# Utility of bronchoalveolar lavage for COVID-19: a perspective from the Dragon consortium.

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August 16, 2022

**Abstract**

Diagnosing COVID-19 and treating its complications remains a challenge. This review reflects the perspective of some of the Dragon (IMI 2-call 21, #101005122) research consortium collaborators on the utility of bronchoalveolar lavage (BAL) in COVID-19. BAL has been proposed as a potentially useful diagnostic tool to increase COVID-19 diagnosis sensitivity. In both critically ill and non-critically ill COVID-19 patients, BAL has a relevant role in detecting other infections or in supporting alternative diagnosis, and can change management decisions in up to two-third of patients. BAL is used to guide steroid and immunosuppressive treatment and to narrow or discontinue antibiotic treatment reducing the use of unnecessary broad antibiotics. Moreover, cellular analysis and novel multi-omics techniques on BAL are of critical importance for the understanding of the microenvironment and interaction between epithelial cells and immunity revealing novel potential prognostic and therapeutic targets. The BAL technique has been described as safe for both patients and health care workers in more than a thousand procedures reported to date in the literature. Based on these preliminary studies, we recognize that BAL is a feasible procedure in COVID-19 known or suspected cases, useful to properly guide patient management and with great potential for research.

## Introduction

The rapid outbreak of coronavirus disease 2019 (COVID-19), originating from severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection, is a public health emergency of international concern. Diagnosing COVID-19, treating its complications, and predicting how the disease will progress in different patients remains a challenge. The DRAGON project (IMI 2-call 21, #101005122) is drawing on new and existing data

and sample collection efforts to carry out a detailed profiling of patients. In the Dragon consortium, Florence University, Italy and Centre Hospitalier Universitarie de Liege, Belgium have focused their research on the role of interventional pulmonology (IP) and bronchoalveolar lavage (BAL) sample collection in COVID-19. This document reflects the perspective of some of the Dragon research consortium collaborators on the utility of BAL in COVID-19.

Interventional pulmonology in patients with COVID-19 is required to manage complications (atelectasis, haemoptysis, pneumothorax, pleural effusions, etc), and to guide airways management (airway secretion management, intubation or tracheostomy guide). BAL in COVID-19 has been used to obtain samples for both cytology and microbiology purposes (detecting infections, differential diagnosis with other interstitial lung disorders, etc). If the role of IP in treating COVID-19 complications and guiding airway management is well established, the role of BAL in COVID-19 diagnosis and management has been questioned. Bronchoscopy is an aerosol generating procedure, and its routinely use in COVID-19 patients has been discouraged.[1] However, avoiding bronchoscopy in COVID-19 patients expose physicians to risks of misdiagnosis and suboptimal treatment. BAL is a well-established minimally invasive technique that has an important diagnostic role and has been routinely used for decades for the diagnosis of infectious, neoplastic and non-neoplastic diffuse lung diseases. BAL clinical role in the diagnosis of respiratory infection is of utmost importance.[2-5] Therefore, BAL has been used in many expert centres to manage COVID-19 and in several research protocols to investigate COVID-19 pathogenetic mechanisms.

We aimed to review the current evidence supporting the role of BAL in the diagnosis of COVID-19 infection, in the detection of coexisting infections, and in the understanding of COVID-19 features and pathogenetic mechanisms.

**Limits of the current diagnostic approach for COVID-19**

The diagnostic gold standard for COVID-19 is the naso-pharingeal (NP) swab reverse-transcription real-time polymerase chain reaction (rRT-PCR) detection of SARS-Cov-2. However, due to the unavailability of a shared reference standard for COVID-19 diagnosis there are no reliable data on NP swab sensitivity. Clerici et al. assessed nasopharyngeal swab sensitivity in patients with known SARS-Cov-2 infection, based on the presence of symptoms and of [?]1 positive rRT-PCR serial testing, and found a sensitivity of 77% (95% CI, 73 to 81%).[6]Wang et al. evaluated SARS-Cov-2 detectability in different biological specimens in COVID-19 patients and found a NP swab sensitivity of 63%.[7] Pooled data found that the probability of a false negative result was as high as 21% even at the optimal testing window (3 days after symptom onset).[8]

Given the limits of NP swab testing, some experts propose to diagnose suspected cases using the widely available, time-saving and non-invasive imaging approach of chest computed tomography (CT), that could serve as an efficient and effective way to flag, diagnose, and possibly triage COVID-19 patients.[9,10] However, as confirmed by a recent metanalysis of 60 studies (5744 patients), CT has a low specificity compared to NP swabs rRT-PCR, 46% (95% CI, 29-63%).[11] Ongoing studies are evaluating the role of radiomics analysis to identify a diagnostic signature for COVID-19 infection, based on standard-of-care chest CT imaging, with promising preliminary results showing a sensitivity of 69.52%, and a specificity of 91.63%.[12]

In this scenario identifying the false negative cases remains of critical importance to properly manage patients avoiding improper allocation of COVID-19 cases and allowing a timely treatment. Since the early pandemic, BAL has been proposed as a potentially useful diagnostic tool to increase COVID-19 diagnosis sensitivity. Nevertheless, considering the high potential to aerosol exposure generate during BAL, international bronchology societies have universally cautioned for a limited and proper use of this tool in clinical practice during the pandemic peaks. The role of BAL in the diagnostic algorithm of COVID-19 has been debated and explored in several studies.

**Methods**

This review is based on previously published manuscripts that were identified through a MEDLINE search (2020 and 2021) of English-language literature. The literature search was limited to clinical journals with

accessible full texts, and the key phrases used were 'bronchoalveolar lavage and COVID-19'. Pediatric cases were excluded. A total of 430 manuscripts were reviewed, but only a select number were chosen at the discretion of the authors. The literature search and the authors' clinical experiences were used to draft the manuscript and to give practical suggestions.

**Indications of major bronchoscopy societies.**

Several bronchology societies have issued documents regarding bronchoscopy during the early phase of the COVID-19 pandemic.[1,13-16] Based on the risk of aerosol transmitted infection all societies at that time recommended postponing elective procedures, limiting the number of procedures in COVID-19 patients, performing procedures in COVID-19 patients with the minimal sufficient staff and with the use of appropriate personal protective equipment (PPE). Deciding how to stratify elective procedures to minimize the risk of transmission while not compromising time-sensitive medical care has been a major challenge and experts recommended reviewing the need for all procedures on a case-by-case basis to assess the indication and urgency.[14]

Known or suspected COVID-19 infection was considered a relative contraindication to bronchoscopy, given the uncertain benefit and possible risks. Bronchoscopy in COVID-19 patients had three main roles: 1) the diagnosis of SARS-Cov-2 infection when other diagnostic tools were inconclusive; 2) the identification of co-infections or superinfections in patients with worsening respiratory conditions; 3) the treatment of bronchoscopic emergencies (massive bleeding, significant airway stenosis, airway secretions causing tracheobronchial obstruction etc).

The major bronchoscopy societies agreed on the need of limiting the use of BAL in the diagnosis of SARS-Cov-2 infection. However, based on the need to avoid false negatives, the societies made a point for a possible indication to perform BAL in cases of suspected COVID-19 when other diagnostic methods were inconclusive and in those situations in which the identification of coinfections could play an important role in the therapeutic decision.

None of these bronchology societies indications given during the early pandemic phase were comprehensive and significant uncertainty remained regarding in whom to perform bronchoscopy.[17] At that time no data specific to bronchoscopy in COVID-19 were yet available, and the recommendations were experts' opinions derived from observations made during prior respiratory viral outbreaks including other SARS, Middle east Respiratory Syndrome and influenza. However, in the rapidly changing clinical environment of the last two years, many centres equipped with appropriate PPE and experienced in the use of BAL, have performed BAL in known or suspected COVID-19 infection generating new evidence on the utility of bronchoscopy in COVID-19 that needs to be carefully considered.

**BAL in suspected COVID-19 non-critically ill patients.**

As recently reported by systematic reviews and metanalysis several retrospective and few prospective observational studies have investigated the role of BAL in suspected or known COVID-19.[18,19] To the best of our knowledge, all studies performed in the non-critically ill patients are retrospective. A summary of BAL findings in non-critically ill patients is reported in Table 1 and 2. Between January and February 2020 Chinese scientists reported 5 cases of suspected COVID-19 investigated with BAL showing positivity for SARS-Cov-2 in all cases.[20]Subsequently, between March and May 2020, Italian virologists confirmed a higher positivity in BAL compared to other specimens (15%, 55/367 positive BAL, compared to 8%, 769/9461 positive NP swabs).[21] A small retrospective case series reported a 19% prevalence (3/19 cases) of SARS-Cov-2 infection in BAL performed in patients with negative NP swab.[22] During the first COVID-19 wave (March-April 2020), De Clercq et al conducted a retrospective monocentre study in Belgium aimed to evaluate the feasibility of their local diagnostic protocol that included BAL in patients' diagnostic workup.[23] They performed 27 BAL in non-critically ill patients with HCRT changes suspected for COVID-19 and two negative NP swabs and found 26% (7/27) positive BAL for SARS-Cov-2. They also identified one coinfection in SARS-Cov-2 positive (E cloacae) and 63% of other pathogens in negative BAL for SARS-Cov-2 including *Mycoplasma pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae, Pneumocysitis jirovecii* , and

other viruses.[23] Another retrospective study conducted in two Belgian centres during the first wave confirmed the utility of BAL in detecting SARS-Cov-2 in 25% (14/55) of non-critically ill patients with negative NP swabs.[24] The Authors also underlined the utility of BAL in therapeutic management that was changed after BAL in 60% of cases (33/55), either because other pathogens were identified (one coinfection with *Serratia marcescens* in SARS-Cov-2 positive cases and 42%, 23/55 of other pathogens in SARS-Cov-2 negative cases including *Mycobacterium tuberculosis, Pneumocystis jirovecii, Haemophilus, Serratia, Escherichia coli, virus Influenza type A, Metapneumoviruses, Herpes viruses and Aspergillus fumigatus* ) or because an alternative diagnosis was made (18% of cases, 10/55, including rheumatoid arthritis, hypersensitivity pneumonitis, cardiogenic oedema, cryptogenic organizing pneumonia, hepatopulmonary syndrome).[24] During the first COVID-19 wave, Mondoni et al. carried out in Italy an observational, retrospective, multicentre cohort study aimed to evaluate the diagnostic yield of bronchoscopy in patients with two negatives NP swabs and suspected COVID-19.[25] A total 109 adults, 71% males, age 60 (SD 13.6) years were enrolled, 108 bronchoscopies (99%) performed with flexible scope and 13 with rigid. Two-third of the procedures (N=78) were performed to confirm a COVID-19 diagnosis, and one-third were urgent/life-saving procedures. Only 10% of the procedures were carried out in the ICU setting (8.2% invasive ventilation, 1.8% ECMO). The diagnostic yield of bronchoscopy to detect SARS-Cov-2 in patients with previous negative swabs and a clinical and radiological suspicion of COVID-19 pneumonia was 55.1% (43/78). 1.8% (2/109) patients with both NP swabs and BAL negative for SARS-Cov-2 showed a late NP swabs positivity. Coinfections were detected in 4 cases (3,6% of the total):*Haemophilus influenzae, Aspergillus fumigatus, Aspergillus spp. and Candida albicans* .[25] In the same period (March-April 2020) Patrucco and coworkers conducted a similar Italian observational, retrospective, multicentre cohort study including 131 suspected COVID-19 with two negative NP swabs (male 71%, age 65, range 54-74 years) the majority in Internal Medicine ward (63%), 27.5% in sub-intensive unit and 9% in ICU. SARS-Cov-2 was isolated in 43 (32.8%) BAL.[26] Positive patients were younger compared to the negative ones (56 vs. 67, p=0.004) and showed a higher HRCT involvement (ground-glass, peripheral, posterior and multilobar involvement).[26] Other microbiological findings were identified in 26 cases (19.8%) and included *Herpesviruses, Cytomegalovirus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and fungi* . Considering both the identification of COVID-19 and the detection of other causal agent, BAL microbiological analysis was considered clinically useful in 67% of cases.[26] Barberi et al in a population of hospitalized patients for suspected COVID-19, negative NP swabs and mild-moderate disease severity (PaO2/FiO2 307, range 254-362), confirmed a BAL positivity of 16% (32/198), 9% (5/54 in patients with negative HRCT).[27] Moreover, BAL detected 12.5% (4/32) of coinfections in SARS-Cov-2 positive patients and 33% of other infections in SARS-Cov-2 negative patients. The logistic regression analysis detected two factors predictive of BAL positivity: fever (OR 1.94 per additional °C, 95% CI 1.13-3.33, p=0.016) and HRCT scan involvement grade 2 or more (OR 7.36, 95%CI 2.10-25.77, p=0.002).[27] Contrarily to those results, three Italian single centre observational retrospective studies on BAL conducted in the same time period (March-May 2020) in suspected COVID-19 with negative NP swabs (N=81, N=79 and N=28 patients respectively), showed poor BAL performance in detecting SARS-Cov-2 infection with 3/81 (3.7%), 2/79 (2.5%) and 0/28 positive BAL.[28-30] In those studies BAL negative for SARS-Cov-2 was still useful to identify other microorganisms (*mycobacteria, Pneumocystis, Haemophilus parainfluenzae, Staphylococcus, Pseudomonas, Streptococcus, Enterobacterales, Klebsiella, Candida, and other viruses* ).[29,30] Two American studies found a 100% concordance between negative NP swabs and BAL conducted in patients that were screened for SARS-Cov-2 before an elective bronchoscopy for suspected diseases other than COVID-19 (obstructive diseases, interstitial lung disease, lung transplant surveillance etc).[31,32] In the study conducted by Oberg et al, all but one patient had HRCT non suggestive for COVID-19 (negative HRTC in 58% and indeterminate or atypical in the remaining cases) and none had clinical-laboratory features of COVID-19.[31] This study suggests that when the clinical-radiological scenario is not suggestive of COVID-19 and the NP swabs is negative, BAL for COVID-19 is unlikely to be useful, even during a pandemic peak.

Among these small retrospective studies there is a notable variably in the reported utility of BAL for the detection of SARS-Cov-2. This suggests that several factors may influence BAL diagnostic accuracy in detecting SARS-Cov-2, including the heterogeneity of the populations, the variability in BAL technique and

sample processing. It is important to mention that BAL diagnostic yield for COVID-19 detection is also influenced by the epidemiological incidence of the disease and may be influenced by the viral variant. With the changing epidemiological scenario and novel omicron variant the BAL diagnostic yield could significantly change. In Figure 2 we present a paradigmatic case in which BAL allowed the diagnosis of COVID-19 (Omicron variant), aspergillosis and transbronchial biopsy detected lung metastasis from melanoma. Prospective studies conducted in larger and more recent populations are needed, particularly considering that the clinical scenario is rapidly changing due to the emergence of the Omicron variant in the vaccinated population.

**BAL in COVID-19 in the critically ill patients.**

Several studies have evaluated the utility of BAL in the critically ill patients, two were prospective.[18,19,33-36] A summary of BAL findings in critically ill patients is reported in Table 1 and 2. The highest positivity for SARS-Cov-2 detection in BAL performed in critically ill patients has been reported by Wang W et al, 93% (95%CI 074-1.00; N total BAL = 15) and Yang Y et al, 68% (95% CI 056-0.79; N total BAL = 44).[7,19,37] The latter study reported a 100% SARS-Cov-2 positivity in the more severe patients in whom BAL was collected within the first 2 weeks. After 15 days the positivity od nasopharyngeal and oropharyngeal swabs decreased while BAL maintained a high positive rate of 63%.[37] Gao et al designed a retrospective study to evaluate the diagnostic accuracy of nasopharyngeal swab (NP) compared to BAL for the detection of SARS-Cov-2.[38] They reviewed 123 intubated patients who underwent both tests (time interval median 1day, IQr 1-2.75 days) showing that 9 cases with negative NS had positive BAL, 7% of the total. The remaining cases were: 70 positive for both, 39 negatives for both, and 5 cases with positive NS and negative BAL. Bacterial pneumonia was identified in 34% of total cases, with significantly more bacterial coinfections in the non-COVID-19 (24/44, 54%) than in the COVID patients (18/79, 23%).[38] Similar results were achieved by Mahmood et al in 55 critically ill patients, in the subgroup of 37 negative NS they found one positive BAL for SARS-Cov-2 (3%) and in the overall cohort found 16% of positive cultures other than COVID-19 (*Staphylococcus, Pseudomonas, Fungi, Mycobacterium avium and Pneumocysist jirovecii* ).[39] In the ICU setting BAL allows the detection of coinfections in a significant proportion of COVID-19 (Table 2). In several studies conducted in the ICU setting BAL was mainly performed for a microbiological purpose, with a significant impact in subsequent medical decisions. Baron et al performed BAL in 24 patients for microbiological purposes and only in 2 (7%) BAL was performed to confirm COVID-19 after negative NS.[40] The Authors describe the use of BAL mainly for a suspicion of ventilator associated pneumonia (N=11, 39%), invasive aspergillosis (N=4, 14%) and to rule out superinfection before starting a steroid course. In this study, BAL had an impact on medical decisions in 20 cases (71%), with introduction (n = 6), continuation (n = 3), switch (n = 2), or withdrawal (n = 4) of antimicrobial therapy in 14 cases (50%) and/or decision to start (n = 6; 21%), or not (n = 6, 21%), corticosteroid therapy.[40] Pickens et al conducted a retrospective single centre study in COVID-19 mechanically ventilated patients, documenting by early BAL (48h within intubation) 21% (28/133) of bacterial superinfection pneumonia. Streptococcus species and methicillin-susceptible *S. aureus* (MSSA) combined accounted for 79% (22/28) of cases.[33] Polymicrobial infections were common, three patients, previously treated with antibiotics had pathogens resistant to standard CAP antibiotics—one*Stenotrophomonas maltophilia* and two methicillin-resistant*S. aureus* (MRSA) and Pneumocystis was found in one patient with HIV on antiretroviral treatment. For each day of mechanical ventilation they measured the Narrow Antibiotic Treatment (NAT) score and found a clinically and statistically significant difference between positive and negative BAL results (NAT score median difference -1, 95% CI -1 to 0; p=0.001). These findings suggest that negative BAL analysis was used to narrow or discontinue antibiotic treatment and that in the absence of BAL ventilator associated pneumonia may be underrecognized yet overtreated with unnecessary broad antibiotics.[33]

**Comparison between BAL, mini-BAL and bronchial wash.**

Currently there are no studies designed to compare the diagnostic yield and complications of BAL, mini-BAL and bronchial wash. BAL consists in the instillation of approximately 120ml of saline solution with the flexible scope wedged into a segmental bronchus. This technique allows to collect the distal (broncho-alveolar) cellular and acellular component of the lung. The instillation of at least 100ml of saline solution is required to reach

the alveolar component and achieve a BAL of sufficient quality for microbiology, cytology, immunological and molecular studies.[41] For patients with severe respiratory failure or poor general conditions bronchial wash or mini-BAL are possible alternative methods for microbiology studies. Bronchial wash collects the bronchial component and is performed with approximately 20ml of saline solutions within the main or lobar bronchi. This technique does not allow to study the alveolar component but given the lower instilled volume it is considered to be less invasive compared to BAL. Mini-BAL is poorly standardized. Has been reported as the instillation of a variable volume of saline solution (between 20 and 60ml) using either the bronchoscope or a blind catheter advanced into a distal airway.[42,43]As for bronchial wash this technique is suitable for microbiologic studies, but not to study the alveolar component. Given the variability of the technique used in different studies it is difficult to evaluate the diagnostic accuracy of this techniques, but they are all reported to have a good safety profile.

In COVID-19 intubated patients mini-BAL has been described in at least two studies. Vanbellinghen et al retrospectively compared the prevalence of aspergillosis in COVID-19 diagnosed using mini-BAL (20ml of saline instilled through a blind catheter) to that of BAL.[42]The Authors performed mini-BAL in 40 cases, BAL in 20 and both in 16 cases, showing a good agreement between the two methods and a similar prevalence of overall positive *Aspergillus* results using PCR and/or galactomannan and/or culture (16.7% BAL and 21.4% for mini-BAL).[42] Torrego et al performed mini-BAL in 63 severe COVID-19 patients (all intubated, PaO2/FiO2 111, IQr 103-125) instilling 60ml of saline with a wedged scope according to the radiological features.[43] One third were performed in prone position. They had 28.6% (18/63) of positive microbiology results, with a profile of pathogens similar to what observed in a retrospective pre-COVID-19 cohort of patients seen at their centre (*Pseudomonas aeruginosa* n = 7, *Staphylococcus aureus* n = 2, *Klebsiella aerogenes* n = 2, *Enterobacter cloacae* n = 2,*Enterococcus faecalis* n = 2, *Escherichia coli* n = 1,*Streptococcus anginosus* n = 1, or *Prevotella melaninogenica* n = 1).[43]

To the best of our knowledge, only Mondoni et al. published a retrospective study that attempted to compared BAL to bronchial washing (BW) in suspected COVID-19 non critically ill patients. The Authors reported an overall diagnostic yield for SARS-Cov-2 detection of 55% (43/78), 57% (35/61) with BAL and 47% (8/17) with BW, statistical difference wasn't reached (p=0.45).

All these bronchoscopy procedures are similarly well tolerated, but safety studies designed to compare these different methods are lacking.

**BAL in COVID-19: cellularity, immunophenotype, and cytokine profile.**

BAL characteristics and cellularity can be extremely useful in clinical practice, helping to identify possible differential diagnosis, and to guide the diagnostic and therapeutic choice of clinicians. BAL and lung cryobiopsy represent unique specimens to investigate the excessive inflammatory pulmonary response to SARS-Cov-2 that represent a major cause of disease severity and death.[44,45] Doglioni et al elegantly described the histological and immunohistochemical features observed in the early-phase COVID-19, in cryobiopsies performed in non-intubated patients, with perivascular CD4-T-cell infiltration, capillary and venular changes, florid alveolar type II cells hyperplasia, no hyaline membranes.[45] The T-cell perivascular infiltrate was CD 4 positive, but negative for functional activation markers (T-BET, FOXP3, CD25 and CD 30). Few interstitial PD1 + and TCF 1+ T CD8+ lymphocytes were detected. NK cells (CD 56+) and B-cells (CD 20 +) were rare or absent.[45] BAL studies can provide precious data on the cellular and molecular component from the distal lung, that nicely integrate histology findings. Compared to lung biopsy BAL is much more easily performed, therefore a considerable number of recent studies have used BAL to evaluate the alveolar cellular profiles that could correlate with clinically meaningful outcomes (e.g. disease severity and mortality) and that could help the understanding of COVID-19 pathogenesis. Dentone et al, described the BAL characteristics and cellularity of 64 COVID-19 patients admitted during March and April 2020 to the Intensive Care Unit (ICU) of Genoa Hospital. 34,4% had coinfections detected by BAL (Candida, Psedumononas, Enterobacter aerogens, Staphylococcus aureus and Klebsiella Pneumoniae).[46] BAL samples from individual patients were taken and their total cellularity, subpopulations, and T lymphocytes activation as HLA-DR expression.[46] The median cellularity was 68 x $10^3$/ml (IQR 20-145). The majority cells in BAL were neutrophils (70%, IQR 37.5-90.5),

followed by macrophages (27% IQR 7-49). Eosinophils were less than 1% (IQR 0.9-3). Lymphocytes were a minority, 1%, with CD3+ 92% (IQR 82-95). Among CD3+ T lymphocytes 52% were CD8+ (IQR 39.5-62.7), with a T CD4+/CD8+ ratio of 0.6 (IQR 0.4-1.2). 20% where HLA-DR+ (IQR 13-32). At multivariate analysis only the percentage of macrophages in the BALF at the time of ICU entry correlated with higher mortality (OR 1.336, 95% CI 1.014-1.759, p = 0.039). The duration of mechanical ventilation was correlated with percentage of TCD8+ in BALF (r = - 0.410, p = 0.008), TCD4+/CD8+ ratio (r = 0.425, p = 0.006) and total lymphocytes TCD3+ (r = 0.359, p = 0.013) in BALF, respectively. The Authors speculate that the lack of lymphocytes in the BALF in patients admitted to the ICU could partly explain a reduced antiviral response. The reason for this depression of lymphocytes could be related to both direct virus damage to the lymphocyte and by cytokine storm induced damage.[46] That innate immunity is extensively activated has been confirmed also by Pandolfi et al, that in the BALFs of 33 adults admitted to the ICU reported a marked increase in neutrophils (1.24 X 10^5 ml, 0.85-2.07), reduced numbers of lymphocytes (0.97 X 10^5 ml, 0.024-0.34) and macrophages (0.43 X 10^5 ml, 0.34-1.62) with viral particles inside mononuclear cells (seen by electron transmission microscopy and immunostaining).[47] The majority of BAL showed coinfections (26/28). The burden of pro-inflammatory citokines was associated with clinical outcome, IL-6 and IL-8 were significantly higher in ICU patients than in Internal Medicine Ward (IL6 $p < 0.01$, IL8 $p < 0.0001$), and also in patients who did not survive (IL6 $p < 0.05$, IL8 p = 0.05 vs. survivors).[47] A recent study by Reynolds and co-workers showed that inflammatory immune dysregulation of the lower airways during severe viral pneumonia (both severe influenza and SARS-Cov-2 were included) is distinct from that of non-severe illness, with an influx of non-classical monocytes, activated T cells and plasmablasts B cells. BAL cytokines were elevated in severe cases, but not in moderate patients. Largest elevation were observed in IL-6, IP-10, MP-1 and IL-8.[48] Contrarily to previous reports, Gelarden et al reported in 83 patients intubated for severe COVID-19 a lymphocytosis (i.e. > 15%) in 74.7% of cases (62/83) with a high prevalence of atypical lymphocytes in BAL (72.3%, 60/83).[49] BAL lymphocytes, including plasmacytoid and plasmablastic cells, were composed predominantly of T cells with a mixture of CD4+ and CD8+ cells. Both populations had increased expression of T-cell activation markers, suggesting important roles of helper and cytotoxic T-cells in the immune response to SARS-Cov-2 infection in the lung. BAL lymphocytosis was significantly associated with longer hospital stay ($p < 0.05$) and longer requirement for mechanical ventilation ($p < 0.05$), whereas the median atypical (activated) lymphocyte count was associated with shorter hospital stay ($p < 0.05$), shorter time on mechanical ventilation ($p < 0.05$) and improved survival.[49] All these data should be interpreted with great caution because are derived from small, retrospective and monocentric studies with an evident heterogeneity between cohorts in terms of phenotypes, disease severity, duration of intubation, presence of coinfections. Moreover, there is a critical lack of BAL data in non-intubated patients with less severe COVID-19, that limit our ability to understand disease pathogenesis in the early phase of the disease. Besides those evident limits, the current body of evidence suggests that BAL cellular analysis is an invaluable tool to provide useful information for diagnostic and prognostic workup and potentially to expand our understanding of COVID-19 pathogenesis.

**COVID-19 single cells studies in BAL.**

The majority of single-cell studies to date were performed on peripheral blood mononuclear cells (PBMC), a minority on NP swabs and BAL. Few studies have dissected the epithelial and immune profiles of BAL derived from severe COVID-19 patients at a single-cell level. Wauters et al revealed infected lung epithelial cells, a significant proportion of neutrophils and macrophages involved in viral clearance.[50] They performed single-cell deep-immune profiling BAL from 5 patients with mild and 26 with critical COVID-19 (compared to non-COVID-19 pneumonia and normal lung) showing divergent immunologic profiles. In mild COVID-19, CD8+ resident-memory (TRM) and CD4+ T-helper-17 (TH17) cells undergo active expansion with good effector functions, while in critical cases they remain more naive. Vice versa, CD4+ T-cells with T-helper-1 characteristics (TH1-like) and CD8+ T-cells expressing exhaustion markers (TEX-like) are enriched halfway their trajectories in mild COVID-19, where they also exhibit good effector functions, while in critical COVID-19 they show evidence of inflammation-associated stress. Monocyte-to-macrophage trajectories show that chronic hyperinflammatory monocytes are enriched in critical COVID-19, while alveolar macrophages, oth-

erwise characterized by anti-inflammatory and antigen-presenting characteristics, are depleted. Moreover, in critical COVID-19, monocytes contribute to an ATP- purinergic signaling-inflammasome footprint that could enable COVID-19 associated fibrosis and worsen disease-severity.[50]Liao et al evaluated BAL from 3 moderate and 6 severe COVID-19 and found abundant pro-inflammatory monocytes derived macrophages in patients with severe COVID-19, whereas highly clonally expanded CD8+ T cells characterized moderate COVID-19 cases.[51] Patients with severe/critical infection had much higher levels of inflammatory cytokines, particularly interleukin (IL)-8, IL-6 and IL-1β, expressed by macrophages that in severe patients may contribute to local inflammation by recruiting monocytic cells and neutrophils thought CCR1 and CXCR2, while in moderate cases can produce more T cell attracting chemokines through CXCR3 and CXCR6.[51] He et al performed single-cell RNA sequencing (sc-RNA-seq) in the leukocytes and epithelial cells of 3 SARS-Cov-2 induced ARDS.[52] They detected 23 cells with viral mRNA reads, but minimal number of expressed genes, thus indicating that SARS-Cov-2 suppresses host gene expression. These cells were identified as monocytes/neutrophils and club cells. Compared to healthy controls club cells showed a significantly elevated mucins genes expression (MUC5AC, MUC5BMUC4, MUC16 and MUC20). The mucin secretion seems stimulated through the innate immune regulators IL-1β and TNF-α (were found 6 transcription factors involved in IL-1β and TNF- α induced MUC5B promoter activation). Four critical surfactant proteins (SPs)— SP-A, SP-B, SP-C, and SP-D, known to maintain the structural integrity of alveoli, were down-regulated in COVID-19 disease and the level of NKX2-1, the transcription factor required for surfactant synthesis was also reduced, thus indicating the loss of alveoli integrity and the possible pathogenesis of ARDS in COVID-19.[52] The transcriptomic signature of major regulators of innate immunity (monocytes, neutrophils and macrophages) in severe COVID-19 indicates different immune profiles among COVID-19 patients: Liao et al. showed abundant macrophages expressing FCN1 in BAL of COVID-19 patients, whereas He et al. noticed only a minor increase in FCN1+ macrophages, with a significant decrease in FCN1+ monocytes/neutrophils.[51,52] By analyzing scRNA-seq data of BAL from 6 severe COVID-19, 3 recovered COVID-19 with mild symptoms and 10 heathy controls, Chen et al. showed high expression of SARS-Cov-2 receptor ACE2 and TMPRSS2 in club and ciliated cells of patients.[53] In severe COVID-19 high neutrophils with excessive expression of cytokines were noted and the dysregulated cytokines/receptors interplay among lung epithelial cells and immune cells correlated with disease severity (ANXA1/FPR2 and TNFSF13/TNFRSF1A interactions between club and macrophage or neutrophils, CXCL2/DPP4 interaction between club and T/NK cells, and ANXA1, C3, CXCL2, SAA1, TNFSF13 expressions in lung epithelial cells).[53] In conclusion scRNA-seq studies can reveal information of critical importance in the understanding of COVID-19 pathogenesis. However, current data on BAL are limited, mostly derived from small sample sizes studies and with large difficulties in validating most conclusions across datasets, possibly due to inconsistent mapping between different diseases stages and different protocols used. Therefore, conclusions from these early scRNA-seq studies of COVID-19 patients may not always be robust and need to be validated before fully relied upon.[54]

**Bronchoscopy complications.**

BAL is reported to be safe, a transient drop in oxygen saturation is occasionally reported in the more severe patients. No major adverse events were reported to date and no deaths were recorded. The most frequent adverse events, described in a minority of patients, were transient hypoxaemia and fever. Mondoni et al reported complications related to bronchoscopy in 5/109 (4.5%) patients. Fever was recorded after BAL in 2/109 (1.8%). 3/109 (2.7%) patients with a known mild respiratory failure had a transient worsening of their gas exchange after bronchoscopy performed during oxygen supplementation. When bronchoscopy was performed in patients who required non-invasive mechanical ventilation (NIV), severe hypoxia and subsequent intubation has been reported in 6 patients.[18]

BAL in COVID-19 is reported as a safe and feasible procedure in all studies, with a safety profile that is similar to what previously reported in non-COVID-19 patients. The risk-benefit profile should be carefully evaluated in severe patients in NIV, because of the possible risk of hypoxemia leading to intubation. The small numbers and the wide heterogeneity of studies prevent us from drawing any firm conclusion on possible differences in terms of safety and diagnostic accuracy between BAL and other sampling techniques, such as mini-BAL and bronchial washes. Future prospective trials are needed to address the safety and accuracy of

these methods.

**Health care workers safety.**

In the published studies all bronchoscopies were performed in accordance to current guidelines using appropriate personal protection equipment (PPE) including gown, face shield, eye protector, shoe cover, double gloves and filtering masks (FFP2/FFP3).[18] Negative pression rooms and disposable bronchoscopes weren't universally available although were used in the majority of centres (negative pressure rooms in 57%, 4/7 studies; disposable scopes in 67%, 6/9 studies).[18] Among all published studies (646 patients, 1,034 bronchoscopies), only Torrego et al reported one case of infection in one bronchoscopist.[18,43] Based on current evidence we can conclude that, if performed with appropriate PPE, bronchoscopy and BAL can be safely performed with minimal risk of infection for the health care workers.

**Conclusions.**

BAL has been widely used during the SARS-Cov-2 pandemic for both clinical and research purposes. In clinical practice BAL can change management decisions in up to two-third of patients confirming a suspected SARS-Cov-2 infection when the NP swab is negative, detecting other infections or supporting alternative diagnosis. Although studies have a wide variability, a pooled estimates of 11% positive cases suggest that BAL can be used to confirm suspected SARS-Cov-2 infection when negative NP swab is negative.[19] The prevalence of false negative BAL for SARS-Cov-2 detection can't be accurately drawn from current studies, but seems to be very low (<2%).[25] In both critically ill and non-critically ill patients, BAL detects coinfections a significant proportion of patients. BAL can help clinicians in difficult differential diagnosis including acute exacerbations of interstitial lung diseases (ILDs), connective tissue related ILDs, hypersensitivity pneumonitis, cryptogenic organizing pneumonia. BAL analyses are used to guide steroid and immunosuppressive treatment and to narrow or discontinue antibiotic treatment reducing the use of unnecessary broad antibiotics. Moreover, cellular analysis and novel multi-omics techniques on BAL are of critical importance for the understanding of the microenvironment and interaction between epithelial cells and immunity revealing novel potential prognostic and therapeutic targets. The BAL technique has been described as safe for both patients and health care workers in more than a thousand procedures reported to date in the literature. Based on these preliminary studies, we recognize that BAL is a feasible procedure in COVID-19 known or suspected cases, useful to properly guide patient management and with great potential for research. Based on the evidences here summarized, we propose a simplified diagnostic algorithm in which BAL can be used in suspected COVID-19 cases when the NP swab is negative, and in COVID-19 cases to guide antimicrobial and steroid treatment when a coinfection is suspected (Figure 1). We acknowledge that this algorithm reflects the clinical practice only in selected centres properly equipped and experienced in the use of BAL and that further large prospective studies are needed to corroborate current knowledge before BAL can be widely recommended.

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**Table 1. Summary of BAL findings in critically and non critically ill COVID-19 patients.**

| | CRITICALLY ILL COVID-19 | NON CRITICALLY |
|---|---|---|
| % of SARS-Cov-2 positive BAL in negative NP swab (ref) | 3-18% (ref 34,35) | 0-55% (ref. 22-30) |
| % of coinfections detected by BAL in COVID-19 patients | 21-54% (ref 32, 34, 36,38) | 2-37% (ref 23-26) |
| % of infections detected by BAL in negative SARS-Cov-2 | 16-54% (ref 34-36) | 19-63% (ref 22-26) |
| % of diagnosis of non infectious diseases in which BAL was helpful | N/A | 18% (ref 23) |
| Overall % of cases in which BAL was considered clinically helpful | 71% (36) | 60-67% (ref 23, 25) |

| | CRITICALLY ILL |
|---|---|
| BAL SARS-Cov-2 | positive |
| Bacterial infections | Enterobacteriacee (Escherichia choli, Klebsiella pneumoniae, K aerogenes, E. cloacae, E fecalis, K |
| | Pseudomonas |
| | Stenotrophomonas maltophilia |
| | MRSA |

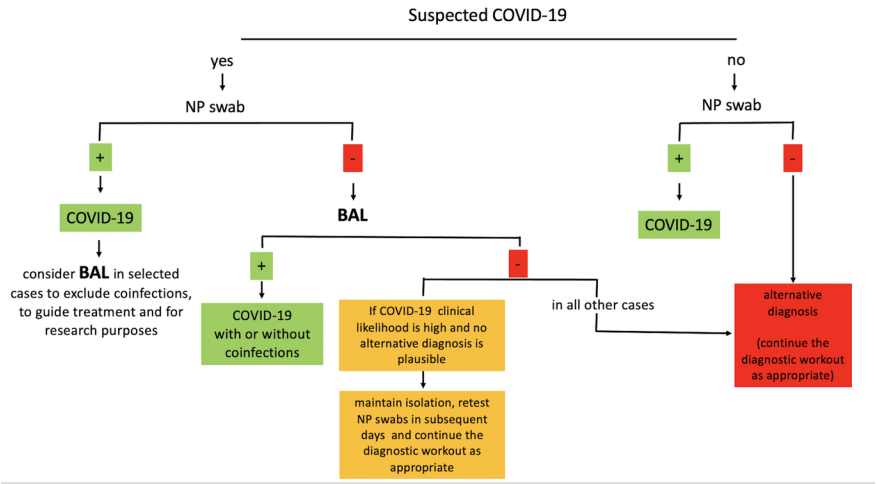
| | CRITICALLY ILL |
|---|---|
| Mycobacterial infections | |
| Fungal Infections | Pneumocystis jirovecii |
| Viral infections | |

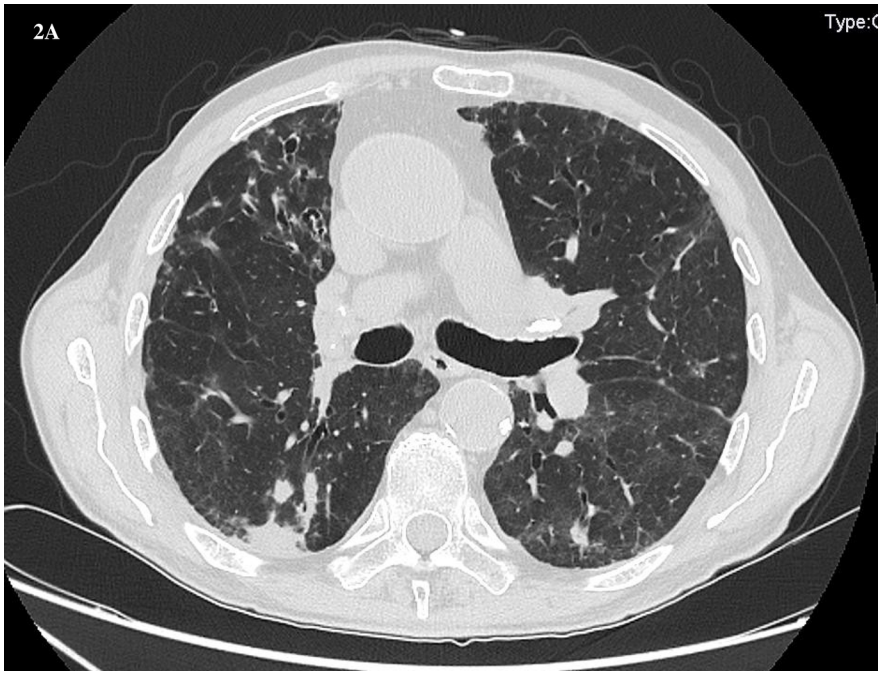
**Table 2. Reported infections** in BAL of critically and non-critically ill patients, with positive or negative SARS-Cov-2 BAL findings.

Abbreviations: Methicillin-resistant S. aureus (MRSA)

Figure 1. Simplified diagnostic algorithm for the use of BAL in suspected COVID-19. BAL, bronchoalveolar lavage; NP nasopharyngeal.

Figure 2. Paradigmatic clinical case showing COVID-19 and Aspergillus coinfection in BAL with concomitant lung metastasis from cutaneous melanoma detected by transbronchial biopsy. 84 years old gentlemen, with metastatic melanoma, vaccinated for SARS-CoV-2 that developed low grade fever, cough and dyspnea. A) The HRCT shows mild diffuse ground glass, with bronchiectasis particularly in the middle lobe and lung nodules. B) The BAL showed a lymphocytosis (52%, with CD4+ 33% and CD8+ 57%). The microbiology and virology panel on BAL detected SARS-CoV-2 positivity with Aspergillus coinfection. Radial-EBUS guided transbronchial biopsies also documented melanoma lung metastasis.



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