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The Importance of a Stepwise Unified Protocol in the Management of Hospitalized COVID-19 Patients: Clinical and Virological Evidence

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Abstract

Objective: Outcome of a stepwise medical protocol in COVID-19 pneumonia patients admitted to hospital.

Methods: A prospective cohort study was conducted between March 3rd, and April 16th, 2020 at Hotel-Dieu de France University Medical Center in Lebanon. Forty patients confirmed with COVID-19 and stratified into eight categories received a quadruple pharmacological therapy (hydroxychloroquine, azithromycin, pitavastatin, zinc). Other medications and procedures were added according to disease severity. QTc and Tisdale score were assessed at baseline and monitored regularly. Outcomes included mortality, hospital length of stay, ICU discharge, and time to negative PCR.

Results: Mean age was 53•1 ± 18•3 years, and most patients (60%) were male. At database lock date, 32 (80%) patients were discharged with a median length of stay of 8 days (5; 11), five remained in isolation unit, one in ICU, and two patients were dead. Viral PCR outcome confirmed a long period of shedding (median 17 days). For ICU patients, median time from disease onset to invasive mechanical ventilation was nine days (6; 10). Length of stay was 13 days (7.2; 18•8) with prolonged need for mechanical ventilation, reaching a median of 8 days (5; 11).

Conclusion: Stratifying patients at presentation and putting them early on adapted treatment was associated with favorable outcome.

Keywords: Stepwise protocol; COVID-19; Pneumonia; ICU; Hydroxychloroquine; Azithromycin

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Introduction

The coronavirus disease 2019 (COVID-19) outbreak has spread rapidly across the globe [1]. Most patients in Wuhan's initial reports had either no symptoms or self-limited respiratory symptoms typical of a viral illness. The severe disease includes florid pneumonia, which may progress to Acute Respiratory Distress Syndrome (ARDS) along with cardiogenic or distributive shock [2]. The severity of the disease is also associated with co-morbidities, such as diabetes and cardiovascular disease [2], requiring Intensive Care Unit (ICU) admission [3] and resulting in higher mortality rates [4]. Mortality rates associated with severe COVID-19 pneumonia are high (8% to 25%) despite aggressive supportive measures and can reach 60% in critically ill subjects [2,4-6].

COVID-19 seems to evolve in two phases: an infectious phase that lasts for a week, mainly in the upper respiratory tract, eventually followed by an inflammatory phase characterized by the progression of lung infiltrates associated with a hypercoagulable state [7].

To date, COVID-19 therapy is not standardized. Treatment should target viral proliferation during the first week of the disease, and later on, should halt the progression toward severe lung disease associated with cytokine storm and hypercoagulable state. More than 300 active clinical trials are ongoing, and various agents are assessed for COVID-19 pneumonia.

Zinc is positively charged and cannot get through the cellular membrane without a ionophore, among which Chloroquine (CQ) [8]. High concentrations of intracellular zinc inhibit RNA-dependent RNA polymerase (RdRp) elongation. HCQ alone (200 mg TID from five to ten days for HCQ, and 500 mg on Day 1) or in combination with azithromycin (250 mg QD for four days) is widely used off-label [9]. The US Food and Drug Administration has issued an emergency use authorization of HCQ in adults hospitalized for COVID-19, particularly when enrollment in clinical trials was not feasible, and the American College of Cardiology has suggested monitoring QTc parameters in this context.

The clinical efficacy lopinavir/ritonavir is controversial): It reduced viral load [10] and shortened ICU and hospital stay, but did not accelerate clinical improvement nor reduced mortality in severe COVID-19 cases. Remdesivir, a novel nucleotide analogue active against SARS-CoV-2 *in vitro*, is currently investigated on moderate and severe cases [11-13]. Tocilizumab, a recombinant humanized monoclonal antibody against human Interleukin 6 (IL-6), is also used to counter Cytokine Release Syndrome [14], and was associated with a favorable outcome [15]. Nevertheless, data on the use of combination therapy in critically ill patients remain scarce [6]. Therefore, the current prospective cohort study aims to report the outcome of a stepwise medical protocol in COVID-19 pneumonia patients.

Methods

Study design and participants

This prospective cohort study, conducted between March 3rd, and April 16th, 2020, included 65 patients suspected with COVID-19 hospitalized at Hotel-Dieu de France Hospital of the Saint Joseph University (USJ) in Beirut. Of these, only confirmed cases were enrolled. The diagnostic criteria applied were based on the World Health Organization interim guidance. The molecular diagnosis was made on nasopharyngeal swabs by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The study was approved by the institution's Ethical Committee (CEHDF #1631 and CEHDF #1642).

Data collection

Upon admission, sociodemographic characteristics and medical history were collected through a standardized questionnaire, and laboratory and imaging tests were performed. Collected data were reviewed by three physicians (pulmonary and critical care, infectious diseases, and clinical immunology) to resolve any discrepancy in interpretation.

Laboratory procedures

All suspected patients were tested for COVID-19 by RT-PCR. The test was performed at the Rodolphe Mérieux laboratory, within USJ Faculty of Pharmacy. Viral RNA was extracted from 140 µL of nasopharyngeal and oropharyngeal swab fluid (viral transport medium), using the QIAmp® Viral RNA Mini Kit (Qiagen®, Courtabœuf, France) as per the manufacturer's instructions. RT-PCR for SARS-CoV-2 was performed on RNA extracts, targeting the envelope protein (E)-encoding gene, and the RdRp-encoding gene, as described by [16]. This method uses a synthetic RNA positive control (supplied by the Charité virology institute - Berlin, Germany, *via* EVAg).

Routine blood examinations included complete blood count, coagulation profile, renal and liver function tests, creatine kinase, lactate dehydrogenase, electrolytes, cardiac enzymes, serum ferritin, C-reactive protein (CRP), D-dimer, and procalcitonin, in addition to a tuberculin skin test, baseline Electrocardiogram (ECG), Tisdale score (to identify patients at risk of QT prolongation), and a high-resolution CT scan.

Diagnostic criteria

The severity of illness was defined according to the SOFA (Sequential Organ Failure Assessment) score and to National Early Warning Score 2 (NEWS2) [17,18]. Pneumonia severity was determined by the CURB-65 (confusion, urea, respiratory rate, blood pressure, age >65). The Urgent Guidance for Navigating and Circumventing the QTc Prolonging and Torsadogenic Potential of Possible Pharmacotherapies for COVID19 was used to follow up on QTc prolongation. Neutrophil-to-Lymphocyte Ratio (NLR) was used to conclude the severity of illness in early- stage detection, while Platelet-to-Lymphocyte Ratio (PLR) served as a prognostic tool. Chest CT reports were evaluated based on the consensus statement endorsed by the Society of Thoracic Radiology and the American College of Radiology, classifying CT findings into four categories for a language standardized reporting.

Patients were considered febrile when experiencing a temperature of at least 38.3°C. Sepsis and septic shock were diagnosed according to the 2016 Third International Consensus Definition for Sepsis and Septic Shock. Secondary infections were examined through clinical symptoms or signs of pneumonia or bacteremia and by analyzing sputum and blood culture samples. Ventilator-Associated Pneumonia (VAP), Acute Kidney Injury (AKI), and ARDS, were diagnosed according to the guidelines for the treatment of hospital-acquired and ventilator-associated pneumonia, KDIGO clinical practice guidelines and the Berlin definition, respectively.

Patient Classification

Before any inclusion, a prospective protocol was developed to classify patients upon hospital admission into eight categories. Table 1 presents the categories, ranging from A (asymptomatic) to G (severe and intubated). Patients with mild disease were assigned to levels B and C based on the absence or presence of risk factors, i.e., age >70 years, a glomerular filtration rate <30 ml/min or on chronic dialysis, heart failure with NYHA III or IV, advanced chronic respiratory failure (COPD GOLD>B, asthma GINA>3, long term oxygen therapy or non-invasive ventilation), cirrhosis (child >B), diabetes mellitus, immunosuppressive treatments. Moderate disease was characterized by additional radiological Ground-Glass Opacities (GGO), and divided into three levels (D1, D2, and E), depending on the clinical severity assessed by the CURB-65. D1 patients had a CURB-65<2 and D2 patients had a CURB-65>2. Level E patients had pneumonia with CURB-65>2 with other associated risk factors. Level F included patients with severe disease experiencing marked desaturation (SPO2<92%), shallow breathing (Respiratory Rate (RR) >24/min), high oxygen requirements (O2>6 L/min to reach SpO2>94%), or organ failure. Patients requiring intubation were categorized at level G. The criteria for discharge included absence of fever for at least three days, substantial improvement of chest CT in both lungs, and

clinical remission of respiratory symptoms.

Treatment protocol

The patients followed an isolation circuit protocol and management according to their level of lung involvement and/or comorbidities: Those in level A to D1 were advised to stay home; those complaining of disproportional dyspnea were hospitalized for temporary monitoring. Level D2 and E patients were admitted to COVID-19 specialized unit (UIP) and transferred to the ICU when their NEWS2 score [18] was higher than 10. Level F and G patients were treated in the ICU.

Level A and B patients received symptomatic treatment and were advised to avoid non-steroidal anti-inflammatory drugs, oseltamivir, and corticosteroids. Level C and D1 patients received a quadruple therapy including: 1) hydroxychloroquine 400 mg BID on day 1 followed by 200 mg TID for ten days, 2) a loading dose of azithromycin 500 mg on day 1 followed by 250 mg QD for four days; 3) pitavastatin 2 mg QD for 14 days; and 4) zinc (as acetate) 20 mg QD for 14 days.

The rationale for adding statins is their ability to maintain normal levels of MyD88 to limit the release of inflammatory cytokines [19], during hypoxia and oxidative stress. Pitavastatin was preferred as it has less drug-drug interaction and muscular toxicity, and better liver tolerability.

For level D2 and higher, lopinavir 400 mg/ritonavir 100 mg BID for ten days was added. For level F and G patients, tocilizumab 8 mg/kg IV (one dose, maximum 800 mg) was added in the event of cytokine release syndrome (IL6>30 pg/ml), bilateral interstitial lung infiltrates, PaO2/FiO2<300, NEWS2 ≥ 8 [18], or two of the following: CRP>100 (or >50 with doubling time within 48 h), LDH>250, Ferritin >500, D-Dimer >1000, Lymphocytes <0·6 × 10^9. For level F and G patients, oxygen therapy was administered through a nasal cannula up to 6 L/min, then with high-flow oxygen therapy.

Patients who developed respiratory failure were intubated and put on mechanical ventilation in volume-assisted mode. Preserved tidal volume of 8 ml/kg with PEEP of 8 cmH2O to 10 cmH2O was applied for L- type patients with preserved lung compliance (>40 ml/cmH2O), and low tidal volume of 6 ml/kg with high PEPP 14 cmH2O to 16 cmH2O for H-type ARDS patients with reduced compliance (<40 ml/cmH2O). Noninvasive ventilation and nebulizer therapy were forbidden. In more severe cases (PaO2/FiO2<150), a prone position for 16 h per day was considered. Extracorporeal Membrane Oxygenation (ECMO) was applied in young patients with refractory hypoxemia after the approval of two intensive care physicians [20].

Outcome and monitoring

Database was locked on April 16th, 2020. The main outcomes included mortality, length of stay in hospital, discharge from ICU, and time to negative PCR. Three types of mortality events were defined:

- Per protocol mortality, a death event occurring in patients taking at least one component of the treatment protocol.

- Intention to treat mortality, defined as a death event occurring in patients either before inclusion in the treatment protocol or during treatment.

- ICU mortality, defined as a death event occurring in patients who stayed in the ICU. The ICU outcomes also included the length of stay, mechanical ventilation, prone position, and the use of ECMO.

For safety outcomes, side effects were collected and graded according to the "Common Terminology Criteria for Adverse Events" (CTCAE) v5.0 [21].

Inflammatory markers were also monitored. A patient was considered in a severe inflammatory state when the NLR was higher than 3.1. The PLR was also assessed as a prognostic value during follow-up. The H-score assessed every 48 h the risk for any reactive hemophagocytic syndrome.

Since patients receiving quadruple therapy are at high risk for QTc prolongation, QTc and the Tisdale score were assessed at baseline and monitored regularly. Tisdale score had to be less than 11 and QTc less than 460 ms for a safe administration [22,23]. A 2 h to 3 h ECG was performed every 72 h for UIP inpatients. QTc measurements were taken every 12 h on leads II and V5 2 h to 3 h after drug infusion on ICU patients. If a patient had QTc ≥ 500 ms or an increment of 60 ms or more from baseline, QTc-prolonging drugs were definitively withdrawn.

Liver Function Tests (LFTs) and lipase were performed every 48 h. Any increase in LFTs at baseline or later during therapy prompted a reconsideration of treatment.

Statistical analysis

Categorical variables were presented as frequencies and percentages with their respective 95% confidence intervals (95% CI). Continuous variables not departing from normality assumptions were presented as mean ± Standard Deviation (SD). Most of the continuous variables were skewed and expressed as median with interquartile range (1st quartile to 3rd quartile). The Spearman correlation coefficient was used to explore the association between

Table 1: Stratification of COVID-19 patients.

Level	Clinical presentation	Radiological findings	Risk factors
A	Asymptomatic	No	No
B	Nonspecific symptoms	No	No
C	Nonspecific symptoms	No	Yes
D1	Mild symptomatic patients: CURB-65<2	Yes	No
D2	Moderate symptomatic patients: CURB-65 ≥ 2	Yes	No
E	Moderate symptomatic patients: CURB-65 ≥ 2	Yes	Yes
F	Severe symptomatic patients: a. Respiratory criteria: SpO_2< 92%, RR >24/min on room air or 6 L/min oxygen and SpO2 >94% b. Organ failure	Yes	Yes/No
G	Same as F+ intubated patient	Yes	Yes/No

continuous variables and their 95% confidence intervals (CI) derived by bootstrapping, based on 10,000 samples. The Mann-Whitney U test was used to compare continuous variables between independent groups. The Kaplan-Meier method was used to estimate median time for censored variables at database lock (discharge from hospital, discharge from ICU, and time to negative PCR).

Results

A total of 40 adult patients with confirmed COVID-19 were included in the analysis. The mean age was 53.1 ± 18.3 years, with male preponderance 60%, (Table 2a). The majority (87%) were admitted to UIP first and only 5 patients were directly referred to the ICU. Nearly one-third of patients had comorbidities, mainly hypertension. The most common symptoms upon admission are listed in Table 2a. The median duration of pre-hospital symptoms was 6 (2; 9) days, and the median time from onset of illness (i.e., before admission) to discharge was 15.5 (8 to 20) days. Most patients (70%) had an indolent pattern, while the remaining presented with a hyperacute or biphasic disease.

At admission, 33 (82.5%) patients had symptomatic pneumonia and were classified in stage D1 and above (Table 2b); high-resolution CT scan showed GGO covering a median of 19% (12%; 30%) of the lung surface. Absolute lymphopenia with a median of 975/mm^3 was observed, without leukocytosis and with moderately elevated CRP. Inflammatory markers (ferritin, cardiac troponin, D-dimers) were moderately elevated. The RT-PCR Cycle Threshold (Ct) median value was 27 (22; 31).

Four patients initially admitted at the UIP deteriorated and were transferred to the ICU. At the database lock date, 32 patients were discharged, five remained in the UIP, one in the ICU, and two patients were dead.

Different outcomes were analyzed at database lock: 32 (80%) patients were discharged from the hospital after a median length of stay of 8 days (5; 11) (Table 3). Viral PCR outcome confirmed a long period of continuous shedding, reaching a median of 17 days (14.5; 19.5). For the nine ICU patients, the median time from onset of disease to invasive mechanical ventilation was 9 (6; 10) days. The length of stay was 13 days (7.2; 18.8) with a prolonged need for mechanical ventilation, reaching a median of 8 days (5; 11). Two patients who presented with a hyperacute pattern died during their course at our institution. The first was 82 years old, diagnosed with severe COVID-19 three days after a coronary surgery, died of multiple organ failure on day 5 without treatment. The second was 78 years old and suffered from coronary artery disease, atrial fibrillation, and hypertension. He was admitted directly to ICU for severe COVID-19 pneumonia (level D2) five days after the onset of symptoms, with rapid deterioration. He received the quadruple therapy, but it was stopped on day 4due to QTc prolongation (571 ms). Multi-organ failure occurred on day 8. Cytomegalovirus viremia developed, evolving into hepatitis. He died on day 13 sec to myocarditis and superimposed infection.

The median SOFA score for ICU patients was 6 (4; 9). The main reason for admission to ICU was hypoxemic respiratory failure. Seven patients required mechanical ventilation with a median PO2/FiO2 of 162 (127; 187) and a median duration of 13 days (9; 20). The median lung compliance remained high 26 (20; 39). Prone position was applied in 3 patients with high degree of reversibility. ECMO was required for eight days in one critical patient with a refractory hypoxemia and evolved favorably.

Table 2a: Demographic, clinical and treatment characteristics of the subjects included in the study.

Variable	Statistic	Value
Age	m ± sd	53.1 ± 18.3
Female gender	n (%)	16 (40%)
BMI	Me (Q1; Q3)	27.6 (23.7; 29.8)
Medical history		
Arterial hypertension	n (%)	13 (32.5%)
Pulmonary disease	n (%)	5 (12.5%)
Cardiac disease	n (%)	6 (15%)
Cancer	n (%)	1 (2.5%)
Other	n (%)	13 (32.5%)
Symptoms		
Day of appearance	Me (Q1; Q3)	-6 (-9; -2)
Cough	n (%)	24 (60%)
Fever	n (%)	29 (72.5%)
Dyspnea	n (%)	19 (47.5%)
ENT	n (%)	8 (20%)
Gastro-intestinal	n (%)	4 (10%)
Asymptomatic	n (%)	2 (5%)
Initial unit of admission		
Pulmonary unit	n (%)	35 (87.5%)
ICU	n (%)	5 (12.5%)
PCR before hospital admission		
Day of first positive PCR result	Me (Q1; Q3)	0 (-2; 0)
Corresponding Ct value	Me (Q1; Q3)	27 (22; 31)
Contamination source		
Travel	n (%)	7 (17.5%)
Family	n (%)	19 (47.5%)
Workplace	n (%)	4 (10%)
Healthcare professional	n (%)	8 (20%)
Unknown	n (%)	5 (12.5%)
Treatment		
Pitavastatine, duration (days)	n (%) /Me (Q1-Q3)	33 (82.5%) /14 (14;14)
Hydroxychloroquine, duration (days)	n (%) /Me (Q1-Q3)	33 (82.5%) /10 (7; 10)
azithromycine, duration (days)	n (%) /Me (Q1-Q3)	29 (72.5%) /5 (5; 5)
lopinavir/ritonavir, duration (days)	n (%) /Me (Q1-Q3)	25 (62.5%) /10 (7; 14)
tocilizumab, duration (days)	n (%) /Me (Q1-Q3)	5 (12.5%) /1 (1; 1)
zinc, duration (days)	n (%) /Me (Q1-Q3)	20 (50%) /10 (7; 14)
Antibacterial therapy, duration (days)	n (%) /Me (Q1-Q3)	17 (42.5%) /10 (7; 14)
Clinical pattern		
Hyperacute	n (%)	7 (17.5%)
Indolent	n (%)	28 (70%)
Biphasic	n (%)	5 (12.5%)

n (%): Frequency and Percentage; m ± sd: Mean ± Standard Deviation; Me (Q1; Q3): Median (1st quartile - 3rd quartile); Ct: Cycle Threshold value (E-gene)

Table 2b: Baseline radiological, biological, clinical scores and staging data of the subjects included in the study.

Variable	n	Me (Q1-Q3) or (%)
Extent of pulmonary injury (CT scan)*	26	19% (12%; 30%)
White blood cells count (.10^{-6} L)	38	5050 (4300; 7100)
Polyneutrophiles count (.10^{-6} L)	38	3745 (2760; 5300)
Lymphocytes count (.10^{-6} L)	38	975 (810; 1480)
Platelets count (.10^{-6} L)	38	206500 (148000; 255000)
CRP (mg/l)	38	42.85 (7; 79.8)
Ferritin (ng/ml)	34	384.5 (125; 894)
LDH (U/l)	31	219 (192; 367)
Troponin (ng/l)	31	6 (3; 10)
D dimers (microg/l)	29	0.62 (0.32; 1.09)
CURB 65		
1	10	66.7%
2	1	6.7%
3	3	20.0%
4	1	6.7%
Clinical severity index	40	2 (1; 5)
Stratification at admission		
Stage A	1	2.5%
Stage B	5	12.5%
Stage C	1	2.5%
Stage D1	12	30.0%
Stage D2	12	30.0%
Stage E	3	7.5%
Stage F	2	5.0%
Stage G	4	10.0%

n (%): Frequency and Percentage; Me (Q1; Q3): Median (1st quartile - 3rd quartile)
(*) extent of pulmonary injury on CT is expressed as a fraction of the total pulmonary parenchyma with ground glass aspect. The fraction could be calculated for 26 patients out of 40

The treatment protocol was generally well tolerated. Few side effects of mild severity were observed, including gastrointestinal symptoms and a slight QTc increase. The vast majority of patients completed their course of treatment as scheduled. However, HCQ and azithromycin were discontinued in two patients because of a grade 3 QTc prolongation that resolved rapidly, and lopinavir/ritonavir stopped in two other patients due to increased liver enzymes (grade 3).

Baseline clinical staging correlated with baseline PCR Ct value (Spearman's Rho 0.4 p=0.02), ICU admission (p<10^{-3}), need for mechanical ventilation (p<10^{-3}), and the length of hospital stay (r 0.516, p<0.001) (Table 4).

The temporal trend of main laboratory parameters is depicted in. Median NLR was high at baseline and peaked at 5.9 on Day 5. Lymphocyte count remained low throughout hospitalization with a nadir around 700 between day 5 and day 9. CRP was moderately high and remained stable. The H-score peaked on Day 9 at 79 (63; 96). For patients receiving tocilizumab, CRP decreased abruptly, and they were followed with the sedimentation rate. For the patients whose condition worsened, the nadir of NEWS2 score occurred between Day 3 and Day 7.

Table 3: Main outcomes of the study.

Outcomes	n (%)	95% CI	Me (Q1 ; Q3)
ICU outcomes (up to DB lock)			
Admitted patients	9 (22.5%)	(11.8% to 37.1%)	
Length of stay (days)			13 (7.2; 18.8)*
Need for mechanical ventilation	7 (17.5%)	(8.2% to 31.3%)	
Duration of mechanical ventilation (days)			13 (7; 17)
Patients remaining in ICU	1 (2.5%)	(0.3% to 11.1%)	
Hospital outcomes (up to DB lock)			
Discharge from hospital	32 (80%)	(65.8% to 90.1%)	
Length of stay (days)			8 (5; 11)*
Mortality outcomes			
Per protocol mortality	1 (2.5%)	(0.3% to 11.1%)	
ITT mortality	2 (5%)	(1.1% to 15.1%)	
ICU mortality	2 (22.2%)	(4.9% to 54.4%)	
PCR outcomes (up to DB lock)			
Patients with negative PCR	30 (75%)	(60.2% to 86.4%)	
Time until negative PCR (days)			17 (14.5; 19.5)*
Safety outcomes (up to DB lock)			
Treatment withdrawal due to AE	4		
Adverse events:	**Grade 3**	**Grade 4**	
Diarrhea	-	-	
QTc increase	4	-	
SGPT	2	-	
SGOT	1	-	
γGT	1	-	
PA	-	-	

DB lock: Database lock; n (%): Frequency and Percentage; Me (Q1; Q3): Median (1st quartile to 3rd quartile). ITT: Intention to Treat. 95% CI: 95% Confidence Interval
(*) as determined from Kaplan-Meier estimator

Discussion

Relying on a pre-specified protocol that combines early clinical stratification and a multifaceted stepwise treatment strategy, the current study successfully stratified SARS-CoV-2 patients at presentation and adapted timely treatment, translating into favorable outcomes: An ITT mortality rate of 5%, comparing favorably with the Chinese experience [24] reporting an 11.7% mortality rate in similar patients; ICU outcomes with 11% remaining in ICU, 67% discharged, and 22% deceased, comparing favorably with the Italian experience reported by [25] with 58% of the patients who remained in the ICU, 16% discharged, and 26% deceased.

The patients presented in majority with an indolent pattern, and received, soon after admission, the combined therapy of HCQ, azithromycin, lopinavir/ritonavir, pitavastatin, and zinc, to hit as many targets in the SARS-CoV-2 virus RNA, while avoiding possible resistance, and taking into account potential side effects. The milder the disease, the simpler the treatment; molecules were added as the condition worsened.

The baseline stratification strategy showed to be useful in these settings: It correlated fairly with length of hospital stay, correlated

Table 4: Association of clinical staging at admission with outcomes and with clinical, radiological, and biological independent variables.

Stages	A	B	C	D1	D2	E	F	G	test	p-Value
Categorical variables										
Ward	3.2%	16.1%	3.2%	35.5%	35.5%	6.5%			MW	<0.0001
ICU				11.1%	11.1%	11.1%	22.2%	44.4%		
No mechanical ventilation	3.0%	15.2%	3.0%	36.4%	33.3%	9.1%			MW	<0.0001
Mechanical ventilation					14.3%		28.6%	57.1%		
Alive	2.6%	13.2%	2.6%	31.6%	28.9%	7.9%	2.6%	10.5%	MW	0.256
Deceased					50.00%		50.00%			
Continuous variables		**n**	**Spearmans' non-parametric correlation with 95%**							
Extent of pulmonary injury (CT scan)		26	**CI*** 0.311 (-0.152; 0.671)							0.121
Duration of ICU stay		9	0.568 (-0.058; 0.927)							0.11
Duration of MV		7	-0.110 (-0.744; 0.491)							0.799
Duration of pre-hospital symptoms		38	-0.170 (-0.524; 0.182)							0.289
Baseline PCR Ct value		33	0.401 (0.070; 0.674)							0.021
Length of hospital stay		40	0.516 (0.188; 0.767)							0.001
Time until negative PCR (days)		30	-0.050 (-0.396; 0.325)							0.791
Lymphocyte count on Day 0		38	-0.216 (-0.506; 0.118)							0.192
NLR ratio on Day 1		38	0.419 (0.024; 0.740)							0.009
PLR ratio on Day 1		38	0.038 (-0.341; 0.400)							0.826

MW: Mann-Whitney U test; n: Frequency; 95%: 95% confidence interval
(*) 95% CI calculated by bootstrapping based on 10,000 samples, using bias corrected accelerated method

with mechanical ventilation duration in ICU patients, and correlated well with baseline Ct values. Of note, most ICU patients had high Ct levels, corroborating [26].

For the PCR outcomes, the initial clinical stratification was not correlated with the duration of SARS-COV-2 RNA detection in the upper respiratory tract, a finding consistent with a recent US study importantly, the median time (17 days) to negative RT-PCR results was relatively long. In one series RT-PCR could detect low levels of the virus in the upper respiratory tract, even after symptoms had fully resolved [26]. Conversely, in Chinese series, mild cases had an early viral clearance, with 90% of the patients repeatedly testing negative on RT-PCR by day 10 post-onset [27], while severe cases had a longer virus-shedding period [4,27].

HCQ and azithromycin were used to reduce viral shedding. HCQ reduced the length of hospital stay and improved the evolution of COVID-19 pneumonia in China. HCQ 500 mg BID was recommended, irrespective of COVID-19 pneumonia severity [9]. HCQ with azithromycin was associated with a higher rate of undetectable SARS-CoV-2 RNA in nasopharyngeal specimens at Day 6 [28].

Lopinavir/ritonavir antiviral combination therapy was given early to all the patients with baseline stage D2 and sicker, in accordance with the Chinese recommendations [29]. Lopinavir/ritonavir can be recommended in relatively high-risk groups of COVID-19 pneumonia at an early stage despite its safety, future clinical trials should verify its efficacy.

Tocizulimab was used in 5 of the ventilated patients, with a favorable outcome. Severe cases with sustained lymphopenia and increased inflammatory cytokines can benefit from early tocilizumab after excluding any concurrent bacterial infection [14].

Contrasting with several studies reporting ICU mortality rates reaching 60% [5,6], the current ICU 22% fatality rate is strikingly lower, presumably attributable to the standardized hierarchical strategy being applied early, improving the outcome of valid non-pharmacological treatment bundles (including mechanical ventilation, prone position and ECMO), the mainstay of ICU supportive care. ARDS pattern was heterogeneous (2 L-type patients, 3H-type patients) and ventilation strategy was initially adapted to the ARDS Net trial to H-type patients. However, in the L-type category, subsequent hypoventilation led to a more liberal tidal volume (7 to 8 mL/kg) with a preserved driving pressure between 13 and 15 cmH2O, suggesting a relatively preserved lung compliance. It is noteworthy that deceased patients classified as having critical disease had persistent and low Ct values, consistent with the findings of Liu et al. [26,27,30].

Limitations

All efforts were put to minimize the risk of bias, including a prospective study design with a unified treatment protocol, exhaustive data collection, and use of standardized definitions for exposure, treatment, and outcomes. Right censored observations were kept to a minimum. Given the ascending nature of the treatment protocol, with most patients getting at least one treatment modality, and given the relatively modest sample size, no meaningful post-hoc analyses were performed to differentially assess particular treatment modalities. Another limitation is that SARS-CoV-2 RNA detection does not necessarily reflect the presence of infectious virus and RT-PCR Ct values may have varied due to specimen collection or handling.

Nonetheless, the study has two merits: First, it was thought and implemented as one unified ascending protocol with no pre-specified intention to compare in-protocol modalities, which can be best achieved with suitable randomized trials. Second, it relied on a pre-specified staging system that showed to be valuable during subsequent hospitalization.

Conclusion

Early clinical stratification and a multifaceted stepwise treatment strategy are cornerstone to improve patient outcomes. Our study demonstrated that stratifying patients at presentation and putting them early on adapted treatment, with a predefined stepwise medical protocol, was associated with a favorable outcome.

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