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The usage of immunosuppressant agents and secondary infections in patients with COVID-19 in the intensive care unit: a retrospective study

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Although COVID-19 infection is an immunosuppressant disease, many immunosuppressant agents, such as pulse methylprednisolone (PMP), dexamethasone (DXM), and tocilizumab (TCZ), were used during the pandemic. Secondary infections in patients with COVID-19 have been reported recently. This study investigated these agents' effects on secondary infections and outcomes in patients with COVID-19 in intensive care units (ICUs). This study was designed retrospectively, and all data were collected from the tertiary intensive care units of six hospitals between March 2020 and October 2021. All patients were divided into three groups: Group I [GI, PMP (−), DXM (−) and TCZ (−)], Group II [GII, PMP (+), DXM (+)], and Group III [GIII, PMP (+), DXM (+), TCZ (+)]. Demographic data, P_aO/FiO_2 ratio, laboratory parameters, culture results, and outcomes were recorded. To compare GI-GII and GI-GIII, propensity score matching (PSM) was used by matching 14 parameters. Four hundred twelve patients with COVID-19 in the ICU were included in the study. The number of patients with microorganisms ≥ 2 was 279 (67.7%). After PSM, in GII and GIII, the number of (+) tracheal cultures and (+) bloodstream cultures detected different microorganisms ≥ 2 during the ICU period, neuropathy, tracheotomized patients, duration of IMV, and length of ICU stay were significantly higher than GI. The mortality rate was similar in GI and GII, whereas it was significantly higher in GIII than in GI. The use of immunosuppressant agents in COVID-19 patients may lead to an increase in secondary infections. In addition, increased secondary infections may lead to prolonged ICU stay, prolonged IMV duration, and increased mortality.

Keywords COVID-19 infection, Immunosuppressant, Methylprednisolone, Dexamethasone, Interleukin, Intensive care unit, Pandemic, Retrospectively, Propensity score matching, Secondary

Abbreviations

CCI	Charlson comorbidity ındex
APACHE-II	Acute physiology and chronic health evaluation II
NLCR	Neutrophil–lymphocyte count ratio
IMV	Invazive mechanical ventilation
MV	Mechanical ventilation

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ICU	Intensive care unit
CRP	C-reactive protein
BMI	Body mass index
IL	Interleukin
TGFß	Transforming growth factor beta
IFN	Interferon
CRS	Cytokine release syndrome
PMP	Pulse methylprednisolone
DXM	Dexamethasone
IL-B	Interleukin-blockers
ARF	Acute respiratory failure
ARDS	Acute respiratory distress syndrome
CMV	Cytomegalovirus
CFS	Clinical frailty score

Pneumonia caused by SARS-CoV-2 infection may lead to life-threatening acute respiratory failure (ARF) and acute respiratory distress syndrome (ARDS). In addition to risk factors such as advanced age, male gender, presence of malignancy, and chronic organ failures, secondary infections are also mentioned among the causes of mortality in COVID-19[1]. During this challenging period, different clinical treatment modalities have been intensively focused on increasing survival. Although the primary treatment goal of infectious diseases is to eradicate the virus with antiviral agents, there were no effective antiviral agents in our clinical practice for a remarkable period. Many studies in the literature were generally concentrated on immunological treatments for damage control, and numerous studies examined the positive effects of immunosuppressive agents such as Tocilizumab (TCZ), dexamethasone (DXM), and pulse methylprednisolone (PMP)[2–9]. In these studies, the reason for using IL blockers was based on cytokine release syndrome (CRS), whereas anti-inflammatory effects were featured for steroids[5,10]. However, it was challenging to interpret the effects of these treatments on outcomes in patients with COVID-19 because they were frequently combined due to clinical worsening. On the other hand, recent studies have started to report the relationship between the usage of immunosuppressant agents and secondary infections in patients with COVID-19[11,12].

In this study, we investigated the effects of immunosuppressive therapies on secondary infections and mortality, in COVID-19 patients in the intensive care unit (ICU).

Methods

Study population

Following Acibadem University and Acibadem Healthcare Institutions Medical Research Ethics Committee (ATADEK) approval (ATADEK-2021-21/35), the study was designed retrospectively in each tertiary intensive care unit of six hospitals between March 2020 and October 2021, in accordance with the Declaration of Helsinki. Informed consent has been waived off by the same ethics committee, Acibadem University and Acibadem Healthcare Institutions Medical Research Ethics Committee (ATADEK), due to the retrospective design of the study. The hospitals included in the study belong to the Acıbadem Health Group, which provides healthcare services in our country, and each intensive care unit is managed by different intensive care teams and is inspected by the hospital infection committee chaired by separate infectious diseases and microbiology doctors.

The data of 496 patients admitted to the ICU due to COVID-19 pneumonia were evaluated. Patients who were under the age of 18 or over the age of 90, transferred to another center, had terminal oncological diseases, were undergoing any immunosuppressant therapy before ICU admission, did not have microbiological culture samples taken or the antibiogram studied, and whose length of ICU stay was less than 24 h were excluded.

Database

All data were obtained and anonymized from the Acibadem Health Group Cerebral Database.

At ICU admission, demographic data of patients (i.e., age, sex), body mass index (BMI), Charlson Comorbidity Index (CCI), Acute Physiology and Chronic Health Evaluation (Apache II), PaO_2/FiO_2 ratio, C-reactive protein (CRP) (mg dL^{-1}) and procalcitonin (µg L^{-1}) values were recorded. In the first week of the ICU duration, maximum (max)-leukocyte count ($\times 10^3$ μL^{-1}), max-neutrophil count ($\times 10^3$ μL^{-1}), max-ferritin (ng mL^{-1}), max-D-dimer (mg L^{-1}), and minimum (min)-lymphocyte count ($\times 10^3$ μL^{-1}) were recorded. Duration of the ICU stay, the need for invasive mechanical ventilation (IMV) and the timing of its initiation, administered immunosuppressant agents [PMP, DXM, and tocilizumab (TCZ)], and additional therapy applications [i.e., therapeutic plasma exchange (TPE), IVIG and ECMO] were recorded.

Outcomes

Infections (i.e., superinfections, reactivations) developed during the ICU stay were recorded in patients who were followed up due to COVID-19 pneumonia as a primary outcome.

Neuropathy, tracheotomized patients, the clinical frailty scale (CFS)[13] at ICU discharge in survivors, length of stay at the ICU (LOS-ICU), and mortality were recorded as secondary outcomes.

Grouping of patients according to immunosuppressive treatments

All patients were divided into three groups according to immunosuppressive therapy administration. Group I (GI) consisted of patients who did not receive PMP, DXM, or TCZ. Group II (GII) included patients who received PMP and DXM but not TCZ. Group III (GIII) included patients who received all three: PMP, DXM, and TCZ.

Culture samples

Combined oropharyngeal and nasopharyngeal swab samples were studied in the laboratory with a *Light Cyler 96 (Roche®, Switzerland*) device using *Bio-Speedy® COVID-19 RT–qPCR (BioEksen®, Istanbul/Turkey)*. Reverse transcriptase-quantitative polymerase chain reaction (PCR) was performed in combined oropharyngeal and nasopharyngeal swab samples to diagnose COVID-19 in patients using a *Light Cyler 96 (Roche®, Switzerland)* device using *Bio-Speedy® COVID-19 RT–qPCR (BioEksen®, Istanbul/Turkey)*.

Blood, urine, and respiratory secretion samples were taken from the patients for a culture antibiogram during the first 24 h of intensive care unit admission. Depending on WHO guidelines, bacterial and fungal infections detected after admission to the intensive care unit were considered secondary infections[14]. Positive cultures that developed 48 h after hospitalization were considered healthcare-associated. Infections detected in the first 48 h after hospital admission and in patients coming from their homes or nursing homes were defined as "community-acquired infections." Sputum culture was studied in non-intubated patients who could give sputum samples. The culture antibiogram was studied from the secretion sample taken by endotracheal aspiration or bronchoalveolar lavage from the patients who were followed up with invasive mechanical ventilation support.

For the duration of stay in intensive care, patients' blood, sputum-bronchoalveolar secretions, and urine cultures were restudied in the presence of newly developed fever (equal to or higher than 38 °C), leukocytosis ($\geq 10{,}000\ mm^{-3}$), increasing sputum or tracheal secretions, deterioration of respiratory parameters, and increasing vasopressor need.

According to CDC guidelines, purulent sputum was accepted as secretions from the lungs, bronchi, or trachea that contained ≥ 25 neutrophils and ≤ 10 squamous epithelial cells per low power field ($\times 100$)[3]. The presence of $\geq 10^5$ CFU mL^{-1} in the endotracheal aspirate culture and $\geq 10^4$ CFU mL^{-1} in the bronchoalveolar lavage sample were accepted as positive cultures for pneumonia[15].

Before taking blood samples from a percutaneous vein or catheter, the skin was fully prepared with alcoholic chlorhexidine (> 0.5%), as recommended by the IDSA Guidelines. If blood samples were taken from the central catheter, blood samples were taken from two different lumens of the catheter[16]. Detection of *S. aureus, Streptococcus pneumoniae, Group A Streptococcus, Enterobacteriaceae, Haemophilus influenzae, Pseudomonas aeruginosa, Bactroidaceae,* and *Candida species* in blood cultures was always considered clinically significant. Detection of *coagulase-negative Staphylococci, viridans streptococci,* and *clostridium species* in blood cultures was evaluated with clinical correlation in terms of contamination[17].

The specimens included blood, urine, wound swabs and aspirates, biopsies, and tracheal aspirates. Patients under mechanical ventilation for at least 48 h who presented with new or progressive pulmonary infiltrates on chest X-rays plus at least two of the following criteria were included in the study: fever ≥ 38 °C, purulent tracheal secretions, leukocytosis ≥ 10,000 mm^{-3} or leukopenia ≤ 4000 mm^{-3} and positive cultures, i.e., $\geq 10^4$ CFU mL^{-1} in BALF cultures and $\geq 10^5$ CFU mL^{-1} in quantitative endotracheal aspirate cultures. Each patient participated only once in the study. All specimens were Gram-stained. Positive cultures underwent microorganism identification using both conventional and automated biochemical methods *(VITEK-2, bioMérieux, Marcy l'Etoile, France)* and *matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).* All culture and susceptibility tests were performed at the Acıbadem Central Microbiology laboratory (LABMED, Istanbul, Turkey) with the *Kirby-Bauer disc diffusion method* using *EUCAST (The European Committee on Antimicrobial Susceptibility Testing)* recommendations. *Pneumocystis jiravecii* DNA and quantitative *CMV (Cytomegalovirus)* DNA were detected using a *Rotor-Gene Q Real-time PCR* instrument *(Qiagen, Hilden, Germany)*. The (1–3)-β-D-glucan assay (D-BDG) (Dynamiker Biotechnology (Tianjin) Co., Ltd., Singapore) was used to test for Pneumocystis jiravecii. The (1–3)-β-D-glucan assay (D-BDG) (Dynamiker Biotechnology (Tianjin) Co., Ltd., Singapore) was used for screening of invasive fungal pathogens. *Aspergillus Galactomannan (GM) Ag VIRCLIA@ Monotest* was used to detect GM (Vircell, Spain) qualitatively. According to the Revised EORTC/MSGERC Definitions of Invasive Fungal Diseases, galactomannan antigen index > 0.5 in plasma/serum and/or galactomannan antigen > 0.8 in BALF was considered significant in clinically suspected cases[18].

CMV reactivation was investigated by detecting viral DNA using real-time polymerase chain reaction (PCR) from tracheal aspiration or BAL samples. *CMV* reaction was defined as CMV DNA ≥ 1000 copies mL^{-1} in bronchoalveolar lavage samples. Based on the recommendations of The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation, we considered only changes in viral load exceeding 0.5 $\log_{10}$ IU/mL (threefold) significant[19]. Cases with CMV PCR positivity were accepted as viral reactivation in our COVID-19 patient[20].

For the diagnosis of Pneumocystis jirovecii, real-time PCR was used to detect DNA from patients' sputum or BAL samples. In cases with suspected invasive candidiasis, the FDA-approved Fungitell® Beta-D-Glucan test was studied from blood samples. A value > 80 pg mL^{-1} was considered positive. All patients were followed with a silicone urinary catheter, and culture samples were taken from the sampling port after the disinfection procedure[21].

Clinical signs and symptoms, laboratory tests, and imaging methods were evaluated together with infectious disease specialists; results of culture-positive colonization were excluded, and results of infectious agents were included.

Statistical analysis

All descriptive data are presented as mean ± sd, median (quartiles), and percentages. The Kolmogorov–Smirnov test was used to detect normal distributions. Student's t-tests, Mann–Whitney U tests, and chi-square (Fisher's exact) tests were used to compare two groups, whereas ANOVA and Kruskal–Wallis tests were used to compare three groups. For comparison between GI-GII and GI-GIII, propensity score matching was used by matching 14 parameters [age, sex, BMI, CCI, APACHE II, PaO_2/FiO_2 ratio, CRP, procalcitonin, leucocyte count, neutrophil

count, lymphocyte count, ferritin, D-dimer, and IMV requirements, caliper:0.2]. The *p*-value was accepted as < 0.05 for significance. All data were analyzed using SPSS version 29.

Results

For all patients

Four hundred twelve patients with COVID-19 were included in the study. The median values of age, BMI, CCI, APACHE II, and PaO_2/FiO_2 ratio were 63, 27.6, 3, 16, and 103, respectively. IMV was required in 92.5% of the patients. The median values for the day of intubation and the duration of IMV were zero (the first day of ICU admission) and 15 days, respectively. 28.6% of all patients had not received PMP, DXM, or TCZ. The median values of LOS-ICU and CFS at ICU discharge in survivors were 18 days and 4. For all patients, the mortality rate was 40.3% (Table 1).

In the first 48 h of intubation, the tracheal and nontracheal culture positivity rates were 35.4% and 31.8%, respectively (Table 2). Except for COVID-19, the median day of the first detected microorganism in the ICU was the 2nd day. During the ICU period, only 12.9% of patients had negative cultures, whereas 74.8%, 63.3%, 31.6%, and 35.7% of patients had tracheal, bloodstream, urine, and central catheter culture positivity, respectively (Table 2). Gram-positive and gram-negative microorganisms, fungi, and *CMV-DNA PCR* were identified in 66%, 54.1%, 52.9%, and 26.5% of patients, respectively (Table 2). The most common microorganisms in tracheal cultures were *Candida albicans* (37.3%), *Acinetobacter baumanii* (31.8%), *non-Albicans Candida* (29.9%) *and Klebsiella pneumonia* (15.9%), whereas *methicillin-resistant staphylococcus (MRS)* (98.1%), *Acinetobacter baumanii* (14.2%) *and Klebsiella pneumonia* (11.5%) were the most common microorganisms in the bloodstream (Table 2).

Included patients	412
Age, year	63 (52–73)
Male, n (%)	303 (73.5)
BMI (kg m^{-2})	27.6 (25.5–30.8)
CCI	3 (2–5)
APACHE II	16 (13–20)
At the ICU admission	
PaO_2/FiO_2 ratio	103 (84–126)
CRP (mg dL^{-1})	11.0 (5.9–17.9)
Procalcitonin (ug L^{-1})	0.19 (0.08–0.59)
In the first week	
WBC (max.) ($\times 10^3$)	15.5 (11.7–20.1)
Neutrophil count (max.) ($\times 10^3$)	13.8 (10.3–18.4)
Lymphocyte count (min.) ($\times 10^3$)	0.39 (0.26–0.57)
Ferritin (max.) (ng mL^{-1})	1274 (665–1743)
D-Dimer (max.)(mg L^{-1})	3.7 (1.8–7.7)
The usage of immunosuppressants, n (%)	
PMP (−), DXM (−) and TCZ (−)	118 (28.6)
PMP plus DXM	184 (44.7)
PMP plus DXM plus TCZ	110 (26.7)
Patients with IMV, n (%)	381 (92.5)
On the day of intubation, the day	0 (0–1)*
Duration of IMV, days	15 (9–27)
Additional therapies, n (%)	
TPE	94 (22.8)
IVIG	47 (11.4)
ECMO	14 (3.4)
Outcomes	
Neuropathy, n (%)	112 (27.2)
Tracheotomized patients, n (%)	109 (26.5)
CFS at the ICU discharge in survivors	4 (3–5)
LOS-ICU, dyas	18 (12–29)
Mortality, n (%)	166 (40.3)

Table 1. Patients' characteristics, therapies, and outcomes. APACHE, acute physiology and chronic health evaluation; BMI, body mass index; CCI, Charlson comorbidity index; CFS, clinical frailty scale; CRP, C-reactive protein; DXM, dexamethasone; ICU, intensive care unit; IMV, invasive mechanical ventilation; LOS, length of stay; PMP, pulse methylprednisolone; TCZ, tocilizumab; TPE, therapeutic plasma Exchange; WBC, white blood cell. $^{\Phi}$, 'zero', negative, and positive values refer to the day of ICU admission, the days before ICU admission, and the days after ICU admission, respectively.

Total patients	412
The day of the first detected microorganism except for Covid-19 in the ICU	2 (0–6)
The number of the (+) tracheal culture in the first 48 h of intubation	146 (35.4)
The number of the (+) non-tracheal culture in the first 48 h of intubation	131 (31.8)
The total number of detected different microorganisms during the ICU period	2 (1–4)
No detected microorganism	53 (12.9)
Only one detected microorganism	80 (19.4)
The detected different microorganisms ≥ 2	279 (67.7)
Area of (+) cultures, n (%)	
Tracheal	308 (74.8)
Bloodstream	261 (63.3)
Urine	130 (31.6)
Catheter	147 (35.7)
Type of detected microorganisms, n (%)	
Gram (+)	272 (66.0)
Gram (−)	223 (54.1)
Fungus	218 (52.9)
Detected microorganisms in tracheal cultures, n (%)	308 (74.8)
Candida albicans	115 (37.3)
Acinetobacter baumannii	98 (31.8)
Non-albicans candida	92 (29.9)
Klebsiella pneumonia	49 (15.9)
Pseudomonas Aeruginosa	35 (11.4)
MRSA	33 (10.7)
E. Coli	23 (7.5)
Stenotrophomonas Maltophilia	19 (6.2)
Aspergillus spp.	17 (5.5)
Streptococcus Pneumonia	16 (5.2)
Corynebacterium striatum	12 (3.9)
Detected microorganisms in bloodstream cultures, n (%)	261 (63.6)
Methicillin-resistant staphylococcus	256 (98.1)
Acinetobacter Baumanii	37 (14.2)
Klebsiella Pneumonia	30 (11.5)
Corynebacterium Striatum	17 (6.5)
Stenotrophomonas Maltophilia	15 (5.7)
Enterococcus Faecium	14 (5.4)
Candida albicans	13 (5.0)
Enterococcus Faecalis	11 (4.2)
Non-albicans candida	11 (4.2)
(+) Antigens and PCRs	
(+) CMV-DNA PCR	109 (26.5)
(+) Beta-glucan antigen	67 (16.3)
(+) Aspergillus antigen	24 (5.8)
(+) P. Carinii-DNA PCR	3 (0.01)

Table 2. Detected microorganisms and their distributions for all patients. ICU, intensive care unit; CMV, cytomegalovirus; PCR, polymerase chain reaction.

Comparisons among groups

GI, GII, and GIII consisted of 118, 184, and 110 patients, respectively (Table 3). In all groups, age, gender, BMI, CCI, APACHE II, PaO_2/FiO_2 ratio, performing IMV, and the day of intubation were similar (Table 3). In GIII, the initiation day for PMP was significantly earlier than that in GII (1 day and 3 days before ICU admission, respectively), whereas the initiation days for DXM were similar in GII and GIII ($p = 0.038$ and $p = 0.792$, respectively) (Table 3). In the GII and GIII groups, the dosages of PMP and DXM were similar ($p = 0.281$, $p = 0.196$). In GIII, TCZ was initiated 2 days before ICU admission (Table 3). All infection and clinical outcomes were significantly higher in GII and GIII than in GI (Table 3).

	Group I	Group II	Group III	
	[PMP (−), DXM (−), TCZ (−)]	*[PMP (+), DXM (+), TCZ (−)]*	*[PMP (+), DXM (+), TCZ (+)]*	
	(n = 118)	(n = 184)	(n = 110)	*p*
Patients' characteristics				
Age, year	65 (51–76)	64 (52–73)	61 (50–68)	0.175
Male, n (%)	81 (68.6)	132 (71.7)	90 (81.8)	0.06
BMI (kg m^{-2})	27.6 (25.5–30.4)	27.6 (25.5–30.9)	27.6 (25.5–30.0)	0.998
CCI	3 (2–5)	4 (2–5)	3 (1–5)	0.21
APACHE II	16 (12–19)	16 (13–21)	16 (13–20)	0.213
Patients with IMV, n (%)	105 (89.0)	171 (92.9)	105 (95.5)	0.171
The day of intubation$^{\Phi}$	0 (0–2)	0 (0–1)	0 (0–1)	0.246
The initiation days of immunosuppressants$^{\Phi}$				
PMP	–	− 1 (− 4;0)	− 3 (− 5;− 1)^{ß}	***0.038***
DXM	–	0 (− 3;1)	0 (− 5;1)	0.792
TCZ	–	–	− 2 (− 4;0)	
The dosages of immunosuppressants				
PMP (mg kg^{-1})	–	3.1 (3.0–3.4)	3.0 (2.8–3.5)	0.281
DXM (mg kg^{-1} day^{-1})	–	0.13 (0.10–0.18)	0.16 (0.10–0.19)	0.196
TCZ (mg)	–	–	800 (600–800)	
Laboratories				
PaO_2/FiO_2 ratio (at the ICU adm.)	113 (87–139)	102 (86–120)	102 (78–126)	0.089
CRP (at the ICU adm.)	14.4 (8.0–20.9)	10.3 (6.0–16.4)$^{\#\#}$	9.6 (3.3–18.1)**	***0.005***
Procalcitonin (at the ICU adm.)	0.21 (0.10–0.52)	0.18 (0.07–0.65)	0.18 (0.08–0.51)	0.48
WBC (max) (in the 1st week)	13.9 (10.3–17.2)	16.5 (12.1–20.5)$^{\#\#\#}$	16.5 (12.9–22.2)***	***< 0.001***
Neutrophil count (max)(in the 1st week)	11.6 (9.1–15.2)	14.7 (10.9–18.7)$^{\#\#\#}$	14.7 (11.1–20.0)***	***< 0.001***
Lymphocyte count (min) (in the 1st week)	0.37 (0.26–0.59)	0.39 (0.27–0.53)	0.42 (0.27–0.56)	0.950
Ferritin (max.) (in the 1st week)	1200 (547–1650)	1286 (701–1723)	1399 (713–2101)	0.096
D-Dimer (max.) (in the 1st week)	3.4 (1.8–6.7)	3.6 (1.7–7.1)	4.2 (2.0–9.6)	0.281
Infection outcomes				
Detected different microorganisms in ICU period	1 (1–3)	2 (1–3)$^{\#\#\#}$	3 (2–4)***,ßßß	***< 0.001***
No detected microorganisms	27 (22.9)	20 (10.9)$^{\#\#\#}$	6 (5.5)***	***< 0.001***
The detected different microorganisms ≥ 2	59 (50.0)	130 (70.7)$^{\#\#\#}$	90 (81.8)***	***< 0.001***
(+) tracheal culture in the first 48 h of intubation	29 (24.6)	66 (35.9)$^{\#}$	51 (46.4)***,ß	***0.003***
(+) tracheal culture	68 (58.5)	145 (78.8)$^{\#\#\#}$	94 (85.5)***	***< 0.001***
(+) bloodstream culture	58 (49.2)	119 (64.7)$^{\#\#}$	84 (76.4)***,ß	***< 0.001***
(+) CMV-DNA-PCR	18 (15.3)	40 (21.7)	51 (46.4)***,ßßß	***0.001***
(+) Beta-glucan antigen	12 (10.2)	34 (18.5)	21 (19.1)	0.104
(+) Aspergillus antigen	2 (1.7)	16 (8.7)$^{\#}$	6 (5.5)*	***0.024***
Clinical outcomes				
Neuropathy, n (%)	13 (11.0)	60 (32.6)$^{\#\#\#}$	39 (35.5)***	***< 0.001***
Tracheotomized patients, n (%)	12 (10.2)	52 (28.3)$^{\#\#\#}$	45 (40.9)***,ß	***< 0.001***
CFS in survivors at the ICU discharge	3 (3–5)	4 (3–6)$^{\#\#}$	4 (3–6)**	***0.007***
Duration of IMV, days	12 (7–16)	17 (9–30)$^{\#\#\#}$	20 (11–36)***,ß	***< 0.001***
LOS-ICU, days	15 (11–21)	20 (11–32)$^{\#\#}$	22 (14–37)***,ß	***< 0.001***
Mortality, n (%)	32 (27.1)	73 (39.7)$^{\#}$	61 (55.5)***,ß	***< 0.001***

Table 3. Comparisons between patients' characteristics, the use of immunosuppressants, and laboratories of three groups. APACHE, acute physiology and chronic health evaluation; BMI, body mass index; CCI, charlson comorbidity index; CFS, clinical frailty scale; CRP, C-reactive protein; DXM, dexamethasone; ICU, intensive care unit; IMV, invasive mechanical ventilation; LOS, length of stay; PMP, pulse methylprednisolone; TCZ, tocilizumab; WBC, white blood cell. $^{\Phi}$, 'zero', negative and positive values refer to the day of ICU admission, the days before ICU admission and the days after ICU admission respectively. $^{\#}$, comparison between GI and GII. *, comparison between GI and GIII. ^{ß}, comparison between GII and GIII. $^{\#}$, * and ^{ß}, $p = 0.05–0.01$. $^{\#\#}$, ** and ^{ßß}; $p = 0.01–0.001$. $^{\#\#\#}$, *** and ^{ßßß}; $p < 0.001$. Significant values are in bolditalics.

Comparisons of groups after propensity score matching

GI versus GII

One hundred and eighteen patients from each group were compared after propensity score matching (Table 4). In GII, the total number of and more than 2 detected different microorganisms during the ICU period, (+) tracheal culture in the first 48 h of ICU admission, (+) tracheal and bloodstream cultures in all ICU periods, recurrent (+) CMV-DNA PCR, (+) Aspergillus antigen, neuropathy, tracheostomized patients, CFS in survivors, duration of IMV, and LOS-ICU were significantly higher than those in Group I ($p < 0.001$, $p < 0.001$, $p = 0.025$, $p < 0.001$, $p = 0.008$, $p = 0.049$, $p = 0.017$, $p < 0.001$, $p = 0.006$, $p = 0.007$, $p < 0.001$ and $p = 0.01$, respectively) (Table 4). In GII, *Candida albicans* in tracheal samples and *methicillin-resistant Staphylococcus aureus (MRSA)* in bloodstream samples were significantly higher than in GI ($p = 0.005$ and $p = 0.033$, respectively) (Table 4). Although the mortality rate in GII (34.7%) was higher than that in GI (27.1), there was no significant difference between the mortality rates of the groups ($p = 0.205$) (Table 4).

GI versus GIII

Ninety-three patients from each group were compared after propensity score matching (Table 4). In GIII, the total number of and more than 2 detected different microorganisms during the ICU period, (+) tracheal culture in the first 48 h of ICU admission, (+) tracheal and bloodstream cultures in all ICU periods, (+) CMV-DNA PCR, recurrent (+) CMV-DNA PCR, recurrent (+) beta-glucan antigen, neuropathy, tracheostomized patients, duration of MV, LOS-ICU and mortality rate (53.8%) were significantly higher than those in Group I ($p < 0.001$, $p = 0.003$, $p = 0.009$, $p = 0.004$, $p < 0.001$, $p < 0.001$, $p = 0.003$, $p = 0.049$, $p = 0.002$, $p < 0.001$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) (Table 4). In GIII, *Candida albicans*, *Acinetobacter baumanii*, *MRSA, and Stenotrophomonas maltophilia* in tracheal samples and *MRSA and Enterococcus spp.* in bloodstream samples were significantly higher than in GI ($p = 0.049$, $p = 0.045$, $p = 0.028$, $p = 0.032$, $p = 0.040$ and $p = 0.014$, respectively) (Table 4).

Discussion

This study showed us some important results: 1. Different immunosuppressant agent combinations were used in patients with COVID-19 pneumonia, especially in the first year of the pandemic. 2. All immunosuppressant agents were already administered before ICU admission. 3. Although these agents were used to prevent the worst clinical situation, obviously, neither severe ARDS was improved (PaO_2/FiO_2 ratios were 102 in both GII and GIII at ICU admission) nor the requirement of IMV was reduced (275 of these 294 patients [GII + GIII] required IMV on the first day of ICU admission). 4. Moreover, secondary infections were much more detected in groups administered immunosuppressant agents. 5. lastly, any of the outcomes, such as mortality, LOS-ICU, and duration of IMV, were positively affected. In contrast, they were higher in GII and GIII than in GI.

Many studies have claimed that COVID-19-related ARDS develops due to an uncontrolled inflammatory response[22–24]. This uncontrolled inflammatory response is associated with the release of large amounts of inflammatory cytokines (IL-1β, IFN-α, IFN-γ, IL-12, IL-6, IL-18, TNF-α, IL-33, TGF-β, etc.)[24]. This response was named a cytokine storm, and it was believed that this cytokine storm was the primary reason for the worst clinical status of patients with COVID-19 pneumonia[22–25]. Yet, systemic inflammatory response syndrome was removed from sepsis guidelines long before this pandemic[26]. Even in the sepsis guideline update for COVID-19, the cytokine storm was not mentioned by strongly highlighted[27]. Already, the guidance of biomarkers and inflammatory mediators in this process is always unclear[28]. Nevertheless, since IL-6 and IL-1 are important cytokines that aggravate macrophage activation syndrome, these cytokines are held responsible for the worst clinical status[25,29]. Therefore, IL-6 and IL-1 blockers were used, although there was no threshold level for either of them. Because cytokine storms are the main mechanism for the COVID-19 pathophysiology, DXM and PMP were inevitably added to the treatments, and their positive effects on mortality were mentioned[5,6,30]. However, some questions remained unanswered in this period: Was a cytokine storm the only reason for the worsened clinical status really? Was there a risk for secondary infection due to the usage of immunosuppressive agents in COVID-19, which is known as an immunosuppressive disease?[31] Could the worsened clinical status ensue from added secondary infections to the clinical course? In those days, there were unconvincing results about secondary infections in studies[5,32]. This study reveals the relationship between using immunosuppressive agents before ICU admission and secondary infections. Detecting positive culture results at ICU admission is important evidence indicating secondary infections as the underlying reason for the worsened clinical status (Tables 2, 4). Recent studies also support our results on this topic[33,34].

In the first wave of the pandemic, the use of MP was at the forefront of our clinical practice because of the emphasis on better lung penetration in previous ARDS studies[35]. DXM usage significantly increased in the following period, particularly after the published RECOVERY study[5]. However, this study had two important limitations: 1. There were no results about secondary infections. 2. The authors said that any laboratory parameters, including hemogram parameters, ferritin, and d-dimer levels, were not recorded and evaluated. Therefore, their mortality result was questionable because it was not adjusted with laboratory parameters. Furthermore, based on their mortality results, DXM was widely used without hesitation, as if there were no limitations or complications. Similarly, IL-blockers were used more often in clinical settings after the REMAP-CAP study[32]. Interestingly, in this study, secondary bacterial infection was observed in only one patient in the IL-blocker group (in 401 patients). Additionally, mortality was not adjusted by any laboratory parameters as in the RECOVERY study. Guaraldi et al.[4] also presented a secondary infection in only one of 125 patients administered TCZ on those days. Actually, Bayo et al. clarified the reason for all these results in their study: blood culture collection in a tertiary hospital during the pandemic period was lower than in previous years[36]. Contrary to these results, recent studies have found a relationship between using immunosuppressive agents and secondary infections in patients with COVID-19[33,37]. Meynaar et al.[37] mentioned that they did not eliminate doubt about the effect of IL-6 blockers on

	Group I	Group II		Group I	Group III	
	PMP (−), DXM (−), TCZ (−)	PMP (+), DXM (+), TCZ (−)		PMP (−), DXM (−), TCZ (−)	PMP (+), DXM (+), TCZ (+)	
	(n = 118)	(n = 118)	*p*	(n = 93)	(n = 93)	*p*
Infection outcomes						
Detected different microorganisms	2 (1–3)	3 (1–3)	***< 0.001***	2 (1–3)	3 (2–5)	***< 0.001***
No detected microorganism	27 (22.9)	9 (7.6)	***< 0.001***	18 (19.4)	6 (6.5)	***0.003***
The detected different microorganisms ≥ 2	59 (50.0)	84 (71.9)	***< 0.001***	53 (57.0)	74 (79.6)	***0.003***
(+) tracheal culture in the first 48 h of intubation	29 (24.6)	46 (39.0)	***0.025***	25 (26.9)	43 (46.2)	***0.009***
(+) tracheal culture	69 (58.5)	96 (81.4)	***< 0.001***	60 (64.5)	78 (83.9)	***0.004***
(+) bloodstream culture	58 (49.2)	79 (66.9)	***0.008***	46 (49.5)	69 (74.2)	***< 0.001***
Microorganisms in tracheal cultures						
Multiple different microorganisms	31 (26.3)	46 (39.0)	***0.026***	30 (32.3)	56 (60.2)	***< 0.001***
Recurrent microorganisms	24 (20.3)	44 (37.3)	***0.006***	23 (24.7)	48 (51.6)	***< 0.001***
Candida albicans	*21 (17.8)*	*41 (34.7)*	***0.005***	20 (21.5)	31 (33.3)	***0.049***
Acinetobacter baumannii	*19 (16.1)*	*27 (22.9)*	*0.25*	18 (19.4)	29 (31.2)	***0.045***
Pseudomonas aeruginosa	*4 (3.4)*	*11 (9.3)*	*0.107*	4 (4.3)	11 (11.8)	0.052
MRSA	*3 (2.5)*	*11 (9.3)*	*0.05*	3 (3.2)	11 (11.8)	***0.028***
Stenotrophomonas maltophilia	*1 (0.8)*	*3 (2.5)*	*0.622*	1 (1.1)	7 (7.5)	***0.032***
Microorganisms in bloodstream cultures						
Multiple different microorganisms	*23 (19.5)*	*36 (30.5)*	0.071	22 (23.7)	35 (37.6)	***0.028***
Recurrent microorganisms	*21 (17.8)*	*27 (22.9)*	0.419	19 (20.4)	26 (28.0)	0.152
Methicillin-resistant staphylococcus	*45 (38.1)*	*60 (50.8)*	***0.033***	37 (39.8)	52 (55.9)	***0.040***
Enterococcus Faecalis/Faecium	*3 (2.5)*	*5 (4.2)*	0.722	3 (3.2)	12 (12.9)	***0.014***
Antigens and PCRs						
(+) CMV-DNA PCR	*18 (15.3)*	*27 (22.9)*	0.136	17 (18.3)	42 (45.2)	***< 0.001***
Recurrent (+) CMV-DNA PCR	*9 (7.6)*	*18 (15.3)*	***0.049***	9 (9.7)	24 (25.8)	***0.003***
(+) Beta-glucan antigen	*12 (10.2)*	*19 (16.1)*	0.124	10 (10.8)	18 (19.4)	0.075
Recurrent (+) Beta-glucan antigen	*2 (1.7)*	*4 (3.4)*	0.342	2 (2.2)	8 (8.6)	***0.049***
(+) Aspergillus antigen	*2 (1.7)*	*10 (8.5)*	***0.017***	2 (2.2)	5 (5.4)	0.222
Recurrent (+) Aspergillus antigen	*1 (0.8)*	*3 (2.5)*	0.311	1 (1.1)	3 (3.2)	0.310
Clinical outcomes						
Neuropathy, n (%)	13 (11.0)	35 (29.7)	***< 0.001***	12 (12.9)	31 (33.3)	***0.002***
Tracheotomized patients, n (%)	12 (10.2)	28 (23.7)	***0.006***	11 (11.8)	36 (38.7)	***< 0.001***
CFS in survivors at the ICU discharge	3 (3–5)	4 (3–6)	***0.007***	3 (3–5)	4 (3–6)	0.253
Duration of IMV, days	11 (6–15)	16 (8–25)	***< 0.001***	12 (7–16)	17 (10–33)	***< 0.001***
LOS-ICU, days	15 (11–21)	20 (12–28)	***0.01***	16 (12–22)	21 (14–36)	***< 0.001***
Mortality, n (%)	32 (27.1)	41 (34.7)	0.205	24 (25.8)	50 (53.8)	***< 0.001***

Table 4. Comparisons between GI and GII, and GI and GIII after propensity score matching. CFS, clinical frailty scale; CMV, cytomegalovirus; ICU, intensive care unit; IMV, invasive mechanical ventilation; LOS, length of stay; MRSA, methicillin-resistant staphylococcus aerosa. Significant values are in bolditalics.

infection. Somers et al.[38] showed that there was no significant effect on mortality but an increase in superinfections in patients receiving TCZ. Kacmaz et al.[39] also demonstrated that COVID-19 was a predictor for secondary infection; however, they did not examine the effect of immunosuppressive agents such as interleukin blockers on secondary infections in patients with COVID-19 and did not mention how COVID-19 increases secondary infections. As for Conway et al.[33], they detected bacterial coinfection in 716 (14%) patients with COVID-19 at ICU admission. In this study, the median value of infections per infected patient was 2, and ICU-acquired infection was 54% of all infected patients. Our results were similar to those of Conway et al. In our patients, tracheal culture positivity was found in 35.4% in the first 48 h of admission to the ICU, and a positive nontracheal culture was found in 31.8% of them, which was above our expected coinfection rates (Table 2). Even in GI, the number of patients with detected microorganisms ≥ 2 was 59 (50%), whereas they were 84 (71.9%) and 74 (79.6%) in GII and GIII, respectively, after PSM ($p < 0.001$ and $p = 0.003$, respectively) (Table 4). Additionally, *Candida albicans, MRS, Acinetobacter baumannii, Stenotrophomonas maltophilia, Enterococcus, CMV, beta-glucan, and Aspergillus antigens* were significantly higher in GII and GIII (Table 4).

The timing of immunosuppressant agent administration to patients is presented in Table 3. In general, both steroid therapy and TCZ therapy were administered before the patients were admitted to the intensive care unit. The first place where the patient's culture was taken was the intensive care unit. It was possible to look at this issue from two perspectives. Firstly, tocilizumab and steroids might have been administered to these patients in the

presence of a superinfection that has not yet been detected. The second possibility is that this treatment was given to critically ill patients who were thought to be more severe and fragile, and the risk of superinfection increased. We think that the second possibility reflects the situation of a more significant majority, as the patients did not undergo invasive procedures before the intensive care unit, did not have long-term hospitalizations, and did not use long-term antibiotics. Table 4 presents the comparison of microorganism growth rates in cultures taken in the first 48 h in the groups. A significant difference was detected between the groups. Therefore, it is necessary to discuss the place and reliability of these treatments in the presence or suspicion of infection, regardless of the situation. Thinking that this was the case, we determined our groups and examined the infection rates.

We think it is important to remember step by step how immunosuppressant agents can be a risk factor for secondary infection, along with their mechanism of action.

Tocilizumab suppresses the IL-6 signaling pathway, has a negative effect on neutrophil number and function, has a negative effect on macrophage activity, and suppresses the production of acute phase proteins such as CRP in the liver, impairing the ability to respond early to infection, and inhibits the activation and proliferation of T cells and B cells. It causes weakness in the adaptive immune response, weakening the body's immune system for secondary infection[40–42].

On the other hand, steroid use suppresses humoral immunity through B-cells, which is managed through T-cells, reduces phagocytic cell activation and production of inflammatory cytokines, and reduces the skin and its barriers[43,44].

For this reason, it is important to evaluate the immunological process when using drugs, especially in combination treatments, and to be alert to immunodeficiency, even if it is partial or temporary.

Based on all these results, we believe that the use of drugs such as PMP, DXM, and TCZ should be alert in terms of secondary infections. In addition, we observed that intensive care-related chronic problems such as IMV and LOS-ICU duration, neuropathy, and tracheostomy rate were more common in patients administered immunosuppressive agents. Apparently, adverse effects and complications of immunosuppressive agents were ignored in this pandemic period.

The first limitation of our study is its retrospective design. This design may restrict us from establishing a definitive causal relationship between the use of immunosuppressant agents, secondary infections, and clinical outcomes. Another limitation is the diversity in patients' treatments. Antiviral and immunomodulator treatments were carried out in our hospitals included in the study according to the current guidelines and health recommendations at that time[45,46]. TCZ and steroids were started before ICU admission in severe COVID cases as recommended by the hospital infectious diseases committee. However, not all of our patients started all these treatments after meeting the same criteria. In general, individual clinician preference has led to differences in treatment selection and timing. It was observed that the treatment recommendations in the guidelines were unclear, frequently updated and varied, and the limited data obtained in the first year of the pandemic caused these individual approaches. For example, steroids were only recommended in the presence of ARDS whereas Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with Coronavirus Disease 2019 didn't recommend the use of TCZ due to limited data[27]. Even so, in order to minimize bias in retrospectively collected cases, we tried to eleminate confounding factors by using propensity score matching.

Despite all these limitations, the infection rates encountered in groups receiving immunosuppressive drugs show the importance of questioning risk factors. Therefore, focusing only on a viral infection will cause us to remain blind to the reasons for patients' deterioration in the presence of these immunosuppressive therapies. In this study, surviving patients in the intensively immunosuppressed group who faced secondary infection had worsening discharge status, and almost all required intensive physiotherapy and home care support and were exposed to long-term complications, as Rasula et al.[47] indicated. Lastly, we can emphasize that when immunosuppressive drugs are added to the treatment, things that should be remembered in these fragile patients are the possible risk of infection and regularly collecting culture samples. A suspicious approach and follow-up, as in hematology and oncology patients, will be possible to provide early diagnosis in terms of superinfection and contribute to survival in this fragile patient group.

On the other hand, the risk of developing muscle weakness and polyneuropathy may cause us to be vulnerable to superinfections, as we may encounter with steroid use, and it will be possible for us to encounter patients who are more fragile and require more extended rehabilitation periods, with intensive care processes that may be prolonged and difficult. For this reason, we believe that these agents should be used in the right patient at the right time, and we need standards regarding the indications for starting treatment. Therefore, prospective studies to be designed in the future will allow us to both protect our patients from undesirable effects such as infection, which is at the center of our study and get effective results.

Conclusions

In conclusion, intense immunosuppressive medicines may increase the risk of infection in patients with COVID-19. Therefore, these medications and their complications may negatively affect the duration of IMV, LOS-ICU stays, and CFS at discharge. It should not be forgotten that these patients are more vulnerable and necessitate extended rehabilitation. Hence, these adverse effects and complications should be considered before immunosuppressive treatments are used in these patients.

Data availability

Reasonable requests for access to the datasets used and/or analyzed during this study can be made to the corresponding author.

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Author contributions

ZTS and BG have given substantial contributions to study conception and design, data collection and manuscript writing, FT, OD, CS, LD, SA and RZ to data collection, ASK manuscript writing, LT and IOA to study conception . All authors read and approved the final version of the manuscript.

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Competing interests

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Additional information

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