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DATABASE ANALYSIS

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A Retrospective Population Study of 385 191 Positive Real-Time Reverse Transcription–Polymerase Chain Reaction Tests For SARS-CoV-2 from a Single Laboratory in Katowice, Poland from April 2020 to July 2022

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Background: This retrospective population study identified 385 191 positive real-time reverse transcription–polymerase chain reaction (RT-PCR) tests for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a single laboratory in Katowice, Poland, from April 2020 to July 2022.

Material/Methods: The material was nasopharyngeal, nasopharyngeal swab or bronchial lavage, and bronchoalveolar lavage (BAL) to confirm or exclude SARS-CoV-2 infection with the RT-PCR technique. Personal data are use according to the Provisions on the Protection of Personal Data by the Gyn-Centrum laboratory.

Results: In 9 months of 2020, the number of SARS-CoV-2 results was 88 986; in 2021, it was 168 439, and in the first 7 months of 2022, it was 12 786. In 2020, the highest number of positive results was recorded in the third quarter (83 094 cases); 2021, in the 1st, 2nd, and 4th quarters (58 712; 37 720; and 71 753 cases, respectively), and in 2022, in the 1st quarter (127 613 cases) of the year. A positive result was observed more often in women and people aged 30-39, followed by those 40-49 years. Patients aged 10-19 years comprised the smallest population of SARS-CoV-2-positive cases.

Conclusions: In the Polish population studied, from April 2020 to July 2022, the detection rates of SARS-CoV-2 positivity were significantly higher for women than for men and in the 30-49 age group for both sexes. Also, the infection detection rate of 385 191 out of 1 332 659 patient samples, or 28.9%, supports that the Polish society adhered to public health recommendations for infection control during the COVID-19 pandemic.

Keywords: Adult Multisystem Inflammatory Disease, COVID-19 Related • SARS-CoV-2 Variants

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Background

In November 2019, a case of viral pneumonia was reported in Wuhan, Hubei Province, China. In 2020, the World Health Organization (WHO) named this novel pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by it was named coronavirus disease 2019 (COVID-19) [1-3]. The virus is transmitted mainly through respiratory droplets [4]. Infected patients usually manifest symptoms such as dry cough, fever, shortness of breath, upper airway congestion, and sputum production. The loss of smell and taste is considered specific to SARS-CoV-2 infection [5]. The disease course and outcome depend on other patient features, especially comorbidities [2,6]. Shortly after starting in China, the virus spread worldwide, and on March 11, the WHO announced COVID-19 as a global pandemic [1,7]. In Poland, as of July 2021, there were 2.9 million confirmed cases of COVID-19, and 75 095 people had died of this disease [6,8,9].

The main reason for the rapid spread of the disease is asymptomatic transmission [10]. Initial strategies deployed to fight the virus spread involved closing public places, self-isolation, and wearing a mask in public [11]. People were encouraged to practice social distancing and work from home when possible. Governments established travel restrictions, particularly in regions with a high rate of new cases [11].

The first case of COVID-19 was reported in Poland on March 4, 2020 [12]. A week later, the first restrictions were introduced. Briefly, public events were canceled and schools and universities were closed [12]. At the end of a month, it was forbidden to leave homes for other than essential purposes. Wearing masks in public places was obligatory from April 16 to May 30, 2020 [12]. As the first wave of COVID-19 faded, restrictions were lifted [12]. In autumn 2020, followed by school openings, the second wave of the coronavirus hit [12]. The daily increase in new infections and the number of deaths was greater this time [12]. Restrictions were reintroduced, and on December 26, the first individual was vaccinated [12].

One of the most useful tools for viral detection is polymerase chain reaction (PCR) [13]. This method is considered to have high sensitivity and specificity. Real-time reverse transcription PCR (RT-PCR) has proven useful for detecting SARS-CoV-2 infection even in the early stages of the disease [13]. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) has been widely used to fight coronavirus disease outbreaks worldwide [13-15]. In early 2020, RT-qPCR-based assays were established as a "gold standard" for SARS-CoV-2 diagnostic testing [13-15] and soon, laboratories in many countries started to perform RT-PCR testing for SARS-CoV-2 detection [16,17]. However, the risk of obtaining a false-negative result of real-time RT-PCR is still an issue, accounting for 3% of the results [18,19]. Furthermore, SARS-CoV-2 is known to have high genetic diversity [16,20], which can create a problem with primer and genome region mismatch, resulting in a false-negative result [16,20]. To reduce this problem, primers used for RT-PCR were designed to target an invariable SARS-CoV-2 genome region [13]. The quality of the tests used for diagnostics is also extremely important – the speed of their update, due to the changeability of the virus, and the ability to detect at least 2 viral genes/regions, increasing their sensitivity and effectiveness [13,16]. False-negative results could also be caused by inappropriate sample transportation, storage, incorrect sample acquisition, suboptimal timing (sampling should be done at peak viral load), or incorrect sample type [13,16].

Therefore, this retrospective population study identified 385 191 positive real-time reverse transcription–polymerase chain reaction (RT-PCR) tests for SARS-CoV-2 from a single laboratory in Katowice, Poland from April 2020 to July 2022.

Material and Methods

Ethics

This study was performed following the guidelines of the 2013 Declaration of Helsinki for human experimentation. Data confidentiality and patient anonymity were maintained at all times. Patient identification information was deleted before the database was analyzed. Emilia Morawiec, PhD, has access to the full database of patients as an employee of the Gyncentrum laboratory and by decision of the bioethics committee. The center agreed to share patient data and anonymize them by Emilia Morawiec. In addition, Emilia Moraweic is obliged to observe professional secrecy (in accordance with the rules of ethics), related to the duties of a laboratory diagnostician. Therefore, it was not possible to identify patients at an individual level, either in this article or in the database. Informed consent was obtained from all patients. It only contained information that the patient consents to the nasopharyngeal or nasopharyngeal swab, and in the case of materials provided from hospitals, also bronchial lavage and bronchoalveolar lavage (BAL) in order to confirm or exclude SARS-CoV-2 infection with the RT-PCR technique and to process personal data in accordance with the provisions on the Protection of Personal Data by the Gyncentrum laboratory. The consent did not include information on whether the patient had a history of SARS-CoV-2 infection or COVID-19 vaccination status. Those patients with suspected SARS-CoV-2 infection were tested. Approval of the Ethical Committee at the University of Technology in Katowice (Academy of Silesia, Poland), no. 04/KEBN/2021, December 17, 2021, was obtained for this study.

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Study Design and Participants

In this retrospective study, 1 332 659 patient samples were analyzed from April 2020 to July 2022 (27 months), of which 385 191 (28.9%) were confirmed SARS-CoV-2-positive cases, including 210 138 women (54.55%) and 175 053 men (45.45%). All data (test dates and results of the RT-PCR assay) were collected up to the final follow-up date (July 31, 2022). All determinations were performed in a single laboratory – Gyncentrum, Laboratory of Molecular Biology and Virology – in Katowice, Poland. Cases where a doctor ordered the examination, as well as when performed at the request of a person, were considered. Data such as age, sex, and obtained results were collected from the electronic medical record system.

Real-Time Reverse Transcription–Polymerase Chain Reaction Assay (RT-PCR) for SARS-CoV-2

To extract total ribonucleic acid (RNA), nasopharyngeal swabs were taken, which were then placed in a test tube with virus preservation medium (VPM/VTM) or in saline, and RNA was isolated using the following kits: Kurabo QuickGene Mini480 Nucleic Acid Isolation System (Kurabo, NY, USA), Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega, Madison, USA), Chemagic 360TM Viral DNA/RNA Kit (Perkin Elmer, Massachusetts, USA), and Biomek RNA Advance Viral Isolation (Beckman Coulter, California, USA). In addition, the RT-PCR reaction was performed based on diagnostic kits with CE, IVD certification: Viasure SARS-CoV-2 Real-Time PCR Detection Kit (Certest Biotec S.L., San Mateo de Gallego, Spain), MediPan 2G + Fast COVID Kit (Medicofarma, Radom, Poland), 2019 Novel-Coronavirus [2019-nCOV] Triplex RT-qPCR Detection Kit (Vazyme, Nanjing, China), MutaPlex Coronavirus Real-Time-RT-PCR Kit (Immunodiagnostic, The Boldons, Great Britain), Xpert Xpress SARS -Co-2 and Xppert Xpress SARS-CoV-2/Flu/RSV (Gene Xpert, Sunnyvale, California, USA), in suitably recommended by the manufacturer thermal conditions, on BioradCFX 96, BioradCFX Opus thermal cyclers (BioRad, California, USA), Aria MX and Aria DX (Agilent Technologies, California, USA). The test was interpreted according to the manufacturer's recommendations.

The RT-PCR tests used had the necessary certification (CE IVD), allowing use for routine diagnostics. Several diagnostic tests were used due to: (1) the lack of availability of one type of diagnostic test on the market that would be able to satisfy the need for mass testing, and (2) the need to confirm the result (in the case of a diagnostically doubtful result) with a test from another manufacturer. The Gyncentrum laboratory was approved by the Ministry of Health and was placed on the list of laboratories performing routine diagnostic tests for SARS-CoV-2 in Poland. It met the premises and personnel criteria as well as the standards of equipment and apparatus required for units conducting testing. It is also essential that the Gyncentrum laboratory was subject to regular and independent external laboratory control carried out by the National Institute of Public Health (NIPH), National Institutes of Health (NIH), Laboratory of respiratory viruses operating at the Central Clinical Hospital of the Medical University of Lodz, Poland, and the European Molecular Genetics Quality Network (EMQN).

Statistical Analysis

Experimental Groups Comparison

Statistical analysis was performed using the publicly available statistical program Social Science Statistics [21]. The nominal data is represented by each comparison's number (n) and percentage (%) of cases. Chi-square (χ^2) or Fisher's exact test was used to assess the relationship between the variables in each comparison.

Sample Size Calculation

According to the data published by the Ministry of Health in Poland on August 4, 2022, the number of infections caused by SARS-CoV-2 since March 4, 2020, was 6 094 876 [9].

For 6 094 876 participants, the maximum error value was 1%. Therefore, assuming a P value of <0.05, the required number of subjects in the study was 9589 (P value <0.05).

Results

This retrospective population study identified 385 191 positive real-time reverse transcription–polymerase chain reaction (RT-PCR) tests for SARS-CoV-2 from a single laboratory in Katowice, Poland, from April 2020 to July 2022. In this section, we decided to present the profile of SARS-CoV-2-positive results from April 2020 to July 2022, broken down by sex and age.

Analysis of the Total Number of SARS-CoV-2-Positive Results from a Single Laboratory in Katowice, Poland, from April 2020 to July 2022

In the first stage of the research, we analyzed the changes in the number of SARS-CoV-2-positive cases in the individual months of 2020-2022. It is worth noting that we obtained 16 658 questionable results, which corresponded to 1.25% of all results. Re-running RT-PCR allowed the classification of 12 987 results as positive (87.96%) and 3671 as negative (22.04%). In 9 months of 2020, the total number of SARS-CoV-2 results was 88 986; in 2021, it was 168 439; and in the first 7 months of 2022, it was 12 786. A total of 385 321 patients were SARS-CoV-2-positive (**Figure 1**).

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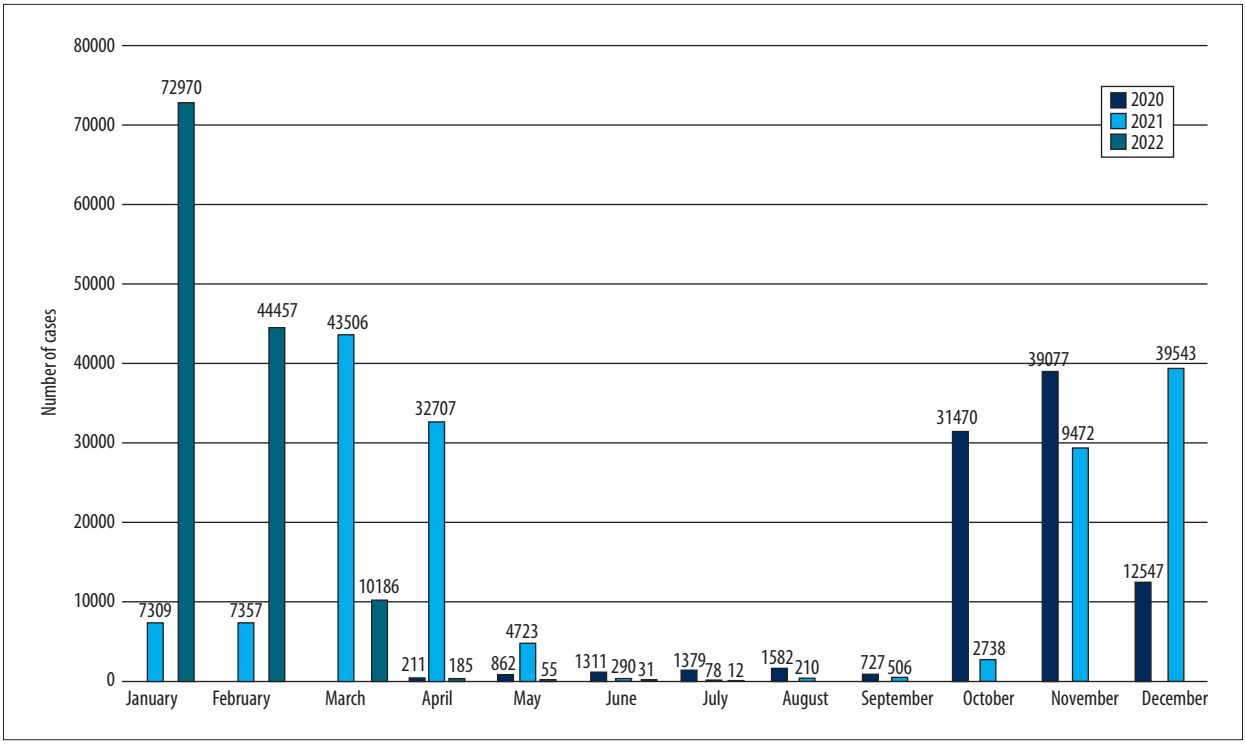

Figure 1. The dynamics of SARS-CoV-2-positive results in 2020-2022.

When assessing the change in the dynamics of infections year/year, month/month, it can be concluded that in 2020, the highest number of favorable results was recorded in the third quarter of the year – a total of 83 094 cases. In 2021, the peak incidence occurred at the turn of the 1st, 2nd, and 4th quarters. However, by 2022, the most significant SARS-CoV-2 results were obtained in Q1, 127 613 cases (**Figure 1**). Chi-square analysis showed significant differences in the infection rate profiles in Q2 and Q3 in 2020-2022 (χ^2=20712.1818; *P*<0.00001). In addition, the number of positives in Q1 and Q2 in 2020 and 2021 differed significantly, confirming that the number of positives in 2020 and 2021 depended on the assessed quarter (the Fisher exact test statistic value was <0.00001). Detailed results presented in this section are shown in **Figure 1**.

Evaluation of the Number of SARS-CoV-2-Positive Results from a Single Laboratory in Katowice, Poland, from April 2020 to July 2022 by Sex

When assessing the structure of the distribution of the number of infections in individual years depending on sex, such a relationship was noted (χ^2=42.7259; *P*<0.00001). In most of the months of 2020-2022, women had a positive result more often than men (**Table 1**).

Table 1 presents the number of SARS-CoV-2-positive cases in particular months of observation in specific years, depending on sex (*P*<0.05).

Evaluation of the Number of SARS-CoV-2-Positive Results from a Single Laboratory in Katowice, Poland, from April 2020 to July 2022 in Individual Age Groups

It was concluded that the group with the highest SARS-CoV-2-positive results were people aged 30 to 39 years, followed by 40- to 49-year-olds. Patients aged 10 to 19 years comprised the smallest population who were SARS-CoV-2-positive. In 2020, the maximum positive results were obtained in the 30 to 39 years old group. In 2021, the percentage of positive results among people aged 30 to 39 and 40 to 49 was similar, at approximately 20%. Nevertheless, in the first 7 months of 2022, the highest percentage of positive results was also in the 30 to 39 years group (**Table 2, Figure 2**). Statistical analysis showed a significant relationship between age and the number of positive SARS-CoV-2 results (χ^2=11187.874; *P*<0.00001). Detailed results presented in this section are presented in **Figure 2 and Table 2** (*P*<0.05).

Discussion

In the present large-scale study, we assessed changes in the incidence of SARS-CoV-2-positive results based on the outcomes obtained using the RT-PCR technique in 385 191 patients. The data and results from this study are noteworthy, as they provide concrete evidence of the evolution profile of SARS-CoV-2 infection rates from the start of the pandemic to July 2022,

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Table 1. Distribution of the number of SARS-CoV-2-positive results in 2020-2022 by sex.

Gender	Month	Number of cases (%)			χ^2 results
		2020	2021	2022	
Female	January		3968 (54.29%)	40258 (55.19%)	χ^2=2.1692 p=0.140795
Male			3341 (45.71%)	32689 (44.81%)	
Female	February		3918 (53.26%)	24499 (55.12%)	χ^2=8.8235 p=0.002974
Male			3438 (46.74%)	19945 (44.88%)	
Female	March		23111 (53.13%)	5799 (56.95%)	χ^2=8276.4804 p<0.00001
Male			20390 (46.87%)	4383 (43.05%)	
Female	April	104 (49.29%)	17421 (53.27%)	87 (47.03%)	χ^2=4.1914 p=0.122985
Male		107 (50.71%)	15282 (46.73%)	98 (52.97%)	
Female	May	393 (45.59%)	2517 (53.30%)	26 (47.27%)	χ^2=17.8824 p=0.000131
Male		469 (54.41%)	2205 (46.70%)	29 (52.73%)	
Female	June	438 (38.73%)	163 (56.21%)	19 (61.29%)	χ^2=33.2997 p<0.00001
Male		693 (61.27%)	127 (43.79%)	12 (38.71%)	
Female	July	440 (31.98%)	35 (44.87%)	6 (54.55%)	χ^2=1.2804 p=0.527196
Male		936 (68.02%)	43 (55.13%)	5 (45.45%)	
Female	August	543 (34.45%)	100 (47.85%)		χ^2=14.3612 p=0.000151
Male		1033 (65.55%)	109 (52.15%)		
Female	September	387 (53.38%)	271 (53.98%)		χ^2=0.0436 p=0.834559
Male		338 (46.62%)	231 (46.02%)		
Female	October	17860 (56.81%)	1565 (55.18%)		χ^2=0.4867 p=0.485417
Male		13756 (43.19%)	1172 (42.82%)		
Female	November	21246 (54.38%)	16426 (55.65)		χ^2=0.2201 p=0.638952
Male		17823 (45.62%)	13036 (44.25%)		
Female	December	6681 (53.25%)	21857 (55.29%)		χ^2=15.9828 p=0.000064
Male		5866 (46.75%)	17677 (44.71%)		

χ^2 – Chi-square; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; % – percentage.

for 27 months. All the results were obtained using the RT-PCR technique, which is the most sensitive of the currently available SARS-CoV-2 virus RNA detection methods. The use of several different tests in the diagnosis of SARS-CoV-2 was necessary. On the one hand, facing the challenge of mass testing of patients and ensuring access to the appropriate quantity and quality of diagnostic tests was challenging. This made it impossible to rely on one type of test produced only by one company. The lab testing at peak times of 20 000 samples per day required the continuous availability of diagnostic tests. During the pandemic, none of the manufacturers who supplied several testing laboratories in the country and abroad could meet this challenge. In addition, it should be taken into account that in the case of doubtful results, it was necessary to re-run the test, enabling the unambiguous determination of the result (as positive or negative). This procedure requires using a different manufacturer's RT-PCR reagent test, which differs in the sequence of the primers. In the vast majority of cases, this allowed for an unambiguous assessment of the material, saving time in diagnosing patients and reducing waiting queues without moving them back in the chain waiting for recollection. Since the number of SARS-CoV-2-positive cases has increased rapidly worldwide, it has become essential to detect the infection quickly and accurately to control and prevent sources of infection. Moreover, a disease caused by each genetic variant of the SARS-CoV-2 coronavirus is characterized by the appearance of other symptoms. An asymptomatic course is also possible; therefore, it is challenging to determine SARS-CoV-2 virus infection based on clinical signs alone, especially in patients in the early stage of the disease. Considering the main advantages of RT-PCR, i.e., the specificity and sensitivity of the method, as well as the ease of preparation of the material for testing and its performance, it can be considered the "gold standard" in SARS-CoV-2 detection [22-24]. However,

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Table 2. Profile SARS-CoV-2-positive results in different age groups.

Age group (years)	Total number (%)	Year	Total per year	January	February	March	April	May	June
<10	23771 (3.35%)	2020 (1.32%)	1174				3	44	26
		2021 (5.93%)	9969	140	195	1411	993	205	7
		2022 (9.91%)	12628	7954	4027	639	3	2	1
10-19	12885 (12.04%)	2020 (2.98%)	2648				11	48	52
		2021 (3.86%)	6487	220	238	1879	1381	221	26
		2022 (2.94%)	3750	2814	731	203	1	0	1
20-29	46327 (20.67%)	2020 (11.12%)	9893				19	102	164
		2021 (10.85%)	18249	739	665	4565	3466	524	22
		2022 (14.26%)	18185	10513	6294	1359	13	4	1
30-39	79525 (19.71%)	2020 (20.39%)	18143				39	224	331
		2021 (19.44%)	32709	1227	1359	8406	5575	873	48
		2022 (22.49%)	28673	16982	9523	2102	47	15	2
40-49	75830 (14.74%)	2020 (21.91%)	19501				42	159	250
		2021 (19.30%)	32459	1236	1429	8807	5885	865	61
		2022 (18.72%)	23870	14426	7741	1656	26	13	6
50-59	56708 (12.50%)	2020 (18.32%)	16306				46	128	137
		2021 (14.32%)	24083	1200	1187	6889	5289	708	31
		2022 (12.80%)	16319	8729	6189	1372	23	2	3
60-69	48097 (6.88%)	2020 (12.62%)	11232				31	77	106
		2021 (14.65%)	24643	1197	1206	7015	5832	718	54
		2022 (9.59%)	12222	5948	4916	1327	19	4	6
70-79	26457 (3.92%)	2020 (6.70%)	5958				11	50	42
		2021 (7.86%)	13217	838	678	3395	3079	406	27
		2022 (5.71%)	7282	3426	2972	861	14	8	1
80-89	15086 (6.18)	2020 (4.64%)	4131				9	30	23
		2021 (3.80%)	6399	512	400	1139	1207	203	14
		2022 (3.57%)	4556	2007	1952	577	17	1	1

Age group (years)	Total number (%)	Year	Total per year	July	August	September	October	November	December
<10	23771 (3.35%)	2020 (1.32%)	1174	59	52	11	338	405	236
		2021 (5.93%)	9969	4	11	34	173	2715	4081
		2022 (9.91%)	12628	2					
10-19	12885 (12.04%)	2020 (2.98%)	2648	56	84	20	1188	899	290
		2021 (3.86%)	6487	4	2	11	88	1202	1215
		2022 (2.94%)	3750	0					

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Table 2 continued. Profile SARS-CoV-2-positive results in different age groups.

Age group (years)	Total number (%)	Year	Total per year	July	August	September	October	November	December
20-29	46327 (20.67%)	2020 (11.12%)	9893	213	276	110	3459	4252	1298
		2021 (10.85%)	18249	15	23	64	298	3237	4631
		2022 (14.26%)	18185	1					
30-39	79525 (19.71%)	2020 (20.39%)	18143	399	378	138	6673	7782	2179
		2021 (19.44%)	32709	12	44	88	530	6264	8283
		2022 (22.49%)	28673	2					
40-49	75830 (14.74%)	2020 (21.91%)	19501	317	328	161	7661	8309	2274
		2021 (19.30%)	32459	11	44	100	598	6021	7402
		2022 (18.72%)	23870	2					
50-59	56708 (12.50%)	2020 (18.32%)	16306	185	215	112	5843	7429	2211
		2021 (14.32%)	24083	8	28	64	358	3558	4763
		2022 (12.80%)	16319	1					
60-69	48097 (6.88%)	2020 (12.62%)	11232	92	136	96	3357	5292	2045
		2021 (14.65%)	24643	8	23	63	338	3355	4834
		2022 (9.59%)	12222	2					
70-79	26457 (3.92%)	2020 (6.70%)	5958	38	65	43	1639	2786	1284
		2021 (7.86%)	13217	5	13	51	214	1932	2579
		2022 (5.71%)	7282	0					
80-89	15086 (6.18)	2020 (4.64%)	4131	20	48	36	1312	1923	730
		2021 (3.80%)	6399	4	10	25	129	1106	1650
		2022 (3.57%)	4556	1					

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; % – percentage.

this technique has drawbacks, such as obtaining uncertain or false positives/negatives [25] due to the high variability of the virus's genetic sequence and its evolution. This translates into the lack of complete complementarity of primers and probes to the target nucleotide sequence of the gene whose expression we want to determine. Therefore, amplifying several or at least 2 target sets is reasonable to reduce the risk of obtaining incorrect results [26,27]. This was also the case in our analysis, where the false-negative rate was 0.975% of all samples. Furthermore, amplifying 2 target genes and repeating uncertain results significantly reduced the number of false negatives [28].

The impact of demographic factors on the dynamic profile of SARS-CoV-2 in the Polish population has not been previously studied. When assessing the number of favorable results in individual years of our analysis, 2 periods of increased incidence of SARS-CoV-2 positivity could be distinguished – from January to March/April and from October to December – when there was a visible disproportion in the number of positive RT-PCR results. An increase in viral infections in the fall/winter and spring months is typical [29,30].

Undoubtedly, the incidence of SARS-CoV-2 infections was influenced by the decisions of governing bodies at the international, national, and regional levels regarding the imposition and revocation of individual restrictions, such as the order to cover the mouth and nose in closed rooms, open spaces, no assembly, remote work, and distance learning. In Poland, the epidemic and the lockdown were announced on March 20, 2020, and restrictions were lifted on April 20 [31].

The relatively small number of favorable results in April to September 2020 was due to the strict restrictions introduced

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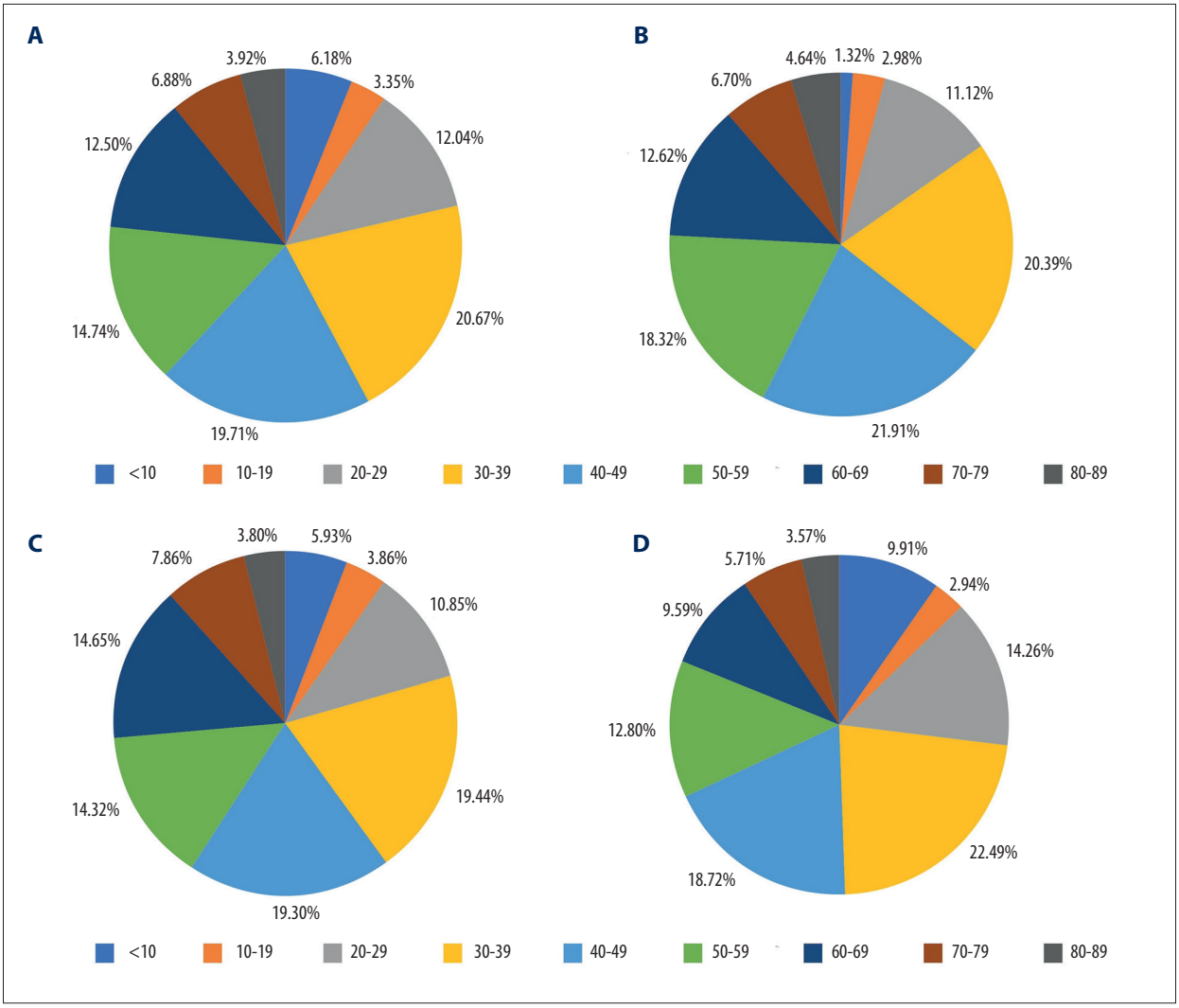

Figure 2. Percentage distribution of the frequency of SARS-CoV-2-positive results in the analyzed period, considering age ranges. (**A**) Percentage of SARS-CoV-2 positive results in age groups in the period 2020 to 2022. (**B**) Percentage of SARS-CoV-2-positive results in age groups in 2020. (**C**) Percentage of positive SARS-CoV-2 positive results in age groups in 2021. (**D**) Percentage of positive SARS-CoV-2 positive results in age groups in 2022.

by the government, with the simultaneous understanding and fulfillment of obligations by society. The low level of SARS-CoV-2 testing and the limitation of movement without significant reasons were probably also necessary. From October 2020, a substantial increase in the number of infections was recorded, which began to slow in December 2020. On October 10, Poland was included in the so-called "Yellow zone," and some points were even in the "red zone." Hence, recommendations were introduced to facilitate travel to the European Union [31].

In November, a month after the significant increase in SARS-CoV-2-positive cases, it was decided to introduce further safety rules, including learning and working remotely, describing them as the last stage before the national quarantine. This introduction contributed to the reduction in positive results in December 2020, along with the limited migration during the holiday season and the smaller number of tests performed during this period. Vaccination with the first dose of the SARS-CoV-2 vaccine was initiated in December. The beginning of 2021 witnessed a gradual loosening of restrictions, adopting solutions regulating travel, and promoting vaccinations [32]. Nevertheless, infections increased in March and April 2021, when variant B.1.1.7 (alpha) dominated, and again in November and December 2021 [33].

It is worth noting that the first 2 months of 2022 had the highest number of SARS-CoV-2-positive results since the start of the pandemic. This is probably related to the resumption of international travel, which in many cases required performing the RT-PCR test before departure, as well as the dominance

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of various SARS-CoV-2 variants with greater infectivity and transmissibility.

The changes in the number of infections noted by us at the end of 2021 and the beginning of 2022 corresponded to the epidemiological situation worldwide. In the fall of 2021, European countries saw a sharp increase in infections, with most cases caused by B.1.617.2 (delta). This variant of SARS-CoV-2 has been classified as a variant of concern (VOC). It became dominant in the US and the European Region by June/July 2021 [34]. By October 2021, the European Center for Disease Prevention and Control had identified 2 other VOCs, B.1.351 (beta) and P.1. (gamma), and both showed community spread. In addition, 3 variants of interest (VOI) were also distinguished – B.1.621 (mu) and C.37 (lambda) – both of which were detected sporadically. Variant B.1.1.7 (alpha), dominant at the beginning of 2021, was considered non-existent in Europe [35].

The increase in positive results from November 2021 to February 2022 is unrelated to the loss of vaccine immunity from the previous entire vaccination course [36]. It has been suggested that the antibody titer is reduced over time after vaccination [37,38]. However, the minimum threshold levels of IgG and IgM antibodies that protect against infection have not been identified. The total levels of anti-S or anti-S1-RBD IgG antibodies, most commonly measured to assess vaccine response, are not directly correlated with the neutralizing power. Vaccine-induced adaptive cellular immunity plays a critical role in the antiviral response [39,40]. In November 2021, a new variant of the SARS-CoV-2 Omicron virus was identified and classified by the WHO as a VOC due to high transmissibility, also obtained by vaccination against SARS-CoV-2. This argument supports the size of the wave recorded in early 2022.

On further analysis, we assessed the importance of demographic data (i.e., sex and age) for susceptibility to SARS-CoV-2 infection. We found significantly more positive results in women than in men (210 138 women (54.55%) vs 175 053 men (45.45%); χ^2=42.7259; P<0.00001), although the available literature does not directly indicate sex differences in susceptibility to infection.

Jin et al concluded that there was no difference in susceptibility to SARS-CoV-2 infections after they assessed the data deposited with the Chinese Public Health Science Data Center [41].

In a study evaluating 44 672 confirmed cases of infection up to February 11, 2020, in the Chinese population, it was shown that 51.4% of those infected were men [42].

Stokes et al analyzed the profile of SARS-CoV-2 infections in the USA in the first 5 months of 2020, showing a similar incidence in both sexes (48.9% men vs 51.1% women) [43].

Gebhard et al indicated that the incidence of SARS-CoV-2 infection was not related to sex after evaluating data from China, Italy, Spain, France, Germany, and Switzerland on April 1, 2020. At the same time, they noted that SARS-CoV-2 infection was more common in men aged >60 years compared to women. The above-mentioned absolute number of older men is lower than that of women [42], although our analysis did not confirm this (50 372 women aged >60 years; 56.2% vs 39 203 men aged > 60 years; 43%). Nevertheless, the incidence of SARS-CoV-2 infection in Poland by sex presented by us is very similar to that published by the Chief Sanitary Inspectorate, which indicated that 55.2% of cases occurred in women and 44.8% in men [44]. Furthermore, a similar incidence of SARS-CoV-2 was recorded in South Korea between January 2019 and March 2020 – 2621 women (62%) vs 1591 men (38%) [45]. However, no scientific studies have analyzed the susceptibility to SARS-CoV-2 in the Polish population according to sex. Therefore, our study is a milestone for a better understanding of the incidence of SARS-CoV-2 and its diversity.

First, people aged 30 to 49 years tend to have young school-aged children who are more susceptible to viral infections. They also constitute a viral reservoir, as the course of COVID-19 is often mild or asymptomatic. Despite the introduction of distance learning for an extended period of the pandemic in Poland, these children had contact with their peers, such as during time spent playing together. We cannot forget the group of children who, due to their age, do not have compulsory schooling [46-49]. This group of people is the most professionally and socially active. Even with the possibility of remote work, people aged 30 to 49 have a social role, shop, help older parents/people, and come in contact with groups of people. Our observations are consistent with those of Sobotka et al, who showed a significantly higher rate of infections among people of working age, including women, because of their higher participation in healthcare-related occupations [50]. The smaller share of senior patients, including people aged >80 years, suggests confirmation of social responsibility, adhering to the guidelines, leaving the place of residence only in critical situations, and awareness of the importance of vaccinations and prophylaxis. Interestingly, some of them can be considered both in the category of limitations of the conducted study and its strengths.

The work's strengths include that the results came from a certified laboratory and cover up to 27 months. During the SARS-CoV-2 pandemic, the Gyncentrum laboratory achieved a record throughput, playing an extremely important role in testing and epidemiological prevention in the regional arena (southern Poland) and on a national scale. To the best of our knowledge, this is the first study on the dynamics of SARS-CoV-2 infections in Polish society, differentiated by sex and age range. An important fact is that the collection of the samples for research and the individual stages of molecular analysis were

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always carried out by the same team of specialists, which significantly increased the reliability of the obtained results.

Nevertheless, it should be emphasized that this was a single-center study; therefore, the results should be applied with caution to the entire Polish population. Nevertheless, a significant limitation of our analysis is its retrospective nature and, therefore, the inability to evaluate the obtained results using criteria other than sex and age. In implementing similar projects, it would be reasonable to collect data on the person who ordered the study and its purpose. In addition, the information for the patient regarding consent to swab collection and SARS-CoV-2 detection did not require an indication of whether the person is vaccinated or not, which to some extent may affect the structure of the results.

Conclusions

Our analysis is the first study of this kind in the Polish population. We showed a significantly higher risk of SARS-CoV-2-positive results in women than in men and in the 30 to 49 years working age group compared to other groups. Our results showed that Polish society has developed a sense of responsibility for its health and that of others and that it adheres to the recommendations. Comparing the infection profile during the 27 months of observation in Poland with other countries, it seems that the policy of restrictions in our country was similar to that in the European Union, and the incidence peaks probably depend on the dominant variant of the coronavirus in that period and its virulence.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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