Original Article

Lower respiratory tract sampling in COVID-19 acute respiratory distress syndrome: A focus on microbiology, cellular morphology, cytology, and management impact

Sameer Bansal[1], Hariprasad Kalpakam[1], Ashwin Kumar[2], Amogha Varsha[2], Anmol Thorbole[1], Ravindra M. Mehta[1]

[1]Department of Pulmonary and Critical Care Medicine, Apollo Specialty Hospitals, Bengaluru, Karnataka, India, [2]Department of Pulmonary and Critical Care Medicine, Kempegowda Institute of Medical Sciences, Bengaluru, Karnataka, India

ABSTRACT

Background: Lower respiratory tract (LRT) sampling through bronchoscopy has been done sparingly in COVID-19 acute respiratory distress syndrome (ARDS) due to the high aerosol risk for the health-care workers (HCWs). Valuable information can be gained by a detailed evaluation of bronchoscopic LRT samples. **Methods:** LRT samples were obtained by bedside bronchoscopy severe COVID-19 ARDS patients on mechanical ventilation. Microbiological, cellular, and cytological studies including LRT COVID-19 reverse transcription-polymerase chain reaction were analyzed. **Results:** A total of 100 samples were collected from 63 patients, 53 were males (84%). Forty-three patients (68%) had at least 1 comorbidity. 55% of cases had a secondary bacterial infection, commonly with multidrug-resistant organisms (94.5%). The most common organisms were *Klebsiella pneumoniae* and *Acinetobacter baumannii* in 56.3% and 14.5% of cases, respectively. Fungal superinfection was observed in 9 patients (14.3%). Bronchoscopy helped confirm COVID-19 diagnosis in 1 patient and helped rule out COVID-19 in 3 patients. The median bronchoalveolar lavage fluid (BALF) white blood cell (WBC) count was 953 (inter quartile range; 400–2717), with mean neutrophil count 85.2% (±13.9) and mean lymphocyte count 14.8% (±13.9). Repeat sampling done in some patients showed a progressive increase in the total WBC count in BALF, an increase in neutrophil percentage, and a higher chance of isolating an organism on the culture. Rate of superinfection increased with a longer duration of illness. Bronchoscopic LRT sampling contributed significantly to modifying antibiotic coverage and discontinuing steroids in 37% of cases. **Conclusions:** Our study provides a detailed analysis of bronchoscopic LRT sampling in critically ill COVID-19 patients, augmenting disease understanding and contributing to clinical management.

KEY WORDS: Bronchoalveolar lavage, bronchoscopy, COVID-19, cytology, mechanical ventilation, microbiology

INTRODUCTION

Bronchoscopic lower respiratory tract (LRT) sampling in COVID-19 patients has been done sparingly due to the high aerosol-related risk to health-care workers (HCWs).[1,2] Very few studies have described microbiological, cellular, and cytological aspects of LRT samples and their relationship

Access this article online

Quick Response Code:

Website:
www.lungindia.com

DOI:
10.4103/lungindia.lungindia_532_21

with inflammation and infection in COVID-19 patients. This study analyzed LRT samples obtained by bronchoscopy with a focus on the above aspects in mechanically ventilated (MV) critical COVID-acute respiratory distress syndrome (C-ARDS) patients.

METHODS

This is a retrospective observational descriptive study conducted at a tertiary committed COVID center between August 25, 2020 and December 03, 2020. Approval was granted by the Institutional Ethics Committee Apollo Hospitals, Bangalore. The study group included all MV intensive care unit (ICU) patients with initially proven or later confirmed COVID-19 who underwent bronchoscopy for various clinical indications. Bronchoscopy was deferred when any of the following were present; positive end-expiratory pressure ≥ 10 cm H_2O, hemodynamic instability, or operator's perception of life-threatening deterioration during the procedure.

The following variables were recorded: demographic and clinical parameters including age, gender, duration of symptoms before hospitalization, presence of comorbidities (diabetes mellitus, hypertension, chronic kidney disease, ischemic heart disease), and duration of ventilatory support before procedure. Procedure details included indications, findings, relevant microbiological and cytological tests, and management changes following bronchoscopy. Safety aspects from both the patient and the HCW perspective were also studied.

Procedure

Bronchoscopy after informed consent was performed by three different operators. A bronchoscopy technician, a respiratory therapist, and an ICU nurse were present for every procedure. All HCWs used adequate personal protective equipment PPE (P-100 respirator, impermeable coverall, face shield, and double-layered gloves). Periodic nasopharyngeal (NP) swabs were tested for COVID-19 reverse transcription-polymerase chain reaction (RT-PCR) in HCWs.

The procedure was performed at the bedside in the ICU, with >20 air exchanges/hour. Negative pressure isolation rooms were not available. Sedation included midazolam and fentanyl and short-acting neuromuscular blockade with atracurium to prevent any aerosol generating cough. Preprocedure, FiO_2 was increased to 100% for 20 min. Rapid bronchoscopy was done, with close monitoring of SpO_2 and vital parameters, with brief in-and-out runs with the bronchoscope as needed. As a safety measure, patients in prone position were maintained in the same position to reduce desaturation.

Pooled washings (average 80–100 ml from multiple segments) were done in view of the need for multisegment sampling and concern of desaturation with a larger volume bronchoalveolar lavage (BAL). Samples were collected and analyzed for laboratory investigations including the COVID RT-PCR.

Statistics

Data were tabulated and analyzed using SPSS (ver. 25.0, SPSS Inc., IBM, Armonk, New York, United States). Results were analyzed in a descriptive fashion as number and percentages, mean and standard deviation, median, and interquartile range (IQR). Difference between mean and medians was expressed using Chi-square test and MannWhitney U-test, respectively. Correlation analysis was done using linear correlation, and results expressed using Pearson's correlation coefficient. Statistical significance was taken at $P < 0.5$.

RESULTS

One hundred bronchoscopic LRT samplings were done in 63 MV C-ARDS patients. Forty-three patients had one bronchoscopy procedure, while 20 patients had repeat procedures, for various indications such as clinical deterioration with new radiographic infiltrates, segmental collapse, increased secretions causing difficult ventilation, and hemoptysis.

Microbiology
Gram stain
Gram's stain showed pus cells in 79 cases (83%), with numerous pus cells (>25 per LPF) reported in 51/79 (64.5%) cases. Morphologically, copious purulent endobronchial secretions correlated with increased pus cells on BAL analysis.

Overall cellular analysis showed the median white blood cell (WBC) count in BAL fluid (BALF) as 953 (IQR; 400–2717). In samples where many pus cells were reported, median WBC count was 1628 – the corresponding mean neutrophil count was also higher (89.2% vs. 85.2%).

Gram stain could detect an organism in 36 cases (36%) – Gram-negative bacilli in 34 cases and Gram-positive cocci in 2 cases, with culture positivity in 55 cases (55%).

Culture reports
Of the 100 cases, bacterial culture was positive in 55 cases (55%) with colony counts $>10^5$ CFU/ml and sterile in 45 cases (45%). All these patients were on prior antibiotics. *Klebsiella pneumoniae* and *Acinetobacter baumannii* were most commonly isolated organisms in 31 cases (56.3%) and in 8 cases (14.5%), respectively. Other organisms isolated were *Burkholderia cepacia* in 4 cases (7.2%), *Enterobacter cloacae* in 3 cases (5.4%), and *Acinetobacter iwoffii*, *Providencia stuartii*, and *Serratia marcescens* in 2 cases each. MRSA, *Pseudomonas aeruginosa*, *Morganella morganii*, *Stenotrophomonas maltophilia*, and *Citrobacter freundii* were other sporadically isolated organisms.

Three patients grew more than one organism in the BALF. In 2 cases, these were *K. pneumoniae* with *E. cloacae*, while in one case, it was *A. baumannii* with *B. cepacia*. All but 3/55 cases had grown multidrug-resistant (MDR) organisms (94.5%), implying nosocomial superinfection.

Fungal evaluation

Nine patients (14.3%) had fungal superinfection as determined by KOH mount, fungal cultures, and/or BAL Galactomannan. Seven patients had a positive KOH mount, of which 5 showed budding yeast with septate hyphae, while 2 had the presence of aseptate hyphae. Fungal culture was positive in only two patients, but the majority of these patients were on empirical antifungal medications. One patient grew *Aspergillus niger*, and the other patient grew *Aspergillus fumigatus*. Four of these patients with a positive KOH mount also had bacterial coinfection with MDR organisms, while three were bacterial culture sterile.

BAL galactomannan was sent for 6 patients and was elevated in all the cases. Galactomannan values were 1.65, 2.88, 1.72, 2.03, 1.52, and 2.14 in these six patients, respectively (done by immune-enzymatic sandwich microplate assay; >0.5 ODI considered positive). While three of these cases had a positive KOH mount, three did not stain with KOH. All six were culture negative. Appropriate antifungal agents were added in all the cases. Simultaneous blood cultures (±1 day) sent in these patients grew fungi in 3/9 patients – 1 patient grew *Candida auris* and 2 *Candida albicans*. Table 1 lists the spectrum of infections in COVID patients.

Table 1: Spectrum of infections on bronchoalveolar lavage

	n (%)
Bacterial infection characteristics	
Bacterial culture positive	55/100 cases (55)
MDR organisms	52 (94.5)
Klebsiella pneumoniae	31 (56.5)
Acinetobacter baumannii	8 (14.5)
Burkholderia cepacia	4 (7.2)
Enterobacter cloacae	3 (5.4)
Acinetobacter lwoffii	2 (3.6)
Providencia stuartii	2 (3.6)
Serratia marcescens	2 (3.6)
Citrobacter freundii	1 (1.8)
MRSA*	1 (1.8)
Pseudomonas aeruginosa	1 (1.8)
Morganella morganii	1 (1.8)
Stenotrophomonas maltophilia	1 (1.8)
Fungal infection characteristics	
Fungal infections	9/100 cases (9)
KOH mount positive	7 (77.7)
Galactomannan positive	6 (66.7)
Fungal culture positive	2 (22.2)
COVID-19 RT-PCR	
BAL positive	27/38 cases (71)

MRSA: Methicillin resistant *Staphylococcus aureus*,
MDR: Multi-drug resistant, BAL: Bronchoalveolar lavage,
RT-PCR: Reverse transcription-polymerase chain reaction

A comparative analysis of patients with and without superinfection is presented in Table 2.

COVID reverse transcription-polymerase chain reaction

In one patient, COVID-19 diagnosis was confirmed on BAL RT-PCR after two consecutive NP swabs were negative. In addition, in three patients, COVID-19 was ruled out on LRT RT-PCR. An important observation was the duration of illness and persistence of RT-PCR positivity in the BAL of many patients. In 18/27 (67%) positive cases, BAL RT-PCR positivity duration was >10 days from the beginning of illness, in 14 cases (54%) it was >15 days, in 3 cases (11%) >20 days, while one patient had a persistently positive BAL RT-PCR report for 67 days.

Bronchoalveolar lavage cytology

The median WBC count was 953 (IQR; 400–2717), with mean neutrophils 85.2% ($\pm$13.9), and mean lymphocytes 14.8% ($\pm$13.9). 51% of patients showed $\geq$10% lymphocytes, 25.5% had $\geq$20%, 14.3% had $\geq$30%, while 6% of patients had $\geq$40% lymphocytes in the fluid analysis. The mean neutrophil to lymphocyte ratio (NLR) in BAL was 13.3. The following findings were noted correlating duration of illness with BAL cytology:
1. As the duration of illness progressed, the mean lymphocyte count in BAL reduced, while the neutrophil count increased
2. Pearson's correlation coefficient between duration of illness and BAL lymphocyte count was -0.27, an inverse relationship, though statistically insignificant ($P > 0.05$).

We also analyzed the peripheral blood sample drawn within 24 h of the BAL. The mean peripheral WBC count was 17.4 ($\pm$7.2), with mean neutrophils 90.3% ($\pm$6.0) and lymphocytes 4.8% ($\pm$4.5). Mean NLR ratio was 35. The correlation between BAL NLR and serum NLR was 0.1, a very weak positive correlation.

Patients with repeat procedures

A subgroup of 20 patients had repeat bronchoscopy procedures with LRT sampling, majority for recurrent thick secretions in the endotracheal tube causing ventilation issues. The following aspects were noted. There was a greater chance of isolating an organism on culture when procedures were repeated (81% of repeat procedures were culture positive). Serial changes noted on baseline broad-spectrum antibiotics included the following:
1. Fourteen patients had reduced secretion amount and purulence over serial procedures. WBC count also reduced sequentially in these patients. Four patients turned culture negative on subsequent sampling, while ten stayed persistently culture positive with the same microorganism with a significant colony count
2. Six patients had grown different microorganisms on serial bronchoscopic sampling. In these patients, WBC count in BALF also increased sequentially, with the percentage of neutrophils increasing with repeat sampling.

Table 2: Comparison of patients with and without super-infection

	No super-infection (*n*=35), *n* (%)	Bacterial/fungal superinfection (*n*=28), *n* (%)	*P*	CI
Demographics				
Bacterial infection	0	25		
Fungal infection	0	9		
Mean age	60.7 (±12.4)	64.3 (±11)	0.2	−3.6 (−9.8-2.6)
Males	30 (85.7)	23 (82.1)	0.7	
Co-morbidities				
≥1 comorbidity	23 (67.7)	20 (71.4)	1	
DM	15 (44)	15 (53.5)	0.71	
HTN	13 (38)	14 (51.8)	0.3	
CKD	3 (8.8)	4 (14.8)	0.68	
IHD	5 (15.1)	4 (14.8)	1	
Chronic respiratory illness	4 (11.7)	1 (3.7)	0.37	
Timelines (days)				
DOI to DOMV	9	12	0.08	
DOMV to DOB	1	2	0.054	
DOI to DOB	11	14	0.02	
BAL cell count				
Median WBC count	400	1001	0.02	
Mean neutrophil (%)	81.30	88	0.07	−6.8 (−14.3-0.7)
Mean lymphocyte (%)	18.70	12		

DM: Diabetes mellitus, HTN: Hypertension, CKD: Chronic Kidney Disease, IHD: Ischemic heart disease, DOI: Date of illness, DOMV: Date of mechanical ventilation, DOB: Date of bronchoscopy, CI: Confidence interval, WBC: White blood cell, BAL: Bronchoalveolar lavage

Impact and safety of bronchoscopy

Bronchoscopy significantly impacted management decisions in these patients. As a background, all patients at the time of bronchoscopy were on prior broad-spectrum antibiotics as per the clinical protocol for MV patients. Since the majority of patients grew MDR organisms sensitive only to the polymyxin group of antibiotics, we changed our policy midway to empiric polymyxins for suspected new-onset infection on MV. A new strategy also was addition of nebulized colistin through a closed, in-line nebulization circuit in patients who had copious purulent secretions in the airways. Subsequent culture reports confirmed MDR organisms in all these cases. After nebulized antibiotics, we found a reduction in the quantity and purulence of secretions in the majority of these patients.

Assessing risk to the HCW's, none of the HCWs developed COVID-19 disease during the study period. We observed transient desaturation up to 10% after bronchoscopy, which reversed within 30 min postprocedure. In addition, experienced operators ensured a quick procedure. No other complications were noted.

DISCUSSION

Bronchoscopy in COVID patients has been challenging due to risk of aerosol exposure and infection to the operator and team. Various guidelines exist on the indications, precautions, limiting personnel, and personal protective equipment for performing bronchoscopy in COVID-19 patients. Recently, the Indian Association of Bronchology also published its consensus statement on bronchoscopy during the COVID-19 pandemic.[3]

Limited data exist on detailed aspects of infection and inflammation in the LRT in critically ill C-ARDS patients.

This study of bronchoscopic LRT sampling in such patients describes noteworthy microbiological, cellular, cytological, and RT-PCR aspects and their clinical relevance in the pandemic. In addition, the study reiterates the importance of detailed knowledge of local microbiological patterns in C-ARDS, vital to understand both disease dynamics and critical management issues such as superinfection.

LRT sampling revealed interesting aspects of bacterial superinfection. Reviewing published literature, Torrego *et al.* found culture-proven secondary bacterial infections in 28.6% of cases,[4] while cultures were positive in up to 60% of cases sampled by Bruyneel *et al.*[5] Nearly 86% of samples obtained by Baron *et al.* showed the presence of at least one microorganism on culture.[6] In our study, cultures were positive for various bacteria in 55% of cases. The various microorganisms isolated reflect the local spectrum. In previous studies, the most common organisms isolated were *Pseudomonas* spp. and *Staphylococcus aureus*, while our series had the majority positive for *K. pneumonia* and *A. baumannii*. We also detected some uncommon pathogens, namely, *Burkholderia, Providencia, Citrobacter, Morganella,* and *Stenotrophomonas*, explained by advanced severe C-ARDS, comorbidities, and uniform steroid usage.

Fungal detection in LRT samples also varies depending on the series. COVID-associated pulmonary aspergillosis (CAPA) has been described in multiple studies. Bruyneel *et al.* reported fungal infection in 16 samples, all culture/galactomannan negative.[5] Baron *et al.* isolated *Aspergillus* spp. on culture/PCR in 25% of cases.[6] Case series by Koehler *et al.* and van Arkel *et al.* suggest 20%–25% incidence of Aspergillosis in critically ill COVID-19 patients.[7,8] Studies from Wuhan reported secondary fungal infections in 35.3% of critically ill patients.[9,10] A case series from France reported presumed

CAPA in 33.3% of ICU COVID-19 patients.[11] All-cause mortality was 33.3% in the French CAPA series and 80% in the study by Koehler *et al.*[7,11] Patrucco *et al.* isolated fungi in 13% of cases (*C. albicans* 11 times [64.7%]).[12] Previous studies have reported high rates of influenza-associated pulmonary aspergillosis which is similar to the high incidence of CAPA.[13] We had fungal superinfection in 9 (14.5%) patients. Since all our patients evaluated with BAL galactomannan had a value >1 with proven COVID, it seems reasonable to consider CAPA in these patients, even though they were culture negative.

Few studies describe the utility of LRT samples obtained by BAL for the diagnosis of COVID-19 infection. This is an important clinical issue as the overall sensitivity of the NP COVID RT-PCR swab ranges from 55% to 70%. Wang *et al.* found SARS-CoV-2 RNA in 14/15 (93%) BAL samples in comparison to 126/398 (32%) of NP swabs from patients with COVID-19.[14] Patrucco *et al.* isolated SARS-CoV-2 27.5% times in patients with two negative swabs.[12] We also found LRT sampling especially useful in this regard. BALF helped in diagnosing COVID-19 in 1 patient when 2 consecutive swabs were negative, while it helped in ruling out COVID-19 in 3 patients. These patients had a suggestive CT scan, elevated inflammatory markers, leukopenia, and two consecutive negative swabs. They were diagnosed as non-COVID viral pneumonia and eventually excluded from the study.

The SARS-CoV2 RT-PCR signal in the BAL and its relationship to symptom onset in critical C-ARDS is an important aspect that needs further exploration. Patrucco *et al.* performed BAL on 86 COVID suspects. Of these, 54 were RT-PCR negative and had a median symptom onset to bronchoscopy (SO → B) duration of 20 days. In comparison, 32 tested positive and had a median duration of SO → B of 12 days.[12] In our study, BAL RT-PCR was positive beyond 15 days in 54% of our cases, while the longest it remained positive was 67 days in one patient. The persistence of the BAL SARS-CoV2 signal has interesting implications on disease course, management, and infectivity and needs further study, as it is difficult to assess whether the virus is dead or alive.

The cellular details in the LRT sampling showed interesting variations. The median WBC count in our study group was 953 (IQR; 400–2717), with mean neutrophils 85.2% (±13.9) and mean lymphocytes 14.8% (±13.9). As the duration of symptoms increased, lymphocyte percentage reduced, while neutrophils increased. This finding of BAL neutrophilia has been described in literature. Pandolfi *et al.* observed that alveolitis in severe COVID-19 patients was associated with hyperactivation of macrophages and neutrophils, with an excessive infiltration of neutrophils at the alveolar level.[15] Lymphocytes were significantly reduced in critically ill patients as compared to patients admitted to the wards. Neutrophilia in these patients signifies severe inflammation with possible superinfection and portends worsening of disease with possible

detrimental outcomes. Multiple autopsy reports have suggested the role of neutrophilia as a marker of severe COVID-19.[16] All our patients were critically ill and had a higher neutrophil percentage, with a mean NLR of 13.3. Liao *et al.* characterized BALF immune cells from patients with varying severity of COVID-19 and from healthy people using single-cell RNA sequencing.[17] BALF of patients with critical COVID-19 infection showed a higher proportion of macrophages and neutrophils and lower lymphocyte count as compared to mild ones. Although most of the studies suggest high NLR as a poor prognostic model, none of them have commented on BALF NLR and its correlation with serum NLR. Mean serum and BALF NLR in our patients was 35 and 13.3, respectively. The correlation between BALF NLR and serum NLR was 0.1, a very weak positive correlation.

A unique aspect of our study was the analysis in the subset of patients with repeat procedures, average 5 days apart, for indications as mentioned above. Interesting findings in the LRT sampling in repeat procedures were a progressive increase in the total WBC count in BALF, decrease in the lymphocyte percentage, and higher chances of isolating an organism on the culture (81% repeat samples were culture positive).

Bronchoscopic LRT sampling significantly impacted management. In 31.6% of cases, antibiotics were escalated based on copious purulent bronchial secretions, with subsequent confirmation on culture. Analysis of preliminary culture results also led to a change in antibiotic policy with empirical polymyxin antibiotics introduced with suspicion of superinfection. Another unconventional strategy was the addition of nebulized colistin to systemic therapy. Other important decisions coinciding with antibiotic escalation were to de-escalate or stop corticosteroids when copious purulent secretions were noted as a systematic immunosuppression reduction strategy. Torrego *et al.* based on BAL introduced a new antibiotic in 83% of patients.[4] Bruyneel *et al.* state that bronchoscopy led to antibiotic adaptation in 18% of total and 31% of positive microbiological samples.[5] Baron *et al.* mentioned that BAL impacted decision-making in 71% of cases: Introduction, continuation, switch, or withdrawal of antimicrobial therapy in 50% cases and decision to start (21%) or not start (21%) corticosteroid therapy.[6]

This is one of the few studies with comprehensive LRT sampling through bronchoscopy at the height of the COVID-19 pandemic and correlates clinical, microbiological, cellular, and RT-PCR findings. This is one of the few studies to report all these aspects with uniform steroid use. These data improve C-ARDS disease understanding, as well as help in clinical decision-making in this critically ill population. Our study did have certain limitations. We restricted the procedures to only intubated critically ill COVID-19 ARDS patients and not able to do galactomannan and molecular microbiological testing on all the samples.

CONCLUSION

A fundamental limitation in MV COVID-19 patients was restricted suctioning due to aerosol risk, limiting many aspects of diagnosis, and information to guide therapy. This study describes the detailed analysis and impact of bronchoscopic LRT sampling in critically ill C-ARDS patients at a stage when there was scant information available in the pandemic.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jackson T, Deibert D, Wyatt G, Durand-Moreau Q, Adisesh A, Khunti K, *et al*. Classification of aerosol-generating procedures: A rapid systematic review. BMJ Open Respir Res 2020;7:e000730.
2. Doggett N, Chow CW, Mubareka S. Characterization of experimental and clinical Bioaerosol generation during potential aerosol-generating procedures. Chest 2020;158:2467-73.
3. Chhajed PN, Nene A, Abhyankar N, Akkaraju J, Agarwal R, Arora S, *et al*. Conventional flexible bronchoscopy during the COVID pandemic: A consensus statement from the Indian Association for Bronchology. Lung India 2021;38:S105-15.
4. Torrego A, Pajares V, Fernández-Arias C, Vera P, Mancebo J. Bronchoscopy in patients with COVID-19 with invasive mechanical ventilation: A single-center experience. Am J Respir Crit Care Med 2020;202:284-7.
5. Bruyneel M, Gabrovska M, Rummens P, Roman A, Claus M, Stevens E, *et al*. Bronchoscopy in COVID-19 Intensive Care Unit patients. Respirology 2020;25:1313-5.
6. Baron A, Hachem M, Tran Van Nhieu J, Botterel F, Fourati S, Carteaux G, *et al*. Bronchoalveolar Lavage in COVID-19 Patients with Invasive Mechanical Ventilation for ARDS. United States of America: Annals of the American Thoracic Society; 2020.
7. Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, *et al*. COVID-19 associated pulmonary aspergillosis. Mycoses 2020;63:528-34.
8. van Arkel AL, Rijpstra TA, Belderbos HN, van Wijngaarden P, Verweij PE, Bentvelsen RG. COVID-19-associated pulmonary aspergillosis. Am J Respir Crit Care Med 2020;202:132-5.
9. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, *et al*. Clinical features of 85 fatal cases of COVID-19 from Wuhan. A retrospective observational study. Am J Respir Crit Care Med 2020;201:1372-9.
10. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, *et al*. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically Ill patients with coronavirus disease 2019. Clin Infect Dis 2020;71:1937-42.
11. Alanio A, Dellière S, Fodil S, Bretagne S, Mégarbane B. High prevalence of putative invasive pulmonary aspergillosis in critically Ill COVID 19 patients. Lancet Respir Med 2020;8:e48-e49.
12. Patrucco F, Albera C, Bellocchia M, Foci V, Gavelli F, Castello LM, *et al*. SARS-CoV-2 detection on bronchoalveolar lavage: An Italian multicenter experience. Respiration 2020;99:970-8.
13. van de Veerdonk FL, Kolwijck E, Lestrade PP, Hodiamont CJ, Rijnders BJ, van Paassen J, *et al*. Influenza-associated aspergillosis in critically Ill patients. Am J Respir Crit Care Med 2017;196:524-7.
14. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, *et al*. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843-4.
15. Pandolfi L, Fossali T, Frangipane V, Bozzini S, Morosini M, D'Amato M, *et al*. Broncho-alveolar inflammation in COVID-19 patients: A correlation with clinical outcome. BMC Pulm Med 2020;20:301.
16. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: An autopsy series from New Orleans. Lancet Respir Med 2020;8:681-6.
17. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, *et al*. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2020;26:842-4.