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ORIGINAL RESEARCH

Clinical Characteristics and Prognosis of Patients with Severe Pneumonia with Neurological Dysfunction: A Regional Multicenter Retrospective Study in Mainland China

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Purpose: Pneumonia is common in ICU patients with neurological dysfunction, but differences in pulmonary pathogen distribution in this population remain unclear. This study aimed to compare pathogen profiles, clinical features, and outcomes between ICU patients with and without neurological dysfunction.

Methods: This regional multicenter retrospective study included adult patients with severe pneumonia admitted to intensive care units (ICUs) in 11 hospitals across Zhejiang and Henan Provinces in mainland China between December 2018 and November 2023. All patients required invasive mechanical ventilation and underwent bronchoalveolar lavage fluid metagenomic next-generation sequencing (mNGS). Patients were classified into neurological dysfunction (ND) and without neurological dysfunction (WND) groups. Clinical characteristics, microbiological findings, and outcomes were compared. Propensity score matching (PSM) and Cox regression were used to assess prognosis.

Results: Among 1737 patients, 636 (41.8%) were in the ND group. After PSM, the ND group showed a higher 28-day ICU mortality rate and shorter time to death compared to the WND group. However, ND was not identified as an independent risk factor for 28-day mortality in Cox analysis. The prevalence of *Acinetobacter baumannii, Klebsiella pneumoniae, Burkholderia, Serratia marcescens, Elizabethkingia, Clostridium* spp. and *Ureaplasma* was higher in ND patients. Significant differences in the prevalence of *Haemophilus influenzae, Fusarium oxysporum, Fusobacterium nucleatum, Porphyromonas gingivalis, Mycobacterium abscessus, Escherichia coli*, varicella-zoster virus (VZV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV) were also observed.

Conclusion: ICU patients with neurological dysfunction exhibited distinct pulmonary pathogen profiles and worse outcomes. These findings may inform empirical antimicrobial strategies. Further prospective studies are warranted to validate these results.

Keywords: intensive care unit,ICU, glasgow coma scale,GCS, neurologic dysfunction,ND, pneumonia, clinical characteristics, metagenomic next-generation sequencing,mNGS, ICU length of stay,iculos, ICU 28-day mortality rate

Introduction

As is well documented, severe pneumonia is characterized by high incidence and mortality rates and is a significant cause of ICU admission.[1] In a global multicenter study involving 183 hospitals, HAP and VAP accounted for 22% of all hospital-acquired infections. In addition, a study conducted in the US reported that the incidence rate of HAP among hospitalized patients was 1.6%. Similarly, the European Center for Disease Control and Prevention (ECDC) analyzed

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data from 947 hospitals in 30 countries and reported that the incidence rate of HAP was 1.3% (95% CI, 1.2 to 1.3%).[2] According to earlier studies, patients with HAP experience prolonged hospitalization by 4 to 16 days and have a mortality rate of 13%.[3] Among patients who receive MV for more than 48 hours, 10% to 40% eventually develop VAP,[4] with a higher prevalence in patients with brain injury and coma.[5] Notably, the mortality rate of VAP patients ranges from 24% to 72%.[6,7] Patients with neurological dysfunction (ND) are more likely to develop pneumonia due to factors such as impaired airway protection, lower autonomous sputum production, dependence on ventilators, and long-term bed rest.[8] While numerous studies have described the pathogenic status of ND patients in the ICU,[9] differences in the pathogenic characteristics of patients with different neurological statuses remain to be elucidated. Indeed, large-scale studies investigating lower respiratory tract BALF samples from ND patients are scarce, and data linking microbial communities, clinical features, and clinical prognosis are limited. Therefore, there is a pressing need to analyze differences in the prevalence of pulmonary pathogens associated with different neurological states and pulmonary infections in the ICU. Thus, BALF samples were collected from a multi-center severe pneumonia cohort of ICU patients with varying neurological states undergoing bronchoscopy in 11 comprehensive hospitals. The samples were subjected to mNGS testing to identify risk factors, clinical features, and distribution characteristics of pathogenic microorganisms associated with the development of pneumonia.

Study Design and Methods

Participants and Data Collection

In this regional multicenter, retrospective study, we analyzed the clinical and laboratory data of adult intensive care unit (ICU) patients admitted to 11 medical centers across Zhejiang and Henan Provinces in mainland China between December 26, 2018, and November 9, 2023. Detailed information on each participating hospital, including hospital name, location, ICU size (number of ICU beds), and the number of enrolled cases, is provided in Table S1.

The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (approval number: IT20230222A), along with the Institutional Review Boards (IRBs) or Independent Ethics Committees (IECs) of all participating institutions. The requirement for informed consent was waived by the IRB, as the study utilized anonymized historical data. All procedures adhered to the ethical standards of the institutional and national committees overseeing human research and complied with the principles outlined in the 2024 version of the Declaration of Helsinki. Given that this was a retrospective study, the requirement for informed consent was waived. BALF sample collection was performed in accordance with the standardized operating procedures of the local hospitals.

BALF sample collection in all participating hospitals was conducted strictly in accordance with two national expert consensus guidelines.[10,11] All procedures were performed by respiratory therapists or ICU physicians with more than three years of clinical experience, under the supervision of institutional medical quality control departments at each participating center. Although sample collection was performed independently at each site, all 11 hospitals followed the same standardized protocols based on the national guidelines, ensuring consistency and homogeneity across study sites. The decision to perform BALF sampling was made by the attending physician.

For inclusion in this study, patients were required to meet at least one of the following ICU admission criteria: dyspnea with respiratory rate ≥30 breaths/min, oxygen saturation ≤93% at rest without supplemental oxygen, PaO2/FiO2 (P/F) ≤300 mmHg (1 mmHg = 0.133 kPa), or evidence of other organ dysfunction such as shock.[12]

Definitions and Diagnostic Criteria

Hospital-acquired pneumonia (HAP) was defined as pneumonia occurring ≥48 hours after hospital admission, irrespective of mechanical ventilation (MV) use. Ventilator-associated pneumonia (VAP) was defined as pneumonia developing ≥48 hours after initiation of MV, accompanied by clinical signs of lower respiratory tract infection and radiographic evidence (chest X-ray or CT). These diagnostic criteria were adopted based on the 2016 Clinical Practice Guidelines for the Management of HAP and VAP, issued by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS).[13]

An immunosuppressive state was defined as meeting any of the following conditions:[14]

1. Peripheral neutrophil count $<0.5 \times 10^9$/L lasting ≥10 days after admission;
2. Receipt of immunosuppressive agents (eg, tacrolimus, cyclosporine, mycophenolate mofetil) or monoclonal antibodies (eg, rituximab) within 30 days prior to admission;
3. Diagnosis of acquired immune deficiency syndrome (AIDS);
4. Presence of hematological malignancy (eg, acute leukemia, lymphoma, multiple myeloma);
5. History of solid organ transplantation;
6. Corticosteroid therapy >2 weeks or receipt of pulse steroid therapy within the previous 14 days.

Chronic pulmonary disease (CPD) was defined as airflow limitation caused by chronic pulmonary disorders, including asthma and chronic obstructive pulmonary disease (COPD). Hematological malignancies (HM) referred to neoplastic diseases originating from the bone marrow or hematopoietic system, including but not limited to acute leukemia, lymphoma, and multiple myeloma. Connective tissue diseases (CTD) included systemic lupus erythematosus, polymyositis, mixed connective tissue disease, and moderate-to-severe rheumatoid arthritis. Cerebrovascular disease (CBD) was defined as a history of transient ischemic attack or stroke with no or only mild residual deficits.

All included patients were aged ≥18 years. The severity of illness was assessed using the Sequential Organ Failure Assessment (SOFA) score. According to previous studies, including a multicenter randomized controlled trial published in JAMA Internal Medicine,[15] organ dysfunction was defined as a SOFA subscore ≥2 in any of the six organ systems. The neurological SOFA subscore (range 0–4) was used for patient stratification:

1. Patients with a neurological SOFA subscore ≥2 (corresponding to a Glasgow Coma Scale [GCS] score ≤10) were classified into the neurological dysfunction (ND) group.
2. Patients with a neurological SOFA subscore <2 were classified into the without neurological dysfunction (WND) group.

Exclusion criteria were as follows:

1. Age <18 years;
2. Pregnancy or lactation;
3. Absence of bronchoalveolar lavage and/or mNGS testing during hospitalization;
4. Lack of radiological evidence of pneumonia (on chest imaging);
5. Incomplete clinical records after chart review.

Data Collection

Two researchers independently reviewed the medical records of patients and collected clinical data, laboratory results, and mNGS outcomes. During the clinical metagenomic examination, all patients or their families signed the informed consent form for the examination to be conducted within the scope permitted by Chinese law. Recorded data comprised the patient's age, gender, comorbidities, Sequential Organ Failure Assessment (SOFA) score, GCS score, 28-day ICU mechanical ventilation time, time from admission to mNGS, total length of stay (LOS), ICU length of stay (iculos), 28-day ICU mortality rate, and time to death.

Laboratory Confirmation

The blood samples of patients were analyzed in hospital laboratories for complete blood count (white blood cell, lymphocyte, and neutrophil counts), C-reactive protein levels, and procalcitonin (PCT) levels. Fiberoptic bronchoscopy was conducted for bronchoalveolar lavage, followed by mNGS detection to identify pulmonary pathogens. Sample collection, storage in sterile containers, and dispatch to the laboratory were completed within 2 hours (at room temperature).

Microbiological Analysis

Pathogen interpretation was performed according to the 2017 Chinese Expert Consensus on Pathogen Detection by Bronchoalveolar Lavage Fluid[10] and the 2023 Chinese Expert Consensus on Cytomorphological Examination of BALF.[11] Detected microorganisms were evaluated comprehensively, incorporating patients' clinical presentation, laboratory findings, imaging results, and the clinical judgment of treating physicians.

Notably, the identification of common oral anaerobes or upper respiratory tract flora—such as Fusobacterium nucleatum or Porphyromonas gingivalis—was not automatically excluded. Their clinical significance was assessed in the context of individual patient factors, including history of aspiration, vomiting episodes, and clinical suspicion of aspiration pneumonia. Organisms considered clinically relevant based on these factors were retained in the final pathogen analysis.

Conversely, organisms judged unrelated to the patient's infectious presentation—such as typical oral commensals or environmental contaminants without supporting clinical evidence—were excluded to minimize misclassification of colonization as infection.

mNGS Testing and Data Processing

BALF specimens underwent mNGS either in hospital-based clinical microbiology laboratories or were outsourced to certified third-party clinical laboratories, depending on the institution:

Seven hospitals outsourced sequencing to nationally accredited third-party laboratories, including BGI Genomics, Joygen Biotech, and Difei Diagnostics, which are widely recognized for their reliability in clinical microbiology in China.

Four hospitals, including the lead center, performed mNGS in their own clinical laboratories using validated platforms and established internal protocols.

While full unification of sequencing protocols was not feasible across all laboratories, participating centers were required to follow a standardized data reporting framework and pathogen interpretation criteria, coordinated by the Microbiology Center of the First Affiliated Hospital of Zhejiang University School of Medicine. Each center applied report harmonization prior to data submission. The lead center consolidated and reviewed submitted results to ensure reasonable consistency for pooled analysis.

Criteria for Pathogen Identification in mNGS

Pathogen identification thresholds were standardized across all participating institutions under the guidance of the lead center. The following criteria were uniformly applied:

For common bacteria, identification required ≥3 unique sequence reads aligned at the species level.

For Mycobacterium tuberculosis and fungal pathogens, ≥1 unique read was considered significant due to known challenges in cell wall lysis and DNA extraction efficiency.

Additionally, all detected species were required to show a read count at least 10-fold higher than that in the concurrently processed negative control (reagent blank).

To enhance clinical relevance, final pathogen interpretation integrated clinical presentation, radiologic findings, inflammatory markers, and attending physician judgment. These standards were consistent with national expert consensus guidelines[10,11] and with institutional protocols used by the lead center.

Statistical Analysis

Statistical analyses were performed using SPSS 25.0 statistical software. Stata 16 software was employed for propensity score matching (PSM) to calibrate baseline differences between the two groups, with a caliper value set to 0.1. Baseline characteristics and outcomes were expressed as mean ± standard deviation, median (interquartile range, IQR), or percentage, as appropriate. Continuous variables were compared using Student's *t*-test, whereas categorical variables were compared using the Chi-square test or Fisher's exact test. A multivariate Cox regression model was employed to identify independent risk factors for 28-day mortality, with $P < 0.05$ considered statistically significant. GraphPad Prism 10 software was used to generate forest plots and Kaplan–Meier survival curves.

For pathogen subgroup analyses, no formal adjustment for multiple comparisons was performed. Reported P-values were uncorrected and should be interpreted as exploratory. The findings regarding low-prevalence organisms require cautious interpretation due to the increased risk of type I error.

Results

A total of 2032 pneumonia patients admitted to the intensive care unit were screened. After excluding 295 patients based on predefined inclusion and exclusion criteria, 1737 patients were included in the final analysis. Among them, 1011 (58.2%) were assigned to the WND group and 636 (41.8%) to the ND group (Figure 1).

As shown in Table 1, baseline characteristics revealed no significant differences in gender between groups. However, differences were observed in age (66% vs 68%, p = 0.002), pneumonia type—CAP (61.3% vs 59.1%), HAP (25.9% vs 21.1%), VAP (12.8% vs 19.8%, p < 0.001)—ventilation support mode (IMV: 84.4% vs 91.2%; NIV: 3.3% vs 2.4%), and comorbidities (72.2% vs 66.8%, p = 0.019) between the WND and ND groups.

As shown in Table S2, white blood cell count, neutrophil count, lymphocyte count, and CRP levels upon admission were comparable between the two groups. However, the procalcitonin (PCT) level was significantly lower in the WND group (0.9 vs 1.0, p = 0.000), whereas SOFA scores were significantly higher (6 vs 10, p < 0.001).

Regarding clinical outcomes before matching, patients in the ND group had a longer 28-day duration of mechanical ventilation (10 vs 7 days, p < 0.001), longer average ICU stay (14 vs 12 days, p = 0.000), a higher overall ICU 28-day mortality rate (49.2% vs 36.7%, p = 0.000), and a shorter time to death (20 vs 22 days, p = 0.000).

After propensity score matching (PSM), no statistically significant differences were observed in 28-day mechanical ventilation time (8 vs 10 days, p = 0.058), total length of stay (22 vs 21 days, p = 0.200), or ICU length of stay (14 vs 14 days, p = 0.650) between the WND and ND groups. However, the 28-day ICU mortality rate remained significantly higher in the ND group (46.99% vs 38.73%, p = 0.004), and the time to death remained significantly shorter (20 vs 22 days, p = 0.006) (Table 2).

After adjusting for gender, age, pneumonia category, ventilation support method, and comorbidities, Cox multivariate regression analysis indicated that ND was not an independent risk factor for 28-day ICU mortality (HR = 0.805, p = 0.078) (Table S3).

Subgroup analysis of 28-day all-cause ICU mortality was further conducted to assess the effect of neurological dysfunction across various clinical subgroups, as shown in Figure 2. The results demonstrated that patients receiving invasive mechanical ventilation (IMV) had significantly increased mortality risk compared to those receiving non-

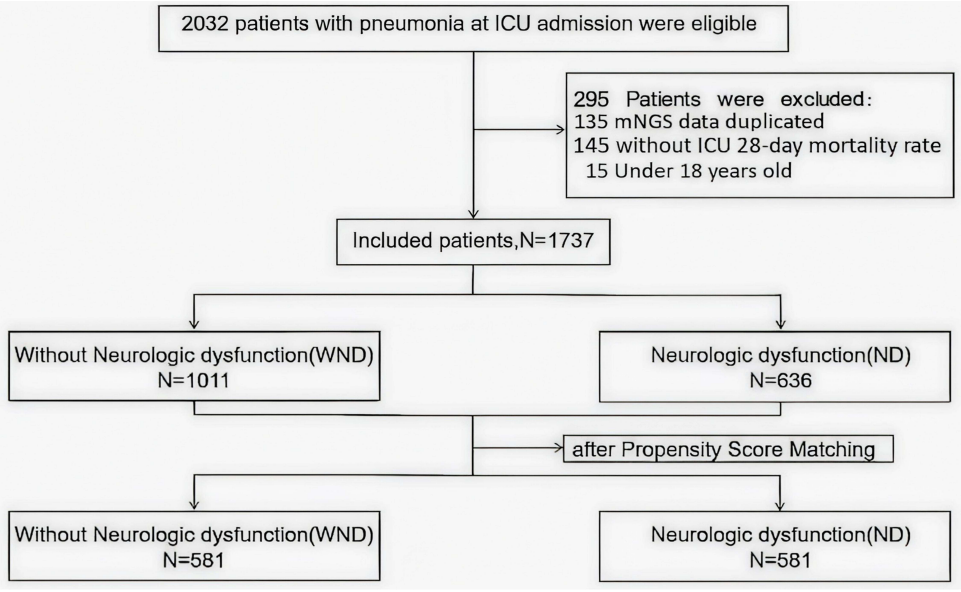

Figure 1 Flow diagram detailing the trial process, illustrating the number of patients in each step and each group.

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Table 1 | Baseline Characteristics Before and After Propensity-Score Matching

Characteristics	All Cases (n=1737)	Unmatched Cohort			After Propensity Score Matching		
		WND (n=1101)	ND (n= 636)	P value (WND vs ND)	WND (n= 581)	ND (n= 581)	P value (WND vs ND)
Age, years	67 (55,77)	66(54,74)	68 (56,77)	0.002	68 (57,76)	67 (55,76)	0.43
Gender							
Male	1216 (70.0%)	759 (68.9%)	457 (71.9%)	0.211	412 (70.9%)	415 (71.4%)	0.85
Female	521 (30.0%)	342 (31.1%)	179 (28.1%)	0.211	169 (29.1%)	166 (28.6%)	0.85
Pneumonia type							
CAP	1051 (60.5%)	675 (61.3%)	376 (59.1%)	<0.001	351 (60.4%)	349 (60.1%)	0.41
HAP	419 (24.1%)	285 (25.9%)	134 (21.1%)		136 (23.4%)	123 (21.2%)	
VAP	267 (15.4%)	141 (12.8%)	126 (19.8%)		94 (16.2%)	109 (18.8%)	
ngs_to_intime	3 (2,7)	3 (2,6)	4 (2,8)	0.016	3 (2,7)	3 (2,8)	0.095
Ventilator mode							
IMV	1510 (86.9%)	930 (84.4%)	580 (91.2%)	<0.001	53 1 (91.4%)	525 (90.4%)	0.49
NIV	52 (3.0%)	37 (3.3%)	15 (2.4%)		18 (3.1%)	15 (2.6%)	
Other	175 (10.1%)	134 (12.1%)	41 (6.4%)		32 (5.5%)	41 (7.1%)	
Comorbidities							
Any	1220 (70.2%)	795 (72.2%)	425 (66.8%)	0.019	412 (67.9%)	407 (67.1%)	0.76
Immunosuppression	430 (24.7%)	330 (29.9%)	100 (15.7%)	<0.001	112 (19.3%)	99 (17.0%)	0.32
Diabetes	427 (24.5%)	260 (23.6%)	167 (26.2%)	0.225	148 (25.5%)	145 (25.0%)	0.84
MI	103 (5.9%)	71 (6.4%)	32 (5.0%)	0.247	37 (6.4%)	32 (5.5%)	0.53
CPD	350 (24.1%)	243 (22.0%)	107 (16.8%)	0.009	112 (19.3%)	104 (17.9%)	0.55
Liver disease	118 (6.7%)	82 (7.4%)	36 (5.6%)	0.166	32 (5.5%)	35 (6.0%)	0.71
CKD	219 (12.6%)	142 (12.8%)	77 (12.1%)	0.653	69 (11.9%)	70 (12.1%)	0.93
Solid_tumor	261 (15.0%)	177 (16.0%)	84 (13.2%)	0.109	88 (15.2%)	81 (13.9%)	0.56
HM	91 (5.2%)	71 (6.4%)	20 (3.1%)	0.003	21 (3.6%)	20 (3.4%)	0.87
CTD	74 (4.2%)	55 (4.9%)	19 (2.9%)	0.049	18 (3.1%)	19 (3.3%)	0.87
Transplantation	88 (5.0%)	72 (6.5%)	16 (2.5%)	0.000	14 (2.4%)	16 (2.8%)	0.71
CBD	279 (16.0%)	141 (12.8%)	138 (21.6%)	0.000	110 (18.9%)	98 (16.9%)	0.36

Abbreviations: CAP, Community-Acquired Pneumonia; HAP, Nosocomial pneumonia; VAP, Ventilator-associated pneumonia; IMV, Invasive mechanical ventilation; NIV, Non-Invasive Ventilation; MI, Myocardial Infarction; CPD Chronic pulmonary disease; CKD, Chronic kidney disease; HM Hematological Malignances; CTD, Connective tissue disease; CBD, Cerebrovascular disease.

Table 2 | Outcome Variables Before and After Propensity-Score Matching

Variables	All Cases (n=1737)	Unmatched Cohort			After Propensity Score Matching		
		WND (n=1101)	ND (n= 636)	P value (WND vs ND)	WND (n= 581)	ND (n= 581)	P value (WND vs ND)
Ventilation time with 28 ICU days	8 (3,16)	7 (3,14)	10 (5,18)	<0.001	8 (4,16)	10 (4,18)	0.058
Los	21 (12,36)	21 (12,36)	21 (11,35)	0.186	22 (13,38)	21 (11,36)	0.20
Iculos	13 (7,23)	12 (7,22)	14 (8,25)	0.000	14 (8,24)	14 (8,25)	0.65
Death_icu_28day[a]	718 (41.3%)	405 (36.7%)	313 (49.2%)	0.000	225 (38.73%)	273 (46.99%)	0.004
Death_time	28 (12,28)	22 (14,28)	20 (10,28)	0.000	22 (14,28)	20 (11,28)	0.006

Note: [a]Death_icu_28day refers to the overall ICU 28-day mortality rate.

invasive ventilation (HR = 2.17, 95% CI: 1.130–4.169, p = 0.020). Additionally, a history of myocardial infarction (MI) (HR = 1.909, 95% CI: 1.368–2.664, p < 0.001) and hematological malignancy (HM) (HR = 1.541, 95% CI: 1.037–2.288, p = 0.032) were significantly associated with increased 28-day mortality. Although age >60 years, immunosuppression, and neurological dysfunction showed elevated hazard ratios, they did not reach statistical significance. No significant associations were observed in subgroups defined by pneumonia type, chronic kidney disease, or connective tissue

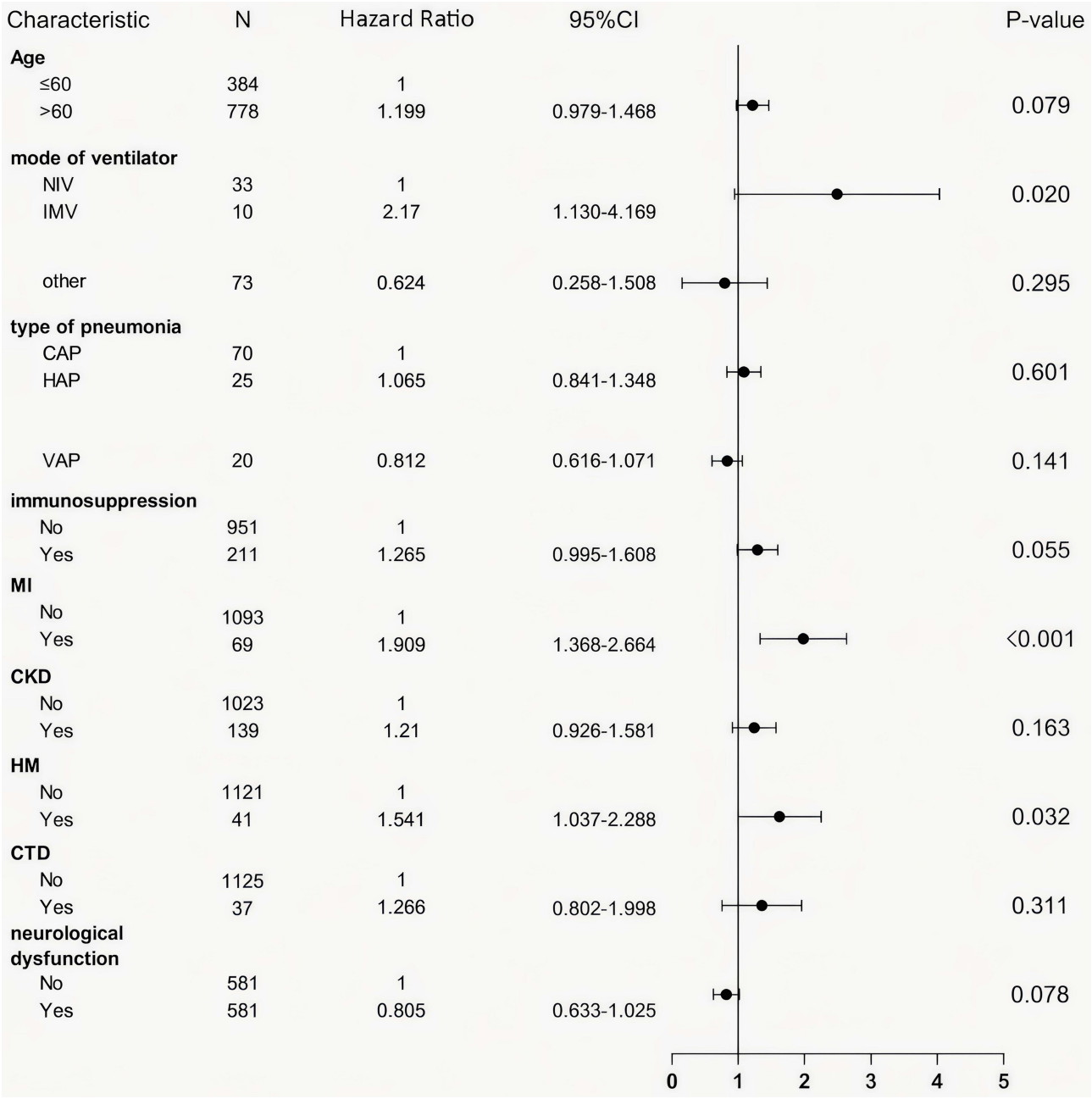

Figure 2 Subgroup analysis of 28-day all-cause mortality.

disease.Kaplan–Meier survival analysis showed that the overall ICU 28-day mortality rate was higher in the ND group compared to the WND group (Figure 3).

As shown in Figure S1, the most prevalent pathogens in the WND group were Acinetobacter baumannii (27.1%), herpes simplex virus type 1 (26.4%), Candida albicans (22.9%), Klebsiella pneumoniae (22.1%), cytomegalovirus (19.3%), EB virus (18.0%), Pseudomonas aeruginosa (14.8%), and Stenotrophomonas maltophilia (14.5%).

As shown in Figure S2, the ND group had higher proportions of patients with hospital-acquired Acinetobacter baumannii (2.0% vs 0.8%, p = 0.049), Klebsiella pneumoniae (32.3% vs 22.1%, p = 0.000), Burkholderia cepacia (9.7% vs 5.6%, p = 0.003), Serratia marcescens (4.5% vs 1.4%, p = 0.001), Elizabethkingia (5.3% vs 2.1%, p = 0.002), and Ureaplasma (2.0% vs 0.8%, p = 0.040).

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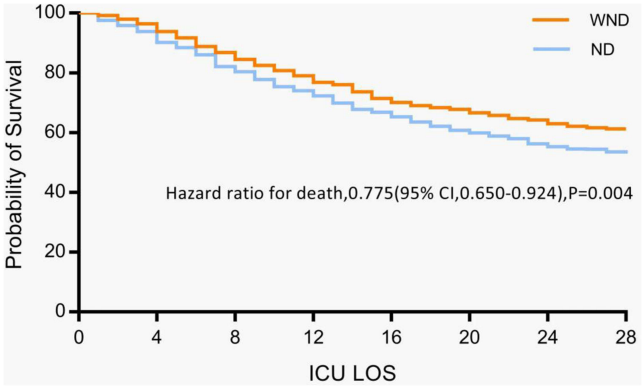

Figure 3 Kaplan-Meier Estimates of Survival Probability.

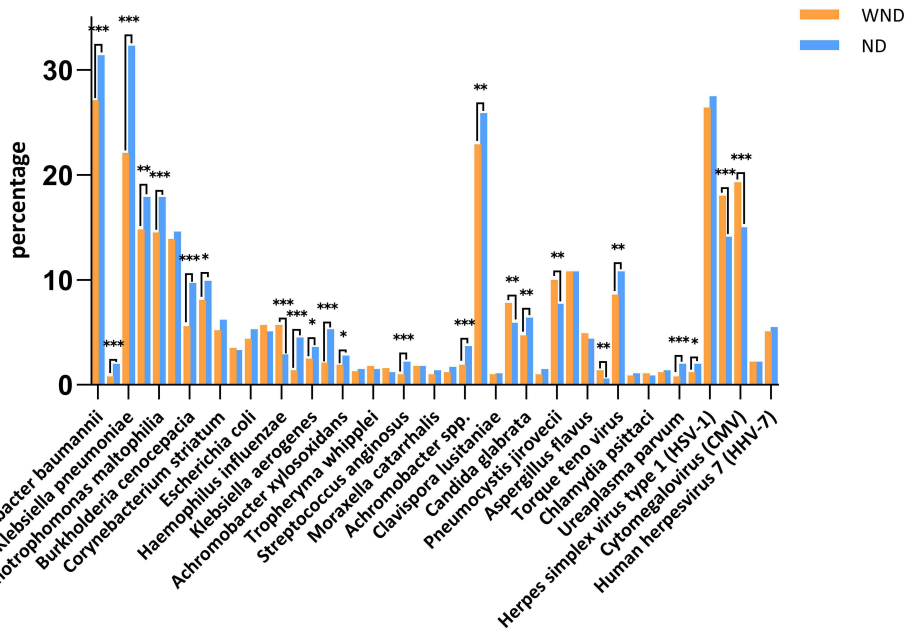

Figure 4 Distribution of pathogens detected by metagenomic next-generation sequencing (mNGS) in the neurological dysfunction (ND) and without neurological dysfunction (WND) groups. Data are presented as histograms showing pathogen detection percentages in each group. Statistical significance: *p < 0.05; **p < 0.01; ***p < 0.001.

Significant differences were also noted in the presence of other microorganisms, as shown in Figure 4: Haemophilus influenzae (2.90% vs 5.70%, p = 0.005), Fusarium oxysporum (0.00% vs 0.30%, p = 0.045), Fusobacterium nucleatum (0.00% vs 0.50%, p = 0.014), Porphyromonas gingivalis (0.00% vs 0.30%, p = 0.045), Mycobacterium abscessus (0.10% vs 0.80%, p = 0.035), and Escherichia coli (0.00% vs 19.30%, p = 0.035).

Discussion

In this regional multicenter retrospective study conducted in Zhejiang and Henan Provinces of mainland China, we identified significant differences in pulmonary pathogen distribution between ICU patients with and without neurological dysfunction (ND). These findings provide important clinical insights for differentiating patient profiles and guiding infection management strategies in critical care settings.

Compared with WND patients, ND patients were generally older, exhibited higher rates of ventilator-associated pneumonia (VAP), required more frequent invasive mechanical ventilation (IMV), and had a higher incidence of central nervous system comorbidities and elevated SOFA scores. These clinical features were associated with higher 28-day ICU

mortality and shorter survival time. In addition, statistically significant differences in the respiratory microbiological profiles were observed between the two groups.

Pulmonary infections are highly prevalent in ICU populations.[1] In recent years, metagenomic next-generation sequencing (mNGS) has become increasingly utilized in clinical microbiology due to its high-throughput, broad-spectrum pathogen detection capabilities.[16] In this study, the application of mNGS to BALF samples enabled comprehensive identification of lower respiratory tract pathogens. Our findings aligned with national surveillance data from the China Antimicrobial Surveillance Network (CHINET), where Klebsiella pneumoniae remains the most prevalent respiratory pathogen,[17] consistent with our observation of K. pneumoniae predominance, especially in ND patients.[18,19]

Importantly, not all organisms detected by mNGS were classified as clinically significant. Pathogen interpretation was performed according to the 2017 Chinese Expert Consensus on Pathogen Detection by Bronchoalveolar Lavage Fluid and the 2023 Chinese Expert Consensus on Cytomorphological Examination of BALF.[10,11] Detected organisms were assessed in conjunction with clinical presentation, imaging findings, laboratory parameters, and physician judgment. Common oral colonizers and environmental contaminants were excluded from the final pathogen analysis. However, due to inherent limitations of mNGS, including its inability to distinguish viable from non-viable organisms, the risk of overestimating certain colonizing species could not be entirely avoided.

Notably, the detection of oral anaerobes and periodontal pathogens (eg, Fusobacterium nucleatum, Porphyromonas gingivalis)[20] suggests that aspiration of oropharyngeal flora may contribute to pulmonary infections in ND patients, likely related to impaired airway protective reflexes. The higher prevalence of multidrug-resistant organisms, including carbapenem-resistant K. pneumoniae and multidrug-resistant A. baumannii, in ND patients further highlights the clinical challenges posed by these infections.[21–23]

Subgroup analyses of low-prevalence organisms, such as Fusarium spp. and Ureaplasma, were exploratory in nature and potentially underpowered. No adjustment for multiple comparisons was applied, and reported P-values were uncorrected; these results should therefore be interpreted cautiously, recognizing the increased risk of type I error. The findings regarding rare pathogens should be considered hypothesis-generating.

In addition, although antibiotic use plays a critical role in infection outcomes, detailed information on empirical versus targeted antimicrobial strategies, de-escalation practices, and treatment modifications was unavailable in this retrospective cohort. As a result, the potential impact of antibiotic management on clinical outcomes could not be assessed in this study.

Furthermore, while the analysis of short-term outcomes such as ICU stay and 28-day mortality was appropriate, the lack of long-term follow-up data—including 90-day mortality, neurological recovery, and functional outcomes—limits the broader clinical applicability of our findings. Future prospective studies incorporating extended follow-up are needed to assess the long-term prognostic implications of neurological dysfunction and associated infections in ICU patients.[24,25]

Taken together, this study highlights distinct microbiological characteristics and worse short-term outcomes in ND patients, providing a foundation for optimized infection management strategies in this vulnerable population.

Limitations

This study has several limitations. First, as a regional multicenter study conducted in Zhejiang and Henan Provinces of China, our findings may not fully represent national ICU epidemiology,[26] and external validation in larger, more diverse cohorts is required. Second, this study focused on short-term outcomes, specifically ICU length of stay and 28-day mortality, without evaluation of long-term outcomes such as 90-day mortality, neurological recovery, or post-discharge functional status. This limits the broader clinical applicability of the findings. Third, although standardized interpretation criteria were applied, the inherent limitations of mNGS in distinguishing colonization from true infection may have affected the accuracy of pathogen identification.[27] Fourth, while prior antibiotic exposure data were retrospectively collected, detailed information regarding empirical versus targeted antibiotic regimens, timing of initiation, de-escalation practices, and susceptibility-guided treatment modifications was unavailable due to inconsistent documentation across centers. Therefore, the potential influence of antimicrobial treatment strategies on pathogen distribution and patient outcomes could not be evaluated.

Fifth, mNGS testing in this study was not centralized; sequencing was performed by different hospital laboratories and third-party clinical laboratories across participating centers. Although all sites followed unified reporting thresholds and contamination control standards supervised by the lead institution, experimental procedures and sequencing platforms were not fully standardized. Despite the central review of all reports before pooled analysis, inter-laboratory variability cannot be entirely excluded and may have influenced microbiological findings. Future studies using centralized sequencing and analysis workflows may help address this limitation.

Finally, no adjustment for multiple comparisons was performed in the microbiological analyses. Subgroup comparisons, particularly for low-prevalence pathogens, should thus be considered exploratory and interpreted cautiously due to the increased risk of type I error and limited statistical power.

Despite these limitations, this study represents one of the largest multicenter investigations applying mNGS to BALF samples in ICU patients with neurological dysfunction. Our findings offer valuable insights into the pathogen spectrum and clinical outcomes of this high-risk population, contributing to evidence-based infection management in critical care settings.

Conclusions

This regional multicenter retrospective study, conducted in 11 hospitals across Zhejiang and Henan Provinces in mainland China, identified significant differences in pulmonary pathogen distribution among ICU patients with neurological dysfunction. Neurological dysfunction was associated with a higher ICU 28-day mortality rate and a shorter time to death in patients with severe pneumonia.

Based on these findings, we recommend strengthening infection prevention and airway management strategies in this high-risk population. Potential measures include aspiration prevention, early assessment of swallowing and expectoration functions, regular microbiological monitoring, and timely initiation of antimicrobial therapy guided by mNGS results or susceptibility testing.

Data Sharing Statement

Data can be obtained from corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the ethics committees of Zhejiang University School of Medicine First Affiliated Hospital (No. IT20230222A) and other participating hospitals. As a retrospective study, the requirement for informed consent was waived.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

All authors declare that they have no competing interests.

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