Pneumonia

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Respiratory dysbiosis as prognostic biomarker of disease severity for adults with community-acquired pneumonia requiring mechanical ventilation

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Abstract

Objetives To ascertain the role of the lung microbiome in the development of severe pneumonia and its potential as a biomarker for disease progression.

Methods BAL samples from 34 adults with severe community-acquired pneumonia (CAP) (17 viral, 8 viral coinfected with bacteria and 9 bacterial) admitted to the ICU for acute respiratory failure between 2019 and 2021 were collected within the first 48 h of admission to the ICU. The microbiome was characterized via the Ion 16S Metagenomics Kit and the Ion Torrent sequencing platform. Clinical factors, including survival, mechanical ventilation duration, blood biomarkers and organ failure in terms of acute respiratory distress syndrome (ARDS), shock or acute renal failure, were correlated with microbiome characteristics.

Results The microbiome diversity in patients with viral pneumonia was significantly greater than that in patients with bacterial or coinfected pneumonia: the Shannon diversity index was 3.75 (Q1–Q3: 2.5–4.1) versus 0.4 (Q1–Q3: 0.2–1.3) and 0.48 (Q1–Q3: 0.3–1.1), respectively ($p < 0.05$). The microbiome diversity index was associated with severity-of-illness (APACHE II), independent of the etiology of pneumonia (B coefficient -1.845; $p < 0.01$). Patients with severe viral CAP who developed ARDS had a lower presence of Proteobacteria, and those who were complicated with ventilator-associated pneumonia had a higher prevalence of *Acinetobacter* at admission. The mortality of patients with bacterial or coinfected pneumonia was 35%. In coinfected patients, the diversity index was associated with the development of shock.

Conclusion Patients with severe CAP have low respiratory microbiome diversity, indicating that respiratory microbiome diversity is a potential biomarker of disease severity.

Keywords Lung microbiome, Diversity, Severe community-acquired pneumonia, Biomarkers

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Introduction

Community-acquired pneumonia (CAP) remains a public health issue with a high clinical burden. Severe cases require hospitalization and admission to intensive care units (ICUs), with a mortality rate reaching approximately 25% of patients receiving mechanical ventilation [1]. Despite the optimization of antimicrobial therapy and support measures, mortality and morbidity due to CAP continue to be very high, and new approaches in respiratory therapy are being developed to improve the outcomes of complicated pneumonia [2].

With the development of culture-independent techniques, many microorganisms have been demonstrated to coexist in the lungs of healthy individuals, constituting the lung microbiome. Research on the lung microbiome has improved our understanding of respiratory diseases, showing that the interactions of the lung microbiota with the host, as well as dysbiosis, likely play a key role in chronic inflammatory respiratory diseases such as chronic obstructive pulmonary disease (COPD) or asthma and in patients who undergo mechanical ventilation [3, 4]. However, few studies have focused on the lung microbiome in CAP, probably due to the great challenge of obtaining a representative sample of the lung microbiota (noncontaminated with upper respiratory tract microorganisms) [5] and the need for techniques capable of studying the microbiome in samples with a very low bacterial load [6].

Our group recently reported a study on the lung microbiome at admission in critically ill patients with severe CAP [7]. In that study, the diversity of the respiratory microbiome among patients with pneumococcal pneumonia was lower than that among patients with severe viral pneumonia. The microbiome of control patients without pneumonia was also evaluated, revealing high diversity, with a predominance of Proteobacteria, Firmicutes and Actinobacteria, as has been shown in other studies [8, 9].

To date, few studies have correlated the respiratory tract microbiome at admission with disease severity in patients with severe CAP. An association between the increase in alpha diversity and the presence of the families Prevotellaceae and Actinomycetaceae with clinical improvements has been shown [10]. Additionally, significant differences in the microbial composition between patients with or without lower respiratory tract infections, with *Klebsiella pneumoniae* and *Bacillus cereus* as potential biomarkers to predict the risk of low respiratory tract infection progress, have been described [11].

The main objective of this study was to analyze whether there is a relationship between the distribution of the lung microbiota and the severity of community-acquired pneumonia. A secondary objective was to analyze the relationship between microbiome composition and the development of organ dysfunction. Our hypothesis was that alterations in the lung microbiota are correlated with the severity of CAP and the onset of organ failure.

Methods

Ethics and consent to participate

The study was approved by the regional (Gipuzkoa) Ethics Committee, reference MOZ-NBI-2017–01. Written informed consent to be included in the study was given by patients or their legal representatives. All the experiments were performed in accordance with the relevant guidelines and regulations.

Patient population

Between January 2019 and May 2021, adult patients with severe CAP admitted to the ICU were prospectively enrolled in the study. The inclusion criteria were as follows: 1) adult patients admitted with clinical and radiological criteria for severe CAP; 2) bronchoalveolar (BAL) samples collected in the first 24 h after admission to the ICU; and 3) no antibiotic treatment for more than 48 h. Pulmonary samples were collected from all patients via a protected mini-BAL system (Combicath™ Plastimed, France), with the exception of 5, which were collected via a nonprotected BAL system. In this study, three groups of patients, on the basis of the etiology of pneumonia, were defined: 1) patients with viral pneumonia, 2) patients with bacterial pneumonia and 3) patients with pneumonia infected with a virus and a bacterium (referred to in this study as "coinfected").

The following clinical data were prospectively recorded: demographic characteristics; severity at admission measured by the APACHE II and SOFA scores during the first 24 h; and outcome variables, mainly the development of organ failure, such as adult respiratory distress syndrome (ARDS), acute renal failure (ARF) and shock; and hospital and ICU stays and mortality. To evaluate ventilator dependence, ventilator-free days during the first 28 days after ICU admission were measured [12].

Blood parameters were recorded at admission and 48 h later. Leukocytes, the percentage of neutrophils, lymphocytes, C-reactive protein (CRP) (at admission and peak) and procalcitonin (PCT) (at admission and peak) were measured as inflammatory biomarkers in all patients. Lactate, creatinine, urea, T-troponin and pro-BNP (b-type natriuretic peptide) levels were also analyzed.

Microbiological procedures

The microbiological diagnosis of pneumonia was performed via routine, well-established techniques: blood-cultures (BD BACTEC™ blood culture systems), urine antigen test for *Streptococcus pneumoniae* and *Legionella*

pneumophila detection (Sofia FIA® Quidel Corporation, San Diego, CA, USA), qRT-PCR targeting viral respiratory pathogens (Allplex™ Respiratory Panels 1, 2 and 3, Seegene, South Korea), PCR of a pharyngeal swab for other less frequent bacterial pathogens (*Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*, CerTest Biotec SL, Spain). The diagnosis of SARS-CoV-2 pneumonia had been commonly done some days before admission using commercial RT-PCR on nasopharyngeal swabs (Allplex™ SARS-CoV-2 assay, Seegene, South Korea).

The analysis of the lung microbiome was determined via direct or nested PCRs if necessary using the Ion 16S Metagenomics Kit (Thermo Fisher Scientific) from nucleic acids extracted from mini-BALs as previously detailed [7]. The resulting PCR products of all the variable regions of the 16S rRNA gene were sequenced via the Ion Plus Fragment Library Kit, and the consensus data were analyzed via Ion Reporter software 5.18.2. Alpha diversity was calculated via the Shannon and Simpson diversity indices. The bacterial load was quantified via real-time PCR using primers and PCR conditions as described previously [13].

The results of metagenomic analysis were not taken into account for the management of patients in this study since it was performed retrospectively on frozen BAL samples originally collected for the etiological study of pneumonia.

Statistics

The normality assumption was evaluated via skewness and kurtosis coefficients, graphical methods, and the Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables are described with the means and standard deviations or with the median and 25th (Q1) or 75th (Q3) percentiles, regardless of their parametric distributions. Categorical variables are described as percentages. To compare means, Student's t test and ANOVA were used in the case of normality, and the Mann–Whitney and Kruskal–Wallis U tests were used otherwise. To compare proportions, the chi-square test and Fisher's exact test were used. Differences in mechanical ventilation dependence were analyzed via the Kaplan–Meier test and the log rank test. To analyze the effects of microbiome diversity and other prognostic factors on severity and evolution, multiple linear regression was used. Considering the APACHE II score as the final outcome, with an expected mean score of 19.6 (SD: 5.8) at the 95% confidence level and a relative precision of 10%, the sample size should be 34.

The significance threshold was 0.05. All analyses were performed via IBM SPSS 29.0 software.

Results

Forty-five critically ill patients admitted with severe CAP were eligible. Eight patients were excluded because they had been treated with antibiotics for more than 2 days, and 3 patients were excluded because they had fewer than 10,000 reads in the sample. Finally, a total of 34 critically ill patients with SCAP with known etiologies were included in the study (Table 1). Three patients were immunosuppressed: two due to chronic lymphocytic leukemia and one due to kidney transplantation. Viral pneumonia was diagnosed in 17 patients: 8 with SARS-CoV-2, 5 with influenza virus, 3 with rhinovirus and 1 with respiratory syncytial virus (RSV). Nine patients had bacterial pneumonia: *Streptococcus pneumoniae* was the most common pathogen ($n=5$), followed by *Legionella pneumophila* ($n=2$), *Haemophilus influenzae* ($n=1$) and *Klebsiella pneumoniae* ($n=1$). Eight patients had coinfected (viral and bacterial) pneumonia (Table 2).

The bacterial load was similar in the BAL samples of patients with viral, coinfected or bacterial pneumonia ($p=0.146$). However, the diversity of the microbiome of patients with bacterial or coinfected pneumonia was lower than that of patients with viral pneumonia (Fig. 1).

The most abundant phylum associated with viral pneumonia was Proteobacteria (median 41.6%, Q1–Q3: 31–51.4%), followed by Firmicutes (21%, Q1–Q3: 10–47.1%), Actinobacteria (16.1%, Q1–Q3: 10.7–28.2%) and Bacteroidetes (4.4%, Q1–Q3: 3.3–12.15%). In patients with viral pneumonia, at the genus level, within the phylum Proteobacteria, Haemophilus stands out, with the absence of *Enterobacteriaceae*. In critically ill patients with bacterial or coinfected pneumonia, the main phylum detected in the lung microbiome was Firmicutes in the case of pneumococcal SCAP and Proteobacteria in patients with SCAP due to *Haemophilus*, *Legionella* or *Klebsiella*, independent of viral coinfection.

Inflammatory biomarkers measured at ICU admission revealed higher levels of CRP and PCT in patients with bacterial and coinfected pneumonia than in patients with viral pneumonia (Table 3). The same was true for the lactate and creatinine levels at admission. No differences were observed in the remaining analytical determinations.

The variables associated with severity at admission, as measured by the APACHE II score, were evaluated via multivariate linear regression analysis. The Shannon diversity index and lactate levels at admission were the only variables associated with the APACHE II score at admission (Table 4).

The median ICU stay was 11 days (Q1–Q3: 7.75–29.25), and the ICU mortality rate was 23.5%. The crude mortality rate was 35.3% for bacteria compared with only 11.8% for viral isolates, although this difference did not reach

Table 1 Demographics and outcomes of 34 critically ill patients admitted to the ICU due to SCAP

	Viral (*n* = 17)	Bacterial (*n* = 9)	Coinfected (*n* = 8)
Age (years)	66 (51.5—70)[1]	65 (50.5 –70)	68 (59.5 –73)
Sex (male)	76.5%	44.4%	87.1%
APACHE II	15 (11.5—18.5)	25 (19.5—31)	24.5 (16.5—27.5)
SOFA	7 (4—8)	9 (7—11.5)	8 (4.5—9.75)
ID[2] (%)	0	11,1	25
COPD[3] (%)	23,5	33,3	12,5
ARDS[4] (%)	47.1	22.2	50
ARF[5] (%)	52.9	66.7	87.5
Shock (%)	29.4	66.7	62.5
ICU length of stay (days)	11 (8.5—35.5)	8 (2—22)	16.5 (11—23)
Hospital length of stay (days)	9 (7—15)	7 (6 – 14)	4 (3—16.5)
Mortality: n (%)	2 (11.8%)	3 (33.3%)	3 (37.5%)

[1] Median. In brackets, percentiles (Q1–Q3)

[2] *ID* Immunodepression

[3] *COPD* Chronic obstructive pulmonary disease

[4] *ARDS* acute respiratory distress syndrome

[5] *ARF* acute renal failure

Table 2 Etiology of viral SCAP episodes coinfected with bacteria

	Viral etiology	Coinfecting bacteria
Coinfection 1	RSV	*H. influenzae*
Coinfection 2	Influenza AH3	*Streptococcus pyogenes*
Coinfection 3	RSV	*S. pneumoniae*
Coinfection 4	Influenza AH1	*S. pneumoniae*
Coinfection 5	Metapneumovirus	*S. pneumoniae*
Coinfection 6	Influenza AH1	*S. pneumoniae*
Coinfection 7	Influenza AH1	*S. pneumoniae*
Coinfection 8	Influenza AH1	*S. pneumoniae*

statistical significance. When assessing mechanical ventilator dependence, a Kaplan–Meier survival curve (Fig. 2) revealed that the etiology of CAP (viral, bacterial or coinfected) was the main factor associated with ventilator dependence that was not affected by the microbiome composition.

Viral pneumonia

The microbiome composition of patients with viral pneumonia influences the severity of the disease, including its evolution to organ failure, complications and outcomes. Seventeen patients had viral pneumonia, of whom 9 (53%) developed ARF, 8 (47%) developed ARDS, and 5 (29%) developed shock. An analysis of the lung microbiome composition in patients with ARDS revealed that the phyla Bacteroidetes and Firmicutes predominated, whereas the phylum Proteobacteria, with *Haemophilus* being the most prevalent genus, was the most frequent phylum in patients who did not develop ARDS (Fig. 3). Among patients who developed shock, a greater percentage of Bacteroidetes during evolution was detected: 8.5% (Q1-Q3: 6.75%−14.7%) vs 3.6% (Q1-Q3: 2.9%−4.4%, $p < 0.05$). No difference at the phylum level was observed in patients with or without ARF.

A total of 35.3% of the patients with viral pneumonia developed ventilator-associated pneumonia (VAP). A significantly greater percentage of *Acinetobacter* was detected in the respiratory microbiome of patients with viral pneumonia and VAP at ICU admission: 9.5% (Q1-Q3: 6.6%−15.5%) vs 3.2% (Q1-Q3: 2.9%−4.4%).

Bacterial infection or coinfected pneumonia

Of the 9 patients with bacterial pneumonia, 2 developed ARDS, 6 developed shock, and 6 developed ARF. No difference in microbiome composition was observed between those who experienced organ dysfunction and those who did not.

Among the 8 coinfected pneumonia patients, 4 developed ARDS, 5 developed shock, and 7 developed ARF. The diversity of the microbiome measured by the Simpson or Shannon index was lower in patients who were complicated with shock ($p = 0.036$).

Mortality analysis

Two patients with viral pneumonia died, both of whom had influenza pneumonia, longer than one month after admission. Six patients with bacterial or coinfected pneumonia died, 4 of whom died within the first 24 h of ICU admission. Three had fulminant evolution with

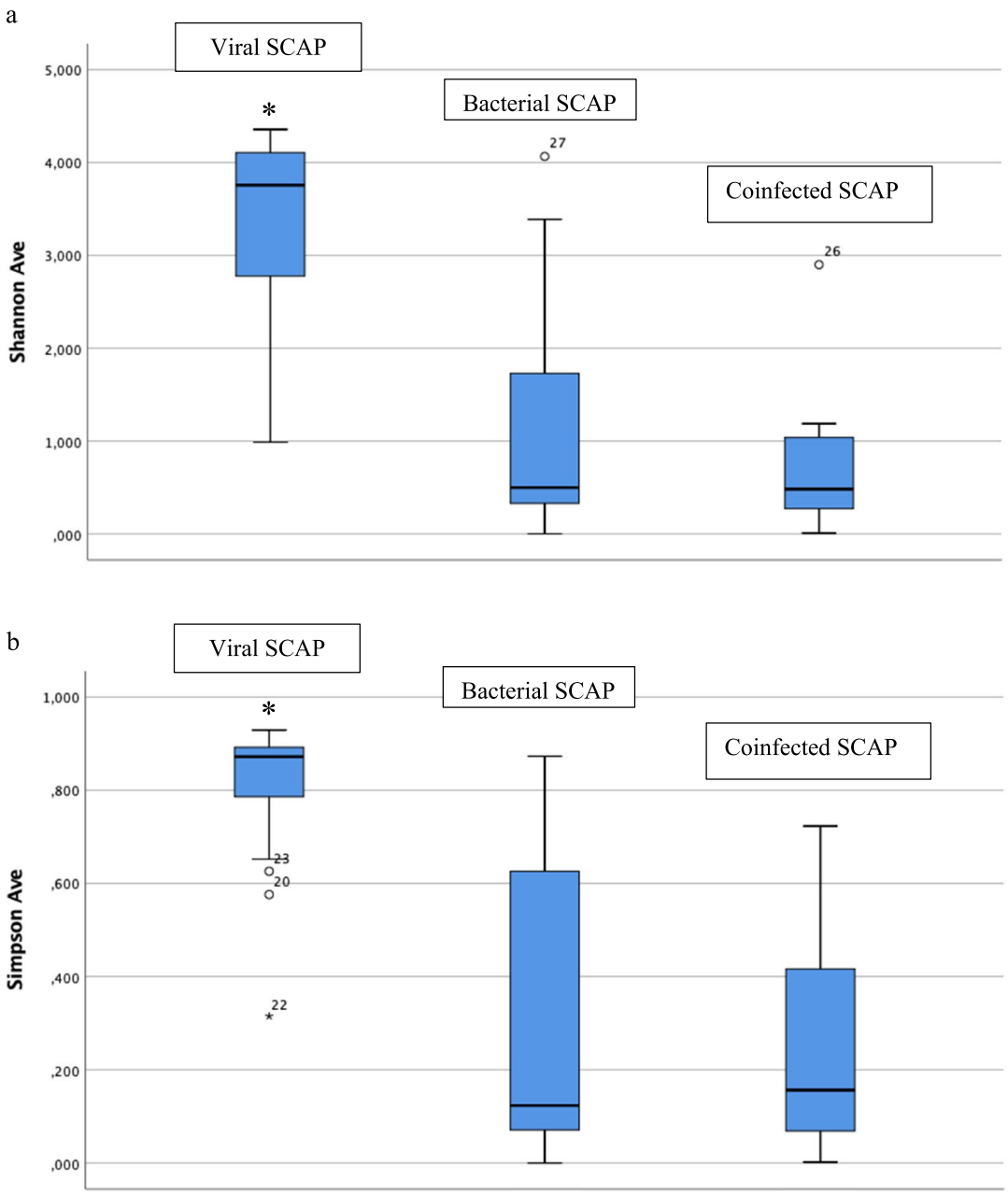

Fig. 1 **a** Shannon diversity index in patients with viral, bacterial or coinfected pneumonia. **b** Simpson diversity index in patients with viral, bacterial or coinfected pneumonia. * $p < 0.05$ compared with the diversity of the microbiome of patients with bacterial or coinfected pneumonia

multiorgan failure. One patient had *Legionella* pneumonia and was treated with cephalosporins before ICU admission. The microbiome analysis revealed a 99.9% predominance of *Legionella,* with a Shannon diversity index of 0.02. The second was a male with influenza H3 virus pneumonia coinfected with *Streptococcus pyogenes* who developed multiorgan failure. According to the microbiome analysis, 99.9% of the reads belonged to *Streptococcus,* with a Shannon diversity index of 0.01. The third was a frail female with pneumococcal pneumonia and suspicion of aspiration who developed fulminant multiorgan failure. In the microbiome analysis, a predominance of Enterobacteriaceae and 23% Streptococcus were documented (46% and *46%, respectively).* The fourth patient had pneumococcal meningitis in addition to pneumococcal pneumonia and died encephalically within 24 h of evolution. The lung microbiome was more diverse than those of the previous three groups.

Table 3 Univariate analysis of blood parameters, the lung bacterial load and the etiology of pneumonia

	Viral Pneumonia	Bacterial pneumonia	Coinfected (viral-bacterial) pneumonia	p[1]
CRP[2] (peak) (mg/L)	77.1 (43.2 –195.4)[3]	361.4 (264.95—559.2)	438.1 (287.6—489.15)	< 0.01
Procalcitonin (ng/L)	0.32 (0.15 –0.95)	8.53(1.83—46.6)	15 (8.4—65)	< 0.01
Lactate (mmol/L)	1.4 (1.05—1.8)	2.3 (1.63—10.9)	3.85 (2.3—5.95)	< 0.01
Leukocytes (per mL)	7360 (5375—12285)	15730 (4520—30510)	12160 (4917—17882)	0.34
Creatinine (mg/dL)	0.8 (0.67—1.33)	1.43 (0.84—2.7)	2.3 (1.5—5.74)	< 0.01
Bacterial load (cfu/mL)	1351 (305.2—7940.4)	22452 (719—85254)	5465 (597.6—25018.7)	0.204

[1] p: ANOVA test for the 3 groups

[2] *CRP* C-reactive protein

[3] In brackets, the percentiles Q1–Q3

Table 4 Multivariate regression analysis of the inflammatory and respiratory tract microbiome variables associated with severity at admission measured by the APACHE II score

	Univariate analysis		Multivariate analysis	
	B coefficient	P	B coefficient	P
CRP max	- 0.04	0.526	-	
PCT max	0.008	0.519	-	
Shannon diversity index	- 1.423	0.155	−1.845	0.014
Lactate levels	0.914	0.015	0.940	0.009

The other two patients who died also had more than 90% reads due to the etiologic pathogen (Table S1).

Among the 5 patients whose lung microbiome contained more than 1% Bacteroidetes with *Prevotella* predominance at the genus level, only one died due to pneumococcal meningitis with a diverse pulmonary microbiome. The other four survived, although one developed multiorgan failure.

Discussion

This study adds new support to the hypothesis that there is a relationship between the severity of CAP and microbiome diversity. Our study supports different models of CAP depending on the viral or bacterial etiology.

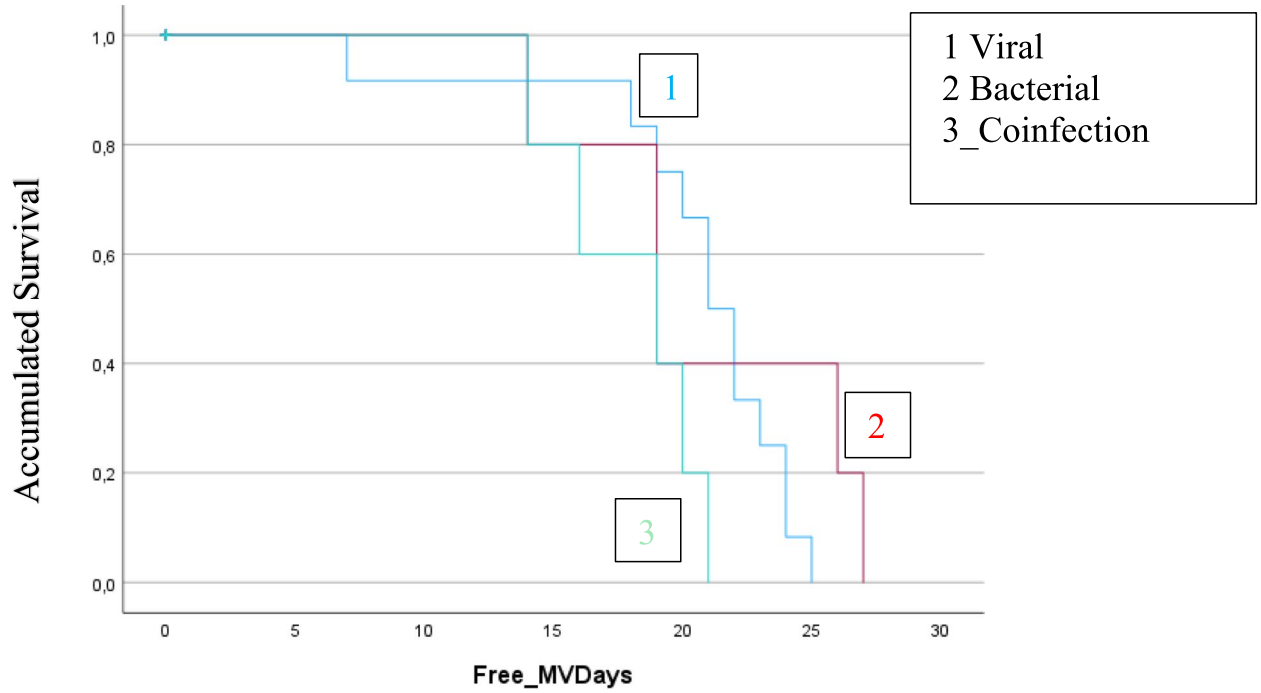

Fig. 2 Kaplan–Meier CAP etiology and mechanical ventilator dependence

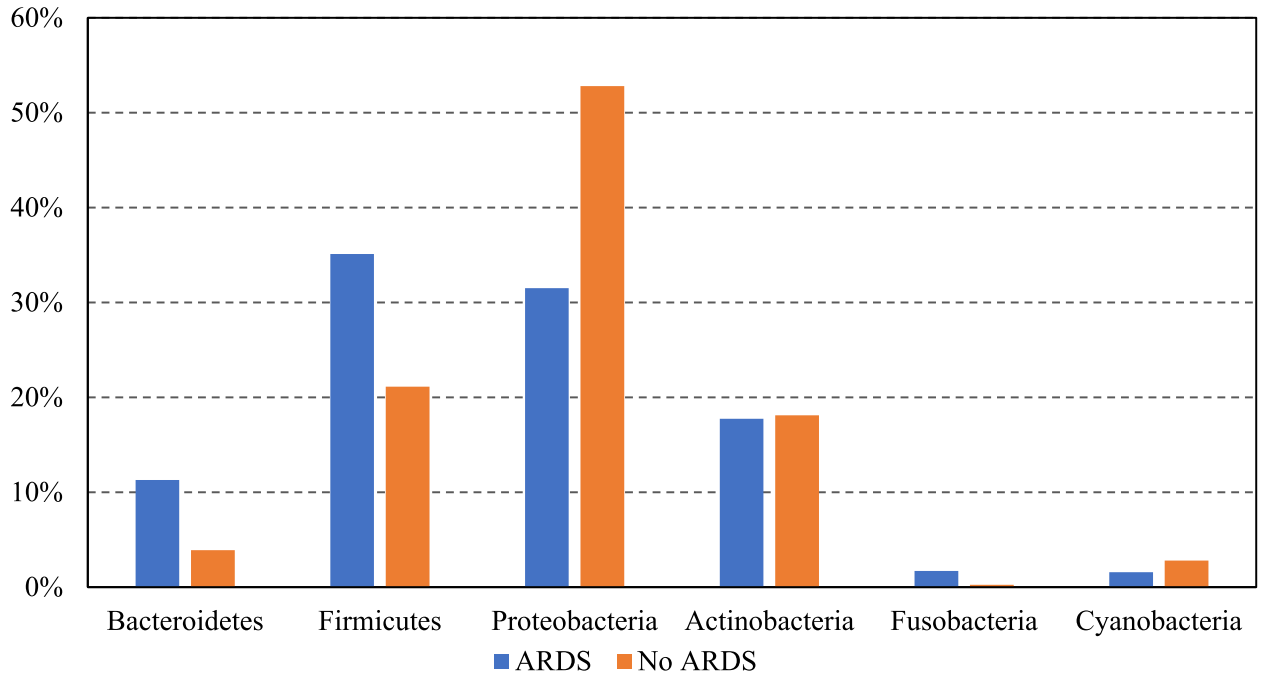

Fig. 3 Distribution of the most prevalent phyla in the lung microbiome of patients with or without ARDS at admission

However, independent of the etiology of pneumonia and host inflammation, the diversity of the microbiome composition was by itself a marker of severity at admission.

In a recent study, an association between the alpha diversity of the lung microbiome and the outcomes of critically ill patients with ARDS due to influenza virus was found, suggesting a potential correlation between microbial diversity and outcome [14]. Moreover, patients with viral pneumonia also had less ventilator dependency than patients with bacterial or coinfected pneumonia did.

The lung microbiome of patients with viral pneumonia shows an overgrowth of Firmicutes and Proteobacteria, characteristic of lung dysbiosis [15]. In patients with viral pneumonia who developed ARDS, a predominance of Firmicutes and Bacteroidetes and a lower proportion of Proteobacteria, mainly *Haemophilus* with an absence of *Enterobacteriaceae*, were demonstrated. A recent study analyzed the microbiome composition of critically ill patients with ARDS and revealed that it was associated with a predominance of Proteobacteria [16]. However, the main taxon within Proteobacteria was *Enterobacteriaceae*, suggesting that the lung microbiome of patients with ARDS was enriched with gut-associated bacteria. This cohort of patients nevertheless had a different profile from those included in our study, as they were critically ill patients with different etiologies, and only 30% of them had pneumonia.

A greater presence of *Acinetobacter* in patients with viral pneumonia was observed in patients who developed ventilator-associated pneumonia. *Acinetobacter* is not a common genus in the healthy respiratory tract microbiome [17] and is a worrisome antibiotic-resistant bacteria [18, 19]. The presence of *Acinetobacter* suggests an altered equilibrium of the respiratory microbiome, which results in complications such as ventilator-associated pneumonia due to a state of dysbiosis, as has been proposed [20]. Viral pathogens can alter the lung microbiota, and a predominance of *Acinetobacter* has been demonstrated in the lung microbiome of patients with influenza infection complicated with ARDS who died [14].

Patients with bacterial and coinfected pneumonia had very low microbiome diversity, a lower abundance of different taxa and a microbiome composition that shifted toward the pathogenic bacterium. In a cohort of patients with *Legionella* CAP, invasive mechanical ventilation was associated with a lower diversity index and a predominance of pathogenic bacteria [21].

A greater severity of pneumonia has been documented in patients with viral infection coinfected with bacteria. In fact, bacterial coinfection has been associated with high mortality rates in patients with viral pneumonia despite the administration of adequate antibiotics [22–24]. In this study, the crude mortality ratio of patients with coinfected pneumonia was 37%, which was higher than the mortality of patients with viral pneumonia (11%). These differences are clinically relevant and related to the ability of viral infection to favor invasive bacterial disease [22, 25, 26]. Recent microbiome studies suggest

a dysregulated immune response after a respiratory viral infection that might lead to the development of secondary bacterial [27, 28] or fungal infections [29].

Prevotella have been associated with improved pneumonia outcomes, and they activate several immune defense pathways in the lower airway [10, 30]. In our study, most patients with more than 1% of reads with Prevotella survived, suggesting that their presence improves the prognosis of patients with CAP.

This study has several limitations. First, this was a single-center study with a low number of patients included, which affects the statistical power of the analysis and the generalizability of the results. SCAP is a medical emergency, so antibiotic therapy cannot be delayed, and most patients have already received at least one dose of antibiotic therapy before being admitted to the ICU. Therefore, the main reason for the low number of included patients was the exclusion of a significant number of patients who had already received antibiotic therapy longer than 48 h before recruitment. Second, although a limit of 48 h of antibiotic therapy was used not to cause significant alterations in the lung microbiome, some of these patients had already been exposed, which could have altered the lung microbiome composition at admission, such as the bacterial load of the respiratory samples. Finally, the correlation between viral pneumonia alone and the severity of illness at admission represents a shortcoming, as patients are exposed to potential confounding factors.

In summary, patients with SCAP had lower lung microbiome diversity, positioning it as a potential biomarker of disease severity. The degree of dysbiosis is different depending on the etiology of pneumonia, and the prevalence of some phyla can increase the vulnerability of patients to worse outcomes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41479-025-00163-1.

Supplementary Material 1.

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Not applicable

Authors' contributions
LV and JM participated in the design of the study and writing the draft paper. AG, OL, JM and LV collected the samples for the study and the clinical and epidemiological data of the patients. AS and JM participated in performing the metagenomic techniques. CS, JR, LV, AG, OL, and JM participated in the analysis of the results. All the authors contributed to the writing and review of the article. All the authors participated in the final approval of the version to be submitted.

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the regional (Gipuzkoa) Ethics Committee, reference MOZ-NBI-2017–01. Written informed consent was given by patients or their legal representatives.

Consent for publication
Not applicable.

Competing interests
JR received consulting fees/speaking bureau from Merck, Pfizer and Roche. All the rest of the authors declare that they have no competing interests.

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