatic infection (Hafeez *et al.*, 2020). In older pediatric age groups (10–14 and 15–19 years), an increase in SARS-CoV-2-positive RT-PCR results were recorded in 2022, with rates of 2.4% and 1.6%, respectively.

Among adults aged 20–54 years, variations in the percentage of positive RT-PCR results were observed over the three years. Individuals of working age in both sexes were more likely to come into contact with infected persons, resulting in a higher incidence rate within these age groups. The highest positivity rates were again reported in 2022, with the 30–44-year age range being the most affected.

A study conducted for the Sofia region found wide variability in age-specific incidence rates of COVID-19 (Marinova *et al.*, 2023). The highest

Table 1. RT-PCR tests for SARS-CoV-2 performed at “St. Sophia” Medical Center and Hospital (2020–2022), percentage distribution by sex and positive results

Period-Year	Total number RT PCR tests	% RT PCR tests in males	% positive RT PCR in males	% RT PCR tests in women	% positive RT PCR in women
2020	694	46.80	38.8	53.17	38.8
2021	3712	50.20	18.5	49.80	23.8
2022	1377	42.05	36.6	58.00	43.9

Table 2. Percentage of positive RT-PCR tests for SARS-CoV-2 at “St. Sofia” Hospital and “St. Sofia” Medical Center (2020–2022), age-adjusted to the total number of tests

Years	Age of patients [years]														
	< 1	1–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	> 65
2020	0.0	0.0	0.29	0.43	0.43	0.29	2.02	2.45	4.61	4.9	2.45	2.88	2.59	3.46	11.96
2021	0.0	0.13	0.19	0.24	0.4	0.73	1.27	1.89	2.21	2.16	1.72	1.56	1.8	1.32	5.47
2022	0.0	0.22	1.23	2.4	1.6	1.89	3.78	4.86	4.94	4.21	3.63	3.34	2.11	2.69	3.92

incidence was reported among individuals aged 50–54 years, reaching 17965.9 per 100 000 populations. Despite the high incidence, this age group often exhibited mild or asymptomatic disease. In contrast, although the incidence was lower among the elderly (65+ years), they experienced a substantially higher disease burden, with more severe clinical manifestations, frequent complications, and elevated mortality (1110.2 per 100 000) (Marinova *et al.*, 2023).

Similarly, in our study, a higher proportion of SARS-CoV-2–positive results were detected among individuals aged 65 years and older—11.96%, 5.47%, and 3.93% for 2020, 2021, and 2022, respectively. The increased incidence of COVID-19 in this population is likely associated with the prevalence of multiple chronic conditions and weakened immune responses, which also contribute to higher mortality in this group. According to Gotseva *et al.* (2024), the Delta variant predominantly affected individuals over 60 years of age, while the Omicron variant was more prevalent among those under 20 years, generally leading to milder clinical outcomes. The Delta variant demonstrated higher virulence and mortality, whereas the Omicron variant, though more transmissible, was associated with less severe disease.

Between 2021 and 2022, a subset of patients tested for SARS-CoV-2 by RT-PCR in our laboratory were also interviewed regarding their COVID-19 vaccination status. The summarized data are presented in Table 3. Reinfection with COVID-19 is not uncommon, particularly with more aggressive variants such as Delta. A previous infection provides partial immunity; however, vaccination offers stronger and more consistent protection against severe disease (Marinov *et al.*, 2022). According to Bergeri *et al.* (2022), global seroprevalence data show a considerable increase over time, with regional variation. Nevertheless, more than one-third of the world’s population remains seronegative for

SARS-CoV-2. Their estimates indicate that global seroprevalence substantially exceeds the number of reported COVID-19 cases.

In 2021, a total of 249 patients were interviewed regarding COVID-19 vaccination and their experiences following immunization. The results showed that 60.6% of respondents had been vaccinated. Among these vaccinated individuals, 17.2% tested positive for SARS-CoV-2 by RT-PCR, indicating relatively good protection post-vaccination. In 2022, the number of interviewed patients increased to 1005, of whom 50.3% reported being vaccinated against COVID-19. Despite the expectation that many individuals had received a booster dose during this period, the percentage of RT-PCR–positive results increased to 26.7%.

Table 3. Interviewed patients vaccinated against COVID-19: percentage distribution of vaccinated individuals and percentage of vaccinated individuals with positive RT-PCR test results for SARS-CoV-2

Year	Interviewed	% vaccinated against COVID-19	% vaccinated with positive RT-PCR for SARS-CoV-2
2021	249	60.6	17.2
2022	1005	50.3	26.7

In 2022, information was also collected regarding the specific type of COVID-19 vaccine administered. The proportion of vaccinated patients who tested positive for SARS-CoV-2 by RT-PCR, relative to the total number of vaccinated positive cases, is presented in Table 4. Among vaccinated individuals who tested positive, the highest percentage (51.1%) had received the Pfizer vaccine, followed by 22.2% for those vaccinated with Johnson & Johnson and 16.3% for those vaccinated with Moderna.

To estimate the proportion of vaccinated individuals who tested positive for SARS-CoV-2 more accurately, their percentages were further calculated

Table 4. Percentage of vaccinated patients with positive RT-PCR results for SARS-CoV-2 in 2022, relative to the total number of vaccinated patients who tested positive

Year	Pfizer	Moderna	AstraZeneca	Johnson & Johnson	AstraZeneca + Pfizer	Moderna + Pfizer	AstraZeneca + Moderna
2022	51.1	16.3	6.7	22.2	3.7	1.48	0.7

Table 5. Percentage of COVID-19–vaccinated patients with positive RT-PCR results for SARS-CoV-2 in 2022, relative to the total number of vaccinated patients tested by vaccine type

Year	Pfizer	Moderna	AstraZeneca	Johnson & Johnson	AstraZeneca + Pfizer	Moderna + Pfizer	AstraZeneca + Moderna
2022	25.1	33.3	36	30.6	27.8	40	25

ed relative to the total number of patients vaccinated with each respective vaccine type (Table 5).

Vaccination without prior infection with Pfizer-BioNTech (BNT162b2) was found by Hall *et al.* (2022) to have an effectiveness of 85% during the first two months after the second dose, which subsequently declined to 51% after six months. It was also established that there was no significant difference in protection between short and long intervals between doses. For AstraZeneca (ChAdOx1), the effectiveness after the second dose was 58%, indicating a lower protection rate compared to Pfizer. Protection against reinfection was 86% during the first year and decreased to 69% after one year. Hybrid immunity (infection plus vaccination) provided the highest and most durable protection - over 90% - remaining stable even 18 months after the initial infection, with no data showing a significant decline in immunity during this period.

According to our study, the combination of the two vaccines from Moderna and Pfizer was associated with the highest percentage (40%) of RT-PCR-positive patients for SARS-CoV-2. Among those who received only one type of COVID-19 vaccine, the highest percentage of positive results was observed with AstraZeneca (36%), followed by Moderna (33.3%) and Johnson & Johnson (30.6%). The results obtained do not allow differentiation of the efficacy between mRNA and vector vaccines. The purpose of administering COVID-19 vaccines is both disease prevention and disease management. However, no definitive conclusions can be drawn regarding the protection provided by individual vaccines, as information on disease severity among positive patients was not available.

Conclusions

The study of sex and age among patients who tested positive by RT-PCR for SARS-CoV-2 showed a consistent trend when compared with the published data for the Sofia region. The highest number of positive cases was recorded in 2021 compared to 2020 and 2022, with patients from the older age groups of 30 years and above being the most affected. The lowest percentage of positive SARS-CoV-2 RT-PCR results was observed in children aged 1 to 9 years, while the highest was found in older individuals, particularly in the 65+ age group. It was confirmed that the proportion of positive results for SARS-CoV-2 in males and females was equivalent, indicating that both sexes were equally affected by COVID-19. In the analysis of data from COVID-19-vaccinated patients, it could not be determined whether mRNA or vector

vaccines provided better protection against the disease, as both vaccine types resulted in approximately 30% positive results when tested by RT-PCR for SARS-CoV-2.

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