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ORIGINAL RESEARCH

Longitudinal Changes in Peripheral and Alveolar Monocyte and Inflammatory Biomarkers are Distinct in Hypercapnia Patients Following Pulmonary Sepsis-Induced ARDS

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Background: Hypercapnia, an Acute Respiratory Distress Syndrome (ARDS) complication after pulmonary sepsis, remains enigmatic in terms of its immunological mechanisms. Our study was designed to compare initial values and longitudinal changes in cellular composition and inflammatory biomarkers between pneumonia sepsis-induced ARDS patients without hypercapnia and hypercapnia patients.

Methods: Between Dec 2022-Apr 2023, we prospectively studied 61 severe pneumonia patients. Eleven non-sepsis pneumonia patients were controls; 50 patients with pulmonary sepsis met ARDS criteria, 26 among them developed hypercapnia. We collected clinical data, respiratory parameters, peripheral blood mononuclear cells (PBMCs), and bronchoalveolar lavage fluid (BALF) at Day 1 and Day 7 post-intubation. Single-cell RNA sequencing (ScRNA-seq) was performed between selected hypercapnia and non-hypercapnia patients to characterize immune and cellular profiles. Specimens were analyzed via flow cytometry and cytokine panel.

Results: By compiling clinical data and specimens, we found that hypercapnia patients with ARDS had poorer outcomes and higher mortality. At day 1, ScRNA-seq and cytometric analysis revealed increase in monocytes and activation of cytokine storm genes with elevated interleukin (IL) −1β, IL-12p40, and IL-23 in peripheral blood. In hypercapnia patients, percentage of CD14+CD16- classical monocyte and concentrations of IL-12p40 and IL-23 increased from day 1 to day 7 in both circulation and airways. However, these alterations of cellular phenotype and cytokine decreased during seven-treatment period in non-hypercapnia patients.

Conclusion: We offer novel perspectives on monocyte-centered clusters and associated biomarkers, which play a pivotal role in driving hypercapnia after pulmonary sepsis-induced ARDS. Our study provides fresh insights into the immunological mechanisms underlying hypercapnia in ARDS, laying the foundation for useful therapeutic targets to improve patient outcomes.

Keywords: hypercapnia, pulmonary sepsis-induced ARDS, monocyte, cytokine storm, chemokine

Introduction

The acute respiratory distress syndrome (ARDS) is the most severe form of acute hypoxemic respiratory failure and affects 10% of all intensive care unit (ICU) patients.[1] Pulmonary infections account for the vast majority of ARDS risk factors and are associated with septic shock in about 70% of cases.[2] Despite advances in patient management during the previous decades, hospital mortality of pulmonary sepsis induced-ARDS remains as high as 40%.[3] Importantly, elevated level of CO_2 (independently of the associated acidosis) has been shown to increase mortality in patients with ARDS and sepsis.[4,5] Numerous studies have attempted to improve outcomes for patients with severe ARDS as outlined by the 2023 ESICM guidelines, but the efficacy of decreasing CO_2 levels remains unclear.[6] Although hypercapnia patients often exhibit signs of

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hyperinflammation,[7] the role of the immune response in the pathogenesis of ARDS-induced hypercapnia has not been determined, and treatment options remain limited.[8] Targeted interventions require a deeper understanding of the relationship between the immune response and hypercapnia outcomes in patients following pulmonary sepsis induced-ARDS.

Sepsis-induced defects in innate and adaptive immune cells were not only observed in blood but also in the lungs of patients dying from sepsis, illustrating that such immune alterations also occurred in situ, although the clinical significance of such regional alterations has not been established.[9] Macrophages and monocytes are important mediators of tissue repair, remodelling, and fibrosis in the different phases of ARDS.[10] Former study reported the accumulation of monocyte-derived macrophages with an enrichment of fibrosis-associated gene signatures is associated with in the development of hypercapnia during severe COVID-19.[11] Furthermore, ARDS progresses rapidly following the initial insult, with a cytokine storm sustaining inflammation and causing tissue injury. However, no consensus is yet reached immune cellular components and regarding inflammatory biomarkers that can be included in the definition of ARDS. Previous studies have been reported that Single-cell RNA sequencing (scRNA-seq) has the potential to uncover pathophysiologic mechanisms and select molecular specific interventions in ARDS patients.[12] In addition, distinct plasma biomarker signatures have been identified in patients with sepsis and ARDS. For example, circulating concentrations of the pleiotropic cytokine interleukin-6 (IL-6) are known to be increased in pro-inflammatory critical care syndromes, such as sepsis and acute respiratory distress syndrome.[13] Taken together, differential gene expression in PBMC may be associated with hypercapnia development and underlie mechanisms of inflammatory injury to the lungs in patients with pulmonary sepsis-induced ARDS.

Therefore, we hypothesized that alterations in the transcriptional state of immune cells and the transcriptional signals for cytokine storm would be distinct in hypercapnia patients, comparing with non-hypercapnia patients after pulmonary sepsis-induced ARDS. In current study, we aimed to identify differences of genomic signatures in hypercapnia patients with pulmonary sepsis-induced ARDS. Our results indicate that monocytes, both CD14+CD16− classical monocytes and CD14+CD16+ intermediate monocytes, play crucial roles in developing hypercapnia. The interrelation of ARDS/sepsis biomarkers, especially interleukin (IL)-12p40 and IL-23, in the blood compartments and alveolar may be indicative of the pathophysiologic changes in hypercapnia patients. Our findings thus underscore the involvement of circulatory and airway myeloid cell subsets and related biomarkers in the pathology of hypercapnia and may be useful therapeutic targets in pulmonary sepsis-induced ARDS.

Materials and Methods

Study Design, Patient Enrollment, Samples and Data Collection

Between December 16th, 2022, and April 30th, 2023, we enrolled about 97 pneumonia patients receiving invasive mechanical ventilation during the study period, and 61 patients satisfied the inclusion criteria. Of these, 50 developed pulmonary sepsis and 11 did not (these should be referred to as the non-sepsis pneumonia group). These patients were carefully selected to participate in a prospective, observational study, with the aim of minimizing confounding signals arising from other sources of infection. Eligible patients were adults aged 18 years or older, exhibiting clinical symptoms suggestive of pneumonia such as fever, radiographic infiltrates, and respiratory secretions. Those who were mechanically ventilated and met the criteria for Sepsis-3.0 definition[14] and ARDS were enrolled in the study. Sepsis patients fulfilled two or more criteria of the quick Sequential Organ Failure Assessment (qSOFA): specifically, low mean blood pressure (≤65 mmHg), tachypnea (≥22/min), and altered mental status (Glasgow Coma Scale, GCS <15).[15] ARDS was defined based on the 2023 ESICM ARDS Treatment Guidelines, utilizing a PO2/FiO2 ratio ≤ 300 mmHg or pulse oximetry SpO2:FIO2 < 315. ARDS Patients were subsequently categorized into non-hypercapnia (arterial PaCO2 ≤ 60 mmHg) and hypercapnia (arterial PaCO2 > 60 mmHg) groups[10] based on results of arterial blood gas. Exclusion criteria included patients under 18 years of age, pregnant women, individuals with decompensated heart insufficiency or acute coronary syndrome, severe liver insufficiency (Child-Pugh score >7) or fulminant hepatic failure, patients with a decision to limit therapeutic interventions, or those with a life expectancy of less than 7 days. As part of the study, serial immunophenotyping of peripheral blood and bronchoalveolar lavage fluid (BALF) samples was conducted, along with the analysis of clinical data. Biological samples were collected 24 hours after enrollment, as well as on Day 7. Demographic clinical

treatment and outcome data were retrieved from the patient electronic medical records system, providing a comprehensive understanding of the patients' condition and response to treatment.

Prior to the collection of any human samples, written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The experimental procedures adhered to the principles outlined in the Belmont Report of the Department of Health and Human Services. The study protocol was thoroughly reviewed and approved by the ethics committee of Tianjin Medical University (ClinicalTrials.gov: ZYY-IRB2023-KY-178) from February 24th, 2023. All participants or their legally authorized surrogates provided written informed consent for the collection of samples and subsequent analyses.

Sample Processing and Cell Isolation

BALF was collected and preserved undiluted from all intubated patients during a bronchoscopy performed, paired blood samples were obtained and repeated at day 7. The bronchoscope was wedged in the segments of interest based on available chest imaging or intra-procedure observations. Aliquots of normal saline (20 mL each) were instilled through the bronchoscope within the selected bronchopulmonary segment. After each aliquot was instilled, saline was retrieved using a negative suction pressure. Approximately 20 mL of BALF was obtained and placed on ice. After passage of BALF through a 100-μm nylon cell strainer to remove clumps and debris, the supernatant was centrifuged for 15 min at 2000g at 4°C. Supernatant was stored at −80°C for cytokine analysis, and BALF cell pellets were collected in phosphate buffered saline solution (PBS) for flow cytometry.

Venous blood was drawn in EDTA and heparin tubes. EDTA blood was centrifuged at 2000g for 30 min at 4°C and supernatant was collected and stored at −80°C for cytokine measurement. Heparin blood was used for peripheral blood mononuclear cells (PBMCs) separation. PBMCs were isolated from whole blood by density gradient centrifugation (Ficoll Plus, Solario Science&Technology CO. Ltd, Beijing, China) according to the manufacturer's instructions. Briefly, 10 mL blood was overlaid over 10 mL of Ficoll-Paque Plus in a 50 mL conical tube and centrifuged at 400g for 25 minutes at 4°C. The PBMC layer was slowly isolated and washed twice with PBS at 450g for 10 minutes at 4°C. Cells were then counted and resuspended with PBS for further analysis.

Single-Cell Gene Expression Analysis

Single-cell capturing and subsequent library construction were executed using the SeekOne Digital Droplet Single Cell 5' library preparation kit (SeekGene DD Chip S3), strictly adhering to the manufacturer's protocol. This was done for age- and gender-matched selected cohort of four patients with pulmonary sepsis-induced ARDS, comprising one patient from the non-hypercapnia group and three from the hypercapnia group.

scRNA-Seq and Quality Control

scRNA-seq was performed using 10× Genomics Single Cell 3′ solution, version 2, according to the manufacturer's instructions, with 10,000 cells loaded for each sample (n = 1 non-hypercapnia and n = 3 hypercapnia ARDS). Libraries were sequenced on the HiSeq Platform (Illumina) from Novogene and raw BCL/fastq files were analyzed using Cell Ranger version 3.14.0 (10× Genomics). Low-quality cells were removed if they expressed <3000 genes/cell and had more than 20% mitochondrial and 10% hemoglobin gene expression. After rigorous quality control, an average of 9,479 cells with a mean read depth of 44,897 reads per cell remained in each sample. These cells were subsequently analyzed using the Seurat 4.0.5 bioinformatics package for downstream bioinformatics analyses.

Data Processing and Bioinformatic Analysis

Samples were analyzed using Seurat (version 3.0, https://satijalab.org/seurat/) for batch correction and cell-type identification. Alignment between samples was achieved by identifying gene-to-gene correlation patterns that are conserved across data sets through canonical correlation analysis (CCA). The cells were then embedded in a shared low-dimensional space and normalized using nonlinear "warping" algorithms. Principal component analysis (PCA) was subsequently conducted on the integration-transformed expression matrix to cluster all cell types. Canonical marker genes were then leveraged to assign major cell lineages to each cell cluster. Expressions of known marker genes for each

cluster were determined using the FindAllMarkers function, with the parameters set to "min.pct = 0.1, thresh.use = 0.25." Cluster visualization was achieved using the Uniform Manifold Approximation and Projection (UMAP) technique for dimension reduction. As a result, we successfully clustered PBMCs into 19 distinct subclusters, allowing for the identification of various immune cell types, including T cells, B cells, NK cells, megakaryocytes, monocytes, neutrophils, erythroblasts, and platelets. In the main text, we presented focused analyses on monocytes isolated from PBMCs. To further enhance our annotation precision, we re-clustered monocytes annotated as CD14+CD16-, CD14+CD16+, and CD14-CD16+ using the methods described previously.[16] We subsequently applied Differential gene expression (DEGs) analysis in each cell type with Wilcoxon rank number test for scRNA-seq data. Pathway enrichment analysis was further conducted with ClueGO based on GO biological process databases.

Functional Analysis of Subpopulations in Monocytes

Differential gene expression analysis was performed in monocyte clusters, and the log-transformed fold changes (log 2 FC) were used to perform hierarchical clustering between genes. Genes related to cytokine storm were identified and demonstrated different expression patterns between non-hypercapnia and hypercapnia ARDS patients. These molecular signatures were subsequently tested for enrichment of biological pathways with ClueGO. Top 8 DEGs in each subcluster Gene regulatory networks were analyzed by SCENIC within monocytes.

Flow Cytometry

The immunophenotyping procedure was performed less than 6 hours after sample collection. Approximately 1×10^6 cells per sample were surface stained for 30 min in the dark with the following fluorochrome-labeled antibodies: PE-conjugated anti-CD16, FITC-conjugated anti-CD14, Percp-conjugated anti-CD45, APC-conjugated anti-CD206 (eBioscience). After washing cells by adding 1 mL PBS and centrifuging at 400 g for 5 min at 4°C, the cells were resuspended in 400 μL PBS and examined by BD FACScantoTM II flow cytometer using the FACSDiva v. 6.1 software. The data were analyzed "offline" by FlowJo software (Version 10.2.1; Tree Star, Inc., Ashland, OR, USA).

Cytokine analysis

Cytokine measurements were assessed by multiplex analysis performed on fluorescently labeled magnetic microsphere beads using the LegendPlex Human Macrophage/Microglia Panel (13-plex) (740503, BioLegend) and the LegendPlex Human Inflammation Panel 1 (13-plex) (740809, BioLegend), according to the manufacturer's instructions. The concentrations of 17 biomarkers in whole samples, including inflammatory markers and cytokines/chemokines (interleukin (IL)-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-23, IL-17, interferon (IFN)-γ, IFN-α, tumor necrosis factor (TNF)-α, IP-10, and thymus and activation-regulated chemokine (TARC)), were quantified and expressed in terms of fluorescence intensities and concentrations (pg/mL). The calibration curve was created separately for each analyte. Briefly, pre-stored supernatant was taken. Then, 25 μL of sonicated beads, 25 μL of sample, and 25 μL of detection antibodies were mixed and placed on a shaker at 500 r.p.m. for 2 h at room temperature. Subsequently, 25 μL of SA-PE was added directly to each tube. The tubes were placed on a shaker at 500 r.p.m. for 30 min. The data were obtained by flow cytometry and were analyzed using LEGENDplex v.10.0 (VigeneTech).

Statistical Analysis

Measurement data were reported as mean ± standard deviation (SD), and categorical variables were expressed as numbers and percentages. We have explicitly addressed the issue of normality testing. Independent samples *t*-test was used to compare data between the two groups, and we adjusted post hoc P values with one-way ANOVA test to compare data among more than two groups. Comparisons between continuous variables on initial day and day 7 values were undertaken with a two-tailed Student's *t*-test. We considered a P-value lower than 0.05 as significant. Statistical analysis was performed using Prism 8 (GraphPad Software) and SPSS (version 22.0).

Results

Hypercapnia ARDS Patients Have Greater Disease Severity and Poorer Outcomes

Between December 2022 and April 2023, we enrolled 61 pneumonia patients receiving invasive ventilation for hypoxemia in our institution. Of these patients, fifty developed sepsis according to specific criteria, other 11 non-sepsis pneumonia patients as control group. Among fifty sepsis patients, 26 developed hypercapnia and 24 without hypercapnia. Airway and blood samples were collected from each patient. Baseline data for all patients are summarized in Table 1.

Baseline demographic characteristics were similar across the non-sepsis pneumonia control, pulmonary sepsis-induced ARDS with non-hypercapnia, and pulmonary sepsis-induced ARDS with hypercapnia groups. Comorbidities and most laboratory test values showed no significant differences between groups (Table 1). Only ferritin levels were higher in hypercapnia patients compared with non-sepsis pneumonia control and non-hypercapnia groups. Evaluation of blood gas and ventilator status revealed significant changes in pulmonary sepsis-induced ARDS with hypercapnia patients. All pulmonary sepsis-induced ARDS patients exhibited low PaO_2/FiO_2 ratio (Figure 1A). Pulmonary sepsis-induced ARDS with hypercapnia patients exhibited respiratory acidosis (pH < 7.30) derived from CO_2 accumulation (Figure 1B). Respiratory compliance had a statistically significant decreased, with elevated driving pressure (DP) (Figure 1B). These findings suggest severe hypoxemia and high respiratory drive occurs in hypercapnia patients during pulmonary sepsis-induced ARDS. Pulmonary sepsis-induced ARDS with hypercapnia patients also showed higher severity scores in the sequential oxygen

Table 1 Baseline Clinical Characteristics and Outcomes of Patients

	Non-Sepsis Pneumonia[a] (n=11)	Pulmonary Sepsis ARDS with Non-Hypercapnia[b] (n=24)	Pulmonary Sepsis ARDS with Hypercapnia[c] (n=26)	Significance, P-value
Demographics characteristics				
Age (years, mean ± SD)	69.2±12.9	73.7±14.2	75.3±9.9	
Male gender (n, %)	6/11 (55%)	15/24(63%)	21/26(81%)	
BMI, (kg/cm^2, mean ± SD)	22.4±3.67	23.73±4.36	23.96±3.62	
Smoker (n, %)	4/11(36%)	8/24(33%)	12/26(46%)	
Comorbid conditions				
Coronary heart disease (n, %)	5/11(45%)	8/24(33%)	10/26(38%)	
Diabetes (n, %)	4/11(36%)	8/24(33%)	10/26(38%)	
Immune dysregulation after Chemotherapy or Autoimmune disease (n, %)	4/11(36%)	7/24(29%)	8/26(31%)	
Chronic lung disease (n, %)	3/11(27%)	3/24(13%)	2/26(8%)	
Hypertension (n, %)	5/11(45%)	11/24(46%)	12/26(46%)	
Neurologic disease (n, %)	3/11(27%)	8/24(33%)	8/26(31%)	
Laboratory test values				
Total white blood cell count (/mm^3,mean ± SD)	15.09±4.82	12.16±5.94	14.59±7.60	$P_{a,b}$=0.049
Arterial blood lactates (mean ± SD)	3.6±1.3	2.9±1.3	4.8±3.5	$P_{b,c}$=0.014
Absolute platelet count (x10^3 /mm^3,mean ± SD)	151±77.3	192±94.7	132±73.8	
INR (mean ± SD)	1.39±0.32	1.39±0.34	1.45±0.49	
APTT (secs, mean ± SD)	35±6.4	32±6.1	32±5.2	
Creatinine (mmol/l, mean ± SD)	90.5±27.6	118.9±125.4	176.4±141.8	
AST (U/l, mean ± SD)	137.8.±183.1	82.6±60.3	124.8±181.9	
ALT (U/l, mean ± SD)	49.6±29.7	116.4±169.0	140.5±281.4	
Ferrin (ng/mL, mean ± SD)	1383.5±762.9	455.6±290.2	1140.9±505.4	$P_{a,c}$=0.015 $P_{b,c}$=0.004

(*Continued*)

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Table 1 (Continued).

	Non-Sepsis Pneumonia[a] (n=11)	Pulmonary Sepsis ARDS with Non-Hypercapnia[b] (n=24)	Pulmonary Sepsis ARDS with Hypercapnia[c] (n=26)	Significance, P-value
ARDS severity and Ventilation parameters				
PaO_2/FiO_2 ratio, mmHg	93.8±20.6	68.9±29.9	57.5±13.3	$P_{a,b}$ =0.003 $P_{a,c}$<0.001
$PaCO_2$, mmHg	45±8.3	39±4.6	85±26.6	$P_{a,c}$<0.001 $P_{b,c}$<0.001
PH	7.43±0.05	7.39±0.09	7.25±0.14	$P_{a,c}$<0.001 $P_{b,c}$<0.001
Driving pressure, cmH_2O	13±2.7	11±2.1	14±3.8	$P_{b,c}$<0.001
Crs, mL/cmH_2O	56.5±13.8	43.8±9.1	32.4±7.2	$P_{a,b}$<0.001 $P_{a,c}$<0.001 $P_{b,c}$<0.001
Quadrant of pulmonary lesions	3±1	3±1	4±1	
Patient severity upon ICU admission				
APACHE II score (mean ± SD)	15.8±6.4	14.5±5.8	17,6±5.9	
SOFA score (mean ± SD)	7.5±2.9	5.0±1.8	6.7±1.9	$P_{a,b}$ =0.003 $P_{b,c}$ =0.006
PSI (mean ± SD)	118±31.6	121±30.5	143±29.4	$P_{a,c}$ =0.021 $P_{b,c}$ =0.008
CCI (mean ± SD)	7.3±1.0	9.0±1.7	10.1±1.9	$P_{a,b}$ =0.007 $P_{a,c}$<0.001 $P_{b,c}$ =0.029
Adjunctive therapy				
Neuromuscular blockers (n, %)	0/11 (0%)	2/24 (8%)	10/26 (39%)	$P_{a,c}$ =0.016 $P_{b,c}$ =0.039
Prone position (n, %)	2/11 (18%)	24/24 (100%)	26/26 (100%)	$P_{a,b}$<0.001 $P_{a,c}$<0.001
Treatment with methylprednisolone (n, %)	6/11 (55%)	2/24 (8%)	1/26 (4%)	$P_{a,b}$<0.001 $P_{a,c}$<0.001
Treatment with dexamethasone (n, %)	0/11 (0%)	15/24 (63%)	25/26 (96%)	$P_{a,b}$<0.001 $P_{a,c}$ =0.011
Renal replacement therapy (n, %)	2/11 (18%)	4/24 (17%)	4/26 (15%)	
ECMO implantation (n, %)	0/11 (0%)	0/24 (0%)	2/26 (8%)	
Outcomes				
MV duration, days	10.9±8.5	17.3±26.5	12.9±8.4	
ICU length of stay, days	15.5±7.7	16.0±11.0	12.4±8.8	
Hospital length of stay, days	15.3±7.9	22.1±11.8	17.0±9.1	
Mortality at day 28 (n, %)	1/11(9%)	5/24(21%)	21/26(81%)	$P_{a,c}$<0.001 $P_{b,c}$<0.001

Notes: Categorical variables are shown as n (%). P values come from *t*-test or one-way ANOVA test, as appropriate. Comparisons with the respective groups by the Student's *t* test. P values are statistically significant ($p < 0.05$).

Abbreviations: BMI, body mass index; INR, international-normalized ratio; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PaCO2, the partial pressure of carbon dioxide in arterial blood; PaO2/FiO2, the ratio of arterial-to-inspiratory oxygen fraction; Crs, compliance of the respiratory system; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; PSI, pneumonia severity index; CCI, Charlson's comorbidity index; ECMO, extracorporeal membrane oxygenation; MV, mechanical ventilation; SD, standard deviation.

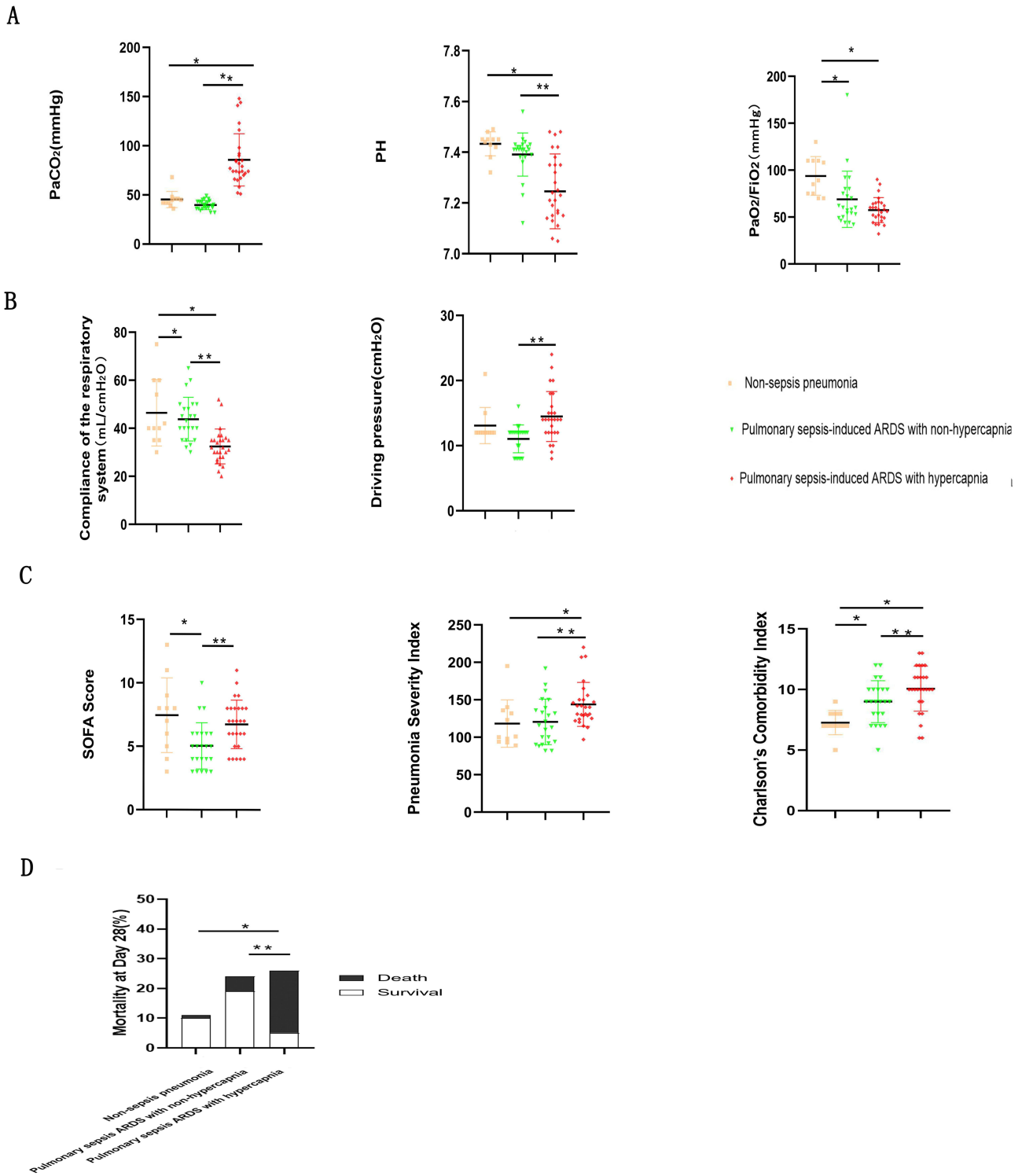

Figure 1 Alterations in $PaCO_2$ correlate with disease severity, ventilator status and higher mortality. Within the initial 24 hours of mechanical ventilation, demographic and clinical features were documented for patients with non-sepsis pneumonia (controls, n=11) and pulmonary sepsis-induced ARDS with hypercapnia (n=26) and pulmonary sepsis-induced ARDS with non-hypercapnia (controls, n=24). (**A** and **D**) Hypercapnic patients exhibited significantly increased severity scores and higher 28-day mortality rates compared to the non-hypercapnic controls. (**B**) Respiratory variables in blood gas were correlated with PaCO2 changes despite similar baseline PaO2:FIO2 ratios. (**C**) On intubation day, pulmonary sepsis-induced ARDS with hypercapnia patients showed decreased respiratory compliance with elevated driving pressure (DP) compared to the non-hypercapnic controls. Mean values with standard errors are represented. A p-value of <0.05 was considered statistically significant. Statistical comparisons are indicated by arrows: *P < 0.05 comparing non-sepsis pneumonia vs pulmonary sepsis-induced ARDS groups, **P < 0.05 comparing pulmonary sepsis-induced ARDS without hypercapnic vs with hypercapnic groups.

Abbreviations: PaCO2, the partial pressure of carbon dioxide in arterial blood; PaO2/FiO2 the ratio of arterial-to-inspiratory oxygen fraction; SOFA, Sequential Organ Failure Assessment; PSI, Pneumonia Severity Index; CCI, Charlson's Comorbidity Index.

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failure assessment (SOFA), pneumonia severity index (PSI), and Charlson comorbidity index (CCI) (Figure 1C) on ICU admission compared with non-sepsis pneumonia control and pulmonary sepsis-induced ARDS without hypercapnia groups. Pulmonary sepsis-induced ARDS patients received more neuromuscular blockers, prone positioning, and steroids. Notably, only 8% of pulmonary sepsis-induced ARDS with hypercapnia patients received venous-venous extracorporeal membrane oxygenation (VV-ECMO). Pulmonary sepsis-induced ARDS with hypercapnia patients also exhibited higher in-hospital mortality (81%, 21/26) (Figure 1D) than non-sepsis pneumonia (9%, 1/11) and pulmonary sepsis-induced ARDS without hypercapnia controls (21%, 5/24), despite similar ICU stay length and ventilation needs. These findings highlight the distinct phenotype and poor outcomes of hypercapnia in pulmonary sepsis-induced ARDS patients.

The Frequencies of Peripheral Monocyte Phenotypes Exhibit Distinct Change in Pulmonary Sepsis-Induced ARDS with Hypercapnia Patients from Day 1 to Day 7

ScRNA-seq (scRNA-seq) was performed as a descriptive study to provide deeper insights of the immune response during pulmonary sepsis-induced ARDS. ScRNA-seq analysis was performed on PBMCs from three hypercapnia patients and one age- and gender-matched non-hypercapnia case. The four patients profiled were male, aged 50 to 70 years. Clustering analysis identified 19 distinct cell clusters (Figure S). Hypercapnia patients exhibited lower neutrophil counts but higher monocyte and T cell counts (Figure 2A). Monocytes were further identified 2 clusters of CD14 + monocytes and 1 cluster

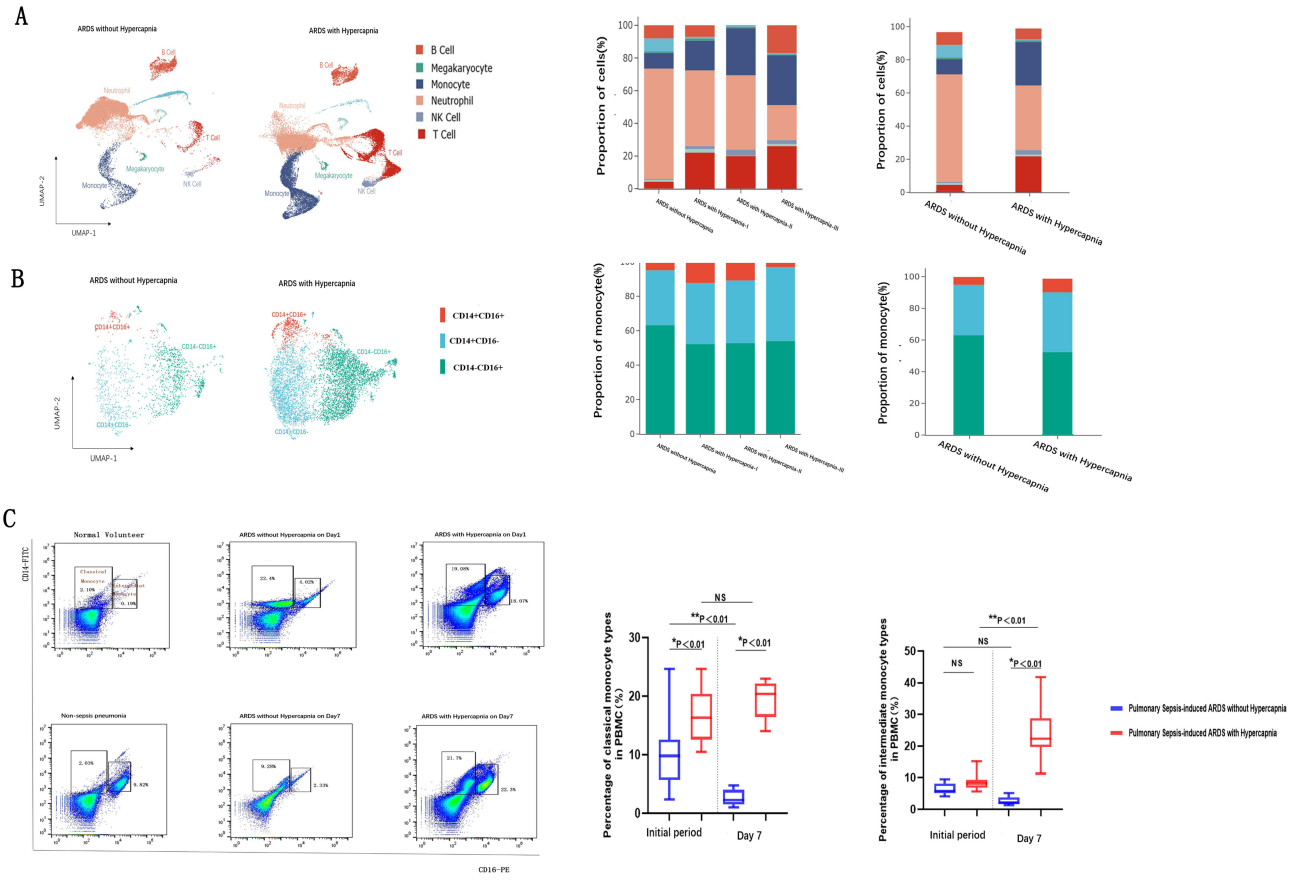

Figure 2 ScRNA-seq analysis of PBMCs in Pulmonary Sepsis-Induced ARDS reveals altered cell type composition and monocyte subset accumulation in hypercapnia group, confirmed by flow cytometry. The ScRNA-seq analysis of PBMCs from pulmonary sepsis-induced ARDS patients (one from non-hypercapnia group, three from hypercapnia group) revealed distinct cell type changes in hypercapnia cases. Subsequently, CD14 and CD16 were utilized to identify percentages of CM and IM via flow cytometry in non-hypercapnia (n = 24) and hypercapnia (n = 26) patients. (**A**) Uniform Manifold Approximation and Projection (UMAP) analysis identified six major cell clusters, with altered proportions of neutrophils, T cells and monocytes in hypercapnia patients. (**B**) Monocyte phenotypes were annotated based on CD14 and CD16 expression. UMAP analysis identified altered proportions of intermediate monocytes (CD14+CD16+,IM) and classical monocytes (CD14+CD16−,CM) subsets in hypercapnia patients. (**C**) Flow cytometry analysis confirmed accumulation of CM and IM monocyte subsets in peripheral blood of hypercapnia patients, from initial day to day 7. The values presented represent the means ±SEM. Differences between groups were statistically analyzed using one-way ANOVA. Significant differences were indicated as: *P < 0.05 when comparing pulmonary sepsis-induced ARDS without hypercapnic vs with hypercapnic groups at same time point, and **P < 0.05 when comparing initial day with day 7 within same group.

of CD16 + monocytes in our patient cohort. Classical monocyte (CD14+CD16−, CM) and intermediate monocyte (CD14 +CD16+, IM) subclusters were categorized, the frequencies of both monocyte clusters were significantly increased in hypercapnia ARDS patients (Figure 2B). These findings suggest that sepsis-induced ARDS with hypercapnia is characterized by monocyte heterogeneity, with potential functional implications.

To further analyze the changes in immune cell composition, monocytes of pulmonary sepsis-induced ARDS with hypercapnia patients were compared with monocytes of pulmonary sepsis-induced ARDS with non-hypercapnia and non-sepsis pneumonia controls (Table S1) by using computational analysis of flow cytometry data. In pulmonary sepsis-induced ARDS with hypercapnia patients, the percentages of CMs ($P<0.001$) and IMs ($P<0.001$) increased significantly in Day 7 compared with pulmonary sepsis-induced ARDS without hypercapnia (Figure 2C). During the disease progression from the initial day to one week later, a decreased percentage of CMs ($P<0.001$) was observed in pulmonary sepsis-induced ARDS without hypercapnia patients, but an increased percentage of IMs ($P<0.001$) in hypercapnia patients (Figure 2C). Our findings suggest a distinct pattern of peripheral blood monocytes kinetics in these patients, which may play important roles in respiratory failure and link to hypercapnia, especially in late stage of pulmonary sepsis-induced ARDS.

Single-Cell RNA Sequencing (scRNA-Seq) Analysis to Identify the Activation of Inflammatory Cytokines and Chemokines in Hypercapnia Patients

We identified 610 immune response–related genes differentially expressed between pulmonary sepsis-induced ARDS with non-hypercapnia and pulmonary sepsis-induced ARDS with hypercapnia subjects in all cells, of which 364 were upregulated and 246 downregulated. Among them, several genes related to inflammatory cytokines and chemokines increased. The expression of genes related to IL-1β pathway stimulus, such as PDE4D, PTGFR and IL1R2 (Figure 3A), were found to be differentially expressed between non-hypercapnia and hypercapnia groups. Other intriguing genes involving monocyte infiltration and recruitment, such as IL-12RB2, IL-12RB1, IL-23A, IL-23R, and CXCL8 (Figure 3A), were increased in hypercapnia blood samples.

Pathways enriched in the top differentially expressed genes (DEGs) of monocyte subpopulation were examined in hypercapnia patients relative to those in patient without hypercapnia. We found enrichment of cytokine storm regulation in Gene Ontology (GO) terms (Table S2). Upregulated terms included those related to cytokine regulation, cytokine stimulus and cytokine production (Figure 3B). Monocyte recruitment was also modulated, with upregulated in chemotaxis and IL-12 production (Figure 3B). These results suggest that promotion of cytokine storm and monocyte recruitment may occur in hypercapnia patients after pulmonary sepsis-induced ARDS.

Levels of Inflammatory Mediators, Especially IL-12p40 and IL-23, are Elevated in the Peripheral Blood of Hypercapnia Patients from Day 1 to Day 7

To investigate the association between levels of inflammatory marker transcripts and secreted proteins, we measured protein levels of cytokines and chemokines in plasma samples from hypercapnia and control patients (Table 2). Within 24 hour of intubation, pulmonary sepsis-induced ARDS patients showed elevated cytokine levels compared with non-sepsis pneumonia controls. Hypercapnia patients had higher levels of inflammatory cytokines (IL-1β, IL-1RA, and IL-5) and chemokines (IL-12p40, IL-23, interferon-inducible protein (IP)-10, thymus and activation-regulated chemokine [TARC]) than pulmonary sepsis-induced ARDS without hypercapnia patients (Figure 3C).

Levels of inflammatory mediators changed in matched pulmonary sepsis-induced ARDS without hypercapnia and hypercapnia patients at day 1 and day 7 are presented in Table S3 and Figure 3D. Similar with initial period, inflammatory cytokines and chemokines in hypercapnia patients became higher than pulmonary sepsis-induced ARDS without hypercapnia group at day 7. In hypercapnia patients, IL-12p40 and IL-23 increased from day 1 to day 7. On contrary, IL-12p40 and IL-23 decreased from day 1 to day 7 in pulmonary sepsis-induced ARDS without hypercapnia patients. Other cytokines were not significantly different between initial day and day 7 in pulmonary sepsis-induced ARDS patients with or without hypercapnia. These findings suggest that IL-12p40 and IL-23 play a crucial role in the development of hypercapnia in pulmonary sepsis-induced ARDS.

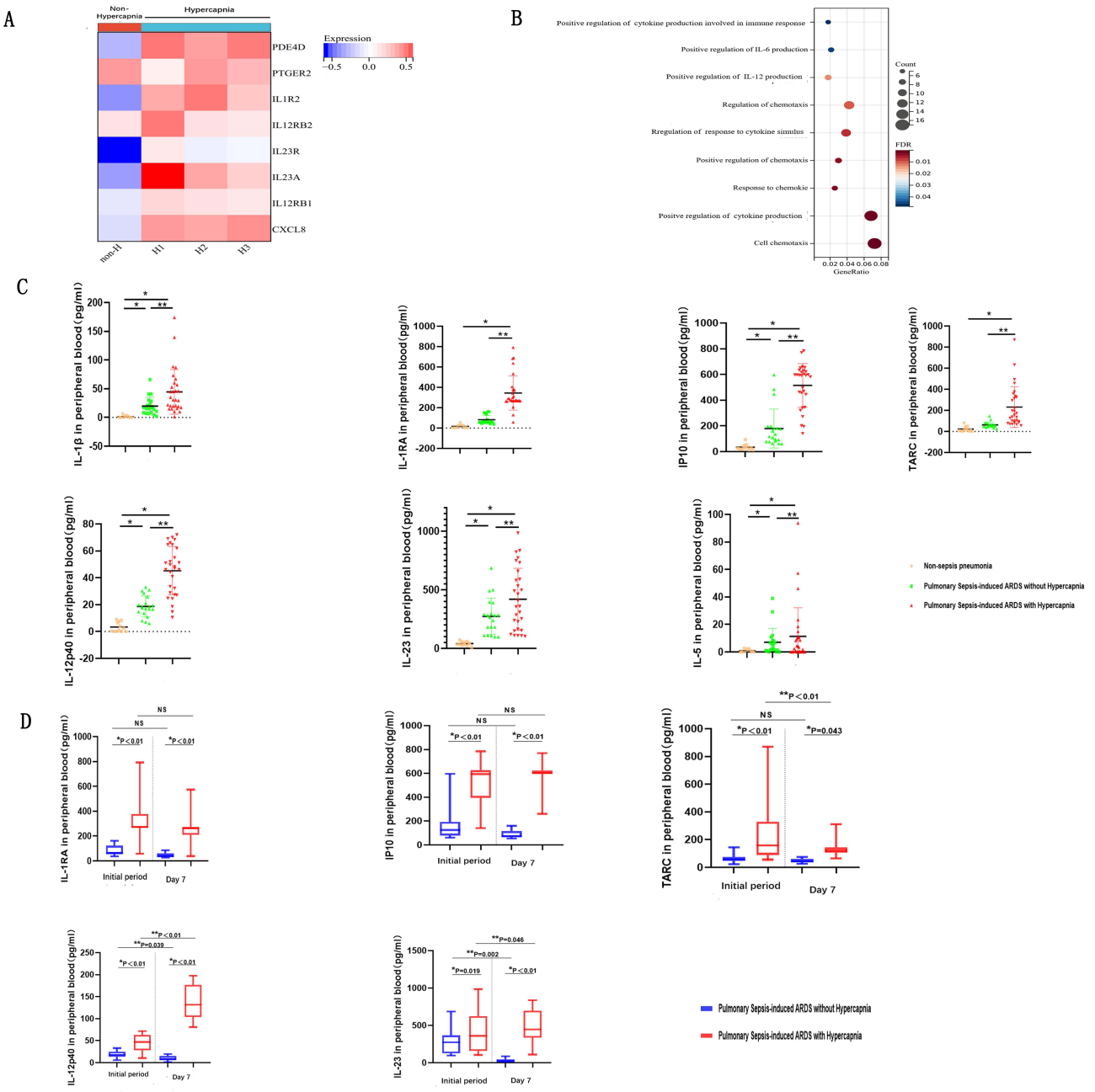

Figure 3 Gene set analysis of hypercapnia monocytes reveals cytokine storm signatures, convinced by increased cytokines and persistent IL-12p40 and IL-23 elevation during disease period. (**A**) Genes associated with cytokine storm upregulated in peripheral blood of hypercapnia patients. Heatmap showing expression levels and weights assigned to each of the 8 genes related to cytokines and chemokines that distinguish between pulmonary sepsis-induced ARDS patients with hypercapnia (n = 3) and those without hypercapnia (n = 1). (**B**) Functional enrichment analysis identified distinct gene signatures related to pathways of inflammatory cytokines and chemokines activation in hypercapnia patients. (**C**) Peripheral blood supernatants revealed elevated cytokines, including serum IL-1β, IL-RA, IP10, TARC, IL-12p40, IL-23, and IL-5, in hypercapnia patients within 48 hours after incubation. (**D**) IL-12p40 and IL-23 levels were significantly higher in hypercapnia than non-hypercapnia controls both at early and late stages of ARDS, and persistent IL-12p40 and IL-23 elevation during study period. (**A** and **B**) Each bubble plot graphically represents genes differentially expressed in hypercapnia samples compared to non-hypercapnia controls, with colors indicating the contributions of individual hypercapnia samples. All P values comparing non-hypercapnia vs hypercapnia for density plots were significantly low, all below 10E-50. (**C** and **D**) Mean values with standard errors are represented. A p-value of <0.05 was considered statistically significant. (**C**) Significant differences were indicated as: *P < 0.05 comparing non-sepsis pneumonia vs pulmonary sepsis-induced ARDS groups, **P < 0.05 comparing pulmonary sepsis-induced ARDS without hypercapnic vs with hypercapnic groups. (**D**) Significant differences were indicated as: *P < 0.05 when comparing pulmonary sepsis-induced ARDS without hypercapnic vs with hypercapnic groups at same point, and **P < 0.05 when comparing initial day with day 7 within same group.

Table 2 Cytokines of PBMC

	Non-sepsis pneumonia[a] (n=11)	Pulmonary sepsis ARDS with non-hypercapnia[b] (n=24)	Pulmonary sepsis ARDS with hypercapnia[c] (n=26)	Significance, P-value
IL-12P70 (pg/mL)	2.03±2.92	11.7±21.3	25.8±39.9	$P_{a,c}$ =0.026
TNF-α (pg/mL)	13.47±39.34	7.43±9.49	15.77±31.94	
IL-6 (pg/mL)	71.43±94.91	256.74±285.52	625.29±1100.25	$P_{a,c}$ =0.044
IL-10 (pg/mL)	8.15±21.5	8.56±11.18	22.9±39.99	
IL-1β (pg/mL)	1.43±2.19	19.88±15.35	44.56±38.94	$P_{a,c}$<0.001 $P_{b,c}$ =0.004
TARC (pg/mL)	1.48±2.19	62.54±27.79	231.17±193.12	$P_{a,c}$<0.001 $P_{b,c}$<0.001
IL-1RA (pg/mL)	16.95±17.51	82.11±42.44	344.90±167.93	$P_{a,c}$<0.001 $P_{b,c}$<0.001
IL-12p40 (pg/mL)	3.34±3.64	18.67±7.86	45.21±21.61	$P_{a,b}$ =0.003 $P_{a,c}$<0.001 $P_{b,c}$<0.001
IL-23 (pg/mL)	41.12±17.25	273.49±156.37	419.79±263.58	$P_{a,b}$ =0.003 $P_{a,c}$<0.001 $P_{2,3}$=0.017
IP10 (pg/mL)	34.77±22.39	179.41±151.52	515.06±169.67	$P_{a,b}$ =0.009 $P_{a,c}$<0.001 $P_{b,c}$<0.001
IL-2 (pg/mL)	18.35±13.12	7.90±9.84	11.03±12.38	$P_{a,b}$ =0.018
IL-4 (pg/mL)	3.32±5.42	7.99±8.71	7,78±11.79	
IL-8 (pg/mL)	1.45±3.36	23.19±23.28	30.45±43.42	$P_{a,c}$ =0.013
IL-5 (pg/mL)	1.45±3.36	7.09±10.07	27.4±42.99	$P_{a,b}$ =0.014 $P_{a,c}$ =0.016 $P_{b,c}$ =0.025
IL-17 (pg/mL)	0.57±0.95	4.34±5.99	3.94±5.28	$P_{a,b}$ =0.045
IFN-γ (pg/mL)	2.13±1.75	5.43±6.10	7.58±11.64	
IFN-α (pg/mL)	5.26±8.72	25.40±18.75	26.80±28.42	$P_{a,b}$ =0.017 $P_{a,c}$ =0.008

BALF Exhibit Similar Cellular Composition of Myeloid Cells in Hypercapnia Patients

After determining that the monocyte-specific transcriptional profiles and expressions of cytokines between hypercapnia and non-hypercapnia were different in early and late pulmonary sepsis-induced ARDS, we sought to characterize whether the same biological programs were activated in lung tissue and associated these programs with hypercapnia.

Monocytes present in increased numbers in the blood of ARDS patients may infiltrate the lungs and differentiate into macrophages.[13] CD45+ immune cells were analyzed after exclusion of doublets and debris from bronchoalveolar lavage fluid (BALF). The cell composition differed between the various pneumonia groups (Table S4). In pulmonary sepsis-induced ARDS patients, the frequencies of CMs and IMs in the airways were increased in the initial phase compared with the airways of non-sepsis pneumonia patients. The frequency of CMs was higher in hypercapnia patients than non-hypercapnia patients in both the initial and late phases of pulmonary sepsis-induced ARDS (P<0.01 for both). Notably, a decreased percentage of CMs (P<0.001) was observed in non-hypercapnia patients, but an increased percentage of IMs (P<0.001) in hypercapnia patients (Figure 4A) from initial day to day 7, just as same as the changes in peripheral blood.

Distinct monocyte/macrophage populations in the lung can be defined as alveolar macrophages (Ams), interstitial macrophages (IMs) and monocyte-derived cells according to their cell surface phenotype. Within the lung, a population of immature and infiltrating CD14+ pulmonary monocyte-derived cells, thought to be recruited into the extravascular space. These can be distinguished from the intravascular CD14+ monocytes by their expression of CD206. In ARDS,

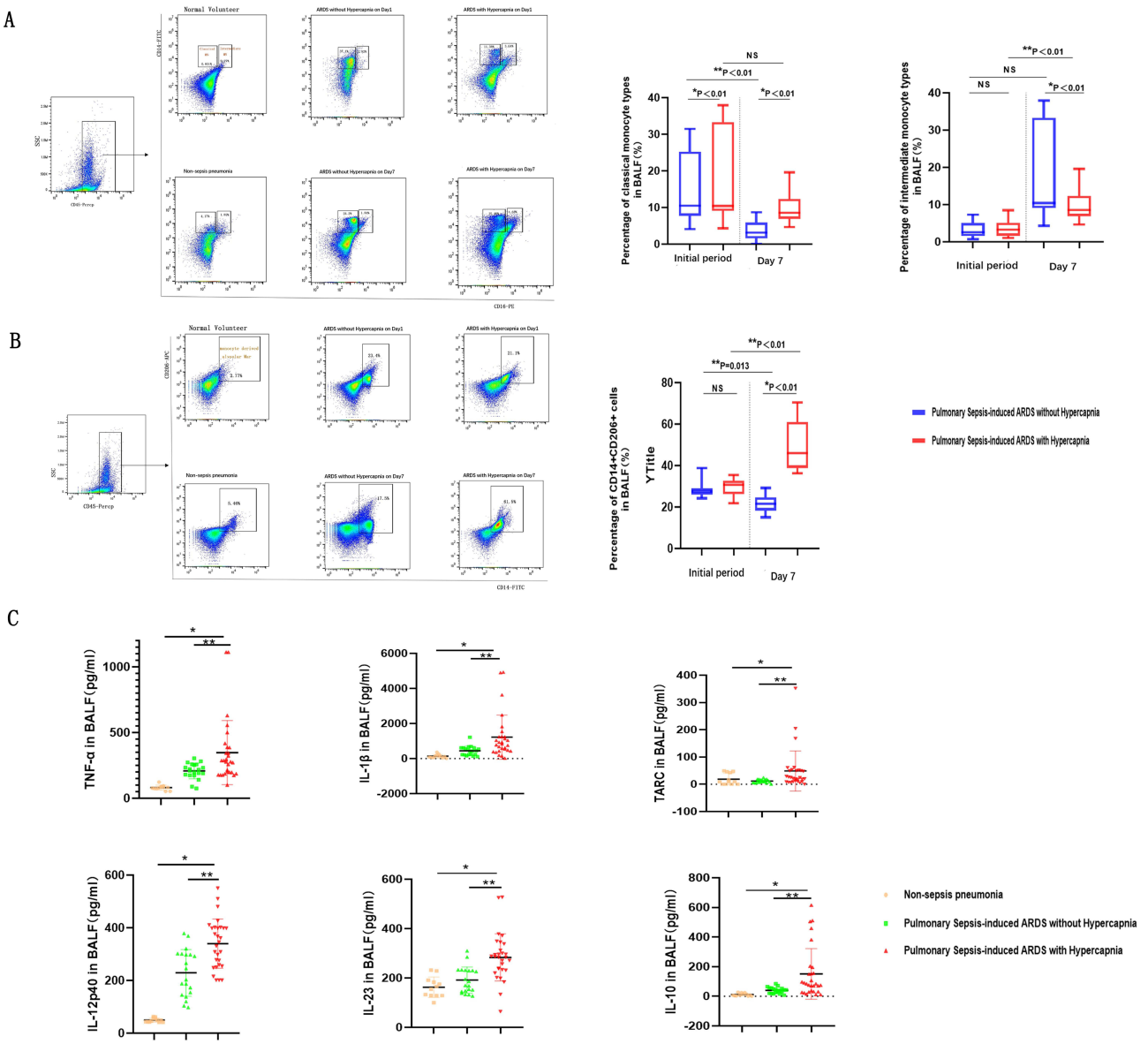

Figure 4 Similar kinetics of cellular composition and activated cytokines storm in BALF from matched hypercapnia patients. Cells and cytokines from bronchoalveolar lavage fluid (BALF) of non-sepsis pneumonia (n=11), pulmonary sepsis-induced ARDS with non-hypercapnia (n=24), and pulmonary sepsis-induced ARDS with hypercapnia (n=26) patients were isolated and analyzed. (**A**) In the airway, CM and IM monocyte subsets showed similar kinetics to those in circulation, with increased percentages in hypercapnia than non-hypercapnia from initial day to day 7. (**B**) Kinetic cytometry revealed elevation of CD14+CD206+ monocyte-derived alveolar macrophages at day 7 in hypercapnia patients. (**C**) BALF supernatants showed similar cytokine elevations in hypercapnia patients, including TNF-α and IL-10. All values are presented as means ± SEMs. Statistical differences between groups were evaluated using one-way ANOVA. (**A** and **B**) Significant differences were indicated as: *P < 0.05 when comparing non-hypercapnia with hypercapnia groups at same point, and **P < 0.05 when comparing initial day with day 7 within same group. (**C**) *P < 0.05 comparing non-sepsis pneumonia vs pulmonary sepsis-induced ARDS groups, **P < 0.05 comparing pulmonary sepsis-induced ARDS without hypercapnic vs with hypercapnic groups.

monocytes migrate to the lungs[17] and transform into monocyte-derived macrophages (MDMs).[18] We analyzed MDMs based on the expression of distinct markers of lung macrophages (CD206) and monocytes (CD14) (Table S4 and Figure 4B).[19] This analysis revealed significantly increased percentage of MDMs in hypercapnia patients (P<0.001) at day 7. Additionally, the proportion of MDMs was significantly lower in non-hypercapnia patients, but higher in hypercapnia patients from day 1 to day 7, just the same as the changes in BALF monocyte phenotype. Infiltration of BALF by activated monocyte and monocyte-derived macrophages were correlated with CO_2 accumulation, indicating these infiltrating myeloid cells play a role in the pathogenesis of hypercapnia in pulmonary sepsis-induced ARDS.

Table 3 Cytokines of BALF

	Non-sepsis pneumonia[a] (n=11)	Pulmonary sepsis ARDS with non-hypercapnia[b] (n=24)	Pulmonary sepsis ARDS with hypercapnia[c] (n=26)	Significance, P-value
IL-12P70 (pg/mL)	21.47±4.83	21.23±8.17	23.54±7.10	
TNF-α (pg/mL)	81.42±18.46	208.03±59.63	347.41±244.34	$P_{a,c}$<0.001 $P_{b,c}$ =0.008
IL-6 (pg/mL)	218.85±108.39	566.80±243.74	603.65±443.36	$P_{a,b}$ =0.007 $P_{a,c}$ =0.002
IL-10 (pg/mL)	11.89±9.88	40.38±20.29	151.13±170.80	$P_{a,c}$ =0.001 $P_{b,c}$ =0.002
IL-1β (pg/mL)	135.58±103.73	444.48±265.25	1221.23±1258.99	$P_{a,c}$ =0.001 $P_{b,c}$ =0.0024
TARC (pg/mL)	11.74±16.15	16.14±12.47	49.09±73.69	$P_{a,c}$ =0.041 $P_{b,c}$ =0.034
IL-1RA (pg/mL)	62.79±61.21	94.85±66.39	116.51±118.32	
IL-12P40 (pg/mL)	49.59±7.79	229.35±88.55	339.88±93.38	$P_{a,b}$<0.001 $P_{a,c}$<0.001 $P_{b,c}$<0.001
IL-23 (pg/mL)	162.63±41.39	191.61±53.29	283.48±95.58	$P_{a,c}$<0.001 $P_{b,c}$<0.001
IP10 (pg/mL)	80.08±83.67	225.71±213.18	314.56±208.52	$P_{a,b}$ =0.043 $P_{a,c}$ =0.001

BALF Exhibit Activated Cytokines Storm in Hypercapnia Patients

We also measured levels of cytokines and chemokines in BALF from pulmonary sepsis-induced ARDS patients. These analyses revealed elevated levels of inflammatory cytokines compared with non-sepsis pneumonia controls (Table 3). Hypercapnia patients had increased levels of IL-1β, TARC, IL-10, and tumor necrosis factor (TNF)-α, which correlated well with transcript expression (Figure 4C). Levels of IL-12p40 and IL-23 in hypercapnia patients were elevated in airways (Figure 4C), in agreement with blood levels of IL-12RB2, IL-23A, and IL-23R transcripts. These findings indicate a severely dysregulated inflammatory milieu in the airways of hypercapnia patients, which leads to a chemokine gradient that facilitates the recruitment of immune cells from the circulation to the site of infection or the activation of myeloid cells.

Discussion

Prior research has established a link between acute hypercapnia and adverse outcomes in ARDS patients.[4] Our present findings strongly suggest that acute hypercapnia, particularly following pulmonary sepsis–induced ARDS, is a risk factor for increased mortality. Furthermore, pulmonary sepsis-induced ARDS patients with hypercapnia exhibited significantly elevated scores on the CCI,[20] PSI, and SOFA, all of which are predictive of comorbidity burden and mortality risk.[21] Additional clinical manifestations among these patients included a significant increase in DP and notable decline in static respiratory system compliance (Crs). A pronounced increase in DP is recognized as a prognostic factor for indicative of high mortality rates.[22] The overall change in Crs following ARDS is affected by the alternation of lung compliance.[23] Compared with non-sepsis pneumonia and non-hypercapnia controls, hypercapnia patients had more severe outcomes and higher mortality rates. Prompt intervention is crucial for minimizing DP and preserving optimal Crs values to prevent acute hypercapnia after pulmonary sepsis-induced ARDS.

The present data further support the dysregulation of inflammatory responses in sepsis and ARDS. While blood has been the most common biological sample used to search immune cells and candidate biomarkers, bronchoalveolar lavage fluid (BALF) is the closest sample to the site of injury and more accurately reflects the local lung environment.[24] The strongest correlation between PBMCs and BALFs was the activation status of peripheral monocyte and monocyte or monocyte-derived macrophages in BALF. In the LPS-induced lung injury model, different studies may have obtained

opposite results on the role of monocytes and monocyte-derived macrophages, with some studies showing that they are important factors in causing injury and others finding that they promote recovery from inflammation, possibly because of the selection of time and choice of experimental method or mouse type. This report provides the first assessment of paired BALF and PBMC cellular and molecular pathogenesis of hypercapnia in patients with pulmonary sepsis- induced ARDS. In hypercapnia patients, monocyte in both the airways and blood exhibited profound phenotypic changes over a seven-day period. We observed notable increasing in the numbers of two monocyte subsets: CD14+CD16+ IMs and CD14+CD16− CMs. It is noteworthy that IM subsets are difficult to detect in healthy individuals.[25] The present study is thus the first to demonstrate a higher percentage of IMs in the peripheral blood and BALF of hypercapnia patients, particularly at day 7 of pulmonary sepsis- induced ARDS. Peripheral blood classical monocytes play a crucial role in LPS-induced lung injury in experimental models.[26] Consistent with prior studies, our results indicate that CMs are the predominant monocyte population in the airways and peripheral blood of hypercapnia patients throughout the early to late stages of pulmonary sepsis- induced ARDS. Importantly, an increasing number of CMs over seven-day period was observed in hypercapnia patients, whereas it significantly decreased during the first week in pulmonary sepsis- induced ARDS with non-hypercapnia patients. The increased number of CM in hypercapnia patients may lead to cytokine storms, which can cause tissue damage, affect the adaptive immune response and lead to lung fibrosis. Accumulating evidence suggests that macrophages play a pivotal role in the pathogenesis of ARDS.[27] In addition, our current findings also indicate that macrophage derived from monocyte increase in number with the development of hypercapnia in the airways. The present observations suggest that monocyte may act as the primary source of migration to the lungs after pneumonia sepsis. Thus, alternations of monocytes represent a distinct phenotypic profile in the circulation and act as pivotal mediators of alveolar homeostasis for developing hypercapnia during pulmonary sepsis- induced ARDS.

As no identified biomarker obtained from blood samples has been shown to be accurately associated with ARDS pathogenesis and monitor response to treatment,[28] this lack of association may be due to an alveolar compartmentalization of biomarkers during pulmonary sepsis- induced ARDS. Thus, our results coordinated the interplay of ARDS biomarkers between the alveolar and blood compartments based on whole-blood transcriptional measurements in pulmonary sepsis-induced ARDS with hypercapnia patients. Top upregulated genes of blood monocytes in hypercapnia patients were mostly related with cytokine activation and myeloid cell recruitment.[29,30] Our data reveal elevated gene expressions of IL-1RA, PTGF2, and IL-1R2, which are associated with the IL-1β signaling pathway.[31] IL-1β and IL-1RA levels were increased in both the circulation and BALF, which correlated positively with hypercapnia outcome. Moreover, while previous investigations have reported on a so-called systemic cytokine storm in lung injury, our study shows that the BALF levels of many cytokines are higher than their plasma levels, indicating that a local rather than systemic cytokine storm during early stage of pulmonary sepsis- induced ARDS. Then, we found significant elevation of IL-12p40 and IL-23 levels in both the circulation and BALF, along with upregulation of recruitment-related chemokines genes (IL-12RB1, IL-12RB2, IL-23A, IL-23R). Prior research suggested that IL-12p40 and IL-23 play a crucial role in regulating the pathogenic activity of myeloid cells.[32,33] Preclinical data over the last 20 years combined with successful clinical trials has identified a clear relationship between IL-12, IL-23 and capable of orchestrating tissue inflammation in lung tissue. IL-12/23 signaling pathways in both mice and humans have been identified to initiate tissue damage induced by monocyte.[34] In COVID-19 patients, IL-12 and IL-23 have been involved in potentially linking the severely decreased capacity of carbon dioxide removal with the inflammatory signature of monocytes. Our current study first observed that the concentrations of IL-12p40 and IL-23 were significantly increased at day 7 in pulmonary sepsis-induced ARDS with hypercapnia patients, but decreased during this seven-treatment period in pulmonary sepsis-induced ARDS without hypercapnia patients. These matched alternations between monocyte percentage and chemokines indicated that IL-12p40 and IL-23 were involved in inflammatory cytokine storm triggered by monocytes migrating to the lungs.[35] The significantly higher level of 12p40 and IL-23 at day 7 may have directly impacted monocyte/macrophage accumulation in the lungs. The increasement of 12p40 and IL-23 seems to be activated pulmonary macrophages for restoring the fibrotic response following the hypercapnia.[36] Our analyses indicated the presence of inflammatory monocytes in pulmonary sepsis-induced ARDS patients and increased inflammatory chemokine levels in the airways, which was correlated with airway monocyte infiltration and hypercapnia development in pulmonary sepsis-induced ARDS.

Limitations of Study

Our study has some strengths, including a prospective design allowing for uniform timing of measurements at a clinically relevant time-point and the combination of clinical, scRNA-seq analysis, flow cytometry and multiplex cytokines data. Although these data identify potentially novel mechanisms that underlie hypercapnia pathogenesis after pulmonary sepsis-induced ARDS, our study still has some limitations. First, it is a monocentric study with a small sample size. Therefore, we could not examine the correlation between the patients' demographic characteristics such as age, gender, ventilation parameters, and therapeutic managements, with aberrant gene expression profiling and dysregulated lung immune response in sepsis patients. A well-designed and multi-center study is needed for further exploration. Second, although our analysis indicated that direct sepsis ARDS induced aberrant gene expression profiling of myeloid cells, validation studies using cell line and animal models are needed in future. Last, studies assessing single-cell transcriptomics of alveolar cell populations are clearly warranted, given prior demonstration of the prognostic utility of studying alveolar myeloid populations.

Conclusions

In summary, the results of our study provide a useful perspective on the dynamic respiratory and systemic immune responses in hypercapnia following pulmonary sepsis-induced ARDS. Genes are differentially expressed in blood of pulmonary sepsis-induced ARDS with hypercapnia patients supporting the observation that accumulation of CO_2 is characterized by enrichment of cytokine and monocyte-dependent chemokine pathways. Together, we show that monocytes are key mediators in regulating lung inflammation, exhibiting distinct inflammation mediators. IL-1β, IL-12p40 and IL-23 derived from BALF and PBMCs are suitable biomarkers for identifying hypercapnia arising from pulmonary sepsis-induced ARDS. During the first week of evolution in hypercapnia but not in non- hypercapnia patients, elevated percentage of monocyte, especially CD14+CD16-classical monocyte phenotype, and related biomarkers in both alveolar and circulation drive accumulation of CO_2, which is associated with more severe disease and increased mortality. Our data provide a clinical and physiologic rationale for predicting the hypercapnia subset in patients of pulmonary sepsis-induced ARDS, and underscore the urgent need for therapeutically targeting CO_2 removal to mitigate the risk of fatal outcomes.

Ethics Approval and Informed Consent

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of Tianjin Medical University (ClinicalTrials.gov: ZYY-IRB2023-KY-178) from February 24th, 2023.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is 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.

Consent for Publication

Written informed consent was obtained from all participants or their legally authorized surrogates.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose.

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