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OPINION REVIEW

Heart failure in COVID-19 patients: Critical care experience

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Abstract

Patients with heart failure (HF) may be at a higher risk of coronavirus disease 2019 (COVID-19) infection and may have a worse outcome due to their comorbid conditions and advanced age. In this narrative review, we aim to study the interaction between COVID-19 and HF from a critical care perspective. We performed a systematic search for studies that reported HF and critical care-related outcomes in COVID-19 patients in the PubMed and Medline databases. From a total of 1050 papers, we identified 26 that satisfied the eligibility criteria for our review. Data such as patient demographics, HF, intensive care unit (ICU) admission, management, and outcome were extracted from these studies and analyzed. We reported outcomes in heart-transplant patients with COVID-19 separately. In hospitalized patients with COVID-19, the prevalence of HF varied between 4% and 21%. The requirement for ICU admission was between 8% and 33%. HF patients with COVID-19 had an overall mortality rate between 20% and 40%. We identified that HF is an independent predictor of mortality in hospitalized COVID-19 patients, and patients with HF were more likely to require ventilation, ICU admission and develop complications. Patients with HF with reduced ejection fraction did worse than those with HF with midrange ejection fraction, and HF with preserved ejection fraction. COVID-19 patients with HF should be identified early and managed aggressively in an attempt to improve outcomes in this cohort of patients.

Key Words: Heart failure; COVID-19; Critical care; Intensive care; Mortality

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Core Tip: Heart failure (HF) can lead to worse outcomes in coronavirus disease 2019 (COVID-19). Moreover, critically ill patients with COVID-19 can develop *de novo* HF. Patients with COVID-19 and HF are more likely to require ventilation, ICU admission and develop complications. HF is an independent predictor of mortality in hospitalized COVID-19 patients and therefore, HF should be identified early and managed aggressively in an attempt to improve outcomes in critically ill patients.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) emerged from China in December of 2019 and continues to be a public health emergency of international concern. As of March 12, 2021, more than 118 million cases have been diagnosed worldwide with 29.3 million patients reported from the United States. Patients with pre-existing comorbidities, cardiac dysfunction and immunocompromised status continue to be at high risk of morbidity and mortality. The literature on COVID-19 infection in patients with heart failure (HF) is limited. Patients with HF may be at higher risk of COVID-19 infection and have a worse outcome due to their comorbid conditions and advanced age.

The currently available literature regarding the role of critical care and a multidisciplinary approach in treating patients with HF and COVID-19 infection, remains scarce. In this narrative review, we aim to study the interaction between COVID-19 and HF from a critical care perspective. We also aim to explore the various outcomes as reported in the literature in this subgroup of patients and to provide a summary of the current evidence and practices in the management of HF in COVID-19 patients in the intensive care unit (ICU).

In this review, we have attempted to summarize all the articles published on the presentation and management of patients with COVID-19 and HF. We searched the PubMed and Medline database for the MeSH terms "COVID-19", "heart failure" and" critical care". Studies published in English, including adults with HF and COVID-19 infection were eligible to be included in this review (Figure 1). All studies published before March 2021 were included. Studies that provided details on patient demographics, HF, ICU admission, management, and outcome were analyzed. Various treatment details including medications such as beta blockers, angiotensin converting enzyme inhibitors (ACEi), aldosterone receptor blockers (ARB), angiotensin receptor-neprilysin inhibitors (ARNi), automatic implantable cardioverter-defibrillator, permanent pace-maker and cardiac resynchronization therapy were included. Critical care details that were obtained were the type of organ dysfunction, the requirement of non-invasive and invasive ventilation, administration of vasopressor support, extra-corporeal membrane oxygenation (ECMO) and outcome. Articles that did not have the patient's details, opinions, comments, letters, and articles not published in English were excluded from the analysis. Studies that included cardiac transplant patients were analyzed separately. Two independent clinicians reviewed all articles.

As of March 2021, a total of 1050 papers were identified (Figure 1). Among these, 26 satisfied the eligibility criteria for our study. One study was a prospective cohort study, while all others were retrospective studies. Studies were principally published from North American and European nations. There were significant differences in the study design, data collection and measured outcomes among the studies which made the comparison of data difficult. Therefore, we divided the studies into four categories and reported the outcomes separately. The four categories were: (1) Studies highlighting prevalence of HF, requirement of ICU level of care and outcomes in hospitalized COVID-19 patients; (2) Studies reporting outcomes in COVID-19 patients admitted to ICU; (3) Studies reporting outcomes in HF patients with COVID-19; and (4) Studies reporting outcomes in heart-transplant patients with COVID-19 (Figure 2).

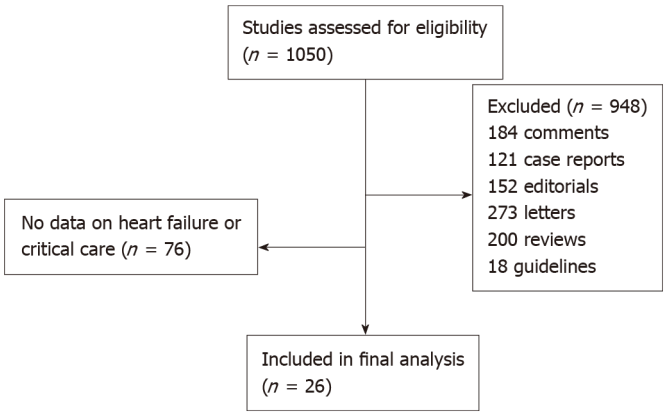

Figure 1 CONSORT diagram.

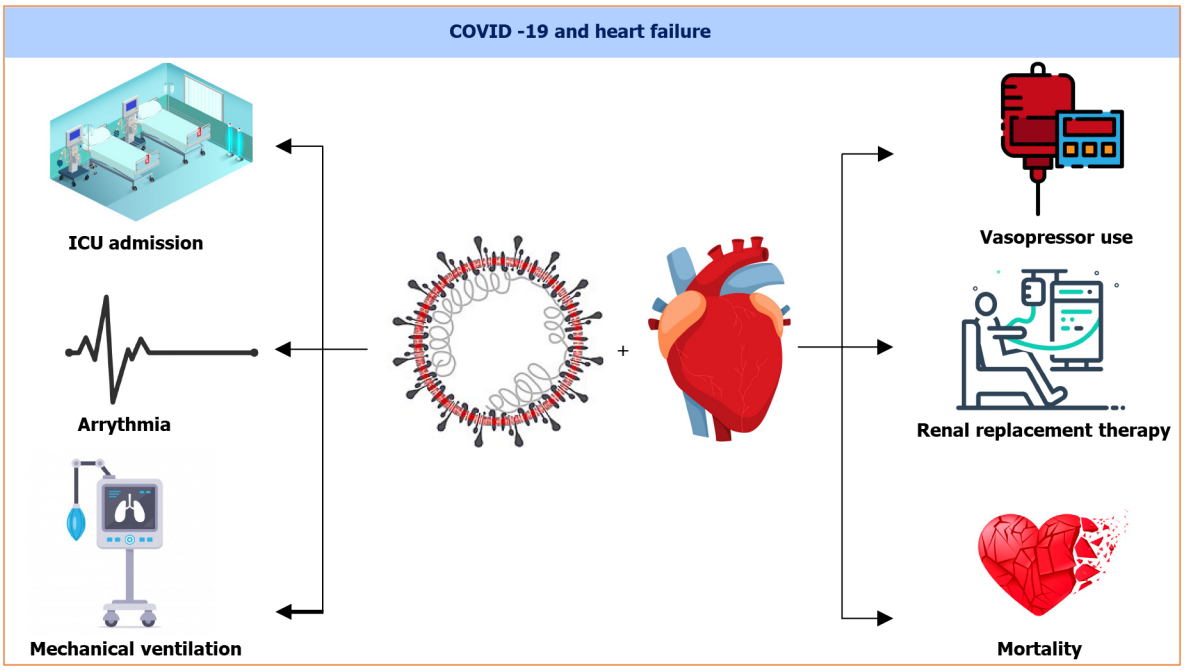

Figure 2 Coronavirus disease 2019 and heart failure.

INCREASED RISK OF HEART FAILURE IN COVID-19 PATIENTS

Among studies that reported outcomes in hospitalized patients with COVID-19, we found 11 studies that provided data on HF and ICU admission (Table 1)[1-11]. The total number of patients in this group was 9420, with studies from the United States contributing the maximum number of patents. The diagnosis of COVID-19 was uniformly established with reverse transcription polymerase chain reaction (RT-PCR) in all of the patients. The proportion of patients with pre-existing HF varied from 1% to 21%, which was almost ten times the community prevalence of HF, as reported by the Framingham study[12]. This suggests that patients with HF are more likely to require hospitalization for COVID-19. Zylla *et al*[3] reported that 3% of patients developed newly diagnosed (*de novo)* left ventricular (LV) dysfunction after admission, while Zhou *et al*[9] and Chen *et al*[10] reported a number close to 25%. This highlights the fact that HF can be both a risk factor, as well as a complication of COVID-19. Most studies reported a male preponderance with a mean age above 60 years. The requirement for ICU admission was between 8% and 33%. Patients with HF were more likely to require mechanical ventilation and develop complications such as thromboembolism, sepsis, stroke and acute kidney injury. The overall mortality rate for hospitalized patients with COVID-19 was between 4% and 40%. Inciardi *et al*[1] noted that chronic therapy with an ACEi, ARB, or ARNI had to be discontinued in 77% of cases

Table 1 Studies highlighting prevalence of heart failure, requirement of intensive care unit level of care and outcomes in hospitalized coronavirus disease 2019 patients, *n* (%)

No.	1	2	3	4	5	6	7	8	9	10	11
Ref.	Inciardi *et al*[1]	Singer *et al*[2]	Zylla *et al*[3]	Russo *et al*[4]	Bhatla *et al*[5]	Peltzer *et al*[6]	Lala *et al*[7]	Shi *et al*[8]	Zhou *et al*[9]	Chen *et al*[10]	Jarrett *et al*[11]
Country	Italy	United States	Germany	Italy	United States	United States	United States	China	China	China	United States
Total number of patients	99	737	166	414	700	1053	2736	416	191	274	2634 (all patients died)
Patients with chronic heart failure	21 (21)	39 (5)	-	46 (11.1)	88 (13)	79 (7.5)	276 (10.1)	17 (4.1)	-	1 (< 1)	291 (11.1)
Newly diagnosed LV dysfunction	-	-	5 (3)	-	-	-	-	-	44 (23)	43/176 (24)	-
Study type	RC, single centre	RC, single centre	RC,multicenter	RC, multicenter	RC, single centre	RC, multicenter	RC, multicenter	RC, single centre	RC, multicenter	RC, single center	RC of in-patients who died of COVID-19 in a single center
Age (mean ± SD)	67 ± 12	60 ± 18	64.1 ± 16.7	66.9 ± 15.0	50 ± 18	62 ± 17	66.4 (median)	64 (range: 21-95)	56 (IQR: 46-67)	62 (IQR: 44-70)	Range: 21-107
Male	8 (81)	423 (57)	108 (65.1)	253 (61.1)	315 (45)	653 (62)	1630 (59.5)	205 (49.3)	119 (62)	171 (62)	1664 (63.2)
LVEF, % (mean ± SD)	48 ± 14	-	53.0 ± 12.3	-	-	HFrEF: 41 (3.8)	-	-	-	-	-
ICU admission	12 (12)	59 (8)	65 (39.2)	-	79 (11.28)	349 (33.14)	-	-	50 (26)	-	1299 (49.3)
NIV	18 (19.1)	40 (5)	39 (23.5)	-	-	-	-	32 (7.7)	26 (14)	102 (37)	-
IV	2 (2)	149 (20.2)	37 (22.3)	-	-	327 (31.05)	307 (11.2)	51 (12.3)	32 (17)	17 (6)	140 (53.2)
ECMO/ICD/CRT/PPM	-	-	PPM:3 (1.8), ICD:2 (1.2), ECMO:3 (1.8)	-	-	-	-	-	ECMO: 3 (2)	ECMO: 1 (<m1)	-
Vasopressor	-	-	30 (18.1)	-	-	323 (30.67)	-	-	-	-	-
Hospital LOS, d, (mean ± SD)	11.4 ± 6.5	4.7 ± 3.0	10.5 (IQR 5-22 d)[ICU stay: 8 (IQR 4-22.5)]	-	-	-	5.75 (IQR :3.36-9.56)	-	11 (7–14) [ICU stay: 8 (4–12)]	-	-
Complications	Venous thrombo-embolism: 12 (12),	-	-	-	-	Bacteremia:100 (9.5), VTE: 54 (5.13),	Hospitalized at time ofstudy	CRRT: 2 (0.5), ARDS: 97 (23.3), Coagulation	RRT: 10 (5), sepsis:112 (59), respiratory failure: 103 (54),	AKI: 29 (11), CRRT: 3 (1),	-

	Arterial thrombo-embolism: 3 (3), septic shock/sepsis: 6 (6)					stroke/TIA:18 (1.71), AKI requiring RRT: 34 (3.23)	publication: 1098 (40.1)	disorders: 12 (2.9), hospitalized at end of study period:319 (76.7)	ARDS: 59 (31), septic shock: 38 (20), coagulopathy: 37 (19), secondary infection:28 (15)	sepsis: 179 (65), DIC: 21 (8), shock: 46 (17), ALI: 13 (5)	
Mortality	26 (26)	68 (9)	26 (15.7)	107 (25.8)	30 (4)	184 (17.47)	506 (18.5)	57 (13.7)	54 (28.2)	113 (40)	2634 (100)

- Signifies that the variable was not reported in the study.

LV: Left ventricle; RC: Retrospective cohort; LVEF: Left ventricle ejection fraction; HFrEF: Heart failure with reduced ejection fraction; ICU: Intensive care unit; NIV: Non-invasive ventilation; IV: Invasive ventilation; ECMO: Extra-corporeal membrane oxygenation; ICD: Implantable cardiovascular-defibrillator; CRT: Cardiac resynchronisation therapy; PPM: Permanent pacemaker; LOS: Length of stay; IQR: Inter-quartile range; VTE: Venous thrombo-embolism; TIA: Transient ischemic attack; AKI: Acute kidney injury; CRRT: Continuous renal replacement therapy; RRT: Renal replacement therapy; ARDS: Acute respiratory distress syndrome; ALI: Acute liver injury; DIC: Disseminated intravascular coagulation.

because of severe hypotension, and patients who died were more likely to have a history of HF. In the same study, the mortality rate remained higher in patients with cardiac disease compared to those without (26% *vs* 9%; $P = 0.039$), even after excluding patients who were denied intubation due to comorbidities or age. Another study used multivariate regression modelling to identify an increased risk of atrial fibrillation among COVID-19 patients with HF (RR 1.88; $P = 0.023$), which in-turn increased the odds of ICU or intermediate care ward admission (OR 2.37; 95%CI: 1.10-5.09; $P = 0.03$) [4,5]. HF was also linked to brady-arrhythmias (OR 9.75; 95%CI: 1.95-48.65) by a separate group of investigators[5]. Three separate meta-analysis identified that HF was independently associated with an increased risk of mortality in patients with COVID-19[13-15].

NEED FOR INTENSIVE CARE UNIT LEVEL OF CARE

We found six studies that reported HF data in COVID-19 patients who were admitted to the ICU, while excluding patients who were hospitalized without requiring ICU care (Table 2)[5,16-20]. These studies had a total patient number of 6539, with a major patient population contributed from the United States. The mean age in this group of patients was above 60 years and there were more men than women. Between 10% and 43% of these patients had pre-existing HF. This range was higher than what was observed among hospitalized patients overall. It was also noted that more patients developed *de-novo* HF in this group (up to 33%)[16]. The average ICU length of stay was between 2 to 5 weeks and a majority of patients required ventilatory assistance. In addition, advanced life-sustaining supportive interventions such as ECMO were also utilized by 3% to 15% of these patients. As a group, these patients had a higher mortality rate, which was as high as 52%. A cross-sectional observational multi-centre nationwide survey in Italy identified that obesity, chronic kidney disease and

Table 2 Studies reporting outcomes in coronavirus disease 2019 patients admitted to intensive care unit, *n* (%)

No.	1	2	3	4	5	6
Ref.	Zeng *et al*[16]	Petrilli *et al* [17]	Bhatla *et al* [5]	Hayek *et al*[18]	Iaccarino *et al* [19]	Arentz *et al*[20]
Country	China	United States	United States	United States	Italy	United States
Total number of patients in ICU	35	990	79	5019	395	21
Patients with chronic heart failure	NR	189 (19.1)	22 (28)	512 (10.20)	60 (15.2)	9 (42.9)
Newly diagnosed LV dysfunction/acute heart failure	5 (14)	-	-	166 (3.3)	-	7 (33.3)
Study type	Retrospective cohort, single centre	Prospective cohort, single centre	Retrospective cohort, single centre	Retrospective cohort, multicenter	Cross-sectional study, multicenter	Retrospective cohort, single centre
Age, (mean ± SD)	64.00 (59.50–68.00)	68 (58-78)	63 ± 16	60 ± 15, 63 ± 14[1]	68.9±0.7	70 (43-92) range
Male	23 (66)	656 (66.3)	40 (51)	3165 (63.06)	291 (73.7)	11 (52)
Risk factors	Hypertension: 13 (37), coronary artery disease: 2 (6), arrhythmia: 2 (6), valvular disease:1 (3), diabetes: 10 (29), COPD: 1 (3)	Diabetes: 389 (39.3), asthma or COPD: 169 (17.1), chronic kidney disease: 259 (26.2), cancer: 138 (13.9)	Coronary heart disease: 21 (27), diabetes mellitus :35 (44), hypertension: 62 (78), atrial fibrillation history: 5 (6), obstructive sleep apnea: 23 (29), COPD: 14 (18), liver disease: 14 (18), chronic kidney disease: 16 (20), current tobacco: 4 (5)	Current or former tobacco use: 2174 (43.31), diabetes mellitus: 2110 (42.04), hypertension:3086 (61.48), coronary artery disease:676 (13.46), chronic obstructive pulmonary disease: 43 (0.85), chronic or end stage kidney disease: 819 (16.31), active malignancy:227 (4.52)	Hypertension: 256 (65.3), obesity: 49 (12.4), diabetes: 90 (22.8), COPD: 41 (10.4), CKD: 34 (8.6), coronary artery disease: 62 (15.7)	Asthma: 2 (9.1), chronic obstructive pulmonary disease: 7 (33.3), diabetes: 7 (33.3), obstructive sleep apnea: 6 (28.6), chronic kidney disease: 10 (47.6), end-stage kidney disease: 2 (9.5), history of solid organ transplant: 2 (9.5), cirrhosis: 1 (4.8), immunosuppression: 3 (14.3)
HFrEF,	5 (14)	-	-	-	-	-
HFpEF	0 (0)	-	-	-	-	-
Drugs	-	-	-	-	ACE-inhibitors: 97 (24.6), ARB: 66 (16.7), beta-blockers: 96 (24.3), ca-antagonists: 31 (7.8), diuretics: 58 (14.7), alpha-blockers: 7 (1.8)	-
ICD	-	-	5 (6)	-	-	-
Ventilation	35 (100)	647 (65.35)	-	-	-	19 (90.5)
NIV	17 (49)	-	-	-	-	4 (19)
IV	18 (51)	647 (65.35)	-	3663 (72.98)	-	15 (71)
ECMO	5 (15)	-	-	176 (3.51)	-	-
Vasopressor	NR	-	-	1617 (32.22)	-	14 (67)
ICU stay duration in days	38 (33–47)	36 (32-40)	-	17 (9-30), 6 (4-10)[1]	-	-
Organ dysfunction	NR	-	-	Acute kidney injury requiring RRT: 1003	-	AKI: 4 (19.1), ALI: 3 (14.3)
Morbidity	acute cardiac injury: 21 (60), atrial or ventricular tachyarrhythmia:3 (9)	86 (8.68) patients being ventilated and 74 (7.47) patients still	-	Still in hospital 30 days after ICU admission: 169	-	Admitted in ICU at end of study: 8 (38.1)

		admitted at the end of study period				
Mortality	3 (9)	485 (49)	-	2043 (40.71)	-	11 (52.4)

[1]Baseline characteristics of patients who did not have cardiac arrest and those who had cardiac arrest respectively.
- Signifies that the variable was not reported in the study.
ICU: Intensive care unit; LV: Left ventricle; COPD: Chronic obstructive pulmonary disease; CKD: Chronic kidney disease; HFrEF: Heart failure with reduced ejection fraction; HFpEF: Heart failure with preserved ejection fraction; ACE: Angiotensin converting enzyme; ARB: Angiotensin receptor blocker; ICD: Implantable cardiovascular-defibrillator; NIV: Non-invasive ventilation; IV: Invasive ventilation; ECMO: Extra-corporeal membrane oxygenation; ECMO: Extra-corporeal membrane oxygenation; RRT: Renal replacement therapy; AKI: Acute kidney injury; ALI: Acute liver injury.

hypertension in men, and obesity (OR 2.564; 95%CI: 1.336-4.920; $P < 0.0001$) and HF (OR 1.775; 95%CI: 1.030-3.057) in women were associated with higher rate of ICU admission[19]. Similar observations were made from a single academic medical centre in New York City and Long Island which found that the strongest risk factors for critical illness besides age were HF (OR 1.9; 95%CI: 1.4-2.5), BMI > 40 (OR 1.5; 95%CI: 1.0-2.2), and male sex (OR 1.5; 95%CI: 1.3-1.8)[17].

OUTCOMES IN CRITICALLY ILL COVID-19 PATIENTS WITH PREEXISTING HEART FAILURE

There were five studies (from North America and Europe) that focused on the outcomes of COVID-19 infection in patients with pre-existing HF (Table 3)[21-25]. Three of them were from the United States, one was from Italy and the last one was from Denmark. Together, these studies included 9191 patients. Maximum number of patients were contributed by the study by Bhatt *et al*[21]. The mean age of patients in these studies were above 70 years, which was about 10 years higher than what was observed in the two previous groups. Two out of the five studies had more women than men. This was in contrast to the uniform male predominance observed in the two previous groups. ICU admission rates were reported by two studies and ranged between 23% and 29%. ECMO was used by three patients in one study[21]. An overall mortality rate between 20% to 40% was observed. Overall mortality variation in multinational studies have ranged from close to 30 % to over 90 %. There was also a significant inter-hospital variability in the outcome of critically ill patients which could not be attributed to the location or performance of the treating facility[26]. Tomasoni *et al*[24] reported more in-hospital complications such as acute HF (33.3% *vs* 5.1%, $P < 0.001$), acute renal failure (28.1% *vs* 12.9%, $P < 0.001$), multiorgan failure (15.9% *vs* 5.8%, $P = 0.004$) and sepsis (18.4% *vs* 8.9%, $P = 0.006$) in COVID-19 patients with a prior history of HF. This suggests that patients with HF and COVID-19 have a poorer outcome than the general population. When compared to hospital admissions for other causes, HF patients admitted for COVID-19 were older, more likely to identify as Black and/or Hispanic, had higher rates of diabetes and kidney disease and used more healthcare resources such as ICU beds (29% *vs* 15%), mechanical ventilation (17% *vs* 6%), and central venous catheter insertion (19% *vs* 7%; $P < 0.001$ for all)[21]. They also had higher in-hospital mortality (24.2% *vs* 2.6%) as well as higher skilled-nursing and rehabilitative care requirement among survivors (13% *vs* 41%)[21]. Similar conclusions were drawn by Alvarez-Garcia *et al*[22], who noted that the history of HF was an independent risk factor for the need for ICU care (adjusted OR 1.71; 95%CI: 1.25-2.34; $P = 0.001$), intubation and mechanical ventilation (adjusted OR 3.64; 95%CI: 2.56 -5.16; $P < 0.001$), and in-hospital mortality (adjusted OR 1.88; 95%CI: 1.27-2.78; $P = 0.002$). Furthermore, the former was the only study to look at outcomes stratified by left ventricular ejection fraction (LVEF) and found that cardiogenic shock (7.8% *vs* 2.3% *vs* 2%; $P = 0.019$) and HF-related causes for 30-day readmission (47.1% *vs* 0% *vs* 8.6%) were significantly higher in patients with HF with reduced ejection fraction (HFrEF) than in those with HF with midrange ejection fraction (HFmrEF) or HF with preserved ejection fraction (HFpEF)[22]. Multivariate cox regression identified older age, more severe HF [baseline New York Heart Association (NYHA) functional classes III and IV], previous mitral regurgitation, lower systolic blood pressure, lower oxygen saturation, lower lymphocyte count, and increased troponin concentrations as risk factors for in-hospital mortality in COVID-19 patients with HF[22].

Table 3 Studies reporting outcomes in heart failure patients with coronavirus disease 2019, *n* (%)

No.	1	2	3	4	5
Ref.	Bhatt *et all*[21]	Alvarez-Garcia *et al*[22]	Caraballo *et al*[23]	Tomasoni *et al*[24]	Andersson *et al*[25]
Country	United States	United States	United States	Italy	Denmark
Patient number	8383	422	206	90	90
Study type	Retrospective cohort, multicentre	Retrospective cohort, multicentre	Retrospective cohort, multicentre	Retrospective cohort, multicentre	Retrospective cohort. multicentre
Age, (mean±SD)	71.7 ± 13.2	72.5 ± 13.3	78 (IQR: 65-87)	73.0 ± 11.4	
Male	4178 (49.8)	236 (55.9)	93 (45.1)	66 (73.3)	
Risk factors	Obesity: 2461 (29.4), morbid obesity: 1425 (17.0), hypertension: 6997 (83.5), diabetes: 5107 (60.9), history of arrhythmia: 4548 (54.3), valvular disease: 1417 (16.9), kidney disease: 5020 (59.9), ESKD: 1689 (20.1), smoking: 3665 (43.7), pulmonary disease: 3539 (42.2), asthma: 628 (7.5), anemia: 628 (7.5), malignancy: 290 (3.5)	Obesity: 169 (40.0), hypertension: 382 (90.5), diabetes mellitus: 269 (63.7), dyslipidemia: 228 (54.0), CAD: 235 (55.7), stroke: 114 (27.0), atrial fibrillation: 160 (37.9), CKD: 177 (41.9), COPD: 94 (22.3), asthma: 58 (13.7), OSA: 57 (13.5)	Hypertension: 164 (79.6)COPD: 67 (32.5)CAD: 73 (35.4)Renal disease:7 9 (38.3)	Smoker: 42 (55.3), hypertension: 68 (75.6), dyslipidaemia: 56 (62.2), diabetes: 37 (41.1), atrial fibrillation: 42 (46.7), coronary artery disease: 55 (61.1), COPD: 22 (24.4), CKD: 49 (54.4)	
LVEF (%), (mean ± SD)	-	-	-	42.1 ± 13.1	-
HFrEF	3318 (39.6)	128 (30.3)	36 (17.5)	64 (71)	-
HFmrEF	-	44 (10.4)	-	-	-
HFpEF	3486 (41.6)	250 (59.3)	-	26 (29)	-
RV dysfunction	-	-	-	16 (28.6)	-
Drugs prior to hospitalization	-	RAAS inhibitors: 260 (61.6), beta-blockers: 354 (83.9), MRA: 60 (14.2), loop diuretics: 318 (75.4), thiazides: 64 (15.2), antiplatelet: 327 (77.5), anticoagulant: 175 (41.5), statins: 351 (83.2)	ACEi/ARB: 58 (28.2), beta-blocker: 94 (45.6), CCB: 69 (33.5), SGLT2i: 1 (0.5), warfarin: 16 (7.8), NOAC: 47 (22.8), diuretic: 99 (48.1), statin: 117 (56.8)	ACEi/ARBs/ARNI: 42 (50.0), MRAs: 23 (34.8), beta-blockers: 69 (81.2), direct oral anticoagulants: 17 (20.5), warfarin: 18 (21.6), statins: 47 (56.0)	-
ICD/CRT	-	-	-	ICD: 20 (22.2), CRT: 8 (8.9) (both prior to hospitalization)	-
ICU	2431 (29)	98 (23.2)	-	-	-
Ventilation	-	96 (22.8)	-	-	-
NIV	-	-	-	28 (31.1)	-
IV	-	96 (22.8)	-	5 (5.6)	-
ECMO	3 (0.04)	-	-	-	-
ICU stay duration	-	5 (2-11)	-	-	-
Mortality	2026 (24.2)	169 (40.0)	41 (20)	37 (41.1)	33 (27)

- Signifies that the variable was not reported in the study.

ESKD: End stage kidney disease; CAD: Coronary artery disease; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; OSA: Obstructive sleep apnea; LVEF: Left ventricle ejection fraction; HFrEF: Heart failure with reduced ejection fraction; HFmrEF: Heart failure with mid-range ejection fraction; HFpEF: Heart failure with preserved ejection fraction; RV: Right ventricle; RAAS: Renin angiotensin aldosterone system; MRA: Mineralocorticoid receptor antagonist; ACEi: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; CCB: Calcium channel blocker; SGLT: Sodium-glucose linked transporter; NOAC: Novel oral anticoagulants; ARNI: Angiotensin receptor II blocker – neprilysin inhibitor; ICD: Implantable cardiovascular-defibrillator; CRT: Cardiac resynchronisation therapy; ICU: Intensive care unit; NIV: Non-invasive ventilation; IV: Invasive ventilation; ECMO: Extra-corporeal membrane oxygenation.

DATA ON CARDIAC TRANSPLANT PATIENTS WITH COVID-19

We analyzed studies that included heart transplant patients separately because they are a distinct subset of patients who are likely to be on immunosuppressive therapy. We found five retrospective studies with a total of 99 patients (Table 4)[27-31]. This was a group of predominantly male patients with a mean age above 55 years and a wide variation in requirements for ICU level of care; ranging from 10.5% to 100%. The patients who were admitted to ICU were sicker as evidenced by increased requirement of vasopressors, mechanical ventilation, and renal replacement therapy (83% *vs* 38%) [28]. The mortality rate was between 18% and 37%. In one study, all patients who required ICU admission died[29]. This was higher than the mortality rate in the general population, but not much more than non-transplanted HF patients or patients in ICU. Bottio *et al*[31] reported that older age ($P = 0.002$), diabetes mellitus ($P = 0.040$), extracardiac arteriopathy ($P = 0.040$), previous percutaneous coronary intervention ($P = 0.040$), cardiac allograft vasculopathy score ($P = 0.039$), lower glomerular filtration rate ($P = 0.004$), and higher NYHA functional classes ($P = 0.023$) were all significantly associated with in-hospital mortality among heart-transplant patients with COVID-19 [31]. We know that steroids are beneficial in severe COVID-19, and cardiac transplant patients are often on multiple immunosuppressive medications that include steroids, calcineurin inhibitors and anti-metabolites[32]. Whether these immunosuppressive medications protected these patients from severe disease is a question that warrants further investigation.

In this review, we summarized the relationship between HF, COVID-19 and the role of intensive care in patients with COVID-19 and HF. Our review of literature revealed many interesting observations. The evidence suggests that patients with HF are more likely to be hospitalized after COVID-19 infection. Exact quantification of risk will require community-level studies and cannot be derived from the hospital-based studies included in this review. Also, patients with COVID-19 are at increased risk of developing *de novo* HF after admission to the hospital; a risk that increases substantially with admission to the ICU. The reversibility and long-term morbidity of COVID-19 related *de* novo HF is unclear at this point and will require future studies with longer durations of follow up. Patients with COVID-19 and HF had increased chance of requiring ICU admission, mechanical ventilation, vasopressors and renal replacement therapy. They also had more complications and a higher mortality rate when compared to non-HF patients. These differences may be due to the effect of additional organ injury and decreased physiologic reserve leading to faster decompensation. This may also be an indirect marker for variation in practices. While it is expected that patients admitted to the ICU are at increased risk for development of complications and mortality, what is interesting is that the presence of HF represents an additional independent risk factor for the same. Furthermore, due to the similarity in clinical presentation, HF in COVID-19 patients is probably underdiagnosed. Therefore, it stands to reason that the risk estimate from the studies reported thus far are lower than the true risk estimate. It was noteworthy that the outcomes in critically ill heart transplant patients with COVID-19 was not very different from critically ill non-heart transplant patients. Whether the immunosuppressive medications that heart transplant patients are on, provides them with a selective advantage in combating the 'cytokine storm' seen in COVID-19 is a question worth asking. What is clear from our analysis of the existing literature is that HF is inextricably linked with the outcomes of COVID-19 infection. What is not known is the exact mechanisms by which they are linked and therefore, this is a field with immense scope for future research. There are many reasons to study HF in COVID-19 patients. Heart-lung interactions dictate that insult to one organ, affects the other. Acute respiratory distress syndrome (ARDS) is the most common manifestation of severe COVID-19 disease. While hypoxia and positive pressure ventilation stresses the right heart, the left heart has to compensate for increased metabolic demand. These problems are compounded in patients with pre-existing HF. Therefore, it is vital that we investigate the interaction between HF and COVID-19 so that we have a better understanding of its pathophysiology, optimal management and outcome.

PATHOGENESIS OF HF IN COVID-19

Cardiac troponins were elevated in 8%-12% of COVID-19 cases and the percentage rose up to 23%-33% in critically ill patients[1,33,34]. COVID-19 is theorized to injure the myocardium indirectly and directly. The systemic inflammatory response and

Table 4 Studies reporting outcomes in heart-transplant patients with coronavirus disease 2019, *n* (%)

No.	1	2	3	4	5
Ref.	Latif *et al*[27]	Ketcham *et al*[28]	Singhvi *et al*[29]	Lima *et al*[30]	Bottio *et al*[31]
Country	United States	United States	United States	United States	Italy
Patient number	28	6	22	5	38
Study type	Retrospective observational	Retrospective observational	Retrospective observational	Retrospective observational	Retrospective observational
Age, (mean ± SD)	64 (53.5-70.5)	57 (34–73)[1]	58.6 (49.1–71.2)[2]	62 ± 9.8	64.9 ± 12.0
Male	22 (79)	6 (100)	14 (63.6)	4 (80)	31 (82)
Risk factors	Hypertension:20 (71), diabetes:17 (61), lung disease: 10 (36), malignancy: 5 (18), chronic kidney disease: 10 (36)	Chronic heart failure: 4 (67), chronic kidney disease: 4 (67), Chronic anemia: 3 (50), coronary artery disease: 4 (67), former tobacco smoker: 1 (17), diabetes mellitus: 4 (67), hypertension: 6 (100), obesity: 3 (50), obstructive sleep apnea: 3 (50)	Hypertension: 21 (95.5), diabetes: 12 (54.5), lung disease: 3 (13.6), chronic kidney disease stage ≥ III: 14 (63.6), end stage renal disease on dialysis: 3 (13.6), malignancy (excluding non-melanoma skin cancers): 6 (27.3), HIV: 1 (4.5), current smoker: 1 (4.5), former smoker: 7 (31.8), permanent pacemaker: 3 (13.6), charlson comorbidity index ≥ 5: 12 (54.5)	Ischemic cardiomyopathy (pre□ HTx): 2 (40), hypertension: 5 (100), hyperlipidemia: 3 (60), diabetes mellitus: 1 (20), obesity: 2 (40), post□ transplant renal insufficiency: 2 (40)	Obesity: 7 (18), arterial hypertension: 25 (66), dyslipidemia: 18 (47), diabetes mellitus: 7 (18), former smoker: 8 (21), peripheral vascular disease: 8 (21), COPD: 3 (8), stroke: 1 (2), malignancy: 3 (8), previous PCI: 11 (29)
NYHA class	-	-	-	-	I:27 (71), II:8 (21), III:3 (8), IV:0 (0)
ICU	7 (25)	6 (100)	4 (18.18)	2 (40)	4 (10.5)
Ventilation	7 (25)	5 (83)	7 (31.81)	2 (40)	17 (44)
NIV	-	0	3 (13.63)	0	15 (39.4)
IV	7 (25)	5 (83)	4 (18.18)	2 (40)	2 (5.2)
ECMO	-	0	-	0	0 (0)
Vasopressor	-	5 (83)	3 (13.63)	-	3 (7.9%)
ICU stay duration in days	-	8.25 (4-12.5)	7 (4-9)	-	-
Organ dysfunction	HD: 3 (10.71)	AKI requiring CRRT: 5 (83)	RRT: 3 (13.63)	AKI requiring HD: 1 (20)	-
Morbidity	4 (18) patients remained hospitalized at the end of study period	2 (33) patients still admitted at the end of the study period	-	One patient developed mild acute cellular rejection	Bacterial coinfection:5 (13), sepsis: 4 (10.5), neurological complication: 1 (2.6), gastrointestinal complication: 1 (2.6)
Mortality	7 (25)	2 (33)	4 (18.18)	0 (0)	14 (36.8)

[1]Range.
[2]IQR.
- signifies that the variable was not reported in the study. All patients had COVID-19 confirmed by RT-PCR.
HIV: Human immunodeficiency virus; Htx: Heart transplant; PCI: Percutaneous coronary intervention; COPD: Chronic obstructive pulmonary disease; NYHA: New York Heart Association; ICU: Intensive care unit; NIV: Non-invasive ventilation; IV: Invasive ventilation; ECMO: Extra-corporeal membrane oxygenation; HD: Hemodialysis; AKI: Acute kidney injury; CRRT: Continuous renal replacement therapy; RRT: Renal replacement therapy; AKI: Acute kidney injury.

cytokine storm increases blood viscosity and coagulability, which causes endothelial dysfunction[35,36]. The sympathetic activation, tachycardia, increased myocardial oxygen consumption and energy expenditure can also injure the myocardium. More cases of takotsubo cardiomyopathy are being diagnosed in patients with severe COVID-19[37]. Elevated positive end-expiratory pressure during mechanical ventilation in COVID-19 patients with severe ARDS increases right ventricular wall stress and can further reduce the cardiac output in a failing heart[38]. In a series of consecutive autopsy cases, Lindner and colleagues documented SARS-CoV-2 in 24 of

39 patients (61.5%), suggesting that direct viral myocardial damage is also possible [39]. SARS-CoV-2 attaches to human cells after binding with its spikes to the ACE2, which are upregulated in patients with cardiovascular disease, diabetes, and those treated with ACEi or ARB[40-42]. In light of this observation, role of ACE2 and ACEi in the pathogenesis of COVID-19 related myocardial injury has also been investigated from a therapeutic point of view.

While there are multiple factors contributing to HF in COVID-19, the incidence of true 'myocarditis' in COVID-19 in unclear[43]. Some authors estimate that myocarditis may account for up to 7% of COVID-19 deaths[44]. This estimate is fundamentally flawed because the diagnosis cannot be confirmed in a vast number of cases. Also, the presentation of acute coronary syndrome, sepsis-related cardiomyopathy and takotsubo cardiomyopathy can mimic myocarditis, making this a challenging diagnosis. Myocarditis, even when subclinical, can worsen patient outcomes. In the short term, it can increase the risk of arrythmias and precipitate decompensated HF, especially in patients with pre-existing chronic HF[45]. In the long term, the resultant myocardial fibrosis and negative remodeling can accelerate the decline of systolic function leading to a limitation of physical activity. Therefore, in the ICU, it is important to screen patients for subclinical myocarditis, by following the AHA recommendation of testing patients with signs consistent with myocarditis with one or more cardiac imaging methods such as echocardiogram or cardiovascular magnetic resonance[46].

SCREENING FOR HF IN THE ICU

Up to one-third of COVID-19 patients admitted to the ICU develop cardiomyopathy, and cohort studies from Wuhan have estimated the proportion of COVID-19 patients with cardiac injury to be between 20% and 28%[8,47]. Therefore, it would be prudent to screen all COVID-19 patients admitted to the ICU for HF. Critically ill patients in the ICU, are not able to communicate their complaints, and physical examination findings are often limited. A screening algorithm such as the one suggested by the Cardiac Society of Australia and New Zealand can be employed in the ICU[48]. Incorporation of such a screening algorithm into the treatment protocol will help identify more patients with HF and optimize treatment.

Although major society guidelines recommend the measurement of natriuretic peptides when the diagnosis of HF is uncertain, they should be interpreted in the context of other clinical information due to their high sensitivity and limited specificity [49,50]. In addition to its diagnostic value, natriuretic peptides also have prognostic significance with higher pro-brain natriuretic peptide (pro-BNP) values associated with increased mortality[51,52]. Therefore, natriuretic peptides can be used for risk stratification of COVID-19 patients with HF. Elevations in cardiac troponins have also been observed in COVID-19 patients and may indicate both coronary and non-coronary disease[53]. Acute coronary syndrome, microvascular ischemia, myocarditis, takotsubo cardiomyopathy and arrythmia are some of the reasons for an elevated troponin in COVID-19 patients[54]. While this makes the measurement of cardiac troponins less useful from the point of view of diagnosing HF, an elevated troponin level cannot be ignored as it may point towards underlying heart disease in an asymptomatic COVID-19 patient.

ROLE OF CARDIAC POINT OF CARE ULTRASOUND

The American Society of Echocardiography defines cardiac point of care ultrasound (POCUS) as 'focused exams with specific imaging protocols based upon suspicion of a specific disease' and differentiates it from ultrasound assisted physical examination [55]. POCUS has multiple uses in the diagnosis and management of HF in COVID-19 patients in the ICU. POCUS has the added benefit of reducing risk of exposure to the health-care worker, when compared to the use of stethoscopes[56]. Adhering to a set-protocol such as the one described by Huang *et al*[57], will reduce inter and intra-observer variability. Documenting POCUS findings is important and if possible, the images should be stored on the device or on a central server. As this technology is relatively new, using mannequins for standardized training in image acquisition and interpretation may be helpful[58].

ECHOCARDIOGRAPHY

The American Society of Echocardiography, in a statement endorsed by the American College of Cardiology, recommends that Transthoracic echocardiography should be performed if it is expected to provide clinical benefit[59]. In the ICU, this can be done at the bedside, with adequate airborne precautions[59]. Echocardiography (ECHO) can provide more information when compared to cardiac POCUS and can also be used to risk stratify patients to aid in follow up. A study of 75 hospitalized patients with COVID-19 showed a significant association between lower LVEF and mortality[60]. The mortality among the patients with LVEF < 50% was 65% compared to 26% in the group with LVEF ≥ 50%. The patients with LVEF < 50% also had higher troponin T and pro-BNP levels[60]. Stepwise modelling demonstrated that mechanical ventilation (OR 22.6; 95%CI: 3.0-170.4), LVEF < 50% (OR 8.2; 95%CI: 1.4-46.9), and pro-BNP above the cohort median value (OR 5.8; 95%CI: 1.4-23.9) were the strongest predictors of mortality[60]. Similar findings were reported by Alvarez-Garcia *et al*[22], as mentioned previously. Both left ventricular global longitudinal strain (HR 1.39; 95%CI: 1.11-1.76) and right ventricular longitudinal strain (HR 1.33; 95%CI: 1.15-1.53) were associated with increased mortality in COVID-19[61,62]. ECHO also allows for more detailed evaluation of right heart function, including tricuspid annular plane systolic excursion/pulmonary artery systolic pressure ratio (HR 0.026; 95%CI: 0.01-0.579; P = 0.019) which was an independent predictor of mortality in one study[63].

MANAGEMENT OF ACUTE HEART FAILURE IN THE ICU

Management of acute HF in COVID-19 patients in the ICU should be done according to established guidelines and protocols. Although questions have been raised about the potential deleterious effects of ACEi and ARBs in COVID-19, a joint statement from the Heart Failure Society of America and American College of Cardiology/American Heart Association recommends continuation of these medications in patients with HF, if hemodynamics allow[64]. An effort must be made to identify HF due to takotsubo cardiomyopathy, which may masquerade as an acute coronary syndrome, but is increasingly being recognized in the context of COVID-19[37]. Since catecholamine-excess is considered to be part of the pathogenesis behind takotsubo cardiomyopathy, this subset of patients may benefit from restricting the use of catecholamine-inotropes such as dobutamine and dopamine and replacing them with non-catecholamine inotropes such as levosimendan and milrinone[65-67]. In patients who develop HF refractory to inotrope support, mechanical circulatory support, *e.g.*, veno-arterial ECMO or other cardiac assistive devices such as Impella (Abiomed, Danvers, MA, United States) may be used. These interventions are resource intensive and may not be available in all centres. In patients with suspected myocarditis or cytokine mediated injury, high dose corticosteroids, intravenous immunoglobulin and even selective cytokine blockade are options that can be considered on an experimental basis, given the absence of strong evidence of their benefit[68].

EXTRA-CORPOREAL MEMBRANE OXYGENATION

The World Health Organization has recommended that ECMO can be used in experienced centres for the management of critically ill COVID-19 patients with ARDS with or without HF[69]. The Extracorporeal Life Support Organization has emphasized that ECMO should be judiciously used as a rescue strategy in severely ill patients since it is a resource-intensive, highly specialized, and expensive form of life support with the potential for significant complications[70]. Key considerations while implementing ECMO include proper patient assessment and selection, personnel assignment, infection control measures before and during ECMO initiation as well as devising protocols for ECMO weaning, decannulation and rehabilitation[71]. Given the resource intensive nature of ECMO, some authors have raised the question of whether it is worth using during a pandemic[72]. Barbaro *et al*[73] used the data from the ELSO registry and determined that the mortality in COVID-19 patients who required ECMO was less than 40%. This shows that in the appropriate setting, ECMO is indeed beneficial in critically ill COVID-19 patients.

The role of ECMO after CPR (E-CPR) is unclear at this point, and as of February 3, 2021, the Extracorporeal Life Support Organization registry has reported 32 COVID-19 patients who underwent E-CPR[74]. Current guidelines recommend judicious use of

E-CPR and only in centres that already have experience in its use[70,75,76]. Candidate selection should be done with due consideration of the patients comorbidities, other organ function, short and long-term life expectancy, availability of ECMO resources and risk of infectious exposure during cannulation and bed-side management[71].

LEFT-VENTRICULAR ASSIST DEVICES

Literature on left ventricular assist devices and COVID-19 are scant. LVADs have been used with varying success in the management of HF in COVID-19. Valchanov *et al*[77] have described a case of a 43-year patient with severe COVID-19 ARDS and HF who was managed with veno-arterial ECMO and an Impella 5.0 ventricular assist device. The patient, however, succumbed to his illness after a 3-wk period. There are case-reports and case-series of patients on long-term LVAD who developed COVID-19[78-82]. It is important to recognize that COVID-19 patients with LVADs are particularly prone to thrombotic complications. This requires intensivists to walk a fine line between potential complications of bleeding and thrombosis. In recipients with COVID-19 infection, daily interrogation of LVAD parameters can help in the early recognition of early signs of hemodynamic compromise, pump thrombosis, right ventricular failure, vasoplegia associated with secondary infection, or innate device malfunction[83].

REHABILITATION OF HF PATIENTS WITH COVID-19 AFTER ICU DISCHARGE

Rehabilitation of COVID-19 survivors who were in the ICU is critical. In those who developed HF, this becomes even more important. COVID-19 survivors who were critically ill often develop respiratory sequelae, cognitive sequelae, deconditioning, critical-illness related myopathy and neuropathy, dysphagia, joint stiffness and pain and psychiatric problems[84,85]. An early physical medicine and rehabilitation consultation, will help identify and address these issues early on. Rehabilitation can be initiated while the patient is still in ICU. However, more holistic rehabilitation will require assessment of respiratory capacity, muscle strength, exercise capacity, gait speed, balance and activities of daily living[86]. This is preferably done in a dedicated rehabilitation facility, after the patient is discharged home[82]. A graded exercise-based cardiac rehabilitation strategy can be prescribed, in accordance with standard HF guidelines[87].

Home based cardiac rehabilitation programs with telemonitoring methods can also be considered[88]. Few authors have reported promising results with remote cardiac care during the COVID-19 pandemic with telemonitoring devices such as the V-LAP™ (Vectorious Medical Technologies, Ltd) device for monitoring left atrial pressure as well as the HeartLogic platform (Boston Scientific, Marlborough, Massachusetts)[89,90]. Although this technology is relatively new, we may soon see its integration into rehabilitation protocols for patients with HF, after ICU discharge.

LIMITATIONS

We identified that only very few studies discussed the medical management of sick patients with COVID-19 in the background of HF. While many studies reported prevalence of 'cardiovascular disease' and 'cardiac injury' (usually defined as troponin I above the 99th percentile upper reference limit or new abnormalities shown on electrocardiography and echocardiography) in COVID-19 patients, the number of studies that reported chronic and *de novo* HF in this cohort was limited. Part of the reason may be the similarity in presentation of severe COVID-19 ARDS and acute decompensated HF. This is particularly challenging in the ICU patients, in whom both conditions often coexist. Moreover, HF is both a risk factor and a complication of COVID-19. Moreover, studies included in this review were retrospective and lack granular details on the severity of the disease, medical treatment, comorbidities, drug interactions, and outcome. Details of COVID-19 infection on the management of HF and vice versa were also not uniformly addressed. Outcomes in different studies were different. Details of treatment of COVID-19, duration of therapy, length of hospital stay, the long-term outcome were not uniformly available. Details of intensive care

treatment, including mode of ventilation, pressors of choice, renal replacement therapies, the role of sedatives and paralytics on this subgroup of populations were also not discussed in all studies. However, the strength of the studies was that it included studies with patients having COVID-19 in the background of HF from all over the world. We also tried to identify the predictors of morbidity and the role of intensive care therapy in these patients, from the literature. More research focusing on this subset of patients is necessary to clarify the pathogenesis, improve screening methods and identify optimal therapeutic strategies.

CONCLUSION

In this review, we identified that HF is an independent predictor of mortality in hospitalized COVID-19 patients. Patients with HF were more likely to require ventilation, ICU admission and develop complications. Patients with HFrEF did worse than those with HFmrEF and HFpEF. COVID-19 patients with HF should be identified early and managed aggressively in an attempt to improve outcomes in this cohort of patients.

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REVIEW

COVID-19: A pluralistic and integrated approach for efficient management of the pandemic

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Abstract

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which triggered the ongoing pandemic, was first discovered in China in late 2019. SARS-CoV-2 is a respiratory virus responsible for coronavirus disease 2019 (COVID-19) that often manifests as a pneumonic syndrome. In the context of the pandemic, there are mixed views on the data provided by epidemiologists and the information collected by hospital clinicians about their patients. In addition, the literature reports a large proportion of patients free of pneumonia *vs* a small percentage of patients with severe pneumonia among confirmed COVID-19 cases. This raises the issue of the complexity of the work required to control or contain the pandemic. We believe that an integrative and pluralistic approach will help to put the analyses into perspective and reinforce collaboration and creativity in the fight against this major scourge. This paper proposes a comprehensive and integrative approach to COVID-19 research, prevention, control, and treatment to better address the pandemic. Thus, this literature review applies a pluralistic approach to fight the pandemic.

Key Words: SARS-CoV-2; COVID-19; Pandemic; Pluralistic approach; Global approach; Efficient management

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Core Tip: Pandemic control requires optimal knowledge of the pathogen, infection routes, mode of transmission, and intervention strategies. The contagiousness of coronavirus disease 2019 (COVID-19) complicates pandemic control or containment because asymptomatic carriers, incubating patients, and recovered patients are all potentially contagious. This literature review proposes and justifies the value of a pluralistic and integrative approach to COVID-19 research, prevention, control and treatment.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative pathogen of the ongoing pandemic, was first detected in late 2019 in Wuhan, Hubei Province, China. This novel respiratory virus causes the infectious coronavirus disease 2019 (COVID-19), which often manifests as pneumonia[1]. At least two previously identified coronaviruses, responsible for SARS-CoV and Middle East respiratory syndrome coronavirus, respectively, have accelerated the understanding of the epidemiology and pathogenesis of SARS-CoV-2[1-4]. Investigations are ongoing to determine the precise origin of the virus.

To control this global scourge, the scientific community and health professionals must invest in understanding the virus, the infection, its spread, the distribution, and its evolution to develop reliable strategies for prevention and/or response. However, public health professionals and/or infectious diseases specialists are often at the front line in the fight against outbreaks or pandemics. However, the statistics provided by epidemiologists often contrast with the information collected by practicing clinicians regarding their patients[5]. Although data collected from travelers coming from areas with a high incidence of COVID-19 may be useful for estimating the incidence, this risk measure is controversial. While epidemiologists use statistical methods or mathematical models to assess the magnitude of the epidemic in the community (*e.g.*, incidence), clinicians focus on patients based on the number of hospitalizations[5]. According to the literature, 80% of confirmed COVID-19 cases did not have pneumonia, approximately 15% had severe pneumonia, and approximately 6% were admitted to intensive care units (ICUs) for the treatment of respiratory failure, shock, or multiorgan damage[6]. Asymptomatic infected persons and incubating patients (potential sources of virus transmission) or patients who have recovered from COVID-19 without showing a reduced SARS-CoV-2 viral load by a factor of $1/10^6$ (*i.e.*, a 6-log reduction), can pose serious challenges for disease prevention and control[1,7].

This raises the issue of the complexity of pandemic control or containment. We believe that a pluralistic and integrated approach will put into perspective the specificities of each discipline and reinforce collaboration and creativity in the fight against this scourge. This paper proposes a collaborative approach of competencies to capitalize on expertise, an integrated strategy for interventions for the control of epidemic or chronic diseases, and a global patient management plan for disease and/or pandemic control.

METHODS

We analyzed the scientific literature according to six main areas of expertise. We searched the PubMed database to construct a clinical scientific bibliography. The basic search term used for the literature search was "covid+19," followed by one or other thematic terms, including virology, epidemiology, prevention, control, Africa, infection, and treatment. Preference was given to the "review" and "most recent" filters. The most appropriate articles for each thematic term were selected for the analysis and discussion. Table 1 summarizes the reference portals according to the corresponding target groups and disciplines. An additional search focused on the

Table 1 Documentary research by target groups and by specialty disciplines

Target groups	Documentary research links	Documentary search dates and periods	Main fields/remarks
Biologists	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+virology&filter=pubt.review	12/10/2020	Medical biology (virology, molecular biology, clinical biochemistry, hematology, immunology, *etc.*)
Public health professionals	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+epidemiology&filter=pubt.review	14/10/2020	Public health (epidemiology, community health, *etc.*)
	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+prevention&filter=pubt.review	12/10/2020	
	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+control&filter=pubt.review	13/10/2020	Communication (transversal)
	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+Africa&filter=pubt.review	12/10/2020	
	https://eu.boell.org/en/2020/08/17/dr-congo-challenge-convincing-people-coronavirus-exists		
Clinicians	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+infection&filter=pubt.clinical study	12/10/2020	Medicine (infectiology, pneumology, cardiology, internal medicine, *etc.*)
	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+treatment&filter=pubt.randomized controlled trial	13/10/2020	
	https://pubmed.ncbi.nlm.nih.gov/?term=covid+19+vaccination&filter=pubt.review	14/02/2021	
Researchers	https://pubmed.ncbi.nlm.nih.gov/32230900/	January 2021	Research (transversal or universal character of science)
	https://www.ncbi.nlm.nih.gov/research/coronavirus/		
	http://www.health.belgium.be/eportal/disclaimer/		
	https://rega.kuleuven.be/if/corona_covid-19		
	https://covid19.sciensano.be/sites/default/files/Covid19/Covid19_fact_sheet_ENG.pdf		
Decision-makers	N/A		A pluralistic approach to inform and guide health policies

basic term "covid+19," followed by "vaccination" with "most recent" as the preferred filter. The PubMed literature search (including the additional vaccination search) was performed from October 12, 2020, to February 14, 2021 (Table 1). Figure 1 presents the flow chart of the search for articles and publications.

RESULTS AND DISCUSSION

Biology and virology

SARS-CoV-2 is a member of the coronaviridae family. It is a beta-coronavirus (subgroup B Sarbecovirus) enveloped with a large single-stranded RNA + that can infect animals and humans[8]. In humans, the structural (spike) protein of the viral envelope recognizes angiotensin-converting enzyme 2 (ACE2) as a receptor and preferentially infects pulmonary epithelial cells. The spike protein binding domain binds to ACE2; the host transmembrane protease serine 2 protease then cleaves the protein to expose fusion peptides that fuse the virus to cell membranes[2,9]. ACE2 is expressed in several human tissues, including the lung, small intestine, kidney, heart, thyroid and adipose tissues, which can be infected by SARS-CoV-2 and cause various symptoms[8,10].

The genome of SARS-CoV-2 is 96.2% and 79.5% identical to the sequences of RaTG13 (bat) CoV and SARS-CoV, respectively. Accordingly, bats are considered the natural host and a potential origin of the virus and may have transmitted the virus to humans through an unknown intermediary or directly *via* the aquatic wildlife market in Wuhan[8,11,12]. In the absence of strong evidence of pangolins as an intermediate host, some authors have suggested the need for coronavirus surveillance in these animals in the wild to minimize human exposure[13]. SARS-CoV-2 may also be transmitted by aerosols or vehicles (hands or soiled objects). Depending on the amount of inoculum, the virus can remain viable and infectious for hours in aerosols and days

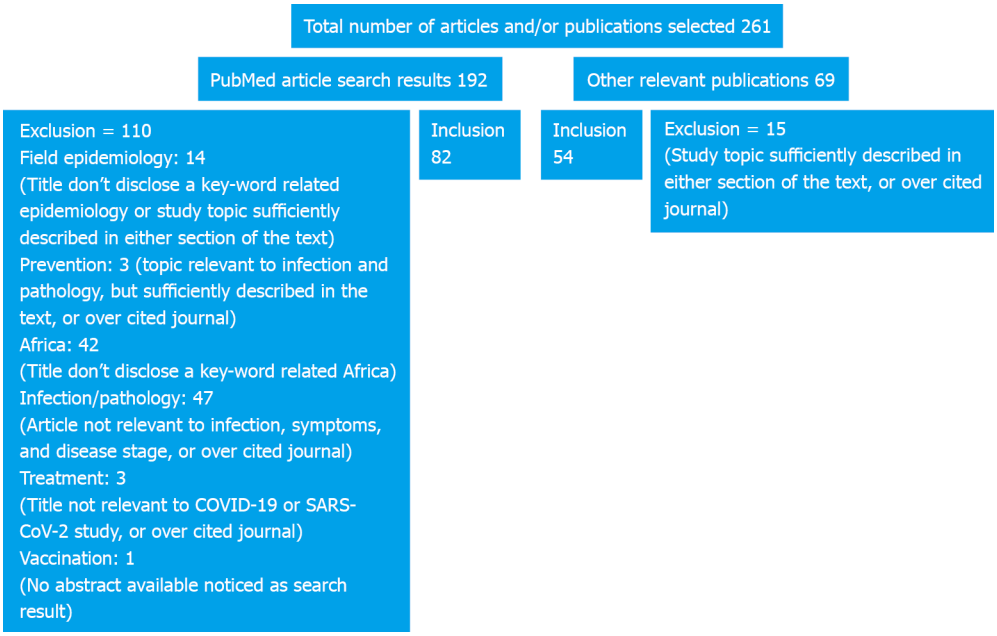

Figure 1 Flow chart of the search for articles and publications. COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

on surfaces[14-16]. Longer SARS-CoV-2 and SARS-CoV-1 viabilities on stainless steel and plastic have been reported. The median half-life of SARS-CoV-2 was 6 h for stainless steel and 7 h for plastic[17]. Hence, universal hygiene precautions such as hand washing with soap and water, wearing masks, and cleaning surfaces have been recommended.

The pre-analytical phase is crucial. The quality of the analysis or results depends on the sample quality. Sputum samples may have viscous consistency due to mucus (purulent or not).

The effective extraction of viral nucleic acid requires the liquefaction of sputum to avoid false-negative results[18]. Moreover, the use of swabs made of non-compliant materials may inactivate the virus particles or inhibit polymerase chain reaction (PCR). For nasopharyngeal or oropharyngeal swabs, swabs (standard or flocked) with a flexible plastic shaft are recommended. Quantitative molecular tests (quantitative reverse transcription-PCR) are used to complement clinical, biological, and radiological investigation tools[19]. Although molecular tests are highly specific for the diagnosis of COVID-19, their sensitivity depends largely on parameters such as the specimen type, time of specimen collection, sampling technique, test quality, and technician qualification[20-23].

Immunoassays measure the levels of antibodies [circulating immunoglobulin M (IgM) and IgG] in patients with COVID-19. However, the usefulness of these tests as an epidemiological tool is questioned in terms of their sensitivity and specificity, since the results may vary depending on the serological window. This window must be neither too early nor too late to produce an interpretable result[24].

One study reported a higher sensitivity for the detection of IgA (about 4-25 d after disease onset), with IgG reportedly better for diagnosis in later stages of the disease [25]. A diagnosis of COVID-19 is suspected in cases in which the symptoms of respiratory infection occur within 14 d (consistent with the incubation time) in an asymptomatic person coming from an epidemic area[26]. The association of SARS-CoV-2 viral load relative to the nasopharyngeal specimen with COVID-19 severity has been reported, in which a higher viral load was associated with a lower lymphocyte count, greater organ damage, and longer time to molecular test negativity[27]. Higher viral loads were detected soon after symptom onset, with higher loads in the nose compared to throat swabs. The same study suggested that the kinetics of SARS-CoV-2 nucleic acid clearance resembled that of influenza and differed from that of SARS-CoV. In addition, the similarity of viral loads in symptomatic and asymptomatic patients suggests that minimally or asymptomatic people may potentially be infectious. Therefore, transmission may occur early during infection. Case detection and isolation may require strategies different from those previously used to control SARS-CoV. The identification of minimally or asymptomatic patients and modest

levels of viral RNA (detectable in the oropharynx) suggest the need for further investigation to determine the transmission dynamics and inform screening practices[28]. Furthermore, the implications of positive (or negative) test results in asymptomatic individuals remain undetermined, as well as the interpretation of these results for immune passports (detection of signals of past infection). The findings seem to contradict much of the popular literature on the use of the test as a tool for COVID-19 management. Although testing is an essential part of disease management, including COVID-19, its inappropriate use may have unintended adverse consequences[29]. Therefore, clinicians must consider other factors when interpreting a patient's test results. The Food and Drug Administration (FDA) issued approval to biotechnology firms to provide COVID-19 tests. One of the major areas of FDA intervention is to increase the availability of tests, treatments, and materials such as ventilators and personal protective equipment (PPE)[30,31]. The World Health Organization (WHO) regularly updates the list of qualified reference laboratories to confirm COVID-19 test results[32].

Epidemiology

To better control the epidemic, scientists are investigating how SARS-CoV-2 is transmitted and spread. Initial data from patients in China provided information on the mode of human-to-human transmission, mainly *via* the respiratory route, most likely through close contact[33,34]. Human-to-human transmission was demonstrated in the first confirmed cases of infection in Wuhan, China[28]. It is generally accepted that the more a person interacts with others and the longer this interaction lasts, the higher the risk of COVID-19 transmission. However, further investigations are needed to understand if and how different animals may be affected by this disease. Researchers at the Friedrich Loeffler Institute in Germany reported that raccoon dogs (*Nyctereutes procyonoides*), an invasive carnivorous animal used for fur, are a potential intermediate host in SARS-CoV-2 transmission. The researchers proposed that the farms where these animals are raised may serve as reservoirs for SARS-CoV-2 and that this risk should be mitigated by effective and continuous surveillance. In their opinion, while it is possible to control the virus on farms, spillover to susceptible wildlife and, in particular, to free-living raccoon dogs would be a major challenge to elimination[35]. Signs of respiratory pathology and increased mortality have also been described in farmed minks (*Neovison mink*) infected with SARS-CoV-2[36,37]. Evidence of animal transmission of SARS-CoV-2 to humans on mink farms was also reported in a phylogenetic study[38]. However, the authors indicated that some farm residents may have been infected within their households and not directly *via* the mink. They added that the survey did not identify common factors that could explain the spread among farms, probably *via* temporary workers not included in the tests. They were concerned that the fur production and trade sector not become a reservoir for the future re-emergence of SARS-CoV-2 in humans.

Globally, incidences ranging from 0.00 to 61.44 per 1000000 persons were reported for COVID-19 at the end of February 2020. Much lower incidences (perhaps due to weaknesses in reporting systems) were recorded in Africa (0.00 for Nigeria and 0.02 for Algeria). In contrast, higher incidences of 55.06 and 61.44 were reported in China and the Republic of Korea, respectively. The numbers of deaths per 1000000 people ranged from 0.00 in Nigeria to 1.97 in China[39]. Africa accounts for less than 1% of the global SARS-CoV-2 mortality[40].

A molecular study reported the early transmission of COVID-19 and a heterogeneous epidemic in South Africa[41]. The study sought to better understand the epidemic heterogeneity of SARS-CoV-2 strains and their introduction during the first month of the epidemic in that country. The early introduction of SARS-CoV-2 into Kwazulu Natal resulted in a localized outbreak in one hospital, which is a likely explanation for the initially high mortality rates in the province. The high rate of COVID-19 transmission in the Western and Eastern Cape highlights the critical need to strengthen local genomic surveillance in South Africa.

All 54 African countries officially reported cases of COVID-19. More than 3000000 people tested positive for COVID-19, with more than 99000 deaths (3.3%). These relatively low levels of mortality may be due to the rapid response in some countries, including South Africa, Uganda and Ethiopia. In addition to the rapid and insightful implementation of stringent response measures, the demographics of the continent (the youthfulness of the sub-Saharan African population) may have conferred some advantage, as a large fraction of deaths caused by COVID-19 occurs in patients over 70 years of age[42,43]. However, the hypothetical effectiveness of containment measures across this continent may be premature. Poor reporting quality, limited communication systems for patients and health professionals, and insufficiency of

surveillance and screening centers across the continent may also contribute to the low reported numbers. The registration rates of all deaths and their causes are incomplete in many African countries because accurate estimates are difficult to obtain, with coverage of registered deaths varying from 5% in Mozambique, 16% in Zambia, 25% in Botswana and Ghana, and 67% in South Africa[44]. In addition, COVID-19 may be confused with other infectious diseases, such as malaria, typhoid, human immunodeficiency virus (HIV)-AIDS, and tuberculosis[45]. These confounding factors can negatively impact the reporting of cases and deaths attributable to COVID-19[42]. Moreover, cultural challenges such as community stigmatization of infected patients, who consequently avoid medical assistance and consult traditional practitioners, further lead to underreporting. Country experience with pandemics and epidemics varies across the African continent, which may influence preparedness (*e.g.*, availability of testing and PPE). West African countries may be better able to respond given their recent experiences with Ebola. Similarly, East African countries have also gained critical epidemic experience from cholera, which has repeatedly affected the region in recent years[46].

The uncertainties regarding the impact of SARS-CoV-2 infection in Africa underscore the need for critical monitoring of the evolution of the pandemic and the factors affecting disease burden. Even in the absence of more effective vaccines and treatments, Africa can lead the fight against this scourge provided that appropriate containment intervention systems are put in place by addressing systematic challenges such as access to water, improved food systems, health education, bed capacity in intensive care hospitals, and increased funding and investment in health care[40].

Infection and pathology

The onset of clinical symptoms seems to favor contagiousness. In some individuals, contagiousness may occur several days before symptom onset. However, contagiousness is more marked in symptomatic persons during coughing. The average incubation period varies from 5 to 6 d, ranging from 2 to 14 d, which justifies the 14-d quarantine period[34]. The initial symptoms (headache, muscle pain and fatigue) are not specific and are followed 2 or 3 d later by fever and respiratory signs.

The clinical manifestations can be severe. Scientists are still seeking to understand COVID-19 severity. Preliminary descriptive studies of databases in China indicated an average time of 1 wk from symptom onset to hospital admission when the disease becomes severe. At this stage, the symptoms include fever, cough, chest pain, and respiratory discomfort. Chest computed tomography (CT) scans almost always show bilateral pneumonia[47]. Since the initial studies, other reported clinical signs include central nervous system involvement (*e.g.*, disorientation, especially in the elderly); sudden loss of taste and/or smell, which occur infrequently but allow confirmation of COVID-19 diagnosis[33,48]. Many patients with COVID-19 present neurological symptoms (including headache, myalgia, and altered consciousness), that are sugg-estive of the disease.

Some patients with SARS-CoV-2 present with symptoms suggestive of acute stroke, epilepsy, encephalopathy, and demyelinating neuropathies and without cough, fever, or other respiratory problems that could provide clues to the underlying pathology. Diagnosing and administering appropriate treatments to these patients is challenging and requires specialized neurologists, which are sorely lacking in Africa[42].

Real-time Assessment of CommunityTransmission-1 study data showed that chills, loss of appetite, headache, and muscle pain were the symptoms most strongly associated with infection, along with the four classic symptoms. The presence of one or more symptoms was associated with SARS-CoV-2 infection, with stronger associations with increasing numbers of symptoms. A loss or change of smell was less predictive of COVID-19 infection, while the proportion of people testing positive with a persistent new cough appeared to be increased[49].

The severity of clinical signs requires hospitalization in approximately 20% of patients, while 5% require admission to intensive care. The most severe forms are mainly observed in people who are vulnerable because of their age (over 70 years) or comorbidities (including diabetes and cardiovascular diseases)[47]. Patients with COVID-19 requiring ICU hospitalization are generally frail and have significant comorbidities. The outcomes in this group were generally poor and did not appear to be influenced by ICU admission. Symptoms of COVID-19 infection occurred during hospitalization for a different medical problem in 38% of the patients analyzed[50].

Observational and modeling studies have shown 30% to 60% of infected patients are asymptomatic (absence of clinical manifestations) or "paucisymptomatic" (presence of few symptoms)[47].

Determination of the clinical, laboratory, and radiological characteristics of patients suspected of COVID-19 infection are essential for early isolation, treatment, and contact tracing[51]. A positive COVID-19 test result in patients with hip fractures was associated with a 2.4-fold increase in 30-d mortality risk[52]. During the peak period of the COVID-19 epidemic in New York city, more than half of patients with emergent large vessel occlusion (ELVO) stroke were positive for COVID-19 and were younger, more likely to be male, and less likely to be white. These findings also suggested an increased incidence of ELVO stroke during the peak of the COVID-19 epidemic[53]. One study suggested that conditions (comorbidities, rheumatic diseases) and abnormal laboratory parameters such as C-reactive protein (CRP), D-dimer, lactate dehydrogenase (LDH), and increased serum ferritin levels were significantly associated with mortality, in contrast to a previous use of antirheumatic drugs. The authors suggested that inflammation was closely related to COVID-19 severity. Their key findings were as follows: (1) Most patients recovered from COVID-19 disease; (2) The use of antirheumatic drugs, corticosteroids, and biological agents did not increase the risk of mortality; and (3) Rheumatic disease activity may be associated with mortality[54].

Advanced age, diffuse distribution, and hypoxemia may help clinicians to identify COVID-19 patients with a poor prognosis. Similarly, aggregated social media data may also influence disease prognosis[55]. Higher troponin T levels and lower lymphocyte counts were predictive of disease progression. Early ventilation may be an effective treatment for severe cases[56]. Severe and consistent lymphopenia with significantly reduced lymphocyte subgroups with normal CD4/CD8 ratio has been reported in critically ill patients. In addition, extremely reduced transferrin saturation at ICU admission and a significant increase on days 3 to 6 with constant hyperferritinemia during the ICU stay have been reported[57]. More severe COVID-19 disease was observed in patients who were older, male, African-American, obese, diabetic, and with a higher overall comorbidity burden. Certain comorbidities paradoxically increased the risk in younger patients in most cases. Among inpatients, male sex was the primary determinant of the need for more intensive care. Further investigations are needed to understand the mechanisms underlying these findings[58]. A cohort study of COVID-19-related deaths in Ontario, Canada, reported a concentrated risk of mortality among residents of long-term care (LTC) facilities, which increased over a short time. A study on preventing the spread of COVID-19 between facilities reported the need for the early identification of risk, which necessitates screening and provision of PPE to staff as well as restructuring of LTC staff[59]. Patients with COVID-19 with an increased ST-segment myocardial infarction (STEMI) picture showed a favorable disease course to a high thrombotic burden and poor prognosis[60]. The authors suggested the need to determine the COVID-19 status in all STEMI cases. They also suggested the need for further work to understand the mechanism of increased thrombosis and identify aggressive antithrombotic therapy. An observational study reported the correlation of amino acid and fatty acid metabolism with COVID-19, providing information on the mechanism, potential markers of clinical severity, and potential therapeutic targets[61]. LDH and CRP may influence respiratory function and may be considered predictive of respiratory failure in patients with COVID-19. The authors suggested the usefulness of these biological markers for the early identification of patients requiring closer respiratory monitoring and more aggressive supportive therapies to avoid a poor prognosis[62]. One study observed that ischemic and hemorrhagic strokes complicated the course of COVID-19. In that series, these events occurred mainly in patients with severe pneumonia and multiorgan failure. Liver enzymes and LDH levels were markedly increased in all cases, and the prognosis was poorer[63]. Another study reported that more than half of the infected patients with cancer were susceptible to severe COVID-19. This risk was exacerbated by concurrent anticancer treatment and predicted poor survival despite COVID-19 treatment[64].

A transient twofold increase in the incidence of out-of-hospital cardiac arrest, associated with reduced survival, was observed during the pandemic, in contrast to data observed in a similar period during previous pandemic-free years. The authors proposed that this finding was partly related to COVID-19 but was also likely due to the indirect effects of the pandemic associated with lock-in and rehabilitation of healthcare services. They suggested that these factors should be considered when reviewing mortality data and public health strategies[65]. While the correlation between the prevalence of heterozygous beta-thalassemia and COVID-19 immunity has been reported, further investigations are required to confirm this finding[66].

Prevention, control, and communication

Communication strategies should focus on the routes of transmission (upper airways), modes of contamination (direct contact with respiratory secretions through airborne droplets and indirect contact through hands or soiled objects), and means of prevention. These strategies must be based on reliable and credible information and data. Prejudices and misinformation about the disease are often based on preliminary observations that are sometimes unreliable and speculative. However, this leads to confusion, panic, and anxiety among citizens[67]. This situation has been described as an "infodemic" by the WHO[68]. Clear and simple coherent messages based on the risk of transmission are preferred for good compliance with barrier measures[69]. Strict compliance with individual protective measures, combined with collective measures (containment, discouragement of gatherings), contributes synergistically to breaking the chain of transmission of both SARS-CoV-2 and other respiratory pathogens[70]. Measures such as containment and discouragement of gatherings help to reduce population density and, thus, reduce viral transmission.

Above all, distracting and/or annoying messages, as well as biased and sometimes unjustified measures, should be avoided. Anxiety-provoking messages should be avoided, as they cause panic and stress, emotional factors that weaken the immune system and, thus, expose the body to pathogens. Collective concerns can influence daily behaviors, economics, prevention strategies, and political decision-making of health organizations and medical centers, weakening COVID-19 control strategies, resulting in high morbidity and mental health needs worldwide[71].

The following means of protection and/or prevention are recommended: (1) Hand washing with soap and water; (2) Antiseptics (hydroalcoholic hand rubs); (3) Disinfection of soiled areas and materials using sodium hypochlorite or glutaraldehyde; (4) Wearing protective equipment (bibs, masks, gloves, lab coat, gowns, *etc.*); (5) Prohibition of activities that encourage gatherings to reduce the risk of viral spread; and (6) Home confinement if possible, especially during the outbreak[33,72-74].

However, frequent hand washing involves prolonged exposure to water and other chemical or physical agents, which results in pathophysiological variations. Undesirable dermatological effects such as excessive skin dryness or contact dermatitis (most often irritating and sometimes allergic), can occur, especially in people with a history of atopic dermatitis. These skin conditions are manageable with the application of a moisturizer immediately after hand washing or disinfectant use to prevent hand eczema[75].

It is important to remember certain public health concepts. The public health system (PHS) plays a key role in both patient management and disease prevention or control. In other words, achieving medico-social objectives requires an efficient, proactive PHS that is well adapted to the realities on the ground. Weak PHSs often face two main challenges in the management of an epidemic or a pandemic: The quality and/or the capacity of response and the compliance of people with the measures prescribed to cut the chain of transmission. Therefore, in the context of COVID-19, one study suggested strengthening the response capacity while recommending adequate prevention measures to avoid the risk of a resurgence of the epidemic[76].

The detection of more COVID-19-positive patients in the community along with compliance with adequate quarantine rules will reduce the number of secondary cases. This requires an increased testing capacity[77]. The limited availability of diagnostic tests makes it almost impossible to detect asymptomatic patients and adds to the uncertainty of the potential impact of SARS-CoV-2 infection in Africa, particularly concerning prevention strategies and economic impact[40]. The implementation of a robust prevention system along with compliance with individual or collective barrier measures (*e.g.*, containment, even if it appears more difficult to bear), is the most effective way to respond to the COVID-19 pandemic[78-80]. The WHO contributes to regularly updated guidelines for the home care of patients with COVID-19 with minor symptoms and the management of contacts, as well as operational guidelines for the management of patients in health facilities and communities, the quarantine of individuals in the context of COVID-19, the clinical management of severe acute respiratory infection when COVID-19 is suspected; and laboratory testing of suspected cases of COVID-19[81]. In addition, in the context of microbiological biosafety, the PPE guidelines are regularly updated[82]. In general, these guidelines are intended to provide information on PPE options in relation to safety and effectiveness to ensure better protection of healthcare workers and patients[74].

In addition to national and international guidelines, special attention must be paid to chemical or physical agents. Chemical agents exist in liquid or gaseous form; physical agents are, among others, heat, UV, and gamma rays. While chemical agents are used for antisepsis, disinfection, and/or sterilization, physical agents are generally

used for sterilization[83]. As PPE remains insufficient and decontamination methods are less cost-effective because they are complex, slow, expensive, and particularly unsuitable for low- and middle-income countries where the need is greatest, some researchers are investigating a new PPE decontamination option. They suggested a low-temperature, low-ambient humidity (WASP-D) decontamination method based on the 30-min or shorter half-life of SARS-CoV-2 (and other common pathogens) at temperatures > 45 °C, combined with the fact that most PPE is designed to be transported and stored safely at temperatures < 50 °C. They concluded that the decontamination of PPE at 12 h, 46 °C, and ambient humidity reduced the SARS-CoV-2 viral load by a factor of 10^{-6} (*e.g.*, $1/10^{6}$), without adversely affecting PPE materials or performance[84]. A test of three mask models purchased from supermarkets and drugstores showed that surgical masks, normally intended to be discarded after 4 h of use, retained very good filtration capacities after 10 machine washes at 60 °C. These masks also remained breathable enough to be worn for several hours without excessive discomfort. Finally, even after several washing cycles, these masks exceed the minimum requirements for fabric masks with an official filtration guarantee[85, 86]. Results in the literature showed that a universal face mask could help to reduce disease severity and strengthen the immunity of the wearer, since high doses of viral inoculum can overwhelm and deregulate the innate immune defenses, aggravating the disease[87]. While there has been apprehension regarding the accumulation of carbon dioxide during prolonged face mask wear, experimental studies have refuted this hypothesis. An observational clinical study reported that wearing face masks neither significantly restricted gas exchange (oxygen flow) nor contributed to carbon dioxide accumulation, even in individuals with pulmonary insufficiency. Nevertheless, prolonged use of face masks can negatively impact breathing, leading to heat stress, drowsiness, breathing difficulties (restricted flow of fresh air), and unusual heart rates. The discomfort experienced with the use of a surgical mask has also been attributed to neurological reactions or associated psychological phenomena such as anxiety, claustrophobia, or affective responses to a perceived difficulty in breathing. In addition, if a face mask is worn for a longer time, the filter becomes wet due to facial sweat and vapor from breathing, promoting particle clogging. Wearers may also experience a false sense of security, encouraging them to spend more time in public places. The other potential side effects of wearing face masks include skin irritation, discomfort from exhaled air entering the eyes, and speech quality and volume during conversations[87].

In addition to the classical measures (barriers or prevention), other factors can optimize COVID-19 prevention or control. Vitamin D is a promising agent for COVID-19 control, as it is involved in various pathophysiological mechanisms that occur during SARS-CoV-2 infection. High-dose vitamin D supplementation, particularly for at-risk groups, is recommended for the maintenance of serum levels between 40 and 60 ng/mL of 25-hydroxy vitamin D needed to prevent or treat COVID-19[88]. Vitamin supplementation or treatment of deficiency may be useful in areas with a high prevalence of hypovitamin D. The role of medicinal plants, including *Allium sativum, Camellia sinensis, Zingiber officinale, Nigella sativa, Echinacea spp., Hypericum perforatum, Glycyrrhiza glabra, and Scutellaria baicalensis*, in enhancing immunity has been reported. Terpenoids show promising effects in inhibiting viral replication, a finding that requires further study. Some alkaloids such as homoharringtonine, lycorine, and emetine have shown potent anti-coronavirus effects. Naturally occurring products such as emodin and baicalin can inhibit protein S production. Other enzymatic targets involved in coronavirus replication, included 3-chymotrypsin-like protease (3CLpro), papain-like protease, helicase, and RNA-dependent RNA polymerase, are inhibited by iguesterin, cryptotanshinone, silvestrol and sotetsuflavone. Consequently, natural products have been introduced as therapeutic agents against COVID-19[89]. A study reported the importance of essential nutrients in the diet for their beneficial effects on immune system function. The intake levels of relevant micronutrients (D, C, B12, and iron) were inversely associated with higher COVID-19 incidence or mortality, especially in subjects genetically predisposed to suboptimal micronutrient levels[90]. The nutrigenetic data obtained from the joint assessment of essential nutrients and the genetic factors that limit their bioavailability can serve as a fundamental tool to help strengthen the immune systems of individuals and prepare populations to fight infectious diseases such as COVID-19[90]. The multiple biological actions of hesperidin and vitamin C suggest that these two major citrus components that modulate systemic immunopathological phases, may be candidates to fight SARS-CoV-2 infections. Experimental studies are needed to corroborate the hypothesis that herbal or plant foods could contribute to COVID-19 prevention[91,92]. The beneficial role of Chinese medicine in the control of respiratory diseases, such as the common cold, has

been reported[93].

The "mandatory Bacillus Calmette-Guérin (BCG)" vaccination approach has shown a reducible effect on COVID-19 infection and mortality rates. Two immunological mechanisms; namely, the heterologous effects of adaptive and innate immunity induced by BCG vaccination, could explain host tolerance to COVID-19 infection. However, no direct evidence supports this biological background. Clinical trials related to BCG vaccination against COVID-19 are currently under investigation. In the absence of strong evidence, BCG cannot be recommended for COVID-19 prevention, although this is not an absolute contraindication[94].

Data suggest that people with epilepsy (PWE) have a low risk of being infected with SARS-CoV-2 and have less severe manifestations of COVID-19 due to their epileptic pathology alone[95]. The mechanisms of the activating effect of hyperventilation (HV), which causes deep and rapid breathing during seizures in PWE, are less well known. Although concrete evidence is lacking, if wearing a face mask can stimulate HV, at least to some extent, this practice should not be indiscriminately recommended to all PWE. However, in the absence of any proven COVID-19 treatment or vaccine, prevention is the best available strategy and it is probably not reasonable to suggest avoiding face masks in PWE under any circumstances[95]. Logically, this population does not need to wear a face mask most of the time, as long as there is no close contact with others, especially during intense physical activities. Instead, it is probably more beneficial to wear a face mask with intermittent breaks in crowded areas in safe, low-density areas[95].

Given the COVID-19 pandemic, there is emerging evidence that, compared to the general population, patients with cancer are particularly vulnerable to infection and adverse events, with correspondingly worse outcomes[64,96]. On admission or before initiating systemic therapy or radiotherapy, confirmation of COVID-19 status is recommended in asymptomatic or paucisymptomatic patients, especially those with high-risk features[23].

Regarding transfusion, the American Blood Bank Association and the Centers for Disease Control and Prevention (CDC) have made no specific recommendations regarding SARS-CoV-2[97].

Although no evidence of the transmission of SARS-CoV-2 through blood transfusion has yet been established, the blood supply has been affected by the COVID-19 pandemic[24,34].

The opportunity now exists for schools and academies to collaborate to advance science and potentially improve student outcomes[98].

SARS-CoV-2 has developed mutations in various parts of its nonstructural proteins (NSPs), particularly NSP2, NSP3, protein S, and RNA-dependent RNA polymerase. Because of the critical importance of mutations in SARS-CoV-2 pathogenicity and the development of serodiagnostics, antivirals, and vaccines, continuous molecular surveillance of the virus is recommended[99]. While seasonal changes, coordinated laboratory testing, isolation/quarantine, and school closures may help to control the COVID-19 pandemic, they are unlikely to stop SARS-CoV-2 transmission. Therefore, effective policies complementary to currently available control measures must be adopted to minimize the exponential spread of infection[100].

Achieving global goals, including the control of pandemics such as COVID-19, requires a strong commitment to impactful public policies and international collaborations, including universal vaccinations against COVID-19, with potential combination with both childhood and adult immunization programs and programs for the treatment of malaria, tuberculosis, HIV/AIDS, and neglected tropical diseases[101]. The core unit (public health office) of the Sri Lankan health system has earned the trust of the community because of its deep-rooted operations on the ground. It has expertise and extensive connectivity with the community. Thus, rigid prevention and control measures have been implemented in the geographical areas assigned to these health facilities. The managerial role of this unit should be further explored for future health system reforms[102] and effective strategies should be developed to strengthen the PHS at its core[103].

It is important to note that information may vary depending on the evolution of the epidemic and research findings[23]. In COVID-19, studies are progressing rapidly and knowledge is changing such that we must realize that today's truths may not be tomorrow's and that we must continue to increase our knowledge of this disease.

Treatment

To avoid patient harm, because of possible coinfections, a diagnosis should be made before starting possible anti-infectious probabilistic anti-influenza, oseltamivir, and/or antibiotic treatment[72]. Other drugs and/or vaccine candidates have been suggested

for treatment, although clinical studies are needed to provide solid evidence of their effectiveness[104].

Tocilizumab improved the clinical status of patients with severe COVID-19[105]. Corticosteroid therapy with high-dose methylprednisolone, followed by tocilizumab when necessary, rapidly restored respiratory function, decreased in-hospital mortality, and reduced the need for invasive mechanical ventilation in patients with COVID-19-associated "cytokine storm" syndrome. However, further investigation of these promising results is required[106]. The role of Chinese medicine as an adjunctive treatment for SARS-CoV-2-induced inflammation has also been reported. Yidu-toxicity could address SARS-CoV-2-induced inflammation by blocking pulmonary syndrome by eliminating inflammatory agents[107]. Moreover, Xuebijing injection effectively improved the levels of inflammatory markers and prognosis of patients with severe COVID-19[108].

Other conventional drugs have also been used. Ruxolitinib showed faster improvement in clinical status, significant improvement in heart tomography, faster normalization of lymphopenia, and a favorable side effect profile in patients with severe COVID-19. These results are informative for testing the efficacy of ruxolitinib in a larger population[109]. Colchicine showed a statistically significant improvement in the time to clinical deterioration in patients hospitalized with COVID-19; however, this result should be interpreted with caution because of the low statistical significance of the results[110]. Remdesivir (RDV) did not show a significant clinical outcome in patients with moderate COVID-19 compared to standard therapy[111]. However, this antiviral agent has shown efficacy against the severe form of COVID-19[111]. This drug also showed favorable pharmacokinetic (PK) and safety profiles in healthy volunteers who were administered the drug once daily[112]. These PK and clinical safety data and preliminary clinical data support further investigation of RDV in patients with COVID-19[112].

Drugs such as hydroxychloroquine are thought to be effective owing to their effects on the ACE2 receptors required for viral entry into the cell. While chronic treatment can lead to heart disease with impaired left ventricular function and conduction disorders with bradycardia, short-term treatment can also cause cardiac damage in some patients. It is important to consider parameters such as age, female sex, ionic disorders, renal insufficiency, and the combination of many products, which are risk factors for cardiac damage. Thus, it is prudent to follow recommendations for safe treatment with "chloroquine" to minimize damage and/or adverse reactions[24,113]. In patients with persistent (mild to moderate) COVID-19, the rate of negative conversion of hydroxychloroquine was comparable to that of standard treatment alone [113].

Regarding adverse drug reactions, a hospital-based pharmacovigilance study reported a high prevalence of adverse reactions in patients with COVID-19, a fortiori caused by drugs inducing gastrointestinal and hepatic disorders. The length of hospital stay, number of drugs used, and underlying diseases were risk factors for the occurrence of adverse reactions in patients with COVID-19[114].

Researchers believe that "the" treatment will require a combination of drugs to effectively control emerging diseases, including COVID-19, HIV, and hepatitis C infections[104]. Early triple antiviral therapy has shown superiority over lopinavir-ritonavir to suppress symptoms and shorten the duration of viral clearance and hospitalization in patients with mild to moderate COVID-19. Future investigation of dual antiviral therapies is warranted, with interferon beta-1b as the background regimen [115].

A ligand-protein interaction study in Africa reported that more than half of the 20 major alkaloids and terpenoids interacted favorably with 3CLpro, which controls coronavirus replication, and had higher binding affinities than those to lopinavir-ritonavir. The study identified substances such as 10-hydroxyusambarensine, cryptoquindoline (alkaloids), 6-oxoisoiguesterin, and 22-hydroxyhopan-3-one (terpenoids), which bind to the receptor and 3CLpro catalytic dyad of SARS-CoV-2. These compounds were identified by predictive analysis of the (absorption, distribution, metabolism, and excretion)/tox and Lipinski filters. However, further experimental analysis of these leads is required for the discovery of natural anti-COVID-19 therapeutic agents to combat the pandemic[116].

Immunotherapy *via* the administration of the plasma of people cured of COVID-19 may be a useful treatment method in countries in which this practice is possible[117].

A Jewish business news source reported that 96% of patients administered an innovative drug (EXO-CD24) were cured[118]. Testing of this technology in the first clinical phase in humans showed that 29 out of 30 patients with moderate to severe disease were discharged from the hospital within 3-5 d. EXO-CD24 is an innovative

preparation based on exosomes enriched with the CD24 protein. According to Nadir Arber, one of the leading physicians and researchers on the team that developed the drug, "Even if the vaccines work and no new mutations are produced, SARS-CoV-2 will stay with us". He adds that a drug was developed within 6 mo from the time the idea was conceived and the technology was developed until it was first tested in humans in the first clinical phase. The Ichilov Medical Center reported that EXO-CD24 uses exosomes - tiny carrier sacs that shuttle between cells - to deliver a protein called CD24 to the lungs and has been the subject of decades of research by Dr. Arber. CD24 is located on the cell surface and plays an important role in regulating the immune system. The protein helps to modulate the immune response and curb the lethal hyperreactivity of the immune system known as a cytokine storm. The administration of EXO-CD24 by direct aspiration into the lungs inhibited immune hyperreactivity resulting from cytokine amplification following SARS-CoV-2 infection[118]. Cytokine storms are a physiological reaction in humans, in which the innate immune system causes uncontrolled and excessive release of pro-inflammatory signaling molecules called cytokines. EXO-CD24 is moving into further testing phases[118].

Vaccination

Vaccination has generated significant interest among people in general, and researchers in particular. People with severe mental illness are at risk of SARS-CoV-2 infection because of the morbidity and mortality associated with COVID-19. Therefore, this population requires early access to safe and effective vaccines. However, further studies are needed to evaluate the efficacy, safety, and interactions of the vaccine with psychotropic drugs, specifically in patients with COVID-19, so that they can be properly informed about the benefits and risks of vaccination[119]. The rapid development of vaccines can have adverse effects, prompting long-term studies and years of post-vaccination treatment. However, for most treatments, the benefits outweigh the risks and many more people - and in most countries - need the vaccine, due to the collapse of economies and the subsequent crippling of livelihoods. With families losing large numbers of relatives to the virus, vaccination may help to restore the quality of life for hundreds of millions, if not billions, of people worldwide. Innovative strategies have improved the efficiency of processes within research models for novel therapies, ultimately accelerating the administration of potential novel treatments to patients [120]. Because the types or incidence of the side effects of COVID-19 vaccines in individuals with Parkinson's disease (PD) do not appear to differ from those observed in the general population, COVID-19 vaccination with approved vaccines can be recommended to patients with PD unless there is a specific contraindication. However, some caution is warranted in the vaccination of very frail and terminally ill elderly patients with PD living in LTC facilities[121].

The Standing Committee on Vaccination (STIKO) vaccination recommendations are recognized as medical standards. The current instructions for the vaccination of immunocompromised patients and the recommendation for COVID-19 vaccination, together with the scientific knowledge and rationale of STIKO, represent a valuable basis for medical action in the field of vaccination against infectious diseases[122]. To date, vaccine-related allergic reactions are rare. Current CDC reports suggest that anaphylactic reactions related to Pfizer-Bio-NTech mRNA vaccines may occur more frequently than with other vaccines. Therefore, to support large-scale COVID-19 vaccine delivery programs, allergists should offer clinical phenotyping, risk stratification, and clear recommendations based on reliable and credible information[123]. At present, there are insufficient data on COVID-19 co-infections with influenza or how these cases would evolve clinically, although they could place a significant burden on an already stressed healthcare system. Until an effective and proven COVID-19 vaccine is available, high influenza vaccination coverage should be the highest priority[124]. The obese population is vulnerable to COVID-19, requiring special attention during this pandemic to avoid complications. In the absence of COVID-19 vaccination, regular physical activity and a healthy diet are recommended, with special attention paid to mental health. Extended quarantine and prophylactic vitamin D administration should also be considered[125]. A study conducted in Australia reported that successful COVID-19 vaccination requires that the government consider elements in its vaccination policy such as the estimation of herd immunity thresholds, vaccine delivery strategies, vaccination clinic locations, provisions for health personnel and training, and strategies for prioritizing vaccines. Moreover, pharmacists should play a key role in the delivery of mass COVID-19 vaccination programs[126]. For the pediatric population, before a safe and effective COVID-19 vaccine is available, the focus should be on making the best use of already available childhood vaccines. Vulnerable or healthy children must be vaccinated according to

the recommended schedules to protect young patients and avoid future epidemics caused by vaccine-preventable diseases such as measles[127].

Independent groups of experts must be involved in life-saving actions to counter anti-vaccine propaganda and provide scientific information to the general public. If the pandemic is to be controlled to benefit the public interest, academic and medical societies and policymakers must speak the same language. Otherwise, the battle will be lost to those who oppose scientific evidence while offering no solution to the problem[128]. The key to success in promoting vaccine uptake is a strategic program, including local capacity building, to build and maintain trust. A critical factor in implementing such a confidence and demand-building approach is the need to invest in communication, especially related to influencing behaviors and the capacity for community engagement[129].

COVID-19 vaccines are expected to induce high-affinity neutralizing antibodies. They should also polarize the T-cell response towards type 1 immunity and avoid the stimulation of cytokines that induce T-helper 2 immunity. To avoid type 2 inflammatory responses, careful selection of the vector and antigen is mandatory. The addition of toll-like receptor ligands (TLRs) and other type 1 immunity-stimulating molecules could be useful for obtaining sufficient CD4+ T cells for antibody production as well as suppression of undesirable type 2 immunity leading to eosinophilia. However, it is only somewhat possible to predict vaccine efficacy and safety. Due to its urgency, COVID-19 vaccination should receive the highest priority[130].

Research

The COVID-19 outbreak is a striking reminder of the need for constant epidemiological surveillance, prompt diagnosis, and robust research[28]. Mapping of the structure of the SARS-CoV-2 spike protein at the atomic scale has allowed the development of therapies to combat the virus[131]. An international research effort is ongoing to understand the COVID-19 pandemic to answer questions regarding epidemiology, clinical epidemiology, biology, therapy, and vaccination. Researchers are also publishing the results of epidemiological, biological, and clinical trial studies in peer-reviewed journals. Moreover, clinical trials are underway to identify reliable, effective, and safe therapeutics[132-135]. Immunological and epidemiological data on endemic human coronaviruses (HCoV) showed that infection-blocking immunity wanes rapidly but that disease-reducing immunity is long-lived. In other words, anti-infectious immunity that prevents pathogen replication to render the host refractory to reinfection (*i.e.*, immune efficacy in relation to susceptibility), declines rapidly, while disease-reducing immunity due to reinfection and/or transmissibility-reducing immunity or infectiousness, with possible reinfection, lasts for a long time. This may be evidenced by the current severity of SARS-CoV-2 and the benign nature of HCoV, suggesting that once the endemic phase is reached and following primary exposure during childhood, SARS-CoV-2 may not be more virulent than the common cold. A different scenario is foreseeable for an emerging coronavirus capable of causing more severe disease in children. These results support the importance of behavioral compliance during pandemic vaccine use and suggest the need to evaluate scenarios for continued vaccination during the endemic phase[136].

CONCLUSION

The development of a robust prevention and/or response system relies on the capitalization and judicious use of knowledge in fields including epidemiology, infectious diseases, pathophysiology, biology, virology, in addition to scientific research. Hence, local or international collaboration in pluralistic teams may guarantee success. The dissemination of simple, coherent, and reliable messages reassures the population and reinforces compliance with individual or collective barrier measures (*e.g.*, confinement, even if it is difficult to bear). An integrative pluralistic approach coupled with efficient communication may be a more effective way of responding to an outbreak or pandemic, especially that caused by SARS-CoV-2. A pluralistic collaborative approach; in other words, the capitalization and judicious use of knowledge, will help to over-come the pandemic in the short or medium terms. Our results suggest the benefits of a pluralistic approach in managing COVID-19 a fortiori in relation to health and related fields. This work would be even more comprehensive if the search source was larger, the collaborative approach was more detailed, and the pluralistic approach extended to complementary disciplines such as biochemistry (vaccines), statistics, and mathematics, biomedical science and biotechnology, inventions (respirators, ven-

tilation equipment), physical therapy, *etc.*

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REVIEW

Animal models for SARS-CoV-2 and SARS-CoV-1 pathogenesis, transmission and therapeutic evaluation

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Abstract

There is a critical need to develop animal models to alleviate vaccine and drug development difficulties against zoonotic viral infections. The coronavirus family, which includes severe acute respiratory syndrome coronavirus 1 and severe acute respiratory syndrome coronavirus 2, crossed the species barrier and infected humans, causing a global outbreak in the 21stcentury. Because humans do not have pre-existing immunity against these viral infections and with ethics governing clinical trials, animal models are therefore being used in clinical studies to facilitate drug discovery and testing efficacy of vaccines. The ideal animal models should reflect the viral replication, clinical signs, and pathological responses observed in humans. Different animal species should be tested to establish an appropriate animal model to study the disease pathology, transmission and evaluation of novel vaccine and drug candidates to treat coronavirus disease 2019. In this context, the present review summarizes the recent progress in developing animal models for these two pathogenic viruses and highlights the utility of these models in studying SARS-associated coronavirus diseases.

Key Words: Animal models; SARS-CoV-1; SARS-CoV-2; COVID-19; Mice; Hamster;

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Core tip: In this review we discuss the importance of various animal models of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1). SARS-CoV-2 is the causal agent of coronavirus disease 2019 (COVID-19) and the World Health Organization declared the outbreak of COVID-19 as a public health emergency of concern. Due to the inadequate knowledge in analyzing the mode of action of COVID-19 infection, we must be thoroughly familiarized with the available animal models. Therefore, we discuss the pros and cons of various animal models, and emphasize the use of humanized mice to study the biology of viral diseases because it is convenient to mimic the human immune system in humanized mice.

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INTRODUCTION

The World Health Organization (WHO) declared the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) outbreak as an epidemic in November 2002 in China, where 8098 confirmed cases were reported, with 774 total deaths. Recently, a new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak in December 2019 in China. At the end of January 2020, WHO announced that SARS-CoV-2 was responsible for the coronavirus disease 2019 (COVID-19) pandemic, leading to a global health emergency of international significance. According to WHO, as of November 5, 2021, 249.48 million SARS-CoV-2 cases were confirmed in 223 countries with 5.05 million confirmed deaths, with a case mortality ratio of 2.2% and differential transmissibility rate R_0 was 1.5–5.5. Although the overall SARS-CoV-2 mortality rate is still low (3%), it has become one of the most rapidly spreading pandemics globally. The Coronaviridae family of viruses affects a wide range of animal species, and the infection range depends upon the type of host getting infected. There have been two major outbreaks caused by viruses belonging to the Coronaviridae family, SARS-CoV-1 and SARS-CoV-2. These viruses crossed the species barrier, adapted themselves to infect humans, resulting in an unprecedented and unexpected high fatality rate. SARS-CoV-1 and SARS-CoV-2 cause respiratory tract syndromes and can cause severe pneumonia among older adults[1]. Although both viruses share a similar mode of transmission and cause similar clinical symptoms [2], SARS-CoV-1 has a higher pathogenicity and mortality rate, whereas SARS-CoV-2 infection has a lesser mortality rate but is more contagious because of its high transmissibility[3]. SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor to enter the cells and infects the upper respiratory tract, and the infection then spreads to the lower respiratory tract. Viral replication continues, resulting in apoptosis of host cells, with loss of type I and II pneumocytes. The damage of alveolar epithelial cells leads to acute respiratory distress syndrome (ARDS). The infection results in a cytokine storm, and other immune cells are attracted as a host defense mechanism to clear the virus[4]. The complex pathophysiology of COVID-19 can only be understood by reproducing tissue-specific and systemic virus–host interactions, which can be studied using animal models.

Animal models are required to completely understand virus evasion strategies, disease etiology, and host responses. Both *in vitro* and *in silico* techniques can be used for examining the intricacies of the virus, especially at the molecular level. The immune responses playing key roles in the viral infection can be studied only in live models[5]. To reduce the risk to humans, animal models are used for the evaluation of vaccination and antiviral agents. The development of animal models should focus on

two key purposes: (1) To evaluate antiviral agents and vaccines; and (2) To characterize viral etiology[6]. The ideal animal models would reflect the pathology, clinical signs, and viral replication observed in humans[7]. A single model cannot reflect every feature of the virus infection; hence, different species are needed to study the various aspects of etiology. Before selecting an animal model for a virus infection, careful consideration is required since each species has its advantages and disadvantages based on the virus being studied. Therefore, researchers should select animals carefully[5]. This review provides a detailed comparison of the available animal models for the two human coronaviruses, SARS-CoV-1 and SARS-CoV-2. The lack of suitable small-animal models for studying the pathogenesis and development of vaccines and antivirals is one of the most serious obstacles to research progress. Several animal models have been used to study coronavirus infections and test the efficacy of vaccines and candidate therapeutic compounds. Reviewing animal models also has an important perspective of selecting rational animal models to evaluate drugs, vaccines and immune responses for tackling COVID-19.

DIFFERENCES AND SIMILARITIES BETWEEN SARS-CoV-1 AND SARS-CoV-2

The family of coronaviruses has been known for the associated risk of respiratory illness after the outbreak of SARS-CoV-1 in 2002 in Guangdong province, China, and the recent outbreak of SARS-CoV-2 in 2019 in Wuhan, China[8]. It is believed that SARS-CoV-2 originated from bats and was transmitted to humans *via* the seafood market in Wuhan. SARS-CoV-1 also originated in bats and was transmitted to humans from market civets[8]. SARS-CoV-1 is a beta coronavirus that belongs to lineages B and C[6]. As indicated by the genome groupings accessible to date, SARS-CoV-2 infection is caused by the strain BatCoVRaTG13, isolated from a bat in China's Yunnan region. Thus, SARS-CoV-2 is not an immediate relative of SARS-CoV-1[9]. Both the viruses are enveloped, nonsegmented, with a positive-strand RNA genome and a spherical shape, characteristic of species of the Coronaviridae family and order Nidovirales[6,10]. SARS-CoV-2 shares a total genome sequence similarity of 79.5% with SARS-CoV-1[11-13], whereas their spike proteins show a nucleotide similarity of 75% to 81%[13]. Both SARS-CoV-1 and SARS-CoV-2 bind to the host cell ACE2 with the help of the spike glycoprotein (S)[14,15]. S is a class I viral fusion protein; a trimeric protein that is proteolytically processed into two subunits S1 and S2[12]. The first difference between these two viruses is that the receptor binding domain (RBD) of SARS-CoV-2 has a higher affinity to ACE2 than that of SARS-CoV-1, making the former more infectious [16]. However, the binding affinity of the entire SARS-CoV-2 S protein to ACE2 is lower when compared to the entire S protein affinity of SARS-CoV-1[16]. Another critical difference is that SARS-CoV-2 RBD always remains in the lying-down position, leading to ineffective receptor binding[15].

In contrast, the SARS-CoV-1 RBD primarily exists in the upright position[17]. Although SARS-CoV-2 RBD is less accessible, it depends on a second strategy called host protease activation to maintain its high infectivity[3]. Another difference between SARS-COV-2 and SARS-CoV-1 is that the former has a furin-like cleavage site in the S protein[18,19]. S protein is cleaved by furin which is essential for cell–cell fusion and entry, whereas preactivation of furin enhances efficient transmission of SARS-CoV-2, allowing entry into host cells with low expression of transmembrane protease serine 2 (TMPRSS2) and cathepsins[19,20]. The SARS-CoV-1 S protein is cleaved at S1/S2 and S2 sites by host cell proteases such as TMPRSS2 and lysosomal cathepsins[17]. The presence of high arginine content at the S1/S2 site of SARS-CoV-2 results in higher cleavability than that observed in SARS-CoV-1[16]. In addition, inhibition of both proteases is required to block SARS-CoV-1 entry into the cells, whereas the blocking of TMPRSS2 is sufficient to inhibit viral replication[17]. Apart from these similarities and differences between SARS-CoV-1 and SARS-CoV-2, they share similarities in their pathogenesis. Both SARS-CoV-1 and SARS-CoV-2 cause host cell apoptosis, activation of immune cells, and an increase in the levels of inflammatory cytokines, leading to a cytokine storm. Finally, diffuse alveolar damage of alveolar epithelial cells has been reported in infections with both viruses, resulting in ARDS[21-23]. Damas evaluated the ACE2 diversity and its correspondence to human ACE2 in 410 vertebrates and developed a scoring system based on the 25 conserved amino acids. This study suggested that nonhuman primates are more susceptible, whereas rodents are less susceptible to the infection[24]. Table 1 list the available animal models for SARS-CoV-2.

Table 1 Comparison of available animal models for SARS-CoV-2 infection

No.	Animal model	Upper respiratory tract	Lower respiratory tract	Feces/Fecal swab	Contact transmission	Airborne transmission	Weight loss	Ref.
1	Cat (6 to 9 mo)	Infectious virus	Nil	Present	Not reported	33%	Not reported	[72]
2	Chicken	Nil	Nil	Not reported	Nil	Not reported	Not reported	[72]
3	Dog	Nil	Nil	Present	Nil	Not reported	Not reported	[72]
4	Duck	Nil	Nil	Not reported	Nil	Not reported	Not reported	[72]
5	Ferret	Infectious virus	Infectious virus	Present	100%	30%	Not reported	[72, 73]
6	hACE2 mouse	Not reported	Infectious titer	Infectious titer	Not reported	Not reported	Present	[57]
7	Hamster	Infectious titer	Infectious titer	Infectious titer	100%	Not reported	Present	[59]
8	Kitten	Infectious titer	Infectious titer	Not reported	Not reported	33%	Not reported	[72]
9	Macaque	Present	Present	Not reported	Not reported	Not reported	Nil	[80]
10	Pig	Nil	Nil	Not reported	Nil	Not reported	Not reported	[72]

MOUSE MODELS

Mouse models are preferred owing to their low cost, convenient husbandry requirements, and ease of availability. However, the drawbacks in using mouse models for human viruses are species tropism, species specificity, immune response factors, *etc.*[25]. Mouse models help us to study the host immune factors by promoting virus infection, making them important for identifying therapeutic targets and developing novel vaccine strategies[26]. Wild-type mouse models, knockout models, transgenic mice, and humanized mice are commonly used in animal studies to study pathogenic diseases, understand the role of specific genes in inhibiting or promoting the disease, and identify therapeutic targets[27].

Wild-type mouse models

BALB/c and C57BL/6 are the most preferred animal models for viral studies so far. However, when infected with SARS-CoV-2, these models showed no clinical signs, mortality, and weight reduction, and there was an absence of viremia. Viral RNA was detected in both types of mice in the lungs only on the first day, while the other organs did not show the presence of the viral RNA. These models tested negative for the anti-SARS-CoV-2 IgG antibodies[28]. These results suggest that BALB/c and C57BL/6 mice models remain uninfected when inoculated with SARS-CoV-2 due to the difference in the ACE2 receptor. Gu *et al*[29] used the mouse-adapted SARS-CoV-2 strain to infect BALB/c mice to overcome these difficulties, and once infected, the BALB/c mice showed inflammation and injury in both young and old mice[29]. Antibody blockade of interferon-α/β receptor alpha chain (IFNAR) in these mice resulted in weight loss and lung inflammation[30]. This study showed that old BALB/c mice were more prone to the disease than the younger ones and can be used to develop candidate vaccines. This was confirmed by the appearance of bronchiolitis in histopathological examination[29]. Likewise, Dinnon *et al*[31] remodeled the spike and RBD of SARS-CoV-2 (SARS-CoV-2 MA) to enable it to bind to the mouse ACE2 receptor[31]; thus, improving the virulence. Several passages were performed, and a virulent strain was generated at P10 (SARS-CoV-2 MA10). When young BALB/c mice were infected with a mouse adapted SARS-CoV-2 MA10 strain, it resulted in weight loss, diffuse alveolar damage, hyaline membrane formation, alveolar septal thickening, and neutrophil presence in alveolar space; whereas 100% mortality was observed in old mice after infecting them with 10^4 and 10^5 plaque-forming units (PFU) of SARS-CoV-2 MA10. However, when infected at 10^3 PFU, the old mice showed weight loss similar to that observed in the young mice, although only rare survival[32]. The infected mice showed inflammatory responses identical to those seen in humans[33]. For the vaccine

study, the Venezuelan equine encephalitis viral vector vaccine was developed to express SARS-CoV-2 S, nucleocapsid, and GFP reporter and primed in BALB/c mice. An initial dose and a booster dose were administered, and the mice were challenged with the SARS-CoV-2 MA10 strain, with the mice vaccinated with S showing neutralizing activity. However, the polyclonal sera had neutralization titers for both SARS-CoV-2 MA10 and SARS-CoV-2, which showed that SARS-CoV-2 MA10 could be used to test vaccine efficacy[32]. Similarly, C57BL/6J young and adult mice were infected with the SARS-CoV-2 MA10 strain.

In comparison to BALB/c mice, significant weight loss was observed with no mortality. Histological changes were observed to be similar in young BALB/c and C57BL/6J mice, but the acute lung injury scores were reduced in C57BL/6J mice[32], which may have been due to its dominant Th1 response, whereas BALB/c mice expressed a Th2 response dominantly[34]. Both BALB/c and C57BL/6J mice, upon infection with SARS-CoV-2 MA10, showed cellular tropism similar to humans, but instead of secretory cell infection, ciliated cells were infected in these mice[32]. Apart from all the other reported mouse models, this model showed limited use for studying alveolar disease pathogenesis. The SARS-CoV-2 MA10 model exhibited several COVID-19 symptoms, such as morbidity and mortality difference with age and host genetics, defects in lung function, and other etiologies[32]. These results show that both BALB/c and C57Bl/6J mice can be used to study mild SARS-CoV-2 MA10 strain, its etiology, and the efficiency of vaccines. Wild-type mouse models remain unaffected by SARS-CoV-2 due to their ACE2 receptor, so mouse-derived viral strains are required for further studies.

SARS-CoV-1, which is similar to SARS-CoV-2, has also been tested in these mouse models. In a study, 4–6-week-old female BALB/c mice were inoculated intranasally with 50 μL of diluted SARS-CoV-1 (Urbani strain). The microscopic examination showed mild and focal bronchiolitis[1]. Tseng *et al*[35] suggested that the viral doses of 103 and 105 median tissue culture infectious dose (TCID50) of the Urbani strain of SARS-CoV-1 were required for initiation of infection[35]. Upon infection, the mice did not develop pulmonary pathology, had no signs of clinical disease, and did not lose weight. Besides, the virus showed high levels of replication in the lower and upper respiratory tract without any symptoms[36]. Upon infection in BALB/c, 129WT and C57BL6 mice, SARS-CoV-1 did not show lethality, but it could replicate in the lungs 2 d post-inoculation (dpi)[2]. The BALB/c mice were also used for vaccine study by Du *et al*[37], where the RBD of SARS-CoV-1 S protein was fused with human IgG1 Fc (RBD-Fc), then injected into mice twice at 3-wk intervals and boosted once again after 1 year. In this study, neutralizing antibodies were found in the mice vaccinated with RBD-Fc, assuring protection from SARS-CoV-1 without any immunopathological damage[37]. However, SARS-CoV-1 replication was not efficient in wild-type mice due to a lack of efficient interaction between the spike protein (S) and murine ACE2[38].

Infection of immunocompetent mouse strain 129SvEv with SARS-CoV-1 showed infection in the conducting airway epithelial cells followed by clearance of the virus from the lungs, which later led to the development of self-limited bronchiolitis[1]. During clinical trials, the infection in young mice showed rapid virus clearance; however, weight loss was followed by several complications in older mice[7]. The 129S mouse strain was more susceptible than the BALB/c strain[1,6]. In addition, the 129S mouse strain showed pneumonitis and mild weight loss after SARS-CoV-1 infection [39]. In the case of weight loss in 129WT, Urbani SARS-CoV-1 virus infection led to morbidity[2]. Upon vaccination of 129S6/SvEv mice with the whole killed virus vaccine (adenovirus-based vaccine), viral replication was inhibited in the murine respiratory tract[40]. However, SARS-CoV-1 IgA antibody was detected in the sera of vaccinated mice, with the vaccine expressing both S protein and nucleocapsid protein (N)[40]. Thus, these studies explain that SARS-CoV-2 and SARS-CoV-1 cannot affect the inbred mouse models due to the difference in ACE2. However, these mice can be used for studying mouse-adapted strains of both viruses for the development of vaccines and antiviral drugs. Young models can be used to study the immune responses to infection, whereas old models can be used to study age-related diseases.

Knockout mouse models

The knockout models are devoid of certain specific genes to study the immune response involved in viral infections and are widely used to study the function of specific genes in inhibiting the disease[24]. Knockout models such as TMPRSS2$^{-/-}$ C57BL/6, IFNAR1$^{-/-}$hCD46, and STAT1 have been used so far to study SARS-CoV-2. The TMPRSS2$^{-/-}$C57BL/6 mice infected with SARS-CoV-2 showed reduced body weight loss and viral replication in the lungs. The absence of TMPRSS2 in the mice might have affected the priming of viral S protein and its subsequent fusion and thus

contained the virus spread within the mice, emphasizing the involvement of TMPRSS2 for the successful establishment of SARS-CoV-2 infection[41]. Knockout mice lacking *IFNAR1* gene but expressing human CD46 (IFNAR1$^{-/-}$hCD46), upon immunization with recombinant measles virus vaccine that expressed stabilized prefusion S protein (rMeV-preS), showed good antibody response[42]. The IFNAR$^{-/-}$mice, upon immunization with the same vaccine, showed an antibody response higher than that detected in human sera from convalescent COVID-19 patients[42]. Thus, the TMPRSS2 knockout model can be used to study pathogenesis, whereas STAT1 knockout mice can be used for study of both pathogenesis and antiviral drugs, but the IFNAR knockout model cannot be used for vaccine study due to its immunodeficiency.

In the case of SARS-CoV-1, the strains used were CD1 (Swiss outbred) and RAG1 (non-leaky SCID mice), which did not develop clinical disease[2]. However, STAT1$^{-/-}$ mice showed bronchiolitis and progressive weight loss[2]. These symptoms progressed to mediastinitis and interstitial pneumonia[2]. In these mice, the development of type 1 interferon (IFN) was indicative of control of SARS-CoV-1 infection, which showed viral replication on day 3. Rag1$^{-/-}$, CD1$^{-/-}$ and STAT1$^{-/-}$mice, which are in the 129S background, were tested against the immunological effectors of the disease. STAT1$^{-/-}$mice supported prolonged viral replication and histopathology similar to that observed in humans[2,6,36]. However, CD1$^{-/-}$mice (lacking natural killer cells) from B6 background, when infected with SARS-CoV-1, showed replication as observed in the lungs of B6 mice[1]. STAT1 knockout mouse models can be used to study the functions of cytokines in immune responses and IFN-mediated responses and analyze inflammation mechanisms[2]. When STAT1$^{-/-}$mice were infected with the same Urbani SARS-CoV-1 strain or with a recombinant isogenic mouse-adapted virus (rMA15), the infection could not be cleared even at 22 dpi[2]. After infection, STAT$^{-/-}$ mice initially lost 15% of their weight, followed by a 30% loss in weight, and the mice were moribund and paved the way for lethal infections[2]. Frieman *et al*[2] reported that epithelial cells of noncartilaginous conducting airways were the primary site of infection in STAT$^{-/-}$mice infected with SARS-CoV-1 of the Toronto-2 strain *via* intranasal inhalation (6×10^6 PFU/30 μL). The conducting airways of epithelial cells had focal intracellular aggregates[2], whereas 129 mice with type I IFN receptor knockout mice (IFNAR1$^{-/-}$) and type I/II double IFN receptor knockout mice (IFNAR$^{-/-}$) showed weight loss followed by morbidity after infection with the Urbani SARS-CoV-1 strain[2]. In ACE2 knockout mice, the copy numbers of S protein RNA were greatly reduced, and only a low number of infectious SARS-CoV-1 could be recovered from the lungs, showing that ACE2 is required for the effective replication of SARS-CoV-1[43]. Thus, STAT1 knockout mice can be used to study pathogenesis, whereas ACE2 knockout models can be used to study SARS-CoV-1-related ARDS. They are not suitable for vaccine and antiviral drug studies due to the immunodeficiency nature of knockout models.

Transgenic mice/genetically engineered models

Several transgenic models have been used widely for investigating the mechanisms related to viral pathogenesis[25]. The limitations of knockout mice in studying SARS-CoV-1 were overcome by developing transgenic mice that expressed human (h)ACE2 [34]. hACE2 transgenic mice may serve as a potential research model. To develop the model for SARS-CoV-1 and SARS-CoV-2 infection, an animal model of transgenic mice that expressed hACE2 had to be developed[44] as the severity of disease development in the transgenic mice model was correlated with the expression of hACE2[45]. The human *ACE2* gene was cloned and inserted into a plasmid, and the mouse ACE2 promoter was also retrieved and inserted upstream of hACE2 coding sequences. The fragments having *hACE2* gene driven by mouse promoter were microinjected into the pronuclei of fertilized mouse ova[44]. Increased expression of hACE2 indicated 100% mortality with severe lung and brain infection, while low levels of hACE2 caused illness without associated mortality[45]. The other requirement in developing the hACE2 mice was controlling the mice's ACE2 receptor that expressed hACE2, which would result in limited tissue distribution of hACE2, making the mice lethargic but surviving the infection. Even after survival, the mice showed interstitial pneumonia with extrapulmonary organ damage, which is indicative of the human model for coronavirus infection[45]. Likewise, using human cytokeratin (CK)18 as a promoter, transgenic mice expressing hACE2 were developed. The CK18 promoter helps efficiently express hACE2 in airway epithelial cells and other organs but not alveolar epithelia. K18-hACE2 mice showed alveolar dysfunction upon infection with SARS-CoV-1[46]. Infected mice showed evidence of perivascular and peribronchial inflammation and lung injury. An increase in the level of chemokines and cytokines was detected in the lungs of K18-hACE2 mice[46]. Extensive studies on this model showed

neuroinvasion by the virus, which started from an olfactory bulb and progressively spread to subcortical and cortical regions of the brain. However, this route of transmission could not be applied to the other infected regions that were not connected to olfactory bulbs[47]. These mouse models have been used for the study of vaccine development, etiology and therapeutics. The studies on SARS-CoV-1 and SARS-CoV-2 have shown that these viruses can infect mice expressing hACE2[44]. Inoculation of SARS-CoV-2 into the transgenic mice showed a reduction in weight, superficial and histological evidence of antibody responses, and lung inflammation, although lung injury was limited[48,49]. The reports on SARS-CoV-2 infection state a lower mortality rate compared to that of SARS-CoV-1[50]. Using hACE2 for further studies encountered whether the expression of hACE2 level and tissue distribution in mice could fully reflect the level and distribution in humans. The murine models usually have ACE2 expression in the bronchial epithelium, whereas humans generally have its distribution in the lungs[47,51,52]. The distribution of hACE2 also depends on the species. A better model for severe SARS-CoV-2 infection can be developed by targeted positioning of hACE2 into the endogenous mouse locus[50]. Using CRISPR/Cas9, hACE2 was inserted into the endogenous mouse ACE2 locus, and these mice were susceptible to SARS-CoV-2 infection and showed greater lung neutrophil infiltration with increasing age. Infection in these mice also occurred *via* the intragastric route[53]. Transgenic mice expressing hACE2 under the CK18 promoter showed an increased viral titer in the brain when infected intranasally by SARS-CoV-2 [54]. Remarkably, García-Arriaza *et al*[55] developed COVID-19 vaccines using modified vaccinia virus Ankara (MVA) as vectors, which expressed the entire SARS-CoV-2 spike protein (MVA-CoV2-S). Upon administration of one dose of this vaccine to k18-hACE2 models, the mice were protected from a lethal dose of SARS-CoV-2. After two doses of vaccine, the viral replication in the lungs was fully inhibited[55]. The same results were observed when the researchers used recombinant MVAs as vectors for delivering SARS-CoV-2 S protein in k18-hACE2 mice[56]. In a comparative study between SARS-CoV-1 and SARS-CoV-2 pathogenicity, SARS-CoV-2 was found to be milder than SARS-CoV-1 in the hACE2-expressing mice. In the case of SARS-CoV-1, extrapulmonary organ damage, cerebral vasculitis, and hemorrhage were observed. In the case of SARS-CoV-2, only interstitial pneumonia was observed. Viral replication was seen in both the upper and lower respiratory tracts. More studies are required in this knock-in hACE2 mouse model for a better understanding of the pathogenesis of the infection[57]. Following all the results available, it is inferred that the transgenic mice expressing hACE2 had a more severe infection when compared with wild-type mice. These mice are a better choice for testing the vaccine potential and antiviral drug efficiency when compared to all other available mice models. In addition, the use of mouse-adapted SARS-CoV-2 strains can be replaced with the use of hACE2-expressing mice.

HAMSTERS

Hamster ACE2 shows a large degree of genome sequence similarity to human ACE2 [58]. When golden Syrian hamsters were inoculated with SARS-CoV-2 *via* the nasal route, viral replication was observed in the lungs, along with the development of inflammation, massive leukocyte infiltration, marked lesions of lung congestion, necrotizing bronchiolitis, and necrosis[59]. Infected hamsters also infected the cohoused hamsters along with causing weight reduction in mice and an increased respiration rate[50]. Quantitative polymerase chain reaction (PCR) was used to measure inflammation in the lungs, which revealed a quick response of IFNs and an increase in interleukin (IL)-16 levels. However, lung pathology and the other symptoms were resolved at 14 dpi[50]. STAT2 knockout hamsters, when infected with SARS-CoV-2, displayed high viremia, lung titers, and systemic spread when compared to the wild-type models. This showed that STAT2 knockout mice exhibited limited systemic spread of the infection, whereas the knockout hamsters showed limited leukocyte infiltration, no pneumonia, and attenuated lung pathology. Transgenic strains of hamsters can be used to restrict systemic viral dissemination by studying the molecular pathways[59]. Monchatre-Leroy *et al*[60] conducted a comparative study between hamsters and ferrets using a single strain of SARS-CoV-2 for infection, which suggested that the hamster model was more relevant than the ferret model because of its systemic lung infection, less maintenance, and ease of supply[60]. When vaccinated for SARS-CoV-2 with the patient isolates of early passages, the Syrian hamster exhibited protection in a harsh challenge setup[61]. In another study, hamsters

immunized for SARS-CoV-2 with recombinant measles virus that expressed the perfusion S protein of SARS-CoV-2 (rMeV-preS) exhibited high levels of Th1-based immunity, proving that the recombinant attenuated vaccine could act as an efficacious bivalent vaccine[42]. The Th1-based antibody response can reduce the risk of antibody-dependent enhancement, which is a challenge in vaccine development. Hamsters have also been used in a study evaluating the protective efficacy and immunogenicity of the whole-virion inactivated vaccine candidates, namely BBV152A, BBV152B and BBV152C. These vaccine candidates, along with Algel adjuvant, either alone or chemisorbed with imidazoquinoline, were found to be safe in the preclinical tests on mice, rats and rabbits[62]. BBV152, when injected into hamsters, produced SARS-CoV-2-specific IgG and neutralizing antibodies 3 wk post-inoculation. In the other two candidates of this vaccine, neutralizing antibodies increased until 7 wk after SARS-CoV-2 challenge. However, this study had some limitations, including the cell-mediated immune response elicited by the vaccine candidates, which need to be explored further, along with the period of antibody response and the cross-neutralizing potential of the neutralizing antibody with other coronaviruses[63]. These results suggest that hamsters can be used as a model for vaccine studies. Similarly, a drug study was conducted in the golden Syrian hamster for SARS-CoV-2. When treated with a combination dose of methylprednisolone and remdesivir, the infected hamsters were relieved of the tissue inflammation, and viral replication was reduced in the early stages of infection[64]. In contrast, treatment with methylprednisolone alone prevented weight loss, reduced anti-RBD antibody development, and improved tissue damage and inflammation[65,66], but the tissue viral RNA loads and viral titers were observed to increase. Similarly, for the treatment of severe COVID-19, either the humanized monoclonal antibody tocilizumab (anti-IL-6 receptor) could be used against the IL-6 receptor[67], or anakinra (antagonist) could be used against the IL-1 receptor[68]. Thus, hamsters can be used to study the SARS-CoV-2 vaccine and antiviral drug efficiency, transmission, and immune response of the host.

For SARS-CoV-1, the golden Syrian hamster is preferred as a model as it exhibits viral loads and mild and transient pneumonia followed by pulmonary histopathology similar to those observed in humans[69]. Hamsters, when infected with SARS-CoV-1, showed high levels of viral replication in pulmonary tissues, severe interstitial inflammation, and pulmonary consolidation. The initial infection of SARS-CoV-1 can elicit strong neutralizing antibody responses, which protects the animals from subsequent infection[69]. In a study evaluating the immunogenicity and preventive efficiency in hamsters, the respiratory virus BHPIV3 was used as a vector to express SARS-CoV-1, and it was found that the S glycoprotein acted as a protective antigen and neutralizer against SARS-CoV-1. Thus, the preventive and high immune response against SARS-CoV-1 can be obtained by a single intranasal administration of recombinant vectors that express the S protein[70]. For SARS-CoV-1, recombinant measles virus vaccine conferred protection to immunized Syrian hamsters at viral titers of more than 100-fold; this vaccine encodes the unmodified SARS-CoV-1 S protein, which can induce high titers of neutralizing antibodies and IFN-γ T cell responses[61]. Together, these results suggest that golden Syrian hamsters can be used to study the transmission, drug efficiency, vaccine efficiency, and modeling mechanism for both SARS-CoV-1 and SARS-CoV-2, along with the study of host defense against severe infection. However, it is not used widely because of the lack of research tools, but it can act as a better alternative for transgenic mice models because the ACE2 of hamsters has a remarkable similarity to hACE2.

FERRETS

Ferrets are commonly used animal models for viruses causing respiratory illness in humans. Ferrets can be used for both viral transmission and pharmacological studies. They are also used to study mucoviscidosis[71]. Ferrets are more susceptible to SARS-CoV-2 infection compared to dogs[72]. Ferrets also show the same symptoms as humans after inoculation with SARS-CoV-2, like elevated temperature suggestive of pyresis, coughing between 2 and 12 dpi, reduced activity, and loss of appetite[72,73]. In ferrets, replication occurred in the soft palate, nasal turbinates, tonsils, and digestive tract, while the virus was absent in the lung lobes, even when inoculation was intratracheal[74]. Severe pulmonary lymphoplasmacytic perivasculitis and vasculitis were detected in the lungs of infected ferrets when observed histologically[72]. The viral shedding profile of ferrets resembled that of asymptomatic human patients who efficiently transmit SARS-CoV-2[75]. Ferrets were infected with SARS-CoV-2 and

treated with certain FDA-approved antiviral drugs, which revealed that emtricitabine–tenofovir showed antiviral efficacy in the respiratory and gastrointestinal tract[76]. Thus, the ferret is a suitable animal model for studying mild and asymptomatic SARS-CoV-2 infection, transmission, and pathogenesis.

When ferrets were inoculated with SARS-CoV-1, a subset showed clinical illness, while the remaining animals did not show infection[77]. The ferret models were characterized by higher cytotoxicity in the upper respiratory tract with fever and sneezing associated with histological changes in the lungs, including lymphohistiocytic bronchopneumonia[69,78]. Viral replication was not detected in the lower respiratory tract in the ferrets, making it a candidate model for antiviral and vaccine testing[79]. Naïve ferrets were used for studying viral transmission by placing them in direct and indirect contact with infected ferrets. Ferrets left in direct contact showed symptoms of infections at 2–6 dpi, whereas those left in indirect contact remained asymptomatic, with only some ferrets showing viral RNA indicating transmission *via* air. Ferrets can also be used to study the immune responses against infection[50,73]. Thus, ferrets can be used to study transmission, immune response against infection, and effect of antivirals and vaccines for both SARS-CoV-1 and SARS-CoV-2.

NONHUMAN PRIMATES

Among the various nonhuman primate models for SARS-CoV-2, cynomolgus macaques and rhesus macaques are used the most, and the common marmoset has shown resistance to infection[48,80,81]. For SARS-CoV-2, the most convincing model that has been suggested by Yu *et al*[81] is the rhesus macaque, which, when inoculated intratracheally, orally, intranasally, and in both eyes, showed asymmetrical breathing patterns and tachypnea in a few animals, suggesting a certain degree of ARDS development[81]. Since age is said to be the major threat factor for COVID-19, mature rhesus macaques (15 years old) were compared with younger macaques (3–5 years old), and an increase in viral load at 7 dpi was seen in the older animals[81]. Thus, aged rhesus macaques can be used as a model for acute disease. Another study was conducted with rhesus macaques on the development of protective immunity after the initial infection. Two animals were inoculated intratracheally and then again after 28 d. Bao *et al*[48] observed the development of protective immunity in macaques with the lack of viral shedding[48]. Rhesus macaques were used for studying the BBV152 vaccine (Covaxin). The animals were given two doses of vaccine at an interval of 14 d and then challenged with SARS-CoV-2. SARS-CoV-2-specific IgG and neutralizing antibodies were produced, showing the protective efficacy of the vaccine. Virus clearance was observed at 7 dpi in the macaques, and this vaccine is now in phase III of its trial[82]. A comparative study on the etiology of SARS-CoV-2 and SARS-CoV-1 was conducted in nonhuman primates, and it was found that cynomolgus macaques remained uninfected after inoculation with SARS-CoV-2. This model shed the virus for an extended period, and the virus was capable of replicating efficiently in both the upper and lower respiratory tract of the model. This model can be used for studying the etiology of SARS-CoV-2 and the analysis of therapeutic approaches to the disease [80]. SARS-CoV-2 was inoculated in both young and mature cynomolgus macaques. The lesions showed pulmonary alveolar edema, formation of hyaline membrane, and other signs of acute lung injury[80]. Koo and workers observed acute interstitial pneumonia with endotheliitis in both rhesus and cynomolgus macaques infected with SARS-CoV-2[83]. These observations showed that cynomolgus macaques could be used as a model for studying the mechanism of severe SARS-CoV-2 infection, and rhesus macaques can be used to study the etiology, immune response, and vaccine efficiency.

Based on previously published studies, SARS-CoV-1 was reported to infect old and new world monkeys, including common marmosets, squirrel monkeys, rhesus macaques, mustached tamarins, cynomolgus macaques, and African green monkeys [69]. SARS-CoV-1 infections in these nonhuman primates showed symptoms such as diarrhea, fever and pneumonitis[69]. The pneumonitis, which was observed in each species, varied with the inoculum dose and route[7]. The highest viral replication was seen in the cynomolgus monkeys followed by African green monkeys, with the findings affected by many factors, including dose, age, route of infection, animal source, inoculation of the virus, and history of the environment[38]. The SARS-CoV-1 Urbani strain showed mild symptoms followed by infection in cynomolgus macaques, rhesus macaques and green monkeys[69]. Replication of SARS-CoV-1 did not occur in mustached tamarins and squirrel monkeys[6]. African green monkeys, when

immunized with recombinant attenuated parainfluenza virus (BHPIV3) that expressed the SARS-CoV S protein, showed the production of SARS-CoV neutralizing serum antibodies, indicating the effectiveness of mucosal immunization[84]. Thus, nonhuman primates can be used to study age-related effectiveness, pathogenesis, and vaccines for both SARS-CoV-1 and SARS-CoV-2. However, they are not used largely because their maintenance and handling are difficult and not available easily.

CATS

Domestic cats were found to test positive for both SARS-CoV-1 and SARS-CoV-2, which were presumed to be infected by their owners[85]. Cats can be infected experimentally with SARS-CoV-1 and SARS-CoV-2, and they show pulmonary changes, viral shedding, and infection similar to humans[86]. The cats inoculated with SARS-CoV-1 *via* the intranasal route showed viral replication in the lungs followed by pneumonitis[38]. Rudd *et al*[87] found that when cats were intratracheally infected with SARS-CoV-1, they showed pulmonary disease with diffuse alveolar damage[87]. They also observed predominant clinical signs, including fever, cough, lethargy, and increased respiratory effort in the cats inoculated intratracheally with SARS-CoV-2. They also found pulmonary lesions such as diffuse alveolar damage and evidence of vascular injury[85]. In another study, cats infected with SARS-CoV-1 developed pulmonary lesions, and active infection and shedding were also observed, which were similar to those occurring in humans. However, they also developed tracheo-bronchoadenitis, which has not been reported in humans[77]. The infected cats were also capable of transmitting the virus to other cats[86]. In the case of SARS-CoV-2, Zhang *et al*[79] infected 8-month-old cats intranasally and found infectious virus in the upper respiratory tract, small intestine, and feces. The same symptoms were observed in 14-week-old kittens, and they also showed histopathological changes suggesting that infection is more severe in younger cats. The mode of transmission of SARS-CoV-2 from infected cats to adjacent uninfected cats could be through feces or respiratory droplets[72]. Likewise, another study on transmission was done on three cats that were inoculated with SARS-CoV-2 and cohoused in pairs with uninfected cats after 1 d of inoculation. After 1 dpi, the shedding of viral particles was confirmed from the inoculated cats that infected the cohoused cats as the shedding of virus from the inmates was recorded after 3–5 d, ensuring the transmission of SARS-CoV-2. However, none of the cats showed clinical signs and no virus detection in the rectal swabs, although all cats developed antibodies[88]. In the United States, zoo-housed tigers and domestic cats belonging to the Felidae family were also positive for SARS-CoV-2[38]. Finally, all these studies prove that cats are susceptible to SARS-CoV-2 and SARS-CoV-1 infection and its capability to develop neutralizing antibodies, which protected them from reinfection[86]. More studies on cats are required to develop medicines for veterinary animals[50]. Further studies must be done on domestic cats to study transmission crossing the species barrier, and the studies should be focused on specific antibody production in cats.

ANIMALS INFECTED MINIMALLY: DOGS, CHICKENS, PIGS AND TREE SHREWS

Few results are available for infection in tree shrews, pigs, chickens and dogs, none of which have shown signs of COVID-19, except for dogs displaying shedding of virus in feces[72]. No respiratory disease was seen in domestic dogs with positive PCR results. Live virus isolation and viral RNA detection were reported for one dog, although there was no transmission to other dogs in the same household[89]. Also, no infection has been observed in pigs or their cell lines[90]. Similarly, chickens are found to be resistant to SARS-CoV-1 and SARS-CoV-2. Chickens inoculated with the virus showed viral RNA, but it was not possible to isolate the replicating virus from them[91]. Chickens also did not transmit the infection to cohoused chickens[74]. When embryonated eggs were injected with these viruses, no replication was observed[72, 92]. The same results were reported for tree shrews, with no clinical signs except for an increase in temperature that was observed only in females[93]. Based on the evidence mentioned above, these animals are not preferred for SARS-CoV-1 and SARS-CoV-2 related studies.

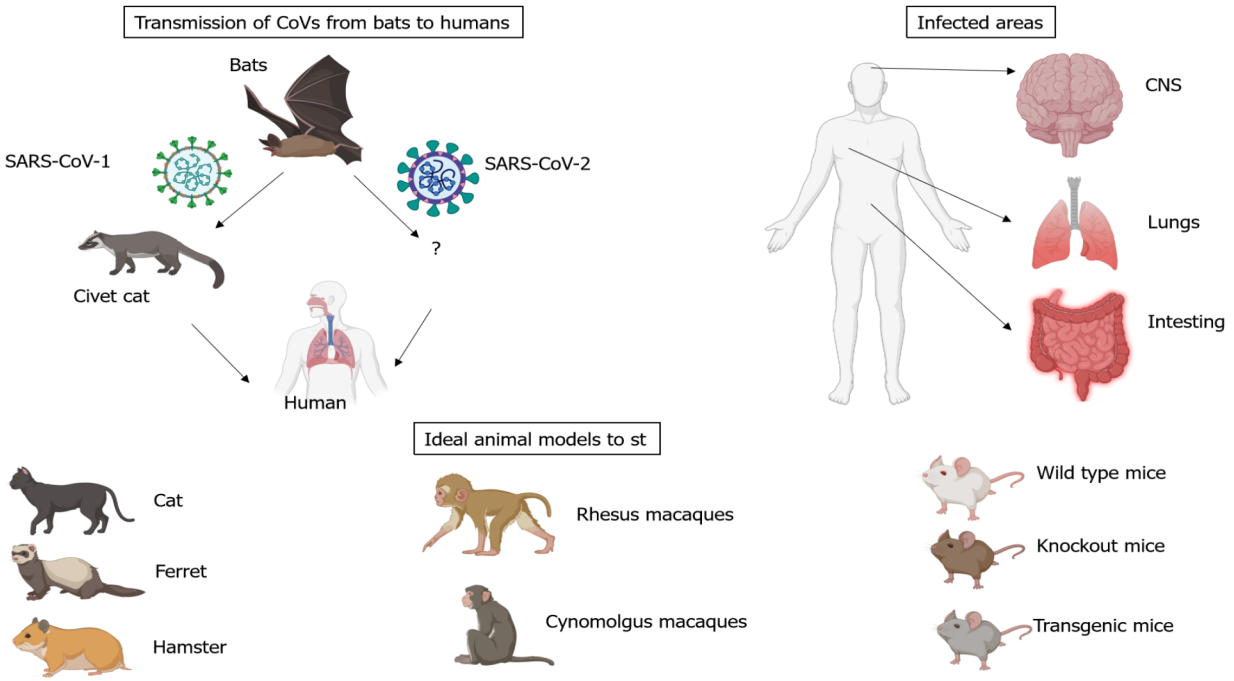

Figure 1 Searching for an ideal animal model to study COVID-19 transmission and pathogenesis. CNS: Central nervous system.

WILDLY CAUGHT POSITIVE ANIMALS

During the disease outbreak, a few wild animals were found to be positive for SARS-CoV-2, including the *Rhinolophus* and *Hipposideros* species of pangolins, bats, palm civets, bamboo rats, raccoon dogs, hog badgers, and hedgehogs[94]. In the Netherlands, some studies on farms revealed that the strain passed from humans to mink, spreading to other humans and the mink population. Viral RNA was detected in air–dust particles in mink farms[95]. Respiratory diseases were detected in infected mink, with interstitial pneumonia, lung inflammation, and little mortality. This shows that minks can serve as a more nuanced model than ferrets, but controlled studies would be needed. Infected lions and tigers showed loss of appetite and respiratory symptoms, but they recovered[96]. From these observations, it is clear that more measures should be taken to stop the transmission of SARS-CoV-2 to other species and to protect the wild animals.

CONCLUSION

Animal models for SARS-CoV-2 and another human coronavirus are used extensively. Animal models that accurately reproduce the severe COVID-19 symptoms exhibited by humans are required to design novel therapeutic approaches. The existing animal models are currently preferred (Figure 1), but efforts must be made to assess them with proper *in vitro* experiments and generate reliable scientific data before being put into use. It is impossible to study the etiology, transmission, therapeutic approaches, drug treatment, and vaccine development in a single animal model due to their inborn differences. Furthermore, there are many differences in biology, behavior, genetics, adaptability, and receptor expression level; all of which influence the infection rate. Thus, various animal models are required to develop a good understanding of the disease and obtain better results. The preferred animal model for each study would depend on reproducibility, efficacy, etiology, *etc.* Laboratory mice and hamsters are preferred due to their ease of availability, easy handling, low cost, small size, and possibility of manipulation at the genetic level. In SARS-CoV-2, cynomolgus macaques and rhesus macaques are better models than all other models discussed. Based on available studies, Lakdawala and Menachery[72] suggested that hamsters, ferrets and cats can serve as alternatives for nonhuman primates and transgenic mouse models [72]. Cats and ferrets can be used as models for studying the transmission and effectiveness of antivirals to limit viral spread. The nonhuman primates that showed reduced viral loads and hamsters that produced neutralizing antibodies and specific

immune responses can be used as models for evaluating the effectiveness of vaccines and antivirals before deployment to humans[72]. For SARS-CoV-1, all nonhuman primate models are suggested to be the best, and among them, ferrets and hamsters are the preferred ones. Various studies have been done on the neuroinvasive capacity of SARS-CoV-2, revealing that SARS-CoV-2 can directly infect neural cells and cause neurological symptoms. These have also given the strategy of using human brain organoids to study SARS-CoV-2 effects on the central nervous system[54,97-99]. Nonetheless, more studies must be conducted in animal models to study the neuroinvasive mechanisms. The recent pandemic is a major threat to global human health, and to overcome this situation, RNA virus-inactivating drugs, and broad-range vaccines are needed. Hydroxychloroquine, remdesivir, and lopinavir/ritonavir are under evaluation for COVID-19 treatment as multiple-target direct antivirals[100]. Developing more antivirals and vaccines against various viruses requires complete information on virus replication and etiology, which requires a detailed study on animal models before testing on humans.

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MINIREVIEWS

Chronic hepatitis B: New potential therapeutic drugs target

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Abstract

Chronic hepatitis B (CHB) infection remains the most causative agent of liver-related morbidity and mortality worldwide. It impacts nearly 300 million people. The current treatment for chronic infection with the hepatitis B virus (HBV) is complex and lacks a durable treatment response, especially hepatitis B surface antigen (HBsAg) loss, necessitating indefinite treatment in most CHB patients due to the persistence of HBV covalently closed circular DNA (cccDNA). New drugs that target distinct steps of the HBV life cycle have been investigated, which comprise inhibiting the entry of HBV into hepatocytes, disrupting or silencing HBV cccDNA, modulating nucleocapsid assembly, interfering HBV transcription, and inhibiting HBsAg release. The achievement of a functional cure or sustained HBsAg loss in CHB patients represents the following approach towards HBV eradication. This review will explore the up-to-date advances in the development of new direct-acting anti-HBV drugs. Hopefully, with the combination of the current antiviral drugs and the newly developed direct-acting antiviral drugs targeting the different steps of the HBV life cycle, the ultimate eradication of CHB infection will soon be achieved.

Key Words: Chronic hepatitis B; Hepatitis B surface antigen; Hepatitis B surface antibody; Covalently closed circular DNA; Direct acting antiviral drugs; Functional cure; Entry block; Nucleocapsid assembly modulator; Interfering hepatitis B virus transcription; Inhibiting hepatitis B surface antigen release

Core Tip: Current treatment of chronic hepatitis B infection with nucleos(t)ide analogs causes long-term suppression of hepatitis B virus (HBV) DNA levels, significantly

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improving hepatocellular injury and extrahepatic complications. However, the risk of hepatocellular carcinoma remains increased. New direct antiviral drugs that target the HBV life cycle, including entry blockers, assembly modulators, covalently closed circular DNA (cccDNA) disruptors, and hepatitis B surface antigen release inhibitors, would lead to hepatitis B surface antigen loss and a functional cure. Moreover, a combination of antiviral drugs with an immune-modulator could enhance the elimination of cccDNA and provide a definitive cure.

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INTRODUCTION

Chronic hepatitis B (CHB) virus infection is a significant public health problem and causes substantial morbidity and mortality. It affects more than 257 million people worldwide, and the first-ever global hepatitis report published in 2017 indicated that in 2015, 887000 persons died from cirrhosis and hepatocellular carcinoma (HCC)[1,2]. The cumulative incidence of CHB infection in children less than 5-years-old fell from 4.7% in the pre-vaccine era to 1.3% in 2015. This reduction in incidence is attributable to progress in immunization coverage. Although we have effective vaccines and potential antiviral drugs to treat CHB patients, the mortality rate of CHB infection still increased over the last 10 years.

Antiviral drugs, such as pegylated-interferon (Peg-INF)-α-2a, Peg-INF-α-2b, Peg-INF-α-1b, and nucleoside or nucleotide analogs (NAs), have been used to treat CHB patients. They strongly suppress HBV replication and slow progression to cirrhosis and HCC. A limitation of the current treatments is the low rate of serological responses because covalently closed circular DNA (cccDNA) persists in the hepatocyte nucleus[3,4]. Hepatitis B surface antigen (HBsAg) loss is uncommon with current therapies, causing the majority of CHB patients to need indefinite therapy. The IFN treatment produces a higher rate of HBsAg loss, but most patients cannot tolerate the adverse events caused by it.

The combination of Peg-IFN and NAs may synergize the treatment effect to enable more CHB patients to achieve HBsAg loss[5,6]. However, a recent randomized controlled, open-label trial did not support the advantage of a combination of Peg-IFN and NAs in CHB patients[7]. Moreover, the patients also need frequent clinical and laboratory monitoring. Numerous clinical trials of drugs that interrupt the HBV life cycle in hepatocytes have been conducted. The novel agents for HBsAg loss include the direct-acting antiviral drugs targeting the different steps of the HBV life cycle and the indirect antiviral drugs modulating host immune response to eradicate HBV[8,9].

This review will address the newly investigated therapeutic drugs, and the results of clinical trials that aim to cure HBV.

HBV GENOME AND LIFE CYCLE

HBV is a small virus of the *Hepadnaviridae* family which infects hepatocytes, replicates, and persists in the nucleus. HBV particles include the HBV genome, nucleocapsid, and envelope proteins. The HBV genome is partially double-stranded DNA, with approximately 3200 base pairs that form a relaxed circular DNA (rcDNA) genome. The minus (-) strand is the longer-strand DNA which complements pre-genomic RNA (pgRNA). The plus (+) strand is the shorter-strand DNA. The minus (-) strand has four overlapping open reading frames (ORFs), consisting of PreC/C, P, PreS/S, and X. The PreC/C ORF encodes the hepatitis B e antigen (HBeAg) and hepatitis B core antigen (HBcAg). The P ORF encodes the HBV DNA polymerase. The PreS/S ORF encodes the large (L), the middle (M), and the small (S) envelope proteins. The X ORF encodes the X protein (HBx)[10].

The large envelope protein contains the receptor-binding domain and is involved in viral entry into the cytoplasm by receptor-mediated endocytosis. This process involves the sodium taurocholate co-transporting polypeptide (NTCP) receptor in the hepatocyte membrane. After attachment, two pathways for cell entry include endocytosis and fusion of the HBV envelope with the plasma membrane[11]. Then, individual rcDNAs are modified into cccDNAs, packaged into chromatin by histone and non-histone proteins[12]. The cccDNAs are responsible for viral persistence in the nuclei of infected cells. These cccDNAs also use pre-C mRNA and all other sub-genomic mRNAs that code for the main viral proteins.

An HBe protein is translated from the pre-C mRNA transcripts, which have a longer reading frame than HBc protein, and is finally secreted into the bloodstream as HBeAg, the immunoactive biomarker for HBV infection[13]. This replication cycle is concomitant with the release of incomplete sub-viral particles and infectious viral particles. The most abundant of these exported sub-viral particles are particulate forms of viral envelopes formed with such HBs proteins as HBs antigen (HBsAg), a primary immunoactive biomarker for HBV infection in conjunction with HBeAg.

The encapsulated mRNAs are known as HBcAg, which can be detected in serum, even when HBV DNA cannot.

CURRENT ANTIVIRAL DRUGS AGAINST HBV

Currently, two different therapeutic strategies have been approved to treat CHB patients. These included IFN-α or Peg-IFN-α and direct-acting antivirals comprised of NAs that include nucleoside analogs, lamivudine (LAM), telbivudine (LdT) and entecavir (ETV), or NAs adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF)[14,15].

IFNs

IFN-α suppresses viral DNA synthesis by stimulating antiviral enzyme production, which results in the clearance of infected hepatocytes, enabling a proportion of CHB patients to achieve a sustained virologic response (SVR). Several studies have demonstrated that IFN-α exhibits an SVR of up to 37%, with a mean loss rate of 33% in HBeAg and 8% in HBsAg. However, other factors influencing SVRs following IFN-α treatment comprised low serum levels of HBV DNA, early infection, treatment-naïve status, HBV genotypes, pre-core HBV mutation detection, chronicity, and co-infection with human immunodeficiency virus (commonly known as HIV)[16]. Due to its limited efficacy, low SVRs, and frequent injections, IFN-α has been replaced with the long-acting Peg-IFN-α.

Peg-IFN-α could prolong the effective half-life of IFN-α, reduce functional dose levels, increase efficacy, and lower side effects[17]. However, randomized clinical trials suggest that Peg-IFN-α effects are better in CHB patients who are HBeAg-positive than in those who are HBeAg-negative. Long-term treatment with Peg-IFN-α in CHB patients with HBeAg-positive status led to viral suppression in 10%–40%, HBeAg loss in 30%–35%, and normalization of alanine aminotransferase (ALT) levels in 35%–50%. Moreover, an HBsAg loss was established in 5% of patients 6 mo after stopping treatment and 10% of patients 3 years post-treatment[18]. Unfortunately, the benefits of Peg-IFN-α treatment vary with patient geographical distributions and HBV genotype, resulting in it not being effective in all CHB patients[19,20-22].

Nucleosides or NAs

The NAs are the small molecule drugs that directly inhibit the HBV DNA polymerase reverse transcriptase activity, resulting in reduced virion production[23]. Moreover, they also compete with natural nucleotide substrates for the elongating DNA chain, interrupting HBV DNA synthesis[24]. There are six NAs approved for CHB treatment: LAM, ADV, ETV, LdT, TDF, and tenofovir alafenamide (TAF). Long-term treatment with NAs can reduce the cccDNA pool in hepatocytes infected with HBV by inhibiting nucleocapsid recycling. However, they cannot prevent the initial cccDNA formation in newly infected hepatocytes[25].

The first generations of NAs are LAM, ADV, and LdT. The NA approved by the United States Food and Drug Administration in 1998 for the treatment of CHB is LAM, which can compete for cytosine in the synthesis of viral DNA. The CHB patients who were treated with 100 mg LAM for 104 wk achieved 52% virological response. However, after 5 years of treatment, approximately 70% of the patients developed LAM resistance[26,27]. ADV, a phosphonate acyclic NA of adenosine monophosphate,

was approved in 2002. In 2003, Marcellin *et al*[28] reported that after 48 wk of 10 mg ADV treatment in HBeAg-positive CHB patients, 53% had histologic improvement, 21% had undetectable serum levels HBV DNA, and 12% had HBeAg seroconversion. Furthermore, Hadziyannis *et al*[29] demonstrated that after 48 wk of 10 mg ADV treatment in HBeAg-negative CHB patients, 64% had histologic improvement and 51% had undetectable serum levels of HBV DNA. However, long-term treatment with ADV also results in a high drug resistance rate of nearly 30% after 5 years of treatment [30]. LdT, the unmodified β-l enantiomer of thymidine, was approved for CHB treatment in 2007[31]. In 2009, Liaw *et al*[32] reported that LdT was superior to LAM in patients with CHB. They found that the rates of therapeutic response in HBeAg-positive and HBeAg-negative patients treated with 104 wk of LdT compared with LAM were 63% *vs* 48% and 78% *vs* 66%. However, long-term treatment with LdT led to nearly 35% drug resistance after 3 years of therapy[33].

ETV, TDF, and TAF are second-generation NAs with a high genetic barrier to HBV resistance. They are used as the first-line drugs for CHB treatment. In 2005, ETV, a guanosine NA with selective activity against HBV, was launched. The effective concentration (EC_{50}) of ETV is 4 nM. This EC_{50} is 100-fold more potent than ADV or LAM in HBV suppression[34]. In 2016, Ahn *et al*[35] reported that ETV had shown durable and increasing viral suppression in 84.6% of HBeAg-positive patients and 96.2% of HBeAg-negative patients over 5 years of treatment. However, the cumulative probability of HBsAg loss at year 5 was 5.2% in HBeAg-positive patients and 4.6% in HBeAg-negative patients. TDF, an acyclic NA with activity against retroviruses, was approved for CHB treatment in 2008. Buti and colleagues[36] reported that 437 patients remained on the study at year 7; among them, 54.5% and 11.8% achieved HBeAg and HBsAg loss in HBeAg-positive patients but only 0.3% of the HBeAg-negative patients achieved HBsAg loss. Although TDF resistance is relatively low, it has been associated with dose-dependent renal toxicity and induced Fanconi syndrome[37,38]. Recently, TAF was approved to be an alternative to TDF because it caused fewer side effects and was suitable for the treatment of CHB patients at risk of renal dysfunction[39]. Moreover, TAF has been demonstrated to be more effective than TDF with continued improved renal and bone safety[40].

Combination of NA plus Peg-IFN-a

Although the current monotherapy of anti-HBV drugs can suppress viral replication, prevent the progression of CHB to cirrhosis, and decrease the rates of HBV-related HCC in most CHB patients, long-term anti-HBV monotherapy rarely achieves the higher rate of HBsAg loss. Hence, to accomplish the goal of a functional cure in more CHB patients, the combination of NA with Peg-IFN-α has been evaluated. The reason for this is that the two classes of anti-HBV drugs have different mechanisms of action. Thus, their combination would result in a synergistic anti-HBV effect. Several studies have demonstrated that the combination of NA with Peg-IFN-α can substantially enhance the rates of HBsAg loss, but the benefits are mainly limited to a small proportion of patients and depend on HBV genotype and patient geographical distributions[41-44]. Moreover, NAs and Peg-IFN-α treatment have no direct impact on viral transcription or cccDNA. Thus, there is a very high risk of reactivation of HBV and the emergence of downstream disease symptoms after stopping treatment. Therefore, new therapeutic drugs that target different HBV life cycle steps or modulate the host immune system are needed.

NEW DRUGS TARGETING HBV LIFE CYCLE

HBV entry inhibitors

Bulevirtide (Myrcludex B): NTCP has been demonstrated as a functional receptor for HBV entry into hepatocytes[11]. Therefore, the new drugs targeting viral entry receptors have been proposed as potential agents for preventing uninfected hepatocytes. Bulevirtide (Myrcludex B) is a synthetic lipopeptide of 47 amino acids obtained from the HBV preS1 domain. When bulevirtide binds to NTCP, it will effectively prevent HBV spread among intrahepatic cells and hinder the amplification of intrahepatic cccDNA pool in infected hepatocytes[45,46].

In 2016, Blank *et al*[47] conducted a prospective, open-label, first-in-human, phase 1 clinical trial in 36 healthy volunteers. They found that bulevirtide was well tolerated, with no serious side effects and no immunogenic effects up to the highest dose of 20 mg intravenously. Moreover, the pharmacokinetic model showed that 10 mg and above of bulevirtide subcutaneous injection could reach a target saturation of over 80%

for at least 15 h. Furthermore, Blank *et al*[48] conducted a study to investigate the effects of bulevirtide on plasma bile acid disposition, TDF pharmacokinetics, and perpetrator characteristics on cytochrome (CYP) P450 3A in 12 healthy volunteers. All of the volunteers received 300 mg TDF orally and 10 mg of subcutaneous bulevirtide. They found that bulevirtide increased total plasma bile acid by 19.2-fold without signs of cholestasis, and co-administration of TDF with bulevirtide revealed no relevant changes in TDF pharmacokinetics.

Recently, Wedemeyer *et al*[49] conducted a phase 2b clinical trial in 60 patients with chronic HBV/hepatitis D virus (HDV) co-infection. They randomized 1:1:1:1 into the following four groups: Peg-IFN-α once-weekly (qw) (n = 15, Arm A); bulevirtide 2 mg once daily (QD) subcutaneous (sc) injection + Peg-IFN-α qw (n = 15, Arm B); bulevirtide 5 mg QD sc + Peg-IFN-α qw (n = 15, Arm C); or bulevirtide 2 mg QD (n = 15, Arm D) for 48 wk. They found that HBsAg levels declined by more than 1 Log_{10} in 6/15 (40%) and 2/15 (13.33%) patients from Arm B and Arm C, respectively. Notably, 4/15 (27%) patients from Arm B had undetectable HBsAg levels, and 3/4 (75%) patients established HBsAg seroconversion. Bulevirtide is moving along to phase 3 studies, whereby monotherapy extended or in combination with Peg-IFN-α will be investigated in CHB patients (Table 1).

cccDNA disruptors

The cccDNA plays a crucial role in the viral life cycle, where it acts as the template for viral transcription, while pgRNA is the template for viral replication. It interacts with histone and non-histone proteins, resembling cellular chromatin within the nucleus [50]. Disruption of cccDNA is considered an optimal target of HBV treatment because its persistence in the nucleus of infected hepatocytes is the crucial reason why HBsAg loss is currently not possible. The blocking of cccDNA formation, enhancing its destruction, and silencing its transcription, are currently under exploration.

Gene editing: The four ORFs of the HBV genome (surface, core, polymerase, and X protein) are translated into seven essential proteins for viral replication. The blocking of any one of the seven proteins would likely be essential to inhibit viral gene expression. Several small molecules have been developed as sequence-specific RNA-guided (gRNA) nucleases and proteins which can block the formation, enhance the destruction, and silence the transcription of cccDNA, while stimulating cell division [51]. These comprise cleaving sequence-specific DNA targets using the transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats-associated 9 (CRISPR/Cas9) systems that could demonstrate antiviral efficacy[52-54].

In 2014, Lin *et al*[55] demonstrated that the CRISPR/Cas9 system could disrupt the HBV genome both *in vitro* and *in vivo*. They showed that the HBV-specific gRNAs significantly decreased the production of HBV core and HBsAg in Huh-7 cells transfected with an HBV-expression vector. They also reported that the CRISPR/Cas9 system could cleave the intrahepatic HBV genome-containing plasmid and facilitate its clearance *in vivo*, causing a reduction in serum HBsAg levels. In 2015, Kennedy *et al* [56] reported the effective inhibition of HBV DNA production in *in vitro* models of both chronic and *de novo* HBV infection using lentiviral transduction of a bacterial Cas9 gene and single-guide RNAs (sgRNAs) specific for HBV. They showed that Cas9/sgRNA combinations specific for HBV reduced HBV DNA levels by up to 1000-fold and HBV cccDNA levels by up to 10-fold. Moreover, this method could inactivate the mutation of residual viral DNA. They concluded that CRISPR/Cas9 systems could serve as effective tools for disrupting the cccDNA pool in chronically-infected HBV patients.

Furthermore, Liu *et al*[57] showed that HBV-specific gRNA/Cas9 could inhibit the HBV replication of different genotypes *in vitro* and *in vivo* due to error-prone repair of viral DNA templates. Dong *et al*[58] reported that the CRISPR/Cas9 system could be used for disrupting intracellular cccDNA and viral replication in pre-cccDNA-transfected Huh7 cells and a new mouse model carrying HBV cccDNA. Zhen *et al*[59] studied the effects of the CRISPR/Cas9 system targeted to the HBsAg-encoding region of HBV in a cell culture system and *in vivo*. They found that the concentration of HBsAg secreted in the cell culture and mouse serum was decreased by CRISPR/Cas9 treatment. They concluded that a CRISPR/Cas9 system inhibited HBV replication and expression *in vitro* and *in vivo*, and may constitute a new therapeutic strategy for HBV infection. Seeger and Sohn[60] reported that HBV infections could be inhibited up to 8-fold by HBV-specific guide RNAs in NTCP-expressing HepG2 cells. Ramanan *et al*[61] demonstrated that the CRISPR/Cas9 system could specifically target and cleave conserved regions in the HBV genome, causing robust suppression of viral gene

Table 1 Developing new therapeutic drug targets for chronic hepatitis B

Drugs	Mechanism of action	Therapeutic class	Route of administration	Clinical trial	Results
HBV entry inhibitors					
Bulevirtide (Myrcludex B)[49]	Competition with NTCP	Peptide	Subcutaneous injection	IIb	HBsAg loss in 27% of HBV/HDV co-infected patients after 48 wk of treatment with Bulevirtide + pegIFN-α and 24 wk treatment-free follow-up
cccDNA disruptors					
CRISPR/Cas9[67]	Disruption of cccDNA	Gene editing	*In vivo*	Pre-clinical	Significantly improved survival of human hepatocytes in liver-humanized FRG mice and demonstrated a decreasing of total liver HBV-DNA and cccDNA
ZFNs[69]	Disruption of cccDNA	Gene editing	*In vitro*	Pre-clinical	Efficiently suppress the cellular template for HBV persistence and inhibit active HBV replication
Nucleocapsid assembly modulators					
JNJ-632 and BAY41-4109[73]	Misdirecting the formation of capsid-like structures	Capsid assembly modulators	*In vitro*	Pre-clinical	Induce the formation of morphologically intact viral capsids and prevented formation of cccDNA
NVR3-778[78]	Misdirecting the formation of capsid-like structures	Capsid assembly modulator	*In vivo*	I/II	The largest mean reduction in serum HBV DNA levels was achieved from the combination treatment of 600 mg NVR3-778 BD + pegIFN 180 mg subcutaneous weekly (1.97 log10 IU/mL)
JNJ-6379[76]	Misdirecting the formation of capsid-like structures	Capsid assembly modulators	Oral	II	No clinically significant changes in levels of HBsAg were observed
ABI-H0731[77]	Misdirecting the formation of capsid-like structures	Capsid assembly modulators	Oral	I/II	Dose-dependent reduces in HBV DNA and HBV RNA not HBsAg was seen in both HBeAg-positive and HBeAg-negative patients
HBV transcription inhibitors					
ARC-520[84]	Interference viral mRNA	Transcription inhibitor	Intravenous injection	II	CHB patients with high dose significantly reduced HBsAg and persisted for $\geq$85 d after the last dose
GSK3389404[85]	Interference viral mRNA	Transcription inhibitor	Subcutaneous injection	I	Dose 120 mg for 4 wk was safe and well tolerate
RG7834[87]	Interference viral mRNA	Gene expression inhibitor	*In vivo*	Pre-clinical	Reduced WHsAg by a mean of 2.57 $\log_{10}$ and WHV DNA by a mean of 1.71 $\log_{10}$ from baseline. However, WHsAg and WHV DNA rebounded to baseline after stopped treatment and WHsAb was not observed.
HBsAg release inhibitors					
REP 2055 and REP 2139-Ca[88]	HBsAg release inhibitors	NAPs	Intravenous injection	II	Substantially reduction of HBsAg levels, HBV DNA levels and increasing of serum HBsAb
REP 2139-Mg and REP 2165-Mg[90]	HBsAg release inhibitors	NAPs	Intravenous injection	II	Addition of NAPs to TDF + pegINFα-2a significantly increased rates of HBsAg loss during therapy and functional cure after therapy

cccDNA: Covalently closed circular DNA; CHB: Chronic hepatitis B; CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats/CRISPR-associated 9; HBsAb: Hepatitis B surface antibody; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HDV: Hepatitis D virus; NAPs: Nucleic acid polymers; NTCP: Sodium taurocholate co-transporting polypeptide; pegIFN-α: Pegylated interferon-alpha; TDF: Tenofovir disoproxil fumarate; WHsAb: Woodchuck hepatitis surface antibody; WHsAg: Woodchuck hepatitis surface antigen; WHV: Woodchuck hepatitis virus.

expression and replication both *in vitro* and *in vivo*, and extended this antiviral activity to a virus isolated from patients. They also reported that upon continuous Cas9/sgRNA, a sharp decline of cccDNA and HBV proteins resulted in a *de novo* infection model.

Wang *et al*[62] evaluated the efficiency of each gRNA and 11 dual-gRNAs on the suppression of HBV (genotypes A-D) replication using the measurement of HBsAg or HBeAg in the culture supernatant. They found that all dual gRNAs could efficiently suppress HBsAg and HBeAg production for HBV of genotypes A-D, and the efficacy of dual gRNAs was significantly increased compared to the single gRNA used alone. Karimova *et al*[63] identified cross-genotype conserved HBV sequences in the S and X region of the HBV genome targeted for specific and effective cleavage by a Cas9 nickase. This technique could disrupt episomal cccDNA, and chromosomally integrated HBV target sites in reporter cell lines and HBV replication in chronically and *de novo* infected hepatoma cell lines.

In 2019, Kostyushev *et al*[64] evaluated CRISPR/Cas9 systems from four different species using co-expressed cell lines with gRNAs targeting conserved regions of the HBV genome. They found that the CRISPR/Cas9 systems from *Streptococcus pyogenes* (Sp) and *Streptococcus thermophilus* (St) targeting conserved regions of the HBV genome could block HBV replication and degrade over 90% HBV cccDNA by 6 d post-transfection. They concluded that the St CRISPR/Cas9 system represented the safest system with high anti-HBV activity.

In 2020, Yang *et al*[65] investigated the utility of CRISPR/Cas-mediated "base editors" (BES) in inactivating HBV gene expression without cleavage of DNA. They found that Cas9-mediated base editing is a potential strategy to cure CHB by permanently inactivating integrated HBV DNA and cccDNA without double-strand breaks of the host genome. Recently, Kayesh *et al*[66] evaluated the effects of adeno-associated virus 2 (AAV2) vector-mediated delivery of 3 selected from 16 gRNAs. These gRNAs/Cas9 significantly suppressed HBV replication in cells, with WJ11/Cas9 demonstrating the highest efficacy. Furthermore, AAV2/WJ11-Cas9 also substantially inhibited HBV replication and significantly reduced cccDNA in the tested cells. It also enhanced ETV actions when used in combination due to different modes of action. They concluded that AAV2/WJ11-Cas9 significantly suppressed HBcAg, HBsAg, and HBV DNA along with cccDNA in the liver tissues without significant cytotoxicity in humanized chimeric mice. A pre-clinical study was reported by Stone *et al*[67], in which HBV-specific AAV-*Staphylococcus aureus* (Sa)-Cas9 therapy significantly improved survival of human hepatocytes in liver-humanized FRG mice and demonstrated a decrease in total liver HBV DNA and cccDNA; in addition, a good tolerance profile was found. The investigators concluded that this approach was safe and feasible for *in vivo* gene editing therapy in CHB infections, and it may be a plausible method to cure CHB patients.

In 2010, Cradick *et al*[68] demonstrated the effective cleavage of viral DNA targets by HBV-specific ZFNs within cultured cells. Moreover, the cleaved fragments were mis-repaired, which could potentially inactivate HBV. The authors suggested that AAVs can transfect 100% of mouse hepatocytes and could be used to deliver ZFNs to the human livers. In 2014, Weber *et al*[69] evaluated three ZFNs that target sequences within the HBV polymerase, core, and X genes. They demonstrated that HBV-targeted ZFNs could efficiently suppress the cellular template for HBV persistence and inhibit active HBV replication, causing them to be potential candidates for cccDNA disruptors (Table 1).

Overall, gene editing techniques have demonstrated the usefulness of destroying HBV cccDNA *in vitro* and *in vivo* and shown the therapeutic potential in acute and chronic HBV infection. Gene editing is at an exciting stage, and the future of curative anti-HBV regimens for chronic HBV infection may well entail the use of it combined with other drugs.

Nucleocapsid assembly modulators

HBV capsid has numerous functions in the HBV life cycle, including reverse transcription, genome packaging, and intracellular trafficking. It is an excellent target for the development of new antiviral drugs[70]. The capsid assembly modulators (CAMs) can disturb pgRNA encapsidation and HBV DNA replication by misdirecting the formation of capsid-like structures[71]. There are two categories of CAM: type I represented by heteroaryl-dihydro pyrimidine, which misdirects the formation of aberrant structures; and type II represented by phenylpropenamides and sulfamoyl-benzamides, which accelerate the formation of morphologically intact empty capsids [72].

In 2017, Berke *et al*[73] conducted the study to evaluate the CAM JNJ-632 and CAM BAY41-4109, novel and potent inhibitors of HBV replication, *in vitro* across genotypes A to D. They found that it can induce the formation of morphologically intact viral capsids. They prevented the formation of cccDNA in a dose-dependent fashion when added with the viral inoculum. Moreover, it also reduced intracellular HBV RNA, HBeAg, HBcAg, and HBsAg concentrations in the cell culture supernatant. They concluded that CAMs have a dual mechanism of action that inhibits early and late steps of the viral life cycle, whereas NAs did not. In 2018, Lam *et al*[74] conducted a pre-clinical characterization of CAM NVR3-778 in HepG2.2.15 cells, mice, and dogs. They found that CAM NVR3-778 suppressed HBsAg, HBeAg, and intracellular HBV RNA production in primary human hepatocytes. Furthermore, it can block cccDNA formation during *de novo* infection and the subsequent transcription and viral protein translation steps. Furthermore, Klumpp *et al*[75] performed a comparative study of NVR3-778 to determine the *in vivo* antiviral efficacy and effects on innate and endoplasmic reticulum stress responses alone or in combination with Peg-IFN-α and compared with entecavir in 61 uPA/SCID mice with humanized livers. Mice were infected with an HBV genotype C preparation and then waited for 8 wk. They were randomly assigned to six groups (control, NVR3-778, entecavir, Peg-IFN-α, NVR3-778 + entecavir, or NVR3-778 + Peg-IFN-α) for 6 wk. Ultimately, the mice given NVR3-778 or entecavir alone for 6 wk showed reduced serum levels of HBV DNA compared with controls or mice given Peg-IFN-α. Moreover, the most considerable HBV DNA serum level reduction was demonstrated in mice given NVR3-778 + Peg-IFN-α. Serum levels of HBsAg and HBeAg were reduced in the groups that received Peg-IFN-α.

In 2020, Zoulim *et al*[76] performed a double-blind study of 57 treatment-naïve patients with HBeAg-positive or -negative CHB infection without cirrhosis. They were randomly assigned to five groups to receive either 25 mg (100 mg loading dose), 75 mg, 150 mg, or 250 mg JNJ-6379 or placebo daily for 4 wk, with an 8-wk follow-up period. They found that all doses of JNJ-6379 tested were well tolerated, demonstrated dose-dependent pharmacokinetics, and had potent antiviral activity in patients with CHB. However, no clinically significant changes in levels of HBsAg were observed. Recently, Yuen *et al*[77] conducted a phase 1/2, randomized, placebo-controlled study to explore the safety, pharmacokinetics, and pharmacodynamics of ABI-H0731 in healthy subjects and patients with CHB in two parts. In part 1, healthy adults were randomly assigned to receive single oral doses of ABI-H0731 (100, 300, 600, or 1000 mg) or matching placebo, or once-daily or twice-daily doses ABI-H0731 800 mg or matching placebo for 7 d. In part 2, HBeAg-positive or HBeAg-negative CHB adults were randomly assigned to receive ABI-H0731 (100, 200, 300, or 400 mg) or matching placebo once daily for 28 d. Overall, ABI-H0731 was safe and well-tolerated. There were no serious adverse events, nor clinically significant drug-related, dose-dependent, or treatment-emergent laboratory findings. ABI-H0731 showed dose-related activity with once-daily dosing. The mean maximal HBV DNA reductions from baseline of 1.7 Log_{10} IU/mL at 100 mg to 2.8 Log_{10} IU/mL at 300 mg after 28 d for the HBeAg-positive and HBeAg-negative patients. The authors concluded that dose-dependent reduction in HBV DNA and HBV RNA with ABI-H0731 occurred in both HBeAg-positive and HBeAg-negative patients. There were no serious adverse events related to the 1600 mg daily doses in healthy subjects or patients with CHB infection receiving doses up to 300 mg once daily.

Furthermore, Yuen *et al*[78] also performed a phase 1/2 study to examine the safety, pharmacokinetics, and antiviral activity of NVR3-778 in 73 patients with HBeAg-positive CHB infection without cirrhosis. The study had eight cohorts comprised of one placebo cohort and seven treatment cohorts. The four dose-escalation cohorts received NVR3-778 of 100 mg (10 cases), 200 mg (10 cases), or 400 mg once daily (QD) (8 cases), or 600 mg twice daily (BD) (8 cases). The fifth cohort was treated with 600 mg NVR3-778 BD + Peg-IFN 180 mg subcutaneous weekly (10 cases). The sixth cohort was treated with Peg-IFN 180 mg subcutaneous weekly + placebo (10 cases). The seventh cohort was treated with 1000 mg NVR 3-778 BD (7 cases). The eighth cohort was treated with a placebo. The investigators found that mean HBV DNA decline was minimal with low once-daily doses of NVR3-778, but when daily dosing was increased to 1200 mg/d, HBV DNA reductions became substantial. The fourth cohort (600 mg NVR3-778 BD) showed a mean HBV DNA reduction of 1.72 Log_{10} IU/mL. The most significant mean reduction in serum HBV DNA levels was achieved from the combination treatment of 600 mg NVR3-778 BD + Peg-IFN 180 mg subcutaneous weekly (1.97 Log_{10} IU/mL). They concluded that NVR3-778 treatment for 28 d up to a dose of 1000 mg BD was well tolerated. Substantial and correlated decreases in serum HBV DNA and HBV RNA concentrations were demonstrated with the higher-dose cohorts and were notably most excellent for combination treatment with NVR3-778

and Peg-IFN. They do not evaluate serum HBsAg, HBeAg, immunomodulatory effects, and effects on cccDNA persistence. These encouraging data suggested that CAMs can result in a substantial reduction in HBV DNA and HBV RNA levels. Longer-term treatments alone or combined with other antiviral agents will be needed to investigate whether CAMs will result in HBeAg, HBsAg, and cccDNA loss (Table 1).

HBV transcription inhibitors

After HBV enters the infected hepatocytes, partially double-stranded DNA (pdsDNA) moves to the nucleus and is converted to cccDNA. Furthermore, it is wrapped by histones to form a mini-chromosome. RNA interference (RNAi) and antisense oligonucleotides are mechanisms in which a double-stranded RNA (dsRNA) inhibits gene expression by degrading mRNA or blocking a specific gene's translation pathway. RNAi can directly target HBV transcripts and induce their degradation, causing gene silencing. Antisense oligonucleotides are small nucleic acids complementary to the target transcript, that induce degradation after binding. Hence, targeting the viral mRNA using RNAi and antisense oligonucleotides may be an effective method to control HBV infection. Many studies of RNAi and antisense oligonucleotides are in progress[79-81].

In 2017, Schluep *et al*[82] conducted a phase 1 randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and pharmacokinetics of ARC-520 injection in 54 healthy volunteers (36 ARC-520 *vs* 18 placeboes). They found that ARC-520 was safe and well-tolerated. In the same year, Wooddell *et al*[83] conducted a phase 2 randomized, double-blind, placebo-controlled study to determine the safety, tolerability, and pharmacological effect of ARC-520 in 40 CHB patients with or without preceding nucleos(t)ide viral replication inhibitors (NUC) treatment. They found that ARC-520 resulted in a rapid and potent decrease in serum HBV DNA. However, the reduction of HBsAg was only demonstrated in HBeAg-positive patients. Follow-up studies in chimpanzees showed that the HBsAg being produced in the HBeAg-negative patients was predominantly derived from an integrated virus, which ARC-520 did not target.

In 2020, Yuen *et al*[84] conducted 2 randomized, multicenter studies to evaluate in-depth HBsAg decline using 1 mg/kg or 2 mg/kg ARC-520 compared with placebo at four monthly doses in 58 HBeAg-negative and 32 HBeAg-positive CHB patients concomitantly with NUC. They found that both HBeAg-negative and HBeAg-positive high-dose groups had significantly reduced HBsAg compared with placebo, with mean reductions of 0.38 and 0.54 Log IU/mL, respectively. Moreover, HBsAg reductions persisted for 85 d in HBeAg-negative patients and > 85 d in HBeAg-positive patients after the last dose of ARC-520. They concluded that ARC-520 was active in both HBeAg-negative and HBeAg-positive CHB patients treated by NUC. However, absolute HBsAg reductions were moderate, which may occur due to HBsAg expression from integrated HBV DNA.

In 2019, GSK3389404, an antisense oligonucleotide, was studied by Han *et al*[85]. The investigators conducted a randomized, double-blind, phase 1 study to assess the safety and pharmacokinetics of GSK3389404 in healthy subjects. Four single ascending-dose cohorts (10 mg, 30 mg, 60 mg, and 120 mg subcutaneously) and three multiple ascending-dose cohorts (30 mg, 60 mg, and 120 mg once weekly for 4 wk) each comprised 6 subjects randomized to GSK3389404 and 2 subjects randomized to placebo. They reported that there were no serious adverse events (SAEs) or withdrawals due to SAEs. GSK3389404 dosing has been tested up to 120 mg for 4 wk with an acceptable safety and pharmacokinetic profile and suitable for further clinical evaluation in CHB patients.

In 2018, Mueller *et al*[86] reported that RG7834, a novel oral HBV gene expression inhibitor, could reduce the levels of viral proteins and lower viremia. RG7834 is a small-molecule compound belonging to the dihydroquinolizinones chemical class similar to RNAi but through a different mechanism. They found that oral treatment of HBV-infected humanized mice with RG7834 Led to a mean HBsAg reduction of 1.09 Log_{10} compared to entecavir, which had no significant reduction on HBsAg levels. In 2020, Menne *et al*[87] conducted a study to evaluate the potency of RG7834 alone and in combination with ETV or woodchuck interferon-α (wIFN-α) in the woodchuck model of chronic HBV infection. RG7834 could reduce woodchuck hepatitis virus (WHV) surface antigen (WHsAg) by a mean of 2.57 Log_{10} from baseline and WHV DNA by a mean of 1.71 Log_{10}. ETV + wIFN-α reduced WHsAg and WHV DNA by 2.40 Log_{10} and 6.70 Log_{10}, respectively. RG7834 combined with ETV and wIFN-α significantly decreased WHsAg and WHV DNA concentrations by 5.0 Log_{10} and 7.46 Log_{10}, respectively. However, WHsAg and WHV DNA rebounded to baseline after stopping treatment, and WHsAb was not observed. Notably, both RNAi and antisense

oligonucleotides do not eliminate cccDNA, and rebound of HBsAg levels to pretreatment points after stopping treatment has been reported. Therefore, it is likely to be used in combination with other drugs (Table 1).

HBsAg release inhibitors

HBsAg release inhibitors function under the same exact mechanism as the RNAi and antisense oligonucleotides that block the release of subviral HBsAg particles. Circulating HBsAg is an immunoinhibitory factor that blocks the innate immune response. Clearance of circulating HBsAg is a crucial step in the functional control of HBV infection and permits anti-HBs seroconversion. In 2016, Al-Mahtab *et al*[88] conducted two studies to evaluate REP 2055 and REP 2139-Ca, nucleic acid polymers (NAPs), in 8 and 12 CHB patients, respectively. The results from both studies showed that NAP monotherapy was accompanied by 2-7 Log_{10} reductions of HBsAg levels, 3-9 Log_{10} reductions in HBV DNA levels, and the appearance of serum hepatitis B surface antibody (HBsAb) (10-1712 mIU/mL). Eight of the nine patients treated with the combination of NAP and immunotherapy experienced HBsAg loss, and all nine patients experienced substantial increases in serum HBsAb antibody titers before treatment was stopped. Moreover, 1 year after the REP 2055 therapy was stopped, a rebound of serum HBV DNA > 1000 copies/mL or HBsAg > 1 IU/mL was not observed in 3/8 CHB patients. Suppression of serum HBV DNA > 1000 copies/mL or HBsAg > 1 IU/mL was further maintained for 290 and 231 wk in 2 of these patients. For REP 2139-Ca treatment, 8 patients achieved HBV DNA < 116 copies/mL after treatment withdrawal. The rebound of serum HBV DNA > 1000 copies/mL or HBsAg > 1 IU/mL occurred over 12 to 123 wk in 7 patients but was still absent in 2 patients at 135 and 137 wk of follow-up. The authors concluded that NAP could elicit significant antiviral responses during treatment which may improve the effect of immunotherapy. NAPs may be a potentially useful component of future combination therapies for the treatment of CHB.

In 2017, Bazinet *et al*[89] conducted an open-label, non-randomized, phase 2 trial to assess the safety and efficacy of REP 2139 and Peg-INF-α-2a in 12 patients with CHB HDV co-infection. The results showed that 6 patients had HBsAg concentrations < 50 IU/mL by the end of treatment. Five patients maintained the level of suppression at the end of 1-year follow-up. Six patients had HBsAb titers > 10 mIU/mL at the end of treatment (five had maximum HBsAb levels of 7681-86532 mIU/mL during treatment), which were maintained at the end of 1-year follow-up. By the end of 1-year follow-up, normalization of serum aspartate aminotransferase (AST) and ALT occurred in 9 of 12 patients. They concluded that combined REP 2139 and Peg-INF-α-2a therapy is well-tolerated, safe, and establishes functional control of HBV and HDV co-infection and normalization of serum AST and ALT in a high proportion of patients 1 year after therapy. In 2020, Bazinet *et al*[90] performed an open-label, phase 2 study of the safety and efficacy of REP 2139 or REP 2165 combined with TDF and Peg-INF-α-2a in 40 HBeAg-negative CHB patients. Forty patients were randomly assigned to groups that received 48 wk of experimental therapy (TDF + Peg-INF-α-2a + REP 2139-Mg or REP 2165-Mg) or 24 wk of control therapy (TDF + Peg-INF-α-2a) followed by 48 wk of experimental therapy. At 48 wk, when patients completed the TDF + Peg-INF-α-2a + NAPs regimen, HBsAg concentrations were ≤ 0.05 IU/mL in 24 of 40 (60%) patients, while all of the patient's achieved seroconversion with HBsAb up to 233055 mIU/mL. During 48 wk of treatment-free follow-up, virologic control persisted in 13 of 40 (32.5%) patients, whereas functional cure persisted in 14 of 40 (35%) patients with persistent HBsAg loss. They concluded that the addition of NAPs to TDF + Peg-INF-α-2a significantly increased rates of HBsAg loss during therapy and functional cure after therapy. However, these results should be carefully applied for Asian race because Van Hees *et al*[91] found that Caucasian patients had more than 6-fold increased chance of HBsAg loss compared to other ethnicities. Further studies regarding ethnicity and HBsAg loss are needed. Thus, NAPs alone or combined with TDF or Peg-INF-α-2a may allow better functional control of HBV infection (Table 1). A longer duration of NAPs treatment would be needed to identify their sustained virological effects and potential risk for adverse events.

CONCLUSION

Tremendous progress has been explored in understanding the pathophysiology and treatment of CHB over the past 20 years. The CHB current treatment with a potent and a high genetic barrier NA (ETV, TDF, and TAF) can suppress the viral replication to an

undetectable level in most CHB patients. They also prevent the progression of CHB to cirrhosis and markedly reducing the rates of HBV-related HCC. Regardless of viral suppression by NAs, there are many obstacles to achieve a functional cure or HBsAg loss in CHB patients. HBV could persist in the hepatocyte nucleus by continuously replenishing the cccDNA with a long half-life and the integrated forms of viral DNA. Moreover, the defective immune response and the ine□cient innate immune response prevent HBV-infected hepatocytes from being cleared by host immunity.

HBsAg loss with or without HBsAb seroconversion is one of the most desired endpoints of new drug development. Targeting HBsAg by inhibiting the entry of HBV into hepatocytes, disrupting or silencing HBV cccDNA, modulating nucleocapsid assembly, interfering HBV transcription, and inhibiting HBsAg release are the primary targets for functional cure in CHB patients. However, newly developed drugs still have limitations in being used alone without IFN and NAs to induce HBsAg loss. Interestingly, a new strategic therapy in treating chronic HBV infection is to use a combination of multiple drugs, including a backbone of a NA, one or more new direct-acting antiviral drugs, and at least one immunomodulator. With the collaborative efforts of basic research scientists and clinical experts, the ultimate elimination of CHB infection is likely to be achieved soon.

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ORIGINAL ARTICLE

Observational Study

Rethinking hospital psychiatry in Italy in light of COVID-19 experience

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Abstract

BACKGROUND

Italy retains a distinctive organization of mental health services according to a community-based model of care with a multidisciplinary team serving a well-defined catchment area under the coordination of the local department of mental health. The coronavirus disease 2019 (COVID-19) pandemic is forcing Italian mental health services to develop new organizational strategies at all levels of care in order to face the associated challenges.

AIM

To explore factors associated with changes in psychiatric admissions to an inpatient psychiatric unit located in Lombardia Region, Italy.

METHODS

All hospital admissions (n = 44) were recorded to an inpatient psychiatric unit during a three month national lockdown in Italy in 2020 and compared with those occurring over the same time period in 2019 (n = 71). For each admission, a 20-item checklist was completed to identify factors leading to admission. Statistical analyses were performed using Statistical Package for Social Sciences for Windows, release 11.0. Chi-square test (or Fisher's exact test) and Mann-Whitney U-test were applied, where appropriate.

RESULTS

Hospital admissions dropped by 38% during the COVID-19 pandemic. No significant differences were found in demographics, clinical variables associated with hospital admissions and length of stay between 2019 and 2020. Compared with 2019, a significantly greater proportion of hospital admissions in 2020 were related to difficulties in organizing care programs outside the hospital (chi-square

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= 4.91, df 1, one-way P = 0.035) and in patients' family contexts (chi-square = 3.71, df 1, one-way P = 0.049). On the other hand, logistic and communication difficulties pertaining to residential facilities and programs were significantly more common in 2019 than in 2020 (chi-square = 4.38, df 1, one-way P = 0.032).

CONCLUSION
Admissions to the inpatient psychiatric unit dropped significantly during the COVID-19 pandemic in 2020, with difficulties in organizing care programs outside the hospital and in patients' family contexts occurring more frequently compared with 2019.

Key Words: Mental health services; COVID-19; Italy; Psychiatric; Pandemic

Core Tip: During the coronavirus disease 2019 pandemic mental health services in Italy developed new organizational strategies in order to face the associated challenges. Compared with 2019, hospital admissions dropped significantly and were more frequently related to restrictions posed by the pandemic, like difficulties in organizing care programs outside the hospital and in patients' family context. On the other hand, logistic and communication difficulties pertaining to residential facilities and programs were significantly more common in 2019 than in 2020, due to the reorganization of residential facilities as close communities looking after their own patients with little reliance on hospital during the pandemic.

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INTRODUCTION

The present organization of mental health care in Italy stems from a reform law dating back to 1978. On the assumption that individuals with mental disorder should be offered the same treatment standards as those suffering from other types of illness, a gradual dismantling of old mental hospitals occurred alongside the setting up of new community-based services within the framework of local departments of mental health, each promoting and coordinating mental health prevention, care and rehabilitation in a defined catchment area. Although the Italian experience has attracted international attention and promoted similar changes abroad, it has retained distinctiveness. In comparison with the countries belonging to the Group of 7 (G7) more advanced economies, Italy has lower population rates of mental health professionals and of beds for acute psychiatric care in general hospitals; as opposed to higher rates of beds in residential facilities devoted to rehabilitation and daily support programs[1, 2].

Among the services which are part of departments of mental health, inpatient psychiatric units are located in general hospitals with an emergency department and provide crisis interventions on a short-term basis, with patients being referred back to outpatient care or other types of interventions as soon as possible. Most admissions take place on a voluntary basis and only a minority are compulsory. According to the national mental health information system, mental health service utilization varies considerably across Italian regions[2]. This is due to the substantial autonomy that each region retains in organization of health care within its territory, according to the general principles and recommendations set out by the national government. Moreover, psychiatric admissions were found to be influenced by a wide array of different factors, such as demographics, illness and treatment variables, mental health service organisation and practice, interaction between inpatient psychiatric units and other health services and/or social agencies, and the role of patients' families, leading to significant variation in pathways to care, typologies of admissions, length of

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hospital stay and the care process itself[3-6].

The coronavirus disease 2019 (COVID-19) pandemic is posing huge challenges to the health care system in general, as well as mental health services, driving the implementation of novel strategies and interventions. However, accounts of changes in mental health service organization and activities in Italy during the pandemic have been mainly narrative, indicating the need for a quantitative approach to the effects of COVID-19 pandemic[7].

The aim of this study was to explore changes in number of psychiatric admissions to an inpatient psychiatric unit located in the Italian region most severely affected by the first peak of COVID-19 pandemic in 2020, and to identify relevant factors associated with the detected changes in comparison with the same time period of 2019.

MATERIALS AND METHODS

Study design

All admissions were recorded to a locked, inpatient psychiatric unit within a general hospital in Cittiglio, a small town located in Lombardia Region, North-western Italy, between February 24th and May 24th, 2020 and compared with those occurring over the same time period in 2019.

Although relatively short, the study interval was chosen because it corresponded to a strict lockdown imposed on the country, which marked a definite and profound difference compared to the previous period. Indeed, a Legislative Decree signed by the Italian Prime Minister on February 23rd, 2020, ordered that: people were not allowed to leave home except for work, health needs or urgent reasons; remote working was promoted whenever possible; commercial activities were suspended unless they supplied essential goods or basic necessities; all types of schools were shut down and distance learning education was offered to students; access to public places and social settings favoring crowding and close contacts among individuals was forbidden, including, among the others, pubs, restaurants, cinemas, theatres, museums, concert halls, public gardens, cultural places, swimming pools, fitness centers, gyms; public events of any type were cancelled and civil and religious ceremonies were strictly limited; access of patients' relatives and caregivers to health services and residential facilities was discouraged or forbidden. A subsequent Legislative Decree on May 13th, 2020, allowed a gradual lessening of the strict limitations listed above, which became noticeable by May 24th.

From the very beginning, mental health services continued to pursue their activities as part of essential health care and reimbursement of their interventions was left unchanged. However, at the outset of the pandemic inpatient units reduced their usual number of beds to pursue isolation requirements and interpersonal distancing and to devote staff to treatment of individuals suffering from COVID-19 illness and its complications. Moreover, psychiatric contacts with the emergency department and hospital admissions were discouraged and limited to urgent cases which could not receive adequate treatment outside the hospital and whose admission could not be postponed.

Data included in the paper were collected as part of routine clinical practice not requiring ethical approval, with patients giving their written informed consent at data collection at the time of hospital admission.

For patients admitted to the inpatient unit a 20-item checklist was completed to identify relevant factors leading to admission and including: (1) Clinical variables (illness severity; difficulties in instigating treatment; diagnostic difficulties; co-morbid physical illness); (2) Negative factors affecting quality of care during hospital stay (insufficient patient's evaluation; negative doctor-patient relationship; defensive psychiatry); (3) Difficulties in the care process (unclear reason for admission; insufficient communication between the inpatient unit and the outpatient clinic; difficulties in planning care programs outside the hospital); (4) Logistic variables within the hospital (delay in specialist consultations or diagnostic tests; organizational problems); (5) Logistic and communication difficulties between the mental health department and other agencies (social agencies, rehabilitation facilities, elderly care facilities, legal system); (6) Variables related to the patients' family context (objective difficulties in the family; insufficient or negative relationship between the mental health staff and family members); (7) Legal acts; and (8) Exceptional personal, familial or social events.

Up to mid-April, patients were admitted to the inpatient unit provided that they had no temperature or other COVID-related symptoms, but did not perform a COVID test; from mid-April onwards patients were tested on a COVID test at the emergency

department and only those negative were admitted to the inpatient unit.

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows, release 11.0[8].

Chi-square test (or Fisher's exact test) and Mann-Whitney U-test were applied, where appropriate, to investigate differences between admissions in 2019 and those in 2020 according to sex, age, diagnosis (grouping ICD-10 diagnoses into four categories: schizophrenia and related psychoses; affective disorders; personality disorders; other diagnoses, mainly including substance use disorders or organic conditions), type of admission (voluntary *vs* compulsory), occurrence of mechanical restraints, length of stay, and reasons for admission.

RESULTS

Hospital admissions dropped by 38% during the pandemic, being 44 in 2020 as opposed to 71 during the corresponding period of 2019.

In 2020, admissions by males were 28 and accounted for 63.6% of the sample. Median age (and interquartile range) of admitted patients was 38.5 (29.25-54.75) years. Diagnoses of schizophrenia and related psychoses, affective disorder and personality disorder were evenly distributed in the sample and overall accounted for 82% of the total. Ten (22.7%) individuals reported substance abuse and 8 (18.2%) carried suicidal risk. Only one (2.3%) patient underwent compulsory admission and 4 (9.1%) were restrained to bed. No significant differences were found on the demographic and clinical variables mentioned above according to study year.

In 2020, length of stay ranged between one and 34 d, with a median (and interquartile range) of 10 (4.25-17) d, and did not differ significantly compared to 2019. The effect of diagnosis on length of stay was explored among patients residing in the service catchment area, since they completed their hospitalization at the inpatient unit under study, whereas non-resident patients were transferred to their local psychiatric services within a few days after admission. A significant difference in length of stay was found according to diagnosis (Kruskall-Wallis Chi-square = 19.88; d.f. 3, $P < 0.0001$), with shorter admissions for personality disorder compared with other diagnoses. However, diagnoses accounted for only approximately 6% of the variance in length of stay.

In 2020, no non-resident patients were under compulsory admission or restraint; whereas in 2019, 29.6% of non-resident patients as opposed to 6.8% of resident ones were so restrained, and the difference was statistically significant (Chi-square = 6.65; d.f. 1; $P = 0.01$). A higher percentage of non-resident patients in 2019 were under compulsory admission, but the difference was not statistically significant.

The Table 1 shows the factors associated with hospital admission, derived from the 20-item checklist mentioned above and ranked according to frequency in 2020. Illness severity was far more common, being rated in about two-thirds of patients, and was followed by other clinical factors such as difficulties in instigating treatment and presence of organic co-morbidity, each occurring in 20.5% of hospital admissions. Among non-clinical factors, impaired relationship with patients' family members (20.5%) and difficulties in planning care programs outside the hospital (11.4%) were more common. Illness severity was significantly more common among the factors associated with hospital admission in 2019 compared to 2020, whereas difficulties in planning care outside the hospital occurred more frequently in 2020.

Overall, in 2020 sole clinical factors were reported in 28 (63.6%) of hospital admissions, sole non-clinical factors (*i.e.*, logistic, communication and family factors) in 8 (18.2%), with a combination of the two in the remaining 8 admissions (18.2%). No significant difference was found compared to 2019.

For further analyses, reasons associated with hospital admission were grouped into five broad categories. Findings were in the expected direction. No significant differences were found between 2019 and 2020 in clinical factors and in the care process. Compared to 2019, during the pandemic a significantly greater proportion of hospital admissions were related to difficulties in organizing care programs outside the hospital (chi-square = 4.91, df 1, one-way $P = 0.035$) and in patients' family contexts (chi-square = 3.71, df 1, one-way $P = 0.049$). On the other hand, logistic and communication difficulties pertaining to residential facilities and programs were significantly more common in 2019 than in 2020 (chi-square = 4.38, df 1, one-way $P = 0.032$).

Table 1 Factors associated with hospital admission, derived from the 20-item checklist described in the study design and ranked according to frequency in the year 2020, *n* (%)

	2019		2020		
Reasons	**No**	**Yes**	**No**	**Yes**	***P* value**
Illness severity	12 (16.9)	59 (83.1)	16 (36.4)	28 (63.6)	0.02
Difficulties in instigating treatment	64 (90.1)	7 (9.9)	35 (79.5)	9 (20.5)	0.11
Organic co-morbidity	64 (90.1)	7 (9.9)	35 (79.5)	9 (20.5)	0.11
Negative relationship with patients' relatives	64 (90.1)	7 (9.9)	35 (79.5)	9 (20.5)	0.11
Difficulties in planning care outside the hospital	70 (98.6)	1 (1.4)	39 (88.6)	5 (11.4)	0.02
Insufficient relationship with social agencies	70 (98.6)	1 (1.4)	41 (93.2)	3 (6.8)	0.12
Objective difficulties in the family system	70 (98.6)	1 (1.4)	42 (95.5)	2 (4.5)	0.31
Insufficient relationship with elderly care facilities	69 (97.2)	2 (2.8)	43 (97.7)	1 (2.3)	0.86
Unclear reason for admission	69 (97.2)	2 (2.8)	43 (97.7)	1 (2.3)	0.86
Insufficient communication with outpatient clinic	70 (98.6)	1 (1.4)	43 (97.7)	1 (2.3)	0.73
Negative doctor-patient relationship	70 (98.6)	1 (1.4)	43 (97.7)	1 (2.3)	0.73
Legal acts	71 (100.0)	0 (0.0)	43 (97.7)	1 (2.3)	0.20
Insufficient relationship with rehabilitation facilities	66 (93.0)	5 (7.0%)	44 (100.0)	0 (0.0)	0.07
Insufficient relationship with legal system	68 (95.8)	3 (4.2)	44 (100.0)	0 (0.0)	0.17
Diagnostic difficulties	70 (98.6)	1 (1.4)	44 (100.0)	0 (0.0)	0.43
Insufficient patient's evaluation	70 (98.6)	1 (1.4)	44 (100.0)	0 (0.0)	0.43

Four items out of 20 (defensive psychiatry; delay in specialist consultations and diagnostic tests; hospital organizational problems; exceptional personal, familial and social events) were null in both 2019 and 2020. More than one factor might be operating on each admission.

DISCUSSION

During the first peak of COVID-19 pandemic, hospital admissions dropped by 38% compared to the previous year. This was the result of strict selection criteria limiting hospital admissions to urgent cases with no alternative options as well as of new organizational strategies involving all levels of mental health care, that were quickly implemented under the coordination of the local department of mental health. Specifically, the outpatient clinic serving the same area of the inpatient unit under study increased contacts with patients combining face-to-face and domiciliary visits with remote consultations: overall contacts were 2727 in 2020 *vs* 2495 in 2019 (+9.3%), with greater increases in contacts by psychiatric rehabilitation professionals (+267.7%), social workers (+117.7%) and nurses (+44.2%). Increased emotional support was also provided to patients' family members and contacts doubled during the pandemic. At the same time, residential facilities were organized as close communities, looking after their own patients with little reliance on the hospital. Indeed, during the pandemic hospital admissions due to difficulties pertaining to residential facilities and programs were found to be significantly lower compared with the previous year.

In other words, team working acquired special relevance in order both to provide emotional support to patients and to cater to their practical needs. The team also ensured a first-line support to health professionals, allowing them to express fears, uncertainties and emotional discomfort, to receive mutual support and devise new interventions in patients' interest, where psychiatrists and psychologists could rely on the indispensable help by those health providers working closer to patients, like nurses, social workers or psychiatric rehabilitation professionals. Remote consultations offered a sort of presence in the absence, but introduced a radically new way of working, with a change from a physical to a digital kind of space, a variation in the subjective experience of the time spent during consultations and difficulties of

different nature (*e.g.*, distraction on behalf of the patient and/or the therapist; external and disturbing factors; greater tiredness during on-line consultations; dehumanization)[9-11].

A reduction in admission rates was reported by other inpatient services across Italy as a consequence of fear of hospitals, seen as potential sites of contagion, and a heightening in the severity threshold of psychiatric symptoms leading to hospital admission upon request by patients' family members or referral by treating clinicians. In most mental health services, outpatient contacts tended to decline during the pandemic though, in some services, they were preserved and, in the catchment area of the inpatient unit under study, increased, as a consequence of different choices in the application of restriction criteria and in service activity[12-14]. These findings suggest that the distinctive organization of mental health services in Lombardia Region, each established according to a community-based model of care with a multidisciplinary team serving a well-defined catchment area under the coordination of the local department of mental health, had the potential: (1) To face and overcome the limitations imposed by the pandemic by changing allocation of human resources and remodeling interventions in order to meet patients' new and different needs; and (2) To implement a shared recommendation that all patients, and especially so those with severe mental disorder, were not left alone and forgotten during the COVID-19 crisis and received regular assessment, emotional support and treatment (*e.g.*, long-acting antipsychotics) by telephone consultations, face-to-face interviews or, in selected cases, domiciliary visits[15,16].

During the pandemic non-resident patients, who were transferred for hospital admission, were likely to be less severely ill and did not require compulsory interventions or restraint. This was probably due to the fact that the Police members were more involved in other tasks of public order during the pandemic and could not provide routine support to health personnel on patients' transfer. Data on admissions in 2019 under standard care showed that non-resident patients were more likely to be restrained, pointing to a delicate ethical issue. In order to promote an efficient use of health resources, Lombardia Region does not pose any limitation on patients' referral to inpatient units other than the local facility, challenging the longstanding practice of a well-defined catchment area pertaining to each department of mental health. However, the lack of reciprocal enduring knowledge by both patients and the health staff-continuity of care-is likely to affect negatively the quality of care and a consequence may be the increased risk for non-resident patients to be restrained at the outset of their hospital admissions.

Although a strong emphasis was placed on trying to shorten hospital admissions during the pandemic in order to ensure ongoing bed availability, avoid patients' transfer and keep interpersonal distancing during hospital stay, no significant difference was found compared to care under standard conditions in 2019. Length of stay varied widely and meaningful variations occurred within each diagnostic group, though individuals with personality disorders tended to have shorter admissions. About one-third of admissions lasted longer than the threshold of 14 d recommended by local health authorities on the basis of regional standards and reimbursement considerations.

These observations suggest that about one-third of patients need longer time periods to achieve clinical improvement and be discharged from hospital, in keeping with the reported limited clinical effectiveness of short hospital admissions that was suggested in patients with severe mental disorders[17] and undermining claims of systematic early onset of action of psychotropics, namely antidepressants and antipsychotics[18-20]. As a result, prediction of resource use in hospital psychiatry can hardly rely on diagnosis and the derived diagnosis-related groups (DRGs), which are inaccurate and explain a very limited proportion of variance in psychiatric length of stay. For this reason, prognosis, rather than diagnosis, has been suggested to provide a better estimate of prospective reimbursement for psychiatry[21-23]. Indeed, among prognostic factors, illness severity was found to be the main reason for psychiatric admissions lasting longer than two weeks, irrespective of diagnosis, which no longer retained any statistically significant effect[6]. Other factors, like those listed in the present paper and related to the care process, logistic and communication aspects of the institutional network, or the patients' social and familial context, could also prove useful as additional variables alongside illness severity to reach a better prediction of hospital length of stay and associated costs. Indeed, in the present investigation non-clinical factors occurred together with clinical ones in 18% of admissions during pandemic; and, in a further 18% of admissions, were the sole reasons, suggesting their specific relevance, even more because psychiatrists during pandemic were invited to give definite priority to clinical factors in deciding hospital admissions. It follows that

imposing strict limitations in length of stay in order to contain costs contradicts findings from everyday clinical practice and carries the risk of increasing inappropriate discharge of patients and/or exposing to financial risks the inpatient care units treating more severe cases. In order to reach a more accurate prediction of the economic impact of psychiatric admissions it would be useful to move from mere length of stay to consider also severity and complexity of clinical picture as well as other context-related factors.

During the pandemic a significantly greater number of hospital admissions were related to difficulties in organizing care programs outside the hospital and in patients' family contexts. The reduction of community-based interventions, the absence or strong limitations of interpersonal relationship and social experiences and the exacerbation of conflicts within families may be responsible for psychiatric crises and reveal that an effective functioning of the mental health system cannot rely on clinical settings only[24]. In this regard, Pelizza&Pupo[25] brought attention to the crucial role of patients' caring communities, mainly represented by family members and local social agencies, and suggested the actual need of a transition from an institutional context, centered on mental health services, to a so-called post-institutional system, where individuals and communities are connected through a rich and articulated set of social ties and patients' settings are not distant and isolated, but connected to mental health services *via* innovative clinical interventions based on new technologies.

Since the COVID-19 pandemic is not decreasing and continues to provide ever-growing and alarming figures over time, the changes mentioned above are likely to last and might even turn into the usual way of working for mental health professionals to come, with the pandemic marking a definite difference between a before and an after[26]. The essential role assigned to mental health services at the outset of the pandemic according to national guidelines and local protocols and their well-established attitude to deliver comprehensive interventions to individuals with mental disorder, covering subjective well-being, daily living, material needs, and social activities, contributed to support mental health professionals' motivation, energy and creativity in planning and implementing interventions during the pandemic. However, if the current situation is lasting for long, a critical evaluation of mental health service organization and requirements (especially, in terms of personnel and technical equipments for online consultations) is mandatory in order to sustain actual efforts.

Some limitations of this study should be acknowledged. Firstly, data were collected in a single inpatient psychiatric unit and this may reduce generalization of findings. However, gender and diagnostic distributions and age at admission in our sample closely resembled those recorded across other inpatient psychiatric units located in Lombardia Region[27] as well as those detected in a representative national sample of inpatient psychiatric units[28], suggesting that the inpatient unit under study was comparable to similar units operating in Italy.

Moreover, the check-list of factors associated to each hospital admission was filled in by a psychiatrist who was also caring for patients, allowing a detailed recording of all the factors involved, though this might reduce objectivity in the estimate of those factors more related to the care process.

CONCLUSION

In conclusion, the COVID-19 pandemic in 2020 forced a re-organization of mental health service activities at all levels of care. Hospital admissions dropped significantly and were more likely to be related to restrictions posed by the pandemic, like difficulties in organizing care programs outside the hospital and in patients' family contexts.

At the same time, community contacts with both patients and their relatives increased through a combination of face-to-face and domiciliary visits with remote consultations.

Finally, residential facilities turned into close communities looking after their own patients with limited reliance on the hospital.

It follows that an accurate evaluation of the effects of the pandemic on psychiatric admissions (with the associated economic impact) should devote concomitant attention to other treatment settings as well (*i.e.*, outpatient services and residential facilities) and include context-related factors alongside severity and complexity of clinical picture.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic forced a re-organization of mental health services at all levels of care. However, most accounts of changes occurring in Italy during the pandemic have been mainly narrative with little reliance on data.

Research motivation

The present study was based on a quantitative data-driven approach to the effects of COVID-19 pandemic on admissions to an inpatient psychiatric unit in Italy.

Research objectives

To explore changes in number of psychiatric admissions to an inpatient psychiatric unit during the COVID-19 pandemic in 2020 and to identify relevant factors associated with the detected changes in comparison with the same time period of 2019.

Research methods

All admissions were recorded to an inpatient psychiatric unit between February 24 and May 24, 2020 and compared with those occurring over the same time period in 2019. A 20-item checklist was completed to identify relevant factors leading to hospital admission.

Research results

During the COVID-19 pandemic hospital admissions dropped significantly compared to 2019 and were more likely to be related to difficulties in organizing care outside the hospital and in patients' family context. On the other hand, admissions related to logistic and communication difficulties pertaining to residential facilities were more common in 2019, due to the re-organization of these facilities as close communities looking after their own patients during the pandemic.

Research conclusions

Mental health services in general, and hospital psychiatry in particular, were forced to face new and different challenges during the COVID-19 pandemic. The Italian community-based model of care with a multidisciplinary team serving a well-defined catchment area had the potential to ensure a proper and rapid re-organization of mental health service activities.

Research perspectives

Since the COVID-19 pandemic is slowly decreasing and the associated limitations persist, the detected changes are expected to last and turn into the usual way of working. Therefore, an ongoing evaluation of mental health service organization, activities and requirements is mandatory to sustain and improve actual efforts.

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LETTER TO THE EDITOR

Repurposing the antioxidant and anti-inflammatory agent N-acetyl cysteine for treating COVID-19

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Abstract

Although several considerations have been raised suggesting a beneficial effect of N-acetyl cysteine (NAC) for the treatment of severe acute respiratory syndrome coronavirus 2 infection, there is currently no clinical evidence that NAC truly prevents coronavirus disease 2019 (COVID-19), reduces the severity of the disease, or improves the outcome. Appropriately designed clinical trials are warranted to prove or disprove a therapeutic effect of NAC for COVID-19 patients.

Key Words: N-acetyl cysteine; SARS-CoV-2; COVID-19; Reactive oxygen species; Cytokines

Core tip: N-acetyl cysteine (NAC) is a well-known antioxidant and anti-inflammatory agent that has been considered beneficial in the treatment for coronavirus disease 2019 (COVID-19). Although previous studies in patients with chronic lung disease, chronic heart disease, immune-mediated disease, viral infections, and malignancy have shown promising results, there is currently no clinical evidence that NAC prevents COVID-19, alleviates the severity of COVID-19, or improves the overall outcome of COVID-19 patients.

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TO THE EDITOR

With interest, we read the review article by Dominari *et al*[1] about the putative therapeutic effect of N-acetyl cysteine (NAC) in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients. The authors raise several arguments in favour of a beneficial effect of NAC for coronavirus disease 2019 (COVID-19), discuss preliminary results about ongoing studies with NAC in COVID-19, and conclude that the results of available trials are not clear. The study is appealing but raises the following comments and concerns.

We do not agree with the notion that NAC is an agent for curing SARS-CoV-2 infections[1]. There are several arguments against the antiviral effect of NAC. First, NAC is primarily an antioxidant and a precursor of reduced glutathione (GSH) that replenishes GSH stores[2]. NAC reduces oxidative stress as it scavenges and neutralises reactive oxidative species, such as OH, HOCl, or RO_2[3]. Thus, NAC is approved as a preventive/therapeutic agent in disorders associated with GSH depletion, as an antidote in paracetamol intoxication, and as a mucolytic agent[2]. Since SARS-CoV-2 infections are associated with oxidative stress, NAC can, at best, reduce oxidative stress and thus reduce secondary effects of the infection[2]. Although NAC additionally has an anti-inflammatory effect by reducing cytokine production *via* blocking of matrix metalloproteinase (MMP)-1, MMP-4, intracellular adhesion molecule 1, nuclear factor B, NF-E2-related factor 2, and tryparedoxin-1b[2], NAC cannot neutralise the virus and cannot reduce the virus load. Thus, NAC may have, at best, a complementary but no curative effect in SARS-CoV-2 infections as all infections are associated with increased oxidative stress and cytokine activation. Second, there are no reports that NAC is capable of reducing viral load, preventing infection, alleviating severity of COVID-19, or reducing mortality. Third, many patients are regularly taking NAC for the treatment of bronchitis, bronchiolitis, pneumonia, asthma, or chronic obstructive pulmonary disease. However, there are no indications that patients regularly taking NAC have a decreased risk of SARS-CoV-2 infection, or that morbidity or mortality of SARS-CoV-2 infection in these patients is lower compared with that in patients not taking NAC. Fourth, NAC did not prevent the presence of SARS-CoV-2 in sputum[4]. Arguments in favour of a promising role of NAC in the management of COVID-19, however, are that it generally enhances immunocompetence[5] and that it inhibits the replication of the influenza virus H5N1 [6]. A potential beneficial effect of NAC for treating COVID-19 may also derive from its capacity to increase glutathione, improve T-cell responses, and modulate inflammation[7-12]. Currently, a protocol for using NAC together with heparin has been developed[13] but no results have yet been published. Since several studies concerning the role of NAC in COVID-19 are under way, final conclusions about its contribution for treating COVID-19 cannot be reliably drawn. Future studies may demonstrate that NAC can reduce replication of SARS-CoV-2. Overall, agents that appear beneficial theoretically need to be thoroughly investigated by appropriately designed clinical trials for their putative beneficial effect. This is particularly the case for anti-COVID-19 agents, as there is strong pressure from healthcare authorities, industry, and the global community to provide a safe and effective cure of this global threat that currently influences all segments of social, economic, scientific, and political life. Effective and safe agents are needed as several drugs that were proposed to be beneficial at the beginning of the pandemic turned out to be harmful or inefficient, such as chloroquine, azithromycin and tocilizumab.

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LETTER TO THE EDITOR

Role of vitamin D deficiency and comorbidities in COVID-19

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Abstract

Recent manuscripts described the incidence of vitamin D hypovitaminosis in coronavirus disease 2019 (COVID-19) patients. Vitamin D deficiency is also common in patients with comorbidities that are associated with a poor COVID-19 prognosis. In this letter, we review the literature regarding the association of comorbidities, vitamin D deficiency, and COVID-19.

Key Words: COVID-19; SARS-CoV-2; Comorbidities; Vitamin D

Core Tip: Vitamin D deficiency is a worldwide problem, and investigations on the benefits of regulating vitamin D levels and the immune response should be performed. Nevertheless, the association between low levels of vitamin D and coronavirus disease 2019 (COVID-19) needs to be further explored, especially investigations on the immune response to COVID-19 and COVID-19 vaccines in patients with and without comorbidities.

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TO THE EDITOR

We read with great interest the article entitled "Association between population vitamin D status and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) related serious-critical illness and deaths: An ecological integrative approach" recently published by Papadimitriou *et al*[1] in the *World Journal of Virology*[1]. This manuscript raised important questions and the authors performed an extensive analysis on vitamin D levels and COVID-19 incidence and severity in Europe, and the potential benefits of vitamin D supplementation to enhance the immune response to the SARS-CoV-2[1]. In the light of these results, we humbly want to state a few points for consideration.

Severe coronavirus disease 2019 (COVID-19) patients present a systemic inflammatory response with a coagulation disorder, possibly evolving to death[2]. Several comorbidities have been identified as risk factors for poor disease prognosis, such as old age[3], co-infections[4], obesity and diabetes mellitus[5], severe asthma, alcohol drinking[6], chronic obstructive pulmonary disease[7], chronic liver disease[8], and cancer[9].

Vitamin D deficiency is associated with poor response to respiratory infections[10], and few reports have identified vitamin D deficiency in moderate and severe COVID-19 patients with conflicting results[1,11,12].

Vitamin D receptor is expressed in many immune cells, including monocytes, macrophages, dendritic cells, neutrophils, and lymphocytes[13-15]. Vitamin D increases the antimicrobial activity of monocytes and macrophages[16] and has anti-inflammatory effects due to the induction of T regulatory cells and reduction in the T helper-17 immune response and pro-inflammatory cytokine production[15].

Papadimitriou *et al*[1] performed an important investigation on the association of vitamin D deficiency and COVID-19[1]. Vitamin D levels can be influenced by many factors such as sun exposure, genetics, supplementation, and comorbidities[17-20].

Vitamin D hypovitaminosis is associated with several comorbidities that are also related to poor COVID-19 prognoses such as old age[21], co-infections[18], obesity [22], diabetes mellitus[23], alcohol drinking, and smoking[24-26], uncontrolled asthma, but not controlled asthma, chronic obstructive pulmonary disease[25-28], cancer[29], and solid organ transplant recipient patients[30].

Besides comorbidities, vitamin D hypovitaminosis is associated with poor glycemic control[23], which is also associated with poor COVID-19 outcomes in diabetic and non-diabetic patients[31]. Cancer patients present low circulating levels of vitamin D [29] and experimental models have identified that vitamin D can modulate the disease development by regulating cell cycle and inflammatory response[32].

Vitamin D deficiency is a worldwide problem[33,34], and vitamin D supplementation has the potential to enhance the immune response to microorganisms[1]. Vitamin D supplementation has been investigated for the treatment and prevention of severe COVID-19, indicating a potential reduction in COVID-19 severity[35].

A recent investigation found that prophylactic vitamin D supplementation in elderlies improved the SARS-CoV-2 immune response[36], and another investigation identified that the treatment with vitamin D reduces COVID-19 severity[37]. Nevertheless, another report found no additional benefit in vitamin D supplementation during COVID-19[38].

Low vitamin D levels also modulate the Renin-Angiotensin-System, which could increase the susceptibility to COVID-19[39], since SARS-CoV-2 uses the angiotensin-converting enzyme 2 and Transmembrane Protease Serine 2 (TMPRSS2) to invade the host's cells[40]. In addition, the lack of vitamin D is a risk factor for the development of autoimmune and neuropsychiatric disorders[41].

Lakkireddy *et al*[42] identified that increasing the serum levels of vitamin D to 80–100 ng/mL significantly reduced inflammatory biomarkers such as interleukin-6, C-reactive protein, and neutrophil-to-lymphocyte ratio during COVID-19, without side effects[42].

In addition, Papadimitriou *et al*[1] recommendation for vitamin D supplementation should also be considered in a broader context[1], outside the COVID-19 pandemic situation, due to the high incidence of vitamin D hypovitaminosis worldwide, the vast associations with other diseases, and the proposed doses do not require medical supervision[1].

COVID-19 vaccination is ongoing worldwide[43-45], since vitamin D can modulate the immune response to vaccines[46,47], investigations on the vaccines should consider evaluating vitamin D levels and the effects of supplementation on the immune response to vaccines.

In summary, vitamin D hypovitaminosis is associated with comorbidities that are known to affect COVID-19 severity and outcome. Further investigations should focus on patients with low vitamin D levels with and without comorbidities and supplementation trials to investigate the effects of vitamin D on the immune response to COVID-19 and COVID-19 vaccines.

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