Universitatea
Transilvania
din Brașov

INTERDISCIPLINARY DOCTORAL SCHOOL

Faculty of Medicine

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Inflammatory and hematological findings in patients with schizophrenia and SARS-CoV-2 infection

SUMMARY

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BRAȘOV, 2023

Universitatea Transilvania din Brașov

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At the same time, we invite you to take part in the public hearing of the PhD thesis.

ACKNOWLEDGEMENTS

The whole scientific process and the elaboration of the doctoral thesis could not have been completed without the support of some special people who have been with me throughout my doctoral studies.

To Prof. Dr. Petru Iulian Ifteni (scientific supervisor),
My special thanks and deep respect for his careful scientific guidance, his effort, his unconditional support, trust, understanding and patience with which he was constantly with me during my research and until the completion of my PhD thesis.

To the regretted Prof. Victoria Burtea†,
A special tribute to the esteemed Professor, for the exceptional human quality that led me to start this scientific work.

The Scientific Advisory Committee,
Warm and sincere thanks to Prof. Dr. Lorena Dima, Prof. Dr. Alina Pascu and Conf. Dr. Andrea Neculau, for their involvement in shaping the research structure and methodology, for the attention with which they have analysed the development of the PhD thesis, for the observations, suggestions and proposals expressed during the years of research.

I thank my family, for their constant support and understanding, for being by my side during my doctoral studies.
Thanks also to my colleagues and friends who have encouraged and supported me during all these years.

List of abbreviations

ACE-2 - angiotensin-converting enzyme 2

ALT - alanine aminotransferase

ANC - absolute neutrophil count

aPTT - activated partial thromboplastin time

ASA/AAS - acetylsalicylic acid

AST - aspartate aminotransferase

BEN - benign ethnic neutropenia

CBC - complete blood count

DIC - disseminated intravascular coagulation

CLZ - clozapine

COVID-19 - coronavirus infection

CoVs - coronaviruses

COX - cyclooxygenase inhibitors

CPZ - chlorpromazine

CRP - C-reactive protein

CT - computer tomography

CTL - cytotoxic T lymphocytes

CTPA - computer tomographic pulmonary angiogram

DAMP - trauma-associated molecular protein

DDI - other drug interactions

DOAC - direct oral anticoagulants

DSM-5 - Diagnostic and Statistical Manual of Mental Disorders

ECG - electrocardiogram

PE - pulmonary embolism

FFP - fresh frozen plasma (FFP)

GM-CSF - granulocyte-macrophage colony-stimulating factor

HLH - haemophagocytic lymphohistiocytosis

HPS - haemophagocytic syndrome

HSV-1 - herpes simplex virus type 1

HSV-2 - herpes simplex virus 2

IFN-γ - interferon-gamma cytokine

IL - interleukin

CSF - cerebrospinal fluid

LDH - lactate dehydrogenase

LMWH - low molecular weight heparin

MAC - membrane attack complex

NET - neutrophil extracellular traps

NK - natural killer cell

NLR - neutrophil to lymphocyte ratio

NMDA - N metal D aspartate

NO - nitric oxide

PaCO2 - partial pressure of arterial carbon dioxide

PAI 1 - plasminogen activator inhibitor 1

PAMP - circulating viral pathogen-associated molecular proteins

PANSS - Positive and Negative Syndrome Scale

PCR - polymerase chain reaction

PET - positron emission tomography

PT - prothrombin time Quick

RT-PCR - real-time polymerase chain reaction

SARS-CoV-2 - acute respiratory syndrome coronavirus 2

CIS - sepsis-induced coagulopathy

MS - multiple sclerosis

CNS - central nervous system

SRT/TRS - treatment-resistant schizophrenia

VTE - venous thromboembolism

TFPI - tissue factor pathway inhibitor

TNF - tumour necrosis factor

DVT - deep vein thrombosis

UFH - fondaparin or unfractionated heparin

vWF - von Willebrand facto

ABSTRACT

Introduction: Inflammation is a necessary response to infection, chemical substances, and tissue damage, whichcan sometimes exert harmful effects. The vulnerability-stress-inflammation model underlies the inflammatory theory of schizophrenia. Patients with severe conditions are at increased risk of hematological abnormalities. COVID-19 also affected the cardiovascular system, leading to the occurrence of arrhythmias, cardiac lesions, myocarditis, heart failure, pulmonary embolism and disseminated intravascular coagulation. This has further complicated the treatment of patients with schizophrenia, who are generally more prone to somatic comorbidities, and whose antipsychotic treatments may increase the risk of myocarditis.

Objectives: The main objective of the thesis is to highlight the complex relationship between COVID-19 infection and the evolution of patients with schizophrenia. Secondary objectives were: highlighting the anti-inflammatory effect of antipsychotic treatment in the context of the COVID-19 infection in patients with schizophrenia; highlighting the inflammatory response in patients with schizophrenia and COVID-19 infection treated with clozapine; elucidating the inflammatory response following COVID-19 vaccination in schizophrenic patients who have experienced COVID-19 infection, and assessing the risk of myocarditis in clozapine-treated schizophrenic patients with prior COVID-19 infection and vaccinated against COVID-19.

Materials and methods: The doctoral research includes 4 studies and was approved by the Ethics Committee of the Clinical Hospital of Psychiatry and Neurology from Brașov. The database was created using Microsoft Excel 2021 software. Statistical analysis was performed using GraphPad Prism 9 and MedCalc software. The chosen statistical significance α threshold is 0.05, with a confidence interval, CI = 95%. Statistical analysis includes elements of descriptive statistics. Study I – Inflammatory response in patients with schizophrenia infected with SARS-COV-2 under chronic antipsychotic treatment, was a prospective cross-sectional study. Study II – Clozapine and neutropenia in patients with schizophrenia and SARS-COV-2 infection. Study III – Outcome of COVID-19 mRNA vaccination in clozapine-treated patients with prior SARS-COV-2 infection, was analytical cross-sectional. Study IV- Assessment of the risk of myocarditis in schizophrenic patients on clozapine treatment, who were vaccinated with COVID-19 mRNA vaccine.

Results: Study I compared 101 patients with schizophrenia treated with antipsychotics to a control group of 101 consecutive patients without major psychiatric disorders, all of whom tested positive for SARS-CoV-2. The study did not reveal significant differences in inflammatory markers, duration of hospitalization or severity of the form of the disease COVID-19. Study II included 105 patients with schizophrenia, on clozapine treatment, who became infected with SARS-CoV-2. About 10% of patients experienced significant neutropenia. Changing the antipsychotic (clozapine) caused relapse in 70% of cases, resulting in longer hospital stay compared to those without neutropenia. Study III compared the results of hematological analyses of patients with schizophrenia, who passed through SARS-CoV-2 infection and were subsequently vaccinated against COVID-19 with mRNA vaccine (Pfizer BioNTech), under treatment with clozapine versus other antipsychotics. The study did not reveal statistically significant differences in hematological parameters between the two groups of patients. We did not find a decrease in lymphocytes and neutrophils. There were no cases of moderate or severe granulocytopenia or agranulocytosis. We found one case of leukopenia (in the non-clozapine group) and a few cases of lymphopenia, none requiring further monitoring. Study IV followed a group of 50 patients with schizophrenia, treated with clozapine, after vaccination against COVID-19, to assess the risk of myocarditis in this high-risk population. There was no significant increase in the risk of myocarditis, no such cases.being identified.

Conclusions: Vulnerable individuals with schizophrenia under antipsychotic treatment showed a lower risk of severe SARS-CoV-2 infection and a likely better prognosis in a protective environment. COVID-19 may be associated with a temporary reduction in ANC levels that is mild, transient, and statistically insignificant in most patients, including those treated with clozapine. The use of Pfizer-BioNTech vaccines against COVID-19 was safe in clozapine-treated patients who were previously infected with SARS-CoV-2. Vaccination against COVID-19 does not appear to further increase the risk of myocarditis in patients with schizophrenia treated with clozapine.

INTRODUCTION. MOTIVATION FOR THE CHOICE OF THEME

Inflammation is a necessary physiological response to infection, chemicals or tissue damage, but it can sometimes have harmful effects. In the central nervous system (CNS), inflammation can have a neuroprotective or neurotoxic role. Pro-inflammatory cytokines, microglial cells, astrocytes and immune cells of the peripheral immune system, including monocytes, macrophages and T and B lymphocytes are involved in mediating inflammation in the CNS. Sensitisation is a process whereby an initial immune response to a stimulus, for example to stress or infection, lowers the threshold for a response to future exposure to the same stimulus, i.e. a weaker stimulus is required to activate the immune response or the release of cytokines is greater than at initial exposure. Sensitization may provide support for the hypothesis that infection in early childhood may generate increased cytokine release, may lead to activation of the immune system upon reinfection or another stimulus later in life, resulting in neurotransmitter disturbances, which is one of the currently accepted theories for the etiopathogenesis of schizophrenia. first proposed for the vulnerability-stress model of schizophrenia. In 1977 the vulnerability-stress model of schizophrenia was first proposed. This model proposes that stress, whether physical or mental, can trigger a psychotic episode. Today, this concept has been expanded to become vulnerability-stress-inflammation, as inflammation is known to play a role in schizophrenia and can be induced by stress. The finding that anti-inflammatory drugs are beneficial in schizophrenia provides perhaps the most compelling evidence that inflammation is involved in schizophrenia.
Haematological manifestations of COVID-19 were originally reported in case studies and descriptive studies of COVID-19 patients in China. Although the etiopathogenesis remains to be elucidated, these widely recognized manifestations of COVID-19 have a significant impact and prognosis. Patients with severe conditions are at increased risk of venous thrombembolism (VTE) due to immobilization, systemic inflammation induced by severe conditions such as sepsis or acute pancreatitis, dehydration, endothelial dysfunction and stasis. COVID-19 also affected the cardiovascular system with the development of arrhythmias, cardiac lesions, myocarditis, heart failure, pulmonary embolism and disseminated intravascular coagulation. This has further complicated the treatment of patients with schizophrenia, who are generally more likely to have somatic comorbidities, and whose antipsychotic treatments may increase the risk of myocarditis.
Since the beginning of the pandemic, the question of the effects of COVID-19 on a vulnerable population has been raised: patients with major psychiatric disorders, especially those with schizophrenia and in particular those treated with clozapine. Patients with schizophrenia, having higher rates of somatic comorbidities, were considered an category at risk of developing severe forms of COVID-19. In addition, clozapine, the gold standard in the treatment of treatment-resistant schizophrenia, can lead to

neutropenia and agranulocytosis, and is therefore a potential aggravating factor in SARS-CoV-2 infection.

The topic of the PhD thesis was chosen because, during the pandemic period, despite the interest shown in the population of patients with schizophrenia, research in the literature does not provide sufficient or conclusive data on the evolution of patients with schizophrenia in a controlled environment and under permanent treatment with antipsychotics. There is also a lack of data on the impact of vaccination with a messenger RNA vaccine in patients with schizophrenia who have experienced the illness. Regarding the efficacy and safety of clozapine, a major treatment in psychiatry, in the context of the COVID-19 pandemic, data in the literature are limited and contradictory. As a result, we have tried, through our PhD thesis research, to provide significant additional information regarding haematological and inflammatory changes in patients with schizophrenia during the COVID-19 pandemic, regarding the safety of vaccination against COVID-19 in this population, and last but not least, regarding the safety of using an essential psychiatric treatment, clozapine, during or after SARS-CoV-2 infection.

CHAPTER 1. INFLAMMATORY THEORY IN SCHIZOPHRENIA

1.4 The vulnerability-stress-inflammation model of schizophrenia

Forty years ago, Zubin and Spring (Zubin & Spring, 1977) first proposed the vulnerability-stress model of schizophrenia. This model proposes that stress, whether physical or mental, can trigger a psychotic episode. Today, this concept has been expanded to become vulnerability-stress-inflammation, as inflammation is known to play a role in schizophrenia and can be induced by stress. For example, if an inflammatory response is stimulated in mothers in the second trimester of pregnancy or in children while the CNS is still developing, children have a greater vulnerability to develop schizophrenia. Animal studies have shown that stress leads to increased levels of pro-inflammatory cytokines (Sparkman & Johnson, 2008). Genetic background also contributes to the level of vulnerability to stress, as described in the pathogen-host defense hypothesis (Raison & Miller, 2013).

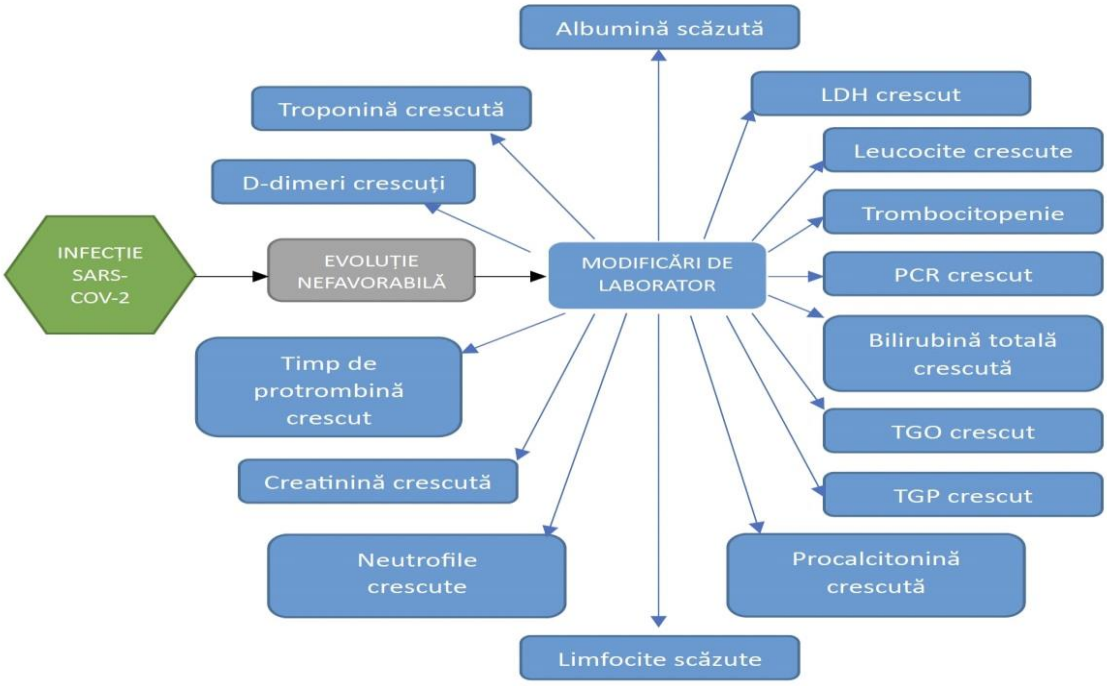
1.5 Markers of inflammation

There are numerous studies on markers of inflammation, some of which report the presence of fibrin degradation products in the brains of schizophrenia patients postmortem (Körschenhausen et al., 1996) and in the cerebrospinal fluid (CSF) in about 50% of them (Wildenauer et al., 1991). Furthermore, studies have observed a decrease in type 1 and an increase in type 2 cytokines in untreated patients (Müller & Schwarz, 2006). A meta-analysis found higher levels of proinflammatory cytokines in the peripheral blood of patients with schizophrenia in both first-episode and relapsing-remitting patients compared to healthy group controls. At the same time they found that levels were also higher for anti-inflammatory cytokines in patients compared to healthy group controls (Goldsmith et al., 2016). A meta-analysis of CSF cytokines showed similar results, i.e. higher levels of pro-inflammatory cytokines and lower levels of anti-inflammatory cytokines (Wang & Miller, 2018). An evaluation of these findings must also take into account possible interfering factors such as smoking, body mass index, gender, sleep, medications, etc. In addition, several cytokines act mainly paracrine and blood levels of these cytokines may not adequately reflect their function. The brain is protected from peripheral inflammation by the blood-brain barrier and an immunological activation, including an increase in blood levels of pro-inflammatory cytokines, does not reflect the situation at the brain level (Müller & Bechter, 2013). However, different means of communication exist between the peripheral system and the CNS immune system (Müller & Bechter, 2013). It is assumed that an inflammatory process is involved in the pathophysiology of at least a subgroup of patients with schizophrenia (Müller & Schwarz, 2010; Potvin et al., 2008).

CHAPTER 2. HAEMATOLOGICAL CHANGES CAUSED BY SARS-COV-2 INFECTION

Haematological manifestations of COVID-19 were originally reported in case studies and descriptive studies of COVID-19 patients in China. Although the etiopathogenesis remains to be elucidated, these widely recognized manifestations of COVID-19 have significant impact and prognosis. Venous thromboembolism (VTE) and its complications have been frequently reported in patients with COVID-19 especially in those with severe forms and are believed to be one of the significant contributors to increased mortality. Autopsy studies have revealed microthrombi not only in the pulmonary vessels but also in other organs. This highlights the importance of effective thromboprophylaxis and treatment of thrombotic complications in patients with COVID-19, especially for those requiring intensive care. The degree of lymphopenia, thrombocytopenia and coagulation profile abnormalities was increased in COVID-19 patients who died. This highlights the importance of early recognition of abnormal haematological phenomena and intervention to improve outcomes.

Figure 2. Main haematological changes in SARS-CoV-2 infection.



CHAPTER 3. DRUG INTERACTIONS BETWEEN ANTI COVID-19 AND ANTIPSYCHOTIC TREATMENTS

At the beginning of the pandemic, COVID-19 patients were treated with chloroquine, hydroxychloroquine (Colson et al., 2020; Shukla et al., 2020), azithromycin (Gautret et al., 2020) and lopinavir/ritonavir (Cao et al., 2020), with risk of QT prolongation and torsades de pointes, with possible other drug interactions in concomitant administration with antipsychotics. These treatments given to patients with severe forms have in some cases led to QT prolongation, partly due to the QT-prolonging clinical condition and partly due to concomitant drugs, and clinicians have had to mitigate this risk (Giudicessi et al., 2020). This use becomes complicated in older patients by decreased drug metabolism (Zanger & Schwab, 2013), high incidence of concomitant somatic diseases, drug interactions, etc. (Gareri et al., 2014; Maher et al., 2011; Rivière et al., 2019). In addition, COVID-19 also affected the cardiovascular system with the occurrence of arrhythmias (atrial fibrillation, ventricular fibrillation and ventricular tachycardia), cardiac lesions, myocarditis, heart failure, pulmonary embolism and disseminated intravascular coagulation (Guzik et al., 2020)

Antipsychotics are associated with a proarrhythmic state and an increased risk of sudden cardiac death, with no substantial differences between first- and second-generation antipsychotics, but with a dose-dependent effect (Ray et al., 2009; Salvo et al., 2016). Antipsychotics (except aripiprazole and lurasidone) appear to be associated with QT prolongation and an increased risk of sudden death (Acciavatti et al., 2017). There are differences between antipsychotics in the degree of cardiotoxicity (Leucht et al., 2013). Sudden cardiac death is described as unexpected natural death from cardiac causes in a short time in a person who often does not have a previous potentially fatal heart condition (Wellens et al., 2014). Many antipsychotics exhibit some degree of potassium channel blockade inducing QT interval prolongation and increasing the risk of polymorphic ventricular tachycardia or torsade de pointes (Roden, 2004; Testai et al., 2004).

The most common acquired cause of prolonged QT syndrome and torsade de pointes is drug-induced QT interval prolongation. Patients requiring intensive therapy are prone to experience QTc prolongation, mainly due to certain drugs that can prolong the repolarization phase, either by their mechanism of action or by interaction with other drugs (Etchegoyen et al., 2017). Elderly patients seem to be more likely to develop arrhythmia during antipsychotic treatments (Vieweg et al., 2009). Also, the action of antipsychotics is mainly mediated by cytochrome P450. Therefore, clinicians need to be aware of concomitantly administered drugs that may inhibit or induce enzymes of this CYP CYP (Conley & Kelly, 2007; Kennedy et al., 2013; King et al., 2004).

Drugs used in the treatment of SARS-CoV-2 (Sanders et al., 2020) such as chloroquine, hydroxychloroquine, lopinavir/ritonavir, remdesivir, tocilizumab, azithromycin, have shown promising results in the beginning but with much controversy later (Gautret et al., 2020). Other compounds have

been considered for therapeutic potential as anti-COVID-19 agents such as favipiravir (Jean et al., 2020), baricitinib (Cantini et al., 2020) and anakinra (Monteagudo et al., 2020).

3.9 Clozapine

Clozapine is a second-generation antipsychotic and is the gold-standard treatment for treatment-resistant schizophrenia. However, it is still underused because of its potential to cause neutropenia and agranulocytosis (Rajagopal, 2005).

The most common side effects of clozapine include neutropenia, agranulocytosis, cardiovascular side effects (tachycardia, orthostatic hypotension or hypertension, myocarditis), gastrointestinal side effects (constipation, ileus, dyspepsia, nausea, sialorrhoea, vomiting, weight gain), nervous system effects (dizziness, drowsiness, insomnia, sedation, vertigo, fever) or metabolic side effects (diabetes, dyslipidaemia, increased body mass index) (Dragoi et al., 2020).

Neutropenia is defined as absolute neutrophil count (ANC) less than 1500/ml; in terms of severity, it can be mild (ANC range 1000/ml-1500/ml), moderate (ANC range 500/ml-1000/ml) and severe (ANC below 500/ml) (Hsieh et al., 2007). It has a variety of causes, such as viral infections, drugs (such as clozapine), therapeutic radiation, autoimmune diseases, malignant diseases, nutritional deficiencies, congenital causes (benign ethnic neutropenia, BEN) and others (Newburger, 2016).

Data are contradictory regarding COVID-19 secondary neutropenia. Some authors show that clozapine patients are at increased risk if associated with COVID-19, other authors (Gee & Taylor, 2020) have shown no statistically significant changes in ANC in clozapine-treated patients testing positive for SARS-CoV-2. In the case of COVID-19 vaccination in clozapine patients, a study of 139 patients showed that clozapine blood levels increased significantly after the second vaccination, and changes in leukocyte counts were limited to mild granulocytopenia, moderate granulocytopenia and leukocytopenia (Veerman et al., 2022).

3.10 Conclusions

Clozapine treatment in patients with COVID-19 may be associated with a temporary reduction in ANC levels, in some cases reaching neutropenic levels, and a slightly increased risk of thromboembolism, pneumonia and toxicity. However, discontinuation of clozapine leads to psychotic relapses, with difficult implications for patients. Since the rates of neutropenia reported in SARS-CoV-2 infected patients are higher than pre-pandemic reports, we assume that in COVID-19 positive patients neutropenia is the result of immunological interference between clozapine and SARS-CoV-2. Future research is needed to clarify this issue. Clozapine-treated patients who develop COVID-19 should continue clozapine whenever possible; the dose will be adjusted if necessary, in relation to blood test results, and treatment will be discontinued if there is a significant decrease in neutrophil counts. Tachycardia, hypotension, fever and

sedation are common side effects of clozapine, which should be managed according to usual recommendations, regardless of COVID-19 infection. Even more frequent monitoring of blood cell counts and plasma clozapine concentration seems to be the general recommendation; however, this is not always possible during a pandemic, when access to healthcare facilities is often limited.

CHAPTER 4. SPECIAL PART

4.2 Objectives/ Working assumptions

The main objective of the present paper is to highlight the relationships between COVID-19 infection and the evolution of patients with schizophrenia.

Secondary objectives are:

- To highlight the anti-inflammatory effect in the context of COVID-19 infection in patients with schizophrenia.
- To highlight the inflammatory response in patients with schizophrenia and COVID-19 infection on clozapine treatment.
- Evidence of inflammatory response following anti-COVID-19 vaccination in patients with schizophrenia who experienced COVID-19 infection and were on antipsychotic treatment.

General working hypothesis:

- COVID-19 infection has a significant impact on patients with schizophrenia.

Secondary working hypotheses are:

- SARS-CoV-2 infection causes an increase in morbidity and mortality in patients with schizophrenia.
- SARS-COV-2 infection causes an exaggerated inflammatory response in clozapine patients.

Vaccination against COVID-19 in patients with schizophrenia who have experienced the disease and are taking clozapine is safe.

4.3 General methodology

4.3.1 SARS-CoV-2 virus

Coronaviruses (CoVs) are RNA viruses belonging to the order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, which infect humans and a variety of animals. The name derives from their structure, the presence of prominent, glycoprotein-like spicules arranged in the form of a crown on the surface of the virus particles. The spicules bind to certain cellular receptors, promoting infection of cells for which they are tropicalized.

The SARS-CoV-2 virus has no capsid, it has an envelope (E) made up of 4 structural proteins S (spires), E (envelope), M (membrane) and N (nucleocapsid). The non-structural proteins of SARS-CoV-2 are RNA polymerase, helicase and proteases (Mariano et al., 2020).

4.3.2 Molecular diagnosis of SARS-CoV-2 infection

This is based on the detection of specific viral RNA sequences by nucleic acid amplification tests such as Real Time RT-PCR.

Polymerase chain reaction end point (PCR) is one of the most innovative technologies in molecular biology.

Using PCR, specific sequences in a DNA or RNA template can be copied and amplified thousands to a million times using specific oligonucleotide sequences, thermostable DNA polymerase and successive thermal cycles. The classical end-point PCR method detects and quantifies amplified material only at the end of the last cycle and involves, in addition, a post amplification analysis involving gel electrophoresis, scanning and interpretation of the amplification curve.

By comparison, RT-PCR, the method we use in our lab, allows quantitative measurement of amplified material after each cycle. By monitoring the reactions during the exponential amplification phase, we can determine the initial amount of the target with high precision. RT-PCR doubles the number of target molecules after each amplification.

Advantages of using the real time technique include: the possibility of accurate amplicon measurement after each thermal amplification cycle, an increased detection range, amplification and detection in a single reaction tube, which eliminates post PCR manipulation and therefore the possibility of errors.

4.3.3 RT-PCR technique

The aim is to determine the presence of SARS-CoV-2 virus in human biological samples by amplification techniques of viral genetic material.

The field of application is the Medical Analysis Laboratory, Molecular Biology Department of the Clinical Hospital of Psychiatry and Neurology Brasov.

4.3.4 Working procedure

Seegene Nimbus is an automated system that performs pipetting of biological samples, reagents and mastermix preparation. The liquid pipetting system performs sample-plate-reagent transfer for extraction and purification of nucleic acids.

Detection and amplification is performed using the Bio-Rad CFX96 equipment to run thermal cycles.

The biological products required for virus detection are: nasopharyngeal exudate, oropharyngeal secretion, nasopharyngeal aspirate, nasal aspirate, collected using sterile swabs mounted on a flexible rod with a breakpoint, as well as bronchoalveolar lavage, sputum, necropsy material.

SARS-CoV-2 RNA is detectable in respiratory samples during the acute phase of the disease.

4.3.5 RT-PCR testing steps

There are three major steps that make up each cycle in RT-PCR reactions. These are usually carried out in 40 repetitive, successive cycles.

- Denaturation = incubation at high temperature is used to split double-stranded DNA into two single strands and to weaken the secondary structure of the single-stranded DNA. Typically, the highest temperature that DNA polymerase can withstand (95 0 C) is used;
- Abrupt cooling, the stage at which complementary sequences (primers) have the opportunity to hybridise; it is carried out at a temperature usually below 50 C;
- Extension, which is carried out at a temperature between 70-720 C, DNA polymerase activity is optimal at this temperature and primer extension occurs at a rate of up to 100 bases per second.

4.3.6 Working methodology

This thesis involved the study of three distinct batches as follows:

1. The group of patients with schizophrenia admitted to the Clinical Hospital of Psychiatry and Neurology in Brasov during the COVID-19 pandemic.

2. The group of patients with schizophrenia admitted to the Clinical Hospital of Psychiatry and Neurology in Brasov during the COVID-19 pandemic and who received clozapine treatment.

3. The group of patients with schizophrenia admitted to the Clinical Hospital for Psychiatry and Neurology in Brasov during the COVID-19 pandemic who had experienced infection, received clozapine treatment and were vaccinated with Pfizer/Biontech mRNA vaccine.

Our hospital entered as covid support hospital phase II by Order no. 623/2020 for the modification and completion of the Ministry of Health Order no. 55/2020. A number of 90 COVID-19 beds were made available serving an area of more than 500000 inhabitants.

The processing of personal data was carried out in accordance with the European legislation in this field (GDPR). The subjects included in the study were not remunerated. The study was approved by the Ethics Committee of the Clinical Hospital of Psychiatry and Neurology in Brasov.

Due to the aspects mentioned above, we consider that it is necessary to interpret the results in the context of the pandemic, the multiple waves and the viral strains that circulated (afa, delta, omicron, or others not yet identified).

The creation of the database was made possible using Microsoft Excel 2021 software. Statistical analysis was performed using GraphPad Prism 9 and MedCalc software. The statistical significance threshold α chosen, is 0.05, with a confidence interval, CI = 95%. The statistical analysis includes elements of descriptive statistics.

From the data collected, studies were conducted to achieve the primary and secondary objectives.

Study I - Inflammatory response in patients with schizophrenia infected with SARS-COV-2 on chronic antipsychotic treatment.

Study II - Occurrence of neutropenia in patients with schizophrenia treated with clozapine and infected with SARS-COV-2.

Study III - Outcome of COVID-19 mRNA vaccination in clozapine-treated patients who previously experienced SARS-COV-2 infection.

Study IV - Evaluation of the presence of myocarditis as an effect of COVID-19 mRNA vaccination in clozapine-treated patients.

4.4 STUDY 1. INFLAMMATORY RESPONSE IN PATIENTS WITH SCHIZOPHRENIA INFECTED WITH SARS-COV-2 ON CHRONIC ANTIPSYCHOTIC TREATMENT

4.4.2 Working hypothesis and objectives

The study aimed to determine whether health outcomes and care differ between patients with schizophrenia and those without a diagnosis of primary mental illness. The primary objective was to compare inflammatory responses and in-hospital mortality between patients with schizophrenia and those without a diagnosis of primary mental illness after being infected with SARS-COV-2.

4.4.3 Materials and methods

4.4.3.1 Study design

We conducted a prospective, cross-sectional, single-center study including 101 patients with schizophrenia treated with oral antipsychotics, admitted to a long-term inpatient unit at the Clinical Hospital of Psychiatry and Neurology in Brasov, Romania. The hospital is a public hospital with clinical psychiatric wards with 150 acute beds and 300 chronic beds.

4.4.3.6 Statistical analysis

We compared the baseline characteristics of both groups. Analysis of variance (ANOVA) and t-test were used to compare means. Confidence intervals of proportions were calculated using the Wilson method. Data were analyzed using SPSS version 26 for Windows. All P-values were bi-directional, with a p-value < 0.05 indicating statistical significance.

4.4.4 Results

101 patients with schizophrenia were admitted to a long-term inpatient unit belonging to the same hospital between 15 April 2020 and 15 April 2021. The characteristics of the patients and the control group are shown in Table 2.

Table 2. Patient characteristics

Characteristics		Schizophrenia	Control	P-value
		N=101	N=101	
Age	Mean (SD)	54.30 (10.83)	54.31 (10.13)	0.17
Male		51 (50.49%)	53 (52.47%)	0.67
Length of hospitalisation	Average (SD)	15.11 (7.47)	15.50 (7.96)	0.71
Severity of infection COVID-19	Mild	86; 85.14%	73; 72.27%	0.02
	Moderate	12; 11.87%	22; 21.78%	0.05

	Severe	2; 1.98%	6; 5.94%	0.15
Comorbidities	Pulmonary	12; 11.88%	4; 3.96%	0.03
	Cardiovascular	30; 29.70%	42; 41.58%	0.07
	Metabolic	38; 37.62%	25; 24.75%	0.04
	Neurological	3; 2.97%	4; 3.96%	0.70
	Other	11; 10.89%	13; 12.87%	0.66
	None	30; 29.70%	38; 37.62%	0.23
Deaths		0 (0%)	4 (3.96%)	0.04

All patients completed biochemical tests during hospitalization. Values obtained on admission (day 1) were considered as baseline. We observed higher values of some inflammation markers in the control group compared to those with schizophrenia (CRP 39.11±73.04 vs. 21.27±51.28, p=.04; fibrinogen 485.06±176.45 vs. 372.71±121.46, p=0). D-dimer levels were not statistically different. Comorbidities were more common in the schizophrenia group. Of all patients, 5.94% (n=6) were obese, with a BMI (body mass index) ≥ 30, 27.72%, (n=28) were overweight (BMI=25-29) and 66.34% (n=67) had a normal BMI. There were 55 (54.45%) cases with BMI ≥ 25 in the control group. In the schizophrenia group there are some altered values (e.g. anemia, hyponatremia), but these items are commonly found in institutionalized patients. There were no statistically significant differences for BMI. All the results of the laboratory analyses are presented in Table 3

Table 3. Laboratory results

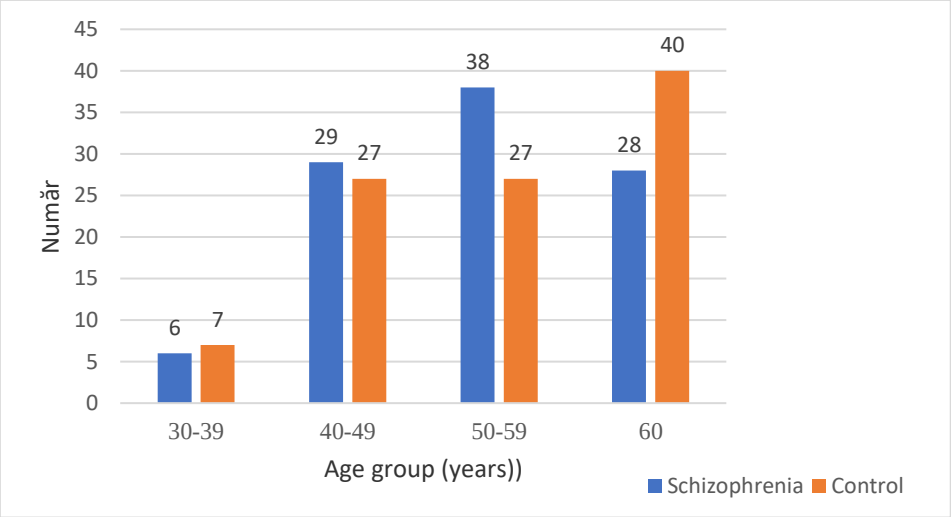
Parameters			Schizophrenia	Control	P-value
Laboratory analysis		Normal values	N=101	N=101	
PCR	Mean (SD)	0-5 MG/L	21,27 (51,28)	39,11 (73.04)	0.04
D-DIMER	Mean (SD)	0-500 MG/ML	858,14 (1253,35)	658,38 (717,27)	0.16
ESR	Mean (SD)	2-20 MM/H	25,39 (20,80)	31,27 (24,66)	0.06
WBC	Mean (SD)	4-10x10^9/L	6,58 (2,62)	7,42 (3,11)	0.03
FIBRINOGEN	Mean(SD)	200-400 MG/DL	372,71 (121,46)	485,06 (176,45)	0.0001

BAS	Mean (SD)	0,0-0,10x10^9/L	0,02 (0,01)	0,04 (0,19)	0.29
NEU	Mean (SD)	2,0 – 7,0x10^9/L	4,38 (3,85)	4,89 (2,94)	0.29
EOS	Mean (SD)	0,02-0,5x10^9/L	0,09 (0,11)	0,05 (0,06)	0.001
LYM	Mean (SD)	0,8-4,0x10^9/L	1,95 (0,07)	1,88 (0,74)	0.34
MON	Mean (SD)	0,12-1,2x10^9/L	0,51 (0,25)	0,54 (0,21)	0.33
RBC	Mean (SD)	4,39-5,5x10^{12}/L	4,33 (0,51)	4,53 (0,61)	0.01
HGB	Mean (SD)	12-16 G/DL	13,45 (1,47)	13,99 (1,36)	0.007
MCV	Mean (SD)	80-100 FL	90,38 (5,37)	89,49 (6,19)	0.27
MCH	Mean (SD)	27-34 PG	31,25 (2,17)	30,70 (2,36)	0.08
MCHC	Mean (SD)	32-36 G/DL	34,52 (0,73)	34,29 (0,89)	0.04
RDW-CV	Mean (SD)	11-16 %	13,97 (1,59)	13,55 (0,94)	0.02
RDW-SD	Mean (SD)	35-56 FL	44,97 (4,65)	43,10 (3,36)	0.001
HCT	Mean (SD)	36-48 %	38,93 (4,2)	40,72 (3,96)	0.002
PLT	Mean (SD)	150-400x10^9/L	220,13 (69,87)	247,44 (99,57)	0.02
MPV	Mean (SD)	6,5-12 FL	9,99 (1,21)	9,98 (0,98)	0.94
PCT	Mean (SD)	0,108-0,282 %	0,21 (0,06)	0,24 (0,08)	0.002
TGP	Mean (SD)	0-31 U/L	23,43 (22,13)	34,11 (25,10)	0.001
TGO	Mean (SD)	0-38 U/L	26,98 (24,18)	29,70 (18,93)	0.37
GLU	Mean (SD)	74-106 MG/DL	120,83 (39,4)	126,98 (43,47)	0.29
CREA	Mean (SD)	0,5-0,9 MG/DL	0,89 (0,43)	0,98 (0,87)	0.35
UREA	Mean (SD)	16,6-48,5 MG/DL	31,42 (18,24)	32,86 (18,03)	0.57
GGT	Mean (SD)	0-40 U/L	45,81 (51,48)	73,60 (110,50)	0.02
PHOSPHATASE	Mean (SD)	35-104 U/L	71,68 (18,58)	83,26 (18,19)	0.0001
HDLC	Mean (SD)	45-65 MG/DL	41,27 (9,26)	42,33 (15,87)	0.56
LDL	Mean (SD)	0-100 MG/DL	116,39 (47,55)	120,68 (54,69)	0.55
TRIG	Mean (SD)	0-150 MG/DL	160,84 (82,06)	175,5 (89,73)	0.22
AMYL	Mean (SD)	28-100 U/L	93,09 (83,10)	64,47 (20,29)	0.0009

K+	Mean (SD)	3,5-5,1 MMOL/L	4 (0,5)	4,05 (0,47)	0.46
NA+	Mean (SD)	136-145 MMOL/L	134,74 (4,12)	136,61 (2,99)	0.0003

The 2 groups analysed (schizophrenia vs. control) did not differ in mean age or age groups (Figure 5).

Figure 5. Distribution of patients by age group



There were no statistically significant differences in D-dimer, fibrinogen, CRP, TGO or TGP values between the two groups in any of the severity forms of COVID-19.

All patients with schizophrenia received antipsychotic treatment. Despite conflicting information at the beginning and during the COVID-19 pandemic, a significant number of patients (n=21; 21.21%) continued clozapine treatment during hospitalization. The reason was a history of aggression, violence or TRS (treatment-resistant schizophrenia). As patients were under supervision and stabilised on treatment, it is justified that they were not on high or maximal doses of antipsychotics. A large number of institutionalised patients are still treated with haloperidol (n=24, 23.76%).

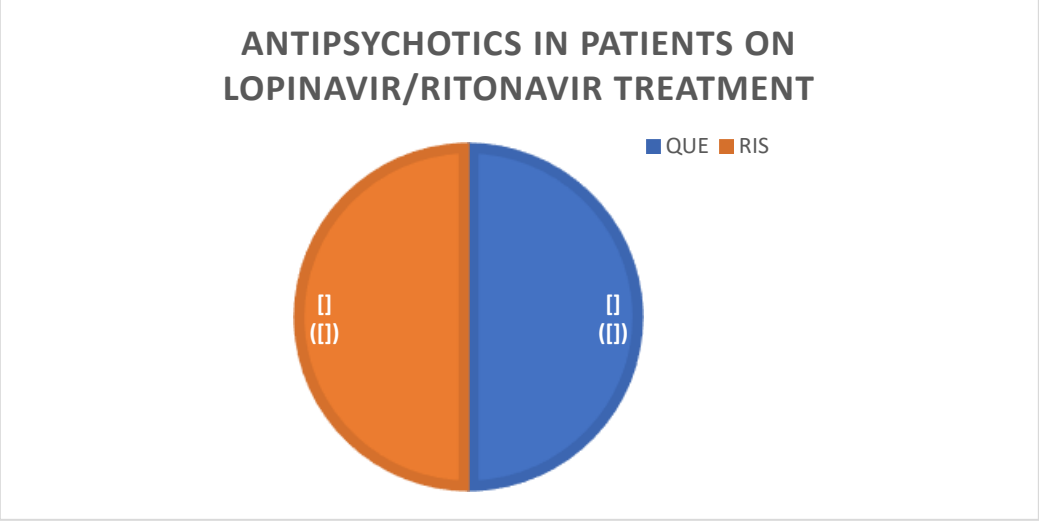
According to protocols, SARS-CoV-2 patients were treated with hydroxychloroquine, lopinavir/ritonavir, azithromycin, enoxaparin, etc. Anti-SARS-CoV-2 treatment was prescribed and monitored by the infectious disease physician. Treatment also included dexamethasone, mucolytics, antitussives as shown in Table 20.

Table 20. Symptomatic treatment

Treatment type	Schizophrenia n=101	Control n=101	P-value
oxygen therapy	1 (0,99%)	7 (6,93%)	**0.03**
hydroxychloroquine	0 (0%)	4 (3,96%)	**0.04**
lopinavir/ritonavir	2 (1,98%)	14 (13,86%)	**0.001**
azithromycin	29 (28,71%)	19 (18,81%)	0.09
paracetamol	79 (78,21%)	77 (76,23%)	0.73
dexamethasone	24 (23,76%)	17 (16,83%)	0.22
anticoagulant	39 (38,61%)	46 (45,54%)	0.31
antibiotics	26 (25,74%)	31 (30,69%)	0.43
mucolytics	15 (14,85%)	1 (0,99%)	**0.0003**
antitussives	15 (14,85%)	2 (1,98%)	**0.001**
analgesics	4 (3,96%)	8 (7,92%)	0.23

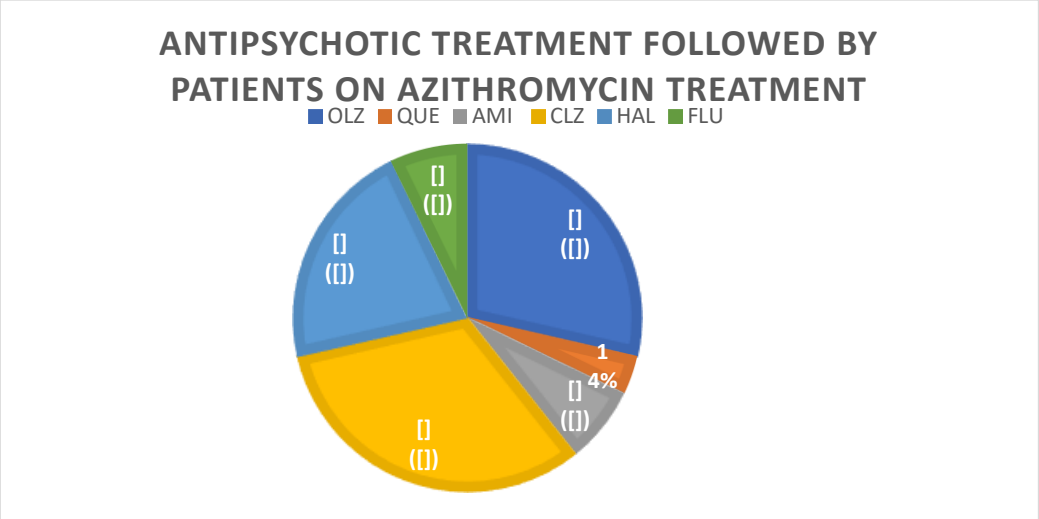
The distribution of patients treated with lopinavir/ ritonavir according to the antipsychotic treatment followed is illustrated in Figure 6.

Figure 6. Antipsychotics in patients on lopinavir/ritonavir treatment



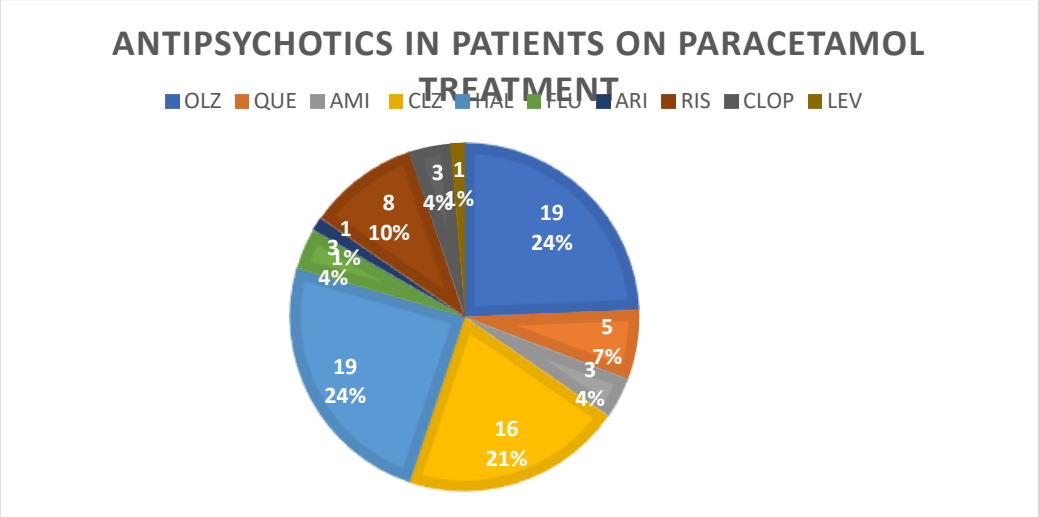
The distribution of azithromycin-treated patients according to the antipsychotic treatment followed is illustrated in Figure 7.

Figure 7. Antipsychotic treatment followed by patients on azithromycin treatment



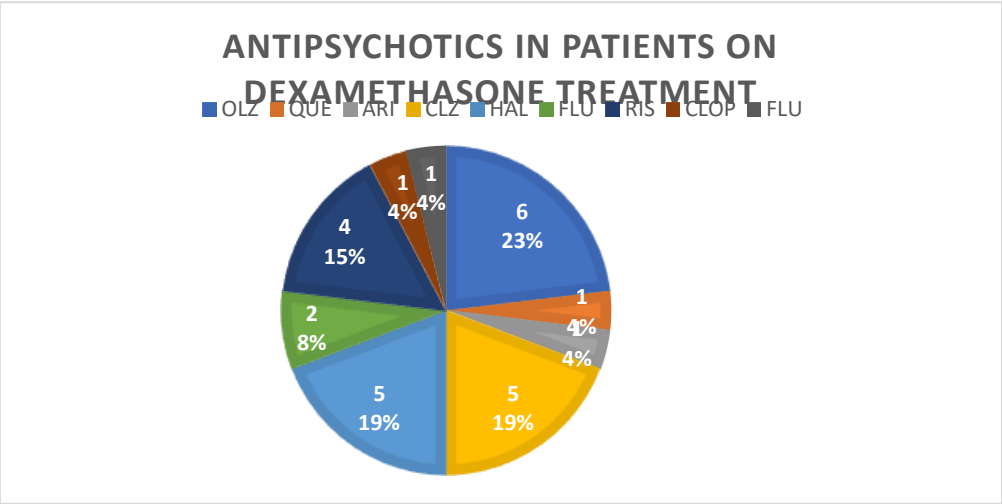
The distribution of patients treated with paracetamol according to the antipsychotic treatment followed is illustrated in Figure 8.

Figure 8. Antipsychotics in patients on paracetamol treatment



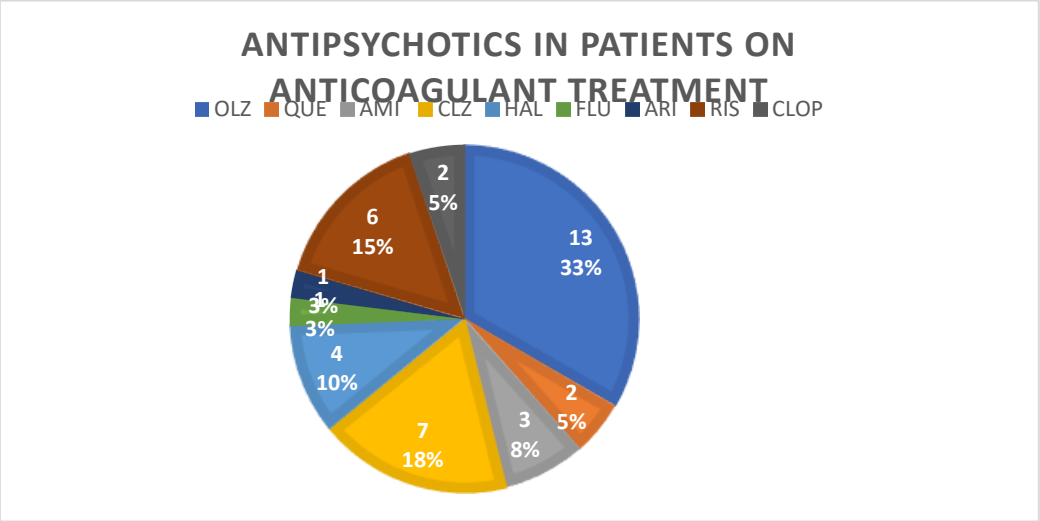
The distribution of patients treated with dexamethasone according to the antipsychotic treatment followed is illustrated in Figure 9.

Figure 9. Antipsychotics in patients on dexamethasone treatment



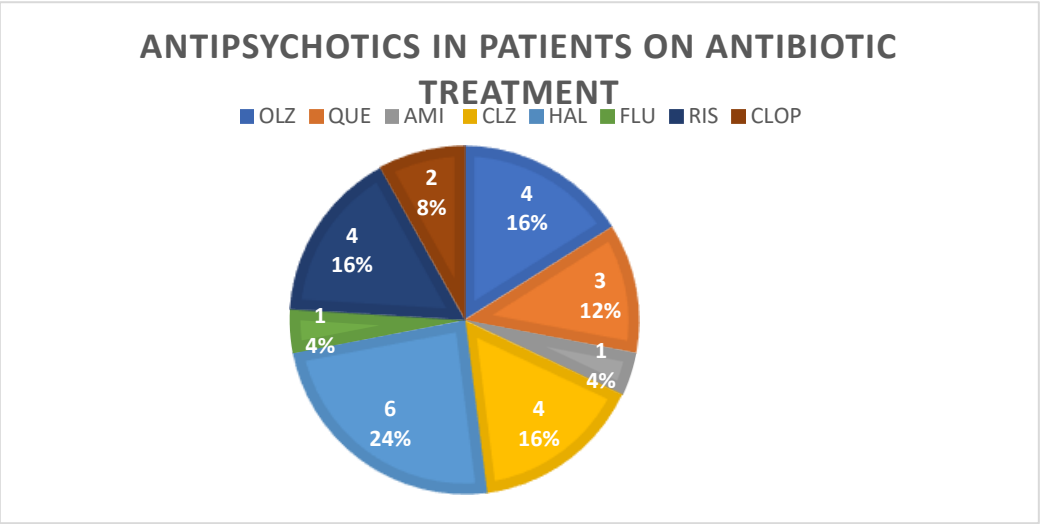
The distribution of patients treated with anticoagulant according to the antipsychotic treatment followed is illustrated in Figure 10.

Figure 10. Antipsychotics in patients on anticoagulant treatment



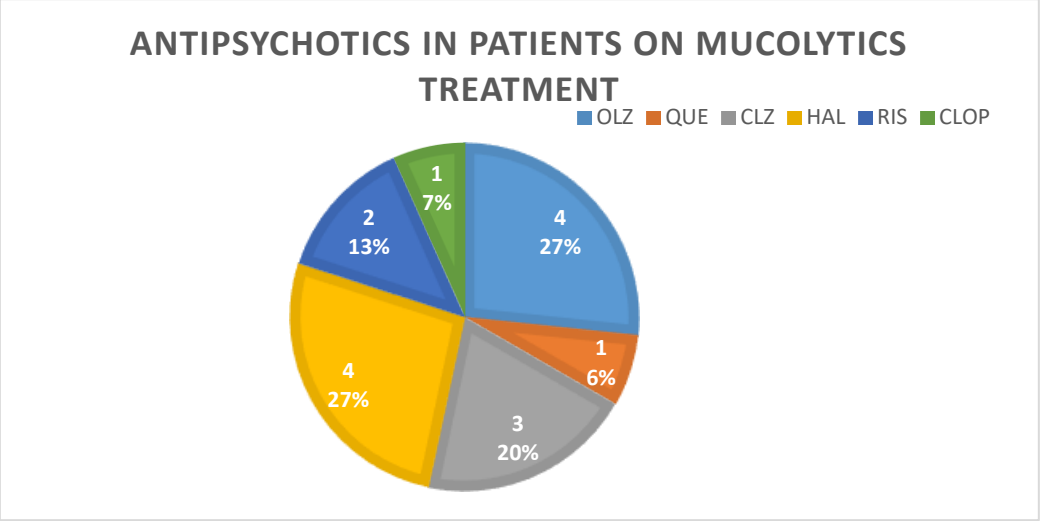
The distribution of patients treated with antibiotics according to the antipsychotic treatment followed is illustrated in Figure 11.

Figure 11. Antipsychotics in patients on antibiotic treatment



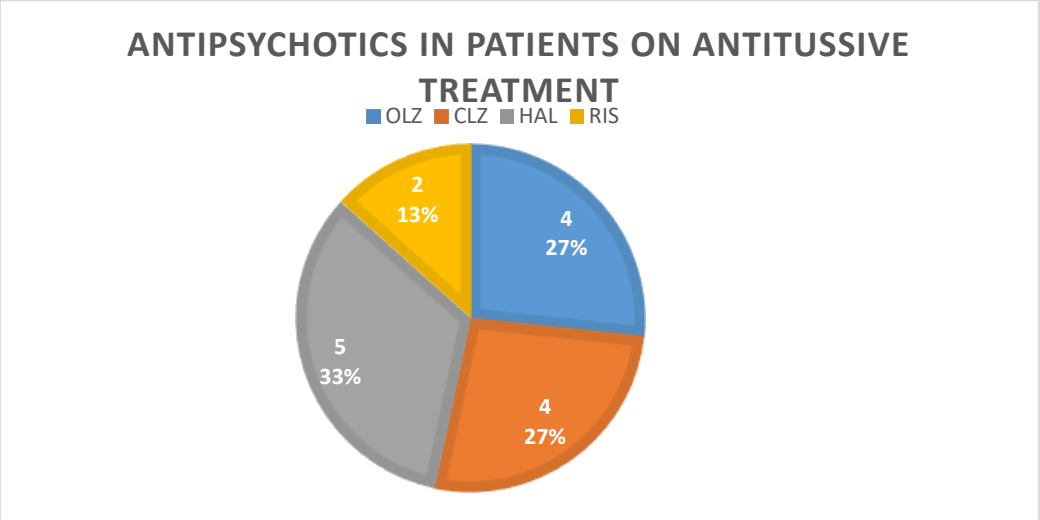
The distribution of patients treated with mucolytics according to the antipsychotic treatment followed is illustrated in Figure 12.

Figure 12. Antipsychotics in patients on mucolytics treatment



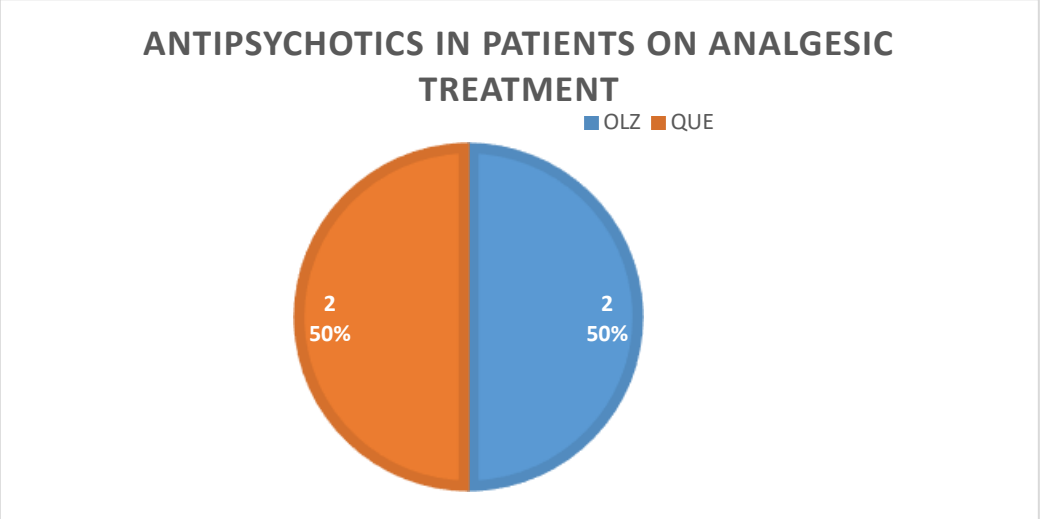
The distribution of patients treated with antitussives according to the antipsychotic treatment followed is illustrated in Figure 13.

Figure 13. Antipsychotics in patients on antitussive treatment



The distribution of patients treated with analgesics according to the antipsychotic treatment followed is illustrated in Figure 14.

Figure 14. Antipsychotics in patients on analgesic treatment



We checked the distribution of COVID-19 severity across age groups. This is detailed in Table 21.

Table 21. Distribution of COVID-19 severity in different age groups

Age groups [years]	Schizophrenia			Control		
	Mild	Moderate	Severe	Mild	Moderate	Severe
30-39	6 (100%)	0	0	6 (85.71%)	1 (14,29%)	0
40-49	24 (82,76%)	3 (10,34%)	2 (6,9%)	23 (85.19%)	4 (14,81%)	0
50-59	31 (81,58%)	7 (18,42%)	0	21 (77,78%)	5 (18,52%)	1 (3,7%)
≥60	25 (89,29%)	3 (10,71%)	0	23 (57,5%)	12 (30%)	5 (12,5%)

4.4.6 Conclusions

The main findings of the study were that vulnerable individuals with schizophrenia on antipsychotic treatment showed a lower risk of severe SARS-CoV-2 infection and a likely better prognosis in a protective environment. It could be speculated that antipsychotics might play an important role in preventing severe manifestation of SARS-CoV-2 and might have protective effects against harmful manifestations of COVID-19. The results of the present study have to be taken judiciously, as not all factors involved in the complex network of COVID-19 infection were considered.

4.5 STUDY 2. OCCURRENCE OF NEUTROPENIA IN PATIENTS WITH SCHIZOPHRENIA TREATED WITH CLOZAPINE AND INFECTED WITH SARS-COV-2

4.5.2 Working hypothesis and objectives

In this study we aimed to assess the absolute neutrophil count (ANC) in a group of schizophrenia patients treated with clozapine and who tested positive for COVID-19 by PCR-RT. The study methodology was approved by the local hospital ethics committee.

4.5.3 Material and method

The evaluation was performed by three board-certified psychiatrists and one laboratory medicine specialist with clinical research experience. Demographic data included age, sex, duration of illness, duration of clozapine treatment and clozapine dose. We compared mean values for ANC, lymphocyte count, and WCC for three data sets: before COVID-19 infection vs. baseline assessment (at the time of confirmed SARS CoV infection; baseline assessment vs. assessment after COVID-19 infection; before infection vs. after COVID-19 infection. According to hospital protocols, all blood samples for laboratory analysis were collected in the morning on an empty stomach. All samples were collected from peripheral venous blood collected in standardized kits. SARS-CoV-2 infection was confirmed by 2 consecutive polymerase chain reaction (PCR) tests (day 1 and day 5) performed by a laboratory medicine specialist.

4.5.3.5 Statistical analysis

The results were analysed using SPSS version 20.00. Adjusted odds ratios (AOR) with 95% CI were calculated and p-values less than 0.05 using the t-test method. Multivariate logistic regression was considered to indicate a significant association.

4.5.4 Results

The study included a total of 105 patients. Of the 95 cases without neutropenia, 59 patients were male (62.1%); the mean age in this group was 43.5 years (SD=12.1) with a mean duration of clozapine treatment of 52.4 months (SD=11.9) (range 2 years to 12 years). At baseline assessment, there was a small reduction in the mean ANC value (4.41×109/l; SD=2.22), which was not a statistically significant decrease from the mean pre-infection COVID-19 value of 4.66×109/l (SD= 2.34; p=0.45). ANC values were also normal in the first month after PCR-negative testing (4.45×109/l; SD=2.35; p=0.91). 21 patients were from the chronic ward, 44 patients from the acute ward and 40 from ambulatory treated patients. There were no deaths during COVID-19 hospitalization. Patient characteristics are described in Table 22.

Tabel 1. Patients characteristics

Characteristics		**Group without neutropenia**	**Group with neutropenia**	**P-value**
		N=95	N=10	
Age	mean (SD)	43.5 (12.1)	45.7 (7.8)	0.57
Male		59 (62.1%)	6 (60%)	0.89
Clozapine treatment duration (months)	mean (SD)	52.4 (11.9)	46 (12.1)	0.11
Clozapine doze	mean (SD)	306 (246)	310 (212)	0.86
Duration of hospitalization on Covid ward (days)	mean (SD)	14.12 (1.6)	18.23 (2.3)	0.001
Severity of Covid Infection	Mild	88; 92.6%	7; 70%	0.02
	Moderate	6; 6.3%	2; 20%	0.12
	Severe	1; 1.1%	1; 10%	0.06
Smokers		63; 60%	7; 70%	0.53
Comorbidities	Pulmonary	15; 15.8%	3; 30%	0.25
	Cardiovascular	20; 21.1 %	2; 20%	0.93
	Metabolic	28; 29.5 %	3; 30%	0.49
	Neurological	3; 3.1 %	1; 10%	0.08
	Other	11; 11.6 %	1; 10%	0.88
	None	18; 18.9 %	0; 0%	0.13
Deaths		0; 0%	0; 0%	-

Clozapine doses were significantly higher in acute patients than in ambulatory treated patients (325 ± 246 mg, vs. 220 ± 120.2 mg, p=0.03). The age group distribution of patients in the two study groups is illustrated in Figure 15 and Figure 16.

Figure 15. Age groups in patients without neutropenia

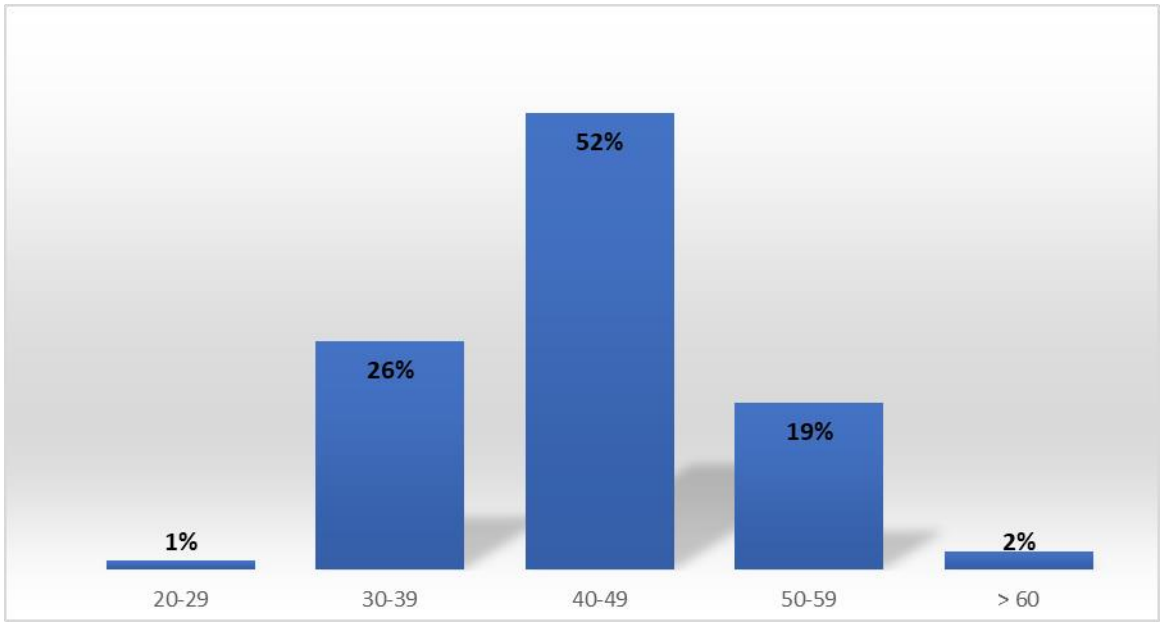
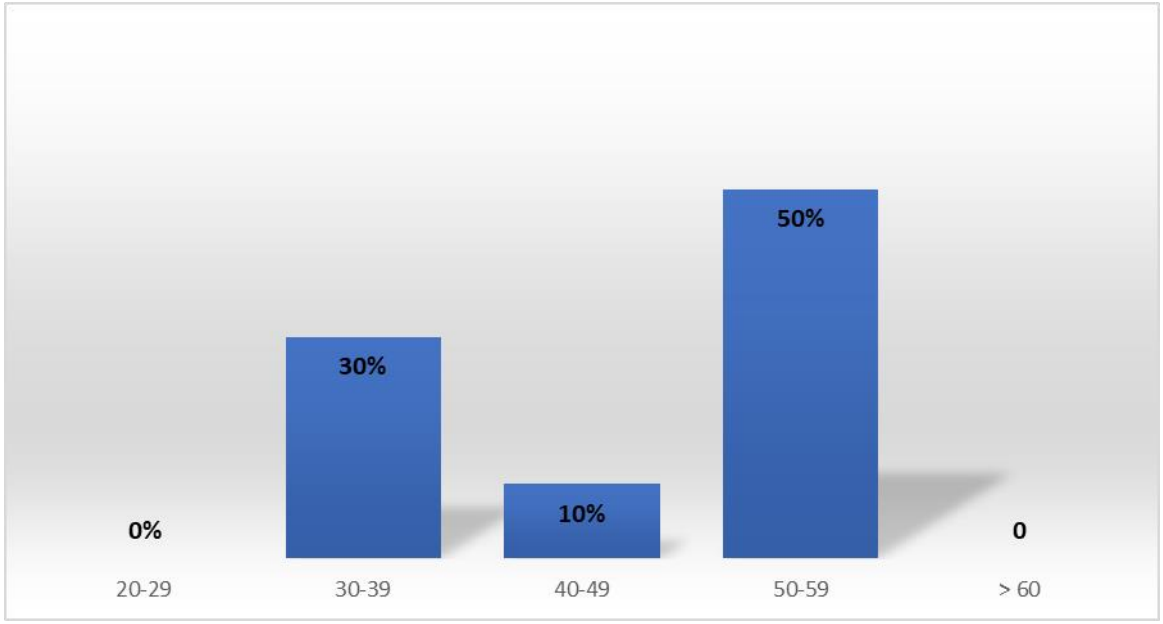


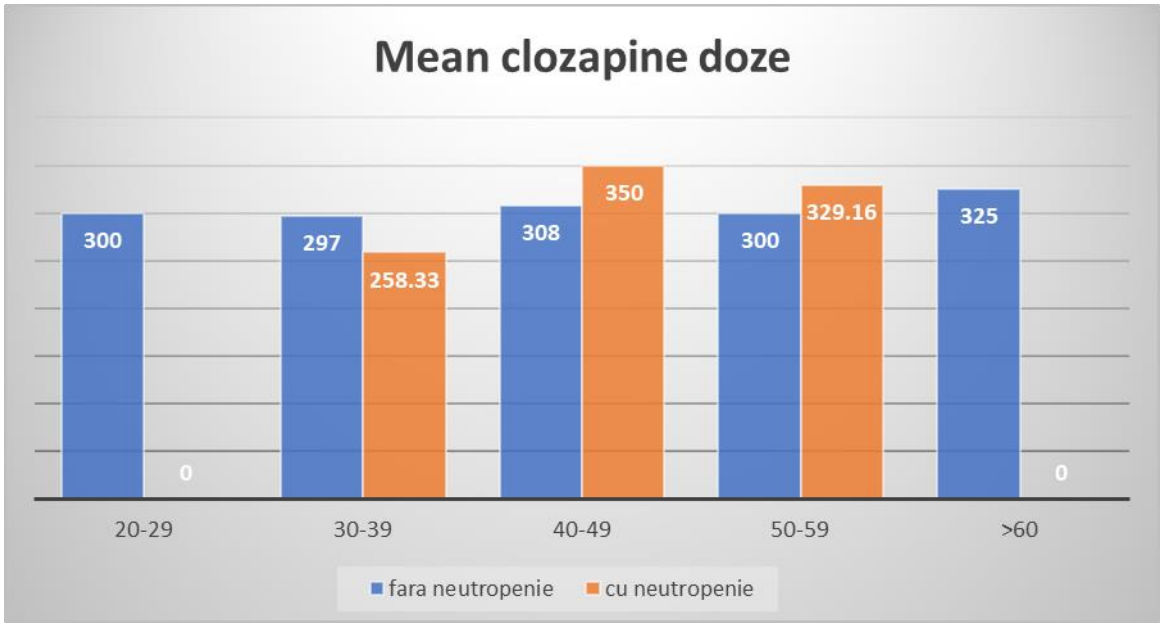
Figure 16. Age groups in patients with neutropenia



From the two figures above it can be seen that most patients with neutropenia came from the age group 50-59 years (50%).

The mean clozapine doses in the two study groups are illustrated in Figure 17.

Figure 17. Mean clozapine dose in the two groups



Regarding the doses of clozapine used, it can be seen that the average dose was similar in the two groups. The highest doses of clozapine were in the group of patients aged 40-49 years for those with neutropenia.

Blood parameters are shown in Table 23 and Table 24.

Table 23. Blood parameters in patients without neutropenia (n=95)

Parameters	Before Covid-19 infection (before first positive PCR test)	Initial assessment (first positive test)	After Covid-19 infection (after first negative PCR test)	P-value before infection vs. Initial assessment	P-value initial assessment vs. after infection
Leukocytes mean; SD; (min și max)	7.33; 2.76; 3.41-16.22	7.28; 2.45; 2.85-15.79	7.11; 2.73; 3.11-15.33	0.56	0.78
Neutrophil mean; SD; (min și max)	4.66; 2.34; 0.73-12.56	4.41; 2.22; 0.72-12.98	4.45; 2.35; 0.76-14.21	0.45	0.91
Lymphocyte mean; SD; (min and max)	1.78; 0.73; 0.5-3.74	1.73; 0.74; 0.5-3.56	1.75; 0.73; 0.6-3.21	0.63	0.77

Neutropenia at initial assessment was identified in 10 cases (Table 24). In 9 cases, neutropenia was mild (1.0-1.5 x 109/l) and in one case it was moderate (0.76 x 109/l), leading to discontinuation of clozapine and switching to another antipsychotic. COVID-19 symptoms were mild (only 2 cases had moderate symptoms). Switching clozapine caused relapse in 7 patients (70%), leading to prolonged hospitalisation compared to those without neutropenia.

Tabel 2. Blood parameters in patients with neutropenia (n=10)

Parameters	Before Covid-19 infection (before first positive PCR test)	Initial assessment (first positive PCR test)	After Covid-19 infection (after first negative PCR test)	P-value before infection vs. Initial assessment	p-value initial assessment vs. after infection
Leukocytes mean; SD; (min și max)	7.14; 2.51; 3.47-13.61	3.91; 1.57; 2.41-5.55	6.12; 2.48; 3.55-16.03	0.002	0.37
Neutrophil mean; SD; (min și max)	4.48; 2.30; 0.76-12,43	1.51; 0.64; 0.72-1.91	4.76; 2.25; 1.7-12.44	0.001	0.78
Lymphocytes mean; SD; (min și max)	2.0; 0.78; 0.64-3.74	1.69; 0.68; 1.7-3.46	1.77; 0.65; 0.5-3.64	0.35	0.49

According to the local protocol, patients were treated with hydroxychloroquine, lopinavir/ritonavir azithromycin and enoxaparin.Patients were not treated with monoclonal antibodies and none were vaccinated against COVID-19 at the time of evaluation. The number of patients requiring oxygen therapy was small.

4.5.6 CONCLUSIONS

COVID-19 might be associated with a temporary reduction in ANC levels, which is mild, temporary and statistically insignificant in the vast majority of patients, including those treated with clozapine. We identified neutropenia in a small group of patients and assumed that this was caused by coronavirus infection and interaction with clozapine. Discontinuation of clozapine treatment could lead to relapses, with severe consequences for patients and their families. It is appropriate to continue clozapine in patients who are stable on this treatment.

4.6 STUDY 3. COVID-19 mRNA VACCINATION RESULTS IN CLOZAPINE-treated PATIENTS WHO PREVIOUSLY EXPERIENCED SARS-COV-2 INFECTION

4.6.2 Working hypothesis and objectives

The haematological effects of Pfizer-BioNTech COVID-19 mRNA vaccine were not investigated in clozapine-treated patients who had also experienced SARS-CoV-2 infection.

The primary objective of our research was to assess the presence of granulocytopenia (mild, moderate or severe or agranulocytosis), leukocytopenia or lymphocytopenia after COVID-19 vaccination in clozapine-treated patients with a history of SARS-CoV-2 infection.

4.6.3 Material and method

4.6.3.1 Study design

We conducted a cross-sectional analytical study. The enrollment period was extended from July 1, 2021 to June 30, 2022. The study was conducted in the Clinical Hospital of Psychiatry and Neurology Brasov, a medical unit with 100 beds for psychiatric emergencies and 315 beds for long-term hospitalizations. Shortly after the declaration of the COVID-19 pandemic, on 11 March 2020, our hospital was declared a medical unit for the treatment of psychiatric and neurological patients infected with SARS-CoV-2. More than 1000 patients have been treated so far.

Participants were divided into two study groups according to the antipsychotic with which they were being treated; patients were treated with either clozapine (clozapine group, CLZ) or other oral antipsychotics (non-clozapine group, NON-CLZ). Patients data were extracted from electronic documents and general cynical observation sheets from the Clinical Hospital of Psychiatry and Neurology Brasov. All data were collected by certified medical staff, including a laboratory physician and four psychiatrists. The following were analyzed: type of COVID-19 infection (mild, moderate or severe), duration since infection, type of vaccine used.

The study was approved by the hospital's Ethics Committee (Approval number: 28014/20.12.2022).

4.6.3.6 Statistical analysis

The results were analysed using SPSS version 20.00 software. T-test was used to compare means and Chi-square test for proportions. We calculated chlorpromazine equivalent doses for clozapine and other antipsychotics using equivalence tables (Atkins et al., 1997; Inada & Inagaki, 2015). A p-value less than 0.05 was considered statistically significant.

4.6.4 Results

A total of 357 patients were initially evaluated. Of these, 255 were excluded because they did not have complete documentation, were not on antipsychotic treatment or were not vaccinated against SARS-CoV-2. Following this initial screening, we selected a total of 100 patients who fully met the inclusion/exclusion criteria. From this population, two initial study groups were formed, namely 50 patients treated with clozapine and 52 patients treated with other antipsychotics. Analysing the types of anti-COVID-19 vaccines administered to the study population, we found that the number of patients vaccinated with Johnson&Johnson was too small to be statistically significant, so these patients were excluded from the final analysis.

Figure 18 shows data on the flow of patient enrolment in the study.

Figure 18. PRISMA chart

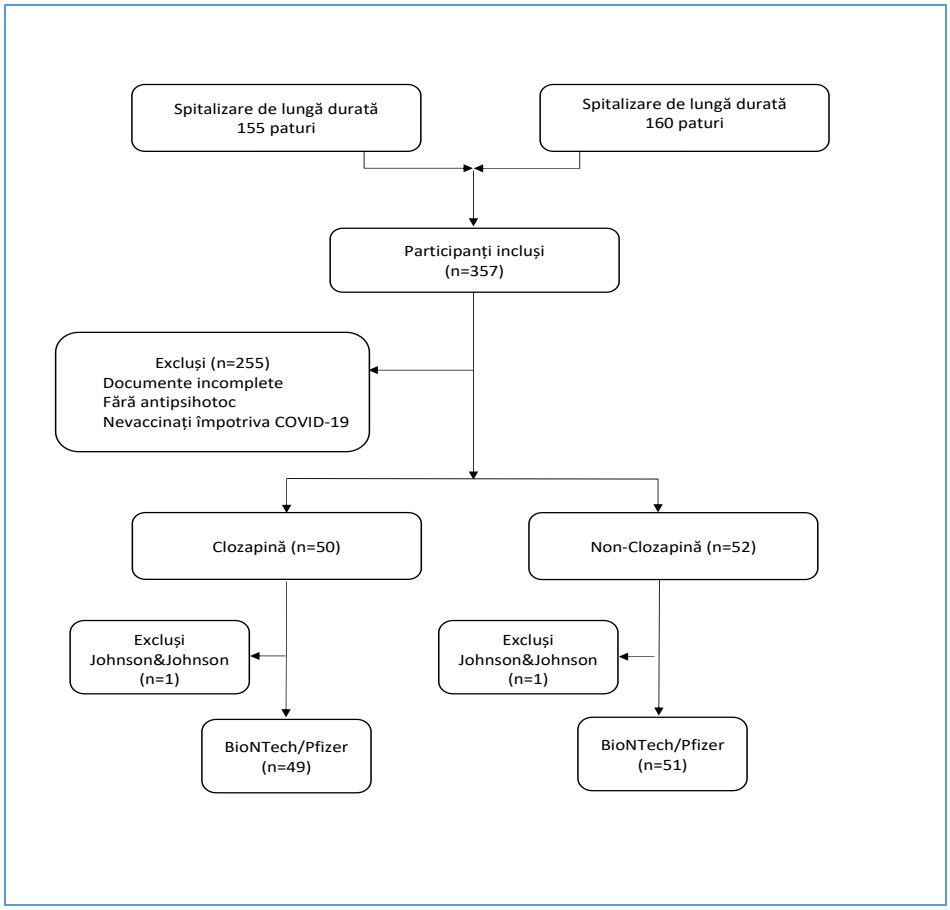


Table 3. Demographic characteristics

Characteristics	**Patients treated with clozapine n=49**	**Patients treated with other antipsychotics n=51**	**P-value**
male (n, %)	28 (57.1%)	22 (43.1%)	0.16
age (mean ± SD)	51.51 ± 10.47	58.06 ± 8.84	0.001

diagnosis			
schizophrenia (n, %)	44 (90%)	43 (84.31%)	0.40
Schizoaffective disorder (n,%)	2 (4%)	3 (5.88%)	0.67
Bipolar affective disorder (n, %)	3 (6%)	5 (9.81%)	0.48
Clozapine doze (mean ± SD, min; max)	273.96 ± 130.81 50; 575	-	-
Chlorpromazine equivalent dose (mean ± SD, min; max)	273.96 ± 130.81 50; 575	352.94 ± 199.55 100; 1066.66	0.02

The results showed a higher proportion of male patients treated with clozapine compared to those treated with other antipsychotics, but without statistical significance (p=0.16), confirming the results of other studies (Si et al., 2012; Szymanski et al., 1996).

We observed a trend of decreasing age in patients with schizophrenia (Szalontay et al., 2015). This phenomenon could be caused by the higher level of stress in today's society as well as the reduced possibilities in recent years to devote time to care and supervision of this category of patients (Uggerby et al., 2011). Clozapine treatment initiation is also easier in younger patients because they generally have fewer comorbidities (Manuel et al., 2012; Stroup et al., 2014).

Our results show that the mean age was significantly lower in the clozapine group (51.51 ± 10.47 vs. 58.06 ± 8.84, p=0.001). No significant difference was observed in time since SARS-CoV-2 infection (6.94 ± 2.04 months in the CLZ group, vs. 7 ± 2.04 in the non-CLZ group, p=0.88), or COVID-19 severity (one severe case in each group, p=0.97).

Mean doses in our study were within the established therapeutic range and were as follows: clozapine-273.96 mg/day (SD ± 130.81, Range 50-575); haloperidol - 10.6 mg/day (SD ± 4.13, Range: 4-16); olanzapine - 12.85 mg/day (SD ± 4.63, Range: 5-20); amisulprid - 480 mg/day (SD ± 178.88, Range: ±400-800); risperidone 3.2 mg/day (SD ± 1.69, Range: 1.5-6); quetiapine - 420 mg/day (SD ± 249, Range: 100-800). Doses of antipsychotics are similar to those reported in most studies of institutionalised patients with schizophrenia (Hagen et al., 2005).

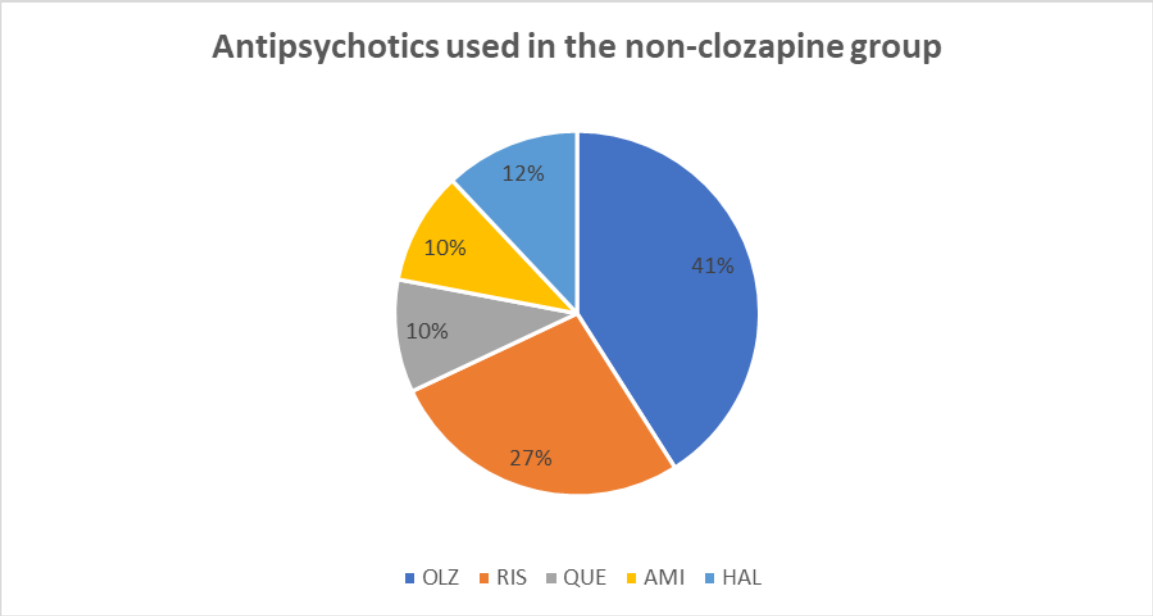
Mean antipsychotic doses and chlorpromazine equivalents are detailed in Table 26. We found that the doses used in the non-clozapine group were significantly higher than the clozapine group.

Table 26. Average antipsychotic dose and chlorpromazine equivalent

Antipsychotic	Equivalent dose (mg)
Chlorpromazine	100
Clozapine	100
Olanzapine	5
Quetiapine	75
Risperidone	1
Amisulprid	278.4
Haloperidol	10

The majority of patients in the non-clozapine group were treated with olanzapine (n=21, 41%) and risperidone (n=14, 27%). The distribution according to the antipsychotic used is shown in Figure 19.

Figure 19. Antipsychotics used in the non-clozapine group



hal- haloperidol, olz- olanzapine, que- quetiapine, ami- amisulprid, ris- risperidone

4.6.5 Haematological findings

All blood samples were taken in the morning, pre-prandial, and were processed the same day in the hospital laboratory. The results showed some significant differences between the two groups, namely in WBC and NEU values, which surprisingly were lower in the Non-clozapine group, while EOS, LYM and

MCV were significantly higher in the Non-clozapine group. The haematological parameters assessed in this study are detailed inTable 27.

Table 27. Haematological parameters

Laboratory analysis	Rage/Unit SI	Patients treated with clozapine n=49	Patients treated with other antipsychotics n=51	P-value
HGB (mean, SD)	12-16 / g/dL	13.19 ± 1.47	13.54 ± 1.51	0.24
HCT (mean, SD)	36-48 / %	39.26 ± 4.17	40.52 ± 4.45	0.15
WBC (mean, SD)	4-10 / 10*9/L	8.54 ± 3.08	7.24 ± 2.14	0.01
RBC (mean, SD)	4-5.5 / 10*12/L	4.43 ± 0.49	4.41 ± 0.50	0.84
PLT (mean, SD)	150-400 / 10*9/L	240.92 ± 67.33	237.65 ± 60.28	0.80
MCV (mean, SD)	80-100 / fL	88.65 ± 3.37	92.01 ± 4.18	< 0.0001
LYM% (mean, SD)	20-40 / %	33 ± 11.71	37.35 ± 8.89	0.04
MON% (mean, SD)	3.0-12.0 / %	7.11 ± 1.81	7.11 ± 1.75	1
BAS% (mean, SD)	0.0-1.0 / %	0.34 ± 0.12	0.31 ± 0.10	0.18
NEU% (mean, SD)	50-70 / %	57.34 ± 12.30	51.75 ± 9.30	0.01
EOS% (mean, SD)	3.5-5.0 / %	1.95 ± 1.80	3.46 ± 2.30	0.0004

A few cases of granulocytopenia were noted: four cases out of 49 patients (8.16%) in the clozapine group compared to two cases out of 51 patients (3.92%) in the non-clozapine group; the difference is not statistically significant (p=0.37). No cases of moderate or severe granulocytopenia or agranulocytosis were identified. Three patients in the clozapine group had lymphocytopenia; the same number of cases of lymphocytopenia was identified in the non-clozapine group. Full results are shown in Table 28.

Table 28. Granulocytopenia, leukocytopenia and lymphocytopenia in the groups analysed

	Mild granulocytopenia (1.5-2.0 x 10^9/L)	**Moderate granulocytopenia (1.0-1.5 x 10^9/L)**	**Severe granulocytopenia (0.5-1.0 x 10^9/L)**	**Agranulocytosis (<0.5 x 10^9/L)**	**Leukocytopenia (<3.5 x 10^9/L)**	**Lymphocytopenia (<1.5 x 10^9/L)**
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
CLZ	4/49 (8.16)	0/49 (0)	0/49 (0)	0/49 (0)	0/49 (0)	3/49 (6.12)
NON-CLZ	2/51 (3.92)	0/51 (0)	0/51 (0)	0/51 (0)	1/51 (1.96)	3/51 (5.88)

	Neutrophil			**Leukocyte**			**Lymphocyte**		
	Mean ($x10^9$/L)	ES	P-value	Mean ($x10^9$/L)	ES	P-value	Mean ($x10^9$/L)	ES	p-value
CLZ	5.08	0.28	0.0035	8.54	0.24	0.0157	2.66	-0.01	0.91
NON-CLZ	3.79			7.24			2.68		

CLZ- clozapine group; NON-CLZ- non-clozapine group; ES=effect size.

4.6.7. Conclusions

The COVID-19 pandemic has brought additional stress to psychiatric patients, especially those treated with clozapine. The fear of combined side effects of treatment, viral infection and vaccination had multiple consequences: switching to other antipsychotics or refusing vaccination. Our results show that the use of Pfizer-BioNTech vaccines against COVID-19 was safe in clozapine-treated patients who were previously infected with SARS-CoV-2. Further research is needed to clarify the effects of other COVID-19 vaccines in this population.

4.7 STUDY 4. ASSESSMENT OF PATIENTS AT RISK FOR ACUTE MYOCARDITIS AFTER COVID-19 mRNA VACCINATION - IN PATIENTS WITH SCHIZOPHRENIA

4.7.2 Working hypothesis and objectives

The cardiovascular effects of Pfizer-BioNTech COVID-19 mRNA vaccine have not been investigated in patients treated with clozapine.

4.7.3 Material and method

4.7.3.1 Study design

We conducted a prospective observational study. The enrolment period was extended from 1 July 2021 to 30 June 2022. The study was conducted in the Clinical Hospital of Psychiatry and Neurology Brasov, a medical unit with 100 beds for psychiatric emergencies and 315 beds for long-term hospitalizations. As mentioned in previous studies, shortly after the declaration of the COVID-19 pandemic, on 11 March 2020, our hospital was declared a medical unit for the treatment of patients with psychiatric and neurological diseases infected with SARS-CoV-2. More than 1000 patients have been treated so far. Patients with schizophrenia who have been treated with clozapine and vaccinated have entered the monitoring period for potential cardiovascular side effects. Patients were followed up for a period of 14 days after administration of the second dose of SARS-CoV-2 vaccine.

The study was approved by the hospital's Ethics Committee (Approval number: 28014/20.12.2022).

4.7.3.6 Statistical analysis

Descriptive statistics were calculated for all study variables. Quantitative variables were summarised with means and standard deviations, and medians with interquartile ranges.

4.7.4 Results

Fifty patients were included in the study whose characteristics are shown in Table 29.

Table 29. Demographic characteristics

Characteristics	**Patients treated with clozapine n=50**
Male (n, %)	29 (58.0%)
Age (mean ± SD)	50.22 ± 11.34
Age at onset of disease (mean ± SD)	23.67 ± 3.45
Diagnosis	
Schizophrenia (n, %)	50 (100%)
Clozapine dose (mean ± SD, min; max)	275.51 ± 132.33; 50; 600
Cardiovascular comorbidities	7 (14%)

Hypertension	7 (14%)
Severity of Covid infection (in those with cardiovascular comorbidities)	
mild, n (%)	6 (85.7%)
moderate, n (%)	1 (14.3%)
severe, n (%)	0
TAS (mean, min, max)	127.77; 95; 180)
TAD (mean, min, max)	80.83; 60; 100)
AV (mean, min, max)	92.57; 56; 119)
Temperature (mean ± SD)	36.5 (1.03)
Oxygen saturation (mean ± SD)	97.62 (5.23)
PCR (mean ± SD), normal values (>5 mg/L)	15.45 (25.35), n=12 (24%)
WBC (mean ± SD), normal values (>11 × 10^9 /L)	4.43 (0.49), n=1 (2%)
Abnormal EKG, number	4 (8%)
CK (mean ± SD), number of abnormal values (>180 U/L in women, >200 U/L in men)	66.14 (53.42), n=2 (4%)
History of myocarditis	0

The vast majority of patients were male n=29 (58.0%) with a mean age of disease onset of 23.67 ± 3.45 and no history of myocarditis. There were no significant changes in vital signs suggestive of myocarditis after the first and second dose of vaccine. In two cases the patients presented somatic symptoms such as palpitations, chest tightness and general affected state, for which they were evaluated cardiologically (EKG, echocardiography and troponins) and had an internal medicine consultation (abdominal ultrasound, amylase). In both cases the diagnosis of indigestion was established and myocarditis was excluded following investigations and consultations.

4.7.6 Conclusion

The overall risk of myocarditis after administration of Pfizer-BioNTech COVID-19 mRNA vaccine is low. Finally, evidence further shows that the benefits of COVID-19 vaccination outweigh its potential effects. Previous studies have shown that concerns about suspected myocarditis could lead to premature discontinuation of clozapine (Patel et al., 2019). Our data support this observation, showing that when myocarditis was suspected, clozapine was reduced or discontinued. Current literature has demonstrated that premature discontinuation of clozapine in treatment-resistant schizophrenia is often associated with poor clinical outcomes (Luykx et al., 2020).

CHAPTER 6. FINAL CONCLUSIONS. ORIGINALITY OF THE THESIS. DISSEMINATION OF RESULTS. FUTURE RESEARCH DIRECTIONS

6.1 Final conclusions

The main findings of the study were that people with schizophrenia on antipsychotic treatment showed a lower risk of severe SARS-CoV-2 infection and a likely better prognosis in a medically controlled environment. Hence the conclusion that antipsychotics could play an important role in preventing severe SARS-CoV-2 manifestation and could have protective effects against harmful manifestations of COVID-19 infection. Early diagnosis, monitoring of psychiatric but also respiratory symptoms, laboratory tests, screening for viral detection were key to the favourable outcome. So was the high level of care given by the medical staff to this category of patients.

COVID-19 could be associated with a temporary reduction in ANC levels, which is mild, temporary and statistically insignificant in the vast majority of patients, including those treated with clozapine. We identified neutropenia in a small group of patients and assumed that this was caused by coronavirus infection and interaction with clozapine. Discontinuation of clozapine could lead to relapses, with severe consequences for patients and their families. We therefore consider it appropriate to continue clozapine in patients who are stabilised on this treatment.

Since the rates of neutropenia reported in SARS-CoV-2 infected patients are higher than pre-pandemic reports, we assume that in COVID-19 positive patients neutropenia is the result of immunological interference between clozapine and SARS-CoV-2. Future research is needed to clarify this issue.

Clozapine-treated patients who develop COVID-19 should continue treatment whenever possible; the dose should be adjusted if necessary in relation to blood test results, and treatment should be discontinued if there is a significant decrease in neutrophil counts. Tachycardia, hypotension, fever and sedation are common side effects of clozapine, which should be managed according to usual recommendations, even if COVID-19 infection is present.

6.2 Originality of the thesis

The PhD thesis has many original components. It presented the effects of SARS-COV-2 infection in patients with schizophrenia on controlled antipsychotic treatment. It showed the impact of this pandemic on morbidity and mortality among these patients after SARS-COV-2 infection.
The study on the impact of vaccination with a messenger RNA vaccine in patients with schizophrenia who have experienced the disease is unique worldwide.

This PhD thesis presents valuable information on the relationship between inflammatory status in patients with schizophrenia who have experienced SARS-COV-2 infection and the results provide a realistic perspective in the context of paucity of information.

According to the author's information, no studies investigating the relationship between schizophrenia, SARS-COV-2 infection, antipsychotic treatment and antiviral vaccination have been conducted in Romania. Moreover, the PhD thesis has brought clarifications regarding the efficacy and safety of clozapine in a pandemic context, the studies conducted being of great interest considering the number of citations in ISI Web of Science so far, some in leading journals such as Schizophrenia Bulletin.

The four studies carried out in the framework of the doctoral research were of utmost importance in the context of the COVID-19 epidemic, as they allowed early diagnosis and appropriate therapeutic approach to patients detected positive for SARS-CoV-2.

Therefore, regular RT-PCR testing and complex batteries of laboratory tests have facilitated access to optimal care and appropriate treatment for patients admitted to the Clinical Hospital of Psychiatry and Neurology Brasov, leading to a low rate of severe COVID-19 and mortality among them. Laboratory evaluations for neutropenia showed that neutropenia did not occur in a significantly increased percentage in patients treated with clozapine during SARS-CoV-2 infection; this result influenced the therapeutic decision of psychiatrists to continue clozapine treatment, leading to a lower rate of relapses and mortality. Also, real-time monitoring of the effects of anti-COVID-19 vaccination in patients with schizophrenia led to the finding that no immediate side effects (such as thrombocytopenia or myocarditis) were evident, leading psychiatrists to recommend vaccination, supported by the evidence provided by our results. As a result, patients with schizophrenia, a vulnerable population and considered to be at high risk of complications, benefited from early and high vaccination rates, leading to decreased morbidity and mortality secondary to COVID-19.

6.3 Dissemination of results

Partial results of the studies carried out in the doctoral research have been published in journals with significant impact factor and presented at conferences with international participation. The published papers are of real interest given the number of citations in ISI Web of Science (Table 30, Appendix 1, Appendix 2).

Table 30. Dissemination of research results

	Papers on PhD thesis topic	**Papers on related topics**
Number of articles	**5**	**1**
Papers presented at conferences	**2**	**-**

6.4 Future research directions

In the future we plan to expand the study to larger samples, more representative of the country's population and including other types of tests.

We propose to develop national treatment strategies for people with severe mental illness in case of disasters (pandemics, natural disasters, conflicts, etc.) in order to maintain the physical and mental health of a vulnerable population.

Given the novelty of COVID-19 vaccines, we propose to prospectively evaluate previously SARS-CoV-2-infected and vaccinated schizophrenia patients on clozapine treatment to assess the risks of long-term neutropenia and myocarditis.

Also, considering the extent of the long COVID syndrome in recent times, we propose, as a future research direction, an evaluation also from this perspective of patients with schizophrenia who have experienced SARS-CoV-2 infection.

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ANNEXES

ANNEX 1. LIST OF PUBLISHED WORKS

1. **Moga, Silvia** & Burtea, Victoria & Andreea, Teodorescu & Lorena, Dima & Ifteni, Petru. (2017). Inflammation in schizophrenia. Archives of the Balkan Medical Union. 52. 328-332. https://www.researchgate.net/publication/321680787_Inflammation_in_schizophrenia
2. **Moga S**, Teodorescu A, Ifteni P, Gavris C, Petric PS. Inflammatory Response in SARS-CoV-2 Infection of Patients with Schizophrenia and Long-Term Antipsychotic Treatment. Neuropsychiatr Dis Treat. 2021 Oct 2;17:3053-3060. doi: 10.2147/NDT.S325062. PMID: 34629871; PMCID: PMC8495225. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8495225/
3. **Moga S**, Teodorescu A, Ifteni P, Petric PS, Miron AA. Clozapine and Neutropenia in Patients with Schizophrenia and SARS-CoV-2 Infection. Neuropsychiatr Dis Treat. 2022 May 4;18:977-983. doi: 10.2147/NDT.S361405. PMID: 35547265; PMCID: PMC9081886. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9081886/
4. **Moga S**, Teodorescu A, Dragan A, Miron AA, Ifteni P. Neutropenia in Patients With Clozapine-Treated Schizophrenia: An Effect of Clozapine or a Consequence of SARS-CoV-2 Infection? A Systematic Review. Am J Ther. 2022 Sep-Oct 01;29(5):e544-e552. doi: 10.1097/MJT.0000000000001532. Epub 2022 Jun 24. PMID: 35749754. https://pubmed.ncbi.nlm.nih.gov/35749754/
5. **Moga, Silvia** & Petric, Paula & Miron, Ana & Ifteni, Petru & Andreea, Teodorescu. (2023). Outcome of COVID-19 mRNA Vaccination in Patients Treated With Clozapine Who Previously Went Through SARS-COV-2 Infection. American journal of therapeutics. Publish Ahead of Print. 10.1097/MJT.0000000000001633. https://pubmed.ncbi.nlm.nih.gov/37097999/
6. Bobescu E, Covaciu A, Rus H, Radoi M, Badea M, **Moga SN**, Benza V, Marceanu LG. Correlation of Cardiovascular Risk Factors and Biomarkers With Platelet Reactivity in Coronary Artery Disease. Am J Ther. 2019 Sep/Oct;26(5):563-569. doi: 10.1097/MJT.0000000000000869. PMID: 30418226. https://pubmed.ncbi.nlm.nih.gov/30418226/

ANNEX 2. LIST OF PAPERS PRESENTED AT CONFERENCES

1. **Moga S**, Petric PS, Teodorescu A, Miron AA, Ifteni P. Outcome of COVID-19 Vaccination in Pattients Treated with Clozapine Who Previously Went Through SARS-CoV-2 Infection, European Conference of Psychiatry and Mental Health “Galatia 2023”, Galați, 17-21 mai 2023
2. Ifteni P, Petric PS, **Moga S.** Potențialul efect protectiv al antipsihoticelor În infecția cu SARS-CoV-2, Conferinţa Zilele Institutului de Psihiatrie “Socola” Iaşi, Iași, 11-13 noiembrie