Clinical significance of culture-negative, PCR-positive bronchoalveolar lavage results in severe pneumonia

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To the Editor:

Pneumonia is an infection associated with substantial morbidity and mortality [1]. The aetiology of pneumonia is often difficult to identify in hospitalised patients, but important to ascertain in order to enhance antibiotic stewardship [2, 3]. There is growing interest in the use of PCR-based diagnostic platforms to improve the diagnosis and treatment of pneumonia in the intensive care unit (ICU). A PCR-based test that facilitates the prompt identification of a pathogen may speed initiation of appropriate antibiotic therapy. Conversely, a negative PCR-based test that predicts a negative qualitative culture may facilitate rapid discontinuation of unnecessary broad-spectrum antibiotics [4, 5]. An area of uncertainty is how clinicians should view discordance between these two testing modalities, specifically cases with a positive PCR-based test and a negative qualitative culture. Examining the cellular characteristics of culture-negative, PCR-positive bronchoalveolar lavage (BAL) samples may support or refute the presence of true bacterial infection. For example, an alveolar neutrophil percentage <50% has a negative predictive value of >90% for bacterial pneumonia [6]. The goal of this study was to describe the characteristics of culture-negative, PCR-positive BAL samples collected from critically ill patients with suspected pneumonia.

This single-centre study included patients enrolled in the Successful Clinical Response In Pneumonia Therapy (SCRIPT) study, a prospective, observational cohort study of mechanically ventilated patients with suspected pneumonia. All participants in SCRIPT have a clinically obtained BAL or nonbronchoscopic alveolar lavage available for analysis. Our standard assessment of BAL fluid includes quantitative culture, multiplex PCR (BioFire FilmArray Pneumonia (PN) Panel), cell count with differential and amylase level. High amylase levels in BAL have been associated with bacterial pneumonia [7]. Full details of the SCRIPT protocol have been described previously [8–10]. We included SCRIPT BAL samples between September 2018 to August 2022 if both culture and multiplex PCR results were available. Clinical and laboratory data were extracted from the Enterprise Data Warehouse. Antibiotic administration was abstracted *via* chart review to determine whether receipt of antibiotics prior to culture collection could explain the culture-negative, PCR-positive result in patients who had subsequent culture-positive BALs. Patients who received at least one dose of an antibiotic that covered the organism detected on culture or PCR in the 24-h period prior to the BAL were considered to have received antibiotics. We examined the 24-h time interval because antibiotic administration between 1 and 21 h prior to culture collection has been shown to decrease the yield of respiratory culture [11].

Statistical analysis was performed using R Studio version 4.1.3 (RStudio 2022.07.0: Integrated Development for R; RStudio, PBC, Boston, MA, USA). Median values and interquartile ranges (IQR) were calculated for continuous variables and compared *via* Wilcoxon rank sum test with continuity correction. Categorical variables were compared *via* Fisher's exact test. Total numbers of patients and numbers of data points available are reported in figure 1b and c.

The following definitions were used.

1) Culture-positive (PCR-positive and PCR-negative): a BAL culture with growth of any bacterial organism at any number of colony forming units (cfu). This category includes growth of bacteria that are not detected by the BioFire Pneumonia Panel and bacteria that are considered oral flora (*i.e. Viridans streptococci*).

Shareable abstract (@ERSpublications)
Some culture-negative, PCR-positive BAL samples may represent true infection. A subset of patients with a culture-negative, PCR-positive BAL result will have a subsequent BAL culture positive for the organism initially identified by PCR alone. https://bit.ly/3DWoFPo

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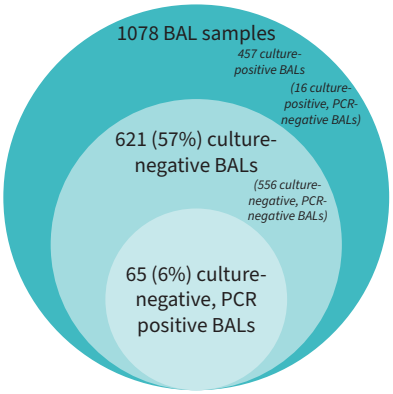
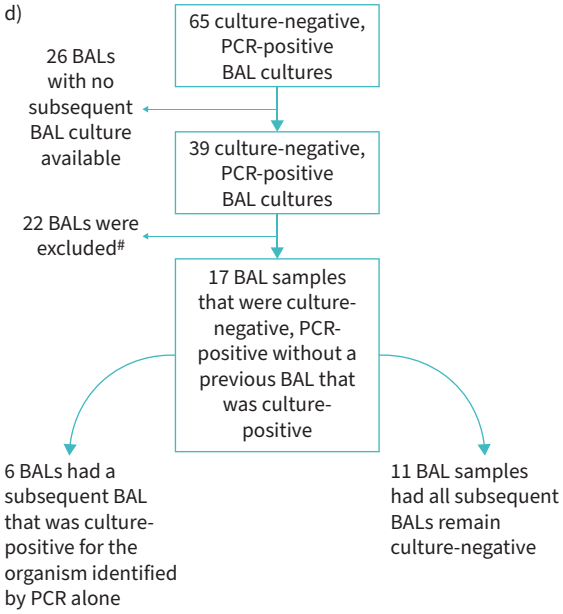
a)



b)

BAL	Culture-negative PCR-negative, n=556	Culture-negative PCR-positive, n=65	p-value
Red blood cell count (cells·μL^{-1})	1580 (354–6225) n=492	2080 (325–7625) n=56	0.54
White blood cell count (cells·μL^{-1})	286 (129–715) n=490	770 (206–1550) n=56	<0.01
Neutrophils (%)	59 (30–81) n=381	76 (58–88) n=41	0.01
Samples with neutrophils ≥50%	60.6% n=381	78% n=41	0.03
Lymphocytes (%)	6 (2–14) n=337	5 (3–9) n=36	0.13
Amylase (units·L^{-1})	39 (16–180) n=345	159 (36–426) n=43	0.01

d)



c)

BAL	Culture-positive n=457	Culture-negative, PCR-positive, n=65	p-value
Red blood cell count (cells·μL^{-1})	1730 (365–7600) n=385	2080 (325–7625) n=56	0.78
White blood cell count (cells·μL^{-1})	879 (244–2517) n=384	770 (206–1550) n=56	0.39
Neutrophils (%)	82 (64–90) n=304	76 (58–88) n=41	0.16
Samples with neutrophils ≥50%	84.9% n=304	78% n=41	0.26
Lymphocytes (%)	3 (2–7) n=254	5 (3–9) n=36	0.18
Amylase (units·L^{-1})	117 (41–610) n=299	159 (36–426) n=43	0.53

FIGURE 1 a) In the culture-negative, PCR-positive samples, the PCR-detected organisms were: *Staphylococcus aureus* (n=25), *Pseudomonas aeruginosa* (n=15), *Escherichia coli* (n=9), *Streptococcus pneumoniae* (n=7), *Serratia marcescens* (n=5), *Streptococcus agalactiae* (n=4), *Klebsiella* species (n=4), *Haemophilus influenzae* (n=2) and *Enterobacter cloacae* complex (n=1). b and c) Cell count, differential and amylase are reported as median (interquartile range). In bronchoalveolar lavage (BAL) fluid, the median white blood cell count, percentage of neutrophils and proportion of patients with a neutrophil percentage ⩾50% are higher in culture-negative, PCR-positive BAL samples compared to culture-negative, PCR-negative samples. There was no significant difference in the cell count and differential of culture-positive BAL samples compared to culture-negative, PCR-positive BALs. d) Flow diagram of the discordant BAL samples included in longitudinal analysis to determine the incidence of a subsequent culture-positive result. #: from the 39 culture-negative, PCR-positive BAL cultures, 21 BALs were excluded because there was a prior BAL culture that was positive for the PCR-identified organism. One BAL was excluded because it was from a patient already included in the analysis; it was obtained within 72 h of the BAL included in the analysis; or it had the same subsequent culture-positive results as the BAL included in the analysis.

2) Culture-negative: a BAL culture with no bacterial growth. Cultures positive for yeast were considered culture-negative.
3) Culture-negative, PCR-negative: a BAL culture with no bacterial growth and a negative BioFire Pneumonia Panel test.
4) Culture-negative, PCR-positive: a BAL culture with no bacterial growth and a positive BioFire Pneumonia Panel test.

1078 BALs with both culture and multiplex PCR results were available from 400 individual patients. The mean age of the cohort was 58.9 years and 159 (39.8%) participants in the cohort were female. The median Sequential Organ Failure Assessment score at ICU admission was 11 (IQR 8–14), and 174 (43.5%) participants experienced in-hospital mortality. The median (IQR) number of days from hospital admission to first BAL was 2 (1–8) days and the median (IQR) number of days from intubation to BAL was 1 (0–2) day. The proportion of BAL samples that were culture-negative, PCR-positive are depicted in figure 1a. As demonstrated in figure 1b and c, the BAL white blood cell count, percentage neutrophils and amylase levels were significantly higher in culture-negative, PCR-positive samples compared to culture-negative, PCR-negative samples.

We sought to determine how often a BAL culture obtained later in the hospital course would grow the organism initially detected on a BAL by PCR alone. To decrease the possibility that the PCR-positive result represented prior or residual infection, we excluded culture-negative, PCR-positive BALs that had an antecedent culture-positive BAL. Out of the 65 culture-negative, PCR-positive samples there were 17 BAL samples without a previous BAL that was culture-positive for the PCR organism (figure 1d). Six (39%) out of 17 had a subsequent BAL that was culture-positive for the organism initially identified by PCR alone. In this group the median time from discordant BAL culture to first follow-up BAL was 5 days. Five (83.3%) out of six patients in this group experienced in-hospital mortality. The other group consisted of 11 (64.7%) out of 17 who had all subsequent BALs remain culture-negative. In this group, the median time from discordant BAL to final BAL was 7.5 days, which was not statistically significant (p=0.36). Seven (63.6%) patients in this group experienced in-hospital mortality, which was not significantly lower than the group with a positive serial BAL culture when proportions were examined using Fisher's exact test (p=0.68).

In addition, we evaluated the number of patients who received antibiotics in the 24 h prior to the culture-negative, PCR-positive BAL. Six (100%) out of six patients with a culture-negative, PCR-positive BAL and a subsequent culture-positive result received antibiotics in the 24 h prior to the culture-negative, PCR-positive BAL. 10 (90.9%) out of 11 patients with a culture-negative, PCR-positive BAL and no subsequent culture-positive results received antibiotics in the 24 h prior to the culture-negative, PCR-positive BAL. This difference was nonsignificant (p=1.0).

In this study we examined the clinical significance of culture-negative, PCR-positive BAL results in patients with suspected severe pneumonia. We found that culture-negative, PCR-positive BAL samples had a significant neutrophilic alveolitis that was less prominent in culture-negative, PCR-negative BALs. The majority of our culture-negative, PCR-positive cases were not *de novo*, but were follow-up BALs from a previous culture-positive BAL. Thus, the BAL cellular profile of these culture-negative, PCR-positive cases may reflect persistent or resolving infection. In 17 patients with a culture-negative, PCR-positive result there was no antecedent culture-positive BAL. In this group of patients, when a subsequent BAL culture was available, the culture was positive for the PCR-identified organism 35% of the time. In these cases, the discordant result might represent early infection.

The strengths of the study are the large sample size and availability of serial BALs during a single hospitalisation. This study was limited by the small number of subsequent BALs in patients with a culture-negative, PCR-positive BAL results and the number of missing values for some of the data points. Despite these limitations, our study demonstrates the cellular profile of patients with culture-negative, PCR-positive BAL results is distinct from culture-negative, PCR-negative cases. Some patients may have a subsequent BAL during hospitalisation that is culture-positive for the organism detected by PCR alone even after appropriate antibiotic therapy for the initial organism detected by PCR. Future studies should examine factors that may contribute to culture positivity in these patients.

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Consent for publication: All participants or their legally authorised representatives provided written informed consent for results to be published.

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Author contributions: All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication. All authors read and approved the final draft of the manuscript. E.E. Rabin and C.I. Pickens performed data collection, data analysis and wrote the original draft of the manuscript. C. Qi provided oversight for multiplex PCR data extraction and analysis. J.M. Walter and R.G. Wunderink edited the manuscript.

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