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The Effectiveness of Hydroxychloroquine Versus Hydroxychloroquine Plus Lopinavir/Ritonavir Therapy in SARS-CoV-2 Pneumonia

SARS-CoV-2 Pnömonisinde Hidroksiklorokin ile Hidroksiklorokin Artı Lopinavir/Ritonavir Tedavisinin Etkinliği

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Abstract

Introduction: There is no specific antiviral treatment with proven efficacy and safety in the management of Coronavirus disease-2019 (COVID-19). We aimed to compare the effectiveness of hydroxychloroquine (HQ) monotherapy and HQ-lopinavir/ritonavir (Lpv/r) combined therapy in patients with laboratory-confirmed COVID-19 and to determine the independent factors predicting mortality.
Materials and Methods: Retrospective observational multi-centered cohort study.
Results: In total, 151 patients (mean age 61±17 years, 66% male) with COVID-19 pneumonia were included: 68 patients received combination therapy, i.e., Lpv/r in addition to HQ, and 83 patients received only HQ. The patients in both groups were similar regarding the majority of baseline variables except for white blood cell count, procalcitonin, lactate dehydrogenase levels, intensive care unit (ICU) admission rates, which were significantly higher, and decreased oxygen saturation in the combination group. The mean duration of symptoms and hospital stay were 5.6±2.3 days and 12.7±9.4 days, respectively. Nearly 43% (n=65) of patients were admitted to the ICU. Patients in the HQ monotherapy group had a shorter stay in hospital than those in the combination group (10 vs. 16 days, p<0.005). The primary end points were 14- and 28-day mortality. Neither treatment group revealed significant differences with respect to 14-day and 28-day survival before and after propensity score matching. Age, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, the Charlson Comorbidity Index (CCI), and ICU stay length were variable predictors of 14-day mortality, while CCI [Hazard ratio (HR) 95% confidence interval (CI): 0.85 (0.43-0.9)] and ICU stay length [HR (95% CI): 1.5 (1.39-1.76)] were the independent predictors of 28-day mortality.
Conclusion: Combination therapy with Lpv/r and HQ did not provide any benefit compared with HQ monotherapy. Charlson Comorbidity Index and ICU stay were independent predictors of 28-day mortality.
Keywords: SARS-CoV 2, COVID-19, treatment, hydroxychloroquine, lopinavir/ritonavir

Öz

Giriş: Koronavirüs hastalığı-2019'un (COVID-19) yönetiminde etkinliği ve güvenliği kanıtlanmış spesifik bir antiviral tedavi yoktur. Laboratuvarca doğrulanmış COVID-19 hastalarında hidroksiklorokin monoterapisi ile hidroksiklorokin-lopinavir/ritonavir (Lpv/r) kombine tedavisinin etkinliğini karşılaştırmayı ve mortaliteyi öngören bağımsız faktörleri belirlemeyi amaçladık.
Gereç ve Yöntem: Geriye dönük gözlemsel çok merkezli kohort çalışmasıdır.

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Bulgular: Toplamda, COVID-19 pnömonili 151 hasta (ortalama yaş 61±17 yıl, %66'sı erkek) dahil edildi. Altmış sekiz hasta kombinasyon tedavisi (hidroksiklorokine ek olarak Lpv/r), 83 hasta sadece hidroksiklorokin aldı. Her iki gruptaki hastalar; lökosit sayısı, prokalsitonin, laktat dehidrojenaz seviyeleri, yoğun bakım ünitesine (YBÜ) yatış oranları haricinde (kombinasyon grubunda önemli ölçüde daha fazla artmış ve oksijen satürasyonu azalmış) temel değişkenlerin çoğu açısından benzerdi. Ortalama semptom süresi 5,6±2,3 gün ve hastanede kalış süresi 12,7±9,4 gündü. Hastaların yaklaşık %43'ü (n=65) YBÜ'ye yatırıldı. Hidroksiklorokin monoterapi grubundaki hastaların hastanede kalış süresi kombinasyon grubuna göre daha kısaydı (10'a karşı 16 gün, p<0,005). Primer sonlanım noktaları 14 ve 28 günlük mortaliteydi. Eğilim skoru eşleştirmesi öncesi ve sonrasında her iki tedavi grubu arasında anlamlı farklılık gözlenmedi. Yaş, hipertansiyon, diabetes mellitus, kronik obstrüktif akciğer hastalığı, Charlson Komorbidite İndeksi (CCI) ve YBÜ'de kalış 14 günlük mortaliteyi öngören değişkenler iken, CCI [Hazard oranı (HR) [%95 güven aralığı (CI): 0,85 (0,43-0,9)] ve YBÜ'de kalış [HR (%95 CI): 1,5 (1,39-1,76)], 28 günlük mortalitenin bağımsız prediktörleri idi.
Sonuç: Lpv/r ve hidroksiklorokin ile kombinasyon tedavisi, hidroksiklorokin monoterapisine kıyasla herhangi bir fayda sağlamadı. Charlson Komorbidite İndeksi ve YBÜ'de kalış, 28 günlük mortalitenin bağımsız belirleyicileriydi.
Anahtar Kelimeler: SARS-CoV-2, COVID-19, tedavi, hidroksiklorokin, lopinavir/ritonavir

Introduction

As of October 27, nearly 44 million cases of Coronavirus disease-2019 (COVID-19) patients infected with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and over 1,170,000 deaths had been reported world wide in the global pandemic[1]. Thousands of new cases are detected in many countries every day. The first COVID-19 case in our country was confirmed on March 11, 2020. There is no specific antiviral treatment with proven efficacy and safety inthemanagement of COVID-19 disease[2], but more than 150 phase 3-4 randomized controlled trials in adults are currently underway in this area because of the urgent need for an effective treatment. Potential treatment options include off-label use of the anti-malarial drugs chloroquine and hydroxychloroquine (HQ) as "viral entry inhibitors", the antiviral drugs lopinavir/ritonavir (Lpv/r) as "viral protein synthesis inhibitors", favipravir and remdesivir as "viral RNA polymerase /RNA synthesis inhibitors", which have been repositioned for COVID-19 therapy, and convalescent-phase immune plasma[3-7]. HQ sulfate inhibits SARS-CoV-2 *in vitro*[8]. Lopinavir/r is a combination of two human immunodeficiency virus (HIV)-1 protease inhibitors widely used in the treatment of HIV infection with a good safety profile. It binds to the SARS-CoV 3C-like protease and inhibits viral protein synthesis[9]. Lopinavir, an HIV protease inhibitor, has been shown to have invitro activity against SARS-CoV-2[10,11]. Moreover, it has been reported to have activity against Middle East Respiratory Syndrome (MERS-CoV-2) *in vitro*[12] and in animal models[13]. Related to the efficacy of Lpv/r treatment, various data, including both *in vitro* and clinical study results, are available in the medical literature[14,15]. A randomized trial with LPV/r including 199 severe COVID-19 patients has shown that it confers no advantage beyond standard care alone[16]. A statistically non-significant decrease in mortality was observed in the LPV/r-added arm (19% vs. 25%). Lopinavir/r (400 mg/100 mg bid) and interferon-alpha (5 million U bid) are recommended as antiviral therapy under Chinese guidelines[17]. Because of the knowledge gap, the Infectious Diseases Society of America (IDSA) guidelines recommend Lpv/r for patients admitted to the hospital with COVID-19 only within a clinical trial[18].

A COVID-19 management guideline prepared by the Scientific Committee of the Ministry of Health in our country has been updated frequently as the related knowledge in the literature increases[19]. In the version of this guide dated February 25, 2020, firstly, HQ 200 mg tb (2x1) (for five days) was recommended in mild COVID-19 cases, while Lpv/r (400 mg/100 mg bid) (for 10-14 days) was recommended primarily in severe cases. In the next version of the same guide dated March 25, 2020, HQ 200 mg tablets (2x1 after 2x2 loading, for five days) and Lpv/r (400 mg/100 mg tablets bid for 10-14 days) were recommended in probable or definitive COVID-19 cases with pneumonia and in patients with a severe course and initial therapy failure, respectively.

In the subsequent guide, dated April 2, 2020, the use of Lpv/r (for 10-14 days) +/- HQ (for five days) was the recommended treatment protocol in pregnant women with COVID-19. All the drugs available in our country have been delivered to hospitals for treatment of COVID-19 patients by the Ministry of Health. Since no specific proven antiviral treatment is available, the combined use of possible treatment options in COVID-19 patients should be considered on a patient basis and by evaluating all currently available literature. In this study, we primarily aimed to compare the effectiveness of HQ monotherapy and HQ-Lpv/r combined therapy in patients diagnosed with COVID-19. Secondarily, we aimed to determine the independent factors predicting mortality.

Materials and Methods

Hospitalized COVID-19 patients older than 18 years whose diagnoses were confirmed by the reverse transcriptase-polymerase chain reaction (RT-PCR) method and who received either HQ monotherapy or HQ-Lpv/r combined therapy, followed-up in pandemic clinics of University of Health Sciences Turkey, Kartal Dr. Lütfi Kırdar City Hospital, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital and

Çekmeköy State Hospitals between March 11, 2020 and April 30, 2020, were included in this study. Demographic features [age, gender, underlying diseases, Charlson Comorbidity Index (CCI)] [20], clinical and laboratory data, vital signs, treatment schemes, side effects, and outcome measures were obtained from patient records or hospital databases. The laboratory data for patients in two treatment groups were recorded on the first, third, and seventh treatment days. The first group included only patients receiving HQ monotherapy, and the second group included patients receiving HQ-Lpv/r combined therapy. The dosing regimes of adjuvant drugs were the same in both groups. The efficacy of the treatment protocols in both groups were evaluated by comparing the clinical outcomes, defined as discharge from the hospital or in-hospital mortality, and independent factors affecting mortality were determined. HIV infection was ruled out in all patients with negative 4th generation enzyme linked immunosorbent assay test results. Electrocardiographic monitoring of patients was performed for any prolongation of the QTc and/or PR interval on the first day in hospital and every other day until discharge. All co-administered medications were checked for any drug-drug interactions.

The primary endpoints were 14-day and 28-day survival, and the secondary endpoints were clinical outcomes and improvement in laboratory parameters.

Upper respiratory tract swab samples were studied byreal-time RT-PCR (Roche Light Cycler 96) to confirm the diagnosis of COVID-19. Only patients with a laboratory-confirmed COVID-19 diagnosis were included in the study. This retrospective study was approved by the institutional review board of University of Health Sciences Turkey, Kartal Dr. Lütfi Kırdar City Hospital on May 13, 2020 with number 2020/514/177/2 and was conducted in compliance with the Declaration of Helsinki Principles.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics, version 17.0 (Chicago: SPSS Inc.) was used for all statistical analyses. Mean, standard deviation, and frequencies were used for descriptive statistics, χ^2 test or Fisher's exact tests were used to analyze categorical variables, and the Student's t-test was used to test for the statistical significance of differences between continuous variables. Cox regression analysis and Kaplan-Meier survival analysis were used to determine the independent predictors of mortality. Results were evaluated as odds ratios and 95% confidence intervals. A two-sided p value <0.05 was considered to indicate statistical significance. In order to adjust for selection bias, propensity score matching (PSM) was performed. The propensity score (PS) was estimated by multinomial logistic regression, which included all of the treatment-related prognostic factors. Patients who had similar PS in the two groups were matched by using the "nearest neighbor matching method". The patients with either PS<0.3 or PS>0.6 were excluded from the groups for matching. After PSM, the groups were characterized similarly in terms of risk factors. The statistical analysis was repeated after PSM. A p value<0.05 was considered to indicate a statistically significant result.

Results

In total, 151 patients were included in this study. The test group (n=68) comprised the patients who received combination therapy (i.e., Lpv/r in addition to standard care consisting of HQ), while the patients in the control group (n=83) were only treated with standard care (HQ). None of the patients received dexamethasone as it was not recommended in the guidelines at the beginning of the pandemic. The mean age of the patients was 61 (minimum: 21, maximum: 100) years. Most of the patients (66%) were male. All of the patients had thorax computed tomography findings compatible with COVID-19 pneumonia. Baseline demographic features, laboratory values, and outcomes of patients receiving two different treatment regimens are summarized in Table 1. Patients in both groups were similar regarding the majority of those variables except for baseline white blood cell (WBC) count, procalcitonin (PCT), lactate dehydrogenase (LDH) levels, and intensive care unit (ICU) admission rates, which were significantly higher in the combination therapy group. Initial oxygen saturation rates were lower in the combination group. Hypertension (42%) and diabetes mellitus (DM) (30%) were the most common comorbid diseases. Sixteen percent of patients had chronic renal disease, and 2.6% had chronic renal failure. The mean duration of symptoms and hospital stay were 5.6±2.3 days and 12.7±9.4 days, respectively. Treatment was initiated within five to six days of symptom onset. Nearly 43% (n=65) of patients (2/3 in the combination treatment group) were admitted to the ICU during follow-up for COVID-19. Patients in the HQ monotherapy group had a shorter stay in hospital than those in the combination therapy group (10 vs. 16 days, p<0.005). Leukocyte counts, creatinine, C-reactive protein (CRP), PCT, LDH, ferritin, and D-dimer levels onthe 3rd and 7th days of therapy were significantly higher, but oxygen saturation was lower in the combination therapy group (Figures 1-5). Six patients had adverse events (1 nausea-vomiting, 1 hyponatremia, 1 hypokalemia, 1 hyperkalemia, 1 elevated transaminase levels) in the monotherapy group, and six patients in the combination therapy group faced adverse events (2 nausea-vomiting, 1 diarrhea, 3 hypokalemia, 1 hyperkalemia, 1 elevated transaminase levels). No serious adverse events (such as prolonged QTc, hepatotoxicity etc.) causing early cessation of therapy occurred in either group. Complications were observed in 22 patients (27%) in the monotherapy group [3 pulmonary thromboembolism (PTE), 19 hepatitis, 1 secondary bacterial infection as pneumonia]. In the combination

Table 1. Baseline demographic features, laboratory values, and outcomes of patients on two different treatment regimens

Characteristic/variable	Standard care (n=83)	Standard care+lopinavir/ritonavir (n=68)	p value
Age (years), median (IQR)	58 (34)	64 (33)	0.721
Gender (male) (n, %)	50 (60)	51 (75)	0.55
Fever (n, %)	53 (64)	45 (66)	0.766
Cough (n, %)	69 (83)	61 (89)	0.245
Dyspnea (n, %)	52 (63)	46 (68)	0.522
CCI (mean±SD)	2.84±2	3.15±3	0.053
Hypertension (n, %)	34 (41)	30 (44)	0.696
Diabetes mellitus (n, %)	21 (25)	25 (37)	0.128
Chronic renal disease/failure (n, %)	12 (15)	17 (25)	0.102
Congestive heart failure (n, %)	0	8 (11)	0.715
Coronary artery disease (n, %)	26 (31)	15 (22)	0.203
COPD (n, %)	13 (16)	9 (13)	0.674
Asthma (n, %)	5 (6)	9 (13)	0.128
Cancer (n, %)	5 (6)	10 (14)	0.076
Immunosuppression (n, %)	6 (7)	8 812)	0.339
Time to initiation of treatment (days), median (IQR)	6.6 (3)	5 (4)	0.135
WBC (/mm^3), median (IQR)	5150 (4400)	5600 (3100)	**0.033**
Lymphocyte (/mm^3), median (IQR)	1050 (950)	1100 (1300)	0.873
BUN (mg/dl), median (IQR)	26 (22)	36 (30)	0.075
Creatinine (mg/dl), median (IQR)	0.7 (0.3)	0.89 (0.76)	0.159
AST (IU/L), median (IQR)	33 (24.3)	34 (28)	0.410
ALT (IU/L), median (IQR)	26 (17.5)	30 (29)	0.845
CRP (mg/L), median (IQR)	41.5 (66)	31.3 (80)	0.192
Procalcitonin (ng/L)	0.1 (0.25)	0.35 (0.41)	0.004
Ferritin (µg/L)	409 (627.5)	320 (247)	0.126
D-dimer (µg/L)	1730 (1310)	1520 (1470)	0.087
LDH (IU/L), median (IQR)	235 (314)	346 (228)	0.001
Creatinine kinase (IU/L) median (IQR)	200 (125)	205 (132)	0.945
O_2 saturation (in ambient air) (%)	92 (4.25)	87 (6)	**0.024**
ICU admission	23 (28)	42 (62)	**0.0001**
Secondary bacterial infection	1[a] (0.1)	4[b] (0.5)	**0.06**
14-day survival (n,%)	69 (83)	51 (75)	0.218
28-day survival (n,%)	64 (77)	44 (65)	0.093
Median hospital stay (days)	10 (5)	16 (12)	**0.0001**
In-hospital crude mortality	19 (23)	24 (35)	0.062

CCI: Charlson Comorbidity Index, COPD: Chronic obstructive pulmonary disease, WBC: White blood cell count, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, IQR: Interquartile range, CRP: C-reactive protein, LDH: Lactate dehydrogenase, LMWH: Low-molecular-weight heparin, a: Pneumonia, b: 3 pneumonia, 1 urinary tract infecion

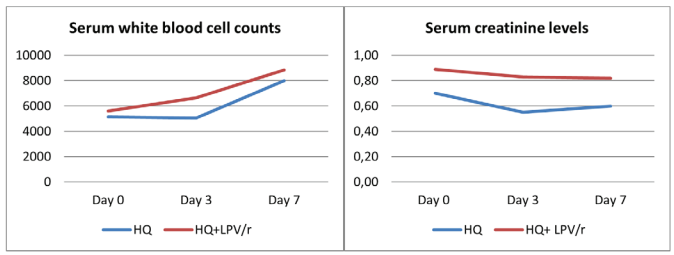

Figure 1. Comparison of serum white blood cell counts and creatinine levels on day 0, 3 and 7 between hydroxychloroquine monotherapy and hydroxychloroquine plus lopinavir-ritonavir combination groups

HQ: Hydroxychloroquine, Lpv/r: Lopinavir-ritonavir

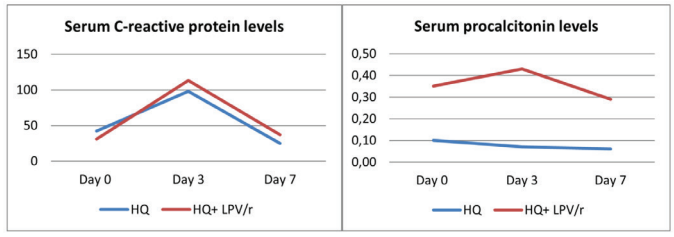

Figure 2. Comparison of serum C-reactive protein (A) and serum procalcitonin levels (B) on day 0, 3 and 7 between hydroxychloroquine monotherapy and hydroxychloroquine plus lopinavir-ritonavir combination groups

HQ: Hydroxychloroquine, Lpv/r: Lopinavir-ritonavir

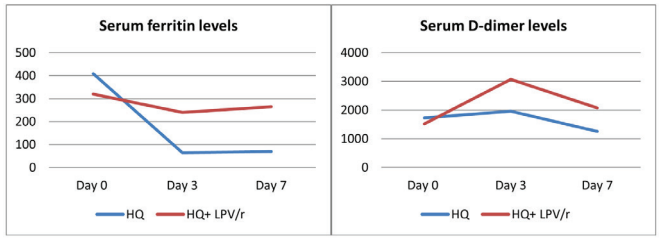

Figure 3. Comparison of serum ferritin (A) and serum D-dimer levels (B) on day 0, 3 and 7 between hydroxychloroquine monotherapy and hydroxychloroquine plus lopinavir-ritonavir combination groups

HQ: Hydroxychloroquine, Lpv/r: Lopinavir-ritonavir

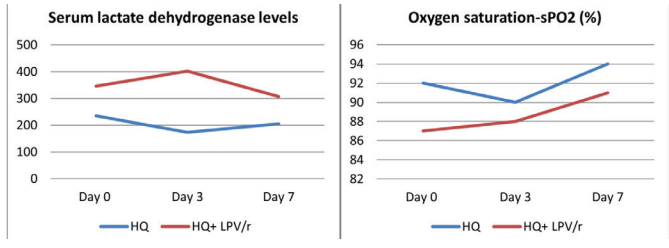

Figure 4. Comparison of serum lactate dehydrogenase levels (A) and oxygen saturation (B) on day 0, 3 and 7 between hydroxychloroquine monotherapy and hydroxychloroquine plus lopinavir-ritonavir combination groups

HQ: Hydroxychloroquine, Lpv/r: Lopinavir-ritonavir

therapy group, 39 patients (57%) developed complications: 4 PTE, 21 hepatitis, 9 acute renal failure, 3 secondary bacterial pneumonia, 1 catheter-associated urinary tract infection, 2 cytokine release syndrome, 1 myocarditis, 1 acute respiratory distress syndrome (ARDS). Secondary bacterial infections were treated with effective antibacterial drugs. In-hospital crude mortality rates in the standard-care group and the combination therapy group were 23% and 35%, respectively. In univariate analysis, age, hypertension, DM, chronic obstructive pulmonary disease (COPD), CCI, and ICU stay were the variables predicting 14-day mortality in the Kaplan-Meier log-rank test and Cox proportional-Hazards model (Table 2). In multivariate analysis, CCI and ICU stay were the independent predictors of 28-day mortality (Table 3). Cumulative survival rates of the patients in two different treatment groups are shown in Figure 5.

Discussion

The main purpose of our study was to compare the clinical efficacy of HQ monotherapy and HQ plus Lpv/r combination therapy. Lopinavir/r is readily available in many countries, including ours, and its effectiveness against SARS-CoV-2 and MERS-CoV-2 has been demonstrated[11,21]. It has also been used in combination with other antiviral drugs in SARS and MERS-CoV-2 infections[11,21,22]. It was suggested that the Lpv/r concentration necessary to inhibit pulmonary SARS-CoV-2 replication might be higher than the serum level obtained by the usual Lpv/r dose[23,24]. The World Health Organization recommends "HQ and Lpv/r not be administered as treatment or prophylaxis for COVID-19, outside of the context of clinical trials" in the interim guidance published on May 27, 2020[25].

Our national guideline published by the Scientific Committee of the Ministry of Health recommends HQ and/or favipravir in the treatment of COVID-19, and Lpv/r in pregnant patients with moderate to severe COVID-19, in the latest version, dated October 8, 2020[26].

The mean age of our patients was 61 years, 2/3 were male, and hypertension and DM were the most common comorbid diseases,

Table 2. Univariate analysis of the risk factors predicting 14-day and 28-day mortality among patients with SARS-CoV-2 pneumonia, before and after propensity score matching

	Before PSM				After PSM			
	14-day mortality HR (95% CI)	p value	28-day mortality HR (95% CI)	p value	14-day mortality HR (95% CI)	p value	28-day mortality HR (95% CI)	p value
Age	1.06 (1.02-1.06)	**0.002**	0.96 (0.92-0.98)	0.37	1.01 (0.9-1.04)	**0.001**	0.71 (0.69-0.88)	0.32
Gender	0.87 (0.43-0.96)	0.35	0.9 (0.81-0.98)	0.48	0.51 (0.12-0.82)	0.67	0.95 (0.3-1.6)	0.94
Hypertension	1.62 (1.3-1.92)	**0.01**	1.53 (1.26-1.98)	0.14	1.02 (1.01-1.73)	**0.01**	1.53 (1.1-1.92)	0.24
Diabetes mellitus	1.54 (1.38-4.09)	**0.01**	1.53(1.36-4.25)	0.39	1.32 (1.28-3.2)	**0.03**	1.53 (1.36-4.25)	0.41
COPD	1.21 (1.11-5.40)	**0.023**	1.1 (0.35-3.4)	0.86	1.2 (1.02-2.4)	**0.012**	1.10 (0.35-3.40)	0.73
CCI	0.75 (0.58-0.97)	**0.03**	0.75 (0.58-0.97)	**0.03**	0.62 (0.53-0.86)	**0.004**	0.75 (0.58-0.97)	**0.03**
O_2 saturation (in ambient air)	2.1 (2.05-6.2)	**0.02**	2.6 (1.9-3.8)	**0.002**	2.9 (1.56-8.2)	**0.01**	**3.2 (2.9-4.9)**	**0.02**
ICU stay	1.4 (1.23-1.53)	**<0.0001**	1.5 (1.39-1.76)	**<0.0001**	1.6 (1.33-1.48)	**<0.0001**	1.5 (1.39-1.76)	**<0.0001**
Median hospital stay	1.4 (1.26-6.5)	**0.001**	1.2 (1.26-3.19)	**0.001**	1.6 (1.3-4.7)	**0.02**	1.5 (0.93-3.8)	**0.02**
Combination therapy	1.56 (1.32-1.91)	0.45	1.49 (1.22-1.65)	0.62	1.63 (1.2-3.6)	0.87	1.53 (1.23-4.2)	0.9

Kaplan-Meier log-rank test and Cox proportional-hazards model, HR: Hazard ratio.
CCI: Charlson Comorbidity Index, COPD: Chronic obstructive pulmonary disease, ICU: Intensive care unit, PSM: Propensity score matching, CI: Confidence interval

Table 3. Factors predicting 14-day and 28-day mortality before and after propensity score matching (PSM)-(Multivariate stepwise Cox regression analysis)

	Before PSM				After PSM			
	14-day mortality HR (95% CI)	p value	28-day mortality HR (95% CI)	p value	14-day mortality HR (95% CI)	p value	28-day mortality HR (95% CI)	p value
CCI	0.75 (0.58–0.97)	**0.003**	0.85 (0.43–0.9)	**0.03**	0.67 (0.53–0.87)	**0.002**	0.7 (0.6–0.9)	**0.02**
ICU stay	1.4 (1.23–1.53)	**<0.0001**	1.5 (1.39–1.76)	**<0.0001**	1.3 (1.03–1.46)	**<0.0001**	1.5 (1.39–1.64)	**<0.0001**
Combination therapy	1.56 (1.32–1.91)	**0.36**	1.49 (1.22–1.65)	**0.52**	1.46 (1.3–1.71)	**0.41**	1.39 (1.2–1.55)	**0.62**

Kaplan–Meier log-rank test and Cox proportional-hazards model, HR: Hazard Ratio
CCI:Charlson Comorbidity Index, ICU: Intensive Care Unit

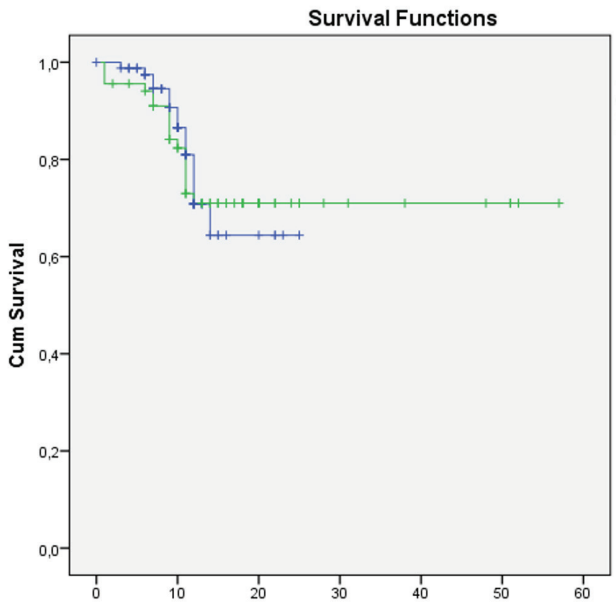

Figure 5. Cumulative survival of patients in two different treatment groups (blue line: monotherapy group, green line: combination therapy group)

similar to the findings in 393 patients reported by Goyal et al.[27]. Sixteen percent of patients had chronic renal disease, and 2.6% had chronic renal failure.

Cao et al.[16] conducted a randomized, controlled, open-label trial for Lpv/r including 199 severe COVID-19 patients. They found no benefit of Lpv/r beyond standard care. The median time between the onset of symptoms and initiation of therapy was 13 days in that trial, while it was nearly six days (one week earlier) in our study. Antiviral drugs are most effective against coronaviruses when they are administered early[28], since the rate of viral replication is high in the earlier stages of COVID-19, but systemic hyperinflammation may ensue in later stages[30]. Lopinavir/r given within 12 days of symptoms may not be as effective as when given earlier in the disease course. The mean duration of hospital stay was six days longer in the combination group in our study. In the course of a pandemic, the length of hospital stay for each patient is important because there may be a shortage of hospital beds, mechanical ventilators, etc. More than half (62%) of the patients in the combination therapy group were followed-up in the ICUs. Similarly, leukocyte counts, creatinine, CRP, PCT, LDH, ferritin, and D-dimer levels on the 3rd and 7th days of therapy were significantly higher, but oxygen saturation was lower in the combination therapy group. Those laboratory parameters have been reported to be prognostic factors of disease severity and worse outcomes[30]. Moreover, in a systematic literature review and meta-analysis including a total of 3,027 patients with COVID-19, WBC, aspartate aminotransferase (AST), creatinine, PCT, LDH, and D-dimer were reported as markers of disease progression[31].

Our national guidelines recommend the administration of Lpv/r in more severe patients, which might be one of the underlying reasons for the increased laboratory values and length of hospital stay in that group. Clinically more severe patients in the combination therapy group required more oxygen support, as expected. Regarding clinical improvement, Lpv/r had no advantage beyond the standard of care. In the trial conducted by Cao et al.[16], the inclusion criteria referred to severely ill patients with COVID-19. However, in our study, all patients with COVID-19 pneumonia were included. Ye et al.[32] conducted a study including 47 COVID-19 patients and compared Lpv/r combined with adjuvant medicine to only adjuvant medicine. In contrast to our results, the body temperature, WBC, lymphocyte and CRP, alanine aminotransferase, and AST decreased faster in the combination group.

In our study, although patients in the combined therapy group required more oxygen support and had worse laboratory results, the 14-day and 28-day mortality rates were not statistically significantly different between the two treatment groups. In the Lpv/r clinical trial, the median time to clinical improvement was not significantly different, and the 28-day mortality rate was similar in both groups. Although statistically non-significant, 28-day mortality was 5.8% lower in the Lpv/r group. Intention-to-treat analysis excluded three patients with early death (in the first 24 hours of therapy) and then clinical improvement was one day shorter in the Lpv/r group. The overall mortality rate was 22% in that trial[16]. In our study, the ICU admission rate in the monotherapy group was 28% with an overall mortality of 23%. In the combination therapy group, the ICU admission rate and overall mortality were 62% and 35%, respectively. Overall, the mortality rate in critically ill patients in the ICU in our study was less than that (62%) reported in the study conducted by Yang et al.[33], but more than that (31%) reported by Auld et al.[34]. Despite a nearly 2-fold greater ICU follow-up requirement in the combination therapy group, only half of the patients in the ICU died of COVID-19. Twice as many complications were observed in the combination group, which was comprised of more critically ill patients.

Only six patients in each group had adverse events in our study. Serious adverse events and respiratory failure or ARDS occurred more commonly in the standard-care group (32% vs. 20% and 27% vs. 13%, respectively) in the clinical trial by Cao et al.[16]. In our study, age, hypertension, DM, chronic obstructive pulmonary disease, CCI, and ICU stay were the variables predicting 14-day mortality. These results were similar to findings in a systematic review and meta-analysis on that subject[29]. Charlson Comorbidity Index and ICU admission were the independent predictors of 28-day mortality in our study.

Study Limitations

The major limitations of our study were its retrospective design and the heterogenity of the patient population regarding disease severity, as combination therapy were provided for more severe patients. Moreover, there were more critically ill patients who had been admitted to the ICU in the combination therapy group, as our national treatment guidelines recommend combination therapy to severely ill COVID-19 patients. Further clinical studies are necessary to determine whether earlier and extended administration of antiviral drugs may improve clinical outcomes in patients with COVID-19 pneumonia. The clinical impact of our study is that we no longer prefer to use Lpv/r as there is no additional benefit to it.

Conclusion

In conclusion, there were no statistically significant differences between the two treatment groups regarding 14-day and 28-day mortality. As combination therapy with Lpv/r and HQ was administered to more severe patients requiring more oxygen support, it did not seem to provide any benefit compared with HQ monotherapy regarding mortality. Charlson Comorbidity Index and ICU stay were independent predictors of 28-day mortality.

Ethics

Ethics Committee Approval: The study were approved by the University of Health Sciences Turkey, Kartal Dr. Lütfi Kırdar City Hospital of Clinical Research Ethics Committee (protocol number: 2020/514/177/2, date: 13.05.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.B., S.T., H.B., Design: A.B., S.T., H.B., N.B., Data Collection or Processing: A.B., S.T., H.B., Analysis or Interpretation: A.B., H.B., Ç.A.K., Literature Search: A.B., S.T., H.B., Writing: A.B., N.B.

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