ORIGINAL ARTICLE

COVID-19 Pandemic: Challenges, Controversies and What we have Learned

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Abstract. Introduction: *The COVID-19 pandemic put us in a very difficult situation and created a lot of challenges for both diagnostics and follow-up of patients with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). We also had difficult problems understanding the virus-host interactions and the progression of the immune response.* ***Objectives:*** *We aimed to point out our experience in the diagnostics of SARS-CoV-2 in patients admitted to Fundeni Clinical Institute. We have also investigated the healthcare personnel in order to have some epidemiologic data about the transmission of the new coronavirus in our institute.* ***Methods:*** *Three different real-time reverse transcription-polymerase chain reaction (RT-PCR) assays were used to screen for the new coronavirus infection. The immunization rate against SARS-CoV-2 was detected by assessing the IgG antibodies in both patients and clinical staff. We have used the chemiluminescence method to assess the anti-SARS-CoV-2 IgG antibodies.* ***Results:*** *Most of the diagnosed patients with SARS-CoV-2 infections were admitted to the surgery wards for hematology and gastroenterology. Our data showed that all the diagnosed patients developed IgG antibodies against SARS-CoV-2, but we have noticed that the immunization against SARS-CoV-2 did not last.* ***Conclusions:*** *Our experience with the SARS-CoV-2 pandemic emphasized that molecular diagnostics by RT-PCR was essential, together with the study of IgG antibodies against SARS-CoV-2, thus enabling us to better interpret PCR test results.*

Keywords*: Covid-19, RT-PCR, SARS-CoV-2, IgG specific antibodies.*

Abbreviations:

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2
RT-PCR: real-time polymerase chain reaction
RNA: Ribonucleic acid
Ig: Immunoglobulins

Introduction

Human coronaviruses (HCoVs) were first detected in 1965 and could induce not only common colds but also severe acute respiratory syndrome (SARS) or Middle East respiratory syndrome (MERS) [1]. SARS used to begin with flu-like symptoms. A few days later, patients could suffer from fever and respiratory distress [2]. In 2002 and 2003, the first SARS pandemic (SARS-CoV-1) occurred, and about 8000 humans were infected. From these, 9.6% died [3]. At the end of 2019, a new variant, the SARS-CoV-2, was detected in Wuhan, China, for the first time. Subsequently, this SARS-CoV-2-causing respiratory disease spread globally, prompting the World Health Organization (WHO) to designate it as coronavirus disease 2019 (COVID-19) and declare it a pandemic on March 11, 2020 [4]. The virus responsible for COVID-19 was initially named 2019-nCoV but was later renamed Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) [5]. The SARS-CoV-2 pandemic threatened healthcare providers and the whole society.

SARS-CoV-2 transmission primarily occurred through respiratory secretions. Infected individuals, whether symptomatic or asymptomatic, transmitted the virus through respiratory droplets. These droplets were released when an infected person coughed, sneezed, talked, or breathed. Close contact with an infected person increased tremendously the risk of transmission [6]. SARS-CoV-2 could also be spread through direct contact with infected individuals or indirect contact with contaminated surfaces. Touching surfaces or objects contaminated with respiratory droplets and then touching the face (eyes, nose, mouth) could lead to transmission [7]. While respiratory droplets are the primary transmission mode, there is evidence that SARS-CoV-2 could remain suspended in the air as aerosols in certain conditions. However, airborne transmission is less common than droplet transmission [8].

Although less common, other potential routes include fecal-oral transmission, bloodborne transmission, mother-to-child transmission, and animal-to-human transmission [9].

At Fundeni Clinical Institute, we have employed real-time reverse transcriptase-polymerase chain reaction (RT-PCR) methods targeting specific genes (RdRp, ORF1ab, E, N, and S) to detect SARS-CoV-2 in both patients and healthcare personnel. Our goal was to contribute to the rapid and effective diagnosis of SARS-CoV-2.

Patients and Methods

The monocentric, prospective, and observational study was carried out at Fundeni Clinical Institute. Nasal specimens were the preferred specimen collection. Tests were performed on 21876 samples by RT-PCR methods collected from both patients and healthcare personnel.

In addition, the presence of SARS-CoV-2 antibodies (in particular of the IgG class) in serum was analyzed in 400 patients with and without previous PCR tests.

We have reported the total number of tests and the overall test positivity and negativity.

Methodology

We have collected samples from both the nasal cavity and oropharynx. Digital health records were utilized to gather a range of epidemiological and clinical information.

Extraction and amplification of SARS-CoV-2

To extract SARS-CoV-2 RNA, swab specimens were obtained from a total of 23,338 individuals, comprising both patients and healthcare staff. A direct virological diagnosis was performed with three

qualitative RT-PCR assays for the detection of SARS-CoV-2. These three different reagents were from Seegene-South Korea, Bosphore-Anatolia Geneworks, Turkey, and GeneXpert, produced by Cepheid-USA. These assays were selected for method validation (interassay validation) and to ensure rapid testing capabilities for organ transplantation emergencies.

The Bosphore kit by Anatolia Geneworks was designed to detect the Orf1ab region of the genome and the N and E genes. Seegene assays were designed to identify the N, RNA-dependent RNA polymerase (RdRp), and E genes. The GeneXpert SARS-CoV-2 test, capable of identifying the E and N2 genes within an hour, was specifically applied to pre-transplantation patients' diagnoses. Procedures for the RT-PCR tests were conducted in strict adherence to the manufacturer guidelines and our laboratory standard operating procedure. The detection of IgG antibodies against the SARS-CoV-2 nucleoprotein in 1,068 individuals was done using the chemiluminescence method (SARS-CoV-2 IgG assay by Abbott in Illinois, USA). We have also analyzed the development of anti-spike antibodies following vaccination with Pfizer, AstraZeneca, and Moderna vaccines.

Statistical Analysis

Statistical analysis was done with Excel (Office 365) and OpenEpi vers.21.

Results

Our research encompassed all patients screened for SARS-CoV-2 prior to their hospital admission at the Fundeni Clinical Institute, along with 2,382 individuals from the Institute's medical staff. Testing for SARS-CoV-2 infection was performed on all study participants. The patient group had a median age of 55.72 years, with men constituting 52.21%. For the medical staff, the median age was 46 years, with 70.44% being female. The majority (35.5%) of admitted patients in our Institute were from the Bucharest-Ilfov region. The wards requesting the highest number of SARS-CoV-2 tests were gastroenterology, haematology, oncology, neurology, and urology, accounting for 75.49% of the total tests conducted (Figure 1).

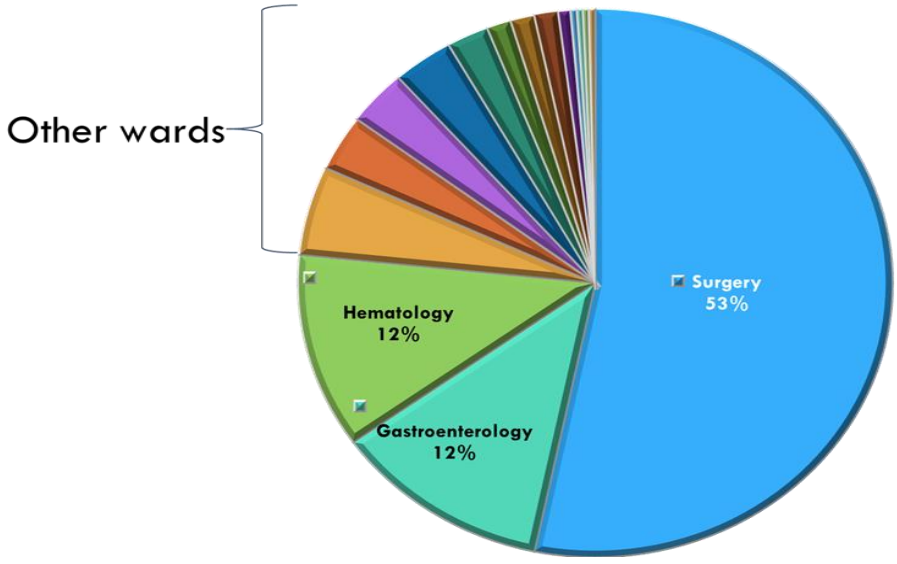

Figure 1. Wards requesting the highest number of SARS-CoV-2 tests

The RT-PCR tests showed a 7.23% positivity rate among patients, all of whom had one or more comorbidities, such as diabetes and obesity, and were between the ages of 1 and 101. The age group 39–74 years saw the highest incidence of positive cases, at 72.42% (Figure 2).

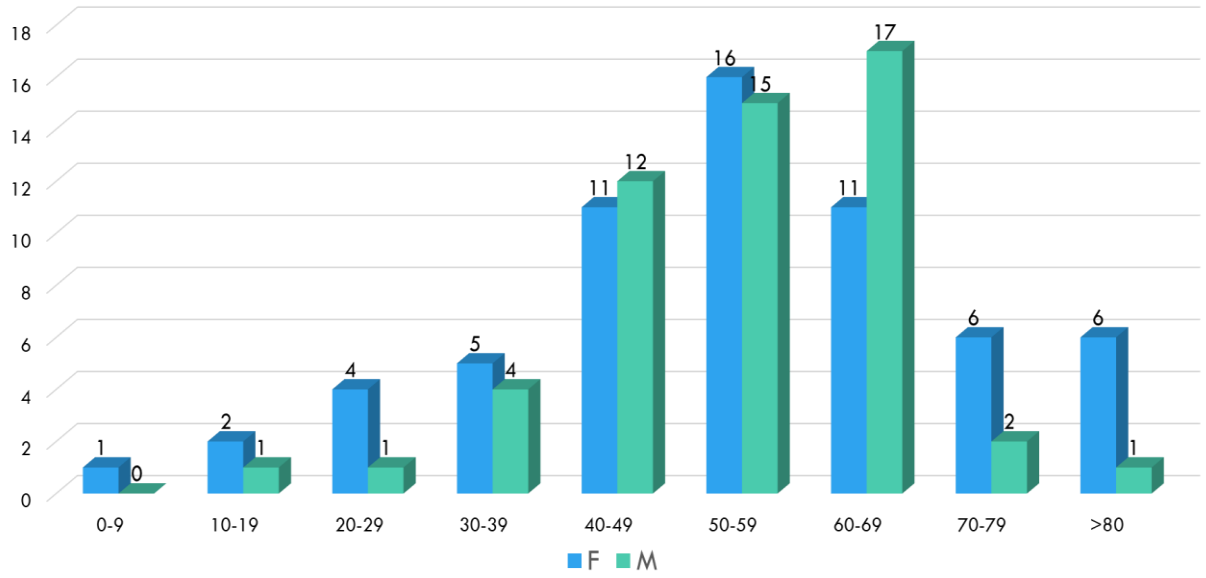

Figure 2. Positive cases by age and sex.
The M / F ratio was 0.84.
71,3% of cases were confirmed in people aged 40-69 years.
There were no positive male cases in the age group 0-9 years.

The largest cluster of positive SARS-CoV-2 cases was identified among patients with hematological malignancies and oncological patients (Figure 3). These types of patients were deeply immunocompromised.

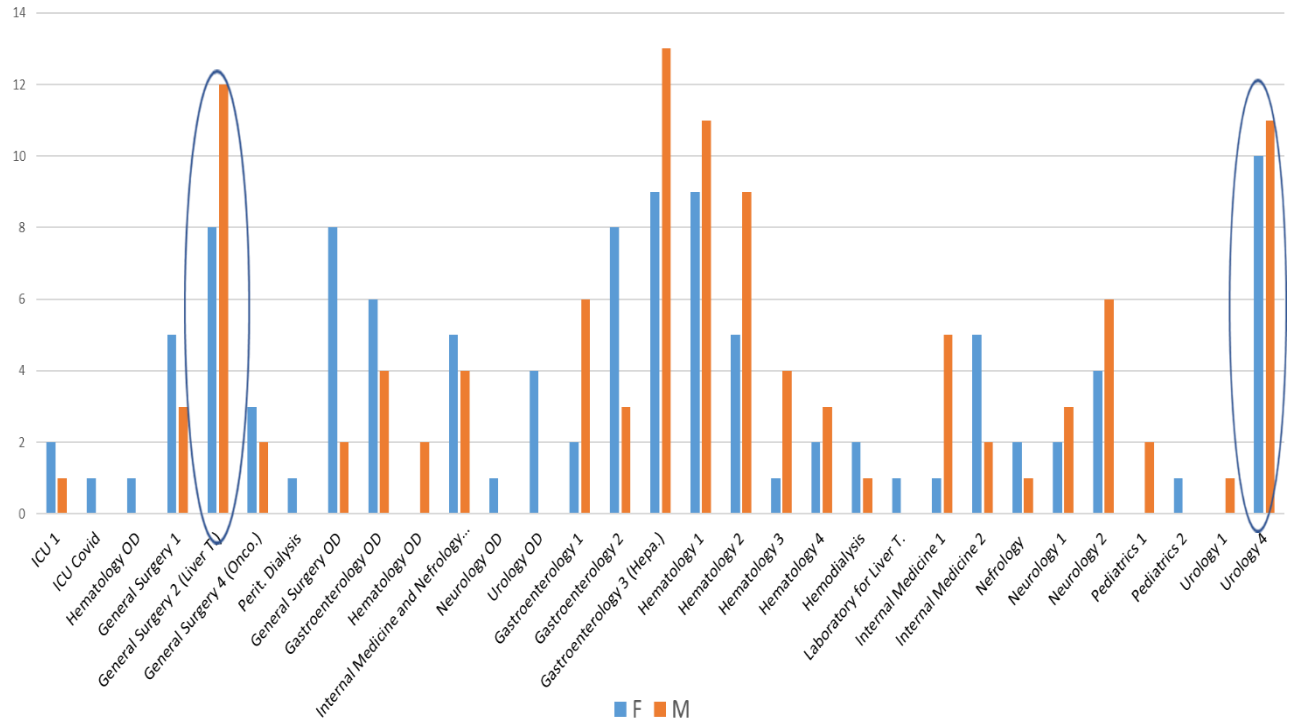

Figure 3. Distribution of positive cases in Fundeni Clinical Institute wards.

Additionally, 389 medical staff members, considered otherwise healthy, have been tested positive for SARS-CoV-2. A significant number of these cases (212) were from departments such as intensive care, general surgery, gastroenterology, and hematology. Female healthcare workers had a higher rate of positive RT-PCR results (293) compared to their male individuals (96) (Figure 4).

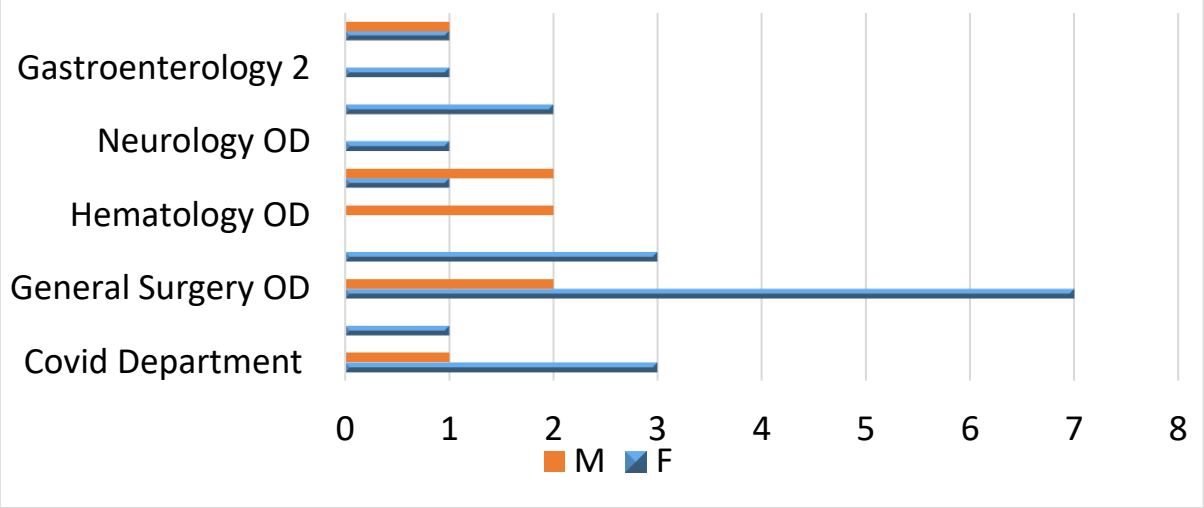

Figure 4. Positive cases among healthcare personnel.

Furthermore, the study used the chemiluminescence method to screen 1,068 individuals, including patients and healthcare workers, for SARS-CoV-2 IgG anti-nucleocapsid antibodies. This screening revealed that 253 patients and 157 staff members had developed IgG antibodies against the virus (Figures 5 and 6).

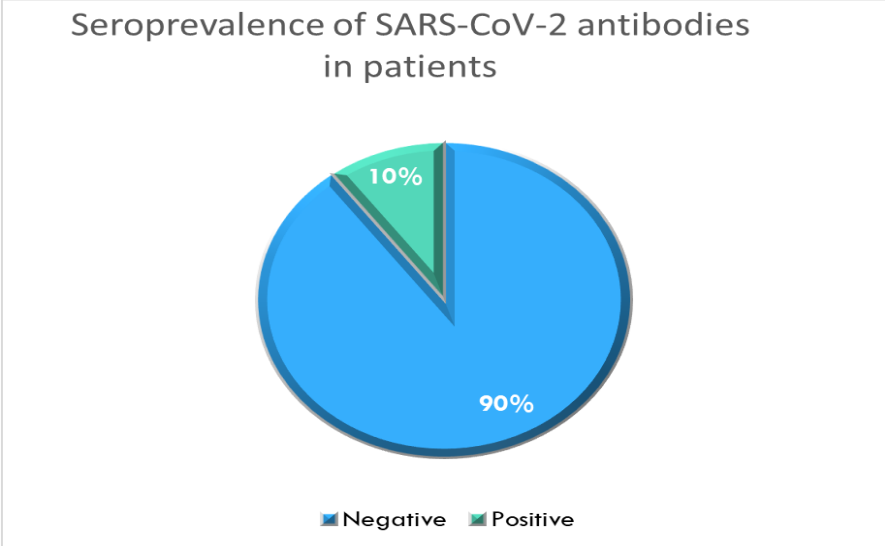

Figure 5. Seroprevalence of SARS-CoV-2 antibodies.

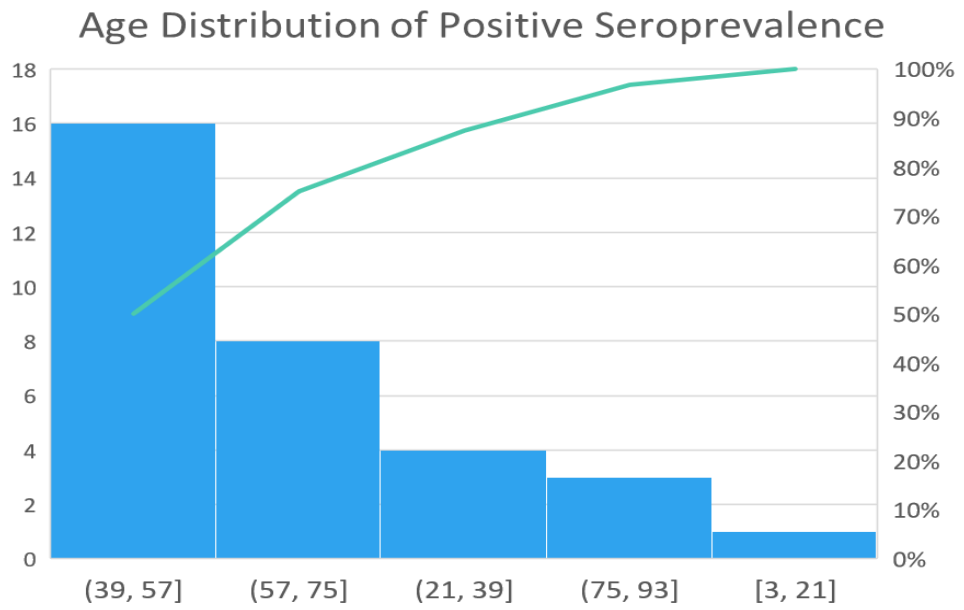

Figure 6. Age distribution of positive seroprevalence.
The ratio of antibodies was between 1.48 and 9.48 (mean = 4.9, median = 4.74, std.dev=±2.32).

An additional number of 50 individuals, comprising patients and medical staff, were tested for SARS-CoV-2 spike (S) protein antibodies post-vaccination. All showed various degrees of antibodies against the SARS-CoV-2 spike protein. Our BSL-2 laboratory recorded the presence of SARS-CoV-2 S protein antibodies in all 50 individuals post-vaccination, tested via chemiluminescence (SARS-CoV-2 IgG assay, Abbott, USA), with over 3000 arbitrary units (A.U.) post-second dose. No reinfections were observed during the study period.

Discussions

SARS-CoV-2 primarily infects alveolar epithelial cells and enterocytes, leading to COVID-19, a condition mainly marked by respiratory symptoms that can escalate to bilateral interstitial pneumonia, respiratory failure, or acute respiratory distress syndrome (ARDS) [10]. In March 2021, the World Health Organization indicated that Romania had 983,217 confirmed COVID-19 cases and 24,386 deaths [11].

In our Fundeni Clinical Institute laboratory, extensive RT-PCR testing for SARS-CoV-2 was conducted among both incoming patients and healthcare staff. Our analysis of RT-PCR outcomes identified four distinct profiles. Detection of the E gene in the absence of the Orf1ab/RdRp and N genes suggested a potential coronavirus infection warranting retesting. Identifying the E, Orf1ab/RdRp, and N genes with a cycle threshold (Ct) value below 30 confirmed SARS-CoV-2 infection. A Ct value above 30 needed SARS-CoV-2 IgG screening to verify immunization status. Immunized individuals with positive RT-PCR results were not classified as an epidemiological threat. Confirmation of SARS-CoV-2 infection was also made when a specific gene was detected with a Ct value below 30; otherwise, retesting was recommended.

Our data showed a slight female predominance in positive cases (59%, M/F ratio – 0.84). The majority were adults, with 71.3% of cases occurring in individuals aged 30–69. Most SARS-CoV-2 diagnoses (83%) were among patients in gastroenterology, hematology, and surgery departments, predominantly with impaired immune responses. This aligns with findings by Chen et al., indicating that SARS-CoV-2 predominantly affects older males with comorbidities such as diabetes and cancer

[12]. Guo et al. found higher positivity rates in intensive care units (ICUs) compared to other wards [13]. Anti-SARS-CoV-2 IgG anti-nucleocapsid antibodies were detected in 38.38% of cases from 1,068 tests. Among confirmed RT-PCR cases, 157 were healthcare workers, and 253 patients had developed immunity to COVID-19. Notably, a significant portion of intensive care unit personnel exhibited immunity without showing symptoms. One-third of tested individuals developed anti-nucleocapsid antibodies, with SARS-CoV-2 IgG seroprevalence unaffected by gender or age, though differences were noted among immunocompromised individuals, mirroring studies from China and Spain [14,15]. In the United States, evidence suggested a large portion of the population remained unexposed to SARS-CoV-2, even in areas with widespread transmission [15]. These observations imply that achieving complete and durable immunity against SARS-CoV-2 may be challenging.

The transmission mechanisms of SARS-CoV-2, a rapidly mutating RNA virus, still need to be fully elucidated. The virus's ability to evolve new strains depends on its replication and spread to new hosts. To curb the epidemic's spread, it's crucial to maintain emergency epidemiological control points such as isolation in special rooms and movement restrictions.

From December to March 2021 in Romania, vaccination was exclusively done with mRNA vaccines, which instructed human cells to produce viral proteins, triggering antibody development.

Nevertheless, a decline in anti-nucleocapsid IgG antibodies was noted over time, similar to post-immunization anti-spike IgG levels. These findings were also met in other similar studies [16-18]. This raises questions about the longevity of these protective antibodies. Like with influenza, we propose that periodic SARS-CoV-2 immunization is necessary to maintain a protective antibody level.

Conclusions

In this work, we have shown our experience in the diagnosis and immunization monitoring of the patients admitted to Fundeni Clinical Institute wards during the COVID-19 pandemic. The COVID-19 pandemic was a challenging period, and we have learned that it was essential to have a rapid and robust diagnosis and to look at the immune response of the patients. Antibodies against SARS-CoV-2 enabled a better and more accurate interpretation of RT-PCR tests. The presence of anti-SARS-CoV-2 IgG antibodies, and in some cases, interleukin-6 serum levels allowed us to understand the real clinical situation of the COVID-19 patients. We have also evaluated our hospital personnel for SARS-CoV-2. Healthcare professionals had a higher risk (13%) of being infected during the SARS-CoV-2 pandemic because they were in front-line contact with infected patients.

Author Contributions: I.C., I.M., A.E.C., A.T., A.I.C., and M.T. conceived the original draft preparation. A.U and M.C. were responsible for conception and design of the review. I.C., I.M., A.E.C., A.T., A.I.C., and M.T. was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

Compliance with Ethics Requirements: *"The authors declare no conflict of interest regarding this article".*

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