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Contents

ORIGINAL RESEARCH

REVIEW ARTICLES

CASE REPORTS

CLINICAL VIGNETTES

LETTERS TO THE EDITOR

Mitsuhiro Abe[1], Kenji Tsushima[2], Daisuke Ishii[1], Kohei Shikano[1], Keiichiro Yoshioka[1], Masashi Sakayori[1], Masaki Suzuki[1], Yasutaka Hirasawa[1, 2], Tsukasa Ishiwata[1], Takeshi Kawasaki[1], Jun Ikari[1], Jiro Terada[1, 2], Koichiro Tatsumi[1]

[1]Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan
[2]Department of Pulmonary Medicine, International University of Health and Welfare, School of Medicine, Narita-city, Japan

Risk factors for acute exacerbation following bronchoalveolar lavage in patients with suspected idiopathic pulmonary fibrosis: A retrospective cohort study

Abstract

Introduction: Bronchoalveolar lavage (BAL) is useful for diagnosing diffuse lung disease and excluding other conditions. However, acute exacerbations (AEs) are recognized as important complications of BAL in patients with idiopathic pulmonary fibrosis (IPF). This study aimed to identify risk factors for BAL-induced AEs in patients with IPF.

Material and methods: We retrospectively analyzed the data of 155 patients with suspected IPF who had undergone BAL between January 2013 and December 2018. BAL-related AE was defined as the development of AE within 30 days after the procedure. We compared clinical features and parameters between patients with AE (AE group) and without AE (non-AE group). We also reviewed the relevant reported literature.

Results: Among the 155 patients, 5 (3.2%) developed AE within 30 days after BAL. The average duration from BAL to AE onset was 7.8 days (2–16 days). Results from the univariate analysis revealed $PaO_2 < 75$ mm Hg ($p = 0.036$), neutrophil content in BAL $\geq 7\%$ ($p = 0.0061$), $\%D_{LCO} < 50\%$ ($p = 0.019$), Gender-Age-Physiology (GAP) stage III ($p = 0.034$), and BAL recovery rates $< 30\%$ ($p < 0.001$) as significant risk factors for post-BAL AE. All five patients who developed AE recovered and were discharged.

Conclusions: Disease severity, high neutrophil levels in BAL, and poor BAL recovery rates may be risk factors for BAL-induced AEs.

Key words: bronchoalveolar lavage, C-reactive protein, idiopathic pulmonary fibrosis, interstitial lung disease, risk factor

Adv Respir Med. 2021; 89: 101–109

Introduction

Bronchoalveolar lavage (BAL) is a standard tool for the diagnostic and prognostic evaluation of diffuse lung diseases [1–3]. BAL is useful for differentiating idiopathic pulmonary fibrosis (IPF) from other fibrosing lung diseases, such as non-specific interstitial pneumonia (NSIP), chronic hypersensitivity pneumonia (CHP), and interstitial pneumonia due to collagen and vasculitis disease. Ohshimo *et al.* [4] reported that 8% of patients with a usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT) might have BAL findings suggestive of such an alternative diagnosis.

Clinical rationale for the study

The American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), and Latin American Thoracic Society (ALAT) 2011 guidelines advocated that the most important application of BAL when evaluating patients with suspected IPF is CHP exclusion; prominent lymphocytosis (> 40%) should suggest CHP [5]. The ATS/JRS/ALAT 2020 guidelines on CHP by Raghu *et al.* described the importance of BAL in diagnosing CHP [6]. Per the ATS/ERS/JRS/ALAT 2018 guidelines [7], BAL is not recommended for patients with a UIP pattern because of the

Address for correspondence: Mitsuhiro Abe, Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan; e-mail: mthrsgnm@chiba-u.jp

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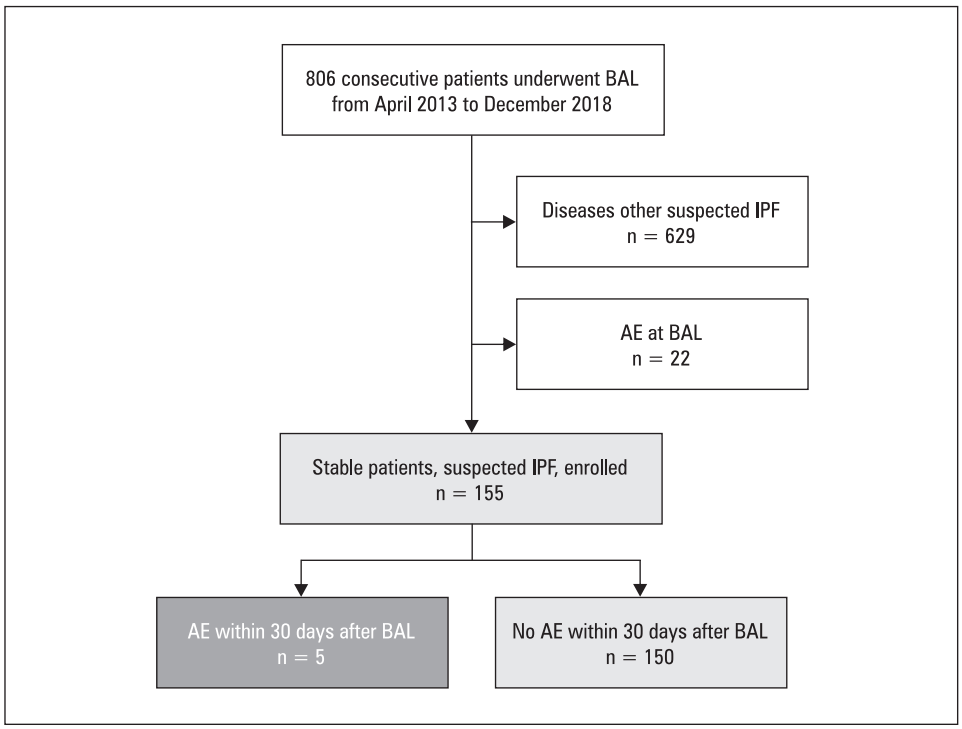

Figure 1. Study flow chart. We identified 806 consecutive patients who underwent bronchoalveolar lavage (BAL) from April 2013 to December 2018. Of them, 629 patients were diagnosed with diseases other than suspected IPF; therefore, they were excluded from the analysis. Additional 22 patients with acute exacerbation were excluded. Therefore, 155 individuals were enrolled in the study. Five patients developed acute exacerbation within 30 days after the BAL procedure. BAL — bronchoalveolar lavage; IPF — idiopathic pulmonary fibrosis; AE — acute exacerbation

risk of acute exacerbation (AE) of IPF (AE-IPF) [8, 9]. Sakamoto *et al*. [8] reviewed 12 cases of BAL-induced AE-IPF and found that functional impairment or active inflammation may be risk factors for BAL-induced AE. Only patient factors were reported; no risk factors were evaluated for bronchoscopic procedures.

Therefore, this study aimed to evaluate the previously proposed risk factors for IPF-AE after BAL and identify other novel risk factors for IPF-AE after BAL.

Material and methods

Our single-center retrospective study was approved by our human ethics committee (protocol number 2083). We obtained informed consent with an opt-out option. The study was conducted in accordance with the ethical principles of the 1964 Declaration of Helsinki and subsequent amendments.

Patients

Between January 2013 and December 2018, 806 consecutive patients underwent BAL at our hospital. Among them, 629 subjects were suspected of having non-IPF diseases, including an "alternative diagnosis" per the 2018 ATS/ERS/JRS/ALAT statement [7]; drug-induced lung injury; interstitial lung disease with collagen vascular disease; acute respiratory distress syndrome; etc.; therefore, they were excluded from our analysis. Twenty-two patients suspected of having AE at the time of BAL were excluded. Finally, 155 individuals suspected of having stable (non-exacerbation) IPF were analyzed (Figure 1). Then, these patients underwent their first BAL procedure.

UIP pattern on HRCT

Radiological diagnosis on HRCT (UIP, probable UIP, and indeterminate for UIP) was determined per the 2018 ATS/ERS/JRS/ALAT guidelines [7]. After discussion between two respiratory specialists, the HRCT pattern was classified as either UIP (n = 68), probable UIP (n = 57), or indeterminate for UIP (n = 30) (Table 1).

IPF confidence

Surgical lung biopsy was performed in eight cases. The final diagnoses were expressed using the four diagnostic confirmation levels proposed by Ryerson *et al*. [10]: a "confident diagnosis" meets ≥ 90% of the guidelines, a "high-confidence diagnosis" meets 70–89%, a "low-confidence di-

Table 1. Baseline characteristics

	n = 155
Age [years]	68.6 ± 7.0
Male, n [%]	110 (70%)
Smoker, n [%]	113 (72%)
HRCT diagnosis [UIP/probable UIP/indeterminate for UIP]	68/57/30
PaO_2 [mm Hg]	80.5 ± 12.5
KL-6 [U/mL]	1353 ± 1200
CRP [mg/dL]	0.4 ± 1.0
%FVC [%]	75.6 ± 18.3
$\%D_{LCO}$ [%]	66.9 ± 23.3
GAP stage [I/II/III]	87/54/14
Confidence level of IPF [confident/high confidence/low confidence/unclassifiable]	70/49/20/16

Data are expressed as mean ± standard deviation. HRCT — high-resolution computed tomography; UIP — usual interstitial pneumonia; KL-6 — Krebs von den Lungen-6; CRP — C-reactive protein; FVC — forced vital capacity; D_{LCO} — diffusing capacity of the lungs for carbon monoxide; GAP stage — Gender-Age-Physiology stage; IPF — idiopathic pulmonary fibrosis

agnosis" meets 51–69%, and an "unclassifiable diagnosis" meets < 50% (Table 1).

Gender-age-physiology stage

Gender-Age-Physiology (GAP) stages were calculated per the criteria reported by Ley *et al*. [11]: sex (female, 0 points; male, 1 point), age (≤ 60 years, 0 points; 61–65 years, 1 point; > 65, 2 points), predicted forced vital capacity (%FVC) (> 75%, 0 points; 50–75%, 1 point; < 50%, 2 points), and predicted diffusing capacity for carbon monoxide ($\%D_{LCO}$) (> 55%, 0 points; 36–55%, 1 point; ≤ 35%, 2 points; cannot obtain D_{LCO}, 3 points). The patients were divided into the following GAP stages based on their total GAP score: I (0–3 points), II (4–5 points), and III (6–8 points).

BAL procedures

BAL procedures were performed using a flexible bronchoscope with a 5.9-mm outer diameter (BF-1TQ290 or BF-6C260; Olympus Corporation, Tokyo, Japan) under intravenous anesthesia. Sterile saline (0.9% NaCl) at room temperature was instilled through the bronchoscope. Per the commonly used methodology in Japan, the total instilled volume of saline was 150 mL (50 mL × 3 times). As the lavage site, 127 cases were in the middle lobe or lingula, and 28 were at other sites. The lavage site was determined by considering the presence of the interstitial shadow. Transbronchial lung biopsy (TBLB) was performed after BAL in 131 cases. We did not perform transbronchial lung cryobiopsy.

Diagnosis of BAL-induced AE-IPF (AE and non-AE groups)

The patients were diagnosed with AE-IPF if they met the following criteria established by Collard [12]: (1) previous or concurrent IPF diagnosis, (2) acute worsening or development of dyspnea (typically of 1-month duration), (3) computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with a UIP pattern that appeared as new shadows on the BAL site and the opposite lung field, and (4) deterioration not fully explained by cardiac failure or fluid overload. BAL-induced AE-IPF was defined as AE-IPF occurring within 30 days post-BAL procedure (AE group). The patients who did not develop AE within 30 days after the BAL procedure were included in the non-AE group.

Statistical analysis

Clinical data are expressed as mean ± standard deviation. We compared the AE and non-AE groups using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Youden's index and receiver operating characteristic curve analysis were used to identify parameters affecting AE within 30 days post-BAL (Figure 2). Univariate logistic regression analyses were used to identify factors affecting BAL-induced AE-IPF. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13]. Statistical significance was defined as $p < 0.05$.

Previous BAL-induced AE-IPF reports

We searched the literature to identify previous BAL-induced AE-IPF reports published from 1977 to 2019. We used PubMed *(https://www.ncbi.nlm.nih.gov/pubmed*) for English reports and J-STAGE (*https://www.jstage.jst.go.jp*) for English and Japanese reports.

Patient and public involvement

The patients were not involved in the design, recruitment, or conduction of the studies included in this analysis.

Results

Table 1 shows the baseline characteristics of the 155 patients. According to HRCT, 68 patients exhibited the "UIP pattern," 57 showed a "proba-

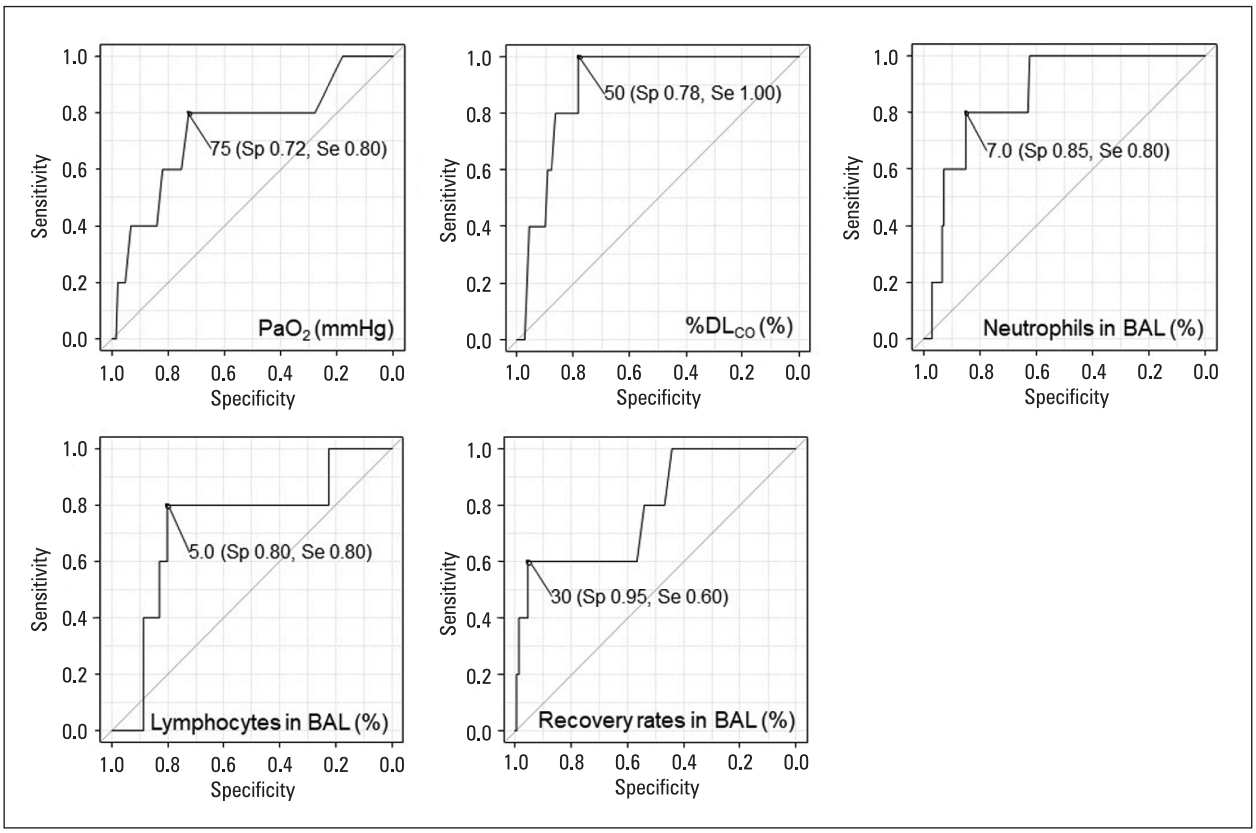

Figure 2. Receiver operating characteristic curve analysis was performed to obtain the parameters affecting acute exacerbation within 30 days of BAL. Sp — specificity; Se — sensitivity; PaO_2 — partial pressure of oxygen; D_{LCO} — diffusing capacity for carbon monoxide; BAL — bronchoalveolar lavage

ble UIP pattern", and 30 were considered "indeterminate for UIP" [7]. The GAP stage was relatively mild [9]. Specifically, 87 patients were classified as stage I, 54 as stage II, and 14 as stage III.

Thirty-one patients had > 25% of lymphocytes in BAL fluid, suggesting the presence of non-IPF diseases, such as CHP and NSIP. One of these patients was diagnosed with NSIP based on surgical lung biopsy. The other 30 subjects did not undergo surgical lung biopsy. Using the Ryerson method, the 155 patients were classified into four groups according to IPF confidence level (confident, 70; high confidence, 49; low confidence, 20; unclassifiable, 16; Table 1).

The median AE-free survival after bronchoscopy was 1057 days (range: 3–2263 days). Among the 155 patients, 19 (12%) developed AE, and 25 (16%) died within the study period. The causes of death were AE (n = 6); respiratory failure (n = 15); and other causes (n = 4), including colon cancer (n = 1), ovarian cancer (n = 1), liver failure due to viral hepatitis (n = 1), and heart failure (n = 1). The overall AE frequency throughout the whole observation period was 0.36% per 30 days and 4.4% per year.

Table 2 shows the clinical data of the five BAL-induced AE-IPF patients and non-AE patients. All five AE-IPF subjects were male, were past smokers, exhibited a UIP pattern on HRCT, and had confident IPF. Although partial pressure of oxygen (PaO_2) ($p = 0.062$) and %FVC ($p = 0.36$) were not significantly different between the two groups, $\%D_{LCO}$ was significantly lower in the AE group than in the non-AE group ($p = 0.0074$). Additionally, the GAP stage was significantly more severe in the AE group than in the non-AE group ($p = 0.0086$). Further, the BAL recovery rate was significantly lower in the AE group than in the non-AE group ($p = 0.029$). There was no significant difference in the lymphocyte fraction in BAL fluid between the two groups ($p = 0.086$), but the neutrophil fraction was significantly higher in the AE group than in the non-AE group ($p = 0.0059$).

Univariate logistic regression analysis results revealed that $PaO_2 < 75$ mm Hg ($p = 0.036$), neutrophils in BAL ≥ 7% ($p = 0.0061$), $\%D_{LCO} < 50\%$ ($p = 0.019$), GAP stage III ($p = 0.034$), and BAL recovery rates < 30% ($p < 0.001$) were the significant risk factors for post-BAL AE (Table 3).

Table 2. Clinical parameters of the AE group and non-AE group

	AE [n = 5]	non-AE [n = 150]	P value
Age [years]	70.0 ± 5.2	68.6 ± 7.1	0.78
HRCT diagnosis (UIP/probable UIP/indeterminate for UIP)	5/0/0	63/56/30	0.14
Confidence level of IPF (confident/high confidence/low confidence/unclassifiable)	5/0/0/0	65/49/20/16	0.14
PaO_2 [mm Hg]	68.6 ± 15.1	80.9 ± 12.2	0.062
KL-6 [U/mL]	1068 ± 258	1363 ± 1219	0.75
CRP [mg/dL]	1.8 ± 3.7	0.4 ± 0.8	0.10
%FVC [%]	70.4 ± 24.2	75.8 ± 17.7	0.36
$\%D_{LCO}$ [%]	39.6 ± 9.1	67.9 ± 23.1	0.0074
GAP stage [I/II/III]	0/3/2	87/51/12	0.0086
TCC in BAL [$10^5/mm^3$]	1.8 ± 1.0	2.4 ± 2.0	0.62
Neutrophils in BAL [%]	13.1 ± 10.5	4.3 ± 8.6	0.0059
Lymphocytes in BAL [%]	8.3 ± 8.9	18.5 ± 17.3	0.086
Recovery rates in BAL [%]	35.2 ± 17.6	52.0 ± 13.4	0.029
TBLB, n [%]	4 [80%]	127 [85%]	0.57

Data are expressed as mean ± standard deviation. AE — acute exacerbation; HRCT — high-resolution computed tomography; UIP — usual interstitial pneumonia; IPF — idiopathic pulmonary fibrosis; KL-6 — Krebs von den Lungen-6; CRP — C-reactive protein; FVC — forced vital capacity; D_{LCO} — diffusing capacity of the lungs for carbon monoxide; GAP stage — Gender-Age-Physiology stage; TCC — total cell counts; BAL — bronchoalveolar lavage; TBLB — transbronchial lung biopsy

Table 3. Univariate logistic regression analysis of risk of acute exacerbation within 30 days post-bronchoalveolar lavage

		HR	95% CI	P value
Age [years]	≥ 65	1.41	0.15–13.0	0.76
	> 65	Ref		
PaO_2 [mm Hg]	≥ 75	0.094	0.01–0.86	0.036
	< 75	Ref		
KL-6 [U/mL]	≥ 800	2.21	0.24–20.3	0.48
	> 800	Ref		
CRP [mg/dL]	≥ 0.3	1.42	0.22–8.78	0.70
	< 0.3	Ref		
%FVC [%]	≥ 60	0.40	0.06–2.55	0.33
	< 60	Ref		
$\%D_{LCO}$ [%]	≥ 50	0.069	0.007–0.64	0.019
	< 50	Ref		
GAP stage	III	7.67	1.17–50.4	0.034
	I/II	Ref		
TCC [$10^5/mm^3$]	≥ 2.0	0.22	0.025–2.10	0.19
	< 2.0	Ref		
Neutrophils in BAL [%]	≥ 7	22.9	2.43–215	0.0061
	< 7	Ref		
Lymphocytes in BAL [%]	≥ 6	0.070	0.007–0.65	0.019
	< 6	Ref		
Recovery rates in BAL [%]	≥ 30	0.037	0.005–0.25	< 0.001
	< 30	Ref		

HR — hazard ratio; CI — confidence interval; Ref — reference; KL-6 — Krebs von den Lungen-6; CRP — C-reactive protein; FVC — forced vital capacity; D_{LCO} — diffusing capacity of the lungs for carbon monoxide; AE — acute exacerbation; GAP stage — Gender-Age-Physiology stage; BAL — bronchoalveolar lavage

Multivariate analysis was difficult to perform because the number of events was as small as five.

From the PubMed and J-STAGE databases, we discovered 12 cases from five reports that evaluated BAL-induced AE-IPF (Table 4) [8, 14–17]. All five of our cases improved and survived with systemic steroid therapy. Although the BAL recovery rates were sometimes inadequate, the average BAL recovery rate of the previous and present studies was 48% (range: 17–80%). Additionally, the average predicted %FVC was 69.5% (range: 42.5–99%), but the average $\%D_{LCO}$ was 45% (range: 16–81%). Furthermore, the C-reactive protein (CRP) level and/or neutrophil count in the BAL fluid were increased in a few cases.

Discussion and conclusions

In this study, we investigated the risk factors for BAL-induced AE-IPF. Our analyses revealed several risk factors for BAL-induced AE-IPF: (1) neutrophils in BAL ≥ 7%, which is an indicator of inflammation and instability in the lung, (2) the BAL recovery rate, which has not been reported as a risk factor previously, and (3) disease severity (PaO_2 < 75 mm Hg, $\%D_{LCO}$ < 50%, or GAP stage III). We first revealed that the BAL procedure itself was involved in the development of AE after the procedure.

Atkins *et al*. [18] reported that the incidence of AE was 4.1 per 100 patient-years based on a meta-analysis from six clinical trials. Additionally, in the INPULSIS trial, a phase III randomized trial of nintedanib (n = 1066), Richeldi *et al*. [19] reported that AE-IPF occurred within 1 year in 7.6% of patients who received a placebo. Among the 155 patients in our study, the incidence of AE within the first 30 days after the BAL procedure was significantly higher than that over the entire observation period (first 30 days: 3.2 per 100 patients-30 days; entire observation period: 0.36 per 100 patients-30 days, p < 0.001), suggesting a risk of AE in patients with IPF for at least 30 days after BAL.

The high neutrophil level in BAL (≥ 7%) was a significant risk factor for AE after BAL (HR, 17.6; 95% CI, 1.17–265; p = 0.038; Table 3). The subjects with elevated neutrophil counts, as indicated by BAL, were predicted to have had increased lung activity at the time BAL was performed. This informed our conclusion that AE was likely to occur in these patients. Kinder *et al*. [20] reported increased neutrophils in BAL fluid to be an independent predictor of early mortality among patients with IPF. Sakamoto et al. [8] focused on the disease's "instability", an elevated CRP level (> 1 mg/dL) and/or increased white blood cell count (> 9000/mm^3), which is unusual for stable IPF cases, were observed in six cases. Careful follow-up is important in patients with "instability," but a high neutrophil level in BAL is a factor unknown before a BAL procedure and thus is not suitable for predicting the onset of BAL-induced AE.

We showed that a low BAL fluid recovery rate was a significant risk factor for AE after BAL (HR, 0.89; 95% CI, 0.81–0.97; p = 0.012; Table 3). Ogata *et al*. [21] reported that the frequency of complications (mainly hypoxemia and fever, not only AE) after BAL increased if the BAL recovery rate was poor. Although the study conducted by Ogata *et al*. [21] included various diffuse lung diseases [i.e., interstitial lung disease (20.7%) and pathologies, including IPF (no description), sarcoidosis (23.9%), infection (11.6%), collagen vascular disease-associated interstitial pneumonia (9.8%), and drug-induced interstitial pneumonia (9.8%)], our findings were consistent with those of their report. Poor BAL fluid recovery implies that much of the saline used for lavage remained in the lungs after the procedure. Saline is thought to be naturally absorbed into the blood vessels in the alveoli. If excessive saline remains in the alveoli, infection is promoted, and it is possible that AE occurs due to lung infection. Some reports have stated that saline lavage per se may cause lung injury. In an animal model, repeated BAL with saline resulted in acute lung injury [22, 23]. Matute-Bello *et al*. [23] reported that repeated lavage with saline reduced the surfactant lipid concentration in alveolar lining fluids and ultimately altered alveolar surface tension. Decreasing the surfactant causes lung injury by facilitating alveolar collapse, increasing mechanical injury, and impairing alveolar host defenses, a likely finding on both animal models and actual patients with IPF.

We found that disease severity (PaO_2 < 75 mm Hg, $\%D_{LCO}$ < 50%, or GAP stage III) was a significant risk factor for AE after BAL only in the univariate analysis. Several risk factors for the development of AE, including low %FVC [24–28], low D_{LCO} [24–26], and poor baseline oxygenation [24], have been previously identified. In a review of 12 case reports, Sakamoto et al. [8] reported that the severity of IPF before the BAL procedure was moderate to severe in patients who met any of the following criteria: (1) %FVC < 65%, (2) desaturation with exertion, and (3) $D_{LCO} \leq 50\%$. The findings support our results and indicate that

Table 4. Cases in which acute exacerbation of idiopathy pulmonary fibrosis developed after bronchoalveolar lavage

Author name	Age	Sex	PaO_2	%FVC (*%VC)	$\%D_{LCO}$	WBC	CRP	BAL to AE	BAL RR	BAL TCC	BAL Ne	BAL Ly	Medication for AE	Oxygen device	Outcomes
			Torr	%	%	/mm^3	mg/dL	days	%	$10^5/\mu L$	%	%			
Our cases	63	M	48	59	59	8200	8.5	6	52	1.9	30.5	5.6	Pulse + PSL, CyA	Nasal	Survived
	67	M	62	63	49	7300	0.2	2	28	1.2	13.6	3.6	Pulse + PSL, CyA	NHF	Survived
	74	M	89	74	63	7200	0.2	16	56	0.74	2.6	4.7	Pulse + PSL	NHF	Survived
	70	M	74	46	81	4200	0.2	12	23	36.3	11.8	3.5	Pulse + PSL	Nasal	Survived
	76	M	70	110	33	9400	0.3	3	17	18.6	7.3	24.3	Pulse + PSL	Nasal	Survived
Sakamoto [8]	51	M	65.7	44*	57	2800	1.6	18	N/A	1.4	4.0	1.7	Pulse + PSL	N/A	Died (47 days)
	68	M	75.8	46*	38	6800	0.4	15	N/A	9.9	3.0	2.0	Pulse + PSL, CyA	N/A	Died (26 days)
	64	M	51.0	75*	20	15100	4.6	12	N/A	16.0	4.0	1.7	Pulse + PSL, CyA	N/A	survived
	70	M	68.5	63*	40	5600	0.1	30	N/A	1.3	9.9	0.03	Pulse + PSL	N/A	Died (80 days)
Yoshitomi [14]	54	F	77.3	42.5*	N/A	6100	0.6	0	54	1.3	6.5	5.4	Pulse + PSL, AZP, CPA	IPPV	Died (36 days)
	75	M	63.5	N/A	N/A	9800	(3+)	0	35	2.7	35	8.8	—	N/A	survived
Suga [15]	67	M	65	59.5*	31	8700	0.1	41	N/A	10	11	46	Pulse	N/A	Died (17 days)
	57	M	61.3	52.5*	16	13800	6.7	3	N/A	10	4.8	2.6	Pulse	N/A	Died (6 days)
Hiwatari [16]	74	M	86	60*	N/A	N/A	(-)	3	63	96	4.0	10	Pulse + PSL	N/A	Died (18 days)
	66	M	64	65	41	N/A	(+)	N/A	67	30	30	2	Pulse + PSL	N/A	Died (104 days)
	79	F	80	99*	58	N/A	1	N/A	54	300	2	3	Pulse + PSL	N/A	Died (56 days)
Amamiya [17]	67	F	104	N/A	N/A	8300	0.4	2	80	2.7	1	23	Pulse + PSL	IPPV	Died (59 days)
average	67	M: 14 F: 3	70.8	69.5	45	8092	1.7	10.8	48	30.7	10.6	8.7			Survived: 7 Died: 10

AE — acute exacerbation,; AZP — azathioprine; BAL — bronchoalveolar lavage; CPA — cyclophosphamide; CRP — C-reactive protein; CyA — cyclosporine; FVC — forced vital capacity; D_{LCO}; diffusing capacity of the lungs for carbon monoxide; IPPV — Invasive positive pressure ventilation; Ly — lymphocytes; N/A — not applicable; Ne — neutrophils; NHF — nasal high flow therapy; PSL — prednisolone; Pusle — steroid pulse therapy; RR — recovery rate; TCC — total cell counts

some patients are already susceptible to AEs at the time of BAL.

We conducted a literature review, but there were no reports of BAL-induced AE since Sakamoto's analysis in 2012 [8]. One reason might be publication bias. Since BAL-induced AE is a major significant complication, it is necessary to reduce the publication bias to evaluate the risk. Our study revealed that the first performance of BAL might cause AE. By contrast, Sakamoto *et al*. [8] reported that the first BAL did not induce AE in any of four cases with AE that had undergone at least one previous BAL.

According to Sakamoto *et al*. [8] only one case survived the AE. In the current study, all five cases were successfully discharged. The death of 10 of the 12 patients reported in the literature (Table 4.) underscores the necessity of addressing AEs due to BAL. The therapies that we administered to the five patients with AEs in our study were similar to those administered to the subjects in the study conducted by Sakamoto *et al*. [8]. These therapies are outlined in Table 4. Additionally, advances in oxygen therapy and medical treatment may have contributed to improved mortality in our cases. Vianello *et al*. [29] evaluated the utility of a high-flow nasal cannula for patients with AE-IPF who did not respond to treatment using a conventional nasal cannula. In our cases, a high-flow nasal cannula was used in two cases. It is not possible to confirm whether the cases we reviewed used a high-flow nasal cannula.

This study's AE incidence (5 in 155 cases, 3.2%) is higher than in previous reports (4 in 202 cases, 1.9% [8]; none of 57 cases, 0% [21]; and two in 104 cases, 1.9% [15]). Although no detailed patient background was reported by Sakamoto *et al*. [8], the average age was 64 years, which was younger than that in our study (68.6 years). In addition, a surgical lung biopsy was performed in 47.3% of cases, suggesting that there are many relatively mild cases in which surgical lung biopsy is possible. Regarding Ogata's report [21], there is no patient background limited to idiopathic interstitial pneumonias, but the overall average age is as young as 58.7 years. These findings are in line with our findings that high severity is a risk factor for AEs. In Suga's report [15], the overall patient background is not mentioned.

Our study had some limitations. First, this was a single-center retrospective study with a small number of cases. Only five patients developed AE after BAL. Since the sample size was small, further studies with more patients are required. Second, the effects of the type of fiber used in bronchoscopy, years of experience of the examiner, examination time, and other bronchoscopic procedures on the development of AEs were not analyzed in the current study. Finally, BAL may not have been the cause of AE in some cases. An increase in the neutrophil level in BAL (≥ 7%) was confirmed in four cases. These results suggest that latent infection or disease progression may have existed before BAL.

Clinical implications/future directions

In conclusion, patients with suspected IPF may develop AE after BAL and should be monitored carefully. Disease severity, high neutrophil levels in BAL, and poor BAL recovery rates may be risk factors for BAL-induced AEs and need to be confirmed in a larger, multi-center prospective study. We will be able to assess some administration parameters, such as the number of years of experience of the examiner and examination times. Other factors to consider are the lavage equipment and its components.

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Conflict of interest

None declared.

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Hossein Kazemian[1, 2*], Hamid Heidari[3*], Jalil Kardan-Yamchi[4], Sobhan Ghafourian[1, 2], Iraj Pakzad[1, 2], Ebrahim Kouhsari[5], Hasan Valadbeigi[1], Nourkhoda Sadeghifard[1, 2]

[1]Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran
[2]Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran
[3]Department of Microbiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[5]Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran
*These authors contributed equally to this work and are co-first authors.

Comparison of toxin-antitoxin expression among drug-susceptible and drug-resistant clinical isolates of *Mycobacterium tuberculosis*

Abstract

Introduction: *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB), is a significant global public health threat. Besides extensive multidrug resistance, MTB possesses several properties for long-term viability in the host as well as stress adaptation and resistance in harsh conditions. The role of toxin-antitoxin (TA) systems in disseminating and maintaining antimicrobial resistance in bacterial populations has also been demonstrated. This study aimed to evaluate differences in expression of MazEF (a well-known TA system) related genes (*mazE3*, *mazF3*, *mazE6,* and *mazF6*) amongst drug-susceptible and resistant MTB isolates in Iran.

Material and methods: A total of 20 confirmed clinical isolates of MTB including 10 drug-susceptible and 10 drug-resistant (nine MDR, and one XDR) species were included in this study. *M. tuberculosis* H37Rv was used as the standard strain. RNA extraction, cDNA synthesis, and relative quantitative real-time PCR were performed according to the standard procedures.

Results: Our analysis indicated significant enhanced expression of the *mazE6* antitoxin gene in drug-susceptible isolates compared to drug-resistant isolates and the standard strain. The expression of the *mazF6* toxin gene was also increased in drug-susceptible isolates compared with the standard strain. In drug-resistant isolates, the expression levels of *mazF3* and *mazF6* genes were significantly higher than that in the susceptible isolates and the standard strain.

Conclusions: In this study, there was significant overexpression of *mazE6* in drug-susceptible isolates. As well, *mazF3* and *F6* were overexpressed in drug-resistant isolates when compared with the standard strain. The changes in expression levels of MazEF6 associated genes were greater than that of MazEF3 in both groups of isolates.

Key words: *Mycobacterium tuberculosis,* toxin-antitoxin system, MazEF, gene expression

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Introduction

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is a significant global public health threat [1]. According to World Health Organization (WHO) reports, there are more than 1.5 million TB-related deaths annually despite the extensive efforts to eradicate TB [2]. Multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB are also major challenges in the elimination of TB [3].

MTB possesses several properties for its long-term viability in hosts such as its special cell wall structure and metabolism [4]. Another important strategy for stress adaptation and resistance in harsh conditions (i.e. poor nutrition, oxidative stress, low pH, and hypoxia) is its Toxin-Antitoxin (TA) system [5-8]. The toxin molecule is

Address for correspondence: Nourkhoda Sadeghifard, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Banganjab, Ilam, Iran; e-mail: sadeghifard@gmail.com

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a stable protein and the antitoxin could either be a labile protein or non-coding RNA. During stressful conditions, the antitoxin is degraded and free active toxins interfere with essential cellular functions [5, 9].

MTB strains harbor several TA systems with MazEF being a well-known type [10]. MazEF consists of MazE (antitoxin) and MazF (toxin), and both components create a complex in normal conditions. This system has 10 loci in the MTB genome which are supposed to be effective in adapting to the environment, programmed cell death, cell cycle inhibition, persistence, and latency. MazF3, MazF6, and MazF9 inhibit mycobacterial growth. Also, they are important in expression of virulence factors. Further, MazF6 plays an essential role in cell survival [7, 9].

The role of TA systems in disseminating and maintaining antimicrobial resistance in bacterial populations has been demonstrated [11]. Moreover, previous studies indicate that *mazE* and *mazF* genes are differentially expressed in drug-susceptible and drug-resistant bacteria [12, 13]. This study aimed to evaluate differences in expression of MazEF related genes (*mazE3*, *mazF3*, *mazE6,* and *mazF6*) between drug-susceptible and drug-resistant MTB isolates in Iran.

Material and methods

Bacterial strains

A total of 20 confirmed clinical isolates of MTB including 10 drug-susceptible and 10 drug-resistant (nine MDR, and one XDR) strains were included in this study [14]. Drug susceptibility testing (DST) and detection of resistance-determinant mutations amongst the isolates were also determined in a previous study [14]. *M. tuberculosis* H37Rv was used as the standard strain. This study was approved by the Ethics Committee of Ilam University of Medical Sciences (Register code: IR.MEDILAM.REC.1395.34).

Total RNA extraction, cDNA synthesis and relative quantitative Real-time PCR

Total RNA of mycobacterial colonies was extracted by an RNA extraction kit (Thermo, Dreieich, Germany) for each strain. The RevertAid First Strand cDNA Synthesis Kit (Thermo) was used for cDNA synthesis according to the manufacture's protocol. Relative quantitative real-time PCR (RT-qPCR) was performed using SYBR Green Low ROX Master Mix (Amplicon, Brighton, UK) and specific primers (Table 1) [12, 14]. Reaction mixtures were made to a final volume of 25μL including 12.5μL of 2X SYBR Green master mix, 1μL of template cDNA (50 ng/μL), 0.4 pM of forward/reverse primers, and 9.5μL of ddH_2O in a 0.2 mL PCR microtube. The PCR reactions were done according to the following protocol: 1 cycle of 95°C for 5 min, 40 cycles of 95°C for 20 s, 58°C for 20 s, and 72°C for 30 s. To ensure the single amplicon production, melting curve analyses were applied. The heat shock protein 65 gene (*hsp65*) was used as an internal control. The genome of H37Rv standard strain was used as the external control. All tests were performed twice.

Table 1. Sequences of primers used in relative quantitative real-time PCR

Gene	Sequence (5'–3')
mazE3	F: CCAGCGTATCCAGATCACC R: GCGGGTGCATACCAAACT
mazF3	F: TATGACACCACCCAATCG R: ACCTATCCACTACGCACAGC
mazE6	F: TCACCACTCATCGTCCTG R: ATGAAGACAGCTATTTCTCTGCC
mazF6	F: GGTCGGTGAGGTCAGTCTTG R: GGTGATTAGTCGTGCCGAGAT
Hsp65	F: AAGTCGGTGGCGGTCAAG R: GCGTTCTCCAGCGTCAGG

Data and statistical analysis

For gene expression analysis, data were analyzed using the Line-Gene K software on the BIOER detection system using the Livak method (Livak. KJ, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{[-\text{Delta Delta C(T)}]}$ method). SPSS 21 was used for statistical analysis. Data are represented as means ± SEM and differences between groups were analyzed using t-test. $P < 0.05$ was considered as statistically significant.

Results

Our analysis indicated increased expression levels of *mazE3* and *mazE6* antitoxin genes in drug-susceptible isolates. It was negligible for *mazE3* but enhanced expression of *mazE6* was significant compared to drug-resistant isolates and the standard strain H37Rv ($p < 0.01$). In drug-resistant isolates, reduced expression of *mazE3* gene was observed, but *mazE6* exhibited

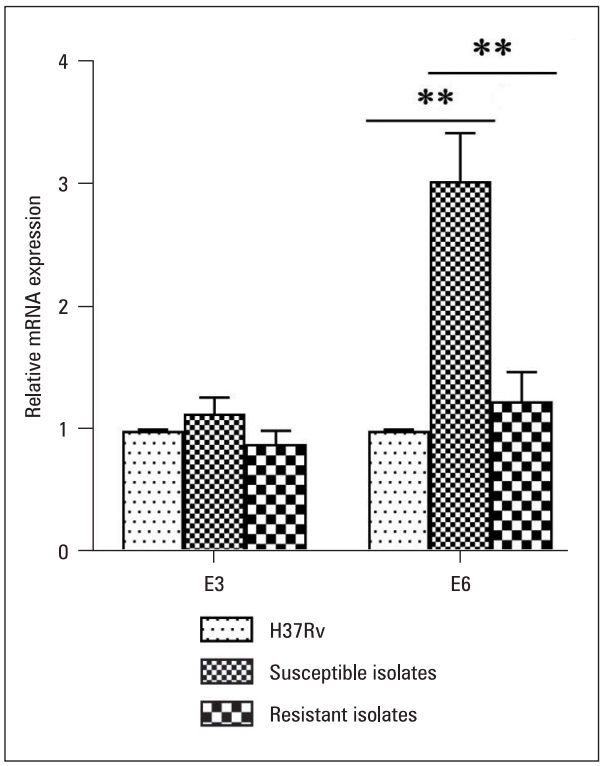

Figure 1. mRNA expression profile of *mazE3* and *mazE6* in drug -susceptible and drug-resistant MTB clinical isolates compared to standard strain H37Rv. **p < 0.01

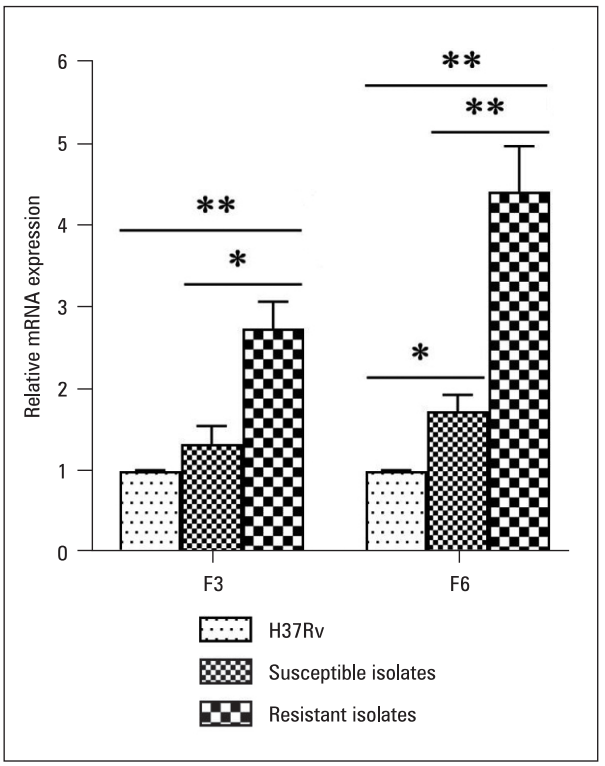

Figure 2. mRNA expression profile of *mazF3* and *mazF6* in drug -susceptible and drug-resistant MTB clinical isolates compared to standard strain H37Rv. *p < 0.05; **p < 0.01

non-significantly increased expression when compared with the standard strain (Figure 1).

Figure 2 shows *mazF3* and *mazF6* expression levels in the clinical isolates compared to the standard strain. The expression of *mazF3* and *mazF6* toxin genes were increased in drug-susceptible and drug-resistant isolates compared with the standard strain. In drug-resistant isolates, the expression levels of both genes were significantly higher than that in the susceptible isolates and the reference strain ($p < 0.05$). The mean ± SEM of the relative expression changes are mentioned in Supplementary Table 1.

Discussion

Drug-resistant TB is still a serious global health problem. Besides extensive multidrug resistance, drug tolerance or persistence are other possible responses to antibiotic treatment of MTB infection [15, 16]. Stabilization in adverse conditions encountered in the host is one of the functions of TA systems and they are important in both the mycobacterial evolution and in the process of infection. MazEF induces a reduction in metabolic activity, persistence, and cell arrest via inhibition of protein synthesis leading to the bacteria being protected from antimicrobial agents [9, 17].

The present study was conducted to investigate *mazE3*, *mazF3*, *mazE6,* and *mazF6* gene expression in drug-susceptible and drug-resistant MTB isolates. We observed no significant increase in the expression level of *mazE3* and *mazF3* genes in susceptible isolates compared with the H37Rv standard strain (Figure 1 and 2). In a study which was conducted by Zhao *et al.*, similar *mazF3* results in susceptible MTB strains were reported [12]. Also, no considerable changes in expression levels of MazEF encoded genes were previously described in susceptible *Staphylococcus aureus* isolates [13].

We found significant enhanced expression levels of *mazE6* and *mazF6* in susceptible isolates when compared with the standard strain. However, overexpression of *mazE6* was higher than *mazF6* (Figure 1 and 2). The *mazF6* upregulation and significant *mazE6* downregulation in drug-susceptible MTB strains was reported in a previous study [12]. Regardless, a higher concentration of antitoxin may justify antibiotic susceptibility amongst our studied isolates. In other words, MazE6 can neutralize MazF6 toxin and prevent its endoribonuclease activity lead-

ing to a normal microorganism metabolism and susceptibility to related antibiotics.

According to our results, no remarkable changes were observed in the expression level of *mazE3* and *mazE6* antitoxin genes in drug-resistant isolates in comparison with the standard strain (Figure 1). However, *mazF3* and *mazF6* were overexpressed significantly when compared to the drug-susceptible isolates and the standard strain. Overexpression of *mazF6* was also higher than *mazF3* (Figure 2). Reduced expression in antitoxin associated genes (*mazE3, E6*) and notable increased expression in both toxin genes (*mazF3, F6*) among drug-resistant strains was shown in previous research [12]. Accumulation of *mazF3* and *mazF6* may be due to various antibiotics and other exposures to stress of drug-resistant isolates. A high existence of MazF3 and MazF6 ribonucleases contributes synergistically to MTB growth inhibition and persistence and mediates resistance to antimicrobial agents [5]. Moreover, these proteins facilitate MTB survival in macrophages, increase resistance to oxidative stress, cause nutrient deprivation, and may cause chronic infection [5, 7, 18].

Conclusion

In this study, upregulation of *mazE6* in drug-susceptible isolates and *mazF3* and *F6* in drug-resistant isolates were observed when compared to the standard strain H37Rv. Expression differences in MazEF6 associated genes were greater than in MazEF3-related genes in both groups of isolates. It seems that the role of MazEF6 in MTB persistence is greater than that of MazEF3. Knowing the role and expression level of the genes encoding TA systems among drug-resistant bacteria may be helpful for the development of novel therapeutic approaches. MazEF associated genes, especially toxin-encoding genes, are a potential target for the treatment of drug resistant and latent TB infections alongside antibiotic therapy.

Limitations

In the present study, investigation of the studied genes (*mazE3, mazF3, mazE6* and *mazF6*) and other MazEF associated genes (*mazE5, mazF5, mazE9, and mazF9)* in various conditions (i.e. presence of several stresses or antibiotics) was required.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Somaye Mohammadi[1], Mohammad Mostafa Ansari Ramandi[2], Ali Safaei[1], Mahsa Mirdamadi[1], Sepideh Taghavi[1], Ahmad Amin[1], Nasim Naderi[1]

[1]Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
[2]Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran

Prognostic significance of lung diffusion capacity and spirometric parameters in relation to hemodynamic status in heart transplant candidates

Abstract

Introduction: Investigations have described a correlation between the severity of heart failure and the severity of pulmonary function abnormalities. In this study, we investigated the association of resting spirometric parameters, lung diffusion for carbon monoxide (DLCO), and the transfer coefficient (KCO) with hemodynamic variables and outcomes in a cohort of heart transplant candidates.

Material and methods: Between January 2018 and January 2020, a total of 100 patients with advanced heart failure who were scheduled for right heart catheterization (RHC) as a pre-transplant evaluation measure were enrolled. Spirometry and D_{LCO} were performed in all patients within 24 hours of their RHC. All selected patients were followed for a median (IQR) time of 6 (2–12) months. The end points of interest were heart failure-related mortality and a combined event involving HF-related mortality, heart transplantation (HTX), and need for the placement of a left ventricular assist device (LVAD).

Results: Among 846 patients scheduled for RHC, a total of 100 patients (25% female) with a mean (SD) age of 38.5 (12.8) were enrolled. There was a significant correlation between FEV_1/FVC and CVP ($\rho = -0.22$, $p = 0.02$), PCWP ($\rho = -0.4$, $p < 0.001$), mPAP ($\rho = -0.45$, $p < 0.001$), and PVR ($\rho = -0.32$, $p = 0.001$). The cardiac output correlated with D_{LCO} ($\rho = 0.3$, $p = 0.008$). Spirometry parameters, D_{LCO} parameters, and hemodynamic parameters did not correlate with the combined event. Among the several variables, only PVR had an independent association with the combined event.

Conclusion: Both mechanical and gas diffusion parameters of the lung were not associated with outcomes in the homogeneous group of heart transplant candidates.

Key words: heart failure, transplantation, spirometry, lung diffusion for carbon monoxide, hemodynamics

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Introduction

Advances in heart failure (HF) treatment have led to an ever-increasing prevalence of end-stage heart failure and it is currently considered a public health priority in most parts of the world. It is estimated that approximately 5–10% of HF patients have advanced (stage D) heart failure [1, 2].

Investigations have thoroughly described a correlation between the severity of heart failure and the severity of pulmonary function abnormalities. Of note, patients with more severe heart failure have more severe abnormalities when compared with those who are at earlier stages of their disease. The abnormal pulmonary capillary hemodynamics in heart failure caused by increases in interstitial and alveolar edema result in impairment of lung mechanics, resistance in membrane conductance, and decreased gas transfer [3–5].

Although both restrictive and obstructive patterns have been seen in patients with heart failure, the mechanical impairment of the lungs in HF is commonly a restrictive lung disease shown by a preserved forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) ratio with a progressively lower FEV_1, FVC, and alveolar volume (VA) as HF severity increases [3, 6]. The severity of mechanical impairment of the lungs correlates with exercise capacity

Address for correspondence: Nasim Naderi, Rajaie Cardiovascular Medical and Research Center, Tehran, Iran; e-mail: naderi.nasim@gmail.com

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[5, 7]. Regarding lung diffusion capacity in HF measured by lung diffusion for carbon monoxide (D_{LCO}), some studies have shown that lung diffusion abnormalities not only correlate with HF severity and exercise performance, but also with HF prognosis [3, 8–10].

The association of spirometric parameters and D_{LCO} with hemodynamic status and outcome in patients with advanced (stage D) heart failure is less clear.

Although some studies investigating the prognostic value of spirometry in patients with stage C heart failure showed that spirometric values predict outcomes in these heart failure populations, other studies' spirometric values did not correlate with outcomes in patients with advanced heart failure awaiting heart transplantation [9, 11]. Furthermore, several lines of evidence suggest that D_{LCO} abnormalities persist in HF after optimal fluid removal or heart transplantation [12, 13].

In this study, we investigated the association of resting spirometric parameters, D_{LCO}, and the transfer coefficient (KCO) with hemodynamic variables and outcomes in a cohort of heart transplant (HTX) candidates.

Material and methods

Study population

The study population enrolled included patients scheduled for right heart catheterization (RHC) in our heart failure and transplantation department between January 2018 and January 2020 according to the following inclusion/ /exclusion criteria.

Inclusion criteria:

— Patients with advanced heart failure according to the European Society of Cardiology [2] who were scheduled for pre-transplant evaluation or left ventricular assist device (LVAD) implantation for the first time;
— On optimal guideline-directed medical therapies (GDMT) [2];
— Patients who had interagency registry for mechanically assisted circulatory support (INTERMACS) clinical profiles of 3 (patients who are stable but inotrope dependent) or 4 (patients who have resting symptoms at home on oral therapy) [14].

Exclusion criteria:

— Pulmonary disease which may cause obstructive or restrictive ventilatory defects;
— Smoker who continued smoking less than 4 days before the test;
— Anemia (hemoglobin less than 12 g/L);
— Chronic kidney disease with a glomerular filtration rate of 60% or less and/or end stage renal disease (ESRD) patients;
— Patients who were unable to perform spirometry/D_{LCO};
— Patients with an INTEMACS profile of 1 (patients with cardiogenic shock) or 2 (patients on inotropic support with progressive decline) [14];
— History of recent heart failure decompensation in the preceding month;
— Patients with significant pleural effusion.

The study was approved by the research and ethics committee of our institute (Ethics code: IR.RHC.REC.1399.081) and written informed consent was obtained from all patients.

Patient evaluations

Right heart catheterization (RHC) was performed via the standard method in all patients using a multipurpose A1 catheter in the catheterization laboratory. The pressures were all averaged out after 10 consecutive heart beats at end expiration in supine position. The following variables were measured for each patient: mean right atrial pressure (RAP); systolic and end-diastolic right ventricular (RV) pressure; systolic, diastolic, and mean pulmonary artery pressure (mPAP); pulmonary capillary wedge pressure (PCWP); mean arterial pressure (MAP); and mixed venous oxygen saturation and cardiac output (CO) measured by the Fick method. Cardiac index (CI) was calculated by dividing CO by body surface area (BSA). PVR was calculated by dividing the transpulmonary gradient (TPG) by cardiac output. The transpulmonary gradient was calculated by subtracting the mean PAP from PCWP.

Spirometry and D_{LCO} (PFTs) measurements are among routine pre-transplantation work ups in our center. Spirometry and D_{LCO} measurements were performed for all patients using the Ganshorn Medizin Electronic pulmonary function testing system with D_{LCO} measurement PowerCube® Diffusion+ within 24 hours of their RHC (just before RHC in more than 80% of them).

Spirometry was performed with the patient in a sitting position using the reproducibility and acceptability criteria. Maneuvers were selected according to the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria.[15] In this analysis, we considered absolute and percent-of-predicted forced expiratory volume in one second (FEV_1 and % FEV_1), forced

vital capacity (FVC and %FVC), and the FEV_1/FVC ratio. A restrictive ventilatory pattern was defined as a combination of FEV_1/FVC that was normal or more than the 5th percentile (lower limit of normal [LLN]) and FVC<LLN with decreased calculated total lung capacity. A spirometric obstructive ventilatory pattern was defined as a combination of FEV_1/FVC below the 5th percentile (LLN) and FEV_1<LLN confirmed by an increased RV size with raw and significant reversibility of airway obstruction.

D_{LCO} and D_{LCO} corrected for alveolar volume (D_{LCO}/VA or KCO) was measured in the standard sitting position with the single breath constant expiratory flow technique according to the ATS recommendations which include rapid inspiration, inspired volume at least 90% of the largest vital capacity, breath-hold time between 9 and 11 seconds, and adequate washout and sample volumes [16].

The mean of all acceptable tests was considered. Calculations were standardized for breath-hold time and adjusted for dead space, gas collection conditions, and carbon dioxide concentration.

D_{LCO} was at STPD (standard temperature, pressure, and dry) and VA was at BTPS (standardized Body Temperature, Pressure, and Saturation)

The predicted values of D_{LCO} (%D_{LCO}) and KCO (%KCO) were calculated using the predictive equations for D_{LCO} and KCO derived by Amra et al. for the Iranian population [17].

Patients' follow-up and outcome measures

All selected patients were followed after the index right heart catheterization until the end of July 2020 with a median follow up time of 6 months. The end points of interest were heart failure-related mortality as well as a combined event of HF related-mortality, heart transplantation (HTX), and left ventricular assist device implantation (LVAD).

Statistical analysis

All analyses were conducted using IBM SPSS statistics 22 for Windows (IBM Corp, Armonk, NY, USA). One sample Kolmogorov Smirnov test was used to assess the normal distribution of variables.

Continuous variables with and without normal distribution are presented as means ± standard deviation and medians (interquartile range), respectively. They were compared using the Student's t-test and the Mann–Whitney U-test, as appropriate. Categorical data are presented as numbers and percentages and were compared by the χ^2 test. The correlations between spirometric parameters, D_{LCO}, KCO, and hemodynamic parameters were assessed via Spearman's rank correlation coefficient. Stepwise binary multiple regression analysis was performed to assess the independent correlation between spirometric parameters, D_{LCO}, KCO, and hemodynamic findings with the outcome measure. All reported probability values were two-tailed and a p value < 0.05 was considered statistically significant.

Results

Among 846 patients scheduled for RHC, a total of 100 patients were enrolled according to our inclusion criteria. The mean (SD) age of patients was 38.5 (12.8) years. One-fourth of the patients were female. More than 90% of patients were already on guideline-directed medical therapies (GDMT). All of them were using loop diuretics, mineralocorticoid receptor antagonists (MRA), and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ARB). Beta-blockers could not be tolerated in 8 patients. The baseline characteristics of patients and their status at the end of the study are summarized in Table 1.

All PFTs were performed within 24 hours of RHC. The median (IQR) of the time interval between RHC and PFTs was 4 (3–5) hours. The PFTs were performed less than 6 hours before RHC in 82% of our study population. In 18% of patients, they were performed within 18–24 hours (the day before or after RHC).

Table 2 depicts hemodynamic variables and spirometry/D_{LCO} parameters.

Seventy percent (70%) of patients had a FEV_1 less than 80% of predicted value, 67% of patients had a FVC less than 80% of predicted value, and all patients had a FEV_1/FVC ratio over 70%. Figure 1 shows the spirometric patterns of our study population. Most patients show a restrictive ventilatory pattern in their pulmonary function tests (PFTs).

Regarding lung diffusion parameters, D_{LCO} and KCO were less than 80% of the predicted values in 71% and 26% of patients, respectively.

The spirometric and D_{LCO} measurement study results did not differ significantly in patients with and without a history of smoking. However, most cases from the smoker group (90%) involved former smokers; only 4 patients were current smokers who were smoking occasionally.

Table 1. Baseline characteristics of patients (n = 100)

Baseline characteristics		Value
Age in years, mean [SD]		38.5 (12.8)
Gender, number [%]	Female Male	25 (25) 75 (75)
BSA, m^2, mean [SD]		1.8 (0.2)
Heart failure type, number [%]	ICMP Non-ICMP	23 (23) 77 (77)
INTERMACS clinical profile, number [%]		
	3	65
	4	35
LVEF, % median [IQR]		10 (10–20)
Smokers, number [%]		31 (31)
Alcohol overuse, number [%]		21 (21)
DM, number [%]		10 (10)
ICD/CRT, number [%]		45 (45)
GDMT, number [%]		92 (92)
NT-Pro BNP, median [IQR], ng/dl		4926 (2613–14102)
Serum creatinine mean [SD], mg/dl		1.23 (0.55)
Hemoglubin, mean [SD], g/L		13.1 (2.1)
Intermittent inotrope therapy, number [%]		43 (43)
Heart transplantation		38 (38)
LVAD		3 (3)
Heart failure mortality		14 (14)

BNP — brain natriuretic peptide; BSA — body surface area; ICMP — ischemic cardiomyopathy; DM — diabetes mellitus; GDMT — guideline-directed medical therapy; ICD — implantable cardioverter defibrillator; INTERMACS — Interagency Registry for Mechanically Assisted Circulatory Support; LVAD — left ventricular assist device; LVEF — left ventricular ejection fraction

Table 2. Hemodynamic variables and PFT parameters (n = 100)

	Value
Hemodynamic parameters	
SBP [mm Hg], mean [SD]	103 (15.5)
DBP [mm Hg], mean [SD]	65 (11.5)
HR [beats/min], median [IQR]	95 (81–100)
CVP [mm Hg], mean [SD]	13.6 (7.4)
MPAP [mm Hg], mean [SD]	32 (11)
PADP [mm Hg], mean [SD]	24.7 (9.2)
PCWP [mm Hg], mean [SD]	25.5 (8.5)
Cardiac index [L/min/m^2], mean [SD]	1.7 (0.52)
Cardiac output [L/min], mean [SD]	3.3 (0.97)
SVR [WU], median [IQR]	20.3 (16–24.6)
PVR [WU], median [IQR]	2.1 (1.05–3.5)
PFT parameters	
FEV_1 [L]	2.6 (2.2–3.2)
Percent of predicted FEV_1 [%]	71.5 (61–84.2)
FVC [L]	3.2 (2.5–3.8)
Percent of predicted FVC [%]	71 (62–83)
FEV_1/FVC	81 (79–83)
DLCO [mL/min/mm Hg]	8.3 (6.7–9.5)
Percent of predicted D_{LCO} [%]	73.9 (63.4–83.2)
KCO [mmol/min/kPa/Lit]	1.85 (1.6–2.1)
Percent of predicted KCO [%]	92.5 (80.2–104)

CVP — central venous pressure; DBP — diastolic blood pressure; D_{LCO} — diffusing capacity of the lungs for carbon monoxide; DPG — diastolic pulmonary gradient. FVC — forced vital capacity; FEV_1 — forced expiratory volume in one second; HR — heart rate; KCO — transfer coefficient of the lung for carbon monoxide; MPAP — mean pulmonary artery pressure; PADP — pulmonary artery diastolic pressure; PCWP — pulmonary capillary wedge pressure; PVR — pulmonary vascular resistance;SBP — systolic blood pressure; SVR — systemic vascular resistance; WU — wood units

Association between lung function parameters and hemodynamic variables

The Spearman's rank correlation coefficient showed a significant negative correlation between FEV_1/FVC and CVP (ρ = -0.22, p = 0.02), PCWP (ρ = -0.4, p < 0.001), mPAP (ρ = -0.45, p < 0.001), and PVR (ρ = -0.32, p = 0.001).

Cardiac output positively correlated with FEV_1 (ρ = 0.2, p = 0.04). There was no association found between any of the hemodynamic variables and %FEV_1, FVC, and %FVC.

Cardiac output positively correlated with D_{LCO} (ρ = 0.3, p = 0.008) in univariate analysis. No correlation was found between %D_{LCO} and the hemodynamic parameters. The multivariable analysis including LVEF, CO, CI, PCWP, MPAP, PVR, history of smoking, diagnosed diabetes mellitus, age, and gender showed an independent association between D_{LCO} and CO (β = 0.7, p = 0.03).

Univariate analysis showed that PCWP (for both variable ρ = -0.2, p = 0.03), mPAP (for both variable ρ = -0.3, p = 0.004), PADP (for both variable ρ = -0.2, p = 0.02), and PVR (for both variable ρ = -0.2, p = 0.02) negatively correlated with both KCO and %KCO.

The multivariable analysis including the variables LVEF, PCWP, MPAP, PVR, history of smoking, diagnosed diabetes mellitus, age, and

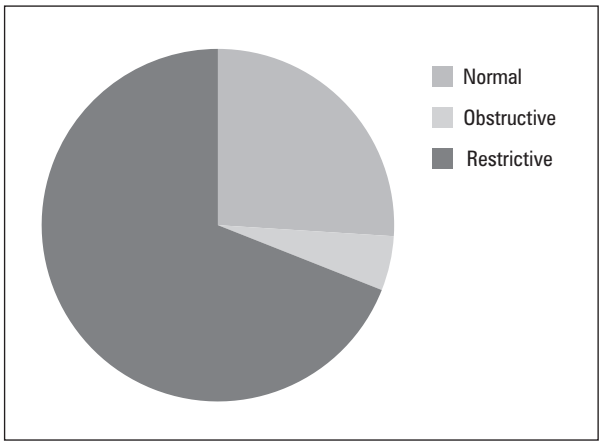

Figure 1. The spirometric patterns of our study population (n = 100)

gender showed no independent association between hemodynamic variables and KCO or %KCO.

Association between PFTs, hemodynamic parameters and outcomes

The median (IQR) follow-up duration was 6 (2–12) months. Outside of 9 patients who had a high PVR (more than 5 wood units), the rest of the patients were listed for HTX. Due to the very limited availability of LVADs in our country, LVAD implantation only became possible for 3 patients as destination therapy during the follow-up period. Heart failure-related mortality was 14%. 38% of patients were eligible to receive a heart transplant. Therefore, the combined event was seen in 54% of the study population.

The median (IQR) amount of time to the end-point (HF-related mortality, HTX, or LVAD implantation) was 89 days (25–120.5). The time to HTX was 31 days (1–108). All of the patients who received HTX had an INTERMACS clinical profile of 3. The median (IQR) amount of time to HF-related mortality was 78 (21–120) days. At the end of the follow-up, 45 patients were still alive.

In univariate analyses, neither the spirometric or D_{LCO} measurement parameters (which included the PFTs patterns) correlated with the combined event. This was also true for the hemodynamic parameters (Figures 2 and 3).

For multivariable analysis, a logistic regression model with a backward elimination method was applied in order to assess the adjusted associations between the combined end-point, PFTs, and hemodynamic parameters. It was found that, among the several variables (which included age, gender, $\%D_{LCO}$, %KCO, $\%FEV_1$, %FVC, FEV_1/FVC ratio, PFT patterns, CVP, PCWP, PVR, mPAP, MAP, and CI), only PVR had an independent association with the combined event ($\beta = 0.25$, p = 0.04, Odd ratio [95% confidence interval] = 1.3 [1–1.6]).

Age (p = 0.02), PCWP (p = 0.02), mPAP (p = 0.02), MAP (p = 0.02), and FEV_1/FVC (p = 0.04) correlated with HF-related mortality in univariate analyses (Figure 4).

Multivariate logistic regression analysis showed that only mPAP had an independent association with HF-related mortality ($\beta = 0.56$, p = 0.05, Odd ratio [95% confidence interval] = 1.7 [1–3]).

Discussion

In this study, we showed that FEV_1/FVC, KCO, and %KCO could be correlated with hemodynamic measures in HTX candidates. Among different mechanical and diffusion parameters of lung function, only D_{LCO} was independently associated with cardiac output in our study population.

Regardless of these associations, neither mechanical nor diffusion parameters of pulmonary function were predictive of outcomes in HTX candidates in the current study.

The relationship between lung function values and hemodynamic measures has been relatively well explained in patients with HF, especially in patients with stage C HF. However, data on the importance of pulmonary function values in patients with stage D HF (advanced HF) have been conflicting [5, 8, 12, 13].

In a study by Georgiopoulou *et al.* [11], the spirometric values significantly correlated with filling pressures in a cohort of stage D HF patients, but none of them were correctly predictive of the adverse outcomes. They have also found no association between the functional capacity of HTX candidates and their spirometric values.

In a study by Lizak *et al.* [3], it has also been reported that spirometry is not useful for the diagnosis and grading of pulmonary diseases in HTX candidates. Another study has shown that, as symptoms of HF worsen, the influence of spirometric values on functional capacity diminishes.

In a recent study, Deis *et al.* have shown that spirometric values ($\%FEV_1$ and %FVC) did not correlate with hemodynamics in advanced HF patients who were candidates for heart transplantation. Also, their association with adverse outcomes was not apparent after adjusting for confounding factors [8]. Furthermore, they found that central hemodynamics were modestly associated with %KCO and that PCWP independently correlated with %KCO in these patients.

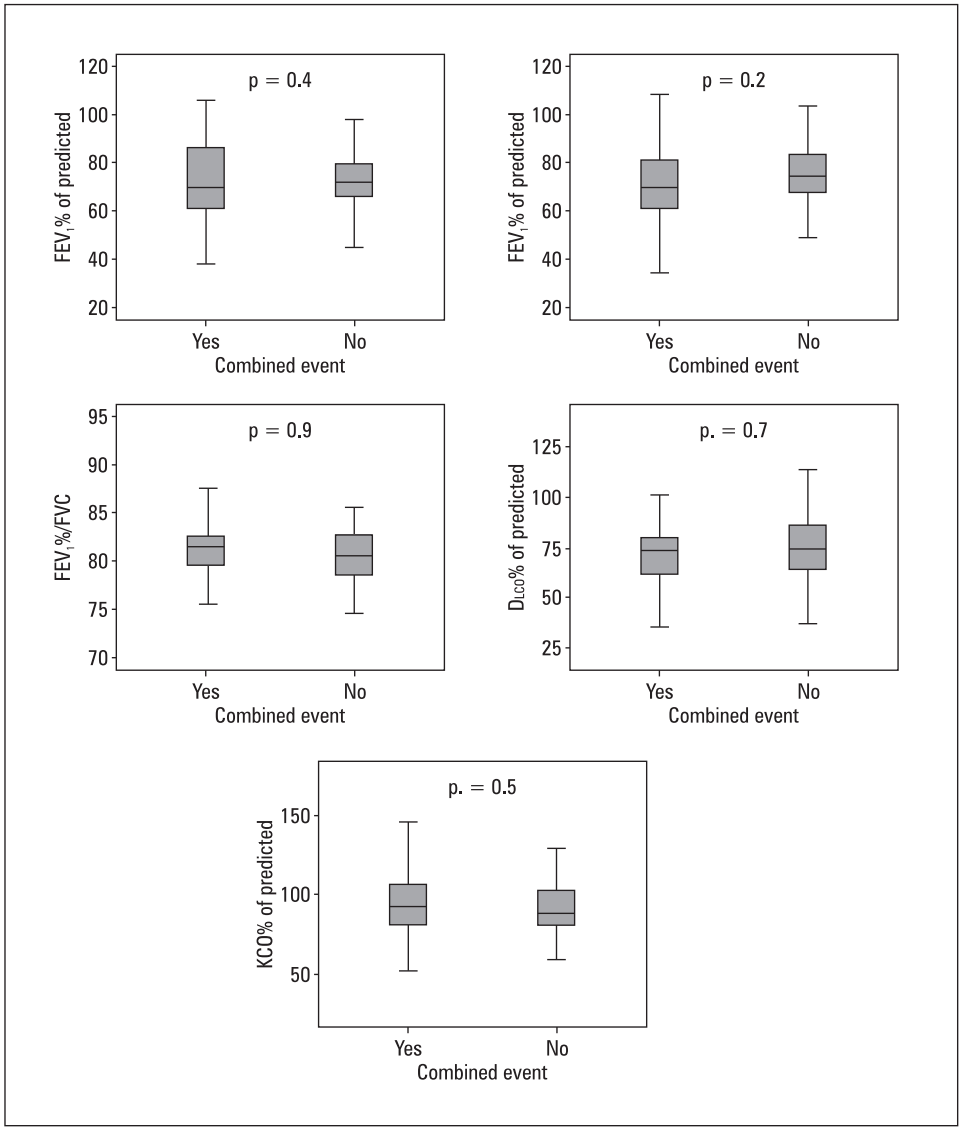

Figure 2. Comparison of PFT parameters in relation to the combined event. D_{LCO} — diffusing capacity of the lungs for carbon monoxide; FEV_1 — forced expiratory volume in one second; FVC — forced vital capacity; KCO — transfer coefficient of the lung for carbon monoxide

They also found a significant association between KCO and adverse outcomes in a cohort of HTX candidates.

There are some differences between our study and the study by Deis *et al*. [8] Although D_{LCO} was similarly reduced in our population, we also found an association between %KCO and PCWP, mPAP, and PVR. However, no association could be found between the %KCO and patient outcome.

There are several potential explanations for our findings. Our study population was a uniform group of HTX candidates who were carefully selected from a group of patients with advanced HF. Most of them were free of severe end organ dysfunction, particularly chronic lung disease, which may considerably attenuate the prognostic value of PFT results.

One of the strengths of our study was that catheterization and PFTs were performed almost concurrently. The cardiac and pulmonary systems are intimately linked physiologically and anatomically [18]. As a result, changes in the hemodynamic status of a patient with HF can have profound effects on the pulmonary system which can cause abnormalities in PFT parameters. Changes in hemodynamic status are more frequent in patients with advanced heart failure. Therefore, the presence of a three-month interval between performing PFTs and RHC in the study by Deis et al. can make their results less conclusive [8].

Furthermore, these pulmonary function abnormalities might just indicate that there is a heart-lung relationship in this specific population of HF patients without providing any underlying

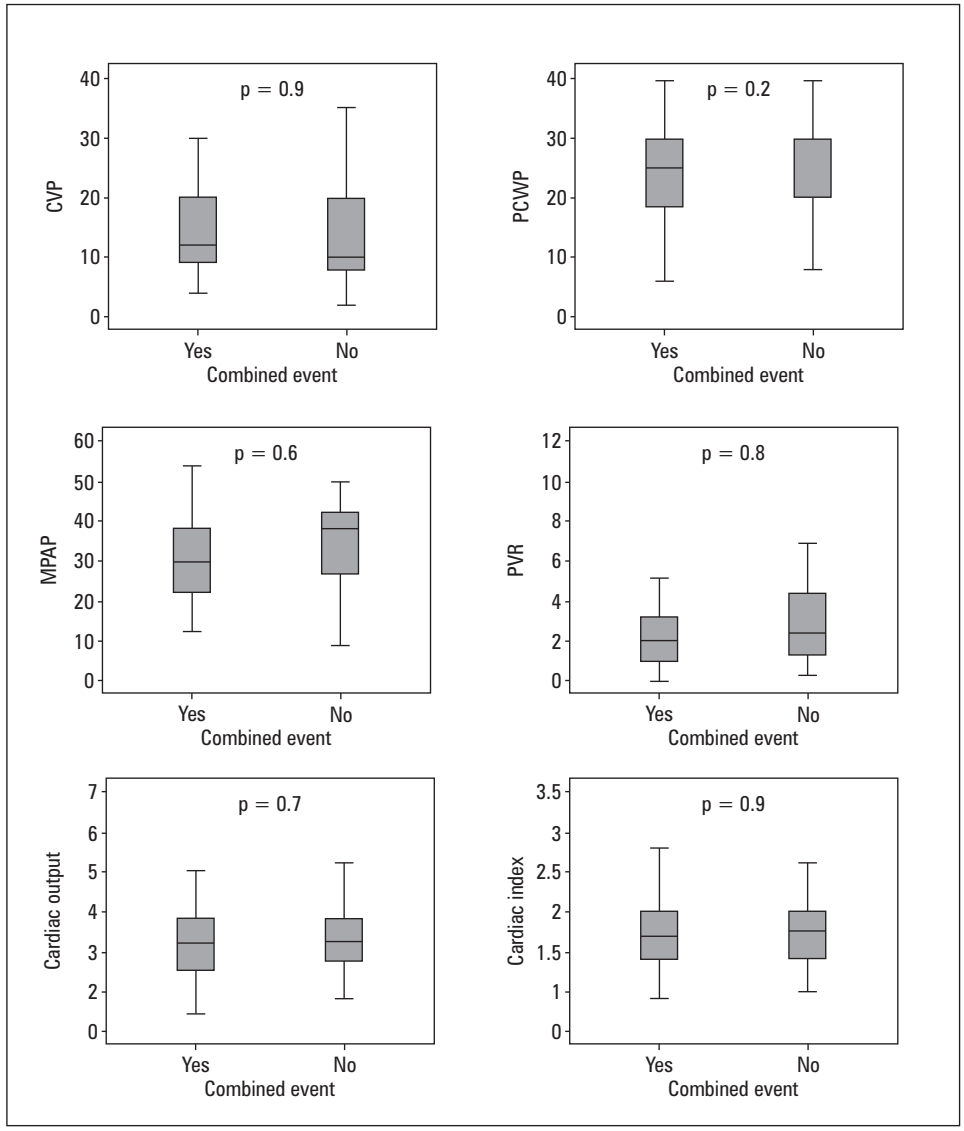

Figure 3. Comparison of hemodynamic parameters in relation to the combined event. CI — cardiac index; CO — cardiac output; CVP — central venous pressure; MPAP — mean pulmonary artery pressure; PCWP — pulmonary capillary wedge pressure; PVR — pulmonary vascular resistance

prognostic importance. Some studies have shown that D_{LCO} abnormalities persist after ultrafiltration or heart transplantation [5, 13]. Chronic damage to the alveolar membrane as a result of long-standing hemodynamic disturbances in HF can lead to decreased D_{LCO} even after optimal HF treatment.

The method of selection of the study population and the definition of the outcomes may be another reason for the different study results. The LVAD is available for a limited number of patients in our country and because of this, HTX is required for the majority of our patients. Many patients with an INTERMACS score of 3 will have a chance to be given a transplant if there are no patients with an INTERMACS clinical profile score of 1 or 2. In summary, more than half of our patients had met the outcome at the end of our short duration follow-up time. As a result, HTX or LVAD implantation may not be considered as an index event or emergent procedure in our population. In fact, this high number of events in our cohort in conjunction with a high prevalence of PFT abnormalities may be the reason for the observed lack of association of PFT values with outcomes (rather than the absence of biologic association).

Study limitations

Although the careful selection of our study population may be a strength of our study, the most important limitation of our study and other similar studies may be acquiring optimal PFTs in patients with advanced HF. The presence of signi-

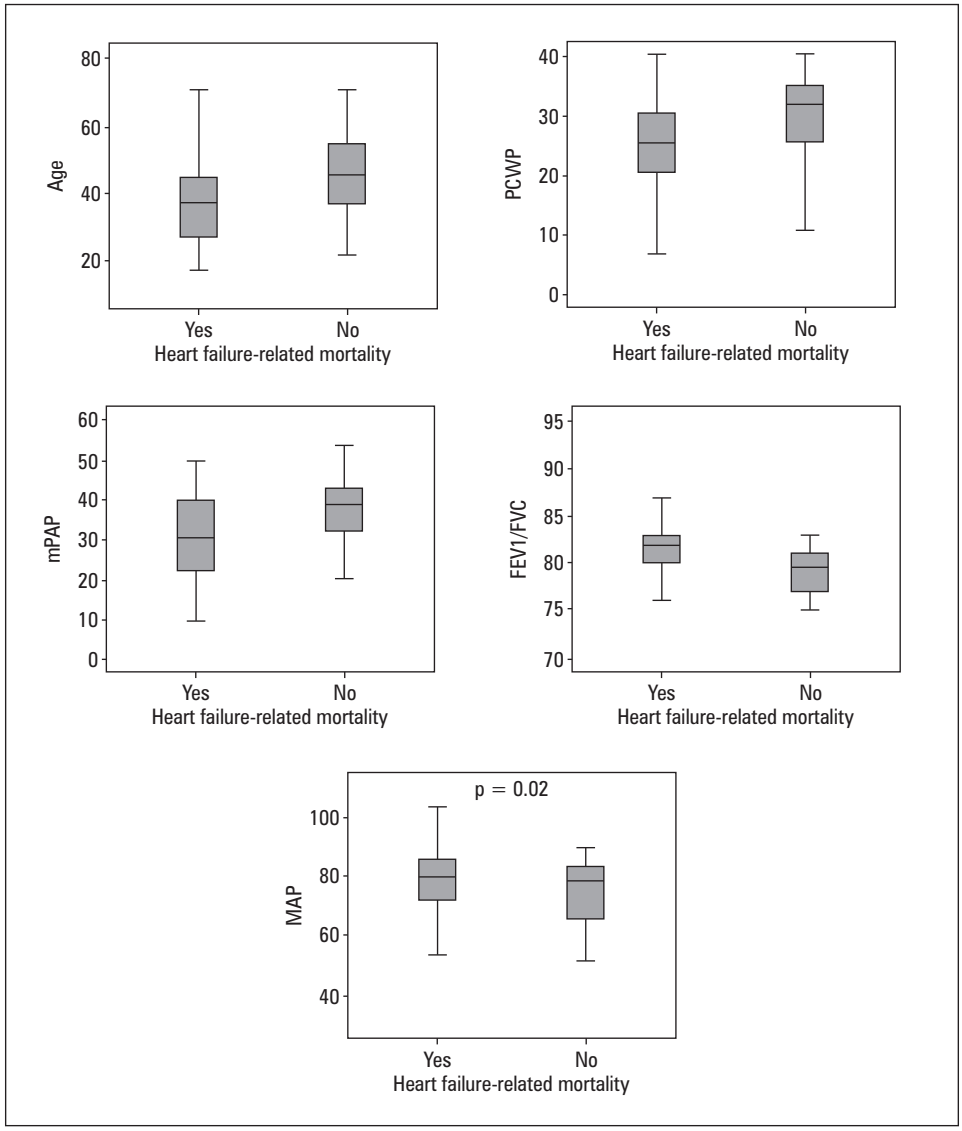

Figure 4. Comparison of PFTs and hemodynamic parameters in relation to heart failure-related mortality. FEV_1 — forced expiratory volume in one second; FVC — forced vital capacity; MAP — mean arterial pressure; MPAP — mean pulmonary artery pressure; PCWP — pulmonary capillary wedge pressure

ficant pulmonary congestion and hypertension, sarcopenia, and respiratory muscle weakness can make the result of our study suboptimal.

In summary, although the prognostic significance of PFTs in patients with chronic lung disease is well known, both mechanical and gas diffusion parameters of the lung were not associated with outcomes in the homogeneous group of heart transplant candidates. Advanced and severe HF leads to significant changes in lung function parameters. Therefore, the usefulness of PFTs to diagnose and grade pulmonary function abnormalities in this population and the importance of pulmonary function abnormalities in heart failure survival needs further evaluation.

The duration of HF, the number of decompensation episodes, and novel heart failure therapies (such as medical and surgical neurohormonal modulations) all may play a role in the development and progression of pulmonary abnormalities. This underscores the fact that more investigations are needed to find definite responses to the remaining questions.

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Conflict of interest

None declared.

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Tomasz Stachura[1, 2], Natalia Celejewska-Wójcik[1, 2], Kamil Polok[1, 3], Karolina Górka[1, 2], Sabina Lichołai[4, 5], Krzysztof Wójcik[2], Jacek Krawczyk[1, 2], Anna Kozłowska[1], Marek Przybyszowski[1, 2], Tomasz Włoch[1, 6], Jacek Górka[3], Krzysztof Sładek[1, 2]

[1]Department of Pulmonology and Allergology, University Hospital in Kraków, Kraków, Poland
[2]2nd Chair of Internal Medicine, Jagiellonian University Medical College, Kraków, Poland
[3]Department of Intensive Care and Perioperative Medicine, Kraków, Poland
[4]Division of Molecular Biology and Clinical Genetics, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[5]Sano Centre for Computational Medicine, Kraków, Poland
[6]Department of Rehabilitation in Internal Diseases, Institute of Clinical Rehabilitation, University School of Physical Education, Kraków, Poland

A clinical profile and factors associated with severity of the disease among Polish patients hospitalized due to COVID-19 — an observational study

Abstract

Introduction: The coronavirus disease 2019 (COVID-19) is one of the greatest clinical challenges of the last decades. Clinical factors associated with severity of the disease remain unclear. The aim of the study was to characterize Polish patients hospitalized due to COVID-19 and to evaluate potential prognostic factors of severe course of the disease.

Material and methods: An observational study was conducted from March to July 2020 in the Pulmonology and Allergology Department of the University Hospital in Kraków, Poland. Consecutive patients with confirmed SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) infection were enrolled, and data about past medical history, signs and symptoms, laboratory results, imaging studies results, in-hospital management and outcomes was prospectively gathered.

Results: The study sample comprised 100 patients at the mean age of 59.2 (SD 16.1) years among whom 63 (63.0%) were male. Among them 10 (10.0%) died, 47 (47%) presented respiratory failure, 15 (15.0%) were transferred to the intensive care unit, 17 (17.0%) developed acute kidney injury, 7 (7.0%) had sepsis and 10 (10.0%) were diagnosed with pulmonary embolism. Multivariable analysis revealed age (OR 1.1; 95% CI 1.01–1.15), body mass index (BMI; OR 1.24; 95% CI 1.01–1.53), modified early warning score (MEWS; OR 3.95; 95% CI 1.48–12), the highest d-dimer value (OR 1.73; 95% CI 1.03–2.9) and lactate dehydrogenase (LDH; OR 1.16; 95% CI 1.03–1.3) to be associated with severe course of COVID-19.

Conclusion: This observational study showed that almost half of hospitalized patients with COVID-19 developed respiratory failure in the course of the disease. Increasing age, BMI, MEWS, d-dimer value and LDH concentration were associated with the severity of COVID-19.

Key words: clinical characteristics, coronavirus disease 2019, respiratory failure, risk factors, SARS-CoV-2

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Introduction

Clinical presentation of COVID-19 is highly variable and involves multiple organs, however, the respiratory system seems to be predominantly affected. There are several reports describing an increased incidence of injuries to the heart, kidneys, muscles, gastrointestinal tract and the nervous system among COVID-19 patients [1–3]. Another significant factor influencing outcomes of persons infected with SARS-CoV-2 is an increased incidence of thromboembolic complications which is secondary to severe disturbances in coagulation and fibrinolysis as well as endothelial damage, sometimes referred to as COVID-19-associated coagulopathy [4, 5]. It is estimated that

Address for correspondence: Kamil Polok, Department of Pulmonology and Allergology, University Hospital in Kraków, Kraków, Poland; e-mail: kj.polok@gmail.com

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SARS-CoV-2 infection remains mildly symptomatic in about 80% of patients. However, approximately 15% of cases are severe, warranting hospitalization and the remaining 5% of patients are critically ill and require management in the intensive care unit (ICU) [6]. At the time of the study, only systemic glucocorticoids were recognized to reduce mortality in several randomized controlled trials [7].

The objective of this study was to characterize the first COVID-19 patients treated in the Pulmonology and Allergology Department of the University Hospital in Kraków, Poland in terms of patients' clinical, laboratory, diagnostic outcomes and factors associated with the severity of the disease.

Material and methods

Study design

This is an observational study conducted from March to July 2020 in the Pulmonology and Allergology Department, University Hospital in Kraków, Poland. The study was approved by the Ethics Committee of Jagiellonian University Medical College, Kraków, Poland (KBET 1072.6120.145.2020), and written informed consent was obtained from all participants.

Study sample and data collection

We enrolled all consecutive patients with SARS-CoV-2 infection confirmed using reverse transcription polymerase chain reaction, admitted to the Pulmonology and Allergology Department. We collected basic demographic (age, sex) and detailed clinical information about the patients (symptoms, comorbidities, medications, smoking status) based on the interviews and comprehensive analysis of medical records. In each patient, modified early warning score (MEWS) was calculated on admission. We recorded laboratory results on the day of admission, on the 2nd to 4th day of hospitalization and the 6th to 8th day of hospitalization as well as results of imaging studies (chest X-ray and computed tomography of the chest).

Outcomes

The patients were observed during the entire hospitalization in the University Hospital for the occurrence of the following outcomes: death, myocardial injury, myocardial infarction (both defined according to the Fourth Universal Definition of Myocardial Infarction [8]), stroke (defined as a new focal neurological deficit with signs and symptoms lasting more than 24 hours), acute kidney injury (AKI, diagnosed according to the Kidney Disease Improving Global Outcomes definition) [9], sepsis, septic shock (both defined according to the 2016 Surviving Sepsis Campaign International Guidelines for Management of Sepsis and Septic Shock) [10], pulmonary embolism (diagnosed with computed tomography pulmonary angiogram) as well as transfer to the ICU and a need for mechanical ventilation.

Statistical analysis

Categorical variables were presented as numbers (percentages), whereas continuous variables were reported as medians (interquartile range) or means (standard deviation) depending on variable distribution. Associations of quantitative data were analyzed with Student t test and with the nonparametric Mann–Whitney U test. To assess the association between selected factors and a severe course of the disease (defined as necessity to administer oxygen therapy), a multivariable logistic regression model was created. The variables for the model (age, sex, the highest d-dimer value during hospitalization, MEWS on admission, baseline LDH concentration) were selected based on the available evidence. The model was validated using the Hosmer-Lemeshow test. This was a complete-case analysis. After application of Bonferroni correction due to multiple testing, a p-value < 0.00062 was considered statistically significant. Statistical analysis was performed with STATISTICA software (Statsoft, Tulsa, USA).

Results

Baseline characteristics

The study group consisted of 100 patients with the mean age of 59.2 (SD 16.1) years among whom 63 (63.0%) were male. The most common comorbidities were hypertension (49.0%), obesity (31.0%) and dyslipidemia (19.0%). Twenty-seven patients (27.0%) had history of smoking. The most commonly used medications were β-blockers (35.0%), diuretics (24.0%), ACE inhibitors and statins (both 19.0%). Detailed demographic and clinical characteristics are presented in Table 1.

Signs and symptoms

The most common symptoms described by the patients on admission were fever (73.0%), cough (67.0%) and dyspnea (44.0%). Additionally, diarrhea and dysgeusia or dysosmia were reported by 29 (29.0%) and 15 (15.0%) patients, respectively. The median time from the onset of symptoms

Table 1. Baseline characteristics

Characteristic	Total (n = 100)
Demographic	
Age [years], mean (SD)	59.2 (16.1)
Sex, male	63 (63.0%)
Smoking	27 (27.0%)
Symptoms	
Time from first symptoms to positive swab [days]	5.0 (1.0–7.0)
Time from first symptoms to admission [days]	7.0 (3.0–9.0)
Fever prior to admission	73 (73.0%)
Sore throat	16 (16.0%)
Dyspnea	44 (44.0%)
Pleuritic chest pain	13 (13.0%)
Ischemic chest pain	0 (0.0%)
Hemoptysis	1 (1.0%)
Cough	67 (67.0%)
Myalgia	27 (27.0%)
Syncope	2 (2.0%)
Diarrhea	29 (29.0%)
Dysgeusia or dysosmia	15 (15.0%)
Measurements & vital signs on admission	
Resting baseline SpO^2 on admission [%]	95.0 (93.0–96.0)
BMI [kg/m^2]	27.7 (25.2–30.8)
Systolic blood pressure on admission [mm Hg]	130.0 (123.5–145.5)
Diastolic blood pressure on admission [mm Hg]	82.5 (77.0–90.0)
Heart rate on admission [beats/minute]	86.5 (77.0–95.5)
Respiratory rate on admission [breaths/minute]	16.0 (14.0–20.0)
Fever on admission	3 (3.0%)
MEWS on admission	1.0 (0.0–2.0)
Comorbidities	
Hypertension	49 (49.0%)
Atrial fibrillation	9 (9.0%)
Chronic heart failure	11 (11.0%)
Coronary artery disease	15 (15.0%)
History of myocardial infarction	9 (9.0%)
History of stroke	3 (3.0%)
Peripheral artery disease	3 (3.0%)
Dyslipidemia	19 (19.0%)
Hyperthyroidism	0 (0.0%)
Hypothyroidism	9 (9.0%)
Diabetes mellitus	16 (16.0%)
Obesity	31 (31.0%)
Chronic kidney disease	6 (6.0%)
Asthma	10 (10.0%)
COPD	7 (7.0%)

Table 1. cont. Baseline characteristics

Active neoplastic disease	6 (6.0%)
History of DVT/PE	7 (7.0%)
Medications	
Acetylsalicylic acid	14 (14.0%)
Oral anticoagulants	3 (3.0%)
Direct oral anticoagulants	4 (4.0%)
β-blockers	35 (35.0%)
ACE-I	19 (19.0%)
ARB	16 (16.0%)
Calcium channel blockers	15 (15.0%)
Statins	19 (19.0%)
Diuretics	24 (24.0%)
Insulin	5 (5.0%)

Data is presented as n [%] for categorical variables and median (interquartile range) unless otherwise specified. ACE-I — angiotensin converting enzyme inhibitors; ARB — angiotensin receptor blockers; BMI — body mass index; COPD — chronic obstructive pulmonary disease; DVT/PE — deep vein thrombosis/pulmonary embolism; MEWS — modified early warning score; SD — standard deviation

to admission to the hospital was 7.0 (IQR 3.0–9.0) days. Hypotension (defined as systolic blood pressure < 90 mm Hg), tachycardia (defined as heart rate > 100/min) and tachypnea (defined as respiratory rate > 20 breaths/min) were observed on admission in 0 (0.0%), 11 (11.0%) and 23 (23.0%) patients, respectively. The median MEWS on admission was 1.0 (IQR 0.0–2.0) point.

Laboratory results

Complete blood count most often revealed lymphopenia (35/100, 35.0%), while thrombocytopenia was present in 17 patients (17.0%). Coagulation tests commonly showed an increased concentration of fibrinogen (50/59, 84.7%), d-dimer (53/84, 63.1%) and prolonged APTT (16/89, 18.3%). Renal function, reflected by estimated glomerular filtration rate (eGFR), was normal in the majority of patients (82/100, 82.0%). Serum concentrations of liver enzymes were commonly elevated, i.e. alanine aminotransferase (ALT; 45/100, 45.0%), aspartate aminotransferase (AST; 48/91, 52.7%) and gamma-glutamyl transpeptidase (GGTP; 53/74, 71.6%). The majority of patients had elevated level of lactate dehydrogenase (LDH; 78/96, 81.3%). Among markers of inflammation we observed elevation of C-reactive protein (CRP), procalcitonin (PCT) and interleukin 6 (IL-6) in 79/100 (79.0%), 29/96 (30.2%) and 39/71 (54.9%) patients, respectively. Medians with IQR as well as proportions of patients with abnormalities in laboratory results are summarized in Table 2.

Imaging studies

Chest X-ray was performed in 99 patients (99.0%). There were no discernible pathological findings in 22 cases (22.0%). Interstitial infiltrates and consolidations were described in 22 (22.0%) and 19 patients (19.0%), respectively. The coexistence of interstitial infiltrates and consolidations was reported in 35 patients (35.0%). One subject had extensive neoplastic changes, which made chest X-ray analysis impossible.

Computed tomography was performed in 45 patients (45.0%) — computed tomography pulmonary angiogram in 43 cases and high-resolution computed tomography in 2 cases. Among patients with available CT results, ground-glass opacities were described in 38 cases (84.4%), consolidations were found in 29 patients (64.4%), and pleural fluid was detected in 11 individuals (24.4%).

Management

Low-molecular-weight heparin (LMWH) was administered in 90 patients (90.0%) with the maximal administered dose being prophylactic in 39 patients (39.0%), intermediate in 28 subjects (28.0%) and therapeutic in 23 patients (23.0%). The majority of patients (69/100, 69.0%) received antibiotics while chloroquine and ritonavir/lopinavir were administered in 36 (36.0%) and 2 (2.0%) individuals, respectively. Oxygen therapy was used in 47 patients (47.0%) during hospitalization, with the median maximal FiO_2 accounting for 50.0% (IQR 28.0–90.0).

Table 2. Baseline laboratory tests results

Parameter	Value; median (IQR)	Patients with abnormal values; n(%)
White blood count [$\times 10^3/mm^3$]	5.95 (4.56–7.59)	Leukopenia: 19/100 (19.0%) Leukocytosis: 11/100 (11.0%)
Neutrophils, count [$\times 10^3/mm^3$]	3.96 (2.69–5.69)	Neutropenia: 4/100 (4.0%)
Lymphocytes, count [$\times 10^3/mm^3$]	1.12 (0.88–1.50)	Lymphopenia: 35/100 (35.0%)
Hemoglobin [g/dl]	13.8 (12.7–14.8)	Anaemia: 18/100 (18.0%)
Platelets [$\times 10^3/mm^3$]	197.5 (159.5–257.0)	Thrombocytopenia: 17/100 (17.0%)
D-dimer on admission [mg/L]	0.84 (0.47–1.42)	Elevation: 53/84 (63.1%)
Highest d-dimer [mg/L]	1.20 (0.74–2.44)	Elevation: 67/81 (82.7%)
Fibrinogen [g/L]	4.7 (3.7–5.9)	Elevation: 50/59 (84.7%)
APTT [s]	31.9 (28.7–35.1)	Prolongation: 16/89 (18.3%)
INR	0.95 (0.90–1.02)	Elevation: 4/98 (4.1%)
eGFR [mL/min/1.73 m^2]	87.0 (66.9–104.7)	Decreased: 18/100 (18.0%)
Urea [mmol/L]	5.3 (3.8–6.9)	Elevation: 17/99 (17.2%)
Glucose [mmol/L]	5.7 (5.2–7.0)	Elevation: 48/93 (51.8%)
ALT [U/L]	31.5 (20.5–61.0)	Elevation: 45/100 (45.0%)
AST [U/L]	37.0 (27.0–56.0)	Elevation: 48/91 (52.7%)
Bilirubin [μmol/L]	7.3 (5.7–9.0)	Elevation: 0/85 (0.0%)
GGTP [U/L]	49.0 (26.0–107.0)	Elevation: 53/74 (71.6%)
Myoglobin [μg/L]	72.2 (42.9–118.2)	Elevation: 21/70 (30.0%)
Creatine kinase [U/L]	114.0 (64.0–189.0)	Elevation: 22/90 (24.4%)
Ferritin [μg/L]	400.0 (223.0–949.0)	Elevation: 35/71 (49.3%)
Lactate dehydrogenase [U/L]	270.0 (224.5–361.0)	Elevation: 78/96 (81.3%)
NT-proBNP [pg/mL]	186.0 (69.0–1061.0)	Elevation: 38/71 (53.5%)
Troponin I [ng/L]	5.3 (2.5–14.4)	Elevation: 7/80 (8.8%)
C-reactive protein [mg/L]	37.0 (9.8–91.0)	Elevation: 79/100 (79.0%)
Procalcitonin [ng/mL]	0.04 (0.02–0.11)	Elevation: 29/96 (30.2%)
Interleukin-6 [pg/mL]	19.1 (1.5–52.6)	Elevation: 39/71 (54.9%)

ALT — alanine transferase; APTT — activated partial thromboplastin time; AST — aspartate aminotransferase; eGFR — estimated glomerular filtration rate; GGTP — gamma-glutamyl transpeptidase; INR — international normalized ratio; NT-proBNP — N-terminal pro brain natriuretic peptide

Outcomes

Among 100 enrolled patients, 10 (10.0%) died, 47 (47.0%) developed respiratory failure, 15 (15.0%) were transferred to the ICU, 17 (17.0%) presented AKI, 7 (7.0%) had sepsis, and 10 (10.0%) were diagnosed with pulmonary embolism. The median time of hospitalization was 19.5 days (IQR 14.0-31.5) while median time of ICU stay accounted for 12.0 (IQR 6.0–19.0) days. The majority of patients (52/100, 52.0%) were discharged with positive result of nasal swab for SARS-CoV-2 and were isolated at home. The median time to viral clearance among 81 subjects with available negative result was 31.0 (IQR 20.0–37.0) days. Details concerning management and outcomes are summarized in Table 3.

Factors associated with severity of the disease — the univariable analysis

Patients with severe COVID-19 had a higher MEWS on admission and more often presented with dyspnea. Moreover, in terms of laboratory results, COVID-19 patients requiring oxygen therapy were characterized by a higher white blood cell and neutrophil count, lower lymphocyte count, higher d-dimer levels as well as higher concentrations of inflammatory markers, liver damage enzymes, LDH, myoglobin, NT-proBNP and troponin. Treatment differences between these groups included more frequent administration of antibiotics and chloroquine. Detailed information about between-group differences are summarized in Table 4. A complete comparison

Table 3. Treatment and outcomes

Treatment	Number of patients; n [%]
Treatment	
Prophylactic LMWH	39 (39.0%)
Intermediate dose LMWH	28 (28.0%)
Therapeutic dose LMWH	23 (23.0%)
Direct oral anticoagulants	11 (11.0%)
Antibiotics	69 (69.0%)
Chloroquine	36 (36.0%)
Ritonavir/lopinavir	2 (2.0%)
Oxygen therapy on admission	33 (33.0%)
FiO_2 on admission	36.0 (28.0–40.0)
Oxygen therapy anytime during hospitalization	47 (47.0%)
Highest FiO_2 during hospitalisation	5.0 (28.0–90.0%)
Outcomes	
Mortality	10 (10.0%)
Mechanical ventilation	8 (8.0%)
Transfer to the ICU	15 (15.0%)
Pulmonary embolism	10 (10.0%)
Myocardial injury	7 (7.0%)
Myocardial infarction	1 (1.0%)
Stroke	0 (0.0%)
Acute kidney injury	17 (17.0%)
Sepsis	7 (7.0%)
Septic shock	6 (6.0%)
Length of hospitalisation [days]	19.5 (14.0–31.5)
Length of ICU stay [days]	12.0 (6.0–19.0)
Positive swab at discharge	52 (52.0%)
Time to negative swab [days] (n = 81)	31.0 (20.0–37.0)

ICU — intensive care unit; LMWH — low molecular weight heparin

of demographic, clinical, laboratory data can be found in Supplementary Tables 1–3.

Factors associated with severity of the disease — the multivariable analysis

Multivariable analysis revealed that severe course of COVID-19 is associated with increased age (OR 1.10; 95% CI 1.01–1.15), BMI (OR 1.24; 95% CI 1.01–1.53), MEWS on admission (OR 3.95; 95% CI 1.48–12.0), the highest d-dimer value during hospitalization (OR 1.73; 95% CI 1.03-2.90) and baseline LDH concentration (OR 1.16; 95% CI 1.03–1.30). The validation of the model was performed using the Hosmer and Lemeshow goodness of fit test ($X^2 = 18.12$, $p = 0.02$). Logistic regression results are summarized in Figure 1 and Supplementary Table 4.

Discussion

To our knowledge, this is one of the first manuscripts describing a prospective cohort of Polish COVID-19 patients, and we believe that it may be useful for local clinicians involved in the care of patients with SARS-CoV-2.

The presented study cohort consisted predominantly of males and the mean age amounted to 59.2 years, which confirms the results of several previous studies reporting higher representation of males in hospitalized patients [1, 2, 11, 12]. Elderly persons with COVID-19 are characterized by a high case fatality ratio and symptomatic infection rate [13, 14]. In a Chinese modeling study, the rate of hospitalization due to COVID-19 increased with age, ranging from 1% for patients aged 20 to 29 years, through 8% for those aged 50 to 59 years, up to 18% for individuals older than 80 years [15]. Underlying medical comorbidities are considered important risk factors for severe COVID-19 course and mortality. The most common comorbidities in the presented cohort were hypertension (49%), obesity (31%) and dyslipidemia (19%), which is comparable to several large studies from the United States and China. Upon initial presentation, COVID-19 symptoms are typically consistent with pneumonia [3, 16–18]. The majority of patients in this case series were hospitalized due to a cluster of flu-like symptoms, i.e. fever (73.0%), cough (67.0%) and dyspnea (44.0%). Additionally, we observed a significant representation of gastrointestinal symptoms as well as other typical COVID-19 symptoms, i.e. loss of smell or taste, which was present in 15% of cases. In a report published by the Centers for Disease Control and Prevention, the described symptoms of COVID-19 included: cough (50%), fever > 38°C (43%), myalgia (36%), headache (34%), dyspnea (29%), sore throat (20%), diarrhea (19%), nausea/vomiting (12%), loss of smell or taste, abdominal pain, and rhinorrhea in fewer than 10 percent each [19].

A number of observational studies involving COVID-19 patients revealed a unique pattern of laboratory changes, encompassing hemostatic derangements and pronounced hyperinflammatory state. First, there are numerous reports about significant coagulation abnormalities, including increased concentrations of fibrinogen and d-dimer as well as relatively common prolongation

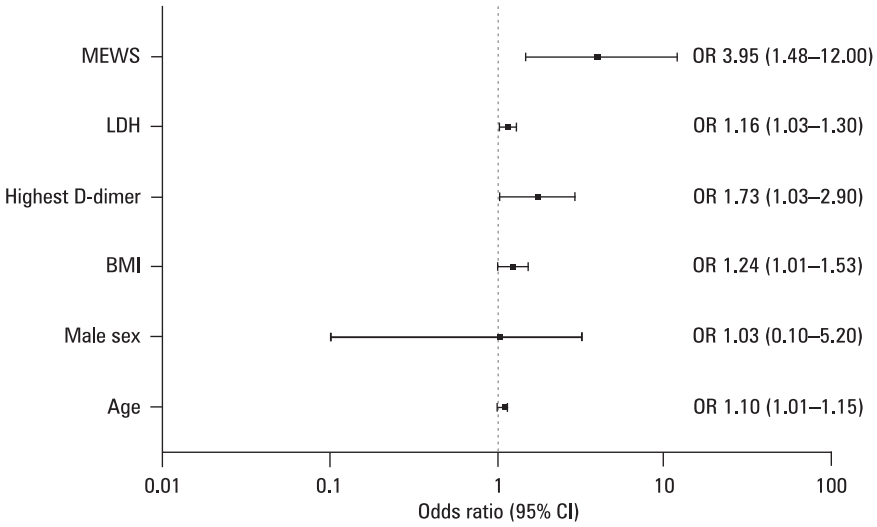

Figure 1. Summary of multivariable regression results. Dots and whiskers represent odds ratios with 95% confidence intervals. BMI — body mass index; LDH — lactate dehydrogenase; MEWS — modified early warning score; OR — odds ratio

of coagulation times. Coagulation tests in the presented cohort showed an increased concentration of fibrinogen and d-dimer in the majority of patients and prolonged APTT in nearly 20% of cases. Another frequently described laboratory anomaly is thrombocytopenia, which was present in 17% of the study group and in 5.0 to 41.7% of patients in the previous reports. According to the studies, utilizing global coagulation tests (rotational thromboelastometry, thrombography) COVID-19-associated coagulopathy represents severe hypercoagulability, which most probably is consistent neither with disseminated intravascular coagulation nor consumptive coagulopathy. Importantly, both abnormalities in coagulation tests and thrombocytopenia seem to be associated with disease severity and mortality [20]. Second, the majority of patients in the current study had laboratory findings suggestive of an exuberant inflammatory response reflected by markedly elevated concentrations of CRP, PCT, ferritin and IL-6. These observations are in line with some previous reports suggesting significant role of hyperinflammatory state with cytokine release syndrome in the pathogenesis of COVID-19 and its association with critical and fatal illness [1, 21]. Finally, recent data suggests that liver injury is quite common among patients with COVID-19 [22]. This was also observed in the current study cohort – serum concentrations of liver enzymes (ALT, AST, GGTP) and cellular damage markers (LDH) were frequently elevated. Importantly, according to the recent meta-analysis, liver injury seems to be more prevalent in severe cases of COVID-19 [23].

Radiological findings may vary depending on disease stage, patients age, immunity status and comorbidities [24] . Normal chest radiographs or CT are found in only 18% of patients with mild disease and this proportion drops to 3% in severely ill patients. Typical CT findings in COVID-19 include bilateral, multilobar ground glass opacities with a peripheral or posterior distribution, mainly in the lower lobes, while consolidation at the initial imaging are less common (more frequent in elderly people). Moreover, pleural effusion is an uncommon but a possible finding, sometimes accompanying the disease progression [25]. In the presented study, chest radiographs were performed in nearly all patients upon admission to the hospital and similarly to data presented above, were normal in 23.9% of cases, while the remaining patients presented interstitial infiltrates, consolidations or both. Important strength of this study is high availability of CT scans which were performed in almost half of the studied patients and most commonly showed ground glass opacities followed by consolidations and presence of fluid in the pleural space.

The frequency of reported complications in COVID-19 is strongly dependent on the studied population and disease severity. The most commonly observed organ involvement in patients with severe COVID-19 is lung injury manifested by acute respiratory failure. In this study, almost half of the studied cohort developed respiratory insufficiency, and 15% of patients were transferred to the ICU due to severe respiratory failure among whom more than a half required endotra-

Table 4. Comparison of selected variables between patients wit severe and non-severe COVID-19

Characteristic	Severe cases (n = 47)	Non–severe cases (n = 53)	P–value
Demographic			
Age [years], mean (SD)	62.3 (15.9)	56.5 (15.8)	0.07
Sex, male	30 (63.8%)	33 (62.3%)	0.87
Symptoms			
Dyspnea	32 (68.1%)	12 (22.6%)	< 0.0001
Measurements and vital signs on admission			
BMI [kg/m^2]	28.4 (26.1–32.1)	26.8 (24.9–28.6)	0.009
Heart rate on admission	90.0 (80.0–100.0)	82.0 (71.0–91.0)	0.007
Respiratory rate on admission	20.0 (17.0–22.0)	15.0 (14.0–16.0)	< 0.0001
MEWS on admission	2.0 (1.0–2.0)	0.0 (0.0–1.0)	< 0.0001
Comorbidities			
Hypertension	29 (61.7%)	20 (38.5%)	0.02
Chronic heart failure	10 (21.3%)	1 (1.9%)	0.003
Laboratory results			
White blood count [$\times 10^3$/mm^3]	6.86 (5.70–9.83)	5.20 (3.87–6.58)	< 0.0001
Neutrophils, count [$\times 10^3$/uL]	5.34 (3.92–8.15)	3.02 (2.03–4.01)	< 0.0001
Lymphocytes, count [$\times 10^3$/uL]	1.00 (0.64–1.20)	1.38 (1.09–1.65)	< 0.0001
D-dimer on admission [mg/L]	1.10 (0.74–2.08)	0.54 (0.35–1.07)	< 0.0001
Highest d-dimer [mg/L]	2.20 (0.96–5.15)	0.91 (0.55–1.43)	< 0.0001
APTT [s]	33.9 (30.2–38.2)	30.8 (27.8–33.1)	0.02
AST [U/L]	46.0 (35.0–68.0)	31 (24.0–41.5)	0.0003
GGTP [U/L]	72.5 (31.0–174.0)	40.0 (23.0–59.0)	0.003
Myoglobin [μg/L]	115.5 (66.4–175.5)	57.9 (38.6–77.9)	< 0.0001
Ferritin [μg/L]	681.0 (379.0–1280.0)	308.0 (145.0–529.0)	0.0004
Lactate dehydrogenase [U/L]	354.0 (284.0–456.0)	232.0 (201.0–275.0)	< 0.0001
NT-proBNP [pg/mL]	482.5 (134.0–2084.0)	93.0 (35.0–321.0)	< 0.0001
Troponin I [ng/L]	12.7 (4.3–29.4)	3.7 (2.5–7.8)	0.0002
C-reactive protein [mg/L]	85.5 (34.5–170.0)	12.1 (2.3–48.2)	< 0.0001
Procalcitonin [ng/mL]	0.10 (0.04–0.20)	0.02 (0.02–0.04)	< 0.0001
Interleukin-6 [pg/mL]	29.3 (19.1–78.0)	1.5 (1.5–21.2)	< 0.0001
Treatment			
Antibiotics	45 (95.7%)	24 (45.3%)	< 0.0001
Chloroquine	25 (53.2%)	11 (20.8%)	< 0.0001
Outcomes			
Pulmonary embolism	8 (17.0%)	2 (3.8%)	0.04
Acute kidney injury	13 (27.7%)	4 (7.6%)	0.014
Positive swab at discharge	19 (42.2%)	33 (62.3%)	0.047

Data is presented as n [%] for categorical variables and median (interquartile range) unless otherwise specified. ALT — alanine transferase; AST — aspartate aminotransferase; BMI — body mass index; GGTP — gamma-glutamyl transpeptidase; MEWS — Modified Early Warning Score; NT-proBNP — N-terminal pro brain natriuretic peptide; SD — standard deviation. The $p < 0.00062$ is considered significant

cheal intubation and mechanical ventilation. In the previous studies from the USA, 12–24% of hospitalized patients required mechanical ventilation [3, 17]. In a nationwide cross-sectional study performed in China including approximately 44,500 cases, one in twenty patients was categorized as critically ill (i.e. with respiratory failure, shock, or multiorgan dysfunction) [6]. Importantly, thromboembolic complications are markers of severe COVID-19 and are associated with multiorgan failure and increased mortality [26]. The evidence to date supports the concept that the thrombotic manifestations of severe COVID-19 are due to the ability of SARS-CoV-2 to invade endothelial cells via ACE-2 (angiotensin-converting enzyme 2). Ten percent of patients in our cohort were diagnosed with pulmonary embolism despite the fact that LMWH was administered in 90% of the analyzed cases. Depending on the population and type of study (retrospective *vs* prospective with active screening for venous thromboembolism), the incidence of thromboembolic complications ranges from 3.9% to 79.4% in the ICU patients and from 1.3 to 14.7% among non-critically ill patients [27]. Kidney involvement in COVID-19 is frequent, with clinical presentation ranging from mild proteinuria to AKI necessitating renal replacement therapy [28]. In our study, a baseline renal function was normal in the majority of patients (82%). In the course of the hospitalization, 7% of our patients have developed AKI, which corroborates the results of the recently published meta-analysis estimating the incidence of AKI among hospitalized patients at the level of 8.9% [29]. It is important to note that a substantial proportion of AKI could have been missed due to limited sensitivity of serum creatinine. The role of secondary infections in COVID-19 may be overestimated, although data is limited [30, 31]. In a review, the rate of bacterial or fungal coinfections was only 8%, whereas in our study, 7% of hospitalized patients developed sepsis or septic shock [30].

Finding an effective treatment for COVID-19 was a long and tortuous road filled with many hopes and even more disappointments. The recruitment to our study was initiated at the beginning of pandemic in Poland, therefore, many patients received medications which are currently known to be ineffective. Chloroquine was administered to 36% of our patients while the combined protease inhibitor ritonavir/lopinavir was used extremely rarely (2% of cases). Unfortunately, the gradually emerging data from controlled trials revealed that these drugs do not improve outcomes in this population [32, 33]. The available evidence shows a benefit associated with the administration of systemic glucocorticoids among patients requiring oxygen therapy. The use of remdesivir is currently suggested in patients with severe COVID-19 who are not critically ill. Despite initial positive signals from observational studies, the use of convalescent plasma proved to be ineffective. Routine use of IL-6 pathway inhibitors (tocilizumab, sarilumab) is not recommended, although these drugs given in the intensive care settings may benefit a selected group of critically ill patients [34].

The mortality rate in our cohort was 10% compared to estimated mortality rate of 2.8% among the entire population of Polish COVID-19 patients [35]. The estimated mortality rate among hospitalized patients accounts for 18.9% and varies greatly depending on a study, ranging from 0.7 to 61.5% [36]. Such variability in the death rate is probably secondary to inter-country differences in population characteristics, healthcare-related factors as well as strategy concerning hospital admissions or outpatient treatment of COVID-19 patients.

Almost half the patients in this study were classified as severe cases based on the development of respiratory failure. Univariable analysis comparing severe and non-severe cases showed that patients with severe COVID-19 more commonly presented with dyspnea and increased baseline respiratory rate as well as higher MEWS on admission, thus suggesting a potential clinical utility of the latter as a risk stratification tool in this population [37]. Analysis of laboratory results revealed that severely ill patients had indicators of coagulopathy, hyperinflammatory state, liver damage and increased cardiac biomarkers [1, 16, 38].

Finding risk factors of severe COVID-19 is particularly relevant for clinician involved in management of COVID-19 patients. There are several papers reporting factors potentially associated with mortality of severe course of the disease, however, none of them is based on a Polish cohort [17, 39]. In a study of 5,279 people with COVID-19 in New York City by Petrilli *et al*. [17], among hospitalized patients, factors associated with critical illness were: age, heart failure, BMI (greater than 40) and male sex, with diabetes being also significant. On the other hand, in a large Chinese study, a multivariable logistic regression model showed 10 potential predictors of critical illness. These variables included chest X-ray abnormalities, age, hemoptysis, dyspnea, unconsciousness, the number of comorbidities, cancer

history, neutrophil-to-lymphocyte ratio, as well as concentrations of LDH and direct bilirubin [39]. A multivariable analysis in our study revealed rather similar results and suggested the increasing age, BMI, MEWS on admission, the highest d-dimer level during hospitalization and LDH concentration as factors potentially related to a severe course of COVID-19. Unfortunately, a relatively small study sample reduces the statistical power of this analysis while single-center character of this study may limit its generalizability.

There is very limited data concerning the clinical characteristics and outcomes of Polish patients hospitalized due to COVID-19. In a retrospective study by Nowak *et al*. [40], the authors reported similar distribution of signs and symptoms as well as comorbidities in their sample. Interestingly, the mortality in our cohort was more than two times lower despite a similar proportion of critically ill patients requiring a transfer to the ICU. This may be partially due to differences in treatment, i.e. our cohort was characterized by more common administration of currently recommended LMWH as well as less frequent use of chloroquine and ritonavir/lopinavir, both of which proved to be ineffective in the treatment of COVID-19. An univariable analysis performed in the aforementioned study suggested age, shortness of breath, cardiovascular disease, malignancy and bilateral patchy shadowing in chest X-ray as potential predictors of mortality in this population, which partially corroborates our results. It is however important to note that data presented in our study was gathered in a prospective manner, and therefore, offers more valuable insight into the clinical profile of Polish patients hospitalized due to COVID-19.

This study has several limitations. First, due to the limited study sample and a relatively low incidence of several outcomes of interest, such as mortality, AKI and pulmonary embolism, we were unable to perform multivariable analysis to assess risk factors for these events. Second, this study was performed in a single ward specialized in treating patients with respiratory failure, therefore, the presented cohort may consist of patients with more severe form of COVID-19 compared to other wards, thus limiting the generalizability of the results. Third, the incidence of several outcomes might be underestimated due to lack of routine screening, i.e. lack of active troponin level monitoring for myocardial injury or lack of routine deep vein ultrasound and CT pulmonary angiogram for venous thromboembolism. Finally, we believe that this cohort of first one hundred patients with COVID-19 treated in our center comprises a relatively large proportion of mild cases compared to later stages of the pandemic.

Conclusion

In this observational study describing the clinical profile of 100 hospitalized patients with COVID-19, nearly half of the analyzed cases developed respiratory failure and approximately 10% died in the course of the disease. The multivariable analysis revealed increasing age, BMI, MEWS on admission as well as higher d-dimer and LDH concentration as factors associated with severe course of COVID-19.

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Conflict of interest

None declared.

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Mahendra M[1], Abhishek Nuchin[1], Ranjith Kumar[1], Shreedhar S[1], Padukudru Anand Mahesh[2]

[1]Department of Respiratory Medicine, Shimoga Institute Of Medical Sciences, Shimoga, Karnataka, India
[2]Department of Respiratory Medicine, JSS Medical College, JSS University, Mysore, Karnataka, India

Predictors of mortality in patients with severe COVID-19 pneumonia — a retrospective study

Abstract

Introduction: The novel coronavirus pandemic has caused significant mortality throughout the world. This study was done as there is scarce data on mortality predictors in severe COVID-19 pneumonia patients admitted to ICU in the Indian population.

Material and methods: A retrospective study was conducted on COVID-19 pneumonia patients admitted to tertiary care center during June–October 2020. The records of patients admitted to ICU were collected and data included demography, symptoms, comorbidites and vital parameters. Laboratory parameters included complete hemogram, random blood sugar, serum ferritin and LDH, renal function test, liver function test. Treatment-associated information such as the use of remdesivir, timing of initiating remdesivir after the symptom onset, the use of steroids, use of anticoagulants, use of HFNC, NIV, ventilator were collected. 30 days mortality data post-discharge was collected via telephonic interview.

Results: 4,012 confirmed cases of COVID-19 were admitted to hospital, of which 560 (13.95%) with severe pneumonia were included in the study. Mean age was 57.75 ± 13.96 years. The mortality rates were 54.64% among severe COVID-19 cases and 5% among mild to moderate COVID-19 cases. The Cox multinominal regression analysis identified $SpO_2/FiO_2 < 400$, age > 50 years, duration of symptom > 4 days, serum ferritin > 450 μg/L, respiratory rate > 23/min, the presence of comorbidities and non-usage of remdesivir were independently associated with increased mortality. Mortality rate at 30 days was 56.60%.

Conclusion: Severe COVID-19 pneumonia is associated with very high mortality, especially in a resource-constrained setting. The use of remdesivir may have to be considered early in the course of disease to prevent excess mortality related to COVID-19.

Key words: COVID-19, pneumonia, mortality, remdesivir

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Introduction

The novel coronavirus disease (COVID-19) has drew worldwide attention by causing the first pandemic by coronavirus leading to international public health emergency. On the 3rd November, 2020, the World Health Organization (WHO) declared coronavirus outbreak as pandemic and public health emergency of international concern [1]. SARS-CoV-2 infection has caused significant morbidity and mortality throughout the world leading to immense health care burden. Currently, worldwide around 55 million people have been infected with SARS-CoV-2, which has resulted in around 1.35 million deaths [2]. In India, around 9 million people have been infected and approximately 132 thousand people have succumbed to SARS-CoV-2 infection [3]. Very little attention has been paid to clinical characteristics and outcomes of severe COVID-19 pneumonia patients in intensive care unit (ICU), data on whom are scarce but are of paramount importance to reduce mortality in a resource-constrained setting such as a government hospital. This study aimed to identify factors associated with mortality in patients with severe COVID-19 pneumonia admitted to a tertiary care COVID-19 hospital in South India.

Address for correspondence: Padukudru Anand Mahesh, Department of Respiratory Medicine, JSS Medical College, JSS University, Mysore, Karnataka, India; e-mail: mahesh1971in@gmail.com

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Material and methods

Study design

We conducted a retrospective study at a tertiary care teaching hospital in South India. The study was approved by institutional ethics committee (SIMS/IEC/503/2020-21).

Participants and eligibility criteria

We retrospectively analyzed consecutive patients with severe COVID-19 pneumonia who had been admitted to our hospital from June to October 2020. Individuals aged 18 years and above were included in the study. Diagnosis of COVID-19 was defined as the patient having a positive result on the oropharyngeal and nasopharyngeal swab for SARS-CoV-2 by reverse transcriptase polymerase chain reaction (RT-PCR). Our hospital used test kits provided by the government of Karnataka.

Classification of severity of SARS-CoV-2 infection was based on the revised national guidelines on clinical management of SARS-CoV-2 infection given by the Ministry of Health and Family Welfare, Government of India [4].

We defined severe COVID-19 pneumonia as an "adult with fever or suspected respiratory infection, plus one of the following; respiratory rate >3 0 breaths/min, severe respiratory distress, SpO_2 < 90% at room air".

Data collection

The records of patients admitted to high dependency unit and intensive care unit were collected and evaluated for predictors of mortality. Data included demographic details, symptoms and the duration of symptoms, comorbidities like diabetes, hypertension, heart disease, obesity, chronic kidney disease, chronic liver disease, malignancy, chronic respiratory diseases like asthma and Chronic Obstructive Pulmonary Disease. Clinical parameters like pulse rate, heart rate, blood pressure, peripheral capillary oxygen saturation/fraction of inspired oxygen (SpO_2/FiO_2) on admission were collected. Laboratory parameters included complete hemogram (Sismex,6 part differential cell counter), neutrophil-lymphocyte ratio, random blood sugar, serum ferritin (Beckman), Serum Lactate Dehydrogenase (LDH) (ERBA EXEL 640), renal function test, liver function test, arterial blood gas, chest radiography. Treatment details like the use of remdesivir, the day of starting remdesivir after the symptom onset, the use of steroids, use of anticoagulants, use of high-flow nasal cannula, noninvasive ventilation, ventilator, incidence of hospital-acquired infections were collected. The outcome variables included the length of hospital stay and mortality.

The patients were managed with supportive care and specific pharmacological protocols created by the hospital's COVID-19 management guidelines committee in accordance with the government of Karnataka. Specific pharmacological treatments included systemic corticosteroids, low-molecular-weight heparin, oxygen and remdesivir. Data collected were cross-checked by the authors, and at the end of data entry - by an independent investigator. Any disagreement between two investigators was resolved by reaching a consensus.

A total of 4,012 patients with laboratory-confirmed SARS-CoV-2 were admitted during the study period. We excluded from the final analysis patients who were still receiving care in the hospital at the time of preparation of this manuscript and those with incomplete information.

Statistical analysis

Descriptive data are presented as frequencies (percentages) of discrete variables and as means (SDs) of continuous variables. For comparisons between the two groups, ANOVA test with the Bonferroni correction was used. χ^2-test was applied to evaluate categorical factors. Fischer's exact test was used in case of low cell frequency. The Cox regression univariate and multinomial analysis and Kaplan-Meier analysis were used for survival investigation. The receiver operating characteristic (ROC) curves were constructed for age, duration of symptoms, SpO_2/FiO_2, serum ferritin, respiratory rate and the cut-off value with the highest sensitivity and specificity selected as threshold. All statistical tests were 2-tailed, and factors were considered statistically significant at $p < 0.05$. IBM SPSS version 22 and CDC Epi Info version 7 were used for analysis.

Results

A total of 4,012 confirmed cases of COVID-19 were admitted to hospital, of which 560 (13.95%) with great severity were included in the study (Figure 1). Mean age of the study population was 57.75 ± 13.96 years. Three hundred sixty-five (65.17%) were men. Hypertension (41.25%) followed by diabetes (41%) was the most common comorbidity. Dyspnea (69.46%) was the most frequent symptom followed by fever (52.5%) and cough (46.78%) (Table 1). Mean duration of symptoms before admission

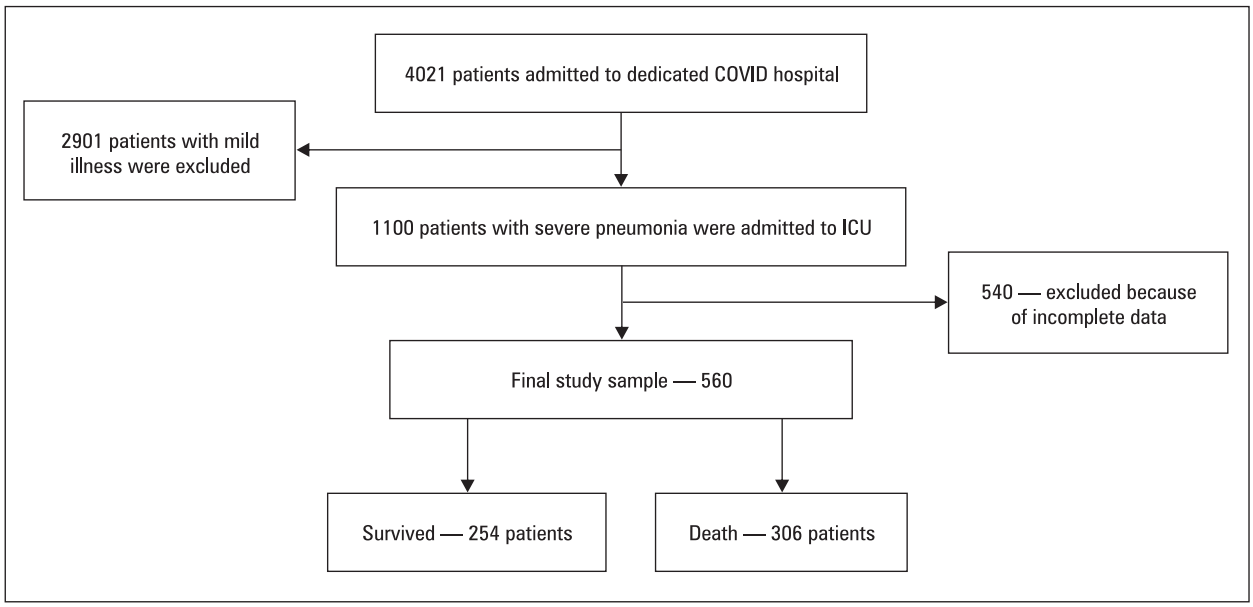

Figure 1. The flowchart depicting enrolment of COVID-19 patients into the study

was 4.11 ± 2.09 days. Remdesivir was given to 298 (53.21%) patients. Mean duration of starting remdesivir after the symptom onset was 5.58 ± 2.78 days. High-flow nasal cannula was given to 245 (43.7%) subjects. Ninety-one (16.25%) needed ventilatory support. A very high mortality, i.e. 306 (54.64%) cases was observed at our hospital. Among the patients who died, nearly quarter of them (82 patients) died within 24–48 hours from admission. Secondary bacterial infection was noted at a late stage of the disease in 8 patients, and organisms isolated were *Streptococcus pneumonia*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. In the Cox regression analysis, we observed age > 50 years, duration of symptoms more than 4 days, SpO_2/FiO_2 < 400 on admission, serum ferritin > 450 μg/L on admission, respiratory rate >23/min on admission, the presence of comorbidities and non-usage of remdesivir to be independent predictors of mortality in patients with severe COVID-19 pneumonia (Table 2). We observed an increased hazard of death by two days after the onset of symptoms which peaked on the 5th day of the symptom onset. The risk of death then decreased, but remained significant till the 8th day (Figure 2). We found steroid usage, use of mechanical ventilation and the day of starting remdesivir after the symptom onset to be independent predictors of prolonged hospitalization in patients with severe COVID-19 pneumonia (Table 3). The presence of chronic liver disease, use of mechanical ventilation, day of starting remdesivir after the symptom onset to be independent predictors of prolonged ICU stay in patients with severe COVID-19 pneumonia (Table 4). The Kaplan-Meier analysis showed statistically significant mortality benefit in patients who received remdesivir and even better survival if used within 4 days of the symptom onset (Figure 3A and 3B). Only a small number of subjects (< 2%) succumbed post-discharge due to COVID-19-related complications with a final mortality rate at 30 days of 56.60%.

Discussion

In the present study, we found elderly patients with male predominance more commonly affected by moderate to severe pneumonia with very high mortality rates of more than 50%. We observed age > 50 years, duration of symptoms more than 4 days on admission, SpO_2/FiO_2 <400 on admission, serum ferritin > 450 mcg/L on admission, respiratory rate >23/min on admission, the presence of comorbidities and non-usage of remdesivir to be independent predictors of mortality in patients with severe COVID-19 pneumonia.

Elderly patients are commonly affected by severe pneumonia due to age-dependent decline in immunity. A Korean meta-analysis of COVID-19 pneumonia found old age to be the risk factor for increased mortality [5]. Old age is an established risk factor for various infections, including viral infections and by far most significant predictor of mortality in COVID-19 pneumonia [6, 7].

Table 1. Baseline characteristics of patients with severe COVID-19 pneumonia admitted to ICU

Variables	Total (n = 560)	Survived (n = 254)	Death (n = 306)	P-value*
Age [years], mean (SD)	57.75 (13.96)	54.39 (14.99)	60.54 (12.39)	**0.004**
Gender, n [%]	365 (65.17)	166 (65.35)	199 (65.03)	0.840
Symptoms, n [%]				
Cough	262 (46.78)	131 (51.57)	131 (42.95)	0.083
Dyspnea	389 (69.46)	140 (55.12)	249 (81.37)	**0.001**
Fever	294 (52.5)	161 (63.39)	133 (43.46)	**0.0001**
Myalgia	61 (10.89)	41 (16.14)	20 (6.54)	0.315
Duration of symptoms before admission, mean (SD) [in days]	4.11 (2.09)	3.27 (1.92)	4.79 (1.98)	**0.0001**
Comorbidities, n [%]	343 (61.25)	115 (45.28)	228 (74.51)	**0.0001**
Diabetes, n [%]	230 (41)	80 (31.50)	150 (49.02)	**0.016**
Hypertension, n [%]	231 (41.25)	72 (28.35)	159 (51.96)	**0.009**
Ischemic heart disease, n [%]	48 (8.5)	11 (4.33)	37 (12.09)	0.118
Chronic kidney disease, n [%]	32 (5.7)	4 (1.57)	28 (9.17)	**0.043**
Chronic liver disease, n [%]	9 (1.6)	1 (0.39)	8 (2.61)	0.171
Morbid obesity, n [%]	13 (2.3)	1 (0.39)	12 (3.92)	0.092
Vitals				
SpO2 at room air [on admission], mean (SD)	78.70 (18.72)	87.74 (12.17)	71.19 (19.87)	**0.0001**
Respiratory rate, breath/min, mean (SD)	21.37 (4.82)	19.94 (2.97)	22.5 (5.67)	**0.003**
Laboratory findings at the time of admission				
Hemoglobin [gm%], mean (SD)	12.35 (2.14)	12.42 (2.18)	12.34 (2.08)	0.121
Total white blood cell count, mean (SD)	9.87 (6.5)	9.04 (4.59)	10.56 (7.74)	0.071
Platelet count [lakh/mm^3]	2.10 (0.93)	2.24 (0.87)	2.03 (0.86)	0.081
Neutrophil Lymphocyte Ratio mean (SD)	8.02 (8.66)	5.87 (4.37)	9.80 (10.71)	**0.029**
Serum ferritin [μg/L], mean (SD)	539.66 (381.78)	367.2 (308.63)	632.29 (385.61)	**0.0001**
Serum Lactate dehydrogenase [LDH] [U/L], mean (SD)	845.73 (593.51)	788.1 (681.62)	866.39 (558.52)	0.160
Serum Creatinine, [mg/dl] mean (SD)	1.66 (2.08)	1.24 (1.33)	1.94 (2.51)	0.002
Random blood sugar [mg/dl] mean (SD)	215.49 (135.3)	181.32 (112.48)	239.92 (144.8)	**0.002**
Treatment				
Remdesivir usage n [%]	298 (53.21)	165(64.96)	133(43.46)	**0.019**
First dose of Remdesivir after symptoms onset, mean (SD)	5.58 (2.78)	5.06 (3.12)	6.01 (2.37)	**0.0001**
Low-molecular-weight heparin, n [%]	365 (65.17)	154 (60.63)	211 (68.95)	0.161
Steroid usage n [%]	454 (81)	185 (72.83)	269 (87.91)	**0.023**
First dose of steroid after admission [in days], mean (SD)	1.22 (1.19)	1.19 (1.25)	1.24 (1.03)	0.931
High-flow nasal cannula, n [%]	245 (43.7)	41 (16.14)	204 (66.67)	**0.0001**
Ventilator, n [%]	91 (61.25)	3 (1.18)	88 (28.76)	**0.0001**
No. of days in ICU, mean (SD)	4.48 (3.23)	5.48 (3.18)	3.64 (3.04)	**0.0001**
No. of days of hospital stay, mean (SD)	8.71 (7.54)	12.53 (8.76)	5.52 (4.28)	**0.00001**

*ANOVA test with Bonferroni adjustment for multiple comparisons

Table 2. Cox univariate and multivariate analysis of factors associated with mortality in patients with severe COVID-19 pneumonia

Variables	Hazard ratio (95% CI)	P–value	Adjusted hazard ratio (95% CI)	P–value
$SpO_2/FiO_2 < 400$	3.35(2.631–4.264)	**0.001**	2.424 (1.869–3.145)	**0.0001**
Age > 50 years	1.01 (1.00–1.02)	**0.0001**	1.589 (1.132–2.228)	**0.007**
Duration of symptoms > 4 days	1.23 (1.18–1.28)	**0.0001**	2.410 (1.659–3.502)	**0.0001**
Serum ferritin > 450 μg/L	1.001 (1.001–1.001)	**0.0001**	2.134 (1.671–2.725)	**0.0001**
Neutrophil to lymphocyte ratio> 7	1.02 (1.01–1.03)	**0.0001**	1.122 (0.880–1.429)	0.354
First dose of remdesivir after symptom onset > 4 days	1.038 (1.003–1.075)	**0.038**	1.234 (0.747–2.036)	0.411
Respiratory rate > 23/min	1.08 (1.06–1.10)	**0.0001**	1.343 (1.046–1.725)	**0.021**
Diabetes	1.46 (1.16–1.83)	**0.001**	0.835 (0.635–1.097)	0.195
Hypertension	1.12 (1.02–1.23)	**0.014**	0.874 (0.754–1.012)	0.072
Ischemic heart disease	1.87(1.33–2.65)	**0.001**	1.233 (0.857–1.774)	0.258
Chronic kidney disease	2.35 (1.59–3.48)	**0.0001**	1.383 (0.886–2.158)	0.153
Presence of any comorbidity	2.32 (1.79–3.0)	**0.0001**	1.822 (1.286–2.581)	**0.001**
Remdesivir usage	0.75 (0.59–0.93)	**0.013**	0.453 (0.342–0.599)	**0.0001**
Steroid usage	1.73 (1.22–2.44)	**0.001**	1.097 (0.749–1.608)	0.633
Creatinine > 1.5 mg/dl	1.08 (1.04–1.12)	**0.001**	1.161 (0.884–1.524)	0.284

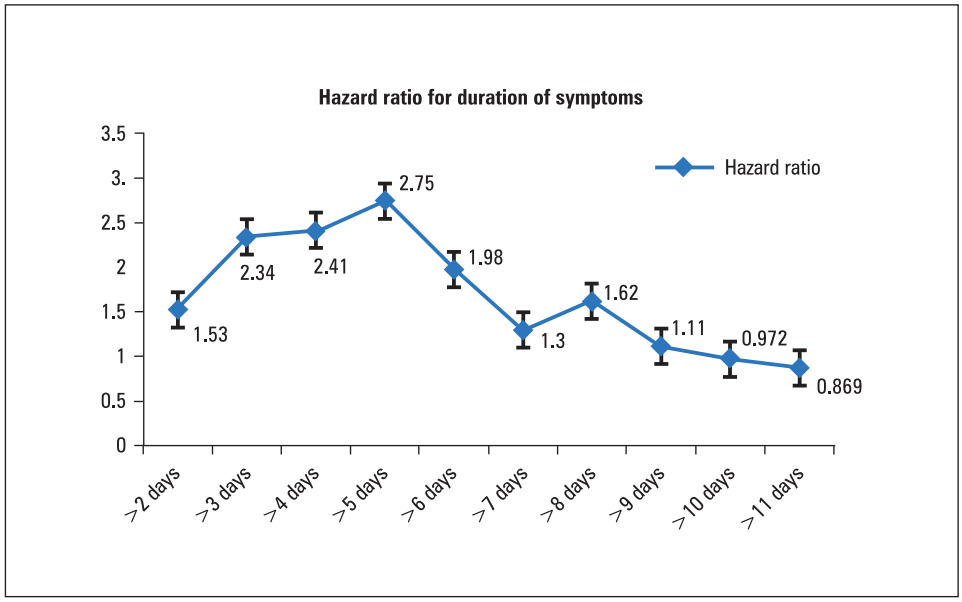

Figure 2. The graph depicting hazard ratio of mortality for each day from the symptom onset and duration of hospitalization

Elderly patients infected with SARS-CoV-2 tend to trigger hyper-activation of the immune system and hypercoagulation in small blood vessels leading to cytokine storm [8]. Though it is still unclear why the elderly are more prone to cytokine storm, possible mechanisms include an increase in activity and abundance of NLRP-3 (Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing protein 3), a component of inflammasome in immune cells and alveolar macrophages in the lungs which upon chronic stimulation cause pulmonary fibrosis [9]. NLRP-3 activity is normally under control of Sirtuin-2 (protein implicated in longevity) which reduces with age. This decline in Sirtuin-2 is exacerbated by SARS-CoV-2 infection and might

Table 3. Cox univariate and multivariate analysis of factors associated with prolonged hospital stay more than 5 days in patients with severe COVID-19 pneumonia

Variables	Hazard ratio (95% CI)	P–value	Adjusted hazard ratio (95% CI)	P–value
Fever	0.72 (0.581–0.90)	**0.004**	0.804 (0.638–1.014)	0.066
Dyspnea	1.26 (1.01–1.58)	**0.036**	1.04 (0.806–1.356)	0.738
Comorbidities	1.28 (1.03–1.59)	**0.025**	1.17 (0.930–1.17)	0.177
First dose of Remdesivir after symptom onset > 4 days	0.959 (0.929–0.989)	**0.008**	0.932 (0.899–0.966)	**0.0001**
Steroid usage	1.29 (0.98–1.71)	**0.06**	1.42 (1.030–1.976)	**0.032**
Use of ventilator	2.38 (1.58–3.59)	**0.0001**	2.17 (1.428–3.313)	**0.0001**

Table 4. Cox univariate and multivariate analysis of factors associated with prolonged ICU stay more than 4 days in patients with severe COVID-19 pneumonia

Variables	Hazard ratio (95% CI)	P–value	Adjusted hazard ratio (95% CI)	P–value
Fever	0.670 (0.520–0.864)	**0.002**	0.77 (0.588–1.008)	0.062
Dyspnea	1.38 (1.07–1.80)	**0.014**	1.18 (0.884–1.598)	0.254
Ischemic heart disease	1.59 (0.998–2.55)	**0.05**	1.45 (0.869–2.44)	0.154
Chronic kidney disease	1.91 (0.981–3.73)	**0.05**	1.50 (0.751–3.019)	0.249
Chronic liver disease	9.49 (2.97–30.35)	**0.0001**	6.58 (1.94–22.27)	0.002
Comorbidities	1.44 (1.12–1.86)	**0.004**	1.20 (0.904–1.614)	**0.202**
First dose of Remdesivir after symptom onset >4 days	0.956 (0.923–0.989)	**0.015**	0.940 (0.904–0.977)	**0.002**
Use of ventilator	3.35 (2.15–5.23)	**0.0001**	3.23 (2.031–5.152)	**0.0001**

promote hyperactivation of NLRP3 and trigger cytokine storm in elderly patients [10]. Another possible reason for increased susceptibility to COVID-19 infection in the elderly could be due to a decrease in T-cells and subsets, which reduces with aging [11]. Mahase *et al.* found that overall death rate from COVID-19 was 0.66% and was sharply rising to 7.8% in elderly people aged over 80 years [12].

Ferritin is an intracellular protein that stores iron and releases in a controlled fashion. Apart from the role of iron store, it has a potential capacity during inflammation following SARS-CoV-2 infection. Ferritin is found to be secreted by alveolar macrophages in the lungs and also stimulated by various cytokines, including IL-6 [13]. Active ferritin in turn stimulates the immune system and activates macrophages leading to an increase in inflammatory process [14]. Various single-center retrospective studies done in China found higher ferritin levels in patients who succumbed compared to survivors and discovered a decrease in ferritin levels with remission of the disease [15–17]. We also found elevated ferritin levels in non-survivors (632.29 μg/L) compared to survivors (367.2 μg/L).

We noted SpO_2/FiO_2 (SF) ratio on admission < 400 to be an independent predictor of mortality in severe COVID-19 pneumonia patients. SpO_2/FiO_2 has been used as a surrogate prognostic marker of PaO_2/FiO_2 in acute respiratory distress syndrome (ARDS) patients with similar characteristics and the outcome in the previous study [18]. According to the Kigali modification, ARDS was defined without the need for positive end-expiratory pressure (PEEP), with the presence of bilateral opacities in the chest radiograph and hypoxia defined with a cut-off of SpO_2/FiO_2 less than or equal to 315 [19]. The study done by Riviello *et al.* using Kigali modification of the Berlin definition had good correlation with the diagnosis of ARDS [20]. SpO_2/FiO_2 is one of noninvasive parameters that might predict a poor outcome in patients with severe SARS-CoV-2 infection [21]. SpO_2/FiO_2 ratio could be used for correct estimation of ARDS in developing countries like India,

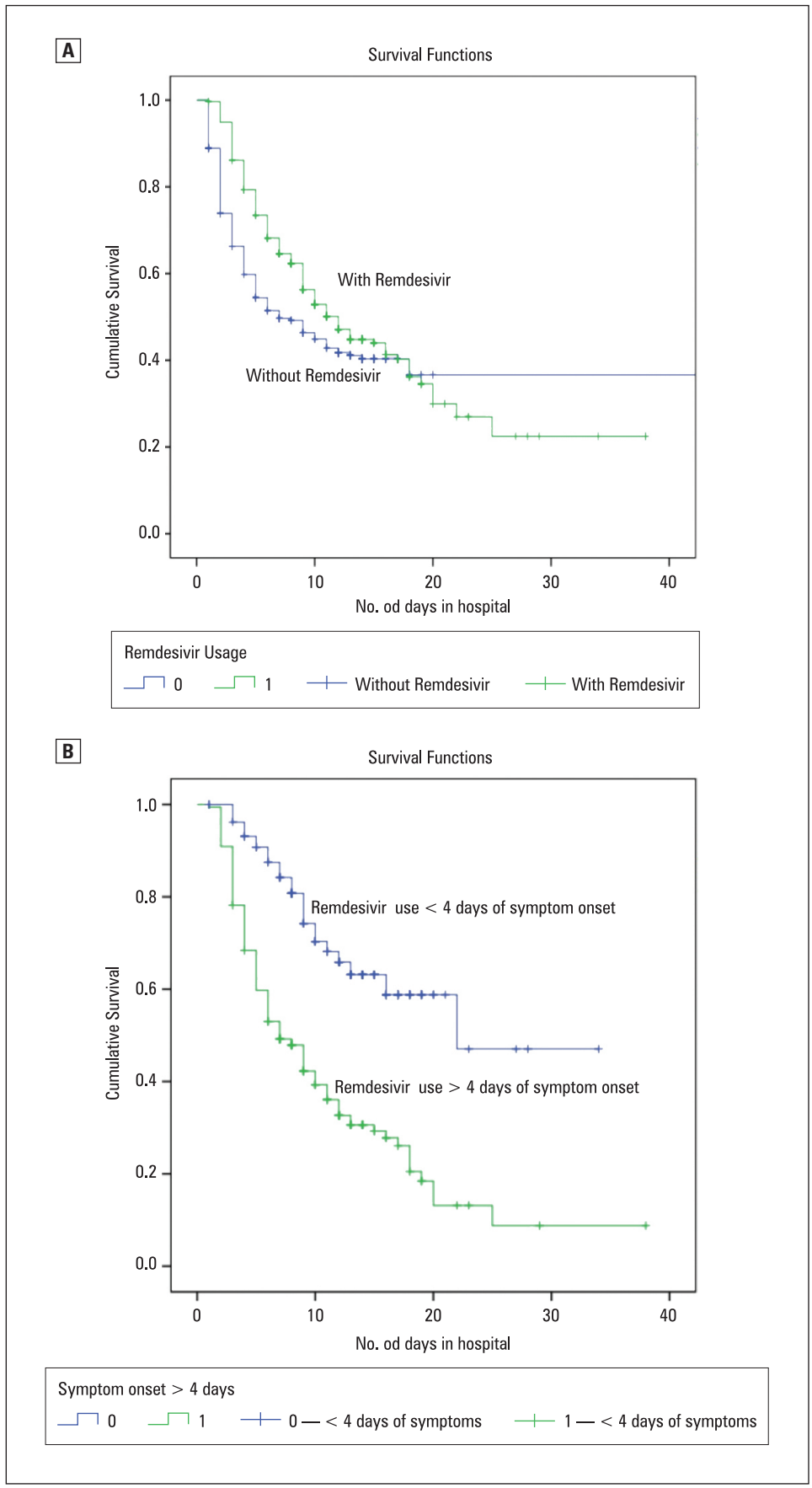

Figure 3. A. The Kaplan-Meier graph for survival with the use of remdesivir in patients with severe COVID-19 pneumonia; **B.** The Kaplan-Meier graph for survival with remdesivir use before and after 4 days of the symptom onset in patients with severe COVID-19 pneumonia

where there is scarcity of critical care specialist and intensive care in the periphery, especially in the COVID-19 pandemic situation.

Several antiviral drugs have been evaluated for the treatment of SARS-CoV-2 infection, but no antiviral agents have shown any mortality benefit. Remdesivir, a nucleoside analog with broad antiviral activity among RNA viruses, including Ebola, has been tried for treatment of SARS-CoV-2 infection. It acts by interfering with non-structural protein 12 polymerase (nsp12) which is a multisubunit of RNA synthesis complex that is responsible for viral RNA genome replication. Remdesivir has shown to decrease time to recovery in adults hospitalized with lower respiratory tract infection in an preliminary study of randomized control trial [22], and may prevent progression to more severe disease. The final report showed that remdesivir improved mortality rates for those receiving supplemental oxygen (4% with remdesivir versus 13% with placebo on day 29 of treatment) [23]. A Chinese study by Wang *et al.* showed numerical reduction in time to improvement with remdesivir compared to placebo, however, it was not statistically significant [24]. A randomized controlled trial (RCT) done by Spinner *et al.* also observed early clinical improvement in patients on remdesivir compared to standard care [25]. None of the RCTs has shown mortality benefit from usage of remdesivir.

The median duration of starting remdesivir in our study was 5.50 days compared to studies done by Spinner *et al.*, Beigel *et al.*, Wang *et al* which was 8 days, 9 days and 11 days, respectively [22, 24, 25]. In the above mentioned clinical trials, the benefit of remdesivir was larger when given earlier in the illness. Like other viral infections (eg.influenza), early use of antiviral drug is associated with improved clinical outcome [26]. Whether the use of remdesivir early in the course of disease when viral replication is the most active and complications have not yet occurred, would improve outcomes - remains to be confirmed by larger RCT studies.

The presence of comorbidities is an established risk factor for mortality in patients with COVID-19 pneumonia in various studies done across the globe [27–30]. We observed comorbidities in nearly 61% of patients. Hypertension (41.25%) and diabetes (41%) were the most commonly noted. Nearly 66.5% of patients with comorbidities succumbed to illness. A recent meta-analysis done in India found the prevalence of hypertension (22.9%) the highest among COVID-19 patients, and diabetes was more prevalent in the Indian population compared to other countries [30]. One of the largest Chinese studies (n = 72,314) found significantly increased mortality in COVID-19 patients with comorbidity [31]. The latest report from the Center for Disease Control, United States discovered cardiovascular diseases (including hypertension, stroke, coronary artery disease, cardiac failure) in 60.9% of patients with COVID-19 [32]. A UK study observed cardiac disease, chronic obstructive pulmonary disease, chronic kidney disease, obesity and liver disorders to be associated with a significant increase in mortality [33]. A recent report by the Ministry of Health and Family Welfare, India that analyzed the death of 15,962 patients with SARS-CoV-2 infection found the presence of one or more comorbidities in 57% of patients [34]. Unlike other studies, our Cox multinominal analysis did not provide evidence for an association between specific comorbidity and mortality.

Vital parameters play an important role in initial assessment and triaging of patients with pneumonia. Respiratory rate being one of the components of many severity scoring systems like CURB65 (Confusion, Urea, Respiratory rate, Blood pressure, Age > 65 years) score, APACHE II (Acute Physiology And Chronic Health Evaluation-II) score shows its importance. We found increased respiratory rate (> 23/min) to be an independent risk factor for mortality similar to a large American study [35].) A Chinese study on 344 critically ill patients also found higher respiratory rate was associated with poor outcome indicating more attention to be paid to vital signs [21].

Due to COVID-19 pandemic, there are accelerated publications without long-term follow-up of patients with mortality data [36].) There is sparse information on mortality rates post-discharge in severe COVID-19 pneumonia in the Indian population. We observed mortality rate of 56.60% (n = 560) in patients with severe COVID-19 pneumonia at 30 days which is lower than that from studies done in Pakistan (n-204, 77%) [37], the United States (n-373, 75.6%) [38] and China (n-344, 88.3%) [21]. However, studies carried out by Graselli *et al.* in Italy (n-1,581, 26%) [39]) and Gupta *et al.* in the United States (n-2,215, 35.4%) [40] had lower ICU mortality than our study. A possible explanation for the disparity in mortality rates is that around 58% and 28% of the study cohort was still in ICU without an outcome projecting falsely low mortality in the studies done by Graselli and Gupta, respectively. In a study conducted by Zhou *et al.* (n-50) who

followed up all patients till the outcome, the mortality rate was 78% [41].

Strengths and limitations

One of the important strengths of our study is the presence of 30 days mortality data post-discharge which is lacking in many studies of patients with severe COVID-19 pneumonia. The evaluation of the timing of remdesivir administration after the symptom onset on mortality rates is an important finding as many studies which did not show any benefit of remdesivir had administered the drug late in the course of the disease. Limitations of our study include the usual limitations of a retrospective study, furthermore, information was collected from a single center.

Conclusion

We found age > 50 years, the duration of symptoms more than 4 days, $SpO_2/FiO_2 < 400$ on admission, serum ferritin > 450 μg/L on admission, respiratory rate > 23/min on admission, the presence of comorbidities and non-usage of remdesivir and late initiation of remdesivir after the symptom onset to be independent predictors of mortality in patients with severe COVID-19 pneumonia.

Clinical implication/future directions

Mortality predictors found in the study could be identified early and treated to possibly reduce mortality in severe COVID-19 pneumonia patients. Mortality benefits of remdesivir with early initiation in the course of the disease need to be relooked with large randomized controlled trials.

Conflict of interest

None declared.

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Teodoro J Oscanoa[1, 2], Jose Amado[1, 2], Xavier Vidal[3], Eamon Laird[4], Rawia A Ghashut[5], Roman Romero-Ortuno[6, 7]

[1]Drug Safety Research Center, Facultad de Medicina Humana, Universidad de San Martín de Porres, Hospital Almenara, ESSALUD, Lima, Peru
[2]Facultad de Medicina, Universidad Nacional Mayor de San Marcos, Lima, Peru
[3]Clinical Pharmacology Department, Vall d'Hebron Hospital, Barcelona, Spain
[4]The Irish Longitudinal Study on Ageing, Trinity College Dublin, Ireland
[5]Academic Unit of Anaesthesia, College of Medical, Veterinary and Life of Sciences, University of Glasgow, Glasgow Royal Infirmary, Glasgow, United Kingdom
[6]Discipline of Medical Gerontology, Mercer's Institute for Successful Ageing, St James's Hospital, Dublin, Ireland
[7]Global Brain Health Institute, Trinity College Dublin, Ireland

The relationship between the severity and mortality of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration — a metaanalysis

Abstract

Introduction: There is increasing scientific interest in the possible association between hypovitaminosis D and the risk of SARS-CoV-2 infection severity and/or mortality.

Objective: To conduct a metanalysis of the association between 25-hydroxyvitamin D (25(OH)D) concentration and SARS-CoV-2 infection severity or mortality.

Material and methods: We searched PubMed, EMBASE, Google scholar and the Cochrane Database of Systematic Reviews for studies published between December 2019 and December 2020. Effect statistics were pooled using random effects models. The quality of included studies was assessed with the Newcastle–Ottawa Scale (NOS). Targeted outcomes: mortality and severity proportions in COVID-19 patients with 25(OH)D deficiency, defined as serum 25(OH)D < 50 nmol/L.

Results: In the 23 studies included ($n = 2692$), the mean age was 60.8 (SD $\pm$ 15.9) years and 53.8% were men. Results suggested that vitamin 25(OH)D deficiency was associated with increased risk of severe SARS-CoV-2 disease (RR 2.00; 95% CI 1.47–2.71, 17 studies) and mortality (RR 2.45; 95% CI 1.24–4.84, 13 studies). Only 7/23 studies reported C-reactive protein values, all of which were > 10 mg/L.

Conclusions 25(OH)D deficiency seems associated with increased SARS-CoV-2 infection severity and mortality. However, findings do not imply causality, and randomized controlled trials are required, and new studies should be designed to determine if decreased 25(OH)D is an epiphenomenon or consequence of the inflammatory process associated with severe forms of SARS-CoV-2. Meanwhile, the concentration of 25(OH)D could be considered as a negative acute phase reactant and a poor prognosis in COVID-19 infection.

Key words: SARS-CoV-2, COVID-19, vitamin D, 25-hydroxyvitamin D, severity

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Introduction

Since the COVID-19 pandemic began in December 2019, and whilst waiting for an effective and safe vaccine, there has been increased urgency to achieve drug therapy with new and old drugs. Among the latter candidates is 25-hydroxyvitamin D (25(OH)D), which has been proposed as a potentially modifiable risk factor for COVID-19 outcomes [1].

Address for correspondence: Teodoro J Oscanoa, Universidad de San Martín de Porres, Facultad de Medicina Humana, Centro de Investigación de Seguridad de Medicamentos, Lima, Peru; e-mail: tjoscanoae@gmail.com
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25(OH)D is a steroid hormone, which comes mainly from the synthesis at the level of the skin, of a precursor that is 7-dehydrocholesterol, which due to the action of ultraviolet light (UVL) B (280–315 nm) exposure is converted into vitamin D3 (cholecalciferol). In some countries at far latitudes, this origin is seasonal only [2]. 25(OH) D can be obtained from diet (e.g., oily fish, eggs, liver) but very few commonly eaten foods contain sufficient amounts, which is why some countries (but only few) have a mandatory vitamin D food fortification policy [3]. The recommended amount of vitamin D intake in the majority of countries is 10 μg or 400 IU of vitamin D daily during winter at least. Vitamin D levels can be affected by obesity, sunscreen, clothing, genetics, gender, smoking and socio-economic status [4]. Vitamin D2 (obtained from dietary intake of mushrooms or some vegetables) and D3 (obtained from sun exposure or diet) are hydroxylated in the liver and kidneys where the active form of this vitamin is generated as, 1,25 Dihydroxycholecalciferol (1,25(OH)2D). Macrophages/dendritic cells and other organ cells also have the ability to convert 25 (OH) D to 1,25(OH)2D via CYP27B1. The biomarker of a patient's VD status is the concentration of total 25(OH)D (25-hydroxyvitamin D) concentration in serum, because vitamin D deficiency correlates better with 25(OH)D than with 1,25(OH)2D [5, 6]. The effects of VD on calcium and phosphate absorption, osteoclast activation, and hence on bone calcification and muscle strength are widely known [7]. The VD receptor (VDR) is very widely expressed, including by all leucocyte classes, and it has been demonstrated that many genes are VD responsive [8], including nearly two hundred genes in monocyte/macrophage cells [9]. Other research has shown that cytokine concentrations and proliferation of immune cells can be modulated by VD [10].

Currently, research designs that have been used to postulate a relationship between hypovitaminosis D and the severity of SARS-CoV-2 infection are either ecological, demographic with risk groups for VD deficiency (Mendelian randomization), or studies on the association of 25(OH) D levels with the risk of having a positive test for the virus [1]. Ecological studies use databases with information on 25(OH)D concentration of populations and countries and relate it to mortality, recovery, severity or susceptibility to SARS-CoV-2 infection. A published metanalysis that included ecological studies in 51 countries found no correlation between 25(OH)D levels and recovery or mortality rates [11]; however, considering latitude, an inverse relationship was found between mortality and 25(OH)D status in Asia, Middle East and Oceania; and surprisingly, in the USA and South America, the correlation was direct [12–14]. Ilie *et al*. found that the Pearson correlation coefficients between mean 25(OH)D levels and COVID-19 cases, and mean 25(OH)D levels and COVID-19 deaths per million population were negative and statistically significant based on data from 20 European countries [15]; however, this study was re-analyzed by Kumar *et al*., adding to the model the life expectancy factor, and the result was the loss of the significance of 25(OH)D levels as a predictor of mortality from COVID-19 [16].

Mendelian randomization studies use genetic variants as markers to evaluate a causal relationship in observational data [17], and have been used in studies of the association between 25(OH)D and severity of COVID-19 infection, based on the fact that the polymorphism of the VD receptor has an impact on the response to 25(OH)D. Mendelian randomization studies use the genetic variant as a surrogate variable for 25(OH)D deficiency, to infer the causal effect of an exposure [25(OH)D concentration] to an outcome (COVID-19 susceptibility, severity or mortality) [18]. Currently, 3 studies have been published using Mendelian randomization on the association of 25(OH)D concentration with the risk or severity of COVID-19; one found a relationship [19]which impedes good immune function, is common during winter and spring in regions of high latitude. There is good evidence that vitamin D deficiency contributes to the seasonal increase of virus infections of the respiratory tract, from the common cold to influenza, and now possibly also COVID-19. This communication explores key factors that make it more likely, particularly in combination, that individuals are vitamin D deficient. These factors include old age, obesity, dark skin tone and common genetic variants that impede vitamin D status. Precision nutrition is an approach that aims to consider known personal risk factors and health circumstances to provide more effective nutrition guidance in health and disease. In regard to avoiding vitamin D deficiency, people with excess body fat, a dark skin tone or older age usually need to use a moderately dosed daily vitamin D supplement, particularly those living in a high-latitude region, getting little ultraviolet B exposure due to air pollution or staying mostly indoors. Carriers of the GC (group-specific component, but the other two did not [18, 20]. The limitations of this type of study is that it

uses a surrogate for 25(OH)D deficiency, and in severe cases of COVID-19, it is necessary to have the serum 25(OH)D concentration at the time of hospitalization for COVID-19; on the other hand, the polymorphism with which the individual was born does not predict numerous other factors that could have affected 25(OH)D status.

The objective of the present study was to perform a systematic review and meta-analytic study on severity or mortality of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration based in observational studies and randomized clinical trials.

Material and methods

This study was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [21].

Search strategy

Two independent investigators performed a systematic search in PubMed, EMBASE, Google Scholar, preprint servers (medRxiv, bioRxiv and Research Square) and the Cochrane Database of Systematic Reviews for studies published between December 2019 and December, 2020. In addition, we conducted a secondary search based on the references lists of the retrieved articles. The PubMed search strategy is detailed in the Supplementary file.

Eligibility criteria

We searched for randomized controlled trials (RCTs) or observational studies reporting data on serum 25(OH)D concentration and SARS-CoV-2 infection severity or mortality. We included studies in English or other (Russian and Spanish) language (all ages) meeting the following criteria: a) COVID-19 patients were diagnosed according to the interim guidance of the World Health Organization [22]; b) inclusion of the mean and standard deviation for laboratory test values of 25(OH)D, and sample size with demographics, comorbidities, and complications; c) the study presented data on hazard ratios (HRs), relative risks (RRs), or odds ratios (ORs) with confidence intervals (CIs) or offered enough data to allow these to be calculated (including via email correspondence with original authors if necessary); and d) SARS-CoV-2 infection severity criteria were described (generally defined as admission to intensive care unit, acute respiratory distress syndrome and/or need for mechanical ventilation). The following exclusion criteria were applied: reviews, abstracts, discussion summaries, and insufficient reported data including absence of vitamin D measurement.

Quality assessment

The quality of observational studies (cohort and case-control studies) and RCTs were assessed according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [23] and the Cochrane Risk of Bias Assessment Tool [24], respectively. Two investigators evaluated the quality of the studies independently. Conflicting results were resolved by discussion and involvement of a third reviewer if necessary.

Data extraction

The following data were extracted from each study: authors, study location, year of publication, study design, number of participants, sex, age at baseline, serum VD level, outcome definition, and effect estimates with 95% confidence intervals (CIs). Targeted outcomes: COVID-19 mortality and/or severity proportions. Even though some studies have considered other 25(OH)D cut-off values [25], in this study we defined Vitamin D deficiency as serum hydroxyvitamin D level <50 nmol/L (< 20 ng/mL) [26]. In a sub-analysis, more severe deficiency was defined as < 30 nmol/L (< 12 ng/mL) [27].

Statistical analyses

Primary analyses evaluated the association (HRs, RRs or ORs) between 25-hydroxyvitamin D concentration and SARS-CoV-2 infection severity or mortality. In the metaanalysis, in order to calculate the effect size of 25(OH)D concentration and gender, the relative risk or odds ratio published by the authors of the included studies were used. We applied random effects with an inverse variance method to calculate the pooled RRs and 95% CIs according to the heterogeneity between the studies [28]. The overall estimates in the pooled analysis were obtained using Stata 13 software (Stata Corp LP, College Station, TX).

Results

After screening 745 citations, 23 studies (5 cohort, 11 cases and controls, 7 cross sectional observational studies) were included (Figure 1), combining to a total sample of 2692 participants. The characteristics of the included studies are summarized in Table 1. The studies were from Belgium [29], China [30, 31], Germany [32], India

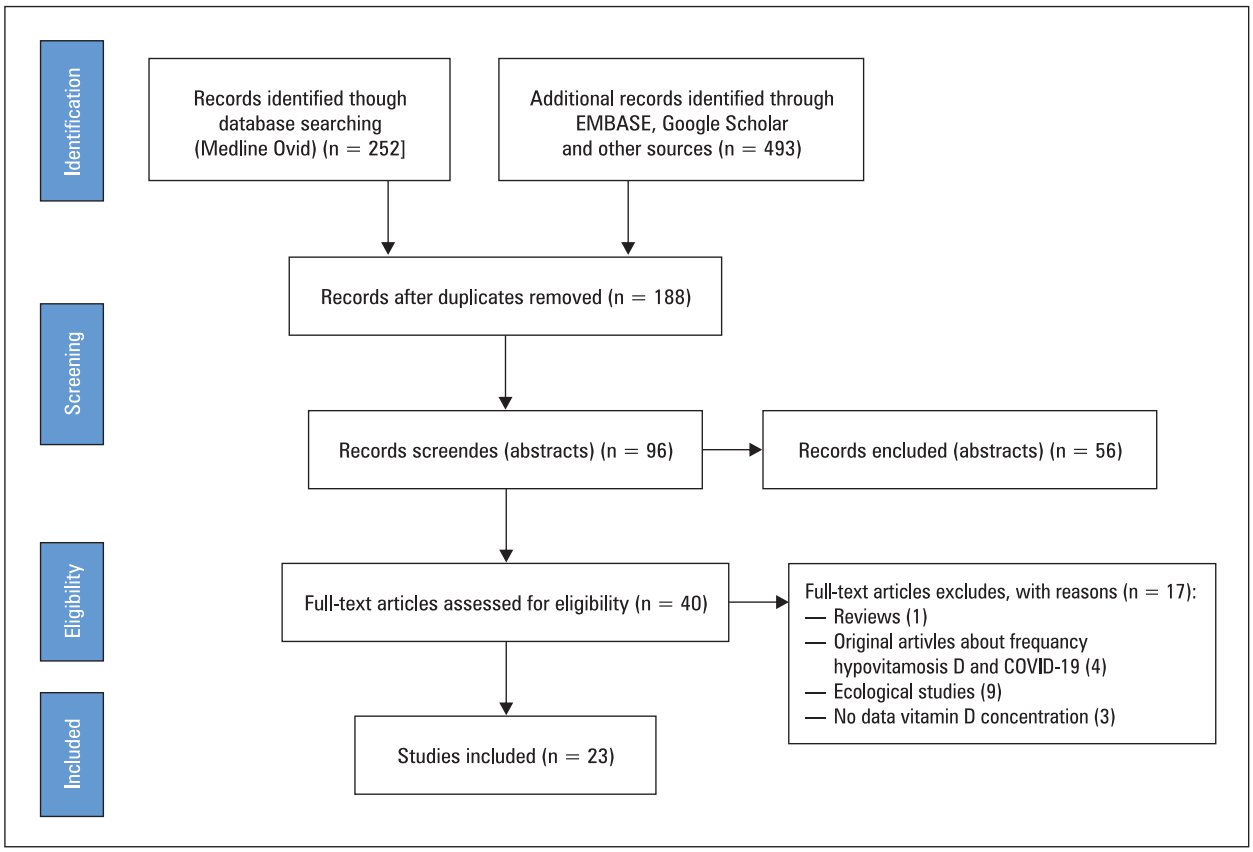

Figure 1. Flowchart of the included studies

[33], Iran [34, 35], Italy [36–38], Philippines [39], Spain [40, 41], Switzerland [42], South Korea [43], Turkey [44, 45], Russia [46], The Netherlands [47], UK [48, 49] and USA [50,51]. Overall, mean age was 60.8 (SD 15.9) years and 53.8% were men. The mean NOS score of the included studies was 8.1 (range: 7–9). The outcomes reported in the included papers are presented in Table 1.

As shown in Figure 2, the metaanalysis suggested that 25(OH)D < 50 nmol/L (< 20 ng/mL) was associated with an increased risk of severe disease (RR 2.00; 95% CI 1.47–2.71, 17 studies) and mortality (RR 2.45; 95% CI 1.24–4.84, 13 studies). Only 7/23 papers reported C-reactive protein values, all of which were> 10 mg/L [31, 38, 41, 44, 52–54].

Subgroup analyses were conducted to assess the effects of age, sex, and the alternative 25(OH) D cut-off value (< 30 nmol/L) separately (Table 2). We found that the severity risk seemed higher in people < 60 years of age (p = 0.040, 4 studies). The severity risk seemed to increase as the cut-off point for 25-hydroxyvitamin D concentration decreased (p = 0.025, 4 studies). Male sex (p < 0.001, 7 studies) also had higher risk of severity and/or mortality.

Two studies analyzed receiver operating characteristic (ROC) curve analyses to find the 25(OH)D cut-off point with the highest sensitivity and specificity for the prediction of severity and/or mortality. Abrishami *et al.* (2020) found the cut-off point of < 62.5 nmol/L (< 25 ng/mL) to have a sensitivity of 75% and a specificity 72%, for differentiating deceased and discharged patients [35]. Ye *et al.* (2020) observed that a cut-off point of 41.19 nmol/L had a sensitivity of 87% and a specificity of 70% for predicting illness severity [30].

Discussion

The main finding of the present paper is that according to the included observational studies, 25(OH)D deficiency (serum 25-hydroxyvitamin D concentration < 50 nmol/L) was associated with an increased risk of severe disease and mortality from SARS-CoV-2 infection. Our findings do not imply causation because they only summarize the conclusions of observational studies. For example, it is not possible to extrapolate that in acute patients with COVID-19 who have hypovitaminosis D, the immediate replacement of vitamin

Table 1. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point25--hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Carpagnano *et al.* 2020	Italy	CC	42	71	65	Mortality	Chemiluminescence immunoassay method	25	Hypertension (62%), cardiovascular disease (38%), chronic kidney disease (38%), diabetes type II (26%), malignancy (12%), COPD (12%).	Mean (SD): VD (50–25nmol/L) = 101 (79.93); VD (< 25 nmol/L) = 102 (79.98)	9
Panagiotou *et al.* 2020	UK	CS	134	29.7	68.5	Severity (admission to ICU)	NR	50	Hypertension (42%), diabetes (28%), malignancy (11%), respiratory (31%), cardiovascular disease (15%), kidney and liver diseases (14%)	NR	7
Alipio *et al.* 2020	Philippines	CS	212	NR	NR	Severity (mild/ordinary vs Severe/critical; CT scan chest y pO2)	NR	50	NR	NR	8
De Smet *et al.* 2020	Belgium	CC	186	58.6	69	Severity (Chest CT)	Elecsys® vitamin D total II (Roche, Switzerland)	50	Chronic lung disease (15%), coronary artery disease (59%), diabetes (14%)	NR	8
Lau *et al.* 2020	USA	CS	20	45	65.2	Severity (ICU vs floor)	UniCel DxI 600 Access Immunoassay System (Beckman Coulter)	50	Hypertension (75%), diabetes (35%)	NR	8
Radujkovic *et al.* 2020	Germany	CC	185	51	60	Severity (Invasive mechanical ventilation)	immunoassay (ADVIA Centaur Vitamin D Total Assay)	30	Cardiovascular disease (31%), diabetes (10%), chronic kidney disease (4%), chronic lung disease (8%), malignancy (9%).	NR	9
Baktash *et al.* 2020	UK	C	70	60	81.3	Mortality and Severity (Ventilation requirement)	NR	30	Hypertension (57%), diabetes mellitus (43%), ischaemic heart disease (25%), chronic respiratory disease (22%), heart failure (20%).	Median: VD (≤ 30 nmol/L) = 191 (108–274); VD (> 30 nmoll/L) = 155 (96–252) p = 0.32	9
Mardani *et al.* 2020	Iran	O	63	55.6	43.3	Mortality	ELISA method (Monobind, USA),	50 and 30	NR	NR	8
Pepkowitz *et al.* 2020	USA	CC	37	65.57	71.5	Severity (admission ICU)	NR	50	NR	NR	8

Table 1. cont. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point25--hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Macaya *et al.* 2020	Spain	CC	80	43.75	69	Severity (death, ICU admission or requirement of high flow oxygen)	chemiluminescent immunoassay, Abbott Diagnostics	50	Hypertension (63%), diabetes mellitus (40%), cardiac disease (24%), advanced chronic kidney disease (33%), chronic respiratory disease (15%)	NR	8
Hars *et al.* 2020	Switzerland	O	160	40.63	85.9	Mortality	NR	50	NR	NR	7
Karahan *et al.* 2020	Turkey	C	149	54.4	63.5	Mortality and severity (Chinese Clinical Guideline for classification)	NR	50	Hypertension (57%), coronary artery disease (22%), diabetes mellitus (41%), chronic kidney disease (20%), congestive heart failure (12%)	Mean (SD): surviving = 62.4 ± 71.2; deceased = 108.7 ± 78.3 ($p < 0.001$)	8
Ye *et al.* 2020	China	CC	60	37	43	Severity (National Health Commission of China classification)	NR	50	Diabetes (8%), hypertension (10%), renal failure (27%), COPD (2%).	NR	9
Yilmaz *et al.* 2020	Turkey	CC	40	47.5	8.48	Severity (experts' consensus statement)	Shimatzu device by high performance liquid chromatography method	50	NR	Median VD (< 50 nmol/L) = 1 (0.2–160); VD (≥ 50 noml/L) = 0.7 (0.2–10.8)	8
Im *et al.* 2020	South Korea	CC	50	42	52.2	Severity (Pneumonias with or without an oxygen supply, and death)	liquid chromatography–tandem mass spectrometry method	50	NR	NR	8
Hernández *et al.* 2020	Spain	CC	197	62.4	61	Mortality and Severity (mechanical ventilation)	automated electrochemiluminescence system	50	Hypertension (39%), diabetes (17%), cardiovascular disease (11%), COPD (8%), active cancer (4%), immunosuppression (8%)	Median overall = 56 (26.3–118.5) VD (< 50 nmol/L) = 61 (31–136); VD (≥ 50 nmol/L) = 32 (23–87); $p = 0.064$	9
Campi *et al.* 2020	Italy	C	103	68	66.12	Mortality and Severity (Admission to ICU)	LIASON 25-OH Vitamin D Total Assay	50	At least 1 comorbidity (61%), CV diseases (12%)	NR	9
Abrishami *et al.* 2020	Iran	CC	73	64.4	55.18	Mortality	Roche Diagnostics "Vitamin D Total" cobas e411immunoassay analyzer	50	Hypertension (25%), chronic kidney disease (22%), ischemic heart disease (18%), diabetes mellitus (15%), asthma/COPD (10%)	NR	8

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Table 1. cont. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point25--hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Walk *et al.* 2020	The Nether-lands	C	133	69	68.2	Severity (the need for invasive ventilation and/or death)	liquid chromatography -tandem mass spectro-metry	50 and 25	Hypertension (36%), diabetes mellitus (22%), cardiac or car-diovascular disease (28%)	NR	8
Karonova *et al.* 2020	Russia	CS	80	53.8	53.2	Mortality	NR	50	Diabetes (15%), hypertension (46%),	NR	7
Luo *et al.* 2020	China	CS	335	44.2	56	Severity (respiratory failure requiring me-chanical ventilation, shock, multiple organ dysfunction)	chemiluminescence immunoassay (DiaSorin, Inc.).	30	NR	Median: severe group = 64.5 (12.3–422); non–severe group = 12 (5.5–30)	8
Cereda *et al.* 2020	Italy	C	129	54.3	77	Mortality and Severity (Severe pneumonia, ICU admission)	chemiluminescence immunoassay	50	COPD (13%), diabetes (31%), hypertension (70%), ischemic heart disease, (41%), cancer (21%), chronic kidney disease (19%)	Median: overall = 10.38 (5.19–16.46); VD (≥ 50 nmol/L) = 6.81 (4.0–14.39); VD (< 50 nmol/L) = 11.15 (5.56–17.14)	9
Jain *et al.* 2020	India	CC	154	66.66	54.42	Mortality and Severity (requiring ICU admis-sion)	automated immunoas-says -Architecti1000sr Make 2015	50	NR	NR	9

25(OH)D — 25-Hydroxyvitamin D; C — cohort; CC — case control; CI — confidence interval; COPD — *chronic obstructive pulmonary disease;* CS — cross-sectional; CV — cardiovascular disease; ICU — *intensive care unit;* NOS — Newcastle -Ottawa Scale;NR — not reported; SD — standard deviation; *VD — vitamin D*

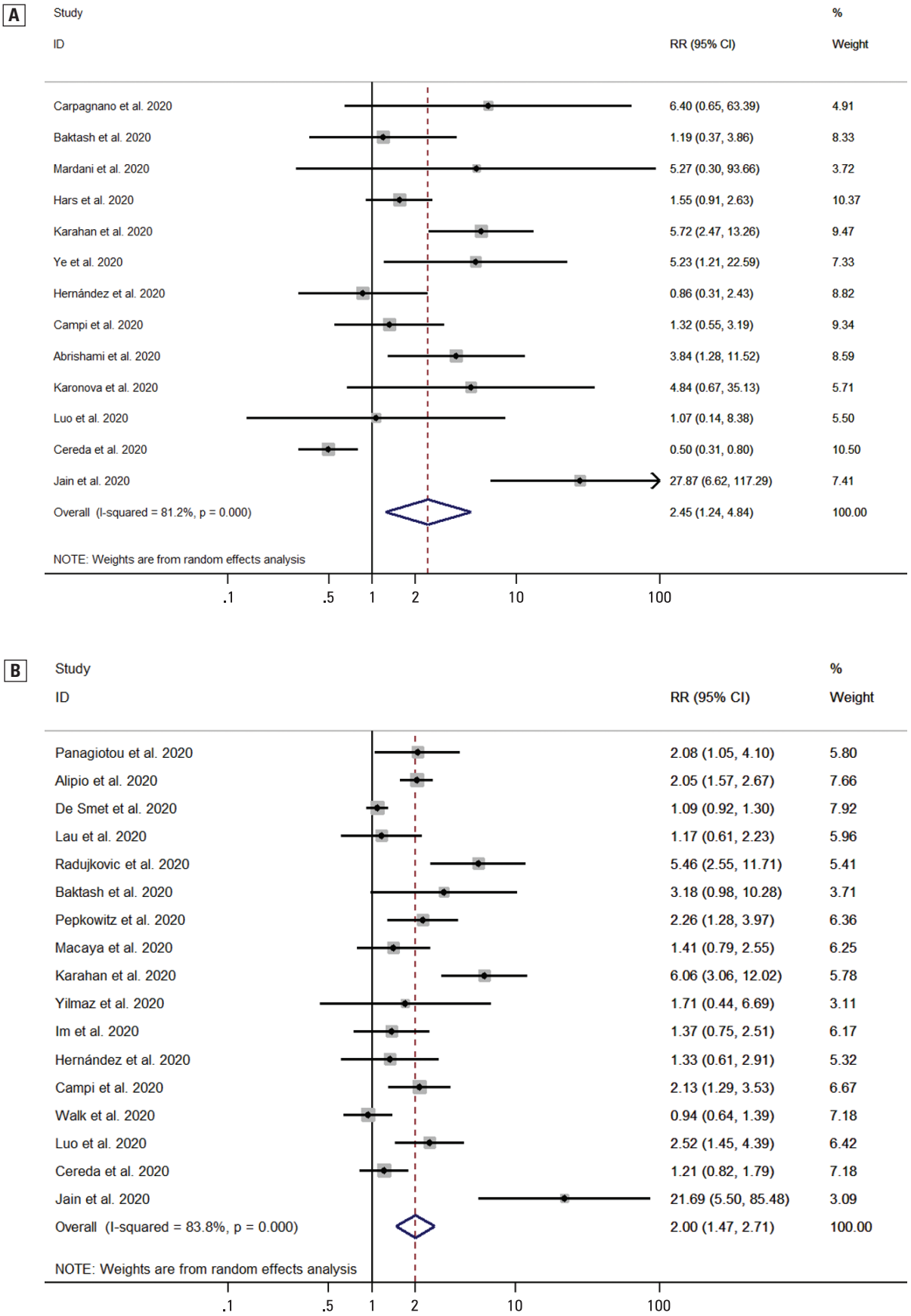

Figure 2. A. Forest plot of the metaanalysis of the association between mortality from SARS-CoV-2 infection and 25-hydroxyvitamin D concentration (< 50 nmol/L); **B.** Forest plot of the metaanalysis of the association between severity of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration (< 50 nmol/L). Analysis model: random effects. CI — confidence interval; RR — relative risk

Table 2. Association between severity of SARS-CoV-2 infection and serum 25-hydroxyvitamin D concentration: summary of subgroup analyses

Subgroup	Studies (n)	RR (95% CI)	P
Severity			
Cut point 25-hydroxyvitamin D concentration [nmol/L]			
< 30	4	2.45 (1.12–5.37)	0.025
< 50	14	1.79 (1.30–2.46)	< 0.001
Mortality			
Cut point 25-hydroxyvitamin D concentration [nmol/L]			
< 30	4	1.85 (0.79–4.37)	0.160
< 50	10	2.67 (1.20–5.94)	0.016
Severity			
Mean age [years]*			
< 60	4	3.05 (1.05–8.86)	0.040
≥ 60	12	1.83 (1.29–2.58)	0.001
Mortality			
Mean age [years]*			
< 60	6	5.47 (2.31–12.95)	< 0.001
≥ 60	7	1.48 (0.71–3.09)	0.298
Gender (severity/mortality)			
Male	7	2.62 (1.77–3.87)	< 0.001
Female	3	1.28 (0.49–3.32)	0.613

*One study (Alipio *et al.* 2020) did not report mean age data

D could improve the prognosis [55]. Another important finding is that there is the possibility that the low concentrations of 25(OH)D reported are an epiphenomenon of the inflammatory process associated with severe SARS-CoV-2 infection, although only 7/23 studies reported C-reactive protein values, all of which were > 10 mg/L.

We found that the severity risk seemed higher in people younger than 60 years of age; however, this finding related to only 4 studies and had a marginal P value. Our conclusion that the severity risk seemed to increase as the cut-off point for 25(OH)D concentration decreased adds biological plausibility to the findings. In addition, higher male risk profile, as clinically expected, is given the known epidemiology of COVID-19 infection [56]. It has to be noted that a tradeoff between our two outcomes exists, as very severe cases who died were counted as mortality and not severity for the purpose of association with 25(OH)D status.

The findings of the present study can be compared with similar meta-analytical studies and others published prior to the pandemic, on risk associated with severity of acute respiratory infections. Ghasemian *et al.* (2020) and Chen *et al.* (2020) published two meta-analytic studies where they found an association between 25(OH) D deficiency and insufficiency with mortality from SARS-CoV-2 infection; however, ecological studies have the limitation that the reported mortality could have varied in each of the countries, as the pandemic evolved [12, 57]. Another meta-analytic study (5 articles included) found a mean 25(OH)D concentration of 18 ng/mL in severe COVID-19 cases (95% CI: 1–35) and 26 ng/mL in non-severe cases (95% CI: 23.9–28.7) [11]. Pereira *et al.* (2020) noted that a 25(OH)D concentration of < 50 nmol/L was associated with an increased risk of hospitalization (3 studies) and mortality from COVID-19 (5 studies) [58]. In a systematic study (7 papers), Munshi *et al.* (2020) found that patients with poor prognosis had significantly lower serum concentrations of 25(OH)D compared to those with good prognosis, representing an adjusted standardized mean difference of -5.12 (95% Cl = -9.14 to -1.10, p = 0.012) [59]. Pham *et al.* (2020) published in 2019 a meta-analytic study where they found that a 25(OH)D concentration of <50 nmol/L was inversely associated with risk and severity of acute respiratory tract infection [60]. Zhou *et al.* published a meta-analytical study in 2019 where they documented an increased risk of community-acquired pneumonia in patients with 25(OH) D deficiency (< 50 nmol/L) [61]. Martineau *et al.* published in 2017 a meta-analytic study with 25 randomized clinical trials, finding that vitamin D supplementation reduced the frequency of acute respiratory infection; however, the benefit was greater in those who were receiving daily or weekly 25(OH)D, and protective effects were

stronger in those with baseline 25-hydroxyvitamin D concentrations < 25 nmol/L; and the benefit was not significant in those who received bolus doses [62].

The possible mechanisms that could explain the inverse relationship between the concentration of 25(OH)D and the frequency of presentation of severe forms of SARS, would be its angiotensin-converting enzyme 2 (ACE2) down-regulation, regulation of IL-6 and prevention of hypocalcemia. Mok *et al.* in *in vitro* studies with Vero E6 cells (African green monkey kidney cells) and hNECs (human nasal epithelial cells) found that calcitriol, the active form of vitamin D, has potent activity against SARS-CoV-2; the hypothesis of the possible mechanism of antiviral action would be in the post-entry phase of viral replication [63]. The mechanism of entry into human cells of SARS-CoV-2 is through ACE2, which is part of the renin-angiotensin system (RAS). It has been postulated that 25(OH)D would have a possible mechanism of protection against acute lung injury (ALI) / acute respiratory distress syndrome (ARDS), through a negative endocrine RAS modulator which inhibits renin expression and generation. This mechanism would be carried out by its inducing action of ACE2 / Ang- (1-7) / MasR axis activity and inhibits renin and the ACE / Ang II / AT1R axis, thereby increasing expression and concentration of ACE2, MasR and Ang-(1–7) [64]. Recently, high levels of ACE have been found in patients with severe COVID-19 with low 25(OH)D concentrations [34]; these findings are compatible with the harmful effect of high levels of ACE in Ang II generation and promote the detrimental effects of the AT1R classical axis (inducing vasoconstriction, inflammation, oxidative stress, and cell proliferation).

Recently, a systematic review found a correlation between premorbid levels of IL-6 and mortality from COVID-19; additionally, the reviewed studies reported concomitant decrease in 25(OH)D concentrations [65]. On the other hand, one of the known actions of concentrations is to modulate the activity of IL-6; therefore, potentially, the control of hypovitaminosis D could reduce the risk of presentation of severe forms of COVID-19 [65]. McGregor *et al.* (2020) found that CD4+ T cells in the bronchoalveolar lavage fluid (BALF) of patients with COVID-19 are Th1-skewed and that VDR is among the top regulators of genes induced by SARS-CoV-2 [66]. Part of the pathophysiology associated with cytokine storm is due to suppression of Th1 cooperative responses, which favors the Th2 type with excessive release of tumor necrosis alpha (TNF-alpha) and interleukins. 25(OH)D causes epigenetic re-modelling, induces and recruits a set of TFs (transcription factor), including STAT3 (signal transducer and activator of transcription 3), c-JUN and BACH2 (BTB Domain And CNC Homolog 2) that collectively repress Th1 and Th17 programs and induces IL-10 via IL-6-STAT3 signaling [66]. Recently, a significant increase in inflammation markers (IL-6, TNFα and serum ferritin levels) has been reported in critically ill COVID-19 patients deficient in 25(OH)D (< 50 nmol/L) [33]. Medical College the current study was undertaken as continuous prospective observational study of 6 weeks. Participants were COVID-19 patients of age group 30–60 years admitted during the study period of 6 weeks. Study included either asymptomatic COVID-19 patients (Group A. Sun *et al.* (2020) found that 74% of patients admitted for severe COVID-19 had hypocalcemia and low concentrations of 25(OH)D and hypoproteinemia, and for these reasons they propose hypocalcemia as a biomarker of clinical severity and prognosis [67]. Actually, the studies on the relationship between the concentration of 25(OH)D and SARS-CoV-2 infection are showing that there is a disturbed parathyroid-vitamin-D axis, which would last up to 8 weeks after the discharge of a patient with SARS-CoV-2 infection with hypovitaminosis D [68]we aimed to investigate associations of VITD status to disease presentation within the CovILD registry. This prospective, multicenter, observational study on long-term sequelae includes patients with COVID-19 after hospitalization or outpatients with persistent symptoms. Eight weeks after PCR confirmed diagnosis, a detailed questionnaire, a clinical examination, and laboratory testing, including VITD status, were evaluated. Furthermore, available laboratory specimens close to hospital admission were used to retrospectively analyze 25-hydroxyvitamin D levels at disease onset. A total of 109 patients were included in the analysis (60% males, 40% females.

It is important to discuss whether low concentrations of 25(OH)D in patients with severe COVID-19 infection is a cause or consequence of severe COVID-19 infection, for three main reasons: absence of baseline 25(OH)D measurement before infection, prior knowledge that the concentration of 25(OH)D decreases as a consequence of an inflammatory process, and most of the studies described on this association did not report the concentration of C-reactive protein (CRP) together with that of 25(OH)D. Before the

COVID-19 pandemic, it was known that 25(OH)D concentration decreases as a consequence of an inflammatory state, that is, it is considered a negative acute phase reactant [69–71] . Additionally, it has been described that this decrease in 25(OH)D during these inflammatory processes can persist for up to 3 months [70]. It has been recommended that a reliable clinical interpretation of the 25(OH)D concentration can be made only if the C-reactive protein (CRP) is < 10 mg/L [71], because it has been described that 25(OH)D concentrations are inversely correlated with CRP concentrations [72]. The mechanism involved in the decrease in serum 25(OH)D during an acute inflammatory state would be associated with the decrease in vitamin D binding protein (VDBP) and increased urinary loss of VDBP that occurs in a systemic inflammatory response (SIR) [69, 70]. In the present paper, only 7/23 studies reported the concentration of C-reactive protein (CRP), and in all of which it was > 10 mg / L. [31, 38, 41, 44, 52–54], therefore, there would be the possibility that one of the causes of the reported decrease in 25(OH)D is an epiphenomenon of the inflammatory process of SARS-CoV-2. Regardless of whether it is its cause or effect, measurement of 25(OH)D concentration should be considered a marker of inflammation, in addition to markers for inflammation and tissue damage in prognostic models for COVID-19 [29].

The present metaanalysis has its limitations, the main one being that it is based on observational studies and not on interventional studies such as randomized controlled clinical trials. Additionally, the studies used different methodologies to assess 25(OH)D status (e.g. LC-MS/MS, ELISA). It is important to emphasize that no causality can be inferred from our results. However, evidence from observational studies is better than that based on the ecological ones. A limitation is that many studies did not report if the 25(OH)D concentration was measured before or during COVID-19 infection, but in the future there may be scope for analyzing vitamin D in hair to solve this issue [73]the number of requests for vitamin D measurement keeps dramatically increasing year-on-year. Currently, the recognised best marker of vitamin D status is the concentration of the 25-hydroxyvitamin D (25(OH. Indeed, it is important to investigate whether SARS-CoV-2 infection, especially the severe forms, cause a decrease in the concentration of 25(OH)D, in patients who previously had them at normal levels. On the other hand, there is a possible 'healthy user effect' confounder, that is, higher concentrations of 25(OH)D could be seen in people who eat well, have healthy lifestyles and spend more time outdoors exposed to sunlight, which in turn makes them more generally resilient in the face of any acute illness [74]. In other words, 25(OH)D status could be epiphenomenally associated with SARS-CoV-2 outcomes.

In conclusion, at present the evidence available supports the hypothesis of increased SARS-CoV-2 risk of infection severity and mortality in patients with 25(OH)D deficiency (< 50 nmol/L). Our findings do not imply causality but support further research in this area, including the conduct of robustly designed randomized controlled trials. On the other hand, new studies should be designed to determine if decreased 25(OH)D is an epiphenomenon or consequence of the inflammatory process associated with severe forms of SARS-CoV-2. Meanwhile, the concentration of 25(OH)D could be considered as a negative acute phase reactant and a poor prognosis in COVID-19 infection.

Conflict of interest

None declared.

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VIA MEDICA

REVIEW

Montaha Al-Iede[1, 2], Eman Badran[1, 2], Manar Al-Iawama[1, 2], Amirah Daher[1, 2], Enas Al-Zayadneh[1, 2], Shereen M Aleidi[3], Taima Khawaldeh[2], Basim Alqutawneh[4]

[1]Department of Pediatrics, Jordan University Hospital, Amman, Jordan
[2]School of Medicine, The University of Jordan, Amman, Jordan
[3]Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, Amman, Jordan
[4]Department of Radiology, Blacktown, Mount-Druitt Hospital, NSW, Australia

Coronavirus disease 2019 (COVID-19): a brief overview of features and current treatment

Abstract

Since the report of the first cases of pneumonia caused by SARS-CoV-2 in December 2019, COVID-19 has become a pandemic and is globally overwhelming healthcare systems. The symptoms of COVID-19 vary from asymptomatic infection to severe complicated pneumonia with acute respiratory distress syndrome (ARDS) and multiple organ failure leading to death. The estimated case-fatality rate among infected patients in Wuhan, the city where the first case appeared, was 1.4%, with 5.1 times increase in the death rate among those aged above 59 years than those aged 30–59 years. In the absence of a proven effective and licensed treatment, many agents that showed activity against previous coronavirus outbreaks such as SARS and MERS have been used to treat SARS-CoV-2 infection. The SARS-CoV-2 is reported to be 80% homologous with SARS-CoV, and some enzymes are almost 90% homologous. Antiviral drugs are urgently required to reduce case fatality-rate and hospitalizations to relieve the burden on healthcare systems worldwide. Randomized controlled trials are ongoing to assess the efficacy and safety of several treatment regimens.

Key words: SARS-CoV-2, COVID-19, ARDS, pandemic

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Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus (2019-nCoV) that first manifested as atypical pneumonia and was distinct from usual pneumonia in terms of symptoms and lethality. On February 11, 2020, 2019-nCoV was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. SARS-CoV-2 is a part of a large family of RNA viruses called *Coronaviridae*, which has four types: Alpha, Beta, Delta, and Gamma. Coronaviruses are known to infect humans and animals, including mammals and birds. Seven coronaviruses, commonly Betacoronavirus HCoV-OC43 and HCoV-HKU1, and HCoV-229E and HCoV-NL63 from Alphacoronavirus genus have been known to cause human infections [1]. Infections caused by these viruses are often mild or asymptomatic. However, severe lower respiratory tract infections have been reported, especially among patients with chronic diseases, immune system dysfunction, and at extreme ages [1, 2].

Zoonotic coronaviruses have caused outbreaks in humans, namely SARS-CoV (2003, in China) and MERS-CoV (2012, in Saudi Arabia). In late 2019, COVID-19 was first reported in Wuhan City, Hubei Province, China, when a group of hospitalized patients with pneumonia of unknown etiology started to be seen [3]. SARS-CoV-2 shares viral structure and genetic sequence with both SARS-CoV and MERS-CoV of 70% and 40%, respectively [4]. This new disease was declared as a global pandemic by the World Health Organization (WHO) on March 11, 2020.

Address for correspondence: Montaha Al-Iede, Department of Pediatrics, Jordan University Hospital, Amman, Jordan; e-mail: montaha95@yahoo.com

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Compared to adults, the number of reported pediatric cases infected with the novel coronavirus (COVID-19) was significantly smaller [5]. Although the figure has been increasing every day, the data on children's disease clinical characteristics are still lacking. According to the clinical severity classification proposed by the COVID-19 guidelines in China, pediatric patients were more likely to have a milder clinical presentation, milder imaging findings, and less severe disease progression [6]. In adults, approximately 5% of patients will require intensive care. Information on pediatric patients needing intensive care is very limited. However, infants under 1 year appear to have an increased risk of severe disease. The infant group had the highest proportion of clinically diagnosed disease, and there remains the possibility that other viruses such as influenza A/B and respiratory syncytial virus may have caused the increased severity of the disease [7].

The pediatric multisystem inflammatory syndrome has been associated with COVID-19; this rare syndrome shares common features with other pediatric inflammatory conditions, including: Kawasaki disease, staphylococcal and streptococcal toxic shock syndromes, bacterial sepsis, and macrophage activation syndromes. It was initially described in Britain but has been reported from the US with increasing frequency. It can also present with unusual abdominal symptoms with excessive inflammatory markers. The case definition has been proposed as the following: I. A child presenting with persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia) and evidence of single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) with additional features. This may include children fulfilling full or partial criteria for Kawasaki disease. II. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus. III. SARS-CoV-2 PCR testing may be positive or negative [8].

Structure and pathogenesis

SARS-CoV-2 virus has spike glycoproteins (S proteins) called peplomers. S protein is the receptor binding site and plays an important role in binding to receptors on the surface of host cells and mediating virus envelope-cell membrane fusion [9]. The S protein has two subunits, S1 and S2, and both are necessary to help the virus invading host cells. SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptors on the targeted cells through its structural spike (S) glycoproteins S1 subunit. The virus uses a transmembrane serine protease 2 (TMPRSS2) for S protein priming as TMPRSS2 activates the spike and helps cleavage of ACE2.

TMPRSS2 acts on S2 subunit of S protein to facilitate the virus fusion to the cell membrane [10]. Once inside the host cells, the virus starts synthesizing RNA using its RNA-dependent RNA polymerase and viral structural proteins to complete virus formation and then virus release (Figure 1) [11].

The pathogenesis of the COVID-19 severity has not been understood yet. However, high levels of serum inflammatory cytokines such as interferon-gamma (IFNγ), interferon gamma inducible protein (IP10), IL-12, IL-6 and IL-1 were noted activating the Th1 cell response [12]. Patients who were critically ill, requiring an intensive care unit (ICU) had higher levels of tumor necrosis factor-alpha (TNFα), monocyte chemo-attractant protein (MCP1), macrophage inflammatory protein (MIP1A) and IP10 than patients who did not require ICU, which supports the relationship between cytokines storm and disease severity [12]. Xu *et al.* [13] have reported the result of first pathologic autopsy of a COVID-19 patient that showed a diffuse alveolar injury and hyaline membrane formation supporting ARDS diagnosis. In addition, the pathological changes seen were similar to MERS and SARS [13]. Flow cytometry revealed a significant reduction in CD4+ and CD8+ T lymphocytes counts in peripheral blood, which affects the immune defense mechanism. However, these T lymphocytes are in an overactive condition as manifested by high Th17 counts and an increased cytotoxicity of CD8+ T lymphocytes leading to a significant immune tissue injury in the patient's lungs and perhaps to the multisystem dysfunction [14]. This has provided a clue for COVID-19 treatmentby using agents directed against Th17 activity (Th17 inhibitors), however, this needs more research to further investigate Th1 and Th2 response in patients with COVID-19 to better understand the disease pathogenesis.

Epidemiology and clinical presentation

Since the initial SARS-CoV-2 virus detection, more than 68 million cases of COVID-19 have been confirmed worldwide, with the majority of cases reported in the United States [15]. Accord-

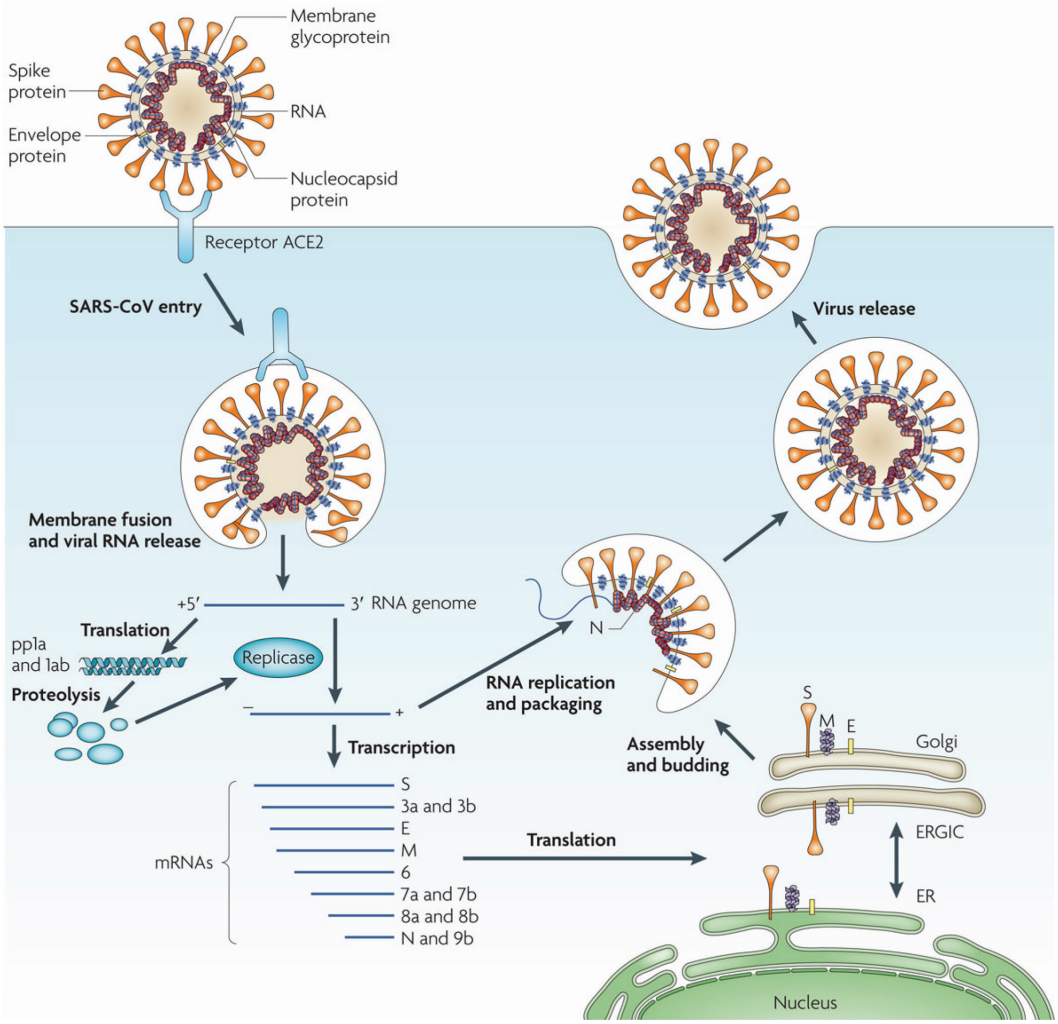

Figure 1. SARS-CoV's life cycle within the host cells. SARS-CoV-2 binds to host ACE2 via spike protein(s). Then cleavage of S protein is facilitated by host transmembrane protease TMPRSS to activate membrane fusion. A released viral genome is translated into nonstructural polyproteins which are processed by viral proteases to create the replicase. This replicase is used to produce several copies of strands and subgenomic mRNAs that are translated by ribosomes into structural proteins. The negative-strand RNA is packaged by structural protiens, followed by budding into endoplasmic reticulum-Golgi intermediate compartmetn's lumen. Finally, the viron is released as an exocytic vesicle 10 [11, with permission]

ing to a report that was published by the Chinese center for disease control and prevention on February 11, 2020, among a total of 72,314 recorded cases, 62% were diagnosed based on a positive nasal swab for viral nucleic acid test, 22% were diagnosed based on their symptoms and history of exposures with no test performed because of insufficient capacity to test all suspected cases in China during the pandemic [16]. The majority of cases (87%) were aged 30–79 years, 3% — 80 years or older, and only 2% were younger than 19 years [16]. In terms of disease severity, the majority of cases (81%) were labelled as mild cases that included patients with no pneumonia or mild pneumonia. Around 14% of the cases who had symptoms that suggest significant respiratory compromise such as difficulty of breathing, tachypnea (respiratory rate > 30/min), hypoxemia with a saturation of blood oxygen less than 93%, with or without the presence of lung infiltrates on chest x-ray were considered as severe. And 5% of the total recorded cases were diagnosed to have a critical illness due to the presence of respiratory failure, hemodynamic instability, with/without multiple organ failure [16]. A possible explanation for the significant deterioration in the patients who become critically ill is thought to be due to a cytokine storm, which is a situation where there is an overproduction of cytokines that consequently leads to mutli-organ dysfunction and failure resulting in death [17].

Patients with COVID-19 typically have flu-like symptoms such as a fever and a dry cough. However, old patients and those with chronic

medical conditions and comorbidities may present with symptoms that suggest lower respiratory tract infection (pneumonia) such as chest pain, chest tightness and shortness of breath. Huang *et al.* [18] first reported symptoms related to COVID-19 among 41 hospitalized patients; fever (98%), cough (76%) and myalgia (44%) were the most common three symptoms observed. Other symptoms such as headache, sputum production, hemoptysis and diarrhea were less frequent [18]. However, some patients predominantly presented with sneezing, rhinorrhea and sore throat. More than half of the infected patients developed dyspnea [18]. Chen *et al.* [19] reported 99 patients with a confirmed SARS-CoV-2 infection diagnosed by rRT-PCR. Most subjects had fever and cough, (82%) and (81%), respectively. Other reported symptoms were difficulty of breathing, headache, chest pain and diarrhea. Furthermore, Wang *et al.* [20] noted symptoms among 138 hospitalized patients with confirmed COVID-19 — fever was the main symptom (98.6%), followed by fatigue (69.6%) and then dry cough (59.4%). Despite being the most commonly reported symptom, fever can be absent in early stages of the illness. A recently published systematic review showed that 36% of 3,470 confirmed cases of COVID-19 had no fever at the onset of symptoms [14].

Mao *et al.* reported neurological manifestations of COVID-19 among 214 hospitalized patients, with hypogeusia and hyposmia in 5.6% and 5.1% of the subjects, respectively [21]. The clinical course of COVID-19 disease displays a wide spectrum of progression patterns. Looking at the timeline of the infected cases from the onset of the disease, a median time of 8 days was noticed to develop dyspnea, 9 days to develop ARDS and 10.5 days to require mechanical ventilation [12]. Severely ill patients with ARDS may quickly progress to multiple organ failure leading to death [22]. Lymphopenia is one of the clinical features of COVID-19 infection, which indicates an immunity suppression that may result in severe complications due to secondary bacterial and fungal infections. Huang *et al.* [18] in their screening study of 41 patients with COVID-19, observed that 26 (63%) of them had lymphopenia and 13 (32%) patients required ICU. The subjects admitted to ICU had higher plasma levels of interleukins (ILs-2, 7 and 10), granulocytes-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α).

The rise in inflammatory markers is the key point underlying the multisystem inflammatory response in COVID-19 [23]. The main inflammatory and immune markers correlating with COVID-19 disease include CRP, ESR, serum ferritin, IL-6, IL-8, IL-10, and low count of lymphocytes, T cell, B cell and NK cell [23].

Transmission of SARS-2

Based on what has been reported about previous outbreaks caused by SARS and MERS, droplets are considered the main mode of transmission. Close contact with an infected person can also transmit the infection. In addition, airborne transmission has been suggested especially when invasive respiratory procedures are performed such as endotracheal intubation [24]. The gastrointestinal symptoms that have been reported with infected patients are related to invade ACE2- expressing absorptive enterocytes from the ileum and colon, which suggests that the digestive system is a potential route for SARS--CoV-2 infection [25].

Diagnostic testing

Detection of viral RNA by RT-PCR

This test is the most commonly used and considered more reliable [26]. It is performed using nasopharyngeal swabs or other upper respiratory tract specimens, including throat swab or even saliva. Viral RNA in the nasopharyngeal swab is measured by the cycle threshold (Ct). The Ct is the number of replication cycles needed to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads. The Ct becomes detectable as early as on day 1 of symptoms and peaks within the first week of the symptom onset. This positivity starts to decline by week 3 and subsequently becomes undetectable. In severe cases, however, PCR positivity may persist beyond 3 weeks after the illness onset when most mild cases will yield a negative result [27]. In a study of 9 patients, it was noted that attempts to isolate the virus in culture were not successful beyond day 8 of the illness onset, which correlates with the decline of infectivity beyond the first week even if the PCR remains positive, thus a "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate the presence of viable virus [28]. The timeline of PCR positivity is different in specimens other than nasopharyngeal swab with PCR positivity declining more slowly in sputum and stool than in nasopharyngeal specimens [28]. Wang and colleagues published a study to compare RT-PCR positivity in different types of clinical specimens

in 205 patients with confirmed COVID-19 infection — it was highest in bronchoalveolar lavage specimens (93%), followed by sputum (72%), nasal swab (63%), and pharyngeal swab (32%) [29]. Specificity of most of the RT-PCR tests is 100% because the primer design is specific to the genome sequence of SARS-CoV-2. Occasional false-positive results may occur due to technical errors and reagent contamination.

Detection of antibodies to SARS-CoV-2

COVID-19 infection can also be proven indirectly by measuring the host immune response to SARS-CoV-2 infection. Serological diagnosis is especially important for patients with mild to moderate illness who may present beyond the first 2 weeks of the illness onset. Serological diagnosis is also becoming an important tool to understand the extent of COVID-19 in the community and to identify individuals who are immune and potentially "protected" from becoming infected. The most sensitive and earliest serological marker is the total of antibodies (IgM and IgG ELISA), which begin to increase from the second week of the symptom onset [30]. These antibodies can be found as early as on the fourth day after the symptom onset but higher levels occur in the second and third week of illness. Antibodies may have cross-reactivity with SARS-CoV and possibly other coronaviruses. Rapid point-of-care tests for detection of antibodies have been widely developed and marketed and are of variable quality. They are considered qualitative in nature and can only indicate the presence or absence of SARS-CoV-2 antibodies but don't confirm the presence of neutralizing antibodies which can only be confirmed by a plaque reduction neutralization test. However, high titers of IgG antibodies detected by ELISA have been shown to positively correlate with neutralizing antibodies [31]. The long-term persistence and duration of protection conferred by the neutralizing antibodies remains unknown.

Radiological changes of COVID-19 pneumonia

Chest X-ray and CT scan are used for early detection of COVID-19 pneumonia. Chen *et al.* [18] reported bilateral pneumonia among 75% of their cohort (99 patients with COVID-19) based on chest X-ray and chest CT scan. A quarter of patients were diagnosed with unilateral pneumonia and only 14% showed a ground-glass appearance on their images. In addition to rRT-PCR, the use of chest imaging as a diagnostic and management tool of COVID-19 is still debatable as chest X-ray in adults has been found to be insensitive in mild or early COVID-19 infection [32]. Similarly, reported cases of children with COVID-19 showed completely normal X-ray on admission [33, 34]; however, in severe and more advanced cases, chest X-ray images were abnormal with bilateral multiple consolidation [35]. Jiehao *et al.* [36] reported multiple patch-like shadows on chest X-ray images of 4 (40%) out of 10 infected children.

Compared to X-ray, a CT scan was shown to be more sensitive in detecting early changes and progression of the disease [37]. In fact, abnormal CT scans have been used to diagnose COVID-19 in suspected cases who initially tested negative on RT-PCR but eventually had positive tests on repeated testing [38, 39].

The main radiological changes seen on chest CT scans of infected patients were bilateral ground-glass opacities [12]. In children, the typical CT findings were unilateral or bilateral subpleural groundglass opacities as well [33, 40–46]. Other studies have reported findings of consolidations with and without air bronchogram and pleural effusion [35, 44, 45]. Furthermore, consolidations with surrounding halo sign were noticed in 50% of cases in a study conducted in children by Xia *et al.* [43]. Moreover, findings such as peribronchial distribution and bronchial thickening were more commonly seen in pediatric patients compared to the adult population [47].

In addition to its role in diagnosis, a high-resolution CT scan (HRCT) was shown by Liu *et al.* [48] to be useful as a potential screening tool as they used it to screen for COVID-19 in five pediatric suspected cases, and it showed multiple ground-glass opacities. However, currently, the society of Thoracic Radiology and the American College of Radiology do not support the use of chest CT for routine screening of COVID-19 [49]. However, Ji *et. al.* [50] reported two pediatric cases whose CT scans were completely normal [50].

Ultrasound (US) has an essential role in differential diagnosis assessment and follow-up of hospitalized patients with COVID-19, especially in intensive care units, where access to CT scan is difficult [51]. However, ultrasound should not be used to replace CT scan [52]. Lung ultrasound can be applied to evaluate many pulmonary conditions such as pleural effusion, atelectasis, consolidations, pleural effusions and pneumothorax. In COVID-19 patients, US allows evaluating the parenchyma inflammation progression, pleural thickening, and subpleural consolidations with or without air bronchograms [52].

Case fatality

Estimating the lethality of COVID-19 disease is challenging [53]. The case-fatality ratio is used to assess the severity of a disease and effectiveness of a treatment [54]. To calculate the CFR, the number of known deaths over a certain period of time is divided by the number of confirmed cases (including deaths and recovered cases) during that time [54]. The CFR does differ from mortality rate which is another measure of death that reflects the portion of a population who dies during a certain period of time. To get an accurate CFR, the true number of infected patients is needed. This means that the CFR can be overestimated if the true number of infected persons is underestimated. Especially, if asymptomatic and mildly symptomatic infected patients do not present to hospitals. Moreover, CFRs can differ between different geographical areas, a reason being different medical services and facilities during the pandemic period [53], the use of inappropriate statistical methods and techniques [54], in addition to the sensitivity and specificity of serologic testing that is used to confirm infection [54].

Wu *et al.* [55] reported a total of 72,314 infected cases with COVID-19 recorded by the Chinese center for disease control and prevention, the overall CFR till February 24, 2020 and among the confirmed 44,672 cases, was 2.3%. There were no deaths recorded in children aged 9 years or younger. However, the CFR was up to 8% among the group of patients aged 70–79 years and even higher (14.8%) in the group of older patients (≥ 80 years). The CFR was the highest in the group of patients with critical illness (49%) [55]. In a recently published systematic review, the overall CFR was 3.7% (3,015 died of 80,565 patients) [14].

Treatment

Repurposed drugs to treat SARS-CoV-2

Currently, there has been no potential therapy shown from randomized clinical trials to improve outcomes or to significantly reduce case-fatality rate among either suspected or confirmed cases of COVID-19. However, the researchers have used drugs targeted at the virus lifecycle steps, viral entry and immunity regulation pathways to provide drug therapy for COVID-19 [56]. Because of the critical need for effective therapies, there has been a clear interest in repurposing available agents for immediate use.

Various drugs that were active against SARS-CoV and MERS-CoV have been considered as potential therapy to treat COVID-19.

Chloroquine and hydroxychloroquine

Chloroquines have been used as antimalarial agents for decades [57]. Because of their immune-modulatory properties, these agents have been considered to treat autoimmune diseases such as rheumatoid arthritis [57]. Chloroquine (CQ) and hydroxychlorquine (HCQ) have a potential antiviral activity against SARS-CoV-2 by blocking several steps required for viral entry to host cells, including host cell receptor terminal glycosylation, proteolytic processing and edosomal acidification [58], in adition to their immune-modulatory properties through inhibition of cytokine production and host cells lysosomal activity [59], both CQ and HCQ affect the viral entrance to the host cell through inhibiting ACE2 receptor binding to viral S protein by impairment of terminal glycosylation of ACE2 on host cells, and inhibiting membrane fusion and uncoating [59]. Once inside the cell, CQ and HCQ concentrate inside the acidic organelles, such as lysosomes, endosomes and Golgi apparatus. Thus, using either CQ or HCQ therapy elevates the PH of the organelle that virus uses to replicate, which may negatively influence the viral entrance [60]. Lysosomal proteases play a role in fusion process between the viral membrane and the host, alkalinization of lysosomes will negatively affect proteases activity causing impairment of fusion process [60]. Preventing virus-host fusion helps blocking the infection. Vincent *et al.* studied the efficacy of CQ on SARS-CoV infection and observed inhibition of SARS-CoV spread in cells treated with CQ prior and after the infection [60]. Thus, they suggested its prophylactic and therapeutic role.

Moreover, Gautret *et al.* reported a 70% viral clearance among 20 infected patients who were treated with HCQ at the sixth day of therapy. The authors also reported an increase in the viral clearance up to 100% after adding azithromycin to HCQ in 6 patients, which supports using this regimen to reduce the length of hospital stay [61]. However, the small size of the patients used azithromycin in this study (6 subjects), and were observed only for a short time (6 days), and taking into consideration the additive risk of developing cardiac complications, mainly QT prolongation with the combined therapy [61], they have encouraged to perform randomized controlled trials to assess this regimen's efficacy.

In addition, surviving sepsis campaign guidelines on the management of critically ill adults with COVID-19 that have been published recently, provided no evidence to support this combination of therapy use in treatment of critically ill patients admitted to ICU [62]. This could be, though, due to multiple organ failure that critically ill patients have, which can influence the metabolism of these agents, and can potentially increase the risk of side effects. Whether to continue using QCs or to stop, RCTs are required. The solidarity and the discovery study are multicenter studies ongoing to provide a better understanding of antimalarial and other antiviral agents' effects.

Safety and side effects

Both CQ and HCQ are distributed well throughout body systems after oral administration and are cheap. The main side effects of these agents include diarrhea and vomiting [59]. Other serious side effects have been reported following a chronic use such as retinopathy and cardiomyopathy [63]. Moreover, toxicity due to CQ therapy has been seen in patients treated with high doses exceeding the therapeutic dosage limits. In contrary, HCQ is associated with fewer side effects than CQ because of its lower level of tissue accumulation [63]. In contrast to HCQ, CQ is considered unsafe to be given during pregnancy due to its teratogenic effect on the fetus. This supports using HCQ rather than CQ in treating pregnant women with SARS-CoV infection. HCQ can cause QT interval prolongation leading to *torsade de pointes* in some individuals. Despite being rare, this side effect can by amplified by using other drugs such as azithromycin that has been suggested to be used in combination with HCQ [62].

Lopinavir/ritonavir combination

The HIV antiretroviral combination lopinavir/ritonavir that is called Kaletra or Aluvia [64] as been used to treat patients with COVID-19. Lopinavir and ritonavir are protease inhibitors that have shown some activity against SARS-CoV-2 *in vitro*, however currently, there is no strong evidence of benefit to use it against COVID-19 [65]. Lopinavir inhibits the activity of 3-chymotrypsin-like protease (3CL) which has a role in viral RNA processing. Ritonavir inhibits the metabolizing enzyme cytochrome P450 3A and that increases the half-life of lopinavir, subsequently, affecting viral replication and release from host cells [66]. Previous studies investigating the activity of lopinavir/ritonavir against SARS and MERS were limited, still they showed a decrease in incubation period and mortality rate [67]. Up till now there has been no published data for this combination supporting its use for SARS-CoV-2. Cao *et al.* investigated the efficacy of Kaletra through conducting a randomized, controlled, open-label study. A total of 199 patients were included, 99 subjects were allocated to the lopinavir/ritonavir group, and the rest (100 patients) to the supportive care group [67]. The results, unfortunately, were not promising, and there were no benefits observed with lopinavir/ritonavir therapy over supportive care. In a different study from China, the authors have investigated the risk factors for prolonged SARS-CoV-2 shedding among 120 patients confirmed to have COVID-19 using tRT-PCR. They have reported a shorter median duration of viral shedding with early administration, within the first 10 days from the symptoms onset, of lopinavir/ritonavir treatment by 6.5 days [64]. However, reported serious adverse effects such as induced transaminase elevation and hepatotoxicity, limit this therapy in treating patients with COVID-19, especially those with liver injury [68]. At present, there is a lack of evidence to recommend the use of this combination for treatment of COVID-19, and more RCTs are required to assess the efficacy and safety of this therapy.

Ivermectin

Ivermectin is an FDA-approved anti-parasitic drug which has shown an antiviral effect on human immunodeficiency virus (HIV) [69]. It is known to inhibit the interaction of integrase protein (IN) of HIV-1 and the importin (IMP) α/β1 heterodimer that is important for IN nuclear transportation of viral proteins [70]. As this process is important for viral replication cycle, affecting the nuclear import can be considered as a therapeutic approach against RNA viruses. Recently, a group of Australian researchers have shown an *in vivo* activity with capability of ivermectin to significantly reduce the virus replication within two days [70]. Further research is required to evaluate ivermectin`s efficacy on treating SARS-CoV-2 infections.

Remdesivir

Remdesivir, which is known as GS-5743, has a broad-spectrum antiviral activity against RNA viruses such as filoviruses, pneumoviridae and paramyxoviruses [71], it has shown an *in vitro* activity against reported cases infected with

SARS-CoV-2 [72]. Remdesivir is intracellulary metabolized to adenosine triphosphate analogue that blocks viral replication by inhibiting the viral RNA polymerases [73]. Initial animal experiments showed some activity against Ebola virus, however, a recent randomized controlled trial in the Democratic Republic of the Congo (DRC) showed that remdesivir was less effective in reducing mortality compared to single and triple monoclonal antibody-based treatments. However, this trial has proven the safety of its use in humans, which led the researcher to consider using it in COVID-19 clinical trials [74]. Recently, Williamson *et al.* have shown that remdesivir treatment was effective in reducing lung damage and disease progression in infected rhesus macaques monkeys with SARS-CoV-2 [75]. Moreover, Grein *et al.* have published their experience with compassionate remdesivir treatment in 53 patients with severe COVID-19, as a 10-day course of remdesivir at a dose of 100 mg intravenously proceeded by a loading dose of 200 mg used and followed up for 28 days. A clinical improvement in terms of respiratory support was observed in 36 (68%) patients [76]. Remdesivir is currently being tested in clinical trials in different countries, two of these trials are randomized phase 3 trials in China.

Favipiravir

Similar to remdesivir, favipiravir inhibits the RNA polymerase activity affecting viral replication [77]. Favipiravir (FPV) is one of the medications approved for treating influenza. However, there has not been strong evidence to support its use to treat patients with COVID-19 compared to remdesivir [77]. Nevertheless, Cai *et al.* evaluated the effects of favipiravir against lopinavir/ritonavir for treatment of SARS-CoV-2. An oral favipiravir was used in a combination with an inhaled interferon-α for the synergistic effect of viral inhibition. Their results were promising as the patients in the FPV arm showed better clinical response in terms of viral clearance and disease progression with minimal adverse effects [78]. Consequently, in March 2020, the National Medical Products Administration of China approved FPV as the first drug to treat COVID-19.

Ribavirin

Ribavirin is a guanosine analog that affects the replication of RNA and DNA viruses. It also inhibits the production of guanosine from the guanine precursor by influencing the function of inosine monophosphate dehydrogenase which further affects virus stabilization [79]. Early administration of ribavirin has been reported to be beneficial in treating COVID-19-related pneumonia [80]. Ribavirin has been used in combination with the protease inhibitor lopinavir/ritonavir given the previously proven efficacy against SARS. Chu *et al.* examined the clinical response of 41 COVID-19 patients who were followed up to 3 weeks to a combination of lopinavir/ritonavir and ribavirin, compared to 111 controls who were SARS infected patients and received only ribavirin. The study subjects in the lopinavir/ritonavir and ribavirin treatment group had lower adverse clinical outcomes represented by ARDS or death compared to the control group. In addition, a reduction in both steroid use and nosocomial infections were noticed as well [81].

Systemic glucocorticoids

Given the high levels of cytokines that are induced by COVID-19, corticosteroids have been used for their anti-inflammatory effect to treat critically ill patients. However, current research suggested no reduction in mortality rate, but delayed viral clearance and high viral load [82, 83]. However, there is an argument for using systemic corticosteroids in patients who develop ARDS as a complications of COVID-19 infection, where in this setup it seems that they decrease the duration of mechanical ventilation and hospital mortality [84].

Corticosteroids were widely used during SARS-CoV outbreak due to their ability to modulate the inflammatory response [85]. At present, there is no clear evidence for or against corticosteroid use in the treatment of SARS-CoV-2 patients. There is some proof that corticosteroid use during early phase of infection may be beneficial [85]. However, corticosteroid should be applied carefully until further evidence that is specific to SARS-CoV-2 infection emerges.

Intravenous immunoglobulin (convalescent plasma therapy)

Immunocompromised patients and individuals with immunological disorders appear to be at higher risk of developing serious complications related to COVID-19 disease compared to healthy individuals. Immunotherapy using immunoglobulin G (IgG) could be used in combination with antiviral agents to treat COVID-19 and to strengthen patients' immune response against

SARS-CoV-2 [86]. Hofmann *et al.* reported that sera from healthy individuals contain anti-coronavirus antibodies [87]. In addition, Pyrc *et al.* in a study on HCoV-NL63, showed that the infection caused by HCoV-NL63 can be inhibited by human sera from healthy adults [88]. Boukhvalova *et al.* reported an improved outcome of RSV infections among immunocompromised patients who were treated with IV Ig obtained from previously infected donors who had a high-titer antibodies against RSV [89]. Thus, immunotherapy using immune IgG antibodies collected from adults recovered from SARS-CoV-2 infection could be a promising modality of treatment for patients with COVID-19. It has been reported that immune IgG antibodies are more efficient in terms of virus neutralization, if collected from patients who live in the same city because of the effect of lifestyle and environmental factors on specific antibodies development against viruses [86]. Using immune IgG antibodies against SARS-CoV-2 infection can help newly infected patients by boosting their immune response to the infection. Thus, a combination of antiviral drugs and immunotherapy can be used as an alternative treatment for COVID-19 until a vaccine is developed.

Interleukin (IL)-6 pathway inhibitors

IL-6 is one of the key cytokines produced by activated macrophages. In their systematic review, Coomes *et al.* demonstrated significantly higher serum levels of IL-6 in patients requiring ICU admissions than non-ICU patients, suggesting that serious complications of COVID-19 can be related to a host immune response and autoimmune damage [90]. Furthermore, Zhou *et al.* reported a correlation between the serum levels of IL-6 and the mortality in patients with COVID-19 [91]. IL-6 is important for production of T helper 17 (Th17) cells. Excessively activated Th17 cells reported in patients with COVID-19 can be explained by the high levels of IL-6 [92]. Elevated levels of IL-6 negatively impact the lung elasticity and are associated with severe bronchoalveolar inflammation [13]. Thus, using agents that inhibit the cytokine pathway at the level of IL-6, such as tocilizumab can be beneficial in managing inflammatory response squeals. Tocilizumab (TCZM) is a recombinant monoclonal antibody that binds to both soluble and membrane-bound receptors [93]. Tocilizumab is not approved for COVID-19 treatment. However, clinicians are using it under emergency use authorization [93]. There is limited high-quality published evidence for IL-6 inhibitor use against COVID-19 [94]. However, a very recent systematic analysis that included sixteen case-controlled and eighteen uncontrolled studies revealed positive evidence for the potential efficacy of TCZM to treat severe cases of COVID-19 [93]. The World Health Organization (WHO) recommends the use of IL-6 inhibitors only in clinical trials. But, many organizations have included IL-6 inhibitors as an option for treating COVID-19 patients with severe disease [94]. Other IL-6 inhibitors such as sarilumab and siltuximab have been evaluated for the management of COVID-19 patients, with no strong evidence to be used in the management of COVID-19 patients [94].

Amantadine

Amantadine is a drug used to treat Parkinson's disease. It could be used to mitigate COVID-19 effects; the researches have shown that patients with Parkinson's disease who are treated with amantadine and have been infected with SARS-CoV-2 virus have been asymptomatic [95]. Its proposed mechanism of action is that it blocks the early stages of viral replication. Moreover, it is hypothesized that amantadine prevents the release of the viral nucleus into the cell cytoplasm by blocking the viroporine channel of SARS-CoV-2 [96]. Very recently, Jiménez-Jiménez *et al.* studied the anti-inflammatory effects of amantadine and its therapeutic influence in treating COVID-19. They have suggested two pharmacological effects: antiviral and anti-inflammatory [97]. Furthermore, Abreu *et al.* have proposed that early use of amantadine could mitigate COVID-19 disease consequences [96]. Further randomized clinical trials are required to prove its usefulness in COVID-19 management.

Systemic anticoagulation

Researchers in several centers caring for adult patients with severe COVID-19 disease noted an increased incidence of thromboembolic events. Those patients typically required ICU admission and mechanical ventilation. Two studies looked at the use of systemic anticoagulation and the impact on in-hospital mortality and reported improved outcomes [98, 99]. In an updated recommendation, the NIH in the US added the use of systemic anticoagulants in its recommendations for the care of hospitalized

critically ill adult patients with COVID-19 [100]. One of the anti-coagulant agents that were tried on COVID-19 patients is sulodexide. It is a natural glycosaminoglycan composed of fast-moving heparin (80%) and dermatan sulfate (20%) [101]. It has an arterial and venous anti-thrombotic action and ani-inflammatory activity through suppression of IL-6 production [101]. Compared with low-molecular-weight heparin (LMWTs), sulodexide is associated with less bleeding risk and is safe to be given to patients with renal insufficiency [102]. This drug may represent an alternative prophylactic agent to LMWH [102]. It was hypothesized that the early use of sulodexide in COVID-19 patients with comorbidities might reduce the severity of the disease and prevent the development of severe complications [103]. Bikdeli *et al*. reviewed 6 randomized controlled trials (RCTs) where the use of sulodexide was compared with placebo. The sulodexide administration was associated with a reduction in the odds ratio of cardiovascular mortality, deep vein thrombosis, and myocardial infarction [104]. However, additional RCTs with this drug are warranted.

Respiratory support

Hypoxemia is common in hospitalized COVID-19 patients. More than a quarter of the hospitalized COVID-19 subjects require intensive care due to acute respiratory failure [105]. Conventional oxygen therapy can be insufficient to meet oxygen needs of individuals with acute hypoxic respiratory failure [106]. Options for treating hypoxic patients, other than conventional oxygen therapy, include high-flow nasal cannula (HFNC), noninvasive positive-pressure ventilation (NIPPV), or intubation and invasive mechanical ventilation. Based on meta-analysis and data from non-COVID-19 clinical trials that showed reductions in the need for intubation in patients who received HFNC or NIPPV, these options are preferable to conventional oxygen therapy [107]. Furthermore, HFNC use is preferred over NIPPV in hypoxic patients due to acute respiratory failure [108]. Patients with COVID-19 should be monitored for signs of respiratory deterioration. Early intubation should be considered when the patients' condition deteriorates and they have additional acute system dysfunction or when HFNC and NIPPV are not available to treat the hypoxic acute respiratory failure [106]. If required, intubation should be performed by experienced staff in a controlled setting to ensure the safety of both patients and healthcare workers.

Use of antibiotic therapy

A meta-analysis of small case series reported that 3.5% of COVID-19 patients had a bacterial co-infection, and 14% had a secondary bacterial infection [109]. Superimposed bacterial infections have been reported in 28% of severely infected and critically ill COVID-19 patients, which may support the antibiotic use in intensive care units [110]. Despite the lack of reported cases of initial superinfections, there is widespread use of antibiotics in hospitalized COVID-19 patients. Antibiotic use in patients with COVID-19 has not been shown to affect clinical outcomes. Contrary, unnecessary antibiotic use has been associated with an increased risk of resistant hospital-acquired bacterial and fungal infections [111]. Antibiotic therapy is not recommended for COVID-19-related pneumonia unless a secondary bacterial infection is suspected.

Vaccine development

Since the full genomic sequence of SARS--CoV-2; the cause of the novel coronavirus pandemic (COVID-19) has been published [112], many countries, institutions, and pharmaceutical companies started racing to develop an effective and safe vaccine, as it's the most reliable and cost-effective method to control any emerging viral infection and flatten its transmission curve as well as to prevent any re-emergences of the disease in the future. According to the WHO, until this date, more than 40 programs are working on a vaccine against SARS-CoV-2 [113], only two of them entered the clinical trials; recombinant adenovirus vector vaccine which is in phase-2 clinical trial [114], and mRNA-based vaccine in phase-1 [115].

In addition to live vector and RNA-based vaccines, candidates using other platforms, such as the whole virus; either killed or live attenuated [116–118], DNA-based [119, 120], and recombinant subunit vaccines, are also under development ; which is currently getting a lot of attention since the surface S glycoprotein has shown to be the main target for subunit vaccines against MERS-COV and SARS-COV [121–124], and it is expected to be the same for SARS-CoV-2 due to the reported high genetic similarities especially with SARS-COV [126, 127]. Several studies have shown that S glycoprotein and its RBD fragment is an ideal vaccine target against SAR-CoV-2 [127–132]. To date all S glycoprotein-based vaccine candidates targeting SARS-CoV-2 are still in preclinical phase [113]. Another interesting

subunit vaccine targets are specific B and T cell epitopes [133–135]. However, as vaccines usually take at least one year to be available, alternative options should be considered.

Existing and widely used vaccines may serve a potential protective effect against SARS-CoV-2 as it has been observed that the incidence of COVID-19 in groups who are vaccinated routinely, especially children, is very low [136, 137], and recently, it has been hypothesized that BCG vaccine may offer a protective shield against COVID-19 based on observations that compared the prevalence of COVID-19 in countries where BCG is a national program with other countries [138, 139]. However, two clinical trials have been started to assess its efficacy in protecting healthcare workers who are in contact with COVID-19 patients [140, 141]. Another interesting short-term protection alternative option is convalescent sera which provide an immediate passive immunity by administering the collected antibodies from recovered patients in susceptible individuals [142, 143], thus, convalescent plasma combined with other potential therapeutic drugs may serve a good alternative treatment until strong options such as vaccines are available. As for any vaccine, in addition to the time it takes to be developed and evaluated, it also poses some challenges regarding its candidate such as antibody-dependent enhancement (ADE); a phenomenon in which the viral antigens used by the vaccine may induce the same disease they're supposed to protect from [144]. Moreover, RNA viruses; which are the big family of coronaviruses have been shown to have a higher rate of mutations when they're compared to DNA viruses [145, 146].

In the past, vaccines were developed through many steps that might have taken several years. Recently, given the urgent need to develop a COVID-19 vaccine, some of the vaccine development steps are happening in parallel while maintaining safety standards. For example, multiple vaccines are evaluated at the same time by some clinical trials [147]. Clinical development of a new vaccine is a three-phase process. During phase-1, small groups of healthy adult volunteers should be enrolled. In phase-2, the vaccine is given to groups of volunteers that reflect the populations for whom the vaccine is intended. In phase-3, the vaccine is given to large groups of people (thousands) to test its efficacy and safety [148]. Multiple vaccines are being tested in early-phase studies, and some vaccine participants are in phase-3 studies assessing efficacy [149].

Conflict of interest

The authors declare no conflict of interest.

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Amina Pervaiz, Firas El-Baba, Kunwardeep Dhillon, Asil Daoud, Ayman O Soubani

Division of Pulmonary, Critical Care and Sleep Medicine Wayne State University School of Medicine Detroit, United States

Pulmonary complications of sickle cell disease: a narrative clinical review

Abstract

Sickle cell disease (SCD) is associated with vaso-occlusive episodes that affect different organs. Pulmonary involvement is a major cause of morbidity and mortality in this patient population.

We performed a literature search in the PubMed database for articles addressing SCD and pulmonary diseases. Acute chest syndrome is defined as a new radiodensity on chest radiograph imaging with a history consistent of the disease. Management includes broad spectrum antibiotics, pain control, and blood transfusions. Microvasculature infarcts lead to functional asplenia, which in turn increases the risk of being infected with encapsulated organisms. Universal vaccinations and antibiotic prophylaxis play a significant role in decreasing mortality from pulmonary infections. Venous thromboembolism in patients with SCD should be treated in the same manner as in the general population. Pulmonary hypertension in patients with SCD also increases mortality. The American Thoracic Society treatment modalities are based on the underlying etiology which is either directed at treating SCD itself, using vasodilator medications if the patient is in group 1, or using long-term anticoagulation if the patient is group 4 (in terms of etiology). Patients with SCD are more likely to suffer from asthma in comparison to controls. Sleep disorders of breathing should be considered in patients with unexplained nocturnal and daytime hypoxemia, or recurrent vaso-occlusive events. Lastly, the utility of pulmonary function tests still needs to be established.

Key words: sickle cell disease, acute chest syndrome, pneumonia, venous thromboembolic disease, pulmonary hypertension

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Introduction

Sickle cell disease (SCD) is one of the most common monogenetic disorders in the world affecting nearly 300 million people worldwide. It is estimated that approximately 100,000 people in the United States have the disease, with a higher prevalence amongst African Americans [1, 2]. The sickle point mutation is a substitution of valine for glutamic acid at the sixth position of the β-hemoglobin gene resulting in sickled hemoglobin (HbS) that is less soluble than normal adult and fetal hemoglobin. Upon deoxygenation, HbS undergoes polymerization resulting in a decreased flexibility of the erythrocyte forming the infamous "sickled" shaped. These changes alter cellular rheological properties, enhance adhesion molecule expression, impair microvasculature blood flow, and promote hemolysis and vaso-occlusive episodes [3].

When the sickle mutation is co-inherited with a mutation at the other β-globin allele, then the production of normal beta-globin becomes obsolete or, at the very least, reduced. The majority of infants with sickle cell disease (60–65%) are diagnosed as having homozygous sickle mutations (HbSS). The second most common affliction is when a patient inherits hemoglobin S and hemoglobin C (HbSC disease), and this is seen in 25–30% of patients. Lastly, there is sickle beta thalassemia with a sickle mutation and a β thalassemia mutation (HbSβ0/+) affecting 9% of patients. People with HbSS and HbSβ0 thalassemia have a more severe disease course in contrast to those with HbSC and HbSβ+ thalassemia [3].

Address for correspondence: Ayman O Soubani, Division of Pulmonary, Critical Care and Sleep Medicine Wayne State University School of Medicine Detroit, Detroit, USA, e-mail: asoubani@med.wayne.edu

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Sickle cell chronic lung disease was the term used to describe pulmonary complications of SCD; however, advances in this field demonstrate that there are different entities of pulmonary conditions with characteristic pathophysiological changes, clinical manifestations, and outcomes. It is discouraged to use the term 'sickle cell chronic lung disease' and efforts should be made to identify the specific phenotype of pulmonary disease in this patient population. This article will describe these conditions which include acute chest syndrome (ACS), pulmonary infections, venous thromboembolism (VTE), and pulmonary hypertension. This article will also review asthma and sleep disorders of breathing in patients with SCD. Lastly, this article will review the abnormal pulmonary function tests seen in SCD. The Pubmed database was used to obtain the relevant references. The database was searched using the keywords 'SCD' and 'pulmonary disease'. English language articles that were deemed relevant by the consensus of authors were reviewed. These articles included original observations, review articles, meta-analyses, and guidelines. When appropriate, references in the bibliography of these articles were also reviewed.

Acute chest syndrome

Acute chest syndrome (ACS) is a severe complication of SCD defined most simply as a new radiodensity on chest radiograph imaging coupled with respiratory symptoms. In fact, in a Jamaican study by Thomas *et al*. [4], they cited ACS as the principal cause of death after 10 years of age in patients with SCD. According to the Cooperative Study of Sickle Cell Disease by Castro *et al*. [5], of the 3751 patients they followed over a decade, approximately 29% (1085 patients) were prospectively witnessed to suffer at least a single episode of ACS [5]. They also found that patients with severe genotypes of the disease (HbSS disease and HbSβ0) were at greater risk of developing ACS when compared to those with milder genotypes (HbSC disease and HbSβ+ thalassemia). Other risk factors include older age, increased white blood cell count, higher hemoglobin levels, lower fetal hemoglobin, and smoking prior to vaso-occlusive pain events.

Pathogenesis

The exact cause of ACS remains unclear in most cases. Vichinsky *et al*. [6] initiated a prospective, multicenter study to explore the causes of ACS. A specific initiating agent was identified in 38% of episodes studied. The etiologies were fat embolism, infection (ranked from most to least common: chlamydia, mycoplasma, viral, bacterial, mixed infection, and legionella), and pulmonary infarct (Figure 1). However, approximately 46% of the cases were found to have no known etiology. Specifically, regarding the pathogenesis of fat emboli, it is believed that bone marrow infarcts, which have been noted in patients with SCD post-mortem secondary to veno-occlusion, lead to bone marrow necrosis and the release of bone marrow fat into the venous circula-

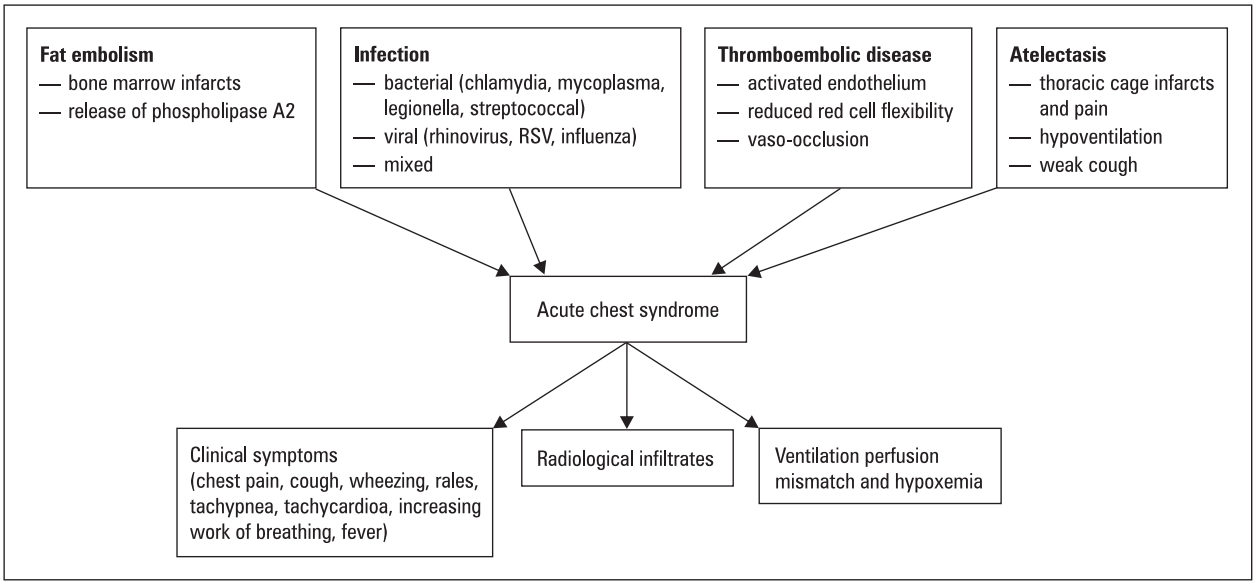

Figure 1. Pathogenesis of acute chest syndrome. PH — pulmonary hypertension; mPAP — mean pulmonary arterial pressure; PAWP — pulmonary artery wedge pressure; PVR — pulmonary vascular resistance; WU — woods unit

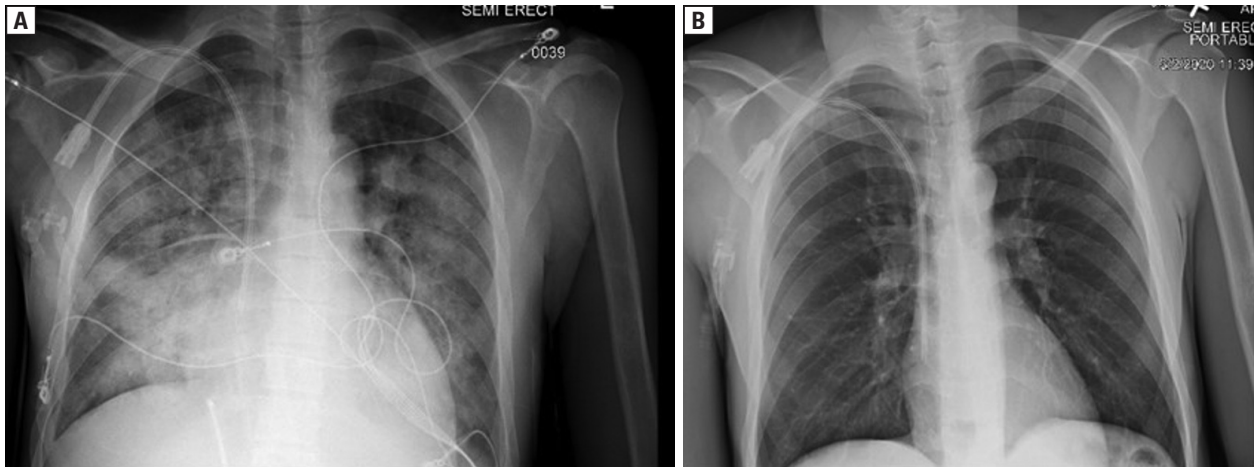

Figure 2. Chest radiograph of a patient during a severe attack of acute chest syndrome (**A**) and after resolution of the episode (**B**)

tion. Secretory phospholipase A2 then converts the neutral fat into free fatty acids, which are highly pro-inflammatory and further propagate tissue injury [7]. This lung injury then precipitates a vicious cycle which results in worsening ventilation-perfusion mismatch and worsening hypoxemia. This hypoxemia then causes HbS deoxygenation, which causes HbS polymerization resulting in decreased erythrocyte flexibility and, in turn, increased vaso-occlusive events. This, again, precipitates more bone marrow infarctions and restarts the vicious cycle.

Clinical presentation

ACS is defined as a new radiodensity on chest radiograph imaging (Figure 2) with at least one of the following clinical findings: fever (≥ 38.5ºC), hypoxia [≥ 3% decrease in oxygen saturation from baseline or oxygen saturation (SpO_2) < 94%], chest pain/discomfort, cough, wheezing, rales, tachypnea, tachycardia, or an increased work of breathing [8]. The severity of ACS has been categorized as: mild, moderate, severe, and very severe (Table 1) [9].

Interestingly, ACS was found to have significant differences in presentation, etiology, and clinical course when comparing adults (≥ 20 years old) to children (< 20 years old). In Vichinsky *et al*.'s study of the National Acute Chest Syndrome Study Group, it was noted that adults were more likely to present with chest pain (55% *vs* 41%), rib/sternal pain (30% *vs* 18%), and shortness of breath (58% *vs* 36%) than children [6]. Multiple other studies found that bone marrow and fat emboli were more common in adults than in children [5, 7], and this is believed to be the reason why adults have worse disease severity (i.e. adults required higher rates of mechanical ventilation (22% *vs* 10%) and higher mortality rates (9% *vs* 1%) when compared to children [6].

Differential diagnosis

ACS can be clinically difficult, if not impossible, to differentiate from an acute pulmonary infection as they both have similar clinical and radiological presentations and may in fact occur simultaneously. Treating all ACS cases as purely infective episodes may lead to progression of the disease and rapid clinical deterioration of the patient as other etiologies such as pulmonary embolism, iatrogenic fluid overload, transfusion related lung injury, opiate narcosis, alveolar hypoventilation, and acute coronary syndrome are overlooked [8].

Diagnostic tests

The diagnosis of ACS can be straightforward when a high level of clinical suspicion is combined with the aforementioned clinical features. The following investigations are essential for all patients presenting with a suspicion of ACS: chest radiograph (most pertinent) (Fig. 2), complete blood count with differential, basic metabolic panel, liver function tests, blood group and screen/crossmatch, blood cultures, arterial blood gas (preferably on room air), respiratory cultures/serology (including atypical respiratory organisms), urine pneumococcal and legionella antigen, and nasopharyngeal aspirate for immunofluorescence or polymerase chain reaction for viruses in patients with coryzal symptoms. It is important to note that computed tomography is not routinely recommended due to high radiation exposure and the tendency of ACS to recur. In addition, there is limited added benefit over chest

Table 1. Severity index of ACS9

Mild*	$tcpO_2$ > 90% on room air CXR showing segmental or lobar infiltrates involving no more than 1 lobe Response to simple transfusion ≤ 2 units of RBC (or 15 cc/kg)
Moderate*	$tcpO_2$ > 85% on room air CXR showing segmental or lobar infiltrates involving no more than 2 lobes Response to simple transfusion ≥ 3 units of RBC (or more than 20 cc/kg)
Severe‡	Respiratory failure (PaO_2 < 60 mm Hg or PCO_2 > 50 mm Hg) Requiring mechanical ventilation $tcpO_2$ < 85% on room air or ≤ 90% despite FiO_2 of 100% CXR showing segmental or lobar infiltrates involving 3 or more lobes Requiring transfusion or exchange transfusion of RBCs to a goal hemoglobin A ≥ 70%
Very severe	Once ARDS is diagnosed. ARDS is defined as: — Acute onset of bilateral infiltrates on CXR — PAWP < 19 mm Hg or lack of clinical evidence of left atrial hypertension — PaO_2/FiO_2 ≤ 200 regardless of PEEP level

*Categories must meet the previously discussed diagnostic criteria above AND all of the following; ‡Must meet the previously discussed diagnostic criteria above AND 1 or more of the following; $tcpO_2$: transcutaneous oxygen saturation. CXR — chest radiograph; RBC — red blood cells; PaO_2 — partial pressure of oxygen; PCO_2 — partial pressure of carbon dioxide; FiO_2 — fraction of inspired oxygen; ARDS — acute respiratory distress syndrome; PAWP — pulmonary artery wedge pressure; PEEP — positive end expiratory pressure

radiography. The only indication for computed tomography is with the addition of pulmonary angiography if there is a high clinical suspicion of pulmonary embolism. Ventilation perfusion (V/Q) scans are also not recommended as they usually reveal diffuse perfusion defects with normal ventilation and can be easily confused with other pulmonary pathologies [8].

Other tests that have been considered include measuring plasma secretory phospholipase A2 (sPLA2) levels and administering bronchoalveolar lavage for lipid laden macrophages via oil red O staining. The enzyme sPLA2 is found in the plasma and it releases inflammatory free fatty acids from bone marrow lipids. Since fat emboli have been noted as a common etiology of ACS, it was previously suggested that measurement of its levels might be useful in the diagnosis of ACS. However, despite its high sensitivity of 100%, its low specificity of 67% and even lower positive predictive value of only 24% makes the test rather weak [10]. Also, bronchoalveolar lavage used to evaluate for lipid laden macrophages via oil red O staining has been proposed for similar reasons as mentioned above [11]; however, the complications of bronchoscopy, including hypoxia and need for mechanical ventilation, outweigh the added benefit. Neither of these tests have sufficient evidence at this point for them to be recommended for routine testing.

Management

The cornerstones for management of ACS include broad-spectrum antibiotics, pain control, and blood transfusion(s) (simple and/or exchange). Firstly, all patients with a diagnosis of ACS should be started on empiric antibiotic therapy with coverage for atypical bacterial organisms as infection is one of the major inciting causes. The antibiotic regimen should then be de-escalated based on culture results. Adequate pain control is pivotal. Usually parenteral opioids are given in the form of patient-controlled analgesia (preferred administration method), which usually avoids over-sedation and minimizes opioid-induced hypoventilation. The mainstay of acute treatment is transfusion therapy. The decision between simple or exchange transfusion is usually dependent on the severity of the episode. The process of exchange transfusion involves phlebotomy to slowly remove the patient's blood followed by replacing it with allogeneic blood, therefore diluting the amount of HbS. It is the preferred modality over simple transfusions. The ability to rapidly give large amounts of blood in order to reduce HbS percentage without increasing the blood viscosity (usually seen at Hb > 11 g/dL) is significantly advantageous; however, it does require trained personnel and equipment which is not readily available at all centers. The general practice is to intervene in cases of mild or developing ACS with a simple transfusion to raise hemoglobin up to 10 g/dL. It has been noted that with early therapy, many episodes of moderate to severe ACS can be avoided. Exchange transfusions are typically reserved for those who show features of severe disease with recurrent vaso-occlusive crises, for those who do not respond

to simple transfusions, or for those with a higher baseline total hemoglobin S concentration [9, 12–14].

In addition to the above therapies, use of supplemental oxygen, incentive spirometry, and intravenous hydration are also pertinent. Patients suffering from ACS can have a low oxygen saturation or a low partial pressure of oxygen and should therefore be provided with supplemental oxygen to avoid hypoxemia, which could propagate ACS. Incentive spirometry decreases the risk of ACS by reducing hypoventilation and atelectasis in patients with bone pain [15]. Incentive spirometry should be encouraged with 10 maximal breaths every two hours while awake to prevent ACS during vaso-occlusive pain episodes. Intravenous hydration is also paramount as individuals with SCD frequently are hypovolemic during pain episodes secondary to poor oral intake and ongoing insensible losses; however, it is prudent to balance volume status to avoid fluid overload and pulmonary edema, which would worsen ACS.

Clinicians should also consider using bronchodilators and venous thromboembolic (VTE) prophylaxis. Bronchodilators are recommended due to the prevalence of wheezing and airway hyperresponsiveness in patients with SCD, but their efficacy has not been appropriately tested in any randomized controlled trials [6]. Patients with ACS are predisposed to VTE events and must receive adequate prophylaxis with unfractionated heparin, low-molecular-weight heparin, or fondaparinux. Systemic steroids have been used previously; however, they are no longer part of standard practice. Previous studies have shown an emergence of rebound vaso-occlusive phenomena after the discontinuation of steroids in children [16].

Prevention

Hydroxyurea has been shown to decrease the incidence of ACS. All patients with a history of ACS with absent absolute contraindications should be started on hydroxyurea [17]. Current practice is to titrate up to a dose of 30 mg/kg or an absolute neutrophil count of 2000/uL. Chronic transfusion therapy is another option. Current practice is to initiate chronic transfusion therapy in patients who have experienced 2 or more moderate to severe episodes of ACS. These transfusions are usually performed every 4–6 weeks with the goal being to maintain a HbS percentage < 50%. Lastly, hematopoietic stem cell transplantation has been proposed as it is a curative option; however, it is not a standard of practice in adults due to the toxicity associated with the myeloablative regimen. Nonetheless, it can be considered in select patients with severe disease who may benefit from the therapy [18].

Pulmonary infections in sickle cell disease

Patients with SCD are at increased risk of infections due to abnormalities in host defenses secondary to functional asplenia. Specifically, they are at increased risk of infection by encapsulated organisms. Pulmonary infections have also been cited as a major etiology of ACS. Despite increased availability of pneumococcal and H influenza vaccines, and the general use of penicillin prophylaxis in children and older adults, patients with SCD are still at risk for invasive infections secondary to pneumococcus and other organisms.

Pathophysiology

SCD patients are prone to infections for a wide variety of reasons. These include splenic dysfunction, defective opsonization, impairment of adaptive immunity, and immunodeficiency. Together, these are likely play a strong role in the higher incidence of pulmonary infections in this patient population [19].

The spleen is a multifunctional organ which plays an important role in the filtration of pathogens and damaged cells, and also aids in the production of antibodies required for adaptive immunity [20]. The spleen plays an important role in the synthesis of tuftsin and properdin that participate in complement activation. Blood flows through the splenic artery and traverses the white pulp (which is composed of lymphocytes) before entering the splenic cords of red pulp to ultimately reach the venous sinuses for removal of defective red blood cells (RBCs), bacteria, and splenic macrophages.

Patients with SCD are functionally asplenic by the age of 3–5 years secondary to ischemia from chronic vascular occlusion and increased blood viscosity from sickling of red blood cells. This results in auto-infarction of the spleen thus increasing the risk of infection by encapsulated organisms such as Streptococcus pneumoniae and *H. influenzae* via the pathophysiology described above [20, 21].

Opsonization refers to the process involving binding of specific antimicrobial proteins called opsonins to the pathogens to enhance the efficiency of phagocytosis. Opsonized bacteria are filtered by both the spleen and the liver; however, poorly opsonized bacteria are only filtered by the

spleen [19]. Splenic opsonization is impaired due to an insufficient availability of splenic immunoglobulins and impaired production of opsonins.

Impairment of B- and T-lymphocyte function results in inadequate memory B-cell function and anti-polysaccharide antibody production [19, 22–24]. There is a diminished humoral response and cell-mediated response in patients with SCD attributed to reduced circulating CD4+ and CD8+ T-lymphocytes. The IgM response after administration of the influenza vaccine is also suboptimal in SCD patients [24].

Infection and acute chest syndrome

One of the major etiologies of the development of ACS is from an acute pulmonary infection. In fact, in Vinchinsky's national acute chest syndrome study group, they identified that 38% of patients with ACS who underwent infectious work-up were found to have a specific infectious organism [6]. In children under the age of 10 years, viral infections were the most common identifiable etiology. In adults, however, atypical bacterial organisms were more commonly identified. *Chlamydia pneumoniae* was the most common, followed by *Mycoplasma pneumoniae*, and then Respiratory Syncytial Virus. It was also reported that these organisms were more commonly linked with ACS than encapsulated organisms such as *Streptococcus pneumoniae* and *H. Influenzae* [6, 8, 25].

Antimicrobials

Due to functional asplenia, patients with SCD are more susceptible to infection with encapsulated organisms than the general population. In addition, if associated with ACS, they are at a higher likelihood of being infected by multiple atypical organisms. Therefore, it is prudent for antibiotic combinations to cover for typical and atypical causes of pneumonia [26]. Clinicians should consider their local biogram and local antibiotic resistance profiles when considering empiric antibiotic therapy. Antibiotics should also be tailored once an appropriate culture and/or sensitivity has been attained.

Prevention

As with all individuals, patients with SCD should be emphasized to practice meticulous hand-washing techniques to protect from typical spread of infections. Also, patients should seek medical attention early if they develop a fever or respiratory symptoms, especially if accompanied by chest pain [21]. However, the two major changes that decreased mortality in patients with SCD were: 1) initiation of antibiotic prophylaxis, and 2) vaccinations [27].

In 1986 and 1995, two groundbreaking studies called the PROPS (Penicillin Prophylaxis in Sickle Cell Disease) and the PROPS-II trial, respectively, showed that prophylactic oral penicillin significantly reduced the risk of invasive pneumococcal infections in children [28, 29] and that penicillin prophylaxis could be safely discontinued at 5 years of age [30]. Also, adherence to lifelong prophylaxis has been called into question further supporting discontinuation of prophylaxis at the age of 5 [31]. In patients with a confirmed penicillin allergy, a macrolide may be used instead [32]. The initiation of a vaccination schedule has also been paramount in decreasing the rate of preventable infections in patients both with and without SCD. The following vaccine series are crucial in preventing pulmonary infections in patients with SCD: pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23), Haemophilus influenza type b (Hib) vaccine, and the seasonal influenza vaccine.

Venous thromboembolism

Venous thromboembolism (VTE) is a significant cause of morbidity and mortality in patients with SCD. Patients with SCD are also at an increased risk to suffer from VTE than the general population. The etiology is multifactorial and relates to both increased traditional risk factors and SCD-specific risk factors. Traditional risk factors that increase the risk of VTE are seen in the general population but are more frequent in patients with SCD due to disease complications such as frequent hospitalizations (some requiring central venous catheter placement), and orthopedic surgeries for avascular necrosis. SCD also has disease specific risk factors such as thrombophilic defects and splenectomy which may modify the risk of VTE. VTE may also increase SCD complications such as acute chest syndrome and pulmonary hypertension [33].

Pathophysiology

All three aspects of Virchow's triad (hypercoagulability, endothelial dysfunction, and hemostasis) have been associated with SCD and result in a highly thrombogenic environment leading to VTE.

The alterations in sickled RBC structure lead to intravascular hemolysis and externalization of

highly procoagulant phosphatidylserines on the RBC membrane. These sickled RBC's become more adhesive to the endothelium. The capture of adhesive red cells, leukocytes, and platelets to the endothelium of the blood vessel wall triggers the vaso-occlusion [34–36]. The endothelial cells and leukocytes also activate proinflammatory molecules such as tumor necrosis factor and interleukin-1b, chemokines, growth factors, eicosanoids, and peptides, all of which can further stimulate cells and induce expression of surface adhesion molecules leading to the continuation of the vicious cycle.

There is extensive laboratory and clinical evidence in literature to safely say that SCD is a condition in which the hemostatic balance is tipped to the prothrombotic state. There are studies showing dysregulation of factors causing the initiation and perpetuation of hemostasis activation. These include studies showing decreased levels of natural anticoagulants such as protein C and protein S [37, 38]. increased expression and/or activity of tissue factor (TF) in whole blood, increased monocytes and circulating endothelial cells, increased levels of von Willebrand factor (vWF) coupled with decreased levels of ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), increased numbers of TF- and phosphatidylserine-bearing microparticles [39, 40] decreased levels of contact pathway factors (factor XII, prekallikrein, and high molecular weight kininogen), and increased markers of neutrophil extracellular trap (NET) formation.

Platelet activation plays a major role in vascular inflammation and thrombosis. A large body of literature suggests that circulating platelets are activated in SCD patients in a steady state as well as in acute painful crises. The following mechanisms are involved in the platelet activation:

The ruptured RBCs release the cell free hemoglobin which reacts with the vascular nitric oxide (NO) leading to vasoconstriction and platelet activation. In addition to the platelet activation by NO depletion, there is ongoing platelet activation in SCD patients evidenced by the increased expression of P-selectin on circulating platelets, plasma soluble factors 3 and 4, β-thromboglobulin, and platelet-derived soluble CD40 ligand [41].

Literature shows that platelets express the pattern recognition receptor nucleotide-binding domain leucine-rich repeat containing protein 3 (NLRP3), apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD), and Bruton tyrosine kinase (BTK), which together control activation of caspase-1 and cleavage of interleukin-1b (IL-1b) within inflammasome complexes. The activation of the NLRP3 inflammasome in platelets promotes platelet aggregation, thrombus formation, and vascular leakage, and can be targeted by BTK inhibitors. The damage-associated molecular pattern molecule high-mobility group box 1 (HMGB1) is a regulatory trigger of the NLRP3 inflammasome16; it stimulates thrombosis and inflammation when released by the activated platelets. The NLRP3 inflammasome in platelets is upregulated in SCD via high-mobility group box protein and toll-like receptor 4 (HMGB1/TLR4) [42].

There is evidence in the literature that bioenergetic dysfunction mechanically contributes to SCD-induced platelet activation. This evidence was found by directly examining mitochondrial function in SCD patients. The bioenergetic alteration is induced by free hemoglobin and is characterized by inhibited complex V activity which leads to augmented oxidant generation. This bioenergetic dysfunction is associated with enhanced platelet activation in vivo. Further, partial inhibition of complex V in healthy platelets in vitro recapitulates the bioenergetic dysfunction observed in SCD patients and results in platelet activation. This proves that a causal relationship is established between this bioenergetic alteration and platelet activation [43].

Diagnosis

In the general population, a D-dimer test is very useful in guiding the diagnosis of VTE due to its high negative predictive value when adjusted for age. Unfortunately, due to the pathophysiology of SCD and the chronic activation of the coagulation cascade, the D-dimer test is unreliable. In addition, some may track the D-dimer to decide whether anticoagulation should be extended; however, due to vasocclusive episodes in SCD, tracking the D-dimer can be deceptive and difficult [44]. Current guidelines recommend starting with a compression ultrasound Doppler for patients with SCD who are suspected of having either upper or lower-extremity DVTs. At this time, there are no studies showing any differences between ultrasound interpretation in patients with SCD from the general population.

If pulmonary embolism is suspected, computerized tomographic pulmonary angiography (CTPA) is currently the test of choice. However, there are current debates whether radionuclide scans (i.e. ventilation-perfusion [V/Q] studies) can be practically advantageous over CTPAs. Specifi-

cally, they minimize radiational exposure and they have no risk of contrast-induced kidney injury. This is particularly important for patients who undergo frequent testing. However, there have been no head-to-head studies comparing CTPA to V/Q studies in patients with SCD at this time [44].

Treatment

There are no current studies or recommendations stating that VTE in patients with SCD should be treated differently from the general population. Antithrombotic therapy guidelines, as per the most recent CHEST guidelines of 2016, state that patients with a proximal DVT or pulmonary embolism should receive a minimum of 3 months of anticoagulation. They also recommend that, in patients with DVT of the leg or pulmonary embolism who do not have cancer, dabigatran, rivaroxaban, apixaban, and edoxaban are preferred over vitamin K antagonist therapy. The duration of therapy can be altered for several reasons including: resolution of the VTE on imaging, bleeding risk, provoked or unprovoked etiology, or if the VTE is recurrent [45]. However, due to the paucity of data, further studies are needed to establish a formalized approach to anticoagulation therapy for first-time VTE in patients with SCD [46].

Pulmonary hypertension

Pulmonary hypertension (PH) is quite prevalent in patients with SCD afflicting approximately 6% to 10.5% of patients based on right heart catheterization [47–49]. There are five different groups of PH: 1) pulmonary arterial hypertension, 2) PH due to left heart disease, 3) PH due to chronic lung disease/hypoxia, 4) PH due to chronic pulmonary thromboembolisms, and 5) PH due to unclear multifactorial mechanisms. PH related to SCD is currently in group 5 as some patients show hemodynamic changes consistent with group 1, while others have features of group 2 or group 4 PH [50, 51].

Pathophysiology

Half of the reported cases of pulmonary hypertension in SCD have precapillary pulmonary hypertension on right heart catheterization, and the other half have postcapillary pulmonary hypertension.

The pathogenesis of precapillary pulmonary hypertension in SCD is well documented in literature and involves the activation of inflammatory and cytokine pathways. A number of factors are involved in the activation of these pathways with abnormal nitric oxide (NO) signaling being the major contributor [52, 53].

Chronic intravascular hemolysis plays a key role in inhibiting NO signaling and impairing vascular endothelial function. Intravascular hemolysis causes the release of cell free hemoglobin, red blood cell microparticles containing hemoglobin, and heme and arginase-1 in the plasma, which in turn inhibit NO signaling. Cell free hemoglobin reacts with NO to form nitrate, and arginase-1 inhibits arginine by breaking it down to ornithine, which is the substrate for NO synthases and further inhibits NO signaling.

Elevated plasma levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, are an independent risk factor for endothelial dysfunction. Furthermore, heme from the hemoglobin molecule in the plasma causes activation of LTR4 and NALP inflammasome pathways and stimulates the systemic and vascular inflammatory response.

Chronic hemolysis also causes platelet and hemostatic activation, generates reactive oxygen species, and activates vascular oxidases which causes an increased oxidant-derived metabolism of NO. As a result, its decreased bioavailability causes it to act as a vasodilator. There is evidence in the literature to support the association of increased levels of markers of hemolysis, including cell free hemoglobin and red blood cell microparticles, with elevated systolic pulmonary artery pressure and precapillary pulmonary hypertension on right heart catheterization [48, 54, 55].

Although hemolysis is thought to play a main role in the pathogenesis of precapillary pulmonary HTN in SCD, it is likely that other factors such as the impact of local hypoxia on vascular remodeling, genetic variability, and thrombosis contribute to this pathogenesis as well.

Chronic hypoxia is a well-recognized cause of pulmonary hypertension because it stimulates various cellular and metabolic processes. There is upregulation of hypoxic responses in SCD via elevated erythropoietin levels. Tissue hypoxia in SCD causes increased expression of the erythropoietin gene resulting in high concentrations of circulating erythropoietin and hypoxia inducible factor (HIF)-α, the major regulator of the body's response to hypoxia [56]. The activation of HIF-1α contributes to the etiology of various forms of pulmonary hypertension through changes in mitochondrial redox signaling, fission, and numbers, and leads to the development of a proliferative, apoptosis-resistant phenotype in pulmonary vascular cells [34,

35]. Furthermore, the elevated levels of placental growth factor in SCD activates HIF-1α in normoxia and has been associated with elevated systolic pulmonary artery pressures in SCD.

Chronic thromboembolic pulmonary hypertension (CTEPH) is a major category of pulmonary hypertension in SCD secondary to the increased predilection for chronic VTE in patients with SCD. Establishing the diagnosis of CTEPH as an etiology of pulmonary hypertension in SCD is imperative in order to offer curative treatment (i.e pulmonary endarterectomy for proximal lesions versus medical therapy for distal lesions).

The pathophysiology of CTEPH is the same as discussed under the VTE section and includes platelet and hemostatic activation pathways via hemolysis and chronic inflammation contributing to the hypercoagulable state. Auto-splenectomy is a well-known risk factor for thrombosis and CTEPH and is commonly seen in patients with hemoglobin SS disease [36]. In one case series which studied 11 SCD patients, acute or organizing thrombi in the distal pulmonary arteries were a common finding [37].

As mentioned earlier, postcapillary pulmonary hypertension comprises almost half of the cases of pulmonary HTN in SCD patients. Chronic anemia and systemic hypertension are common in patients with SCD. This leads to left ventricular diastolic dysfunction from left ventricular dilatation and concentric hypertrophy of the myocardium eventually leading to postcapillary pulmonary hypertension [38, 39].

Clinical presentation

Common symptoms of PH include exertional dyspnea, fatigue, chest pain, lower extremity edema, near syncope, syncope, and palpitations. This makes it challenging to diagnose clinically as there is significant overlap with other complications of SCD. For example, exertional dyspnea could be due to PH or could be due to acute worsening of the patient's chronic anemia. Physical examination findings consistent with PH are a loud P2 heart sound, jugular venous distension, and bilateral lower extremity swelling.

Diagnostic tests

The American Thoracic Society (ATS) has put forth PH screening guidelines for patients with SCD with the rationale that a link exists between elevated tricuspid regurgitant jet velocity (TRV) and mortality [57]. At this time, the ATS recommends a one-time transthoracic Doppler echocardiograph (TTE) for asymptomatic pediatric patients (age 8–18) and follow-up evaluation based on the results. Once an individual reaches the age of 18, they recommend a TTE once every three years or at a shorter interval depending on the results.

The preferred initial test in the diagnosis of PH is TTE (Figure 3). An elevated TRV can be used to estimate right ventricular systolic pressure or pulmonary artery systolic pressure. In adults, a TRV between 2.5–2.9 m/s can identify 25–44% of patients with a mean pulmonary artery pressure ≥ 25 mm Hg. However, values > 3.0 m/s identify about 75% of patients with PH. In adults, even borderline values (> 2.5 m/s) are associated with early mortality [58]. N-terminal-pro-brain natriuretic peptide (NT-pro-BNP) can be used as a screening test for PH when TTE is unavailable or if sonographers are incapable of obtaining adequate images [59]. A serum NT-pro-BNP level ≥ 160 pg/mL detects PH with a sensitivity and specificity of 57 and 91 percent, respectively [42]. However, it is important to note that NT-pro-BNP may be falsely elevated in patients with renal insufficiency or if the patient has pre-existing left heart failure. Obesity may also cause false negatives. Nonetheless, the gold standard for diagnosis of PH is right heart catheterization. The hemodynamic definitions of pre- and postcapillary PH can be seen in Table 3 [60].

Another test used in the evaluation of PH is the six-minute walk test (6MWT). It is intended to evaluate the distance a patient can walk and examines oxygen desaturation with exertion. A study compared the distance walked by 17 patients with SCD alone in comparison to 26 patients with SCD plus PH; they found that the group with SCD plus PH walked a shorter distance during the 6MWT (320 versus 435 meters). They also noted that the distance walked in the 6MWT was inversely correlated to the mean pulmonary arterial pressure (mPAP) measured by right heart catheterization [61].

Management

Patients with SCD have an increased risk of mortality if they develop PH. Risk factors associated with increased mortality are: TRV ≥ 2.5 m/s, NT-pro-BNP ≥ 160 pg/ml, or RHC-confirmed PH. Therefore, the American Thoracic Society has published an evidence-based consensus on guidelines for the management of PH in patients with SCD [57]. These treatment modalities can be divided into three categories: 1) SCD-specific therapies; 2) PH directed therapy; 3) long-term anticoagulation.

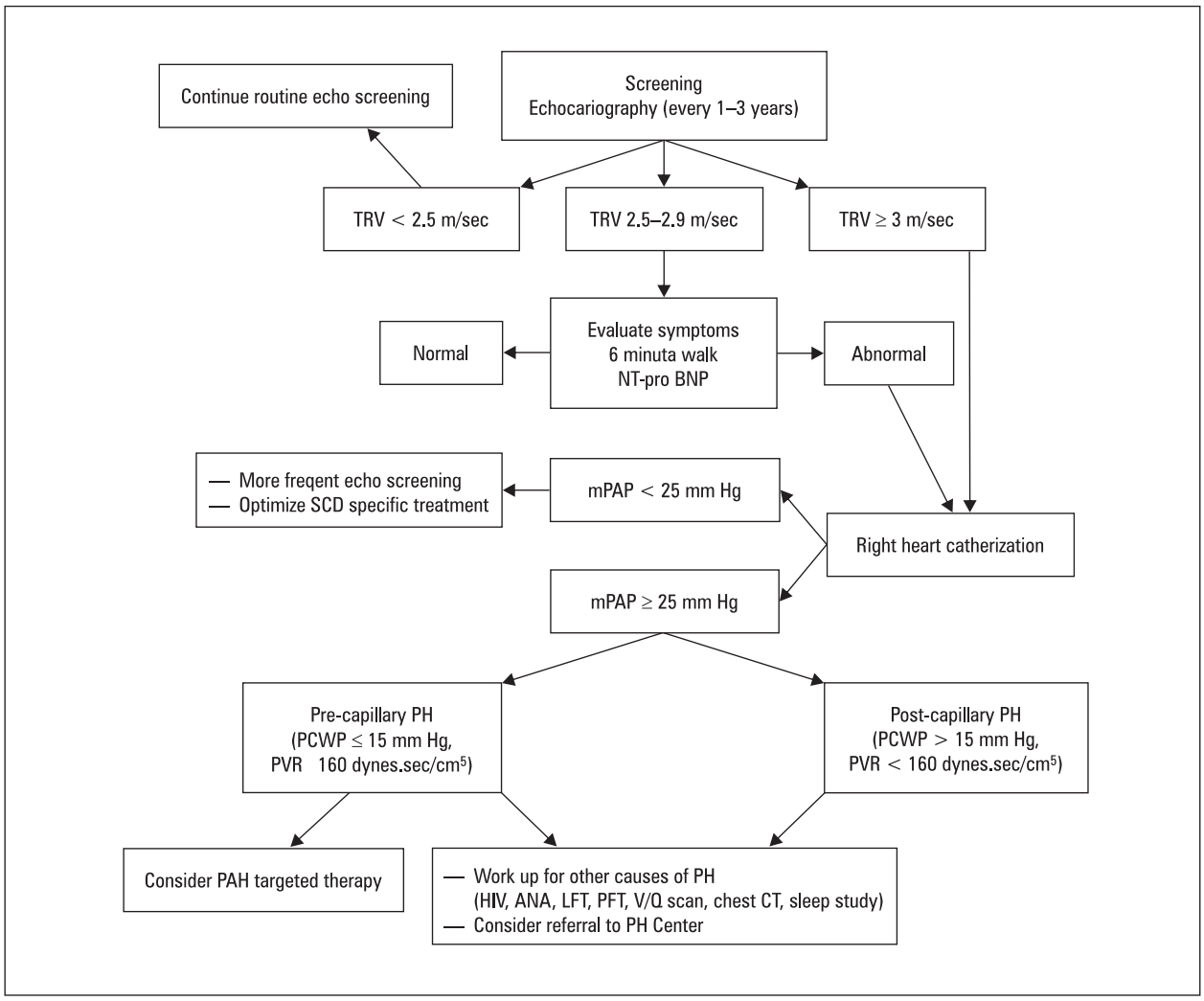

Figure 3. Diagnostic work up for pulmonary hypertension in SCD based on The American Thoracic Society guidelines [36]. TRV — tricuspid regurgitant jet velocity; SCD — sickle cell disease; PH — pulmonary hypertension; PAH — pulmonary arterial hypertension; NT-pro-BNP — N-terminal-pro-brain natriuretic peptide; mPAP — mean pulmonary artery pressure; PAWP — pulmonary artery wedge pressure; PVR — pulmonary vascular resistance; ANA — anti-nuclear antibody; HIV — human immunodeficiency virus; LFTs — liver function tests; V/Q scan — ventilation/perfusion scan

Patients with the aforementioned risk factors should undergo intensive SCD-specific therapies to reduce the severity of their hemolytic anemia via the use of hydroxyurea (HU) or chronic red blood cell transfusion regimens if they cannot tolerate HU. HU increases the concentration of fetal hemoglobin and thus, decreases the frequency of vaso-occlusive crises and acute chest syndrome occurrence thereby improving mortality in HbSS patients. The ATS practice guidelines have a strong recommendation for the use of HU in patients with an increased risk of mortality; therefore, this includes patients with PH. Per the largest trial to date, after 17.5 years of follow-up, it was reported that HU improved patient survival without accompanying serious adverse events [62]. Unfortunately, there are no clinical trials to assess the mortality benefit of chronic transfusions in the management of PH in SCD. However, a recent retrospective study of 13 HbSS patients with precapillary PH reported that chronic transfusion therapy improved their New York Heart Association functional class and hemodynamics, particularly pulmonary vascular resistance ($p = 0.01$) [63].

Pulmonary arterial hypertension (PAH) directed therapies are complicated and unfortunately, there is a paucity of data evaluating their efficacy in patients with SCD. Typical PAH treatment options include endothelin receptor antagonists (bosentan, macitentan, ambrisentan), prostacyclin agonists (epoprostenol, treprostinil, iloprost), soluble guanylate cyclase stimulators (riociguat), phosphodiesterase-5 inhibitors (sildenafil, tadalafil), and calcium channel blockers (nifedipine, diltiazem). However, in PAH patients

with SCD, it is recommended against using phosphodiesterase-5 inhibitors and calcium channel blockers. There is a study titled the Pulmonary Hypertension and Sickle Cell Disease with Sildenafil Therapy (Walk-PHaSST) trial which compared sildenafil to placebo (n = 74). The study was terminated due to increased hospitalizations for pain crises in the sildenafil group [64, 65]. Endothelin receptor antagonists like bosentan are typically used. There were also two randomized control trials comparing treatment with an endothelin receptor antagonist (bosentan) against placebo in patients with SCD with RHC with precapillary PH (the ASSET-1 trial) or postcapillary PH with a PVR of at least 100 dyn seconds cm − 5 (the ASSET-2 trial). Regrettably, the trials were prematurely terminated due to a withdrawal of support from their sponsors because of slow patient enrollment (n = 14) [66]. Therefore, due to the inherent complexity of PH management, it is recommended that these patients be referred to institutions who specialize in PAH therapy.

The American Thoracic Society currently recommends that a patient with SCD who has right heart catheter confirmed PH and venous thromboembolism (VTE), but without additional risk factors for bleeding, be placed on indefinite anticoagulant therapy rather than on therapy over a limited duration of time. This recommendation is regarded as weak with low quality evidence. They came to this conclusion after performing a meta-analysis of four randomized trials that compared long-term anticoagulation therapy to therapy over a limited duration of time. Their analysis showed that indefinite anticoagulation had less recurrent VTEs by 13.8% and, possibly, lower mortality. They believed this outweighed the 2.4% increase in bleeding risk, cost, and burden of monitoring [57].

Asthma in SCD

The diagnosis of asthma in a patient with SCD has been associated with increased rates of pain, acute chest syndrome episodes, and premature death [67]. The etiology is not quite clear; however, it has been demonstrated that patients with SCD were more likely to suffer from asthma and bronchial hyperreactivity in comparison to their ethnic matched and similar aged counterparts. Bronchial hyperreactivity is the measurement of forced expiratory volume in 1 second before and after bronchodilator use (albuterol 200 μg) and it is important to assess seeing as patients with asthma may be asymptomatic [68].

Pathophysiology

The pathophysiology of asthma in SCD is not clear. It is unknown whether its pathogenesis is secondary to the pathophysiology of SCD itself or caused by similar genetic and environmental factors that are found in standard asthma. However, the fact that asthma is more prevalent in patients with SCD patients than in their counterparts lends to the theory that the asthma is a manifestation of SCD. One theory is that the inflammatory pathway implicated in the pathogenesis of asthma is similar to that of pain. Ultimately, it is postulated that leukotrienes (cysteinyl leukotrienes and leukotriene B4), which are lipid mediators of inflammation, are elevated in both asthma and SCD pain crises and are implicated in bronchoconstriction, smooth muscle proliferation, mucous production, and vasoconstriction [68]. There is also the potential role of nitric oxide as exhaled NO has been noted to be a marker for asthma severity; however, no direct study has implicated the NO pathway in the process of asthma in patients with SCD [67].

Treatment

According to the National Institute of Health guidelines, asthma in patients with SCD should be treated similarly to how it is treated in patients without SCD. Acute exacerbations are treated with oxygen therapy, short acting beta-agonists, and systemic steroids. Magnesium sulfate may also be considered in severe refractory cases. However, it is important to note that patients with asthma who are treated with short-term corticosteroids may be at increased risk of suffering a rebound vaso-occlusive crisis within two weeks. Nonetheless, the risk of asthma itself outweighs those of corticosteroid use; therefore, they should not be withheld for these concerns [69].

Sleep-disordered breathing

Sleep-disordered breathing refers to a group of conditions characterized by complete or partial cessation of breathing while sleeping. An evaluation for sleep-disordered breathing should be considered in patients with SCD who have unexplained nocturnal and daytime hypoxemia, recurrent vaso-occlusive events, or enuresis (mainly in pediatrics). Obstructive sleep apnea (OSA) is the most common etiology of sleep-disordered breathing affecting approximately 2–4% of the adult population. Men tend to be most commonly affected; however, women and children are not impervious to this disorder [70]. OSA should be suspected in patients who snore, have witnessed

apneic, gasping, or choking episodes during sleep, or have excessive daytime sleepiness. A common screening tool used by primary care providers for OSA is the STOP-BANG questionnaire.

Sleep-disordered breathing appears to be more prevalent in patients with SCD than in the general population. One prospective study of 32 adult patients with SCD found sleep-disordered breathing in 44 percent with a mean apnea-hypopnea index (AHI) of 17/hour (95% CI 10–24/hour) [71]. Another small prospective study of 20 young adults found that 10 had an AHI > 5 consistent with OSA. This finding did not correlate with symptoms or obesity; however it was associated with reduced health-related quality of life and increased systolic blood pressure [72].

However, it remains uncertain whether the risk factors for OSA in the general population are the same as in patients with SCD. Many studies have reported that increased neck size and a higher body mass index are significant risk factors for sleep disordered breathing in adults with SCD, as in the general population [73]. Something that is more unique to SCD patients is the chronic use of opioids secondary to sickle cell pain, and it has been found that chronic use of opioids is an independent risk factor for sleep apnea [74]. Sleep-disordered breathing in patients with SCD is important to watch out for because intermittent oxyhemoglobin desaturation could result in increased vaso-occlusive episodes. There is evidence of increased rates of both vaso-occlusive episodes and cerebrovascular events linked to nocturnal hypoxemia [75]. Furthermore, sleep-disordered breathing should be evaluated by a formal sleep study in SCD patients with pulmonary hypertension.

The treatment of sleep-disordered breathing complicating SCD is essentially the same as that in patients without SCD. The use of oxygen supplementation and/or bilevel (BPAP) or continuous non-invasive positive airway pressure (CPAP) with sleep is recommended [70].

Pulmonary function tests in sickle cell disease

Screening pulmonary function tests (PFTs) are not recommended in the management of SCD by the National Heart, Lung, and Blood Institute (NHLBI) guidelines [76]. However, PFTs are routinely obtained as part of the evaluation of dyspnea in patients with SCD or for monitoring of a known diagnosis of asthma.

In children with SCD, PFTs tend to show an obstructive pattern which could be confounded by the increased incidence of asthma in children with SCD [77]. In comparison, many adults have restrictive pathophysiological findings [78]. In a multicenter study, PFTs were performed on 310 adult African-Americans with SCD irrespective of symptoms [21]. Only 10 percent of these patients had normal PFTs. The most common finding was a restrictive defect (74%). An isolated reduction in diffusing capacity for carbon monoxide (DLCO) was seen in 13%, while an obstructive pattern was seen in around 1% [79]. It is unclear though if the obstructive defects in childhood transition to restrictive defects in adulthood.

Longitudinal studies of pulmonary function in adults with SCD demonstrate an average annual decline in FEV_1 double that of the general population [78]. Furthermore, there is evidence that reduced FEV_1 in patients with SCD is associated with an increased mortality risk [80, 81].

Recurrent episodes of ACS with pulmonary infarctions may lead to chronic scarring and pulmonary fibrosis. These may result in a restrictive pattern with reduced diffusion capacity for carbon monoxide on PFT, and scattered areas of honeycombing on high-resolution computed tomography (HRCT) of the chest.

Despite these observations, the importance of abnormal PFTs in SCD remains unknown. This could be because of inconsistent study designs and classification strategies when comparing results across studies, and a lack of longitudinal data. Further research is required to find associations between abnormal lung function, respiratory symptoms, and SCD outcomes.

Conclusion

SCD, as demonstrated in this review, results in a spectrum of pulmonary diseases that include acute chest syndrome, respiratory infections, and pulmonary vascular diseases that can worsen other respiratory conditions such as asthma, lung function, and sleep-disordered breathing. There have been advances in the understanding and management of these diseases; however, more transitional research and clinical trials are needed to prevent and find more effective and better tolerated therapies. Knowledge of these pulmonary complications and maintaining a high clinical suspicion are important in preventing long-term complications and improving patient quality of life.

Conflict of interest

None declared.

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VIA MEDICA

REVIEW

Aleksandra Małolepsza[1], Aleksandra Kudrycka[1], Urszula Karwowska[1], Tetsuro Hoshino[2], Erik Wibowo[3], Péter Pál Böjti[4], Adam Białas[5], Wojciech Kuczyński[1]

[1]Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, Lodz, Poland
[2]Department of Sleep Medicine and Sleep Disorder Center, Aichi Medical University, Aichi, Japan
[3]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand
[4]Department of Neurointervention, National Institute of Clinical Neurosciences, Budapest, Hungary
[5]Department of Pathobiology of Respiratory Diseases, Medical University of Lodz, Lodz, Poland

The role of screening questionnaires in the assessment of risk and severity of obstructive sleep apnea — polysomnography versus polygraphy

Abstract

Obstructive sleep apnea (OSA) is a disease of significant importance, which may lead to numerous severe clinical consequences. The gold standard in the diagnosis of this sleep-related breathing disorder (SRBD) is polysomnography (PSG). However, due to the need for high expertise of staff who perform this procedure, its complexity, and relatively low availability, some simpler substitutes have been developed; among them is polygraphy (PG), which is most widely used.

Also, there is a variety of questionnaires suitable to assess the pre-test probability and severity of OSA. The most frequently used ones are the STOP-BANG questionnaire (SBQ), NoSAS questionnaire, and Berlin questionnaire (BQ). However, they have different sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) when being used in various populations.

The aim of this study is to provide a concise and clinically-oriented review of the most frequently used questionnaires, with special attention to its strengths and limitations. Moreover, we discuss whether PSG or PG would be more preferred for confirming OSA diagnosis with the highest likelihood.

Key words: obstructive sleep apnea, polysomnography, polygraphy, STOP-BANG, NoSAS

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Introduction

Obstructive sleep apnea (OSA) is a disease of significant importance, characterized by repetitive pauses in breathing during sleep, caused by upper respiratory tract collapses [1]. Considering the prevalence of OSA and its physiologic consequences, in certain patients, the time to establish correct diagnosis in polysomnography (PSG) is of great clinical importance. According to some previous estimates, OSA affects 3 to 7% of adult men and 2 to 5% of adult women in the general population [2]. There is also an association between the risk of OSA development and age, accounting for even higher disproportions among genders (78% in women to 90% in men) [3, 4].

OSA is characterized by the recurrent cessation of breathing (apneas) or partial upper airway obstruction (hypopneas) during sleep. Apnea-hypopnea index (AHI) is a widely used measurement for indicating the severity of OSA [1, 5]. Depending on the numbers of apneas and hypopneas per hour, OSAS can be classified as mild (AHI ≥ 5, to < 15), moderate (AHI ≥ 15 to < 30) or severe (AHI ≥ 30) [6].

Several risk factors for OSA development have been identified, including obesity, older age, male sex, and neck circumference [7, 8]. Various

Address for correspondence: Aleksandra Kudrycka, Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, Lodz, Poland; e-mail: aleksandra.kudrycka@stud.umed.lodz.pl

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studies also showed the association of OSA and hypertension, highlighting that elevated morning diastolic blood pressure may be one of the symptoms related to OSA [8, 9]. Other symptoms reported by patients which indicate OSA include snoring, breathing pauses noticed by a bed partner, morning headaches, and daytime sleepiness [8]. These measurable and reported features are used in some questionnaires for assessing OSA probability.

OSA leads to severe clinical consequences. Several population-based studies have reported that OSA escalates the risk of hypertension, cardiovascular events, metabolic and endocrine disorders, underscoring the need for a timely diagnosis and treatment [10–13]. Also, a variety of studies show a considerable indirect effect of OSA on traffic accidents, accidents during work and loss of productivity [14–17]. Consequently, patients with a high pre-test probability of OSA should be prioritized to sleep examinations.

According to the American Academy of Sleep Medicine Clinical Practice Guideline, sleep studies have been categorized as Type I, Type II, Type III and Type IV [18]. Type I is in-laboratory full polysomnography (PSG). It includes electroencephalogram (EEG), electrooculogram (EOG), chin electromyogram (EOM), electrocardiography (ECG), respiratory airflow, respiratory movements, leg movements, oxygen saturation and notification of body position [19]. Type II studies use the same monitoring sensors as Type I, but are unattended and can be performed outside of the sleep laboratory [18]. Type III studies use devices that measure limited cardiopulmonary parameters at a minimum of four channels (airflow, respiratory effort, pulse rate and oxygen saturation) [19]. They are divided into cardiorespiratory polygraphy (PG) and portable home monitors. Type IV studies are limited channel devices, which further include oxygen saturation, pulse rate, single respiratory effort signal or airflow. All the above-mentioned studies are collected in Table 1.

The gold standard for OSA diagnosis is PSG. Moreover, the clinical application of this method goes beyond OSA. PSG is recommended not only for the detection of sleep-related breathing disorders (SRBD) like OSA, central sleep apnea syndrome, Cheyne-Stokes respiration and alveolar hypoventilation syndrome, but also for narcolepsy, parasomnias, sleep-related seizure disorders, restless legs syndrome and periodic limb movement sleep disorder [20]. However, PSG is a relatively expensive and not widely available procedure, which requires well-trained personnel. Furthermore, in the time of decreased availability of health service, like during the pandemic of SARS-CoV-2, PSG would be even more unobtainable. Polygraphy (PG) is one of the examples of type 3 devices, and has been proposed to be a substitute for PSG for assessing patients with a high pre-test probability of OSA [21]. These devices do not detect arousals during sleep, and the AHI obtained from them is usually lower than the result achieved from PSG [20]. Therefore, the patients still have to undergo PSG and the time for proper diagnosis extends. The main advantage of using PG, however, is cost-effectiveness and feasibility of use [22].

Review of the literature of the field shows that there is a variety of questionnaires suitable for assessing the pre-test probability and severity of OSA (Table 2). The questionnaires are easy-to-use and low-cost tools used by sleep specialists all over the world, however, they have different sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) when being used in various populations.

Table 1. Categories of sleep studies

Type 1	Type 2	Type 3	Type 4
Stand in-laboratory technician-attended PSG	Full unattended, ambulatory overnight PSG	Cardiorespiratory PG or portable home monitors	Limited channel devices
Consists of: EEG, EOG, EMG, ECG, respiratory airflow, respiratory movements, leg movements, oxygen saturation and notification of body position	Consists of basic channels named in type 1	Minimum four channels: airflow, respiratory effort, pulse rate and oxygen saturation	Consists of: oxygen saturation, pulse rate, single respiratory effort signal and/or airflow
Optional parameters: more EEG channels, leg EMG, body position channel, snoring detection	Optional channels may differ between available technologies	Optional channels: body position, one electrophysiological channel (e.g. ECG or leg EMG), actigraphy	Optional channels: body position, snoring sensor and/or photoplethysmographic pulse wave

Table 2. Questionnaires used to assess pre-test probability and severity of OSA

Questionnaire name	Scoring	Cut-off value	Advantages	Disadvantages
STOP-Bang	From 0 to 8 points	3 points	Helpful as a screening tool for detection of OSA in sleep clinic and surgical population. The greater the score, the greater probability of severe OSA	Composed of subjective and objective responses.
NoSAS	From 0 to 17 points	7 points	Easy to use because of consisting only 5 items. Nearly all of the items can be easily measured and are objective. It can be applied in demanding populations. (e.g. major depression)	
Berlin questionnaire	**High risk:** if there are 2 or more categories where the score is positive. **Low risk:** if there is only 1 or no categories where the score is positive.	—		Nearly all of questions can be subjectively understood.

Therefore, the aim of our study is to provide a brief and clinically-oriented review of the most frequently used questionnaires for OSA examination, with special attention to its strengths and limitations. Moreover, we discuss whether PSG or PG should be used to confirm the diagnosis of OSA with the highest likelihood.

STOP-Bang questionnaire

The STOP questionnaire was developed due to a need for creating a user-friendly, quick and concise questionnaire for OSA screening in surgical patients at preoperative clinics [23, 24]. It includes four „yes/no" questions referring to snoring, tiredness, observed apnea and pressure (STOP). The STOP-BANG was developed to further improve the sensitivity of this questionnaire and to detect patients, especially with moderate and severe OSA [23]. It consists of subjective perception as well as clinical characteristics, with a total of 8 items. The acronym BANG stems from the first letters of the following features: body mass index, age, neck circumference (in male ≥ 43, in female ≥ 41), gender (BANG), which are assessed while completing this questionnaire. These features are also described by „yes/no" answers which make the scale quick and simple to fill out. For each question, answering "yes" scores 1 and "no" response scores 0. Score 1 is obtained for age > 50 years old, neck circumference in male ≥ 43 cm and in female ≥ 41 cm and BMI > 35 kg/m^2. The total score ranges from 0 to 8 points (Table 3).

Numerous studies indicated the widespread use of STOP-BANG questionnaire (SBQ) [25–28]. For example, SBQ has been used for detecting OSA in pregnant women (second trimester), in highway bus drivers, in obese and surgical patients [25–29]. Additionally, SBQ is thought to be an excellent tool for screening moderate to severe OSA in adults with Down Syndrome [30]. Despite validation in multiple various populations, SBQ appears to be less useful in patients with chronic kidney disease and end-stage renal disease [31]. On the contrary, a study conducted on patients with atrial fibrillation reported high sensitivity (89%) at the cost of low specificity (36%) [32]. The PPV was 89%, and the NPV was 36%.

SBQ is also commonly used in the sleep clinic, where the prevalence of OSA is high. In the study conducted by Reis *et al.*, score ≥ 3 had a sensitivity and PPV for all OSA of 93.4% and 86.6%, respectively [33]. The increase in the SBQ score accompanies the rise in the probability of OSA, to 95% with a score of 6. Moreover, with higher SBQ score, the greater the probability of severe sleep apnea would be. Reis *et al.* also showed that an SBQ score of 3 and 2 had an NPV for moderate or severe OSA of 85.3% and 91.7%, respectively. It means that a score lower than 3 showed high discriminative power to exclude moderate to severe OSA. Similar results were obtained by Farney *et al.* because their research probability of having OSA in patients with a score of ≥ 3 was 85.1% [34]. Also, as in a previous study, with any score of > 3, the probability of detecting severe OSA continuously increases. Recently, we assessed

Table 3. STOP-Bang questionnaire

STOP	Do you SNORE loudly (louder than talking or loud enough to be heard through closed doors)?	Yes	No
	Do you often feel TIRED, fatigued, or sleepy during daytime?	Yes	No
	Has anyone OBSERVED you stop breathing during your sleep?	Yes	No
	Do you have or are you being treated for high blood PRESSURE?	Yes	No
BANG	BMI more than 35kg/m^2?	Yes	No
	AGE over 50 years old?	Yes	No
	NECK circumference >16 inches (40cm)?	Yes	No
	GENDER: Male?	Yes	No

the SBQ's accuracy in positional OSA in adults [35]. As in previous studies, we used a cut-off score of 3, and we found high sensitivity (96.9%), but the specificity was only 16.7% in our study population. For the probability of OSAS diagnosis with SBQ≥3, the PPV was 79.2% and NPV was 62.0%. In the study conducted by Boyton *et al*. using a cut-off of ≥ 3 points, for AHI levels of > 5, > 15, and > 30, respective sensitivities were 82.2, 93.2 and 96.8% and specificities were 48.0%, 40.5%, and 33.1% [36]. PPV and NPV for AHI > 5 were 79.2% and 28.3%, for AHI > 15 were 52.2% and 66.7%, for AHI > 30 were 36.4% and 96.3%, respectively.

When it comes to the general population, Tan *et al*. showed the sensitivity of a STOP-Bang score of ≥ 3 was 66.2% for detecting AHI ≥ 15, and 69.2% for detecting AHI ≥ 30. The specificities were 74.7% and 67.1%, respectively. The NPVs were 85% for moderate-to-severe OSA and 94.8% for severe OSA. The PPVs were 50.6% and 20.2%, respectively [37]. Investigations carried by Silva *et al*. [38] revealed that the sensitivity of SBQ score ≥ 3 was 87% to detect moderate-to-severe OSA and 70.4% to detect severe OSA. The specificities were 43.3% and 59.5%, respectively. However, there is an insufficient amount of data in the general population and further investigation is needed.

In a meta-analysis of seventeen studies including a total of 9,206 patients, the accuracy of the STOP-Bang questionnaire was validated by PSG [39]. In the sleep clinic population, the pooled sensitivity of a STOP-Bang score ≥ 3 to predict any OSA, moderate-to-severe and severe OSA was 90%, 94% and 96%, respectively, whereas the pooled specificity was relatively low (49%, 34% and 25%, respectively). The PPVs for any OSA, moderate-to-severe, and severe OSA were as follows: 91%, 72% and 48%, whereas the NPVs were 46%, 75% and 90%, respectively. This review also showed relatively high sensitivity of SBQ in detecting OSA in the surgical population (91%). The specificity at the same cut-off is modest, ranging from 32% in the surgical population to 34% in the sleep clinic. In another meta-analysis, the researchers also observed that the STOP-BANG has great sensitivity for detecting OSA, but its limitation is specificity [40]. They showed that SBQ is superior for detecting mild, moderate, and severe OSA than other questionnaires, but has the significant impact on the population on which it is used. Summarizing, the discussed questionnaire is the best screening tool for the detection of OSA in the sleep clinic and surgical population.

NoSAS

The NoSAS was developed as a new screening tool for recognizing patients at risk of sleep-disordered breathing [41]. The NoSAS score consists of five items and a certain amount of points is given for each item (Table 4). Neck circumference > 40 cm is rated at 4 points, body mass index (BMI) between 25 and < 30 kg/m2 — 3 points, BMI ≥30 kg/m^2 — 5 points, 4 points for being older than 55 years, and 2 points for being male. Consequently, the total score ranges from 0 to 17 points.

In the HypnoLaus study conducted on 2,168 participants, a score of 8 was used as a threshold [41]. The score had an AUC of 0.74, a PPV — 0.47 and an NPV — 0.90. Similar results were obtained from the EPISONO cohort — the NoSAS score had an AUC of 0.81, a PPV of 0.33 and an NPV of 0.98. Additionally, in this research, the NoSAS was compared with the STOP-BANG questionnaire and Berlin questionnaire, and found to have a significantly better outcome. The same threshold was used in a different study by Peng *et al*., and the results were as

Table 4. NoSAS questionnaire

Feature	Points
Neck circumference	4
BMI 25 to < 30 kg/m^2	3
BMI ≥ 30 kg/m^2	5
Snoring	2
Age > 55 years	4
Sex (male)	2

follows: to predict AHI ≥ 5, AHI ≥ 15 and AHI > 30, the sensitivity and specificity were 0.590 and 0.707, 0.649 and 0.626, and 0.644 and 0.562, respectively [42]. When the AHI ≥ 5 was used for diagnosing sleep-disordered breathing, the NoSAS score had the largest area under the curve compared to other questionnaires in the study (the Berlin questionnaire was the second one). Another study in patients referred by primary care physicians to the sleep unit by Coutinho Costa [43] demonstrated the sensitivity and PPV were 94.3% and 87.6% for all OSA severity categories, using a cut-off value of 7 points. With the same cut-off, the NPV for all OSA was 50%. In another study conducted on a group of patients suspected of sleep-disordered breathing, the NoSAS showed 71.6% sensitivity, 68.7% specificity, PPV 89.0% and NPV 40.7% for detecting OSA [44].

The main advantage of the NoSAS questionnaire is its small number of items which can be easily and objectively measured. Additionally, due to its ease of use, it can be applied in demanding populations, for example in patients with major depression [45].

In a study conducted by Tan *et al*. [46] in a multi-ethnic Asian cohort, the sensitivity, specificity, NPV and PPV of the NoSAS score to predict severe SRBM were 69.2%, 73.1%, 95.2%, and 23.7%, respectively. Therefore, the researchers proved that NoSAS performed similarly to the STOP-Bang and Berlin questionnaires. One of the major limitations of this study, however, is that they used type 3 portable monitors.

Berlin questionnaire

The Berlin questionnaire (BQ) was initially developed in 1999 to identify patients at risk for OSA in primary care [47]. The Berlin questionnaire is divided into three categories (Table 5). The first of them is related to snoring, the second part is about sleepiness and fatigue, and the last one is about the presence of hypertension. In category 1, high risk was defined as persistent symptoms in two or more questions about their snoring. In category 2, high risk was defined as persistent waketime sleepiness, drowsy driving, or both. In category 3, high risk was defined as a history of high blood pressure. Patients at high risk in at least two categories are considered to be also at elevated risk for OSA.

There are numerous studies that evaluated the Berlin questionnaire validity for OSA risk in sleep clinic populations [48–52]. Saleh *et al*. showed that the sensitivity, specificity, PPV and NPV were as follows: 97%, 90%, 96% and 93% against AHI > 5 [48]. A similar sensitivity for predicting OSA was found by El-Sayed (95%), but they noted a sensitivity of only 23%. The PPV and NPV in the latter study were 92% and 33%, respectively [51]. The researcher also assessed these parameters at AHI > 15 and AHI > 30 cut-offs. The BQ had high sensitivity for detecting moderate-to-severe (95%) and severe OSA (97%), but very low specificity for detecting moderate-to-severe (7%) and severe OSA (10%). In a study by Amra *et al*., the sensitivity, specificity, PPV and NPV of the BQ for OSA diagnosis with AHI > 5 were found to be 84%, 62%, 96%, 25%, respectively [50]. In contrast, the values at AHI ≥ 15 were 87.9%, 36.7%, 75.3%, 58.0% and at AHI ≥ 30 were 87.8%, 26.5%, 51.5%, 70.9%. The study conducted by Ng *et al*. showed that the BQ was unreliable in patients in predicting OSAS by PSG-AHI [53]. A different study demonstrated that the BQ has a high sensitivity (87.2%), but low specificity (11.8%) with PPV 73.2% and an NPV 25.0% [54].

There was also a study carried out on the general population [55], which concluded that the high-risk group based on the BQ predicted an AHI ≥ 5 with a sensitivity of 69% and specificity of 83%. On the other hand, a study in a generally healthy elderly population revealed that the BQ is not a satisfactory tool to predict OSA [56]. The BQ is also considered to be a poor predictor of OSA in a random group of patients undergoing pulmonary rehabilitation [57].

It is also worth highlighting that OSA was also found to be associated with idiopathic intracranial hypertension (IIH) [58]. The sensitivity of the BQ in IIH patients was 83.3%, the specificity was 58.3%, the PPV was 75%, and the NPV was 70%, respectively [59].

In the meta-analysis conducted by Senaratna et al. [60], the Berlin questionnaire was proven to have good sensitivity for detecting clinically rel-

Table 5. Berlin questionnaire

Category 1					
Do you snore?	Yes	No	Don't know		
Your snoring is...	Slightly louder than breathing	As loud as talking	Louder than talking	Very loud, can be heard in adjacent rooms	
How often do you snore?	Nearly every day	3–4 times a week	1–2 times a week	1–2 times a month	Never or nearly never
Has your snoring ever bothered other people?	Yes	No			
Has anyone noticed that you quit breathing during your sleep?	Nearly every day	3–4 times a week	1–2 times a week	1–2 times a month	Never or nearly never
Category 2					
How often do you feel tired or fatigued after your sleep?	Nearly every day	3–4 times a week	1–2 times a week	1–2 times a month	Never or nearly never
During your wake time, do you feel tired, fatigued, or not up to par?	Nearly every day	3–4 times a week	1–2 times a week	1–2 times a month	Never or nearly never
Have you ever nodded off or fallen asleep while driving a vehicle?	Yes	No			
If yes, how often does it occur?	Nearly every day	3–4 times a week	1–2 times a week	1–2 times a month	Never or nearly never
Category 3					
Do you have high blood pressure?	Yes	No	Don't know		

evant OSA (≥ 15 AHI) in the sleep clinic population. In the other populations, it had modest-high sensitivity for detecting clinically relevant OSA. Additionally, its specificity was low in all populations. In another meta-analysis, the BQ with the Sleep Disorders Questionnaire were the two most accurate questionnaires in preoperative use, but the researchers also observed that no single prediction tool functions as an ideal preoperative test [61].

Sleep Apnea Clinical Score

The Sleep Apnea Clinical Score (SACS) is a relatively new screening tool which aims to predict the presence of OSA, based on snoring, witnessed episodes of apnea, neck circumference and systemic hypertension [62]. Depending on the OSA severities indicated by AHI levels, the SACS had the sensitivity ranging from 39% to 51% and specificity ranging from 90% to 88% in primary care population [63]. In the study conducted on 91 patients with COPD, the SACS performed better than the BQ and ESS in predicting OSA [62]. However, the data regarding this questionnaire are limited and it is required to conduct more studies assessing a predicting role and utility compared with other scales.

Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) consist of 8 items in which patients rate their tendency to falling asleep in certain situations during daytime. Each item is rated from 0 to 3, where '0' indicates no probability of falling asleep and '3' indicates high probability [64]. The score greater than 10 is a predictor of the presence of excessive daytime sleepiness. The studies showed that this questionnaire is not a useful tool neither for OSA diagnosis nor to assess its severity [65, 66]. On the other hand, Hardinge *et al*. measured the intensity of daytime sleepiness before and after continuous positive airway pressure (CPAP) and came to conclusion that it is a great tool for monitoring the effectiveness of OSA treatment [67].

Discussion

It is worth pointing out that all of the presented questionnaires differ from each other in terms of objectivity of the answers. The STOP-BANG has 3 of 8 points which are subjective responses, the NoSAS has only 2 of 17 points which are subjective responses, whereas BQ is practically composed of questions which can be subjectively understood (despite the occurrence of hypertension). That creates a problem in understanding or subjective perception of a certain ailment.

The screening questionnaire for OSA should be accessible to perform, precise and appropriate for different populations. In our review, most of the presented studies focused on the validation of questionnaires in the sleep clinic patients, where the prevalence of OSA is high. Sleep clinics may demand questionnaires of high sensitivity, like SBQ, in order to accurately diagnose patients with OSA. Additionally, when the result of SBQ is 5 or higher, it may prompt clinicians to carry out PSG sooner, because the higher the score, the greater probability of severe OSA would be. In some populations, for example in the surgical population, time of predicting OSA is crucial. SBQ is a quick and verified tool for predicting this SRBM and thus will be clinically convenient and applicable under time-sensitive situation. On the other hand, in the general population, the high specificity of questionnaire may prevent unnecessary referral for PSG.

One of the mentioned studies [41], which was carried out on a sizable population, indicated that the NoSAS, as a new screening tool, had greater diagnostic accuracy than the SBQ or BQ. It consists of only 5 items, practically all of them are objective and it seems to be a very quick, easy and precise tool for prediction of OSA. In a different study, conduced on adult patients referred to the sleep center, the NoSAS showed a better discrimination capacity compared to the Berlin and STOP-Bang [68].

None of the presented questionnaires was sensitive and specific enough to desist further investigations. If PSG is available, it should be used as a gold standard in the diagnostic path. In case of the absence of this expensive and time-consuming examination, PG should be applied as a faster and easier option.

In one of the studies [69], the researchers provided a valuable finding that a symptomatic patient with BMI lower than 25.0 kg/m^2 has a very low chance (< 3%) of AHI ≥ 15 events/h in the lateral sleep position. Therefore, positional treatment can be an alternative applied prior to conducting PSG in that group of patients.

The ESS is a well described tool for assessing daytime sleepiness, but it is not recommended as a questionnaire for OSA diagnosis.

Summary

The SBQ seems to be a useful screening tool in the sleep clinic and surgical population. However, the current literature review shows that studies suggesting which questionnaire can be useful in the general population are sparse. Therefore, further research in this field would be of great clinical importance. The presented questionnaires may have some utility in assessing the likelihood of OSA in patient, albeit they do not give satisfactory level of certainty in the detection or exclusion of this SRBD. PSG remains a gold standard for OSA detection, and PG should constitute the first alternative only in case of its unavailability.

Conflict of interest

The authors declare no conflict of interest.

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Dimitrios Velissaris[1], Diamanto Aretha[2], Konstantinos Tsiotsios[1], Charalambos Gogos[1], Vasileios Karamouzos[2]

[1]Department of Internal Medicine, University Hospital of Patras, Rion, Greece
[2]Intensive Care Unit, University Hospital of Patras, Rion, Greece

Continuous positive airway pressure in the treatment of COVID-19 patients with respiratory failure. A report of six cases with excellent outcome

Abstract

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently considered a significant threat to global health and global economy. This new rapidly spreading virus causes enormous stress to healthcare systems as large number of patients present with respiratory failure, needing intubation and mechanical ventilation. While the industry is racing to meet the rising demand for ventilators, all the alternative respiratory support modalities are employed to save lives in hospitals around the globe. We hereby report 6 patients who were diagnosed with SARS-CoV-2 and treated with continuous positive airway pressure in a negative pressure isolated room in a tertiary center in western Greece. The rapid progression of mild flu-like symptoms to respiratory failure in all patients was controlled with the use of continuous positive airway pressure making this strategy a reasonable alternative to respiratory failure due to SARS-CoV-2 as it may avert intubation and mechanical ventilation.

Key words: respiratory failure, coronavirus disease 19, continuous positive airway pressure

Adv Respir Med. 2021; 89: 197–202

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently considered a significant threat to global health and global economy. This new rapidly spreading virus causes enormous stress to healthcare systems as large number of patients present with respiratory failure, needing intubation and mechanical ventilation. While the scientific community is concentrated on finding a specific treatment and/or a vaccine for the new virus, our only option is supportive therapy resulting in a high demand for intensive care unit (ICU) beds and ventilators. Attempting to mitigate this need, all the alternative respiratory support modalities are employed to save lives in hospitals around the globe. Continuous positive airway pressure (CPAP) devices are frequently used in patients with respiratory failure, and although conflicting data exist for their use in coronavirus infection, in a resource scarce environment, they could be a choice to avert intubation and save patients.

Case series

Six patients suffering from fever, cough, and mild respiratory distress, presented to the Emergency Department (ED) of a tertiary center in western Greece during March 2020. All patients were diagnosed positive for SARS-CoV-2, and on admission were alert, oriented, and hemodynamically stable. Their demographic data, past medical history, and clinical examination findings on admission are presented in Table 1. All patients were

Address for correspondence: Vasileios Karamouzos, Intensive Care Unit, University Hospital of Patras, Rion, Greece; e-mail: vkaramouzos@hotmail.com

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Table 1. Demographic data, past medical history, and major findings of clinical examination on admission

	Age/sex	BMI/tobacco use	Past medical history	Previous medication	Symptoms on admission
Patient 1	44 / M	29.3 / No	Hypertension	Olmesartan 20 mg od	Chest pain, temperature 38.3°C, dyspnea, mild productive cough. Chest auscultation: crackles on middle and lower lobes bilaterally
Patient 2	74 / M	23.4 / Yes (10 packs/year)	Benign prostate hypertrophy, hypertension	Tamsulosin 0.4 mg od, Amlodipine 10 mg od	Diarrhea, temperature 38.8°C , myalgias, non-productive cough. Chest auscultation: lower lung crepitations
Patient 3	74 / F	25.4 / No	Hypertension, hypothyroidism	Thyrohormone 0.1 mcg od, Amlodipine/valsartan 10/160	Fatique, non-productive cough, temperature 38.2°C. Chest auscultation: crackles on middle and lower lobes bilaterally
Patient 4	64 / M	23.8 / No	Coronary disease, hypertension, dyslipidemia	Carvedilol 12.5 mg bd, valsartan 160 mg od, simvastatin 20 mg od, ASA 100 mg od	Temperature 38.3°C , mild dyspnea, non-productive cough. Chest auscultation: crackles on left lower lobe
Patient 5	79 / M	26.9 / Former smoker	Hypertension, dyslipidemia, peripheral artery Disease	ASA 100 mg od, felodipine 5 mg od, rosuvastatin 5 mg od	Temperature 38°C, non-productive cough, fatigue, mild dyspnea. Chest auscultation: crackles on lower lobes bilaterally
Patient 6	50 / M	32 / No	Hypothyroidism	Thyrohormone 0.1 mcg od	Dyspnea, non-productive cough, temperature 38.3°C. Chest auscultation: some crackles on lower lobes bilaterally

ASA — acetylsalicylic acid; bd — twice a day; BMI — body mass index; od — once daily

isolated in the COVID-19 ward of our hospital, and during their stay they developed respiratory failure. Their PO_2/FiO_2 ratio varied between 130 to 160, they were tachypneic with bilateral infiltrations on chest X-rays. Based on the clinical status and single organ involvement, a decision was made to support them with CPAP oxygen therapy via face mask. StarMed's Ventumask 30 CPAP mask with a Venturi flow driver and adjustable PEEP valve was used in all patients, and to minimize the risk of virus dispersion, all subjects were treated in a negative pressure room (Figure 1). Upon admission all patients were on hydroxychloroquine, lopinavir/ritonavir, azithromycin and ceftaroline or ceftriaxone. Oxygen saturation, blood pressure, heart rate and urine output were continuously monitored. Additionally, the respiratory rate and patient's compliance with CPAP therapy was recorded by the staff nurse. CPAP therapy was well tolerated by all patients and no signs of superinfection of any etiology were noticed based on daily clinical examination and laboratory tests. Blood gas analysis was performed twice daily and in case of any clinically significant event. The attending physicians adjusted the fraction of inspired oxygen and the level of CPAP in accordance to patient's respiratory improvement. All persons were monitored with chest X-rays. Patient number 6 was the only one that received a chest CT scan during his hospitalization (Figures 2–4). Their stay in the negative pressure room and CPAP treatment ranged from 3 to 10 days and 3 to 9 days, respectively (Table 2). All 6 patients, representing 10.3% of the total COVID-19 admissions of that period, were discharged from the negative pressure room with nasal cannula or Venturi face mask, clinically improved. Finally, after a short stay (up to ten days) in COVID-19 ward, all patients were discharged from our hospital.

Discussion

The clinical spectrum of COVID-19 is broad, ranging from asymptomatic to severe disease with high mortality. In severely ill patients exhibiting signs of cytokine storm, respiratory function can deteriorate rapidly, and the current evidence proposes that dyspneic patients over 60 years old with comorbidities have to be monitored closely, especially during the first weeks after symptoms onset [1, 2]. Respiratory viruses can cause acute respiratory distress syndrome (ARDS), and in the last decade, zoonotic coronaviruses were

A

Requirement		Setting	Flow delivered
O_2% approx	PEEP cmH_2O	Flow O_2 L/min A + B	Flow approx L/min
40%	5	11 + 8	78
	7.5	11 + 8	72
	10	12 + 6	70
	12.5	12 + 4	65
	15	12 + 4	60
	20	13 + 2	60
50%	5	10 + 18	80
	7.5	10 + 18	77
	10	10 + 16	72
	12.5	10 + 14	65
	15	10 + 12	60
	20	12 + 10	60
60%	5	9 + 26	80
	7.5	9 + 26	74
	10	10 + 24	75
	12.5	10 + 22	72
	15	10 + 22	70
	20	10 + 18	60
100%	Feed with a minimum O_2 flow of 40 L/min		

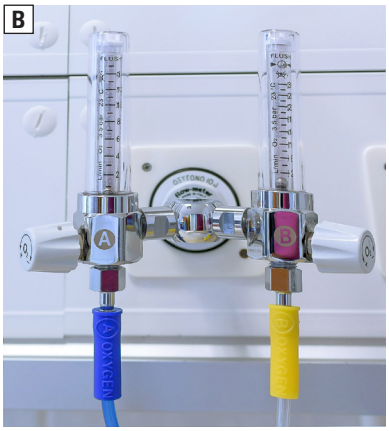

Figure 1. StarMed's Ventumask 30 CPAP mask with Venturi flow driver (black arrow) and adjustable PEEP valve (white arrow). The device is connected to a dual oxygen flow meter (**B**) and using the settings table (**A**), flows can reach up to 80 L/min and FiO_2 can be adjusted from 30% to 100%. The positive end-expiratory pressure can be set up to 20 cmH_2O and is monitored with the pressure gauge (*)

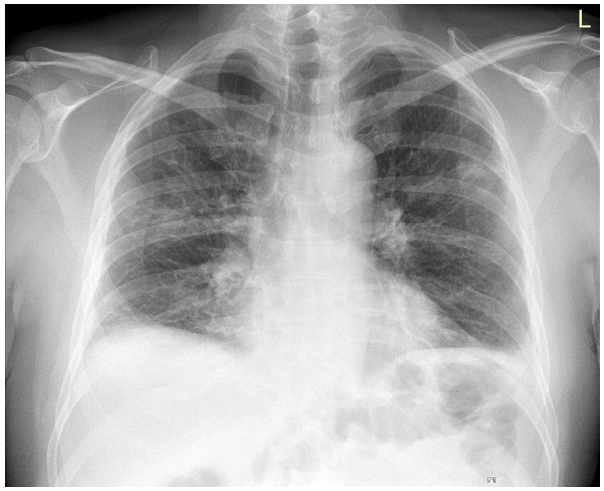

Figure 2. Chest X-ray on hospital admission (day 0)

able to cross the species barrier causing severe acute respiratory Syndrome (SARS), Middle East respiratory syndrome (MERS) and recently the pandemic COVID-19. In the event of respiratory failure, prior to intubation and mechanical ventilation in carefully selected patients, a non-invasive ventilation (NIV) or a CPAP trial could be attempted. CPAP and NIV therapy are well documented in patients with respiratory failure, in immuno-compromised patients, in weaning patients from mechanical ventilation, and in critically ill patients with mild ARDS [3, 4].

A CPAP machine maintains a positive pressure in the airway, which can be adjusted while the fraction of inspired oxygen can be raised up to 100%. A tube carries the oxygen-air mixture

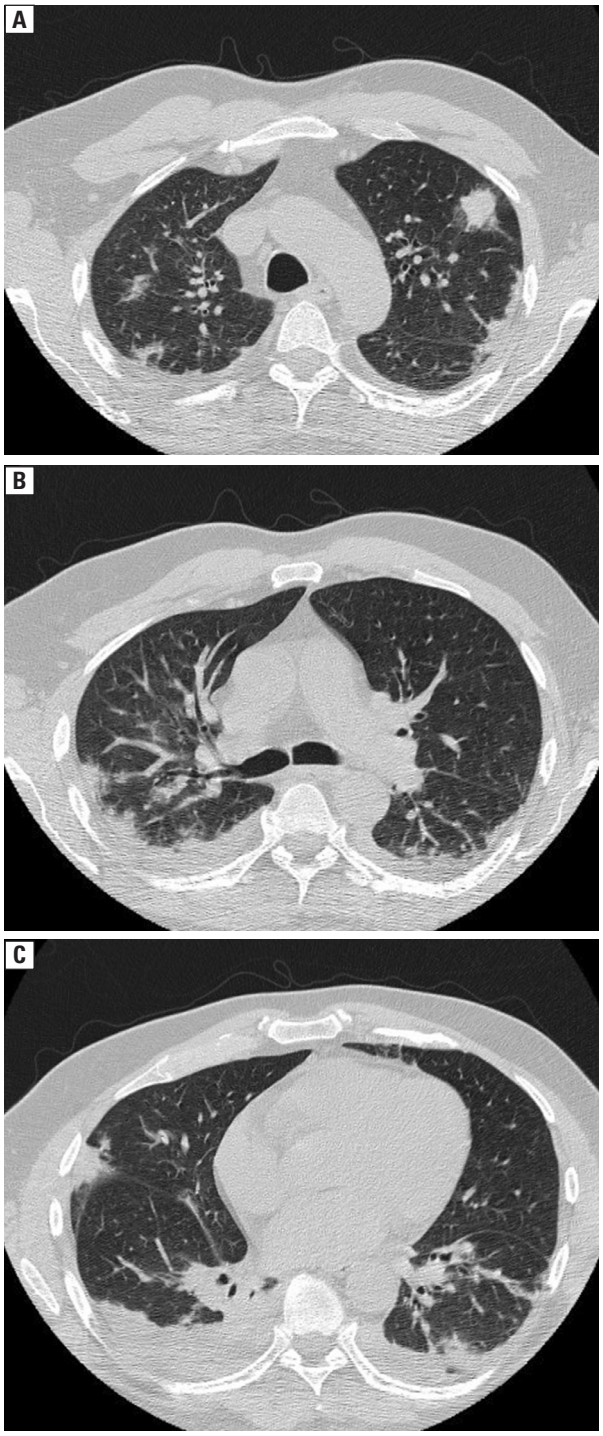

Figure 3. Chest computed tomography scan before his admission to the negative pressure room (day 4)

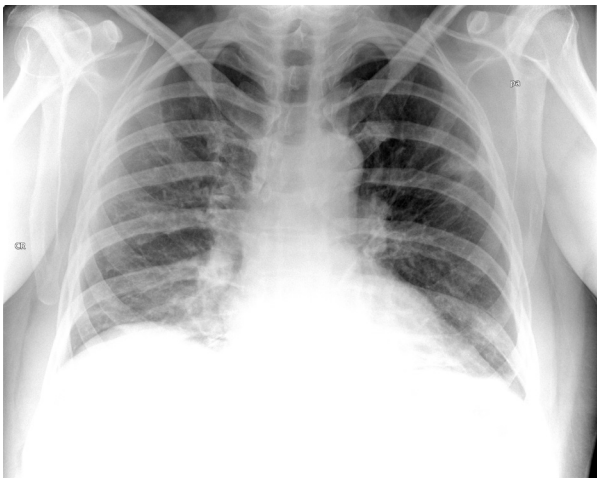

Figure 4. Chest X-ray upon discharge from the negative pressure room (day 8)

to an oronasal mask usually that must create a good seal with the patient's face. Beside oronasal masks, other frequently used interfaces include helmets, nasal masks, and full-face masks. CPAP decreases the work of breathing and improves oxygenation by ameliorating lung compliance, allows alveolar recruitment, counteracts the intrinsic positive end expiratory pressure (PEEP) and decreases preload and afterload in cases of congestive heart failure [4]. In patients presenting to the emergency department (ED) with acute respiratory failure and without signs of neurologic and/or hemodynamic compromise, a trial of CPAP should be attempted before intubation and mechanical ventilation [5]. Continuous positive airway pressure (CPAP) should be preferably used in a negative pressure isolated hospital room when treating cases of SARS-COV-2 infection due to the high dispersion of the virus when using high flow devices. Alternatively, CPAP therapy could be applied with the use of a helmet combined with a filter on the exhalation port [6].

There are limited and conflicting data regarding the use of NIV or CPAP in respiratory viral infections (RVI). In the study by Kumar *et al.*, the use of NIV in patients with severe influenza A (H1N1) showed NIV failure in up to 85% [7]. In a multicenter observational study of critically ill patients due to influenza infection hypoxemic respiratory failure, 806 of 1898 patients underwent initial NIV, and 56.8% of them required finally intubation and invasive ventilation. The more severe cases (SOFA ≥ 5) had a higher risk of NIV failure. Also NIV failure was associated with increased ICU mortality compared to invasive mechanical ventilation [8]. NIV has been shown to have positive results in the management of some patients with SARS, while in a study based on a multicenter cohort of 302 MERS critically ill patients, NIV was used initially in 35% of subjects, but the vast majority of them (92.4%) required invasive mechanical ventilation [9, 10].

In a recent retrospective observational study that included 24 patients with respiratory fail-

Table 2. Patients' data during stay in negative pressure room

	PO_2/FiO_2 on admission	Days in negative pressure room	Days on CPAP	CPAP cm H_2O	Hours per day on CPAP	Side effects	Antibiotic treatment	Anti-viral agent	Hydroxy chloroquine	PO_2/FiO_2 on discharge from negative pressure room
Patient 1	145	10	9	5–7.5	24	Nasal bridge pressure ulcer	Ceftaroline, azithromycin	Lopinavir/ ritonavir	Yes	200
Patient 2	160	6	5	5	24	None	Ceftarolin, azithromycin	Lopinavir/ ritonavir	Yes	320
Patient 3	140	7	7	7.5–10	24	Nasal bridge pressure ulcer	Ceftriaxone, azithromycin	Lopinavir/ ritonavir	Yes	250
Patient 4	150	5	5	7.5–10	24	None	Ceftarolin, azithromycine	Lopinavir/ ritonavir	Yes	180
Patient 5	160	3	3	7.5	24	None	Ceftriaxone, azithromycin	Lopinavir/ ritonavir	Yes	205
Patient 6	120	4	4	7.5	24	None	Ceftarolin, azithromycine	Lopinavir/ ritonavir	Yes	170

CPAP — continuous positive airway pressure

ure type 1 due to SARS-CoV-2, CPAP treatment successfully averted intubation in over half of the patients. All the patients were treated in a negative pressure room at the Royal Liverpool Hospital. 14 patients were weaned off CPAP and discharged. Their median time on CPAP and bed stay were 4.5 and 10.5 days, respectively [11]. Furthermore, a small two period retrospective case control study that included 52 patients (14 controls and 38 cases) showed that CPAP is feasible in deteriorating COVID-19 patients and can avoid intubation at 7 days and at 14 days. More patents in the control group were intubated or died in comparison to the experimental group (57% *vs* 23%, p = 0.043). Median use of CPAP was 5 (2–7.5) days and for 8 (4–11) hours daily [12].

Finally, new data for COVID-19 are becoming available, raising concerns regarding the lack of ICU beds worldwide, and the high mortality rates observed after intubation and mechanical ventilation [13, 14]. The current available evidence indicates that a CPAP therapy can be used in COVID-19 respiratory failure, and this strategy may avert intubation.

Conclusions

In anticipation of new studies that will shed more light on a definitive COVID-19 treatment, management principles for this new clinical entity in case of ARDS are mainly supportive and should be similar to the management of ARDS from other causes. Until a specific antiviral treatment is available, the use of invasive and non-invasive ventilation should be tailored according to patient's needs and clinical status. There is a true need for efficient trial designs to test the role of continuous positive airway pressure support in patients with respiratory failure due to SARS-CoV-2, alone or in combination with other treatment options.

Conflict of interest

None declared.

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Mandeep Singh[1], Lakhvir Kaur[1], Renuka Bajaj[2], Sumeet Arodhia[2]

[1]Pulmonary Medicine, Amandeep Medicity Hospital, Amritsar, India
[2]Amandeep Medicity Hospital, Amritsar, India

Double carbapenem regimen used as salvage therapy to treat multidrug-resistant *Klebsiella pneumoniae* causing ventilator-associated pneumonia

Abstract

Carbapenemase-producing *Klebsiella pneumoniae* is an emerging threat worldwide. The appropriate therapy for infections due to these multidrug-resistant pathogens is not well defined and depends upon the susceptibilities of individual isolates, and the choices are often severely limited. We report a case of a 8-year-old male child with ARDS with left-sided tubercular pleural effusion who developed ventilator-associated pneumonia due to multidrug-resistant *Klebsiella pneumoniae* treated successfully with a regimen comprising a combination of colistin and double carbapenem.

Key words: double carbapenem, ventilator-associated pneumonia, *Klebsiella pneumoniae*

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Introduction

Klebsiella pneumoniae is a frequent cause of nosocomial infections [1]. A rise of antimicrobial drug resistance in *Klebsiella pneumoniae* raises serious therapeutic challenges [2]. Among several mechanisms of drug resistance in *Klebsiella pneumoniae*, carbapenemases are increasingly recognized worldwide. They are particularly prevalent in *Klebsiella pneumoniae* from several geographic areas, including the Indian subcontinent and the Mediterranean countries [3]. New antibiotic options are urgently needed for the treatment of carbapenem-resistant *Enterobacteriaceae* infections. We report a case of a 8-year-old male child with ARDS with left-sided tubercular pleural effusion who developed ventilator-associated pneumonia due to multidrug-resistant *Klebsiella pneumoniae* treated successfully with a regimen comprising a combination of colistin and double carbapenem.

Case report

A 8-year-old male child on mechanical ventilation was shifted to our hospital from another hospital with a history of increasing oxygen requirement and radiological deterioration. He presented to the previous hospital with one-week history of cough, fever and breathlessness. Pleural tap was done at the previous hospital, which was positive for *Mycobacterium tuberculosis* on gene xpert. On presentation, the child was already on meropenem, vancomycin and antitubercular medication for the past 3 days. In spite of treatment, the general condition of the patient was deteriorating, thus he was shifted to our setup. On admission to our hospital, the patient was febrile, on mechanical ventilation with oxygen requirement of 70%, end expiratory pressure of 7 mm Hg and inspiratory pressure of 26 mm Hg. He was generating tidal volumes of around 200 mL. On suctioning of the endotracheal tube blood

Address for correspondence: Mandeep Singh, Amandeep Medicity Hospital, Amritsar, India; e-mail: mandeepsingh27@gmail.com

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clots were seen. His laboratory investigations revealed raised serum bilirubin of 2.1 mg/dL, aspartate aminotransferase of 313 U/L, alanine aminotransferase of 667 U/L, international normalized ratio — 1.5, total leukocyte count of 15 × $10^3/\mu$L and raised C-reactive protein of 23.9 mg/L. Endotracheal secretions showed the growth of *Acinetobacter baumannii* with colony count: > 100000, colony-forming units/ml which was sensitive to colistin, fosfomycin, minocycline and ceftriaxone EDTA sulbactum (Figure 1). The antibiotics and antitubercular therapy were changed to ceftriaxone EDTA sulbactum 1.5 mg 12 hourly, vancomycin 500 mg 8 hourly and levofloxacin 500 mg once daily, amikacin 500 mg once daily, ethambutol 600 mg once daily.

There was sudden worsening of respiratory pattern on the third day of presentation with subcutaneous emphysema. Computed tomography of the chest was done (Figure 2, 3), which showed bilateral patches of consolidation with crazy paving in both the lungs, along with pulmonary interstitial emphysema with extensive pneumo-mediastinum, minimal left pneumothorax and subcutaneous emphysema.

Intercostal chest drain was inserted on the left side. The child was not relieved of fever even after three days of changing antibiotics. Blood, pleural fluid and urine cultures were sterile. Bronchial lavage revealed the growth of multidrug-resistant *Acinetobacter baumannii* which was sensitive to colistin, ceftriaxone EDTA sulbactum and fosfomycin. Then colistin (loading dose of 6000000 IU, then 3000000 IU every 12 hours) was started, and ceftriaxone EDTA sulbactum (1.5 mg every 12 hours) was continued. As liver functions improved, isoniazid 300mg was started and aminoglycoside stopped due to risk of significant side effects in combination with collistin. The patient became afebrile after 48 hours of revising the antibiotics. Subsequently, rifampicin was re-introduced. After two doses of rifampicin 450 mg, the boy started having bleeding through the endotracheal tube, and repeated liver functions showed deterioration (international normalized ratio — 1.8, platelet count — 110000, serum bilirubin — 2.1 mg/dL, alanine aminotransferase — 209 U/L, aspartate aminotransferase- 45 U/L). On ultrasound of the whole abdomen, hepatomegaly with minimal ascites was present. Rifampicin was stopped and fresh frozen plasma was given to control bleeding. As there was a problem with ventilation, therapeutic bronchoscopy was done to remove the clots. Bleeding tendency on re-introducing rifampicin could be due to part of sepsis induced by disseminated intravascular coagulation or some idiosyncratic reaction. There was a gradual improvement in the patient's general condition. Percutaneous tracheostomy was done.

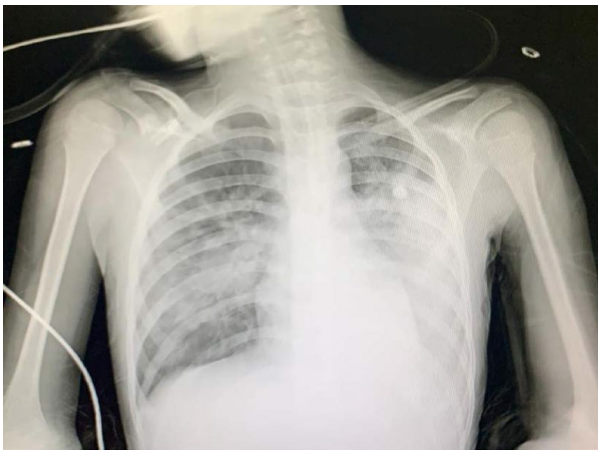

Figure 1. Chest radiography (anterior-posterior view) showing bilateral consolidation

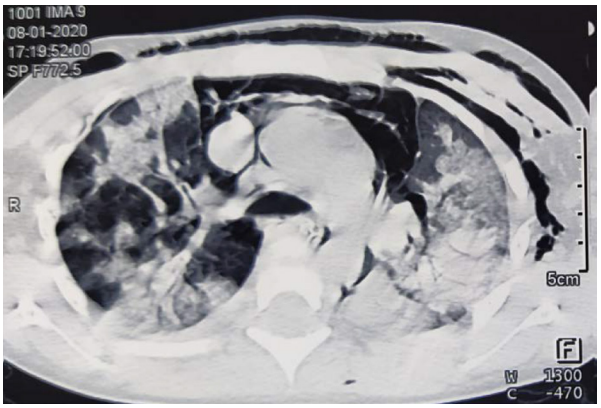

Figure 2. High resolution computed tomography of the chest showing bilateral patches of consolidation

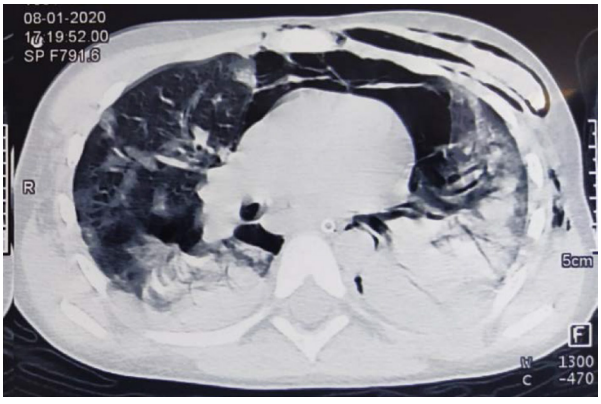

Figure 3. High resolution computed tomography of the chest showing bilateral patches of consolidation with pneumomediastinum, pneumothorax and subcutaneous emphysema

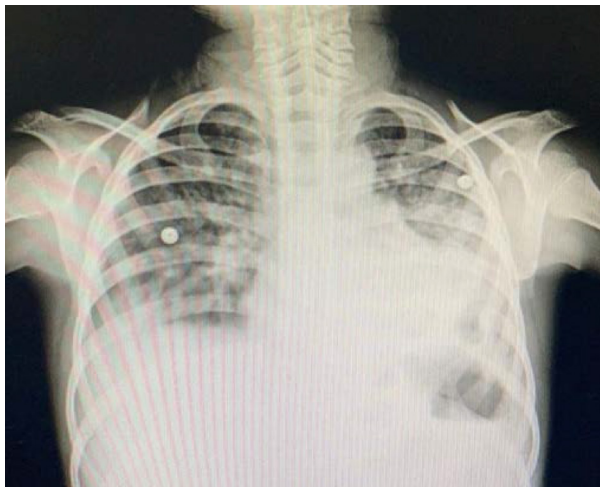

Figure 4. Chest radiography showing an increase in left lower zone infiltrates

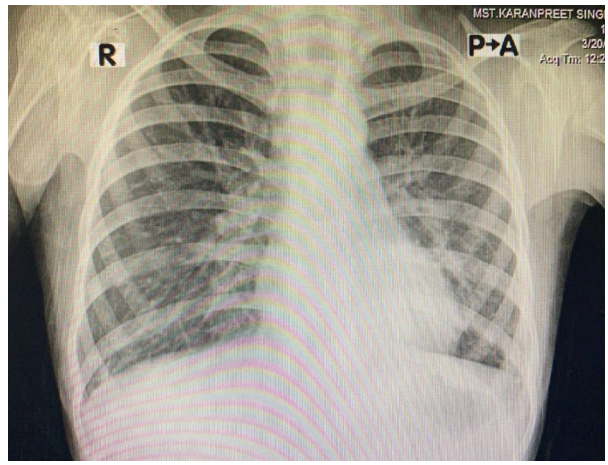

Figure 5. Follow-up chest radiography showing complete resolution of opacities

The boy started improving clinically and radiologically. In due course, pyrazinamide 750 mg once daily was started. Again, after 12 days of ceftriaxone EDTA sulbactum and colistin combination, the patient started having high-grade fever upto 104°F and oxygen requirement increased. There was worsening in inflammatory markers and on radiology (Figure 4) with an increase in left lower zone infiltrates.

Repeat tracheal aspirates showed carbapenemase-producing multidrug-resistant *Klebsiella pneumoniae* which was sensitive only to colistin (minimum inhibitory concentration ≤ 0.5 μg/mL), which was already going on. The patient was started on combination of imipenem (MIC ≥ 16μg/mL) in extended infusion, ertapenem (MIC ≥ 8 μg/mL), and colistin was continued. Fever subsided within 48 hours of starting the combination with gradual normalization of inflammatory markers. In due course, the patient was de-cannulated. Colistin and double carbepenem regimen was continued for 14 days and the child was discharged in good clinical condition. Chest radiograph done at follow-up showed almost complete clearance of infiltrates (Figure 5). Follow-up chest radiographs showed almost complete clearance of the infiltrates.

Discussion

The increasing global prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) combined with the decline in effective antimicrobial therapies is a serious public healthcare problem. According to CDC, CRE is defined as *Enterobacteriaceae* that are resistant to any carbapenem antimicrobial (i.e., minimum inhibitory concentration of ≥ 4 mcg/mL for doripenem, meropenem or imipenem OR ≥ 2 mcg/mL for ertapenem) or documented to produce carbapenemase [4]. Infections caused by these Gram-negative multidrug-resistant organisms resulted in high mortality rates, prolonged hospitalization and increased cost of care [5]. Currently, the available antibiotic options to combat these organisms are limited. So, new therapeutic approaches against these burgeoning organisms are needed. Recently, the double carbapenem regimen has been come up as a valid therapeutic option in severe infections due to pandrug-resistant *Klebsiella pneumonia* [6].

This case confers how the combination of colistin with ertapenem plus imipenem was effective and synergistic against a multidrug-resistant *Klebsiella pneumoniae* causing ventilator-associated pneumonia, even in the presence of high MIC values. The rationale for this combination has not been extensively explored, it is hypothesized that one of the carbapenem compounds distracts the carbapenemase enzyme acting as a suicide inhibitor, thus allowing and preserving the other carbapenem's activity [7]. Carbapenemase enzyme has preferential affinity for ertapenem, due to the ease of hydrolysis versus that of imipenem. Since enzyme is consumed during this interaction with ertapenem, higher concentrations of imipenem are present in the vicinity of the organism that would otherwise be recognized if copious amounts of enzyme were freely available to degrade imipenem. After that disruption caused to the outer bacterial cellular membrane by colistin allowing other drugs to reach adequate intracellular concentrations. Despite hydrolysis of carbapenems by the carbapenemase enzyme, these compounds perpetuate their bactericidal

effect. This has been demonstrated both in vitro and in animal models [8, 9].

A study was conducted to determine therapeutic strategy for pandrug-resistant *Klebsiella pneumoniae* severe bloodstream infection by Oliva *et al.*; it showed combination of colistin with ertapenem plus meropenem manifest rapid bactericidal activity, even at subinhibitory concentrations. Therefore, given the potent in vitro effect and the good clinical outcome of the patient, it suggested that colistin might be useful as an initial therapeutic add-on against pandrug-resistant organisms, rapidly decreasing the bacterial amount and limiting drug toxicity [10].

Another combination, colistin-rifampin may have a role in the treatment of multidrug-resistant *Klebsiella pneumoniae* and may possibly slow the selection of hetero-resistant subpopulations during colistin therapy. A study conducted to determine synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing *Klebsiella pneumonia* by Tascini *et al.* showed that colistin plus rifampin is the most consistently synergistic combination against KPC-producing *Klebsiella pneumoniae* isolates, including colistin-resistant strains [11]. Combination is based on the principle that perturbation of the outer bacterial cellular membrane by colistin may favor the uptake of rifampin, allowing the drug to reach sufficient intracellular concentrations to inhibit protein synthesis. But in our case, we were not able to use this regimen due to rifampicin-induced hepatotoxicity and bleeding tendencies.

A source of these multidrug-resistant organisms could be a prolonged stay in hospital, and in addition, on mechanical ventilation. We treated our patient with colistin and double carbapenem regimen for 14 days, but we stopped colistin 6 days before the completion of regimen as the patient was already on colistin for the previous 8 days and he recovered clinically as well as radiologically.

Conclusions

In conclusion, this case indicates that this regimen is a valid and effective therapeutic strategy in treating severe infections caused by carbapenemase-producing *Klebsiella pneumonia*. Contact precautions and active surveillance are common measures that should be employed for controlling the spread of these microorganisms in hospitals.

Conflict of interest

None declared.

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Zeeshan Hafeez[1], Sarwat I. Gilani[2], Lying Han[2], Gizelka David-West[3], Oleg Epelbaum[1]

[1]Division of Pulmonary, Critical Care and Sleep Medicine, Westchester Medical Center, Valhalla, New York, United States
[2]Department of Pathology Westchester Medical Center, Valhalla, New York, United States
[3]Division of Gynecological Oncology Westchester Medical Center, Valhalla, New York, United States

Malignant pleural effusion from squamous cell carcinoma of the vulva: extremely rare metastatic pattern of a rarely metastasizing cancer

Abstract

The discovery of a malignant pleural effusion indicates metastatic disease and thus invariably results in the highest possible cancer stage. Although the female reproductive tract overall is a common primary tumor site giving rise to malignant pleural effusion, vulvar carcinoma stands out for its propensity for locoregional spread rather than distant metastasis. Our case contributes to the extremely limited number of published descriptions of thoracic involvement by vulvar carcinoma, with malignant pleural effusion being a particularly unusual pattern.

Key words: vulvar carcinoma, squamous cell carcinoma, malignant pleural effusion, pleural metastases

Adv Respir Med. 2021; 89: 207–210

Introduction

Malignancy is second only to infection as the commonest cause of exudative pleural effusion [1]. Lung is the primary tumor site most closely associated with pleural metastasis, followed by breast cancer. Among extrathoracic solid neoplasms, cancer of the female reproductive tract accounts for the highest percentage of malignant pleural effusions (MPE) [2]. Epithelial ovarian carcinoma (EOC) is the most common gynecological malignancy implicated in MPE, but primaries from virtually every female reproductive organ have been reported to metastasize to the pleura [3, 4]. This list includes vulvar cancer, which is the fourth commonest gynecological malignancy in the United States after uterine, ovarian, and cervical cancers [5]. Squamous cell carcinoma (SCC) accounts for the vast majority of vulvar cancer cases. Only six percent of patients present with distant metastases and, among these, pleural involvement is one of the rarest patterns [6]. Herein we report a case of MPE due to metastatic SCC of the vulva. To our knowledge, this is only the second such case description in the English-language literature.

Case presentation

A 63-year-old Caucasian woman presented to our institution with new onset of dyspnea and non-productive cough. Additional history was significant for a 5 × 6 cm nodular right vulvar lesion with central ulceration and early extension into the ipsilateral vagina evaluated and biopsied one week previously. At that time, palpable right inguinal lymphadenopathy (LAN) was also present. Biopsy material demonstrated moderately differentiated, non-keratinizing SCC. She was afebrile, and her oxygen saturation on room air was 94%. Lung auscultation revealed decreased breath sounds at both bases. Routine laboratory evaluation was unremarkable. Frontal radiograph of the chest showed bilateral

Address for correspondence: Zeeshan Hafeez, Division of Pulmonary, Critical Care and Sleep Medicine, Westchester Medical Center, Valhalla, New York, United States; e-mail: zeeshan.hafeez@wmchealth.org

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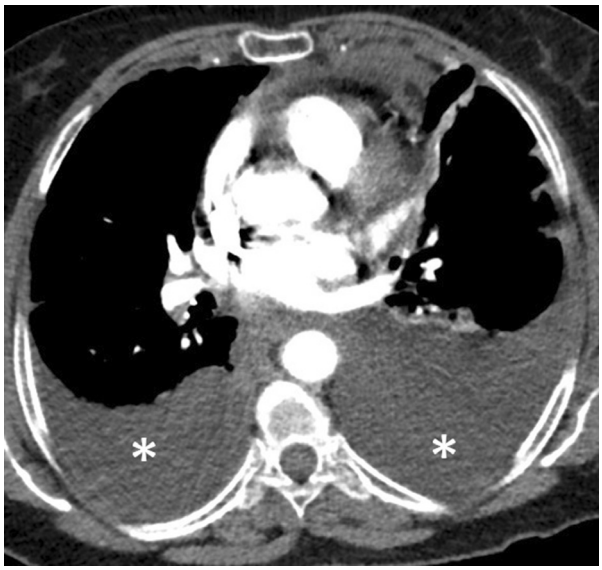

Figure 1. Chest computed tomography axial image set to soft tissue window demonstrating bilateral pleural effusions, left greater than right (asterisks)

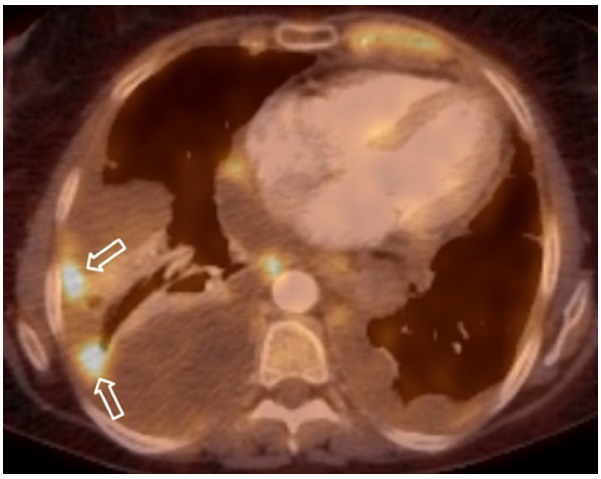

Figure 2. Representative axial fused PET/CT image showing hypermetabolic foci along the right pleural surface (arrows). The left pleural space also demonstrated areas of increased metabolic activity (not shown)

pleural effusions, left greater than right. Subsequent computed tomography (CT) of the chest confirmed this finding (Figure 1). Left-sided thoracentesis was performed, yielding exudative pleural fluid. Microscopic examination revealed scattered atypical cells with high nucleus to cytoplasm ratio, irregular nuclei, and prominent nucleoli. Rare mesothelial cells and abundant mixed inflammatory cells were also identified. Immunohistochemical staining for calretinin and WT-1 highlighted the mesothelial cells. Staining for the squamous cell marker p40 was negative in the atypical cells. These cytological findings were non-diagnostic for establishing the presence of MPE. Repeat left pleural fluid cytology yielded similar results. As the next step, ^{18}fluorodeoxyglucose positron emission tomography (^{18}FDG-PET)/CT scanning was ordered. Besides hypermetabolic enhancement of the vulva with regional LAN, it demonstrated increased ^{18}FDG uptake along both pleural surfaces but more prominently on the right with a maximum standardized uptake value (SUV) of 6.64 (Figure 2). No other suspicious foci of ^{18}FDG activity were detected. In light of concern for metastatic malignancy to the pleura, she underwent video-assisted thoracoscopic surgical pleural biopsy of the more intensely ^{18}FDG-avid right side. On intraoperative inspection, numerous pleural nodules were observed. Histology of the abnormal pleura showed invasive, non-keratinizing, poorly-differentiated SCC with areas of necrosis (Figure 3). These findings were morphologically concordant with the previously obtained malignant vulvar tissue. The patient started and completed six cycles of platinum-based chemotherapy with Cisplatin and Paclitaxel. Computed tomography imaging immediately following this regimen demonstrated a robust tumor response. Unfortunately, during the subsequent treatment holiday, she experienced catastrophic relapse and passed away under hospice care approximately 8 months after the discovery of her MPE.

Discussion

In aggregate, gynecological cancer is the third most common solid tumor to give rise to MPE [2]. As is true of all other solid malignancies, pleural involvement by gynecological cancer signifies stage IV disease and is associated with poor survival [7]. In order to create pleural fluid accumulation, thoracic metastases need to both increase pleural capillary permeability and decrease pleural lymphatic drainage. While gynecological cancers with access to the peritoneal cavity such as ovarian and fallopian primaries are capable of transcoelomic spread into the pleural space, the predominant mechanism of pleural implantation in most cancers, presumably including vulvar carcinoma, is lympho-hematogenous dissemination. Even though the clinical picture and radiology could be highly suggestive of MPE, especially in patients with known malignancy, only positive cytohistology can definitively establish this diagnosis. The least invasive and therefore the most practical initial source of a pathological specimen in suspected cases is pleural fluid

withdrawn during thoracentesis and processed for cytology. The approximate overall cytological yield in MPE is a disappointing 50%, a number that can exceed 80% in exfoliative cancers such as ovarian adenocarcinoma and can fall under 30% in non-exfoliative cancers such as SCC [8, 9]. It is thus not surprising that pleural involvement could not be confirmed by fluid cytology in our patient despite repeat sampling.

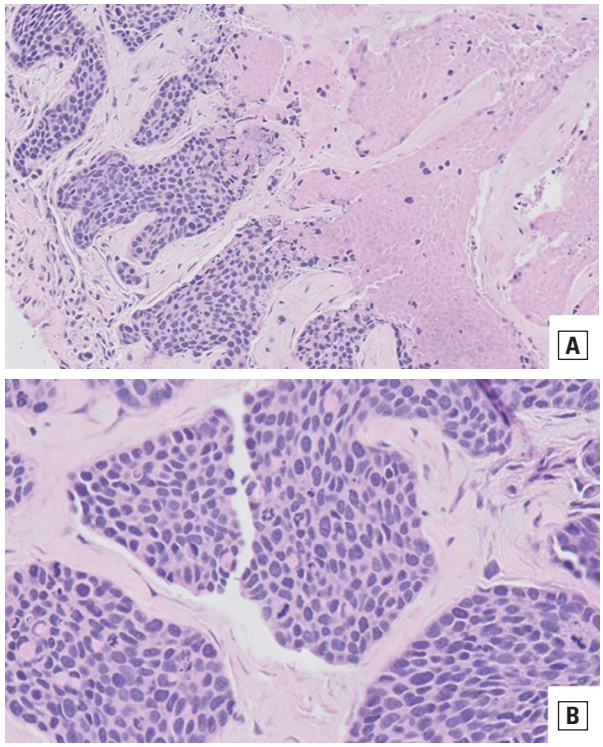

Figure 3. A. Low-power view of the pleural biopsy showing nests of neoplastic squamous cells in a fibrotic background with associated necrosis (Hematoxylin & eosin, original magnification × 200). **B.** High-power view allowing better appreciation of the pleomorphic nuclei and eosinophilic cytoplasm of the neoplastic squamous cells. There is no evidence of keratinization. Numerous mitotic figures are present (H&E, original magnification × 400)

As mentioned, the majority of vulvar carcinoma cases remain localized — with potential invasion of nearby structures — or spread only as far as the regional lymph nodes [6]. The list of metastatic sites reported in the literature includes the central nervous system [10], breast [11], heart [12], lung [13], liver [13], bone [13], skin [13], and muscle [14]. We were able to find only a single published case describing pleural metastases from vulvar carcinoma, also with biopsy-proven squamous histology of pleural implants [15]. In that report, the presence of vulvar carcinoma was likewise known prior to detection of MPE as in our case, but pleural involvement occurred much later in the disease course: it signified recurrence approximately one year after initial diagnosis and vulvectomy. In contrast to our case, however, the original vulvar specimen was not available for correlation. Table 1 summarizes the differences and similarities between our case and the one published previously.

Conclusion

While not an uncommon gynecological malignancy, vulvar cancer is at the same time an exceedingly rare source of pleural metastases. Squamous cell carcinoma, the usual histology of vulvar cancer, is associated with poor cytological yield of pleural fluid sampling, so our case both illustrates a very unusual metastatic pattern and reminds that suspicion for MPE ought to be maintained despite negative pleural fluid cytology and despite a rarely metastasizing cancer.

Table 1. Comparison of features of the present case with those of the single previously published report.

Source	Erra *et al.* [15]	Present case
Publication year	2016	2020
Diagnosis of vulvar carcinoma	Historical	Confirmed at reporting institution
Age at MPE diagnosis	76 years	63 years
Histology of pleural implants	SCC	SCC
Timeline of MPE detection	1 year after diagnosis	1 week after diagnosis
Pattern of pleural involvement	Unilateral (right)	Bilateral
Symptoms	Dyspnea, chest pain	Dyspnea, dry cough
Mode of MPE diagnosis	Thoracoscopy with pleural biopsy	Thoracoscopy with pleural biopsy
Inguinopelvic lymph node involvement	No	Yes (by PET)
Treatment history of primary site	Vulvectomy	None

MPE — malignant pleural effusion; PET — positron emission tomography; SCC — squamous cell carcinoma

Conflict of interest

None declared.

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Fryderyk Zawadzki, Marta Wajda-Pokrontka, Tomasz Stącel, Maciej Urlik, Mirosław Nęcki, Remigiusz Antończyk, Magdalena Latos, Maria Królikowska, Damian Maruszak, Marcelina Łazaj, Małgorzata Kowacka, Alina Kliczka, Marian Zembala, Marek Ochman

Department of Cardiac, Vascular and Endovascular Surgery and Transplantology, Medical University of Silesia in Katowice, Silesian Centre for Heart Diseases, Zabrze, Poland

Emphysema as a possible complication of infant respiratory distress syndrome leading to lung transplantation

Abstract

Infant respiratory distress syndrome (IRDS) develops among premature infants due to structural immaturity of the lungs and insufficient production of pulmonary surfactant. Nowadays, treatment takes place under conditions of intensive care and includes oxygen therapy, mechanical ventilation, exogenous supplementation of pulmonary surfactant and antenatal corticosteroid therapy. The treatment of IRDS, especially mechanical ventilation, may lead to complications which can contribute to developing a severe dysfunction of the respiratory system. Unavailability of pharmacological treatment of IRDS and development of pulmonary barotrauma due to mechanical ventilation in our patient led to the forming of severe pulmonary interstitial emphysema. In this case report, lung transplantation was performed as an only successful therapeutic option.

Key words: IRDS, pulmonary surfactant, pulmonary barotrauma, pulmonary interstitial emphysema, lung transplantation

Adv Respir Med. 2021; 89: 211–215

Introduction

Infant respiratory distress syndrome (IRDS), formerly known as hyaline membrane disease, is a common problem among preterm infants. Its incidence is inversely proportional to gestational age (GA). Extremely preterm infants (GA ≤ 28 weeks) run the highest risk, nevertheless IRDS also occurs among late preterm and even term infants, however, the incidence is adequately lower. A report prepared by the Safe Labor Consortium reveals that IRDS was diagnosed in 10.5% of infants born at the 34th week of gestation (WG), 6% at 35 WG, 2.8% at 36 WG, 1% at 37 WG, and 0.3% at ≥ 38 WG [1]. The disorder is caused by developmental insufficiency of pulmonary surfactant production and structural immaturity in the lungs. Surfactant scarcity leads to inability to maintain open alveoli during end expiration [2].

Antenatal corticosteroid (ACS) therapy and application of exogenous surfactant has lowered the mortality and morbidity associated with RDS [3, 4] but has not eliminated them. Complications due to therapeutic interventions such as supplemental oxygen, positive pressure ventilation, and the use of endotracheal tubes still exist and result in different forms of pulmonary air leaks, which can lead to the development of emphysema.

Case presentation

This case report describes a 38-year-old woman who underwent lung transplantation in 2016 due to extremely advanced emphysema. The beginning of the disease is difficult to determine due to the lack of medical records and inaccurate patient history.

According to our best knowledge, the woman was born as a preterm infant in the 8th month of pregnancy with diagnosis of hospital-acquired pneumonia and IRDS. She required mechanical ventilation, however, the exogenous surfactant

Address for correspondence: Fryderyk Zawadzki, Department of Cardiac, Vascular and Endovascular Surgery and Transplantology, Silesian Center for Heart Diseases in Zabrze, Faculty of Medical Sciences in Zabrze, Zabrze, Poland; e-mail: zawadzkifryderyk@gmail.com

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as well as the antenatal corticosteroid therapy couldn't have been administrated because they were not available at that time. The lack of ACS therapy and employment of postnatal mechanical ventilation without surfactant application were the plausible reasons for major lung immaturity, respiratory impairment and finally, initiated pulmonary interstitial emphysema. At the age of 2, due to recurring respiratory infections and worsening exercise capacity, the patient was diagnosed with asthma. Despite complying accuracy and available pharmacological therapy (inhaled corticosteroid and short-acting ß$_2$-adrenergic-agonist), asthma was not sufficiently controlled. The girl has undergone several pneumonia and bronchitis until she was 10. Asthma treatment was terminated at the age of 11 due to lack of symptoms and achieved remission.

According to the patient medical history, there were no cases of severe emphysema in her family, she was not an active or passive smoker either. The patient had no environmental exposures and before the labor she had an office work.

In 2006, the woman became pregnant and from the very beginning complained of escalating dyspnea. After she went into a labor, a significant deterioration of the respiratory function occurred, and a quickly progressing emphysema was revealed in the chest X-ray examination — a cesarean section was conducted. Since delivery, she has been hospitalized for a few times due to significant deterioration of general condition with increasing exertion and rest dyspnea, every time being treated with antibiotics, steroids and bronchodilators. The bronchial obturation reversibility test was conducted with the following results: before bronchodilator-forced expiratory volume in 1 second (FEV_1) = 18%, forced expiratory volume in 1 second to forced vital capacity ratio (FEV_1/FVC) = 40% and after bronchodilator — FEV_1 = 23%, FEV_1/FVC = 42%.

In 2014, she was admitted to the Silesian Center for Heart Diseases for preliminary qualification to lung transplantation. Alpha 1-antitrypsin deficiency and connective tissue diseases were excepted. Echocardiography did not reveal any abnormalities and did not indicate the presence of pulmonary hypertension (right ventricle systolic pressure = 25 mm Hg, acceleration time = 143 ms, tricuspid annular plane systolic excursion = 21 mm, left ventricle ejection fraction = 55%). During the six-minute walk test (6MWT), the patient reached the distance of 356 m with 2 points in the Borg scale and 8% of desaturation during the test. After 11 months, a decision of the patient's qualification for lung transplantation was made. The woman demonstrated exacerbation of lung dysfunction (FEV_1 = 21%, FEV_1/FVC = 59%) and deterioration of general state. In computed tomography, multiple emphysematous bullae were observed (Figure 1). The 6MWT must have been stopped after 4 minutes — in that time the patient reached the distance of 189 meters with 5 points in the Borg scale, and desaturation from 97% to 91% was observed (after the test oxygen was required).

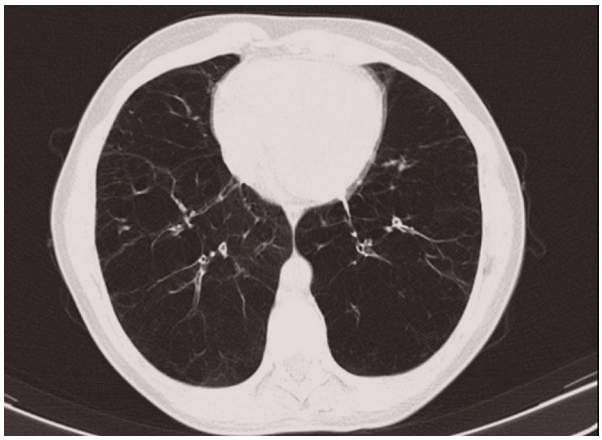

Figure 1. Results of computed tomography scan of the patient's chest from the qualification day for lung transplantation — multiple emphysematous bullae

Nine months after qualification, the woman with an end-stage respiratory failure was admitted to the transplantology ward for lung transplantation due to the availability of a compatible donor. Uncomplicated, sequential double lung transplantation was performed. Immunosuppressive maintenance therapy including tacrolimus and prednisone was administered. One month after the surgery, the patient was admitted to the department due to the warning symptoms suggesting deterioration of lung function. The woman was diagnosed with mycotic infection with *Aspergillus* spp. as a result of immunosuppression therapy - voriconazole was administrated. During the next several months, the bronchiolitis obliterans syndrome occurred. Progression of bronchial stenosis was probably associated with past fungal infection. The patient underwent more than 50 balloon dilatations, more than 30 argon plasma coagulations, 2 laser therapy and 1 cryotherapy as bronchoscope interventions since lung transplantation procedure. Despite postoperative complications and multiple bronchial interventions, the patient's general state and respiratory function have prominently improved, which is reflected in her results. 2 years after transplantation, the woman achieved in spirometry FEV_1 = 63% and

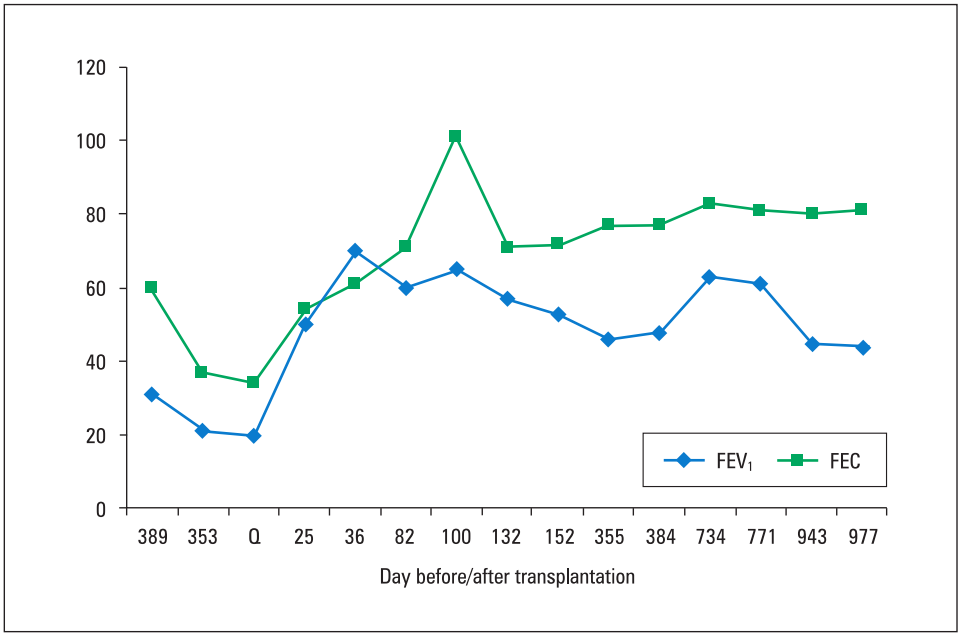

Figure 2. Detailed results of spirometry before and after transplantation (% predicted of FEV_1 and FVC)

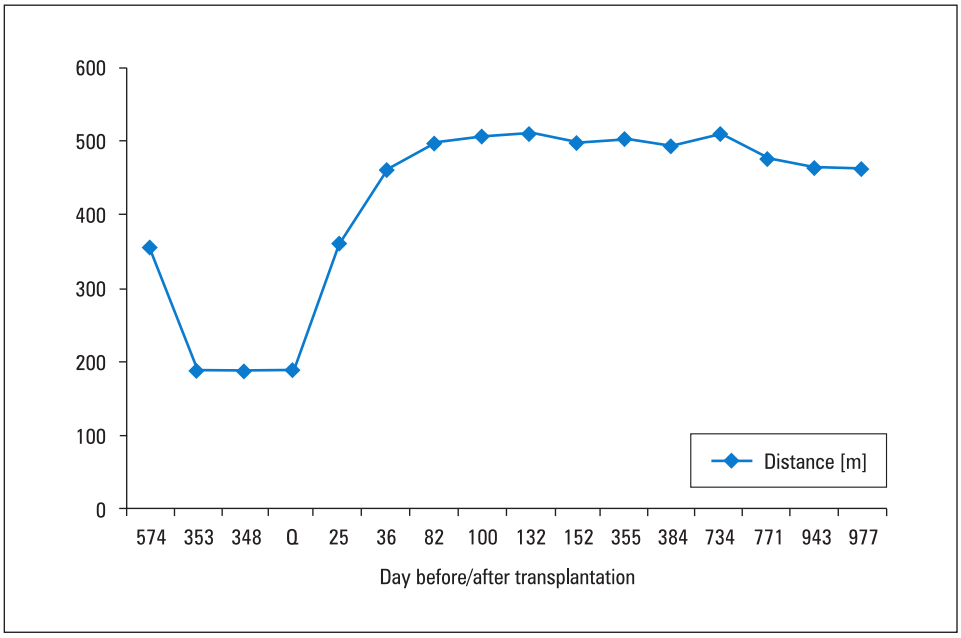

Figure 3. Detailed results of 6 minute walk test before and after transplantation

FEV_1/FVC = 79%. In 6MWT, she reached the distance of 510 meters with 3 points in the Borg scale and without desaturation. The results of spirometry and 6MWT following 5-year observation were presented in Figure 2 and Figure 3, respectively.

Discussion

The patient's condition that contributed to the decision about lung transplantation takes origin in a neonatal period and is a synthesis of several factors such as the absence of antenatal steroids therapy, lack of surfactant supplementation, pneumonia, invasive mechanical ventilation, severe asthma, recurring infections and labor.

Since the core of IRDS is lung immaturity, the best intervention would be to prevent delivery of premature infants. However, if preterm birth cannot be avoided, IRDS may be prevented, its severity decreased or its effects reduced by application

of antenatal steroid therapy, exogenous surfactant supplementation and early administration of positive airway pressure. As a result of these measures, many extremely low birth weight infants do not exhibit the clinical features of RDS [5].

While mechanical ventilation is definitely lifesaving and has led to improvement in neonatal survival, it may cause severe and chronic lung damage. In this case, invasive mechanical ventilation was complicated by an air leak and resulted in pulmonary interstitial emphysema, which is characterized by trapping the gas from the alveoli inside the interstitial spaces of the lung and is diagnosed on the basis of chest radiography [6]. Clinical data suggest that this type of complication is associated with increased mortality and morbidity in preterm infants and can negatively affect long-term pulmonary and non-pulmonary outcomes [7]. The incidence of pulmonary interstitial emphysema in the randomized controlled trials evaluating prophylactic vs rescue surfactant therapy totaled 3% to 5% [8]. According to the one retrospective study, risk factors for developing pulmonary interstitial emphysema are higher maximum inspired oxygen concentration and higher mean airway pressures when compared with that in control subjects. Moreover, in infants weighing less than 1000 g, these factors were associated with an increased risk of death [7].

Neonatal pneumonia may be both the reason for respiratory distress and an additional impairing factor in the preexisting one. Early-onset pneumonia is commonly presented by respiratory distress beginning at birth or soon after it, while the highest risk of late-onset pneumonia exists in the group of preterm infants who require assisted ventilation. Data from adults that are transposable to neonates show the four times higher risk of hospital-acquired pneumonia in intubated than in non-intubated patients [9].

Research efforts have been devoted to developing innovative ventilation strategies aiming to provide sufficient gas exchange along with decreased incidence of complications and damage [10]. According to the European Consensus Guidelines, CPAP should be preferentially started from birth in all infants at risk of RDS until their clinical status can be assessed. nCPAP is the preferred noninvasive alternative to endotracheal intubation and mechanical ventilation for very preterm infants (GA ≤ 32 weeks) who are at risk of IRDS.

Volume-targeted ventilators provide a more consistent tidal volume, which results in lower lung injury rate than in pressure-limited ventilation. Data have demonstrated that volume-targeted ventilation is associated with a lower risk of BPD and mortality than pressure-limited ventilation and is also reported to be superior to pressure-limited ventilation in the management of acute respiratory failure in neonates [11].

Antenatal administration of corticosteroids improves both lung mechanics and gas exchange through accelerating the development of type 1 and type 2 pneumocytes, which leads to structural and biochemical changes. A single course of antenatal corticosteroid therapy administered to women at risk of preterm delivery (PTD) reduce both prevalence and severity of respiratory distress syndrome as well as mortality in offspring, which was shown by Liggins and Howie in a landmark paper [12]. The outcome has been confirmed in over two dozen randomized trials [13].

A significant reduction in the incidence of IRDS among infants exposed to ACS therapy has been consonantly notified in randomized trials performed worldwide. In a 2017, a systematic review of randomized trials that compared antenatal corticosteroid therapy with placebo/no treatment among women at risk of preterm birth, ACS therapy resulted in a reduction in IRDS (RR 0.66, 95% CI 0.56-0.77), reduction in moderate to severe disease (RR 0.59, 95% CI 0.38–0.91) and reduction in need for mechanical ventilation (RR 0.68, 95% CI 0.56–0.84) [13].

Exogenous surfactant replacement therapy is effective in reducing IRDS mortality and morbidity in preterm infants, which has been shown in several clinical trials conducted among preterm infants at the greatest risk of IRDS. In these trials, comparing surfactant therapy versus placebo, surfactant administration was associated with a lower prevalence and advancement of RDS, reduced mortality, and a decreased rate of associated complications such as pulmonary leak, including pulmonary interstitial emphysema [14].

Intubation and administration of surfactant is appropriate for the patients with persistent severe respiratory distress [required fraction of inspired oxygen to maintain oxygen saturation above 90% (FiO_2) ≥ 0.40] or who are apneic [15]. Surfactant therapy is most effective when administrated within the first 30 to 60 minutes of life and preceded by the placement of a pulse oximeter and clinical confirmation of correct endotracheal tube placement as well as balanced with appropriate time of nCPAP [14, 16].

What is remarkable, in the time when this patient was born, neither the antenatal corticosteroid therapy and surfactant replacement nor the

noninvasive ventilation strategies were available, hence couldn't have been applied.

To conclude, premature infants with immature respiratory system demand comprehensive and specialized measures of protection and advanced therapy as early as possible, including antenatal age. Careful attention to many aspects of neonatal care such as antenatal corticosteroids therapy, delivery room resuscitation, ventilatory support and surfactant administration are needed to decrease pulmonary complications. Nevertheless, the results are not always as satisfying as supposed and may be not sufficient enough. By multicasual and long-lasting lung impairment, at some point of age, lung transplantation remains to be the only reasonable and lifesaving. therapeutic option for such patients.

Conflict of interest

None declared.

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VIA MEDICA

CASE REPORT

Harry J Pick[1*], Mark A Faghy[2*], Gareth Creswell[3], Deborah Ashton[1], Charlotte E Bolton[1], Tricia McKeever[1], Wei Shen Lim[1], Thomas Bewick[3]

[1]Nottingham University Hospital NHS Trust, Nottingham City Hospital, NG5 1PB Nottingham, United Kingdom
[2]University of Derby, Kedleston Road, DE22 1GB Derby, United Kingdom
[3]University Hospitals of Derby and Burton NHS Foundation Trust, Uttoxeter Road, DE22 3NE Derby, United Kingdom
*Authors contributed equally to this work.

The feasibility and tolerability of using inspiratory muscle training with adults discharged from the hospital with community-acquired pneumonia

Abstract

Introduction: Patients experience substantial morbidity following discharge from hospital and during recovery from community-acquired pneumonia (CAP). Inspiratory muscle training (IMT) has demonstrated improved functional capacity and reduced patient-reported symptoms. To date the safety and tolerability of these methods have not been determined in CAP patients recovering following hospitalization. Accordingly, this study aimed to assess the safety and tolerability of IMT in adults discharged from hospital with CAP.

Material and methods: Participants received an IMT device (POWERbreathe KHP2) and completed 9-weeks IMT training with weekly follow-up. Frequency (twice daily) and load (50% PImax) were fixed throughout, but training volume increased incrementally (2-week habituation phase, 7-week training phase). Primary outcomes of interest included IMT safety and tolerability.

Results: Twenty-two participants were recruited; 16 were male, mean age 55.2 years (range 27.9–77.3). From 1183 possible training days, side effects were reported on 15 occasions by 10 individual participants. All reported side-effects were assessed as grade 1 and did not prevent further training. Participant-reported IMT acceptability was 99.4%.

Conclusion: Inspiratory muscle training is safe and tolerable in patients following hospitalisation for CAP. Patient satisfaction with IMT is high and it is viewed by patients as being helpful in their recovery. Distinguishing CAP-related symptoms and device-related side effects is challenging. Symptom prevalence declined during follow-up with concurrent improvements in spirometry observed. Further research is required to determine the efficacy of IMT interventions following CAP and other acute respiratory infections.

Key words: recovery; pnuemonia; respiratory muscles; training

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Introduction

CAP is responsible for over 100,000 admissions to hospital each year in England and Wales with an incidence of 8 per 1000 adults aged over 65 [1, 2]. Most hospitalised patients are discharged into the community to recuperate and the burden of disease in recovery is substantial, with 65.7% of patients re-consulting a primary care practitioner, and > 50% of patients failing to return to their usual daily activities in the 4-weeks post-discharge [3]. Whilst the burden of recovery is extensive the physiological basis for recovery remains poorly understood. Previous work in this area has demonstrated reduced skeletal muscle strength, impaired exercise capacity and reduced quality of life following an episode of CAP [4]. A potential mechanism is abnormal and impaired respiratory muscle function in the post-pneumonia period. In healthy volunteers, respiratory muscle strength is associated with exacerbated dyspnoea and fatigue, heightened perceptual discomfort and reduced exercise capacity and functional status. During ventilation,

Address for correspondence: Harry Pick, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; e-mail: harry.pick@NHS.net

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breathing accounts for 1–3% of total oxygen consumption and quiet breathing requires a small fraction of the respiratory muscles maximal pressure generating capacity in healthy individuals. However, in an acutely diseased lung (i.e. CAP) the pressure required to breathe is increased due to changes in airway resistance and chest wall mechanics [5].

Inspiratory muscle training (IMT) improves respiratory muscle strength and reduces markers of physiological stress [6]. IMT techniques have also been demonstrated in patient groups; reducing dyspnoea perception and discomfort in chronic obstructive pulmonary disease, increased strength and endurance of respiratory muscles in patients after a cerebrovascular event and increased lung volumes, respiratory muscle strength, and exercise capacity in elderly patients [7, 8]. The efficacy and tolerability of IMT in CAP patients have yet to be determined.

Material and methods

We undertook a prospective observational feasibility study with adult patients discharged from hospital with CAP. CAP was defined as: the presence of one or more symptom of acute lower-respiratory respiratory-tract infection (cough, increasing breathlessness, sputum production, chest pain, and fever); evidence of acute infiltrates consistent with a respiratory infection on admission plain chest radiograph; , and treated by the clinical team for CAP. Exclusion criteria included; inability to understand verbal or written information in English, hospital admission within preceding 10 days, active tuberculosis, post-obstructive pneumonia secondary to lung cancer, aspiration pneumonia, World Health Organisation performance status 2 or greater prior to admission, inability to provide informed consent, or presence of a contraindication to pulmonary function testing.

Following ethics approval (Ethics reference 17/EE/0043) and informed consent, twenty-two adults (average age was 55.2 years; range: 27.9–77.3 years, mean CAP score 44.3 ± 19.6) and 16 (72.7%) males were recruited to the study and completed 9 weeks of IMT training (described below). One or more co-morbid illness was present in 10 patients. World Health Organisation (WHO) performance status at hospital admission was grade 1 in nine, grade 2 in three, grade 3 in seven, and grade 4 in three patients. A control group was not included within the study design as the primary aim was to determine the safety and tolerability of IMT methods in patients following hospitalisation for CAP. Most patients (21/22) were admitted from the Emergency Department, with three patients taking prescribed antibiotics before admission. Unilobar consolidation was the most common radiographic abnormality identified following admission (17/22 patients), followed by multilobar consolidation (4/22) and unilateral pleural effusion (1/22).

Following informed consent and alongside routine clinical investigations, patients were assessed for symptom prevalence and functional activity weekly for 9 weeks using a combination of hospital review, telephone consultation, and patient diaries. A five-item, CAP specific questionnaire was used to record patient-reported symptoms prior to discharge and at each follow-up consultation, scoring was conducted inline with previous guidelines [9]. Dynamic spirometry (forced expiratory volume in 1 second; FEV_1 and Forced Vital Capacity; FVC) was assessed using a handheld spirometer (MicroPlus; Micro Medical, Buckinghamshire, UK) in accordance with published guidelines [10]. A hand-held mouth pressure meter (Micro R.P.M., Micro Medical, Buckinghamshire, UK) was used to assess both maximal inspiratory (MIP) and maximal expiratory (MEP) mouth pressure. Patients were provided with an IMT device (Figure 1, POWERbreatheKHP2, HaB International, Southam, UK) for nine weeks. Patients conducted two IMT sessions at home each day an intensity equivalent to 50% of the participant's maximum inspiratory muscle pressure (MIP). Participants were trained on using the device and completing sessions effectively

Figure 1. A visual representation of the POWERbreatheKHP2 device that was used by patients as part of the 9-week intervention

Table 1. Mean FEV_1, FVC, MIP, MEP and CAP score at discharge, 6-week and 9-week measurements

	Discharge (n = 22)	6-week follow-up (n = 20)	9-week follow-up (n = 19)	Difference in mean between hospital discharge and 9-week follow-up (95% CI)	P value
FEV_1 (l)	1.89	2.68	2.73	0.84 (0.54–1.14)	< 0.001
FVC (l)	2.27	3.22	3.18	0.91 (0.56–1.27)	< 0.001
MIP (cmH_2O)	65.2	102.9	116.0	50.8 (37.7–64.0)	< 0.001
MEP (cmH_2O)	74.4	100.8	105.6	31.2 (18.0–44.4)	< 0.001
CAP score (AU)	44.3	83.2	92.8	48.5 (30.2–69.8)	< 0.001

CI — confidence interval; FEV_1 — forced expiratory volume in 1 second; FVC — forced vital capacity; MIP — mouth maximal inspiratory pressure; MEP — mouth maximal expiratory pressure. P-value compares the difference in means at discharge versus 9 weeks

was conducted at baseline and during all face follow up sessions.

Session volume was incremental and started with 10 breaths per session in week one, increasing to 20 breaths in week two, and 30 breaths for week's three to nine. Patients were asked to self-report symptom prevalence and functional activity during weekly phone calls for 9 weeks, whilst assessments of respiratory muscle strength and lung function were conducted using standardised ATS/ERS guidelines at discharge, 6-weeks, and 9-weeks. Adherence to the protocol was also confirmed at face to face visits via completed participant diaries and verified against recorded training sessions on the device.

Safety was assessed by recording the frequency and severity of patient-reported IMT side effects during face to face and weekly telephone consultations. IMT tolerability was measured by the proportion of patients who completed IMT training according to the study protocol. Secondary outcomes included changes ion patient-reported symptoms over the study period (CAP score) and markers of pulmonary (FEV_1, FVC, FEV_1/FVC, PEF) and respiratory muscle function (MIP and MEP). Paired t-tests were used to test the significance of the difference between means at baseline and follow-up. Statistical analyses were conducted using Stata/IC 15 (©StataCorp. 2017).

Results

One unexpected and unrelated serious adverse event (death) occurred during study follow-up. Three other participants under active follow-up at the time of this adverse event were asked to stop further IMT whilst awaiting results of an initial internal and additional independent external investigation, as directed by the study sponsor. Subsequently, these participants did not restart IMT as they were outside of the training window at the time of conclusion of the investigation. They were subsequently excluded from the analysis of protocol compliance.

IMT was completed according to protocol in 14/19 (73.7% of patients). Side effects from IMT were reported on 15 occasions (10 individuals) over a total of 1183 training days (range of training days 7 to 63). Side effects related to IMT included chest pain (n = 2), cough (n = 1), increased dyspnoea (n = 4), and dizziness (n = 8). Participants rated these side effects as grade 1 on the Common Terminology Criteria for Adverse Events (CTCAE) scale and it did not prevent patients from completing the current session or continuing with future training. CAP symptom scores improved between discharge (mean 44.3 ± 19.6) and 9 weeks after discharge (mean 88.2 ± 15.0), with a significant average improvement of 43.9 (difference between means, 95% CI: 34.8–52.9, $p < 0.001$). All spirometric measures and respiratory muscle strength measures improved between baseline assessment at discharge and repeat assessment at 9-weeks (Table 1). Sixteen patients had a repeat chest x-ray within 9-weeks of discharge, with complete radiographic resolution in 14, persistent consolidation in one patient, and a unilateral pleural effusion in another.

Discussion

This is the first study of IMT in adult patients recovering following hospitalisation with an episode of CAP. We report that IMT is safe and tolerable in this patient group, but acknowledge the need for further study in this area to determine the full extent of any clinical benefits of using IMT methods during recovery (i.e. with the use of a control group and randomised control design).

The primary outcome of this work was to determine whether IMT methods are safe and tolerable to patients during their recovery from

CAP. This study demonstrates that IMT in adult patients recovering following hospitalisation with CAP is safe and tolerable. We also observed improvement in CAP-symptom scores and lung function tests as would be expected in recovery. The authors acknowledge that the efficacy of IMT methods cannot be determined by this study and the full extent to which IMT may have influenced the rate or extent of improvement in recovery due to the lack of a control group and should be considered with future research. Specifically, the use of IMT methods needs to be evaluated against the low-grade side-effects experienced by patients and the protocol adherence to determine the full extent of the benefits to patients.

We do present the first study that demonstrates that IMT methods are safe and well tolerated by patients following admission to hospital with CAP. Reported side-effects were rare and of low severity, rated as grade 1 on Common Terminology Criteria for Adverse Events scale (CTCAE), and did not prevent further training or deviation from the study protocol. This is consistent with results from a study of IMT in patients with thoracic malignancy which reported IMT-related side effects in only 3 patients during follow-up whilst 2 studies of IMT in differing patient cohorts (pre-operative IMT in patients undergoing coronary artery bypass grafting and IMT in chronic heart failure) both reported no adverse events during or after IMT. On repeated assessment patients reported that they believed IMT was helpful in their recovery. This is consistent with results of IMT from other studies; pre-operative patients and patients with chronic heart failure participating in rehabilitation also reported that IMT was tolerable and that they believed it to be beneficial in their recovery [11] FEV1, 24 +/- 7% predicted.

It is plausible that IMT methods could also have important implications for patients during their recovery from COVID-19 [12]. COVID-19 and other viral infections can cause significant damage to the lungs and airways result in acute respiratory distress syndrome (ARDS). The patients at the greatest risk are likely to have multiple co-morbidities, de-conditioning of the respiratory musculature and increased likelihood of respiratory failure and the need for critical care interventions. Those that develop severe complications from are admitted to intensive care units and require prolonged periods of ventilation. Mechanical ventilation induces rapid atrophy and profound weakness of the respiratory musculature (<18 hours), creating a disparity between the force-generating capability of the respiratory muscles and the pressures required for spontaneous tidal breathing [13]. Interventions that increase respiratory muscle strength are well tolerated by patients with respiratory illness and the data here demonstrates that the inclusion of IMT techniques could prove a useful addition to the recovery from respiratory infections.

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Conflict of interest

None declared.

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Not just skin deep: Multiple cutaneous nodules as the first presenting sign of small cell cancer

Avneet Garg, Harshi Dhingra, Vinita Jindal, Simranjeet Kaur, Vijay Suri

Adesh Institute of Medical Sciences and Resaerch, Bathinda, Punjab, India

A 65-year-old male who was a chronic smoker presented with complaints of dyspnea, cough, weight loss, weakness, and bony pains for the last 2-3 months. His medical history included hypertension and hypothyroidism. On presentation, his vitals were as follows: heart rate 86/min, blood pressure 122/78 mm Hg, respiratory rate 22/min, and an oxygen saturation of 96% on room air. Chest examination revealed a barrel-shaped chest, a hyperresonant note was heard on percussion, and diffuse polyphonic rhonchi were heard on expiration. Contrast-enhanced computed tomography (CT) of the chest showed bilateral extensive emphysematous changes. The right upper lobe had a heterogeneously enhancing soft tissue lesion and there was significant mediastinal lymphadenopathy encasing the lower trachea and great vessels. The patient was at high risk for developing a pneumothorax related to image-guided sampling of the right upper lobe lesion in view of extensive emphysema. Therefore, he was referred to us for EBUS-TBNA (endobronchial ultrasound-guided transbronchial needle aspiration) and guided mediastinal lymph node sampling. Prior to this, a physical examination revealed round, firm, non-tender, skin coloured, 2–3 cm sized skin nodules that were located as follows: 2 on the left lateral chest wall, 1 on the right lateral chest wall, and 1 in proximity to the umbilicus. Fine needle aspiration cytology (FNAC) was attempted from two of skin nodules which were suggestive of lung cancer. EBUS-TBNA was deferred and an excision biopsy was performed on one of the skin nodules. The histopathological report revealed small cell carcinoma and this was further confirmed by IHC. A CECT of the abdomen and brain was performed, and a Tc99m bone scan was completed which revealed bony metastases. The patient was recommended to undergo chemotherapy (cisplatin and etoposide) and palliative radiotherapy but was unwilling and was ultimately lost to follow-up.

In this case, we encountered a patient who presented with metastatic skin nodules that were the first clinical presentation of small cell lung cancer. Small cell cancers constitute about 16% of total lung cancer histological types and 75% present at an extensive stage as shown in the largest lung cancer study from India [1].

Skin metastasis as an initial presentation is rarely reported in lung cancer [2–4]. Although all histological types of lung cancer can metastasize to the skin, it is most commonly reported in adenocarcinomas. Squamous cell carcinomas are the second most common cause; small cell lung cancers rarely have this presentation [5]. Interestingly, skin metastasis has been reported more commonly in upper lobe lesions [3].

Common sites includes the chest, abdomen, back, head, and neck [5]. In our case, nodules were present on the chest and abdomen. The presence of metastasis to the skin makes the disease unresectable and is associated with a poor prognosis. The median survival time is about 5 months [4].

Our case emphasizes the importance of a detailed physical examination in cases of lung cancer in order to look for any manifestation of the disease on the skin, even if it is encountered rarely. Skin nodule sampling can confirm the diagnosis and may obviate the need for a more invasive procedure (e.g. image-guided lung mass sampling or mediastinal lymph node sampling, as in our case) in already debilitated lung cancer patients. Moreover, detection of skin nodules helps in the staging and prognosis of the disease.

Address for correspondence: Avneet Garg, Department of Pulmonary Medicine, Adesh Institute of Medical Sciences and Research, AIMSR, Bathinda, Punjab, India; e-mail: dravneetgarg@gmail.com

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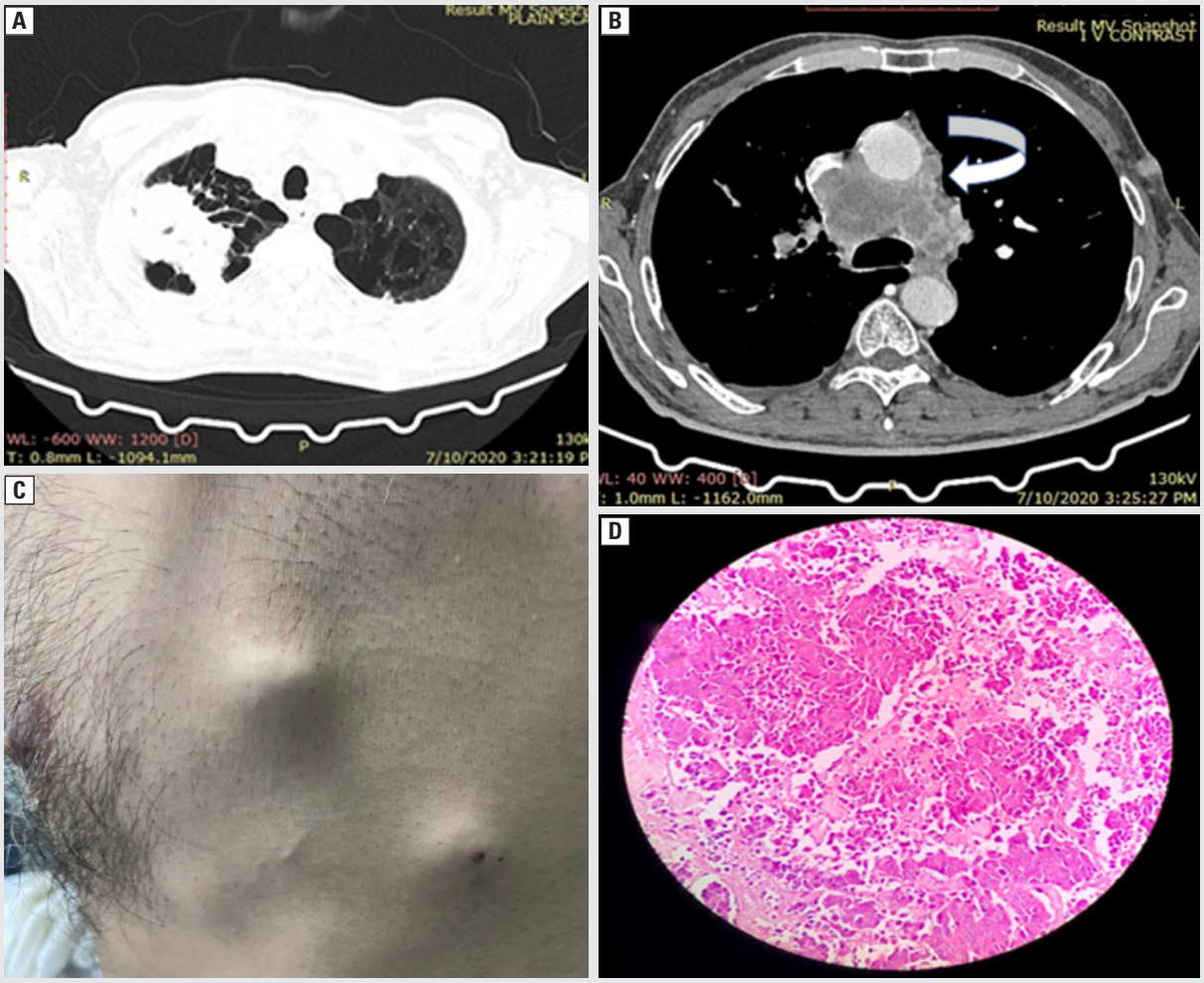

Figure 1. CT of the chest in pulmonary window (**A**) showing a right lung mass in the apical segment and extensive emphysematous changes in bilateral upper lobes. CT of the chest in mediastinal window (**B**) with a curved arrow is at a lower level and shows conglomerated multiple mediastinal lymph nodes. **C.** Skin nodules on the left lateral chest wall. **D.** Histopathological confirmation of small cell carcinoma. (H&E, × 400)

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A rare etiology of diffuse pulmonary hemorrhage

Martin Janík[1], Veronika Rybárová[1], Ľubomír Straka[1], Petr Hejna[2]

[1]Department of Forensic Medicine, Jessenius Faculty of Medicine, Comenius University, University Hospital, Martin, Slovakia
[2]Department of Forensic Medicine, Faculty of Medicine, Charles University and University Hospital, Hradec Kralove, Czech Republic

A 36-year-old drug addict presented to the emergency room after intravenously injecting 20 milliliters of pure gasoline. At the initial evaluation, he had complained of severe "freezing" chest pain and raging thirst. He provided a remote history of intravenously injecting low doses of gasoline. The admission examination was notable for a pronounced scent of gasoline on his breath, hypotension, and respiratory distress with diffuse rhonchi throughout the lungs. The laboratory workup showed severe metabolic acidosis, leukocytosis with neutrophilia, and markedly elevated serum myoglobin. The patient was treated with corticosteroids and maximum supportive care but there was no improvement. A chest radiograph was not performed given the patient's rapid decline. He soon developed large-volume hemoptysis and died without a definitive diagnosis 4 hours after presentation to the hospital. Postmortem examination was significant for diffusely enlarged and blood-filled lungs (Figure 1A, B). The left lung weighed 1780 g and the right lung weighed 1890 g (a normal lung weighs ~ 300–400 g). Microscopically, all lung sections demonstrated diffuse areas of fresh hemorrhage (Figure 1C). Thickened alveolar septa contained many neutrophils with dilated capillaries consistent with capillaritis (Figure 1D). There were also prominent hemosiderin-filled alveolar macrophages that showed evidence of previous alveolar hemorrhage (Figure 1E). Targeted toxicology confirmed a high concentration of hydrocarbons in the postmortem blood.

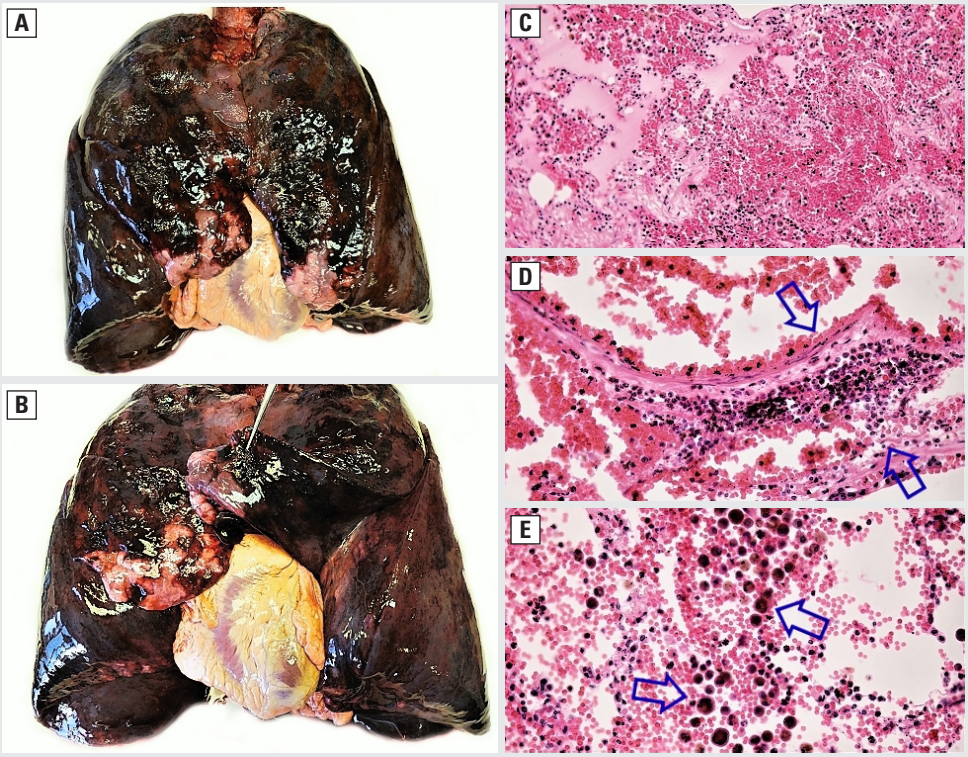

Figure 1 A Anterior surface of the severely blood-filled lungs **B** Interlobular surface with prominent areas of fresh hemorrhage **C** Acute alveolar hemorrhage with fibrin and edematous fluid (H&E ×20) **D** Capillaritis (blue arrows) surrounded by red cells (H&E ×40) **E** Prominent hemosiderin-filled alveolar macrophages (blue arrows) with associated fresh hemorrhage (H&E ×40)

Address for correspondence: Martin Janík, Comenius University, Jessenius Faculty of Medicine, Martin, Slovakia; e-mail: janik.mato@gmail.com

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Diffuse alveolar hemorrhage (DAH) refers to a distinct subset of pulmonary hemorrhage with widespread bleeding into alveoli, presumably because of injury to lung microcirculation [1]. DAH can occur in many clinical settings including vasculitides, autoimmune and coagulation disorders, and infections. Although uncommon, a number of medications and toxins such as anticoagulants, anti-arrhythmic drugs, and cocaine have been reported to cause DAH [2]. Intravenous gasoline is directly toxic to the lung microcirculation and carries a poor prognosis, although survival has been achieved [3]. The radiological features and the symptoms of gasoline toxicity are non-specific and dose-related. Nonetheless, a striking ice cold sensation in the chest (owing to exhalation of the volatile gasoline vapors) may be a useful diagnostic clue in an appropriate clinical context.

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Vidushi Rathi, Karan Madan, Anant Mohan, Vijay Hadda, Saurabh Mittal

All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

Ambulatory management of primary spontaneous pneumothorax: the jury is still out

To the Editor

Pneumothorax is a commonly encountered condition in clinical practice. The drainage remains an essential treatment for most patients with secondary spontaneous pneumothorax; however, the ideal treatment of primary spontaneous pneumothorax still remains unclear. The British Thoracic Society guidelines suggest the use of initial aspiration with the help of a syringe and cannula [1]. As a large proportion will still end up requiring a chest tube insertion, better ways of management are being investigated. One such method is the use of a small valved device which can be left in place and allows the patient to remain ambulatory (Figure 1). In a recent issue of The Lancet, Hallifax *et al*. have published the results of randomized controlled trial comparing the ambulatory device with the standard care for primary spontaneous pneumothorax (PSP) [2]. Although the authors have concluded that ambulatory management of PSP is a reasonable and preferable option, there are few issues which need discussion.

The primary outcome chosen for the study was the duration of hospital stay, which itself is not ideal. The primary goal of PSP treatment is symptom relief and recurrence prevention. Using an ambulatory device is likely to reduce the hospitalization in settings where patients with chest drains are routinely hospitalized. The time to the symptom or radiological resolution, or patient comfort, would be more clinically relevant outcomes. Secondly, the chest drain removal and discharge criteria were not protocolized for the control group, which is a source of bias in primary outcome assessment. Around one-third of patients in the standard arm could be managed with single aspiration; however, all patients in the ambulatory arm underwent device insertion, which could have been avoided in a significant proportion by simple aspiration. A solution for this confounder would be randomization after the failure of simple aspiration. Serious adverse

Figure 1. The valved ambulatory device available for the use for drainage of pneumothorax

Address for correspondence: Saurabh Mittal, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India; e-mail: saurabh_kgmu@yahoo.co.in

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events related to the device were high, necessitating a strict follow-up and daily review. Although it is suggested to use a written action plan for the same, this may not be feasible in all settings, especially in most developing countries. This suggests that results may not be applicable to a large proportion of individuals globally. Given these concerns, we suggest that the ambulatory device should be used with a constant vigil only in a selected group of patients consenting for regular follow-up and monitoring.

Conflict of interest

None declared.

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Arunachalam Meenakshisundaram[1], Anant Mohan[1], Karan Madan[1], Sandeep Aggarwal[2], Pawan Tiwari[1], Vijay Hadda[1], Miying Ering[2], Saurabh Mittal[1]

[1]Department of Pulmonary, Critical Care and Sleep Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi, India
[2]Department of Surgical Disciplines, All India Institute of Medical Sciences (AIIMS), New Delhi, India

Missed diagnosis in the COVID-19 era: Are we losing ourselves?

To the Editor

The current pandemic of coronavirus disease 2019 (COVID-19) has taken a toll on our already overburdened healthcare system [1]. Doctors are scarce in public hospitals, and with the current scenario, the emergency departments (ED) are expected to cater to a large number of patients presenting with respiratory complaints [2]. This has led to less emphasis on clinical examination as it is difficult to perform while wearing personal protective equipment, including face shields. This may lead to missed common diagnoses by overtime working resident doctors in emergencies but may be life-threatening unless managed in time [3, 4]. We need to encourage the medical fraternity to continue using clinical acumen while dealing with the current pandemic. Herein we present a patient, who could have been easily diagnosed if it was not a pandemic situation, but the diagnosis was missed due to COVID-19 panic in emergencies.

A 22-year-old male presented to the Emergency Department with the complaints of high-grade fever, shortness of breath, and dry cough for three days associated with abdominal pain for two days. He was a non-smoker and had no previous medical history of significance. On evaluation in the emergency, he was conscious and oriented. He was febrile with a heart rate of 132 beats per minute, respiratory rate of 24 per minute, blood pressure of 86/56 mm Hg and a pulse oxygen saturation of 83% while breathing room air. A chest ultrasound performed in emergency demonstrated bilateral lung sliding along with the presence of B lines and no pleural or pericardial effusion. Blood investigations revealed haemoglobin of 13 g/dL, total leucocyte count of 5200 cells/mm^3, and platelet count of 76,000 cells/cu.mm. Given the above presentation and symptoms, the patient was suspected of having COVID-19, and an oro-nasopharyngeal swab was sent for RT-PCR for SARS-CoV-2. The patient was managed with intravenous fluids and amoxicillin-clavulanate. After 8 hours, the RT-PCR report came negative, and the patient was shifted to the Pulmonary Medicine ward in view of the respiratory complaints. When received in the ward, the man had gross abdominal distention. On history taking, it was noted that he had not passed stools for three days. The chest radiograph performed revealed air under the left diaphragm (Figure 1), and erect

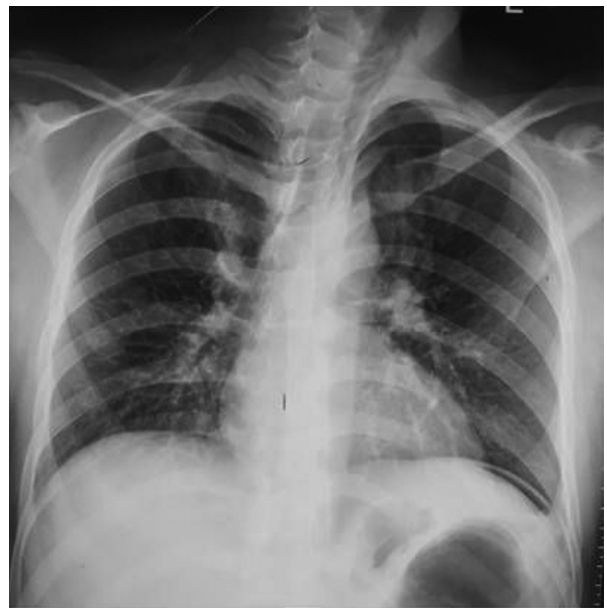

Figure 1. The chest radiograph demonstrating clear lung fields with air under left side of diaphragm suggesting pneumoperitoneum

Address for correspondence: Saurabh Mittal, Department of Pulmonary, Critical Care and Sleep Medicine, All India Institute of Medical Sciences (AIIMS), New Delhi, India, e-mail: saurabh_kgmu@yahoo.co.in

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abdomen radiograph showed dilated bowel loops and multiple air-fluid levels. The patient was immediately taken up for emergency exploratory laparotomy. Intraoperatively, he was found to have an ileal perforation around 30 cm proximal to the ileocecal junction with perforation peritonitis. Peritoneal lavage and a diversion loop ileostomy were performed. Post-procedure period was uneventful, and the patient was discharged after five days.

This case brings us to the issue of learning, teaching and practising the basic clinical skills during the pandemic time. We all need to make efforts to alleviate panic in the emergencies and encourage resident doctors working in emergencies to continue using clinical judgements while deciding plan for the patients with respiratory symptoms. Minimum essential history in the form of the review of all major body systems should form part of the initial assessment. Let us not forget our basics, and we all will succeed in overcoming these hard times.

Conflict of interest

None declared.

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Saurabh Mittal, Karan Madan, Anant Mohan

Department of Pulmonary, Critical Care and Sleep Medicine, All India Institute of Medical Sciences, New Delhi, India

EBUS-TBNA in children: The road less travelled

The ultrasonographic evaluation and sampling of mediastinal lesions by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) or endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is the standard of care for adult patients [1]. It allows for real-time visualization of mediastinal lymph nodes during bronchoscopic needle aspiration and has virtually replaced mediastinoscopy for the evaluation of mediastinal pathologies in adults. The cell block samples obtained during EBUS-TBNA are processed as biopsy samples and significantly add to the yield of EBUS-TBNA. The use of EBUS-TBNA for mediastinal lesions in pediatric patients has revolutionized clinical practice at many centers and is now being used routinely for the evaluation of undiagnosed mediastinal lymphadenopathy and masses [2, 3]. While the reach of mediastinoscopy for mediastinal evaluation is limited, endo-ultrasonic modalities have a more extensive reach. In a recent study by Demir and Onal [4], authors described their experience with mediastinoscopy for the sampling of mediastinal lesions in 22 patients. None of the patients had undergone EBUS-TBNA or EUS-FNA prior to mediastinoscopy which suggests that this modality is still underutilized. Out of the 22 patients in the study population, 20 were ≥ 12 years of age. EBUS-TBNA can easily be performed in this age group as tracheal size in these individuals is sufficiently large enough to allow insertion of an EBUS bronchoscope without causing ventilatory compromise. In such patients, the procedure can also be performed under conscious sedation and does not necessarily need general anesthesia. Younger children usually require the procedure to be performed under general anesthesia with an airway conduit [5]. The endoscope used for endoscopic ultrasound is large and may not be appropriate for small children. As an alternative, the thinner EBUS bronchoscope (6.9 to 7.4 mm) can be introduced transesophageally to perform needle aspiration from esophageal accessible lymph node stations, particularly the subcarinal and left paratracheal ones. This technique is described as transesophageal bronchoscopic ultrasound-guided fine-needle aspiration (EUS-B-FNA) [6].

One of the concerns among pediatricians regarding endosonographic techniques remains the ability to acquire a sufficient sample for histological analysis. Tissue cores can be obtained with the use of the usual 21G or 22G needles. In addition to the standard 21 and 22 G needles for EBUS-TBNA, larger gauge EBUS-TBNA needles (19G) and pro-core needles are also now available. These may allow for the obtaining of a sufficient enough sample for histopathological analysis. Transbronchial forceps biopsy under EBUS guidance from lymph nodes can also be performed in patients with a suspected lymphoma [7]. In this technique, a small path is created in the bronchial tree under ultrasound guidance to allow the small biopsy forceps to enter the lymph node and obtain biopsies for histological evaluation. The EBUS-TBNA and EUS-B-FNA approach can provide a diagnosis in a significant proportion of pediatric patients thereby avoiding mediastinoscopy, especially in patients with granulomatous etiology. In the present era, the endosonographic evaluation of the mediastinum must be considered as the first-line approach for mediastinal lesions in the pediatric population. Mediastinoscopy should be reserved for individuals with non-diagnostic EBUS-TBNA or EUS-B-FNA, and/or for lesions that are not accessible by either of these two approaches.

Address for correspondence: Saurabh Mittal, Department of Pulmonary, Critical Care and Sleep Medicine, All India Institute of Medical Sciences, New Delhi, India; e-mail: saurabh_kgmu@yahoo.co.in

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Conflict of interest

None declared.

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Mayank Mishra, Girish Sindhwani

All India Institute of Medical Sciences, Rishikesh, India

Antifibrotics for COVID-19 related lung fibrosis: agents with benefits?

To the Editor

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an exponentially spreading pandemic with more than 36 million confirmed cases and over one million deaths worldwide, all within ten months of its first case in Wuhan, China [1]. The brunt of the infection affects the respiratory system and may range in presentation from an asymptomatic infection to severe acute respiratory distress syndrome (ARDS). Although more than 25 million cases have been reported to have recovered globally [2], an alarming upcoming trend is that of the long-term sequelae of COVID-19, the most devastating of which is pulmonary fibrosis. Referral for up to 10–15% of non-critically ill moderate to severe COVID-19 patients is sought in view of varying degrees of fibrotic change in the lungs in the authors' growing experience. Irrespective of the underlying etiology, pulmonary fibrosis notoriously jeopardizes the patient's functional capacity, confers chronic respiratory insufficiency, and consequently, compromises quality of life. Due to dearth of conclusive data, it may not be presently possible to compute the actual prevalence of COVID-19 lung fibrosis. However, given the enormity of the pandemic and its predominant and wide range of effects on the lungs, a significant burden of post-COVID-19 pulmonary fibrosis is anticipated [3]. Therefore, long-term follow-up studies will be desperately needed to address this issue.

The pathogenesis of pulmonary fibrosis involves alveolar epithelial damage triggered by genetic predisposition, unchecked chronic inflammation, viral infections, or ARDS. This happens due to the overexpression of pro-inflammatory cytokines (i.e. tumor necrosis factor-alpha, interleukins), proliferation and persistence of pro-fibroblastic cells and mediators (i.e. fibroblasts, transforming growth factor-beta, fibroblast growth factor, platelet derived growth factor), and resultant activation of the profibrotic pathway. Excess collagen and extracellular matrix replace normal lung tissue and produce architectural distortion typical of interstitial pulmonary fibrosis. Recent reports suggest that these mediators are likely implicated in COVID-19 lung fibrosis as well, as suggested by their increased serum levels in these patients [4–6].

The typical sequence of events in COVID-19 patients developing pulmonary fibrosis consists of an upper respiratory viral prodrome, atypical pneumonia, and ARDS culminating in fibrosis. Fibrosis may begin during or after the acute infectious episode and is more likely to develop in patients with a prolonged severe illness due to a cytokine storm, in those with pre-existing lung conditions, and in the elderly. No definitive profibrotic mechanisms are known in COVID-19 patients; however, pulmonary fibrosis in fatal COVID-19 cases characteristically shows the histological picture of diffuse alveolar damage and microthrombosis. Other proposed mechanisms driving fibrosis in these patients include a cytokine storm-related hyperimmune response triggered by the SARS-CoV-2 antigen, severe acute lung injury, fibrosing organizing pneumonia, and drug induced- and/or artificial ventilation-induced lung damage. It may not always be possible to identify which mechanism is at work in a particular patient. Further, even after the virus gets cleared in patients who

Address for correspondence: Mayank Mishra, All India Institute of Medical Sciences, Rishikesh, India, e-mail: virgodrmayank@gmail.com

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have recovered from COVID-19, it does not necessarily mean that fibrosis may not ensue. However, COVID-19-related lung fibrosis is supposedly not a progressive fibrosing interstitial lung disease (PF-ILD).

In the landmark placebo-controlled INBUILD trial, nintedanib was administered to patients who had progressive pulmonary fibrosis due to a wide variety of interstitial lung diseases (ILDs). The drug intervention was associated with a reduction in FVC decline (about 60%) thereby concluding that nintedanib appears to inhibit fibrogenesis across a broad range of pulmonary diseases [7]. Somewhat similar effects were observed with pirfenidone in another phase-2 randomized controlled trial [8]. An autopsy study of ARDS patients noted that longer the disease duration, greater were the chances of fibrosis [9]. Such patients may benefit from antifibrotic drugs if introduced early in the disease course before the need for mechanical ventilation emerges. These studies potentially imply that the early use of antifibrotics in COVID-19 lung fibrosis may possibly reduce immune-mediated fibrotic lung changes. However, other aspects of lung damage like inflammation and thrombosis must also be optimally addressed to maximize the potential benefit.

In light of these facts, the question that is currently puzzling clinicians around the world is whether antifibrotics indicated for other PF-ILDs would be of any benefit in COVID-19 patients developing lung fibrosis. Available anti-fibrotic medications like pirfenidone and nintedanib approved for use in PF-ILDs like idiopathic pulmonary fibrosis (IPF) and scleroderma-interstitial lung disease have broad anti-fibrotic activity irrespective of the underlying etiology. Importantly, the similar cytokine profiles in IPF and COVID-19 possibly suggest similar pathogenic mechanisms of lung fibrosis in both diseases, thus implying the likely utility of antifibrotics used in IPF for COVID-19 patients also, in whom they may be expected to prevent occurrence and/or progression of fibrosis. Therefore, it would be interesting to explore their full potential role, if any, in such patients to fulfil the urgent but largely unmet need for such therapies. Nevertheless, their use must not be outside of experimental studies, and the optimal timing of initiation, dosage, and duration of treatment must be determined.

No evidence currently exists to support empirical off-label use of antifibrotics in COVID-19 patients. Thus, well-designed, prospective, randomized clinical trials of these drugs in this group of patients are warranted. Until conclusive evidence builds up, these patients may probably best be offered aggressive pulmonary rehabilitation, possibly an extended course of low dose steroids on a case-by-case basis, and a trial of antifibrotic agents within a study protocol with periodic assessment of lung function and chest imaging. It is also likely that quite a few of these patients may have their lung changes resolved with time, possibly over a period of months. Such trends were also evident in previous coronavirus outbreaks where spontaneous but gradual resolution of fibrotic sequelae was observed [10, 11].

To conclude, limiting the development of post-COVID-19 lung fibrosis is expected to be a challenge in view of the blistering disease course and the ongoing search for effective antivirals, anti-inflammatory agents, and immunomodulatory therapies. Even a small degree of fibrosis in these patients, especially in the elderly who may quite commonly have other preexisting respiratory comorbidities, may be sufficient to significantly compromise their lung function and quality of life. Insightful evidence on therapeutic options for the treatment of this dangerous disease may bring about a landmark change in its management and, consequently, reduce these devastating sequelae.

Conflict of interest

None declared.

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Karthikeyan P Iyengar[1], Vijay K Jain[2], Raju Vaishya[3], Pranav Ish[4]

[1]Southport and Ormskirk NHS Trust, Southport, United Kingdom
[2]Atal Bihari Vajpayee Institute of Medical Sciences, Dr Ram Manohar Lohia Hospital, New Delhi, India
[3]Indraprastha Apollo Hospital, Sarita Vihar, New Delhi, India., India
[4]Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

Long COVID-19: An emerging pandemic in itself

To the Editor

The novel coronavirus SARS-CoV-2 disease is predominantly a respiratory illness that is highly contagious and is spread by droplet transmission. It causes a spectrum of illnesses from a mild sore throat to serious viral pneumonia requiring hospitalisation [1]. It is estimated that about 80% of people infected with COVID-19 have a mild course of illness. About 20% of the remaining patients require hospitalisation to treat their pneumonia and may need therapeutic assistance with oxygen. In about 5% of cases, the pneumonia becomes so severe that patients may need to be admitted to the intensive care unit for ventilatory support [2]. Currently, the majority of attention has been focused on the management of critical cases. Patients who experience only mild symptoms are being managed on an outpatient basis. However, it has become increasingly recognised that a sizeable third group of people seem to be demonstrating ongoing symptoms pertaining to COVID-19 far longer than expected for the disease pattern. This has raised concern amongst the health community due to the anticipated long-term effect on health care systems [3].

Prevalence is unknown but not uncommon

"Long COVID" is a term being used to describe the long-term effects of COVID-19 in people who have had either suspected or confirmed COVID-19. These people are reporting lasting effects of the infection [4]. Data from the COVID-19 symptom tracker app developed by Kings College London/ZOE COVID Symptom Study estimates that up to 10% of people with COVID-19 take at least three weeks to recover with some experiencing symptoms for 30 days or more [5]. A team of researchers from Italy reported that nearly nine in 10 patients (87%) discharged from a Rome hospital after recovering from COVID-19 were still experiencing at least one symptom 60 days after onset [6].

Presentations are variable and non-specific

The characteristic symptoms of COVID-19 include fever, dry cough, and shortness of breath. Some people also experience aches and pains, a sore throat, and loss of taste and/or smell. Patients suffering from a mild form of the disease might expect to get better after a few weeks. There is growing evidence that, in some patients, the symptoms persist longer than expected. Besides the well-described symptoms of COVID-19, the British Lung Foundation and Asthma UK's post-COVID survey of over 1000 patients (of which over 800 had not been admitted to hospital) found that the top five reported symptoms of long COVID were breathing problems (90%), extreme tiredness (64%), sleeping problems (22%), cough (22%), and changes in mood involving anxiety or depression (22%) [7]. The majority of these people had not experienced these symptoms before COVID. The initial findings from the survey showed that many people who had a mild to moderate course of disease are now on a long road to recovery that is affecting both their physical and mental health. The Italian study from Rome found

Address for correspondence: Pranav Ish, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi, India; e-mail: pranavish2512@gmail.com

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Table 1. Reported clinical characteristics of long COVID

General	• Fatigue • Tiredness • Sleep disturbances
Respiratory	• Dyspnoea • Exacerbation of Asthma or COPD • Persistent cough
Mental Health	• Emotional Disturbances • PTSD • Anxiety • Depression • Mood disturbances
Musculo-skeletal	• Joint pain • Myalgia • Arthritis of small joints
Cardiovascular	• Chest pain • Palpitations
Neurological	• Pins and needles' sensation • Headache • Dizziness

COPD — chronic obstructive pulmonary disease; PTSD — post-traumatic stress disorder

that, in patients recovering from COVID-19, many still reported fatigue (53%), dyspnoea (43%), joint pain (27%), and chest pain (22%) even though none of the patients had fever or any signs or symptoms of acute illness. Two-fifths of patients reported a worsened quality of life [6]. The reported clinical features affect multiple parts of the body and are highlighted in Table 1.

An unknown pathophysiology that will be deciphered with time

As the understanding of COVID-19 is unravelling, it has been acknowledged that COVID-19 is associated with inflammation and a prothrombotic state [8]. People with severe COVID-19 seem to show an altered immune response and suffer an exaggerated inflammatory response (the "cytokine storm"). Whether the triggering of the immune system has any role in the features of long COVID is unclear and hence, the exact underlying pathophysiology of long COVID is still not known. It is evident that clinical features seen in this cohort of long COVID patients is not restricted to patients who had been hospitalized, but is also being observed in patients who have had an initial mild illness.

Huge impact in all dimensions of life

There is a growing body of evidence that a significant minority of patients are suffering from persisting and distressing symptoms that, under normal circumstances, would represent 'red-flag' symptoms requiring urgent investigation. Many people report an emergence of new symptoms late in the course of their illness. They state that these symptoms exhibit a relapsing-remitting pattern even though many had reported a mild initial illness. These factors combine to add to the distress and uncertainty of the condition. The long-term effects of COVID-19, even on people who suffered a mild infection, could be far worse than were originally anticipated according to researchers and doctors in Lombardy, Italy (the worst affected region in the country) [9]. The doctors warn that some victims may never recover from the illness and that all age groups are vulnerable. Some people may find that their ability to properly work, concentrate, and even take part in physical activities will be severely impaired. In the United Kingdom (UK), similar findings seem to have been found in a recent survey conducted by the British Lung Foundation and Asthma UK in people recovering from mild to moderate COVID-19. These patients had reported to have been struggling for weeks with symptoms, raising concerns that there is not adequate support for people who have not been treated on an inpatient basis with the illness [7]. The post-COVID-19 period was also found to be taking its toll on patient's mental health. Over one-half of the people surveyed said that they did not feel they can cope well after the illness. There have been some patients that have reported symptoms of post-traumatic stress disorder.

Strategies

Recognising the growing concerns of patients, medical professionals, and medical organisations, the National Health Service (NHS) of England has launched a new service titled "Your COVID Recovery" in order to support, expand, and provide access to COVID-19 rehabilitation treatments for those who have survived the virus but still have problems with breathing, mental health, or other complications [11]. This post-COVID-19 support program idea can be extrapolated to support people in other countries. This support system consists of:

Multi-disciplinary support plans are being put into place to support patients who have been in hospitals or suffered at home with the virus with access to a face-to-face consultation with their local rehabilitation team (usually comprising of physiotherapists, nurses, and mental

health specialists). The multi-disciplinary team will be able to assess the needs and provide an appropriate level of support.

Online Support: Peer-to-peer community and mental support groups have been developed (e.g. Long Covid.org) to provide online resources and exercise tutorials to help in the post-COVID recovery period.

Social Media Support: Organisations and social media platforms (e.g. Facebook and Twitter) have created virtual support groups such as the "Long COVID Support group" for peer to peer support and information exchange.

Future directions

As we learn more about COVID-19, dealing with the emerging problem of long COVID will require a coordinated response from the government, public health bodies, healthcare systems, scientists, and the medical society in general. Research into the long term effects of COVID-19 on both hospitalized patients and those who initially only had mild symptoms and were treated on an outpatient basis will be necessary to understand and unravel the pathophysiology of long COVID. The Post-hospitalisation COVID-19 Study (PHOSP-COVID) that is being planned in order to assess the long-term effects of COVID-19 in hospitalized patients should be extended to include milder cases in order to understand the full spectrum of the disease [12].

Conclusion

It is important to acknowledge that the effects of COVID-19 are not only acute, but that the disease has long-term consequences as well. The recognition and increased awareness of long COVID is necessary to manage this illness effectively. Rehabilitation, counselling, and mental health support form cornerstones of treating this condition. Establishing scientific studies and research will help us to keep an open mind when dealing with this new disease.

Conflict of interest

None declared.

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Manu Madan, Anant Mohan, Karan Madan, Vijay Hadda, Pawan Tiwari, Randeep Guleria, Saurabh Mittal

Department of Pulmonary, Critical care and Sleep Medicine, All India Institute of Medical Sciences (AIIMS) New Delhi, India

Timing of anti-viral therapy in COVID-19: key to success

To the Editor

Since the onset of the current coronavirus disease 2019 (COVID-19) pandemic, there have been attempts to identify medications for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As there have been no antivirals available for the treatment of this disease, repurposing of drugs has started and various classes of drugs are being tried. Some of the candidate drugs include remdesivir (recently approved by Food and Drug Administration), ivermectin, and interferon β-1b. There is emerging evidence regarding the efficacy of these drugs; however, no definite conclusions are available. A recent study by Shi et al. reported results about the efficacy of antiviral therapies in patients with coronavirus disease 2019 (COVID-19) in China and found no significant impact on improvement [1]. Similar results were reported in a recently published randomized controlled trial (RCT) about the use of interferon β-1a in patients with severe coronavirus disease 2019 (COVID-19) and found no significant difference in the time to clinical response in the experimental arm as compared to the control arm [2]. However, there are a few aspects regarding the timing of the initiation of antivirals which require discussion. In both of these studies, the authors have not reported the timing of initiation of antiviral therapy, which is crucial to patient outcomes. We all understand that the host's immune response plays a crucial role in the prevention as well as containment of any infection; however, when an antiviral agent is sought for patients with disease or for patients who are at risk of severe disease, it should be done in a timely manner [3]. COVID-19 has an initial virological phase which leads the patients into a host inflammatory response phase where they tend to develop a cytokine storm [4]. Based on the report by Wölfel et al. [5] which states that the virus cannot be isolated beyond day 8, it is also likely that antivirals may not be efficacious beyond this time. Thus, it would be best to use antiviral medications relatively early in the illness and anti-inflammatory drugs later. Using antiviral drugs later in the disease course may add to the adverse effects rather than yielding clinical benefits. In the study by Effat *et al.* [2], the mean (standard deviation, SD) duration of starting treatment in the interferon arm was 11.7 (5.71) days. This late initiation of antiviral therapy may be the reason behind no difference in time to clinical response, which was the primary endpoint. However, there was a difference with respect to the percentage of patients being discharged by day 14, favouring the interferon group. Such a result may be owed to the properties of interferon, which endorses more than just an antiviral mechanism (i.e. decreasing vascular leakage and inflammatory biomarkers like IL-6) [6, 7]. They also reported that starting interferon treatment early in the course of the disease showed mortality benefit (odds ratio, 13.5; 95% confidence interval 1.5 to 118) which further emphasizes the importance of early initiation of therapy [2].

Table 1 enlists some noteworthy trials in COVID-19 regarding the use of antiviral medications and timing of treatment initiation for the outcome reported. Remdesivir showed no benefit when treatment was started after ten days of illness. Instead, it was associated with higher

Address for correspondence: Saurabh Mittal, Department of Pulmonary, Critical Care and Sleep Medicine, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India; e-mail: saurabh_kgmu@yahoo.co.in

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Table 1. List of studies with use of antivirals and outcomes reported

Author	Drug	Number of patients	Study design	Day of initiation of treatment from symptom onset	Outcome in comparison to control or standard of care
Wang *et al.* [8]	Remdesivir	237	RCT	11	No benefit Patients started on treatment within 10 days had decreased mortality (11%) vs patients having treatment started after 10 days (14%)
Spinner *et al*. [9]	Remdesivir	584	RCT	8	Higher odds of a better clinical outcome with those randomized to standard care (OR 1.65; 95% CI 1.09–2.48; p = 0.02)
Beigel *et al*. [10]	Remdesivir	1063	RCT	9	The remdesivir group had a shorter time to recovery (median, 11 days, as compared with 15 days; rate ratio for recovery, 1.32; 95% CI 1.12 to 1.55; p < 0.001)
Cao *et al*. [11]	Lopinavir-ritonavir	199	RCT	13	No benefit
Hung *et al.* [12]	Interferon beta-1b, lopinavir–ritonavir, and ribavirin	127	RCT	5	Significantly shorter median time from start of study treatment to negative nasopharyngeal swab in treatment group (7 days [IQR 5–11]) than the control group (12 days [8–15]; HR 4·37 [95% CI 1.86–10.24], p = 0.0010) Clinical improvement was better in the combination group
Zhou *et al.* [7]	Interferon alpha	77	Non randomized	8	Significant accelerated viral clearance (p = 0.002), and reduced circulating levels of IL-6 ($p = 5.7 \times 10^{-10}$) and CRP (p = 0.002)

CI — confidence interval; CRP — C-reactive protein; HR — hazard ratio; Il-6 — interleukine 6; IQR — interquartile range; OR — odds ratio; RCT — randomized clinical trial

mortality than the control arm [8]. However, when used within ten days, it tended to show benefits in other trials [9, 10]. Most other trials tend to start antivirals late and have reported no clinical benefits with their use.

This brings us to essential questions of whether these drugs, if initiated early, can lead to clinical benefits, and whether or not these negative trials are giving us a false portrayal of their efficacy. Based on the available evidence, we suggest that antivirals should be initiated within the first ten days of illness, especially in research settings. In this COVID era, with the limited therapeutic options available to physicians, the appropriate and timely use of therapy can help save lives.

Conflict of interest

None declared.

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