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PROGNOSTIC FACTORS IN THE EVOLUTION OF SARS-CoV-2 INFECTION AND THE IMPACT ON QUALITY OF LIFE

ABSTRACT

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CONSTANȚA

TABLE OF CONTENTS

GENERAL PART

INTRODUCTION

Recently, a global health crisis of unprecedented scale has occurred, with a significant impact, caused by the SARS-CoV-2 viral infection, which has been the primary subject of study for doctors worldwide, since its emergence, before it turned into a pandemic. This virus has tested healthcare systems, both through the diagnosis and treatment of patients, as well as by slowing down or halting the spread. Vulnerabilities in the system have been highlighted, and questions have arisen about the factors influencing the progression of the disease, as well as the way in which the quality of life of infected individuals is affected.

The SARS-CoV-2 pandemic has revealed major weaknesses in diagnosis, treatment, and disease management, leading to significant changes in the global public health system. Although considerable progress has been made, many issues related to the progression of the infection remain unsolved, especially regarding the early identification of patients at high risk for complications and the long-term impact on quality of life. Since this is a viral infection different from all previous forms, with still undiscovered peculiarities, a significant amount of time will pass before all manifestations are identified, both during the acute phase and in the aftermath.

I. Current state of Knowledge

1. Origin, Evolution, and Etiological Agent of SARS-CoV-2 Infection

1.1. Etiological Agent

Coronaviruses (CoVs) are RNA viruses that belong to the order Nidovirales, the family Coronaviridae, and the subfamily Orthocoronavirinae. They infect both humans and a wide range of animals (birds and mammals). Their name derives from the characteristic appearance of the virions observed under electron microscopy, due to the prominent glycoprotein spikes arranged in a crown-like pattern on the surface of the viral particles. The viruses have a diameter of 60 to 140 nm, and the spikes are 9 to 12 nm in length. These spikes bind to specific receptors on the surface of cells, promoting their infection.

The three zoonotic beta-CoVs that have crossed the species barrier and caused fatal pneumonia in humans in the 21st century are: the severe acute respiratory syndrome coronavirus (SARS-CoV), responsible for the SARS epidemic that began in 2002 in Guangdong Province, China; the Middle East respiratory syndrome coronavirus (MERS-CoV), which caused a respiratory infection epidemic in 2012 in the Arabian Peninsula; and the novel CoV, initially named 2019-nCoV and later SARS-CoV-2, discovered

in December 2019 in Wuhan, Hubei Province, China, sequenced and isolated in January 2020, which caused the current pandemic of atypical pneumonia. SARS-CoV-2 belongs to the Betacoronavirus genus, the Sarbecovirus subgenus, just like SARS-CoV, with which it is closely related. [1-6]

1.2. Origin and Evolution of SARS-CoV-2 Infection

SARS-CoV-2 could have been transmitted to humans from bats, as coronaviruses are commonly found in this species. There are approximately 1,500 species of bats, and several hundred coronaviruses have been identified, some very similar to SARS-CoV-2, with a sequencing similarity of over 96%. However, the mechanisms that might be involved in the transmission of the virus from bats to humans, as well as the changes that led to the severe manifestations in the human host, have not yet been explained [7].

There are three hypotheses regarding the evolution of the virus in the scientific community:

1. The "zoonotic" hypothesis – suggests that the virus crossed the species barrier naturally, from an intermediate host at the Wuhan market, since the first cases appeared in that region of China [8,9].

2. The laboratory leak hypothesis – proposes that the virus was introduced to the human population through a "leak" from a laboratory, either deliberate or accidental. It suggests that although these accidents are rare, they can happen, with past events leading to sporadic infections and short transmission chains in laboratory studies [10].

3. The possibility of the virus originating in other regions of the world – In a retrospective study, SARS-CoV-2 was detected in sewage samples in Spain on March 12, 2019, indicating that the virus might have emerged earlier in other parts of the world [11].

2. SARS-CoV-2 Viral Infection (Epidemiology)

2.1. General Epidemiological Data

The first case of SARS-CoV-2 infection appeared in Wuhan, the capital of Hubei Province in China, in November 2019, according to Kpozehouen and collaborators [12], from where it spread worldwide. The WHO [13] classified this infection as a pandemic on March 11, 2020, with COVID-19 becoming a global health issue due to the rapid spread, severity of cases, and numerous uncertainties surrounding the virus. In Romania, the presence of the coronavirus was first confirmed in Gorj County on February 26, 2020 [14].

The COVID-19 pandemic ranks fifth in the list of the deadliest epidemics and pandemics in history, alongside the Justinian Plague and the Black Death, both caused by the same bacterium, Yersinia pestis, the bubonic plague, the Spanish flu, and the HIV/AIDS epidemic. As of August 28, 2023, the pandemic had recorded 769,805,366 infections and 6,955,484 deaths [15].

2.2. Virus Mutations and Waves of Spread

Throughout the pandemic, the virus's transmissibility and virulence have increased, with a notable trend toward mutations, including certain combinations of specific point mutations [16].

Depending on the evolutionary lineage and the mutations involved, the WHO has classified several virus mutations as variants of concern (VoC) or variants of interest (VoI).

Unlike a "peak," which represents a temporary increase in new COVID-19 infections, a "wave" can be defined as a "sustained" period of increase and decrease [17]. Waves are a characteristic feature of a pandemic [18]. The main factors influencing waves are seasonality, human behavior, and collective immunity. Around the world, most countries have experienced three or more waves.

3. Risk Factors and Prognostic Factors in SARS-CoV-2 Infection

3.1. Risk Factors for Severe Infection

Since the emergence of the novel coronavirus, the global scientific community has focused on analyzing and discovering its characteristics, particularly the risk factors for severe disease progression. The data has come from multiple sources and has been validated through repeated studies, making the identified factors considered major risks for severe forms of COVID-19.

Numerous studies have highlighted the involvement of certain genetic determinants in the increased severity of SARS-CoV-2 infection, as well as protective factors influenced by these determinants.

3.2. Prognostic Evaluation in COVID-19

Prognostic evaluation involves estimating future outcomes regarding the risk of developing severe forms of the disease, the need for hospitalization, complications, and the risk of death, by analyzing clinical, biological, or demographic characteristics [19].

Several reviews and meta-analyses support that prognostic factors associated with an increased risk of severe disease, hospitalization or admission to the intensive care unit, unfavorable outcomes, and mortality include [20–26]:

- Patient-related factors: advanced age, male sex, obesity, smoking history, blood group A;

- Presence of comorbidities: arterial hypertension, cardiovascular disease, cerebrovascular disease, peripheral arterial disease, dementia, diabetes mellitus, chronic respiratory disease (e.g., COPD, obstructive sleep apnea), active malignancies, immunosuppression, chronic kidney or liver disease, rheumatologic disease, bacterial or fungal coinfection;

- Symptoms: myalgia, dysphagia, productive cough, chills, nausea, dyspnea, chest tightness, dizziness, headache, hemoptysis, tachypnea, hypoxemia, respiratory failure, hypotension, tachycardia;

- Complications: shock, acute infection or sepsis, acute kidney, liver, or cardiac injury, acute respiratory distress syndrome, venous thromboembolism, arrhythmias, heart failure;

- Laboratory findings: lymphocytopenia, leukocytosis, neutrophilia, thrombocytopenia, hypoalbuminemia, hepatic cytolysis syndrome, and azotemia;

- Elevated inflammatory markers: C-reactive protein, procalcitonin, ferritin, erythrocyte sedimentation rate (ESR), tumor necrosis factor-alpha (TNF-α), interferon gamma, interleukins, lactate dehydrogenase (LDH), D-dimer, neutrophil-to-lymphocyte ratio. Yan X and colleagues demonstrated that a high neutrophil-to-lymphocyte ratio is an independent risk factor contributing to in-hospital mortality in COVID-19 patients;

- Elevated cardiac markers: creatine kinase; $PaO_2/FiO_2 \leq 200$ mmHg;

- Imaging findings: bilateral pneumonia on chest CT with a high extent score at hospital admission, consolidative infiltrates, or pleural effusion on chest CT;

- High prognostic scores: SOFA, APACHE II, NEWS2, COVID-19 Severity Score.3.3. Severitatea infecției cu SARS-CoV-2 și impactul asupra evoluției clinice.

The severity of SARS-CoV-2 infection varies significantly, ranging from asymptomatic or mild forms to severe and critical forms that can be life-threatening. In most cases, the infection follows a course that includes several distinct stages, and the severity of the disease depends on a range of factors, including age, comorbidities, and the patient's immune response [27–32].

4. Paraclinical Diagnosis

Since the early weeks of the pandemic, methods for detecting SARS-CoV-2 infection have expanded significantly, incorporating rapid and accessible techniques.

Molecular diagnosis- is based on nucleic acid testing technology. Nucleic acid detection methods mainly include genetic sequencing, CRISPR, and nucleic acid amplification tests. PCR requires thermal cycling and is highly sensitive and specific for virus detection. Isothermal nucleic acid amplification is a rapid detection method that can be performed at a constant temperature and does not rely on the operation of a thermal cycler [33].

Imaging diagnosis- has been of the highest value in detecting and classifying the severity of lung involvement in COVID-19 infection.

Chest X-ray- has a diagnostic value of approximately 50%, with a normal appearance not excluding SARS-CoV-2 infection [34]. The most commonly encountered changes are increased intensity, irregular opacities, located peripherally, especially subpleurally [35]. The involvement is bilateral, often located in the lower and peripheral areas [36].

Chest CT scan – Several studies have shown that most COVID-19 patients present with characteristic chest CT images, such as "ground-glass" areas and consolidations, predominantly distributed

subpleurally [37,38]. This examination is also used to classify the disease based on the extent of lung lesions.

5. Principles of Treatment and Prevention

5.1. Treatment

Throughout the pandemic, treatment principles have undergone significant changes due to findings from scientific research and the periodic updating of treatment guidelines. Initially, antimalarial drugs were used both internationally and in Romania, but these were later replaced by antiviral therapies and/or monoclonal antibodies for severe forms of the disease.

Common Principles:

- Asymptomatic forms will not receive treatment;

- Patients with mild forms, if there is no risk of severe progression and lung involvement, will receive symptomatic treatment;

- Antivirals are most effective when administered closer to the time of infection, and are primarily indicated for patients at risk of severe disease progression;

- Antibiotics are not recommended, as bacterial coinfections are rare;

- Prophylactic anticoagulation should be administered to all hospitalized patients to prevent microthrombosis, and therapeutic anticoagulation should be given to all patients with a high risk of thromboembolic events or pulmonary embolism.

Controversial Treatments:

- There has been hesitation regarding the use of NSAIDs, as they were considered to inhibit the beneficial effects of inflammation in patients with mild/moderate forms of the disease;

- The need to replace ACE inhibitors and/or sartans in treatment was discussed, but the European Society of Cardiology recommended continuing treatment as of March 13, 2020;

- Ivermectin – it was considered for its immunomodulatory and potentially antiviral effects, though its use was not recommended by guidelines.

5.2. Prevention – Vaccination

Prevention measures in Romania included physical isolation, the declaration of states of emergency and alert. Interactions were restricted, leaving the home was only allowed for justified reasons, and the mandatory wearing of protective masks in public spaces, commercial areas, public transport, and workplaces was introduced [39]. Before COVID-19, developing a vaccine for an infectious disease always took several years, and there was no vaccine for preventing coronavirus infections in humans [40].

In Romania, vaccination began on December 27, 2020. Initially, healthcare personnel were vaccinated, followed by the at-risk population, and then the rest of the population [41].

6. Impact on Quality of Life

6.1. Quality of Life

COVID-19 had an intense and complex impact on quality of life. Quality of life can be assessed through patient-reported measures, being subjective and multidimensional [42].

COVID-19 impacted the global population at several levels:

Physical: through its effect on overall health, both at the time of acute infection, due to the symptoms it causes, and at a distance from this phase, in some patients (long COVID).

Emotional and Social: through global prevention measures, such as social distancing, which limited interactions, leading to feelings of loneliness and isolation for many people. Several studies in the scientific literature have found a significant impact on quality of life, especially among females, job seekers, and young people [43-45].

Mental: the pressures from economic uncertainty, fear of illness, social isolation, and the loss of loved ones led to increased anxiety, stress, depression, and other mental health disorders, affecting individuals of all ages.

Economic: quality of life was diminished due to the intensification of stress and insecurity about the future, especially due to job loss and economic challenges.

6.2. Long-term Impact - Long COVID

It is defined as the persistence or appearance of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months, without another explanation [46-48].

Commonly encountered symptoms include fatigue, dyspnea, and cognitive dysfunction, as well as others that generally impact quality of life. These symptoms may reappear after the initial recovery from an acute episode of COVID-19 or may persist from the original illness. They may also fluctuate or relapse over time [49], having a significant impact on quality of life and potentially lasting for several years, or even for a lifetime [50,51].

6.3. Transition from Pandemic to Endemic - Common Cold/Flu

On May 5, 2023, the WHO declared that COVID-19 is no longer a public health emergency of international concern.

If the virus is not eradicated, it will transition into an endemic state within a few years [52]. Endemicity will be established by observing seasonal variations, without unexpected peaks outside of typical seasons [53].

Personal Contribution

2. Study I - Evolution of SARS-CoV-2 Infection and Mortality Risk: Demographic, Biological, and Behavioral Risk Factors as Predictors of Disease Severity

2.1. Objectives

This study aims to identify and analyze the prognostic factors that can influence the evolution of SARS-CoV-2 infection, particularly the risk of mortality among infected patients. The study examines the correlations between demographic, clinical, biological, and behavioral variables, offering insights into the determinants of disease severity.

1. Analysis of the relationship between demographic and clinical characteristics and disease progression: This objective examines how factors such as age, sex, body mass index (BMI), and other initial clinical characteristics influence the severity of SARS-CoV-2 infection and the risk of mortality.

2. Determination of the influence of exposure to respiratory hazards and smoking behavior on disease progression: The study explores the impact of exposure to respiratory hazards and smoking behavior (including pack-years) on the severity of SARS-CoV-2 infection, assessing the role of these factors in disease progression and the risk of severe complications.

3. Evaluation of the impact of comorbidities on the prognosis of infection: This part investigates whether a history of anti-COVID-19 vaccination and the presence of comorbidities (such as cardiovascular diseases, diabetes, and chronic respiratory conditions) influence the clinical course of patients and the likelihood of developing severe forms of the disease.

4. Evaluation of the impact of vaccination on disease severity.

5. Correlation of biological parameters (blood tests and oxygen saturation) with disease severity: The study analyzes the predictive value of biological parameters, including laboratory tests conducted at the time of admission and before discharge, as well as oxygen saturation levels, to identify relevant markers for patient prognosis.

6. Defining the clinical-evolutionary particularities of COVID-19 infection based on viral variants and waves of spread over time.

2.2. Materials, Methods, and Study Group Structure

The research is based on a non-interventional retrospective study that included patients referred to the Outpatient Department of the "County Clinical Emergency Hospital Constanța" - Tuberculosis Pneumology Department Agigea, the ICU Department of the "Saint Apostle Andrew County Emergency Hospital Constanța," the ICU Department of the "Medgidia Municipal Hospital," and patients from the "Pneumoftiziology Clinical Hospital Constanța" between 2020-2023 for diagnosis and treatment of the SARS-CoV-2 viral infection.

The study was conducted with the approval of the ethics committees of the involved medical institutions, in accordance with ethical principles and national regulations regarding biomedical research (no. 30/2.11.2020).

Information was retrieved from the medical records regarding the following:

- Demographic data (age, sex);
- Anthropometric indices (weight, height, body mass index: BMI);
- Smoking history, exposure to respiratory hazards;
- History of COVID-19 vaccination;
- Presence of comorbidities;
- Blood test results (neutrophil count, lymphocyte count, white blood cell count, platelet count, eosinophils, hemoglobin, hematocrit);
- Systemic inflammation biomarkers (erythrocyte sedimentation rate: ESR, C-reactive protein, D-dimer, Lactate Dehydrogenase);
- Other basic blood tests: AST (TGO), ALT (TGP), blood glucose, creatinine, urea, fibrinogen;
- SpO2 at admission;
- Disease staging based on chest CT scan from the time of patient admission to COVID-19 wards. To quantify the degree of lung involvement, the study used the Total Severity Score (TSS) proposed by Li et al. Considering the extent of lesions in each lung lobe (0%, 1-25%, 26-50%, 51-75%, and 75-100%), each lobe was assigned a score between 1 and 5, allowing the radiologist to classify pulmonary involvement into: mild (0-9 points), moderate (10-17 points), and severe (18-25 points), using the international scoring system [54];
- Result of the rapid antigen SARS-CoV-2 test or RT-PCR SARS-CoV-2 test, confirming the diagnosis of SARS-CoV-2 viral infection;
- Hospitalization period.

The patients' deaths were confirmed by checking the National Health Insurance House database (CNAS), using the SIUI platform (http://siui.casan.ro).

The following inclusion/exclusion criteria were established for the patients:

Inclusion Criteria:

- Age over 18 years;
- Signed informed consent regarding the collection, statistical processing of data, and publication of results in medical journals or scientific events, provided patient anonymity is maintained;
- Confirmed SARS-CoV-2 viral infection;
- Complete data regarding blood count, inflammatory biomarkers, and chest CT examination.

Exclusion Criteria:

- Absence of informed consent in the patient's medical record;
- Incomplete medical records lacking the necessary data;
- Pregnant or breastfeeding women;
- Patients with HIV/AIDS infection or cancer;
- Patients with acute infections of other etiologies.

2.3. Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 25 and Microsoft Office Excel/Word 2021. Quantitative variables were expressed as means with standard deviations or medians with interpercentile ranges, and their distribution was assessed using the Shapiro-Wilk test.

2.4. Results of Study I:

The study group included 318 patients, with data from 30 patients excluded due to not meeting all inclusion criteria.

Key findings:

- No significant differences were observed between disease severity and sex, exposure to respiratory hazards;.

- The majority of patients were infected during wave 2 (36.8%) and wave 3 (26.7%), corresponding to the Alpha and Beta variants.

- The mean survival period was 25.49 days (95% CI: 22.48–28.51), with a median of 25 days.

- Patients with mild disease had fewer comorbidities (30.5% vs. 19.2%), while those with moderate disease had more frequent comorbidities.

- SpO_2 values at admission were higher in patients with mild forms, decreasing progressively with disease severity.

- Patients with severe forms had higher levels of leukocytes, ESR, CRP, AST (TGO), ALT (TGP), LDH, and D-dimer, and lower lymphocyte counts compared to patients with less severe disease.

- Patients infected during wave 1 were significantly younger than those infected in waves 2, 3, and 4.

- Patients from wave 3 had a higher BMI than those in wave 4, indicating that the Delta variant spread less among obese patients in this cohort, contrary to expectations — further investigation is needed.

- Patients from wave 4 were more frequently smokers, while those from wave 5 had occupational exposure to respiratory toxins.

- Oxygen saturation was higher in patients from wave 1 compared to waves 2, 3, and 4; also higher in wave 2 than in waves 3 and 4.

- Mortality rate did not differ significantly based on pandemic wave, sex, BMI.

- Patients who died were older, had lower oxygen saturation, lower lymphocyte counts, and higher levels of ESR, AST, LDH, and D-dimer, compared to survivors.

- Leukocyte count, fibrinogen, and ALT did not significantly affect mortality.

- Patients infected in wave 4 (Delta variant) had higher fibrinogen and D-dimer, but lower LDH than patients from other waves.

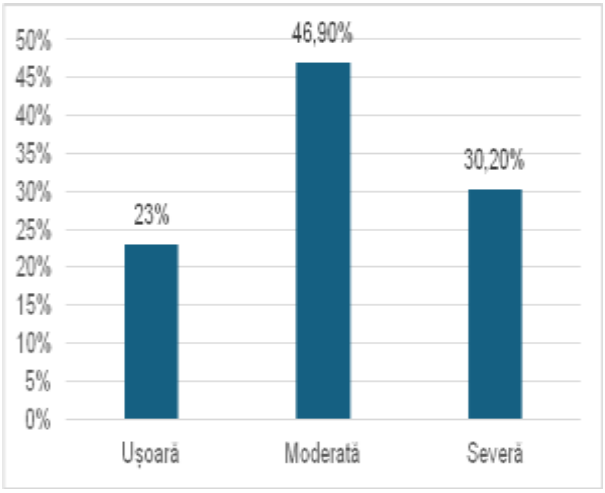

Figure 1. Distribution of patients according to disease form

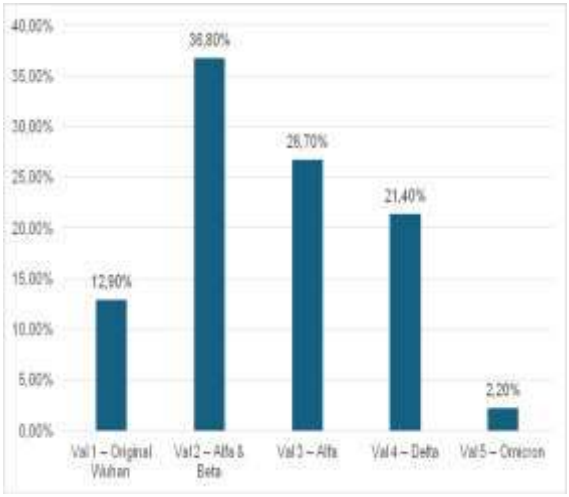

Figure 2. Distribution of patients according to the strain variant involved in the pandemic wave

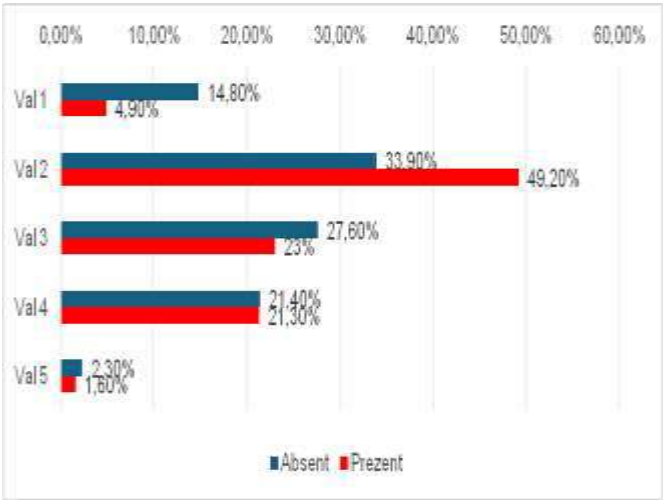

Figure 3. Distribution of patients according to the occurrence of death and the pandemic wave

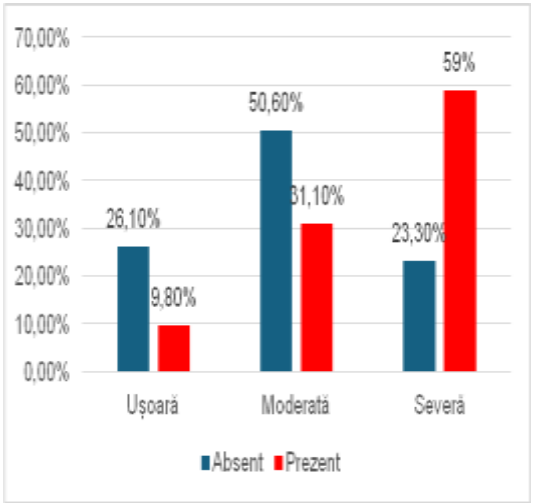

Figure 4. Distribution of patients according to the occurrence of death and the disease form

2.5. Discussion

Regardless of the dominant viral variant, certain risk factors were consistently associated with a higher risk of severe infection and death. These include:

- **Advanced age**: Individuals aged ≥65 years showed a significantly increased risk of developing severe forms of COVID-19, and mortality in this age group remained consistently high across all pandemic waves [55];

- **Sex**: Studies have indicated a higher risk for men in developing severe COVID-19 and a greater mortality rate compared to women. This difference, observed consistently throughout all pandemic waves, may be attributed to both biological and behavioral factors [56];

- **Comorbidities** (diabetes, hypertension, cardiovascular diseases, pulmonary conditions): Comorbidities were correlated with increased severity of COVID-19 across all pandemic waves [57].

3. Study II – The rs12252 IFITM3 Polymorphism and Its Impact on COVID-19 Severity: A Clinical and Imaging-Based Approach

3.1. Objectives

A person's genetic background influences susceptibility to infectious diseases and the severity of their progression [58]. Therefore, through this study, we aimed to investigate whether the rs12252 genetic mutation plays an important role in our population and to observe the differences by comparing other parameters between mild and severe forms of the disease.

3.2. Material and Method

- Study Design

This is an observational study that includes blood samples from 51 patients. The study aims to investigate the correlation between the G allele of the rs12252 single nucleotide polymorphism in the IFITM3 gene and the severity of COVID-19 in a sample from the Romanian population. The patients were divided into two study groups—31 patients with severe forms of the disease and 20 patients with mild forms—by analyzing the frequency of the G allele in these groups.

Blood samples were analyzed at CEDMOG - "Research and Development Center for Morphological and Genetic Studies in Malignant Pathology" in Constanța. Additional parameters were evaluated for patients hospitalized in the Pneumology TBC Agigea Department of "SCJU Constanța" and at the Pneumoftiziology Hospital in Constanța.

- Inclusion Criteria

Patients aged over 18 years who had a positive PCR test for SARS-CoV-2.

- Data Collection

All examinations were performed in the same imaging laboratory and analyzed by the same technician. To classify pulmonary involvement, the Total Severity Score (TSS) proposed by Li et al. was used. This score classifies COVID-19 cases into three categories based on lung involvement: mild (TSS 0-9 points), moderate (TSS 10-17 points), and severe (TSS 18-25 points), according to the international scoring system [59; 60].

Blood samples were taken from venous blood, fasting, from all patients, at admission and before discharge. The analysis included erythrocyte sedimentation rate (ESR), fibrinogen, D-dimer, lactate dehydrogenase (LDH), and C-reactive protein (CRP).

Blood for genetic tests was collected later. The genetic analysis included:

Genomic DNA purification;

Genotyping.

3.3. Statistical Analysis

All the study data were evaluated using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021. To assess normality, the Shapiro-Wilk test was performed for quantitative variables, and these were reported as means with standard deviations or medians with interpercentile ranges. Quantitative variables with a normal distribution were compared between groups using the Welch T-Test (taking into account the inequality of variances between groups, as determined by Levene's tests). Error plot graphs with 95% confidence intervals for means were used to illustrate the comparisons.

3.4. Results

In this study, we aimed to explore the relationship between clinical characteristics, laboratory parameters, and the IFITM3 rs12252 polymorphism in COVID-19 patients with mild or severe infection. The analysis focused on assessing the general characteristics of the patients, levels of inflammatory biomarkers, and genetic predispositions to identify potential contributions to disease severity. These findings provide valuable insights into how the progression of COVID-19 is influenced, highlighting both key differences between mild and severe cases, as well as the potential role of the IFITM3 rs12252 polymorphism in influencing outcomes. Before evaluating the genetic profile, we assessed the general characteristics of the patients and compared the values of inflammatory biomarkers. The characteristics of the patients are presented in **Table I**.

Table I. Characteristics of the analyzed groups related to the severity of COVID-19 infection

Parameter/Infection		**Total**	**Mild**	**Severe**	**p**
Demographic Characteristics					
Nr. (%)		51 (100%)	20 (39.2%)	31 (60.8%)	-
Age	**Average ± SD**	53.31 ± 13.91	42.75 ± 11.26	60.13 ± 10.96	**<0.001***
	Median (IQR))	54 (47-66)	46.5 (30.2-52.2)	62 (50-69)	
Gen (Masculin) (Nr., %)		23 (45.1%)	3 (15%)	20 (64.5%)	**0.001****
BMI	**Average ± SD**	27.37 ± 4.61	25.07 ± 1.53	26.85 ± 5.3	**<0.001***
	Median (IQR))	27.5 (24.2-30)	25.1 (23.9-26.2)	28.9 (27.7-32)	
Genotype (AG) (Nr., %)		5 (9.8%)	0 (0%)	5 (16.1%)	0.072***
Laboratory parameters					
ESR	**Average ± SD**	42.83 ± 24.16	16.11 ± 8.63	50.58 ± 21.5	**<0.001**
	Median (IQR))	39.5 (23-63.5)	19 (7.5-23.5)	48 (35-73)	****
Fibrinogen	**Average ± SD**	4.46 ± 1.73	3.27 ± 0.77	4.8 ± 1.79	**0.001**
	Median (IQR))	4.13 (3-5.54)	3.3 (2.8-3.7)	4.7 (3.2-5.8)	****
PCR	**Average ± SD**	71.12 ± 70.48	7.8 ± 11.62	69.51 ± 69.78	**<0.001***
	Median (IQR))	47 (8.25-106)	4.2 (0.75-8.5)	76.8 (34-130)	
LDH	**Average ± SD**	449.7 ± 298.4	182.56 ± 40.62	527.3 ± 296.1	**<0.001***
	Median (IQR))	344 (225-617)	202 (161-215)	428 (297-660)	
D-dimer	**Average ± SD**	1.22 ± 1.36	0.84 ± 0.71	1.34 ± 1.5	0.332*
	Median (IQR))	0.7 (0.48-1.5)	0.65 (0.38-1.01)	0.75 (0.5-1.6)	
TSS/SCLT	**Average ± SD**	18.52 ± 4.44	4 ± 1.41	19.45 ± 2.46	**0.004***
	Median (IQR))	18 (17-21)	4 (3-5)	19 (17-21)	

***Mann-Whitney U Test, **Fisher's Exact Test, ***1-sided Fisher's Exact Test, ****Welch T-Test**

3.5. Discussions

The response to viral infections can vary significantly between individuals, and this variability is influenced by genetic differences in certain molecules involved in the cellular entry process. The severity of the infection might be associated with single nucleotide polymorphisms (SNPs), considering that these proteins play an essential role in SARS-CoV-2 penetration into host cells and in the host's immune response to the virus.

Our results are in agreement with findings from existing literature [61-63]. The G allele of the rs12252 SNP in the IFITM3 gene has a frequency between 0.01 and 0.05 in Caucasian Europeans. This allele is associated with greater disease severity, including COVID-19, and is less frequent in Caucasian Europeans compared to Africans, Americans, and Asians [64].

Although the G allele reflects a low prevalence in the general population, 9.8% of the patients studied had a heterozygous genotype (AG). Moreover, all of these patients had severe COVID-19. It is extremely important to note that the G allele is rare, and its homozygosity was not identified in our study.

4. Study III - Impact on Quality of Life. Part I - The Impact of the COVID-19 Pandemic on Mental Health: A Retrospective Evaluation of Pandemic Experiences

4.1. Research Objectives

* 1 and 2- Studying differences in anxiety, depression, and stress levels during the pandemic based on professional and living situation: This objective aims to examine how various occupational and living conditions influenced mental health during the pandemic, specifically focusing on anxiety, depression, and stress levels.

* 3 and 4- Studying the correlation between the frequency of deaths of close family members or friends during the pandemic and the anxiety, depression, and stress levels of their relatives: This objective will assess whether the loss of close individuals during the pandemic has a significant impact on the mental well-being of their family members or friends.

* 5- Studying differences in anxiety, depression, and stress levels based on vaccination status: This part will investigate whether individuals who were vaccinated against COVID-19 experienced different mental health outcomes compared to those who were not vaccinated, in terms of anxiety, depression, and stress.

* 6- Studying differences in anxiety, depression, and stress levels based on fear of hospitalization: This objective focuses on exploring how fear of hospitalization due to COVID-19 might have influenced the mental health of individuals, particularly regarding anxiety, depression, and stress.

* 7- Studying differences in anxiety, depression, and stress levels based on the presence of reinfection: This objective will look at the mental health effects of reinfection with COVID-19, analyzing whether individuals who had multiple infections experienced higher levels of anxiety, depression, and stress.

*8- Studying differences in anxiety, depression, and stress levels based on the timing of the infection: This objective examines whether the timing of the infection (e.g., during early, middle, or later stages of the pandemic) had an impact on the mental health of individuals.

*9- Studying differences in anxiety, depression, and stress levels based on the presence of insomnia and age groups: This goal explores how insomnia, a common symptom during the pandemic, interacted with anxiety, depression, and stress levels across different age groups.

* 10- Studying differences in the occurrence of depressive syndrome based on age groups: This will look into how the manifestation of depressive symptoms varied across different age groups during the pandemic.

* 11- Studying differences in anxiety, depression, and stress levels during the pandemic based on the presence or absence of pets in the household: This objective investigates whether having pets at home served as a buffer against the mental health effects of the pandemic, potentially reducing anxiety, depression, and stress.

* 12- Studying the impact on quality of life and the frequency of post-COVID-19 symptoms: This objective focuses on understanding the long-term consequences of COVID-19 on the quality of life and how frequently post-COVID-19 symptoms are experienced by those who had the virus.

4.2. Study Design

This study is a cross-sectional investigation aimed at evaluating the prevalence and severity of psychological distress, including stress, anxiety, and depression, among the population of Romania. The study used an online interview format, distributed via the WhatsApp mobile application, as well as a written version provided to patients in family medicine, general medicine, and pulmonology offices in the southeastern region of Romania. The interview included a psychological assessment tool, as well as questions regarding demographic information and personal history related to COVID-19 and the manifestations of this condition.

To assess the participants' mental health status, the Depression, Anxiety, and Stress Scale (DASS-21) [65] was used.

The population analyzed included socially active adults or pensioners. These individuals were recruited through family doctors and pulmonologists from various cities in Romania. The study was conducted in compliance with relevant legislation regarding the processing and free movement of personal data. Prior to participation, informed consent was obtained from all respondents.

4.3. Statistical Analysis

The data obtained in this study were analyzed using IBM SPSS Statistics 25. Data visualization was done using Microsoft Office Excel/Word 2021. For quantitative variables, distribution was assessed using the Shapiro-Wilk Test, and the results were presented as means with standard deviations or medians with interpercentile ranges.

4.4. Results

A total of 1000 questionnaires were distributed. Of these, 637 forms were completed by respondents. However, only 521 met the inclusion criteria for statistical analysis, while the rest were invalidated for various reasons.

Among the respondents, 201 were men and 320 were women, with ages ranging from 18 to 85 years. To better characterize the cohort, participants were divided into four age groups: young adults (18-24 years), adults (25-35 years), middle-aged individuals (36-64 years), and elderly (over 65 years). Women represented 61.42% of the participants, while men represented 38.58%. The majority of participants (n=296; 56.81%) were in the middle-aged group, followed by adults (n=168; 32.24%). Young adults (n=37; 7.10%) and elderly individuals (n=20; 3.83%) made up a smaller percentage of the sample. The average age of the study group was 41.92 ± 13.04 years.

Prediction of DASS-21 Scores Based on Analyzed Factors

Table II. Generalized Linear Models Used in Predicting DASS-21 Scores

Univariate Models						
Score/Parameter	***Anxiety***		***Depression***		***Stress***	
	B (95% C.I.)	**p**	**B (95% C.I.)**	**p**	**B (95% C.I.)**	**p**
Medical staff*	-3.69 (-7.13 - -0.26)	**0.035**	-2.57 (-5.77 – 0.63)	0.116	-2.52 (-5.96 – 0.918)	0.151
Other Occupations*	-4.73 (-7.79 - -1.67)	**0.002**	-3.85 (-6.7 - -0.99)	**0.008**	-4.19 (-7.25 - -1.12)	**0.007**
Living with family	-2.75 (-5.2 - -0.3)	**0.028**	-3.29 (-5.56 - -1.01)	**0.005**	-3.09 (-5.53 - -0.64)	**0.013**
Death of a Family Member	10.68 (6.69-14.68)	**<0.001**	10.5 (6.79-14.21)	**<0.001**	10.09 (6.09-14.1)	**<0.001**
Death of a closer friend	5.19 (3.16-7.23)	**<0.001**	5.05 (3.16-6.94)	**<0.001**	5.48 (3.45-7.51)	**<0.001**
Hospitalization	10.23 (8.24-12.22)	**<0.001**	10.46 (8.65-12.28)	**<0.001**	10.92 (8.95-12.88)	**<0.001**
Infection – End	-2.97 (-5.47 - -0.48)	**0.019**	-3.26 (-5.61 - -0.9)	**0.007**	-4.24 (-6.74 - -1.74)	**0.001**
Reinfection	4.82 (2.32-7.31)	**<0.001**	6 (3.7-8.3)	**<0.001**	5.19 (2.7-7.68)	**<0.001**

Multivariate Models						
Score/Parameter	***Anxiety***		***Depression***		***Stress***	
	B (95% C.I.)	**p**	**B (95% C.I.)**	**p**	**B (95% C.I.)**	**p**
Medical staff*	-4.12 (-0.25 - -8.)	**0.037**	-3.73 (-7.31 - -0.15)	**0.041**	-2.83 (-6.69 – 1.02)	0.150
Other Occupations*	-3.93 (-0.39 - -7.48)	**0.030**	-3.57 (-6.85 - -0.29)	**0.033**	-3.2 (-6.74 – 0.32)	0.076
Living with family	-2.58 (-5.38 – 0.2)	0.070	-3.05 (-5.63 - -0.46)	**0.021**	-2.78 (-5.57 - -0.002)	**0.049**
Death of a Family Member	8.75 (4.52-12.98)	**<0.001**	8.19 (4.28-12.1)	**<0.001**	7.92 (3.7-12.14)	**<0.001**
Death of a closer friend	2.01 (-0.48 – 4.5)	0.114	2.4 (0.1-4.71)	**0.041**	2.65 (0.16-5.13)	**0.037**
Hospitalization	8.19 (5.82-10.56)	**<0.001**	8.06 (5.87-10.25)	**<0.001**	8.56 (6.2-10.93)	**<0.001**
Infection – End	-1.51 (-3.77 – 0.74)	0.190	-1.8 (-3.89 – 0.28)	0.090	-2.63 (-4.88 - -0.38)	**0.022**
Reinfection	1.4 (-1.13 – 3.93)	0.278	2.45 (0.11-4.8)	**0.040**	1.35 (-1.16 – 3.88)	0.293

*** The predictive variable is occupation, with the reference category being Unemployed/Pensioners.**

4.5. Discussions

As mentioned earlier, the SARS-CoV-2 pandemic had a substantial impact on individuals' psychological well-being. The prolonged duration of the pandemic, alongside factors such as social isolation, fear, and uncertainty, contributed to the increasing challenges related to mental health. These challenges include rising levels of stress, anxiety, depression, and other psychological issues [66,67].

5. Study III – Impact on Quality of Life. Part II – Expanding the Psychological Impact Assessment on Quality of Life: Application of DASS-21, PCL-5, and B-IPQ

5.1. Research Objectives

Studying Differences in Levels of Post-Traumatic Stress, Disease Perception, Anxiety, Depression, and Stress – Based on Age Groups:

- 18-24 years – Adolescents
- 25-35 years – Young Adults
- 36-64 years – Adults
- Over 65 years – Elderly

Studying Differences in Levels of Post-Traumatic Stress, Disease Perception, Anxiety, Depression, and Stress – Based on Sex.

Studying Differences in Levels of Post-Traumatic Stress, Disease Perception, Anxiety, Depression, and Stress in Patients with Confirmed SARS-CoV-2 Infection Compared to Those with Unconfirmed Infection, and Based on the Severity of the Disease.

5.2. Study Design

This study represents a cross-sectional research in which we assessed the occurrence and intensity of psychological distress, including stress, anxiety, depression, post-traumatic stress, and illness perception, in the population of Romania. It involved three psychological assessment tools, along with demographic information and a personal history related to COVID-19 and its manifestations.

The mental health status was primarily measured using the Depression, Anxiety, and Stress Scale, short version (DASS-21), which consists of 21 items, the Brief Illness Perception Questionnaire (B-IPQ), and the PCL-5, a self-assessment tool consisting of 20 items, designed to evaluate the 20 symptoms of Post-Traumatic Stress Disorder (PTSD) according to the DSM-5 manual.

5.3. Statistical Analysis

The data from the study were analyzed using IBM SPSS Statistics 25 and illustrated using Microsoft Office Excel/Word 2021. For quantitative variables, normality distribution was assessed using the Shapiro-Wilk Test, and the data were presented as means with standard deviations or medians with interpercentile ranges. Qualitative variables were expressed as absolute frequencies or percentages, and differences between groups were evaluated using the Fisher's Exact Test. To provide more information about the results of the contingency tables, Z-tests with Bonferroni correction were performed. Independent quantitative variables that did not follow a normal distribution were compared between groups using the Mann-Whitney U test or Kruskal-Wallis H test. Post-hoc Dunn-Bonferroni tests were used to further clarify the results of these comparisons for independent quantitative variables with non-parametric distribution. Generalized linear regression models were used to determine the prediction of B-IPQ and PCL-5 scores, using the disease form as an independent variable. The predictive performance was estimated using the beta coefficient with 95% confidence intervals along with the significance level.

5.4. Results

A total of 521 responses met the inclusion criteria for statistical analysis. Table III contains general data about the study cohort. The average age was 41.92 ± 13.05 years (with a median of 41 years, interpercentile range = 30-52 years), with the majority being middle-aged adults (56.8%). Most respondents were female (61.42%), from urban areas (85.8%), and had a confirmed COVID-19 infection (65.3%).For

334 patients, the form of the disease was observed, with the majority having a mild form of COVID-19 (59.3%). Regarding the analyzed scores: The average anxiety score (DASS-21) was 12.47 ± 11.46 points (median = 10 points, interpercentile range = 2-20). The average depression score (DASS-21) was 10.58 ± 10.68 points (median = 8 points, interpercentile range = 2-16). The average stress score (DASS-21) was 12.08 ± 11.47 points (median = 10 points, interpercentile range = 2-18). The average total B-IPQ score was 36.76 ± 13.97 points (median = 32 points, interpercentile range = 29.5-47). The average total PCL-5 score was 13.81 ± 12.06 points (median = 11 points, interpercentile range = 5-22).

Table III. Characteristics of the patients analyzed in the study

Parameter	***Value***
Demographic Characteristics	
Age (Average ± SD, Median (IQR))	41.92 ± 13.05, 41 (30-52)
Age Groups (Nr., %)	
Young Adults (18-24 ani)	37 (7.1%)
Adults (25-35 ani)	168 (32.2%)
Middle Age (36-64 ani)	296 (56.8%)
Old (≥ 65 ani)	20 (3.8%)
Gender (Feminine) (Nr., %)	320 (61.42%)
Place of origin (Urban) (Nr., %)	447 (85.8%)
Confirmed COVID-19 infection (Nr., %)	340 (65.3%)
Form of disease (Nr., %)	198 (59.3%) Mild, 105 (31.4%) Moderate, 31 (9.3%) Severe
Score values DASS-21, B-IPQ, PCL-5	
Anxiety Score (Medie ± SD, Mediană (IQR))	12.47 ± 11.46, 10 (2-20)
Depression Score (Medie ± SD, Mediană (IQR))	10.58 ± 10.68, 8 (2-16)
Stress Score (Medie ± SD, Mediană (IQR))	12.08 ± 11.47, 10 (2-18)
B-IPQ Score (Medie ± SD, Mediană (IQR))	36.76 ± 13.97, 32 (29.5-47)
PCL-5 Score (Medie ± SD, Mediană (IQR))	13.81 ± 12.06, 11 (5-22)

5.5. Discussion

Current literature indicates that the COVID-19 pandemic led to increased levels of anxiety, depression, and stress in the general population, especially among women, students, and certain age groups. Contributing factors include social media, misinformation, increased time spent thinking about COVID-19, and self-isolation.

Our study highlights the significant impact of the COVID-19 pandemic on the mental health of Romanians. Individuals who experienced a COVID-19 infection reported high levels of anxiety, stress, and depression on the DASS-21 scale, along with high scores on the PCL-5 and B-IPQ, with the severity of the illness being correlated with the intensity of these symptoms.

6. General Conclusions

Clinical and Biological Analysis in **Study 1**- Key Conclusions:

1. Age ≥ 68.5 years and its predictive value for mortality: The analysis demonstrated that age ≥ 68.5 years had an acceptable performance in predicting death, with an Area Under the Curve (AUC) of 0.776, sensitivity of 67.6%, and specificity of 78.4%. This suggests that older age is a significant factor in predicting mortality among infected patients, with a lower survival rate for patients aged ≥ 65 years. The average number of survival days was significantly lower for patients in this age group. Furthermore, age ≥ 68.5 years was identified as a significant predictor of the risk of death (Odds Ratio [OR] = 3.112), meaning that individuals in this age category have a threefold higher risk of death.

2. Smoking prevalence and severity of disease: Smoking was more frequently encountered among patients with mild forms of the disease.

3. Oxygen saturation (SpO2) and disease severity: Oxygen saturation upon admission decreased with increasing disease severity. This indicates that lower SpO2 levels are associated with more severe disease outcomes.

4. Impact of viral strain evolution on disease severity: The evolution of viral strains influenced the severity of the disease. Patients with mild forms were more frequently infected with the original Wuhan strain (present in the first wave of the pandemic), while severe forms were more frequently associated with the Alpha and Beta strains (corresponding to the 2nd and 3rd waves). This suggests that more recent strains (Alpha and Beta) may be associated with a higher risk of severe COVID-19 compared to the original strain. Additionally, patients from the first wave exhibited significantly better health parameters (e.g., oxygen saturation, lymphocytes, erythrocyte sedimentation rate [ESR]), indicating a milder form of infection compared to subsequent waves.

Conclusions from **Study 2**- Key Findings:

1. Association between COVID-19 severity and clinical and laboratory markers: An association was identified between the severity of COVID-19 infection and factors such as age, male sex, Body Mass Index (BMI), and increased laboratory values (e.g., ESR, fibrinogen, C-reactive protein [CRP], and lactate dehydrogenase [LDH]). This suggests that these factors may play a role in the severity of the infection.

2. Genetic involvement of IFITM3 gene polymorphism: Patients with the heterozygous (AG) genotype of IFITM3 were found more frequently among those with severe forms of the disease. However, the homozygous form was not present in the research sample. Although the difference did not reach strict statistical significance ($p = 0.072$), the analysis showed a relatively significant increased risk (Relative Risk [RR] = 1.769), suggesting a potential genetic involvement in the severity of the infection.

Conclusions from **Study 3**:

- **Part I**: The pandemic caused considerable psychological stress, manifested through anxiety, stress, and depression, often reaching clinically concerning levels. In the socio-economic context of Romania, it was identified that elderly individuals, pensioners, the unemployed, those living alone, those who were hospitalized due to COVID-19, those who lost a family member during the pandemic, or those who experienced reinfections, regardless of their vaccination status, remain psychologically vulnerable, even after the end of the pandemic. The results suggest that most respondents experienced significant symptoms of anxiety, depression, and stress, with 59.1% of patients exhibiting an abnormal level of anxiety, 42.4% depression, and 33.2% stress.

- **Part II**: Disease perception and PTSD were not correlated with the age and sex of the patients; however, they had major implications, directly proportional to the severity of the disease. Significant differences were observed between patients with confirmed COVID-19 infection and those without, with the former exhibiting higher DASS-21 and PCL-5 scores, suggesting that COVID-19 infection had a significant impact on levels of stress, anxiety, depression, and post-traumatic symptoms compared to patients who were not infected.

7. Elements of Originality of the Thesis

The thesis addresses a topic in clinical medicine with significant global impact—predicting the severity risk, mortality, and impact on quality of life (especially regarding mental health implications). It provides a deeper understanding of how various markers influence the survival of patients infected with SARS-CoV-2, with an emphasis on integrated clinical data analysis and interpretation. This work adds value to the existing literature by providing new, relevant, and up-to-date data that can be used by researchers and practitioners to improve medical practices.

Study 1: "**Evolution of SARS-CoV-2 Infection and Mortality Risk: Demographic, Biological, and Behavioral Risk Factors as Predictors of Disease Severity**" The originality of this study lies in offering a comprehensive overview of the impact of various pandemic waves in Romania. It highlights the complex relationships between epidemiological factors, virus variants, and demographic characteristics of the population. The study provides a more detailed and personalized approach to assessing mortality risk in the context of SARS-CoV-2 infection. Additionally, threshold values for multiple parameters were identified, which can be directly used in hospitals to guide therapeutic interventions. This study represents a first in the comparative analysis of emerging virus variants and the evaluation of their impact on mortality and survival rates, considering a significant number of risk factors, including comorbidities and lifestyle.

Study 2: "**rs12252 IFITM3 Polymorphism and its Impact on the Severity of COVID-19: A Clinical and Imaging Approach**" The originality of this study is primarily given by the discovery of the heterozygous AG genotype exclusively in severe cases within the research cohort, making it the only analysis of its kind conducted in our region. This finding could potentially open new avenues for genetic research related to COVID-19 severity, adding valuable insights into how genetic predisposition may influence the progression of the disease.

Study 3: "**Impact on Quality of Life—Impact on Mental Health**" The originality of the third study lies in its thorough application of multiple psychological evaluation tools (DASS-21, PCL-5, and B-IPQ) to assess the post-pandemic repercussions on mental health. This study provides valuable information for managing future similar situations and offers a unique perspective on the long-term mental health effects of the COVID-19 pandemic. By combining different psychological scales, the study highlights how the pandemic has shaped the mental well-being of individuals, especially vulnerable groups, and outlines a framework for post-pandemic mental health care.

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