**The clinical anatomy of SARS-CoV-2 variants of concern in central Greece during October 2020 – July 2022**

Ms. Voulgaridi Ioanna1\*,, Ms. Bogogiannidou Zacharoula1\*, Dr. Dadouli Katerina1, Mr. Galanopoulos P. Achilleas1,2, Dr. Kyritsi A. Maria1, Dr. Vontas Alexandros1, Ms. Matziri Alexia1, Ms. Kola Konstantina1, Ms. Vachtsioli Evangelia1, Ms. Anagnostopoulos Lemonia 1, Ms. Tsispara Anastasia3, Dr. Oikonomou G. Katerina 4, Mr. Babalis Dimitris3, Prof. Petinaki Efthimia5, Asst. Prof. Tseroni Maria6, Asst. Prof. Kalala Fani2, Prof. Speletas Matthaios2, Assoc. Prof. Mouchtouri A. Varvara1, Prof. Hadjichristodoulou Christos1

1 Laboratory of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, Larissa, Greece; [ioavoulg@uth.gr](mailto:ioavoulg@uth.gr) (VI); [zbogogiannidou@uth.gr](mailto:zbogogiannidou@uth.gr) (BZ); [adadouli@uth.gr](mailto:adadouli@uth.gr) (DK); [acgalanopoulos@uth.gr](mailto:acgalanopoulos@uth.gr) (GPA); [mkiritsi@uth.gr](mailto:mkiritsi@uth.gr) (KAM); [avontas@uth.gr](mailto:avontas@uth.gr) (VA); [alexmatz@uth.gr](mailto:alexmatz@uth.gr) (MA); [kokola@uth.gr](mailto:kokola@uth.gr) (KK); [evachtsioli@uth.gr](mailto:evachtsioli@uth.gr) (VE); [lanagnost@uth.gr](mailto:lanagnost@uth.gr) (AL); [mouchtourib@uth.gr](mailto:mouchtourib@uth.gr) (MAV); [xhatzi@uth.gr](mailto:xhatzi@uth.gr) (HC).

2 Department of Immunology and Histocompatibility, Faculty of Medicine, University of Thessaly, Larissa, Greece; [fkalala@uth.gr](mailto:fkalala@uth.gr) (KF); [maspel@uth.gr](mailto:maspel@uth.gr) (SM).

3 Emergency Department, General Hospital of Larissa, Larissa, Greece; [tsispara@gmail.com](mailto:tsispara@gmail.com) (TA); [dbabales@yahoo.com](mailto:dbabales@yahoo.com) (BD).

4 Intensive Care Unit, General Hospital of Larissa, Larissa, Greece; [oikonomoukaterina85@gmail.com](mailto:oikonomoukaterina85@gmail.com) (OGK).

5 Department of Microbiology, University Hospital of Larissa, University of Thessaly, Larissa, Greece; [petinaki@uth.gr](mailto:petinaki@uth.gr) (PE).

6 Department of Nursing, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece; [mtseroni@nurs.uoa.gr](mailto:mtseroni@nurs.uoa.gr) (TM).

\*These authors contributed equally.

The study was performed at the Laboratory of Hygiene and Epidemiology, University of Thessaly, Greece.

**Correspondence:** Prof. Hadjichristodoulou Christos, 22 Papakyriazi str, 41222, Larissa, Greece

Tel: (0030) 2410 565007 | Email: [xhatzi@uth.gr](mailto:xhatzi@uth.gr)

**Shortened title/Running head:** Clinical differences among SAR-CoV-2 VOCs.

**Abstract**

The emergence of SARS-CoV-2 variants of concern (VOCs) during the COVID-19 pandemic necessitates investigation into their clinical differentiation and outcomes. This study aimed to examine these differences among VOCs, considering multiple related factors. An observational cohort study was conducted on patients diagnosed with SARS-CoV-2 infection via nasopharyngeal/oropharyngeal swab who visited the emergency department of a public Greek hospital between October 2020 to July 2022, during different VOC circulation in the region. Data on clinical manifestations, outcomes, medical history (comorbidities, prior SARS-CoV-2 infection, vaccination status against COVID-19) were collected through a questionnaire and medical records for those hospitalized. A total of 913 patients were included in the study (813 adults ≥18 years old, 100 children <18 years old). Significant differences were observed across VOCs for both adults and children. Lower proportion of children developed symptoms during the non-Omicron variants, 73.5%, compared to Omicron variants, 86.4%. Fever, dyspnoea, taste and smell disorders were observed more frequently among non-Omicron adult cases, in contrast to upper respiratory symptoms, which were more common symptoms among Omicron infections. The non-Omicron variants were associated with higher rates of hospitalization 30.6%, pneumonia 23.0%, and death 6.1%, compared to Omicron variants: 8.0%, 5.0%, and 1.8%, respectively. Vaccination against COVID-19 was shown to be a protective factor for severe outcomes. Our findings suggest distinct clinical presentations and outcomes associated with different VOCs. Despite the fact that current VOCs circulating less severe, COVID-19 vaccine continues to play a protective role for severe cases.

**Key words:** SARS-CoV-2, variants of concern (VOCs), outcomes of COVID-19, differences in symptomatology, differences in severity, vaccination.

1. **INTRODUCTION**

On 31 December 2019, the first cluster of coronavirus disease 2019 (COVID-19) cases was declared in Wuhan, Hubei Province, China, as cases of pneumonia of unknown aetiology. Twelve days later, the virus's genetic sequence was announced and classified in genus Beta coronavirus.

Similar to other RNA viruses, through mutation of its genetic elements severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has the ability to evolve, resulting in diversification of characteristics such as environmental survival, contagiousness and pathogenicity. SARS-CoV-2 mutations led to the emergence of multiple variants, with different characteristics compared to their ancestral strain, wild type (WT) (Wuhan Hu 1).1 Just one alteration in an amino acid could significantly impact the virus's capacity to evade the immune system, nullifying an existing effective vaccine.1 The World Health Organization (WHO) declared that certain variants were of higher concern due to increased risk and greater impact on public health; this prompted the characterization of specific variants of interest (VOIs).2 According to WHO, a SARS-CoV-2 variant is characterized as a VOI when the first genetic changes are predicted or known to affect virus characteristics such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape; and when causes significant community transmission or multiple COVID-19 clusters in multiple countries, with increasing relative prevalence alongside an increasing number of cases over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health.2 A SARS-CoV-2 variant is characterized as a variant of concern (VOC) when it meets the definition of a VOI, and through comparative assessment has a demonstrated association with one or more of the following changes of global public health significance: 1) increase in transmissibility or detrimental change in COVID-19 epidemiology; 2) increase in virulence or change in clinical disease presentation; 3) decrease in effectiveness of public health and social measures (PHSM) or available diagnostics, vaccines, or therapeutics.2

As of July 2022, nine VOCs were identified from the start of the pandemic: alpha (B.1.1.7) was the first VOC described in the United Kingdom (UK) in late December 2020; this was followed by beta (B.1.351) first reported in South Africa in December 2020, gamma (P.1.) initially documented in November 2020 in Brazil, Delta (B.1.617.2) first reported in India in December 2020, Omicron BA.1 (B.1.1.529.1) initially reported in South Africa in November 2021, and Omicron BA.2 (B.1.1.529.2), Omicron BA.3 (B.1.1.529.3), Omicron BA.4 (B.1.1.529.4) and Omicron BA.5 (B.1.1.529.5). VOCs are associated with enhanced transmissibility or virulence, a reduction in neutralization by antibodies obtained through natural infection or vaccination, the ability to escape the immune system, or a decrease in effective of therapeutics or vaccination.3,1

The clinical impact of VOCs varies, with multiple factors seeming to play an important role in COVID-19 severity. Our study aims to identify differences in the clinical image and outcomes of COVID-19 among different SARS-CoV-2 VOCs, considering the demographic variables of participants, their underlying diseases, and medical history of previous SARS-CoV-2 infection or vaccination against COVID-19.

# MATERIALS AND METHODS

* 1. **Study design and participants**

An observational cohort study was conducted in a region of central Greece from October 2020 to July 2022. Our participants were derived from individuals who tested positive for SARS-CoV-2 infection from nasopharyngeal/oropharyngeal samples. These individuals had presented to a public hospital emergency department due to symptoms compatible with COVID-19 or close contact with a confirmed COVID-19 case. Participants were randomly selected among laboratory-confirmed cases, and were contacted to obtain information regarding symptoms and outcomes. During the study design phase, we aimed to have an equal number of participants in each variant group. Matching among participants was conducted to adjust for age and sex.

The study utilized a closed-ended telephone or paper-based questionnaire to gather information about participants’ demographic data, comorbidities, clinical symptoms due to COVID-19 (fever, cough, sore throat, nasal congestion or runny nose, headache, fatigue, taste or smell disorders, gastrointestinal disorders, dyspnoea etc.) and treatment, SARS-CoV-2 infection outcome (diagnosis of pneumonia, hospitalization, admission to High Dependency care Units (HDU), admission to Intensive Care Units (ICU), intubation, death) and medical history of previous SARS-CoV-2 infection. Telephone interviews were conducted fifteen days following the diagnosis of SARS-CoV-2 infection. In case of persistent symptomology, interviews were repeated one month following the first interview. Information regarding hospitalized patients or participants with a history of hospitalization due to COVID-19 was obtained from their medical records. Following the implementation of the national vaccination strategy, information was also collected regarding vaccine administration, vaccination date, vaccine type and number of doses.

* 1. **Laboratory analysis**

Respiratory samples (nasopharyngeal or oropharyngeal swabs) were collected in transfer tubes containing viral transport medium. Samples were analyzed at the Laboratory of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, which functioned as a Public Health Laboratory to address the heightened demands resulting from the COVID-19 pandemic. SARS-CoV-2 RNA was isolated from samples with the KingFisher Flex System (ThermoFisher Scientific, Waltham, MA, USA) using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems™, Waltham, MA, USA) according to the manufacturer’s instructions. Detection of the virus’s genetic material was performed using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), with primers targeting SARS-CoV-2 specific genes: ORF1ab (Open Reading Frames), N protein (Nucleocapsid protein) and S protein (Spike protein) with the TaqPath™ COVID‐19 CE‐IVD RT‐PCR Kit (Applied Biosystems™, Waltham, MA, USA) on a validated QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific, Waltham, MA, USA). The threshold for positivity ≤37 cycle threshold (Ct) for SARS-CoV-2 infection was established.

Participants’ classification by VOC was first screened using as a marker the detection or absence of the S gene via RT-qPCR, known as “S gene dropout” or “spike gene target failure”.4,5,6 During the study period, a proportion of the total number of each variant was confirmed with Next Generation Sequencing (NGS). The NGS analysis was performed with the Illumina COVIDSeq™ Assay (Research Use Only (RUO)) (96 samples), which incorporates the ARTIC v4.0 [multiplex PCR](https://www.sciencedirect.com/topics/medicine-and-dentistry/multiplex-polymerase-chain-reaction) protocol on a NextSeq2000 sequencing system (Illumina Inc. USA). To identify SARS-CoV-2 variants, Illumina’s DRAGEN COVID [Lineage](https://www.sciencedirect.com/topics/immunology-and-microbiology/lineages) App in BaseSpace™ (pipeline and lineage tool) (RUO) v1.3.0 was used.

RT-qPCR tests and the majority of NGS sample analyses were conducted at the Laboratory of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly located in Larissa, Greece. The remaining NGS sample analyses were performed at the central laboratory of the National Public Health Organization (NPHO) in Athens, Greece.

* 1. **Classification of COVID-19 severity and vaccination status**

We categorized the severity of COVID-19 cases into four classes including: a) severity 0: no referred symptoms; b) severity 1: mild symptoms; c) severity 2: severe disease (hospitalization, including HDU admission and excluding ICU admission); and d) severity 3: critical care patients (ICU admission, intubation) or death caused by SARS-CoV-2 infection.

Participants were considered fully vaccinated with at least the minimum doses required for complete immunization, according to manufacturers’ directions and guidelines from the NPHO for each vaccine type.

* 1. **Statistical analysis**

A total of 180 samples were estimated for each VOC, considering the anticipated proportion of individuals who may decline the interview or not respond. Categorical variables were described using frequency and relative frequency, while continuous variables were described using medians and interquartile range (IQR). Associations between categorical variables were tested using the chi-squared test, while the Mann-Whitney or Kruskal-Wallis test were used to examine associations between categorical and continuous variables. Data were checked for deviation from normal distribution using the Shapiro-Wilk normality test. The “variants” were used as 6-value variables as well as binary variables, as defined by the number of participants related to the under-study outcome. To estimate the effect of age, sex, comorbidities, predominant variant, and vaccination on disease outcomes, we used odds ratios (ORs) with a 95% confidence interval (CI), estimated with binary logistic regression models. Furthermore, to compare the relative incidence of each symptom between one variant and the remaining, we also used ORs with 95% CI estimated with binary logistic regression models. These models were adjusted for age, sex and vaccination status. For all analyses, a 5% significance level was set. Analysis was carried out with R language.7 “Table1”, “sjPlot” and “tidyverse” packages were employed to conduct the analysis. The graphical representations Figure 2, Figure 4 and S. Figure 1 were conducted using the ggplot2 package (version 3.5.0).8 Bar plot in Figure 2 was created through the function geom\_col and area plots in Figure 4 and S. Figure 1 were created through the function geom\_area.

* 1. **Ethical statement**

The research protocol was approved by the Research Ethics Committee of the Faculty of Medicine, University of Thessaly, Greece (84 / 09.12.2022), and was also approved by the Scientific Council of the public hospital from which the sample was derived (19535 / 28.06.2023).

1. **RESULTS**

**3.1** **Characteristics and clinical image of participants with laboratory-confirmed SARS-CoV-2 infection.**

Between October 2020 and July 2022, from a total 1,075 randomly selected individuals, 913 responded positively and participated in the study (response rate 85%).

**3.1.1** **Demographic characteristics of children (< 18 years old) with laboratory-confirmed SARS-CoV-2 infection.**

Among 100 minor participants, 60 (60%) were male; their ages ranged from 2.5 months to 17 years old (median: 11 years, IQR: 8 – 13 years). From the total number of children, 34 were infected by a non-Omicron variant: 11 by WTS, eight by the Alpha variant, and 15 by Delta variant. Meanwhile 66 were infected by the Omicron variant, with 30 cases attribute to Omicron BA.1, 16 to Omicron BA.2, and 20 to Omicron BA.4/ΒΑ.5. S.Table I details the medical history of minor participants, including their vaccination status against COVID-19 and/or previous SARS-CoV-2 infection.

**3.1.2 Characteristics of symptoms due to COVID-19 in children (< 18 years old).**

As presented in Table I, 82 children developed symptomatology (82%). The proportions of symptomatic infected children showed a statistically significant difference among VOCs (p = 0.039). Children infected by Omicron variants developed more frequently symptoms compared to non-Omicron variants (Omicron: 86.4%, non-Omicron: 73.5%) with Omicron ΒΑ.4/BA.5 and Omicron BA.2 presenting the two highest proportions of symptomatic cases (ΒΑ.4/BA.5: 95%, ΒΑ.2: 93.8%). Fever, nasal congestion/discharge and cough were the most common symptoms, with fever developing in 62 of 100 children (62%), nasal congestion/discharge in 37 children (37%) and cough in 36 children (36%). Presence of fever was more common for the Omicron BA.2 and Delta variants (14/16   
(87.5%) and 12/15 (80%)) compared to the remaining variants (p= 0.046). The longest duration of fever recorded was three days, referring to cases of infection by the Delta variant. As presented in Table I, symptoms caused by upper respiratory tract infection such as cough, nasal congestion/discharge and sore throat were primarily observed in cases of Omicron variant infection; 26 of 66 Omicron cases presented cough (39.4%), 31 developed nasal congestion/discharge (47%), and 19 experienced sore throat (28.8%). Smell and taste disorders were recorded more frequently among infection by the Delta variant, observed among five of 15 cases (33.3%). Although clinical manifestations from the gastrointestinal (GI) tract were more commonly reported among Omicron cases (Omicron: 10.6% versus non-Omicron: 2.9%), statistically significant difference was not identified among the VOCs (p=0.662). An overview of the clinical picture is detailed in Table I. Among the pediatric population studied, no cases with severity 3, and few cases from each variant were characterized as severity 2 (Table I).

**3.1.3** **Demographic** **characteristics of adults (**≥**18 years old) with laboratory-confirmed SARS-CoV-2 infection.**

In total, 813 adults (≥18 years old) participated in the study and their age ranged from 18 to 100 years old (median: 47 years old, IQR: 35-60 years old) with 464 of them being female (57.1%). Descriptive data about sex, age, comorbidities, medical history of previous SARS-CoV-2 infection and vaccination per VOC are cited in S.Table II.

**3.1.4 Characteristics of symptoms due to COVID-19 in adults (≥18 years old).**

Among all adult participants, 762 reported experiencing at least one symptom (93.7%). No statistically significant difference was found in the proportions of COVID-19 cases developed clinical manifestations among VOCs (p=0.813). The duration of symptoms was observed to last longer in cases of non-Omicron variants compared to Omicron variants (p <0.001) (Table II). Regarding symptom duration, 14 Delta cases (9.9%) and 15 Alpha cases (9.6%) reported symptoms lasting longer than 20 days, while eight WTS cases (4.5%) and two Omicron cases (0.8%) reported durations exceeding 20 days.

Table II presents the total number of symptoms and their frequency for each variant. Fever, dyspnoea, taste and smell disorders were observed more frequently among non-Omicron cases, in contrast to sore throat and nasal congestion/discharge, which were more common symptoms among Omicron infections. From the total number of cases infected by non-Omicron variants (N=474), 381 experienced fever (80.4%), 111 experienced dyspnoea (23.4%), 164 experienced taste disorders (34.6%), and 146 experienced smell disorders (30.8%). In comparison, among Omicron infected cases (N=339) 226 presented fever (66.7%), 27 experienced breathing difficulties (8.0%), 45 experienced taste disorders (13.3%), and 37 experienced smell disorders (10.9%). Regarding the most commonly developed symptoms among Omicron variant infections, 139 cases experienced sore throat (41.0%) and 168 cases reported nasal congestion/discharge (49.6%). In contrast, among non-Omicron variant infections, only 98 cases experienced sore throat (20.7%) and 101 cases reported nasal congestion/discharge (21.3%).

Figure 1 presents the OR for developing the symptom under study in each variant versus the remaining VOCs. Fever and dyspnoea were more common among the Delta variant (OR= 1.58 and OR= 1.96, respectively). Symptoms from the upper respiratory system (sore throat and nasal congestion) appeared more frequently in the Omicron VOCs. Taste and smell disorders were primarily observed among non-Omicron variants, particularly WTS and Delta (WTS: taste disorders OR= 2.16 and smell disorders OR= 2.39; Delta: taste disorders OR= 1.32 and smell disorders OR= 1.69). As presented in Figure 1, GI symptoms and appetite disorders were more likely to have been reported in infections by the Alpha and Omicron ΒΑ.4/BA.5 variants (Alpha: GI OR= 1.99 and appetite disorders OR= 1.93, Omicron ΒΑ.4/BA.5: GI OR= 2.58 and appetite disorders OR= 1.91).

**3.2 Total findings regarding clinical manifestations in the entire study population.**

Figure 2 illustrates the comparison of symptoms experienced by adults and children. Fever and dyspnoea were more common among adults infected by non-Omicron than by Omicron variants (fever: OR=2.05, p< 0.001, dyspnoea: OR=3.53, p< 0.001), while headache and fatigue were more frequent among Omicron adult cases (headache: OR=1.43, p= 0.012, fatigue: OR=1.41, p= 0.011). In both age groups, upper respiratory symptoms were more commonly observed in cases of the Omicron variant (adults: OR=0.44, p< 0.001, children OR=0.31, p= 0.001). Olfactory and gustatory disorders were more commonly reported in non-Omicron variants (adults: OR=3.44, p< 0.001, children: OR= 5.05, p= 0.024). In S.Figure 1, the percent proportion and the course of the most common symptoms reported by the entire study population, including adults and children, are presented.

**3.3 Severity of COVID-19 in adults (≥18 years old).**

Table III presents the outcomes of COVID-19 among adults categorized as severity 2 or 3 – there is no respective section for children due to the low number of reported cases classified as severity level 2 and the complete absence of cases classified as severity level 3. The Alpha and Delta variants were responsible for higher number of hospitalizations, specifically 58 of 156 (37.2%) and 51 of 142 (35.9%), respectively (p< 0.001). These two variants caused pneumonia and death more frequently than the remaining VOCs (pneumonia: Delta: 41/142 (28.9%) and Alpha: 39/156 (25.0%), p< 0.001; death: Delta: 13/142 (9.2%) and Alpha: 11/156 (7.1%), p= 0.004). HDU admission, ICU admission and intubation were recorded more frequently in Delta and WTS, without a statistically significant difference. The frequency of COVID-19 outcomes categorized as severity 2 or 3 is provided in Figure 3.

The association between specific risk factors and the risk of outcomes in adults with severity 2 or 3 was examined through multivariate analysis (Table IV). For each outcome of severity 3, multivariate analysis was restricted to non-Omicron cases due to few Omicron patients. Age was estimated as a risk factor for every critical outcome. Vaccination was identified as a stable protective agent for hospitalization and pneumonia in both non-Omicron and Omicron variants (p< 0.001). Immunization via vaccine appeared to reduce the possibility of ICU admission and intubation in non-Omicron cases (ICU admission: OR: 0.16, p=0.098; intubation: OR: 0.15, p=0.085). The risk of death among vaccinated cases infected by a non-Omicron variant was approximately five times lower, compared to unvaccinated cases infected by the same variant (OR: 0.22, p= 0.028). Considering the different outcomes of severity 3 as a common outcome, history of vaccination was evaluated as a statistically significant protective factor (OR= 0.19, p= 0.015) (S.Table III). The rest results of this multivariate analysis are presented in S.Table III. Restricting the multivariate analysis in unvaccinated adults, age, male sex, respiratory system disorders, malignancies and metabolic disorders/endocrinopathies were associated with poorer prognosis (S.Table IV).

Figure 4 illustrates the morbidity and variation of COVID-19 severity by VOC among unvaccinated and vaccinated participants. Immunization via vaccines against COVID-19 appeared to reduce the severity. Hospitalization, HDU/ICU admission, intubation and death caused by SARS-CoV-2 infection were more commonly recorded among unvaccinated individuals, while the possibility of experiencing mild symptomology was higher in vaccinated individuals.

1. **DISCUSSION**

In this study, we analyzed data collected from 913 COVID-19 cases in a region of central Greece between October 2020 to July 2022. Clinical manifestations and outcomes among participants infected by different SARS-CoV-2 VOCs were compared. We observed that in adults and children, symptom profiles differed based on etiologic variants.

The majority of pediatric COVID-19 patients developed mild symptoms, with fewer than 20% remaining completely asymptomatic. A similar proportion has been reported in another study, in which 25% of children did not experience symptoms.9 In our study, children infected by Omicron variants developed clinical manifestations more frequently compared to those infected by the non-Omicron variant: Omicron: 86.4%, non-Omicron 73.5%. However, Delta variant was found to cause similar proportion of symptomatic cases (86.7%) as Omicron variants (86.4%), followed by Alpha: 75.0%, and WTS: 54.5. Similar results were reported in another study, where among children seen in an emergency department of a Canadian hospital, 92.7% of those infected with Omicron and 91.6% with Delta developed symptoms, while the proportion of symptomatic children infected by other VOCs were less than 90% (WTS: 86.6%, Alpha: 82.3%).10

In our study, the majority of adults developed symptoms, with only 6% declared completely asymptomatic. No statistically significant differences were found in the proportions of asymptomatic cases among VOCs. A high heterogeneity in the proportion of asymptomatic cases is observed among studies.11,12 A meta-analysis which included data up to 4 February 2021 found the pooled percentage of asymptomatic cases was 40.50% (95% CI: 33.5%-47.5%; I2 = 99%; p < 0.001).12 In this review, the percentage of asymptomatic ranged from 3.7% to 87.5%. The proportion of asymptomatic individuals in our study was estimated among the lowest from those reported in the aforementioned literature. This could be attributed to the fact that our sample was drawn from cases admitted to the hospital emergency department. It is also worth noting that the NPHO organized daily COVID-19 surveillance points. Therefore, a proportion of asymptomatic individuals may have been diagnosed at these facilities as they did not require medical assistance.

Considering that the majority of COVID-19 cases experienced symptoms, their duration could be further studied. Non-Omicron VOCs more frequently caused clinical presentations exceeding 20 days, with the Delta variant being prevalent (9.9%), followed by Alpha (9.6%) and WTS (4.5%). While several studies related to long COVID-19 exist in the literature, the duration for acute infection for each specific variant has not been extensively studied or reported in a systematic manner.

Regarding symptomatology in children, fever and cough were the most common symptoms among Delta and Omicron infections; fever was reported by 80.0% and cough by 60.0% of Delta cases, whereas the corresponding percentages were 62.1% and 39.4% in Omicron cases. Similar findings have been reported in other studies.10,13,14 In adults, fever was the most frequently self-reported symptom in all waves. Incidence of fever ranged from 81.8% during the WTS wave to 64.8% during the Omicron BA.1 wave. Flisiak R et al. also recorded a steady decline of fever until the predominance the Omicron variant.15 Cough was recorded at high rates throughout all stages of our study. This observation is consistent with findings from a study by DeWitt et al., who also noted cough as the most frequently reported symptom, albeit at higher rates.16

Children and adults diagnosed with Omicron more frequently experienced symptoms of the upper respiratory tract. In particular, 28.8% of children with Omicron infection experienced sore throat and 47.0% experienced nasal congestion/discharge, while 41.0% of adults infected by the same variant developed sore throat and 49.6% nasal congestion/discharge. Similar results have been reported in other studies.17, 18 Menni et al. observed that sore throat and hoarse voice were more prevalent among Omicron cases compared to Delta infections.18 This finding may be attributed to mutations primarily occurring in the Spike protein, which enhance the virus's affinity for binding to receptors in the upper respiratory system and decrease tissue tropism of Omicron in lungs compared to previous strains.19,20,21

During the Omicron wave, a decrease in the incidence of taste and smell disorders was observed. The incidence of taste disorders ranged from 44.9% during the WTS wave to 13.3% during Omicron wave, while the incidence of smell disorders ranged from 39.8% during the WTS wave to 10.9% during the Omicron wave. This finding is supported by another study.16 During the initial phase of the pandemic, VOCs caused a higher proportion of cases with smell and taste disorders (55% during the pre-Delta period). However, there was a decline during the Delta phase and a further decrease during the Omicron VOCs (17%).16 Similar to findings by Coelho et al., olfactory and gustatory dysfunctions were more common in patients with the Alpha variant (64%) followed by Delta (57%), and less frequent in Omicron (21%); however, this study did not include patients infected with WTS.22 Data from three prospective household cohorts comparing SARS-CoV-2 symptomatology of WTS/Alpha to Omicron BA.1/BA.2 variants are in line with our results; Omicron infections were associated with lower odds of loss of smell or taste (OR: 0.14).23

Among children, GI symptoms more frequently developed in cases of Omicron (10.6%), but the difference was not calculated as statistically significant among VOCs (p= 0.662). Similar results have been found in other studies, too.24,25 A published article reported that SARS-CoV-2 RNA was more frequently present in anal swabs of patients infected with the Omicron variant compared to previous variants, a finding that may support the higher prevalence of GI symptoms among Omicron cases in our study.26 Conversely, symptoms of the GI tract among adults were self-reported more commonly in the Alpha variant (31.4%), followed by Omicron BA.4/ BA.5 (28.4%). Another study focusing on the same age group claims that pre-Delta variants more commonly resulted in manifestations from the GI tract than the Delta and Omicron variants (pre-Delta: 44.9%, Delta: 35.6%, and Omicron: 30.1%, p< 0.001).27

The frequency of GI symptoms reported in the literature vary by VOC; several possible pathophysiological explanations exist.28 Molecular perspectives support that the high ACE-2 protein expression found in intestinal epithelial cells may facilitate entry of SARS-CoV-2 into host cells.29 Thus, mutations may change virus tropism, as described above in taste and smell disorders. Alteration of gut microbiota may be another reason for GI symptoms.30,31 Since the onset of the pandemic a variety of drugs have been utilized, including antibiotics and antivirals, potentially interfering with the GI microbiota.32

Given the lifting or easing of public and personal health measures during the predominance of the Omicron variant, the higher proportion of upper respiratory tract infection or GI symptoms could be caused by other viral or bacterial co-infections. Additionally, a “test all” approach occurred during the Omicron period as rapid antigen tests were available, compared to the non-Omicron period characterized by a more selective strategy testing individuals who were unwell or experienced core COVID-19 symptoms.

During our study period, we found that cases infected by Alpha or Delta variants had higher risk for hospitalization, diagnosis of pneumonia and death compared to cases infected by the WTS or Omicron variants (during the Alpha wave we observed hospitalizations at 37.2%, diagnosis of pneumonia at 25.0%, death at 7.1%, and during the Delta wave hospitalizations at 35.9%, diagnosis of pneumonia at 28.9% and death at 9.2%; while during the WTS wave we observed hospitalizations at 20.5%, diagnosis of pneumonia at 16.5%, death at 2.8% and during Omicron hospitalizations at 8.0%, diagnosis of pneumonia at 4.1% and death at 1.8%). Our findings are consistent with other studies.33,34,35 Several factors should be considered as contributing to this outcome, which are described below.

The Omicron variant’s intrinsic virologic properties could explain its lower severity. Although the Omicron variant has demonstrated partial vaccine escape and higher transmissibility, it presents lower pathogenicity and a lower replication rate in lung tissues.33,36 This may be due to mutations near the furin cleavage site such as S655Y or the combination of mutations S477N, Q498R and N501Y in the Omicron variants’ Spike protein, as these increase the binding affinity for ACE-2 receptor.37,38 Likely owing to the tropism of Omicron, pro-inflammatory cytokines and chemokines were found lower in the lungs of Omicron infected mice than in those infected by previous variants, further explaining the lower severity in cases of Omicron.39 During the genetic makeup of the virus, it has also been reported that Omicron proved less fusogenic than the Delta variant and WTS, since the Spike protein of Omicron is less efficiently cleaved into two subunits compared to the two other variants.40,41 This could further explain the lower pathogenicity of Omicron, and subsequently the lower frequency of pneumonia diagnosis, respiratory failure/syndromes, hospitalizations, ICU admissions, intubations and deaths.42,43

Despite the variants' characteristics, it should be considered that during the predominance of the Omicron variant, a greater number of individuals were immunized via vaccination or through SARS-CoV-2 infection, either one or multiple times. In our study, vaccination coverage was over 80% during the Omicron period, and the proportion of vaccinated participants with three doses was nearly 70% during the last pandemic wave. Booster shots have proven effective in reducing the risk of severe disease and death, with the protection afforded following a booster unaffected by the initial vaccination.33,34,44 Moreover, previous SARS-CoV-2 infection has proven protective, especially in unvaccinated populations.34 During Omicron predominance, one in ten of our participants mentioned a history of COVID-19, while no participants reported SARS-CoV-2 history in pre-Omicron waves.

We found that Delta and Alpha variants displayed the highest mortality (Delta: 9.2%, Alpha: 7.1%). In other studies, Delta also presented with the highest case fatality rate, followed by Alpha.45,46,47,48 Despite the high virulence of Delta, factors including healthcare worker (HCW) burnout syndrome and exceeding capacities of hospitals/ICUs during the Delta wave should be considered. The lowest mortality was calculated in Omicron variants (1.8%); these results are similar to outcomes of other studies.43,49,50 As the pandemic progressed, lower pathogenicity of the predominant variant, the experience of HCWs, health unit staffing and enhancing ICU capacity, as well as immunity from vaccinations and previous infection could have resulted in lower COVID-19 severity. As the pandemic threatened global public health, strict compliance with public health guidelines may have inhibited other co-infections, which would had worsened clinical outcomes.

1. **LIMITATIONS**

Our study presents a number of limitations. Firstly, study participants are not representative of the general population, since they were primarily sourced from patients visiting the hospital emergency department; this could explain the higher proportion of symptomatic cases. Secondly, excluding hospitalized cases whose data were drawn from medical records, the prevalence of symptoms in non-hospitalized cases were self-reported. Moreover, strains from the Beta and Gamma variants were not included in the study, as there were not enough samples in our region. Another limitation is the continuous downward trend in participants’ response rates during the study period, resulting in a varied number of cases among each variant group. During BA.4/ BA.5 predominance, three months following the start of BA.4/ BA.5 circulation our laboratory did not receive samples for molecular analysis; this further explains the low number of participants in this group. Finally, NGS was not conducted on all samples, with the remaining samples characterized within the same period and the S gene drop out.

However, our study has several advantages. The majority of VOCs are included and compared simultaneously. Furthermore, the inclusion of all age groups provides a macroscopic overview of the clinical characteristics and outcomes of each variant. Our findings and their interpretations our enhanced as the vaccination initiation period coincides with our study period.

1. **CONCLUSIONS**

Clinical manifestations and COVID-19 outcomes appeared to differ during each VOC predominant period. As the pandemic progressed the proportion of severe cases decreased, while mild/moderate cases increased. This could be attributed to characteristics of the virus, the host, the virus-host interaction and the effect of environmental factors. In our study, vaccination lessened severe COVID-19 outcomes. At present, vaccination could be one of the most effective and feasible approaches to prevent adverse health impacts. Despite the fact that current circulating VOCs are less virulent, COVID-19 vaccine continues to play a protective role for severe outcomes.

**AUTHOR CONTRIBUTIONS**

**Voulgaridi Ioanna**: data collection; data curation; original draft preparation; writing - review and editing. **Bogogiannidou Zacharoula**: data collection; data curation; original draft preparation; writing - review and editing. **Dadouli Katerina**: statistical analysis and visualization. **Galanopoulos P. Achilleas**: statistical analysis and visualization. **Vontas Alexandros**: laboratory analysis**. Kyritsi A. Maria**: laboratory analysis and original draft preparation. **Matziri Alexia**: laboratory analysis. **Kola Konstantina**: laboratory analysis. **Vachtsioli Evangelia**: laboratory analysis**. Anagnostopoulos Lemonia**: writing - review and editing. **Tsispara Anastasia**: data curation. **Oikonomou G. Katerina**: data curation. **Babalis Dimitris**: data curation. **Petinaki Efthimia**: supervision; laboratory analysis and writing - review and editing. **Tseroni Maria**: supervision and writing - review and editing. **Kalala Fani**: supervision and writing - review and editing. **Speletas Matthaios**: supervision and writing - review and editing. **Mouchtouri A. Varvara**: conceptualization; methodology; supervision; writing - review and editing. **Hadjichristodoulou Christos:** conceptualization; methodology; supervision; and writing - review and editing. All authors have read and approved the manuscript.

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data supporting the findings are available, only for sections non-infringing personal information, from the corresponding author upon reasonable request.

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**ORCID**

Voulgaridi Ioanna <https://orcid.org/0000-0002-7352-0934>

Bogogiannidou Zacharoula <https://orcid.org/0000-0001-8908-6709>

Dadouli Katerina <https://orcid.org/0000-0002-8821-0795>

Galanopoulos P. Achilleas <https://orcid.org/0009-0008-4692-7027>

Kyritsi A. Maria <https://orcid.org/0000-0002-4689-4623>

Vontas Alexandros <https://orcid.org/0000-0001-5424-0235>

Anagnostopoulos Lemonia <https://orcid.org/0000-0002-0654-1620>

Petinaki Efthimia <https://orcid.org/0000-0002-9702-7925>

Tseroni Maria <https://orcid.org/0000-0003-1463-7056>

Kalala Fani <https://orcid.org/0009-0001-5588-8014>

Speletas Matthaios <https://orcid.org/0000-0003-1287-7734>

Mouchtouri A. Varvara <https://orcid.org/0000-0002-2535-3596>

Hadjichristodoulou Christos <https://orcid.org/0000-0002-4769-8376>

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**REFERENCES**

1. Aleem A, Akbar Samad AB, Vaqar S. Emerging Variants of SARS-CoV-2 and Novel Therapeutics Against Coronavirus (COVID-19). 2023 May 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Accessed April 18, 2024. https://pubmed.ncbi.nlm.nih.gov/34033342/

2. WHO. Tracking SARS-CoV-2 variants. Accessed April 18, 2024. https://www.who.int/activities/tracking-SARS-CoV-2-variants

3. Duong D. Alpha, Beta, Delta, Gamma: What’s important to know about SARS-CoV-2 variants of concern? *CMAJ : Canadian Medical Association Journal*. 2021;193(27):E1059. doi:10.1503/CMAJ.1095949

4. WHO. Enhancing response to Omicron SARS-CoV-2 variant: Technical brief and priority actions for Member States. Accessed April 18, 2024. https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states

5. McMillen T, Jani K, Robilotti E V., Kamboj M, Babady NE. The spike gene target failure (SGTF) genomic signature is highly accurate for the identification of Alpha and Omicron SARS-CoV-2 variants. *Scientific Reports*. 2022;12(1):1-8. doi:10.1038/s41598-022-21564-y

6. Walker AS, Vihta KD, Gethings O, et al. Tracking the Emergence of SARS-CoV-2 Alpha Variant in the United Kingdom. *N Engl J Med*. 2021;385(27):2582-2585. doi:10.1056/NEJMC2103227

7. R: The R Project for Statistical Computing. Accessed May 10, 2024. https://www.r-project.org/

8. Wickham H. ggplot2: Elegant Graphics for Data Analysis (3e). Springer. Accessed May 10, 2024. https://ggplot2-book.org/

9. Dethioux L, Dauby N, Montesinos I, Rebuffat E, Hainaut M. SARS-CoV-2 seroprevalence in children and their family members, July–October 2020, Brussels. *Eur J Pediatr*. 2022;181(3):1009. doi:10.1007/S00431-021-04284-9

10. Sumner MW, Xie J, Zemek R, et al. Comparison of Symptoms Associated With SARS-CoV-2 Variants Among Children in Canada. *JAMA Netw Open*. 2023;6(3):e232328-e232328. doi:10.1001/JAMANETWORKOPEN.2023.2328

11. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis. *PLoS Med*. 2020;17(9). doi:10.1371/JOURNAL.PMED.1003346

12. Ma Q, Liu J, Liu Q, et al. Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis: A Systematic Review and Meta-analysis. *JAMA Netw Open*. 2021;4(12):e2137257. doi:10.1001/JAMANETWORKOPEN.2021.37257

13. Zhu F, Ang JY. COVID-19 Infection in Children: Diagnosis and Management. *Curr Infect Dis Rep*. 2022;24(4):51-62. doi:10.1007/S11908-022-00779-0/FIGURES/2

14. Zimmermann P, Curtis N. COVID-19 in Children, Pregnancy and Neonates: A Review of Epidemiologic and Clinical Features. *Pediatric Infectious Disease Journal*. 2020;39(6):469-477. doi:10.1097/INF.0000000000002700

15. Flisiak R, Rzymski P, Zarębska-Michaluk D, et al. Variability in the Clinical Course of COVID-19 in a Retrospective Analysis of a Large Real-World Database. *Vaccines.* 2023;15(1):149. doi:10.3390/v15010149

16. DeWitt ME, Tjaden AH, Herrington D, et al. COVID-19 Symptoms by Variant Period in the North Carolina COVID-19 Community Research Partnership, North Carolina, USA. *Emerg Infect Dis*. 2023;29(1):207-211. doi:10.3201/EID2901.221111

17. Brewster RCL, Parsons C, Laird-Gion J, et al. COVID-19-Associated Croup in Children. *Pediatrics*. 2022;149(6). doi:10.1542/PEDS.2022-056492/185378

18. Menni C, Valdes AM, Polidori L, et al. Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study. *The* *Lancet*. 2022;399. doi:10.1016/S0140-6736(22)00327-0

19. Hui KPY, Ho JCW, Cheung M Chun, et al. SARS-CoV-2 Omicron variant replication in human bronchus and lung ex vivo. *Nature*. 2022;603(7902):715-720. doi:10.1038/s41586-022-04479-6

20. Meng B, Abdullahi A, T M Ferreira IA, et al. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature*. 2022;603: 706–714. doi:10.1038/s41586-022-04474-x

21. Hui KPY, Ng KC, Ho JCW, et al. Replication of SARS-CoV-2 Omicron BA.2 variant in ex vivo cultures of the human upper and lower respiratory tract. *The Lancet of Discovery Science.* 2022;83:104232. doi: https://doi.org/10.1016/j.ebiom.2022.104232

22. Coelho DH, Reiter ER, French E, Costanzo RM. Decreasing Incidence of Chemosensory Changes by COVID-19 Variant. *Otolaryngology–Head and Neck Surgery*. 2023;168(4):704-706. doi:10.1177/01945998221097656

23. Westerhof I, De Hoog M, Ieven M, et al. The impact of variant and vaccination on SARS-CoV-2 symptomatology; three prospective household cohorts. *International Journal of Infectious Diseases*. 2023;128:140-147. doi:10.1016/j.ijid.2022.12.018

24. Iijima H, Kubota M, Ogimi C. Clinical characteristics of pediatric patients with COVID-19 between Omicron era vs. pre-Omicron era. *Journal of Infection and Chemotherapy*. 2022;28(11):1501. doi:10.1016/J.JIAC.2022.07.016

25. Tian Y, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Aliment Pharmacol Ther*. 2020;51(9):843-851. doi:10.1111/APT.15731

26. Yunjie S, Zubing M, Hao W. Characteristics and implications of Omicron variant associated digestive system infections – Correspondence. *Int J Surg.* 2022;104:106750. doi: 10.1016/j.ijsu.2022.106750.

27. Wang RC, Gottlieb M, Montoy JCC, et al. Association Between SARS-CoV-2 Variants and Frequency of Acute Symptoms: Analysis of a Multi-institutional Prospective Cohort Study—December 20, 2020—June 20, 2022. *Open Forum Infect Dis*. 2023;10(7). doi:10.1093/OFID/OFAD275

28. Jin S, Lu X, Xu C. COVID-19 induces gastrointestinal symptoms and affects patients' prognosis. *J Int Med Res.* 2022;50(10):3000605221129543. doi: 10.1177/03000605221129543. https://journals.sagepub.com/doi/epdf/10.1177/03000605221129543?src=getftr

29. Galanopoulos M, Gkeros F, Doukatas A, et al. COVID-19 pandemic: Pathophysiology and manifestations from the gastrointestinal tract. *World J Gastroenterol*. 2020;26(31):4579. doi:10.3748/WJG.V26.I31.4579

30. Zuo T, Zhang F, Lui GCY, et al. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology*. 2020;159(3):944-955.e8. doi:10.1053/J.GASTRO.2020.05.048

31. Ward D V., Bhattarai S, Rojas-Correa M, et al. THE INTESTINAL AND ORAL MICROBIOMES ARE ROBUST PREDICTORS OF COVID-19 SEVERITY THE MAIN PREDICTOR OF COVID-19-RELATED FATALITY. *medRxiv*. Published online January 6, 2021:2021.01.05.20249061. doi:10.1101/2021.01.05.20249061

32. Kujawski SA, Wong KK, Collins JP, et al. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. *Nature Medicine*. 2020;26(6):861-868. doi:10.1038/s41591-020-0877-5

33. Greene SK, Levin-Rector A, Kyaw NTT, et al. Comparative hospitalization risk for SARS-CoV-2 Omicron and Delta variant infections, by variant predominance periods and patient-level sequencing results, New York City, August 2021–January 2022. *Influenza Other Respir Viruses*. 2023;17(1):e13062. doi:10.1111/IRV.13062

34. Nyberg T, Ferguson NM, Nash SG, et al. Comparative analysis of the risks of hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2) variants in England: a cohort study. *The Lancet*. 2022;399(10332):1303-1312. doi:10.1016/S0140-6736(22)00462-7

35. Paredes MI, Lunn SM, Famulare M, et al. Associations between SARS-CoV-2 variants and risk of COVID-19 hospitalization among confirmed cases in Washington State: a retrospective cohort study. *Clinical Infectious Diseases*. 2022;75(1):e536–e544. https://doi.org/10.1093/cid/ciac279

36. Hui KPY, Ng KC, Ho JCW, et al. Replication of SARS-CoV-2 Omicron BA.2 variant in ex vivo cultures of the human upper and lower respiratory tract. *EBioMedicine*. 2022;83. doi:10.1016/j.ebiom.2022.104232

37. Escalera A, Gonzalez-Reiche AS, Aslam S, et al. Mutations in SARS-CoV-2 variants of concern link to increased spike cleavage and virus transmission. *Cell Host Microbe*. 2022;30(3):373-387.e7. doi:10.1016/J.CHOM.2022.01.006

38. Zahradník J, Marciano S, Shemesh M, et al. SARS-CoV-2 variant prediction and antiviral drug design are enabled by RBD in vitro evolution. *Nature Microbiology.* 2021;6(9):1188-1198. doi:10.1038/s41564-021-00954-4

39. Uraki R, Kiso M, Iida S, et al. Characterization and antiviral susceptibility of SARS-CoV-2 Omicron BA.2. *Nature.* 2022;607(7917):119-127. doi:10.1038/s41586-022-04856-1

40. Suzuki R, Yamasoba D, Kimura I, et al. Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant. *Nature*. 2022;603(7902):700-705. doi:10.1038/s41586-022-04462-1

41. Gupta R. SARS-CoV-2 Omicron spike mediated immune escape and tropism shift. *Res Sq*. 2022. rs.3.rs-1191837. doi: 10.21203/rs.3.rs-1191837/v1.

42. Vieillard-Baron A, Flicoteaux R, Salmona M, et al. EPIDEMIOLOGICAL CHARACTERISTICS AND SEVERITY OF OMICRON VARIANT CASES IN THE APHP CRITICAL CARE UNITS. *medRxiv*. Published online January 28, 2022:2022.01.25.22269839. doi:10.1101/2022.01.25.22269839

43. Relan P, Motaze NV, Kothari K, et al. Severity and outcomes of Omicron variant of SARS-CoV-2 compared to Delta variant and severity of Omicron sublineages: a systematic review and metanalysis. *BMJ Glob Health*. 2023;8(7):e012328. doi:10.1136/BMJGH-2023-012328

44. Bester JC. A Clinician’s Obligation to be Vaccinated: Four Arguments that Establish a Duty for Healthcare Professionals to be Vaccinated Against COVID-19. *J Bioeth Inq*. 2022;19(3):451. doi:10.1007/S11673-022-10182-Y

45. Matsumura Y, Yamamoto M, Shinohara K, et al. High mortality and morbidity among vaccinated residents infected with the SARS-CoV-2 Omicron variant during an outbreak in a nursing home in Kyoto City, Japan. *Am J Infect Control*. 2023;51(7):800-806. doi:10.1016/j.ajic.2022.09.007

46. Ward T, Glaser A, Overton CE, Carpenter B, Gent N, Seale AC. Replacement dynamics and the pathogenesis of the Alpha, Delta and Omicron variants of SARS-CoV-2. *Epidemiol Infect*. 2023;151:e32. doi:10.1017/S0950268822001935

47. Tabatabai M, Juarez PD, Matthews-Juarez P, et al. An Analysis of COVID-19 Mortality During the Dominancy of Alpha, Delta, and Omicron in the USA. *J Prim Care Community Health*. 2023;14. doi:10.1177/21501319231170164

48. Malli F, Lampropoulos IC, Perlepe G, Papagiannis D, Gourgoulianis KI. Analysis of SARS-CoV-2 Cases, COVID-19 Outcomes and Vaccinations, during the Different SARS-CoV-2 Variants in Greece. *Vaccines*. 2023;11(1). doi:10.3390/VACCINES11010126

49. Christensen PA, Olsen RJ, Long SW, et al. Signals of Significantly Increased Vaccine Breakthrough, Decreased Hospitalization Rates, and Less Severe Disease in Patients with Coronavirus Disease 2019 Caused by the Omicron Variant of Severe Acute Respiratory Syndrome Coronavirus 2 in Houston, Texas. *Am J Pathol*. 2022;192(4):642-652. doi:10.1016/J.AJPATH.2022.01.007

50. Johns Hopkins University & Medicine - Coronavirus Resource Center. COMPARING CASES, DEATHS, AND HOSPITALIZATIONS INDICATES OMICRON LESS DEADLY. Accessed April 19, 2024. https://coronavirus.jhu.edu/pandemic-data-initiative/data-outlook/comparing-cases-deaths-and-hospitalizations-indicates-omicron-less-deadly