Causal analysis of plasma fatty acids and pneumonia: identifying diagnostic biomarkers through transcriptome-wide association study

Xubin Chen[1], Xiaoyu Wu[2], Pingan Mao[1], Zijin Guo[1], Xiaomei Wang[3]*

[1]Department of Rehabilitation Medicine, Lishui Hospital of Wenzhou Medical University, The First Affiliated Hospital of Lishui University, Lishui People's Hospital, Lishui, Zhejiang, China
[2]Department of Neurosurgery, Lishui Hospital of Wenzhou Medical University, The First Affiliated Hospital of Lishui University, Lishui People's Hospital, Lishui, Zhejiang, China
[3]Department of Hepatology and Infectious Diseases, Lishui Hospital of Wenzhou Medical University, The First Affiliated Hospital of Lishui University, Lishui People's Hospital, Lishui, Zhejiang, China

Submitted: 14 March 2025; **Accepted:** 18 July 2025
Online publication: 6 September 2025

Arch Med Sci 2026; 22 (5): 2875–2888
DOI: https://doi.org/10.5114/aoms/208380
Copyright © 2025 Termedia & Banach

***Corresponding author:**
Xiaomei Wang
Department of Hepatology and Infectious Diseases
Lishui Hospital of Wenzhou Medical University,
The First Affiliated Hospital of Lishui University,
Lishui People's Hospital,
No. 15 Dazhong Street
Liandu District, Lishui
323000, Zhejiang, China
Phone: +86-18957092292
E-mail:
xmw18957092292@163.com

Abstract

Introduction: Fatty acids mediate pulmonary inflammation through cytokine regulation, interactions with the tryptophan-kynurenine pathway, and mediators such as 18-HEPE that boost interferon-λ. To systematically dissect these mechanisms and their translational implications, we pioneered a novel framework integrating bidirectional two-sample Mendelian randomization (MR) with multi-algorithm machine learning. This approach not only quantifies causal relationships between fatty acids and inflammatory outcomes but also identifies clinically actionable diagnostic biomarkers.

Material and methods: We obtained single nucleotide polymorphisms (SNPs) significantly associated with eight plasma fatty acids from genome-wide association study (GWAS) summary statistics and used them as instrumental variables in bidirectional two-sample MR across six pneumonia phenotypes (pneumonia, asthma-related pneumonia, bacterial pneumonia, critical pneumonia, viral pneumonia, and lobar pneumonia). Causal estimates were calculated using inverse variance weighting (IVW), which combines SNP-specific Wald ratios weighted by the inverse of their variance, with MR-Egger and weighted median approaches for sensitivity analysis. Transcriptomic data were then analyzed by LASSO regression, support vector machine recursive feature elimination and random forest to identify fatty acid metabolism–related biomarker candidates.

Results: MR analysis suggested potential causal associations between omega-6 fatty acids and critical pneumonia (OR = 1.28, 95% CI: 1.01–1.61, p = 0.038), linoleic acids (LA) and bacterial pneumonia (OR = 0.85, 95% CI: 0.73–0.99, p = 0.047), and docosahexaenoic fatty acids (DHA) and pneumonia (OR = 0.83, 95% CI: 0.74–0.93, p = 0.002). Moreover, ACAA1 and OLAH, which are genes involved in fatty acid metabolism, were identified as potential candidate biomarkers for pneumonia.

Conclusions: Our study employed MR analysis to establish a causal association of omega-6, LA, and DHA with pneumonia. Additionally, through transcriptomic analysis, we identified plasma fatty acid metabolism-associated biomarkers that may serve as diagnostic indicators for pneumonia.

Key words: plasma fatty acid, pneumonia, Mendelian randomization, biomarkers, machine learning algorithms.

Creative Commons licenses: This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY -NC -SA 4.0). License (http://creativecommons.org/licenses/by-nc-sa/4.0/).

Introduction

Pneumonia, a global health challenge driven by infectious pathogens, is aggravated by antibiotic resistance and chronic respiratory comorbidities [1]. This study focuses on pathogen-mediated mechanisms, distinguishing pneumonia from non-infectious pneumonitis caused by autoimmune disorders or chemical exposures [2]. Severe cases often progress to systemic complications, including coagulation abnormalities such as disseminated intravascular coagulation, distinct from coagulation necrosis [3]. These abnormalities synergize with cytokine storms, elevating cardiovascular mortality risk through endothelial dysfunction and myocardial suppression [4, 5]. Despite advances in therapy, pneumonia remains a leading global cause of morbidity and mortality, underscoring the urgency to elucidate pathogenic mechanisms and advance novel therapies [6].

Fatty acids are essential membrane phospholipids and bioactive mediators that regulate immune cell function and inflammatory signaling [7, 8]. Dysregulated lipid profiles, particularly imbalances in circulating polyunsaturated fatty acids (PUFAs), influence lung pathology through membrane-mediated immune cell dysfunction and the production of lipid signaling molecules [9]. This mechanistic duality is exemplified in chronic obstructive pulmonary disease, where skewed omega-3 to omega-6 PUFA ratios correlate with sustained airway inflammation [10]. The interplay extends to acute infectious contexts, as observed in severe pneumonia patients exhibiting activation of the tryptophan-kynurenine axis [11]. During this process, indoleamine 2,3-dioxygenase catalyzes tryptophan conversion into immunomodulatory kynurenines that suppress T-cell activity, establishing a regulatory network with PUFA-derived inflammatory mediators, a phenomenon inversely associated with protective PUFA ratios [11–13].

Experimental evidence underscores therapeutic opportunities, with murine models demonstrating that omega-3 fatty acids attenuate bacterial pneumonia severity through coordinated

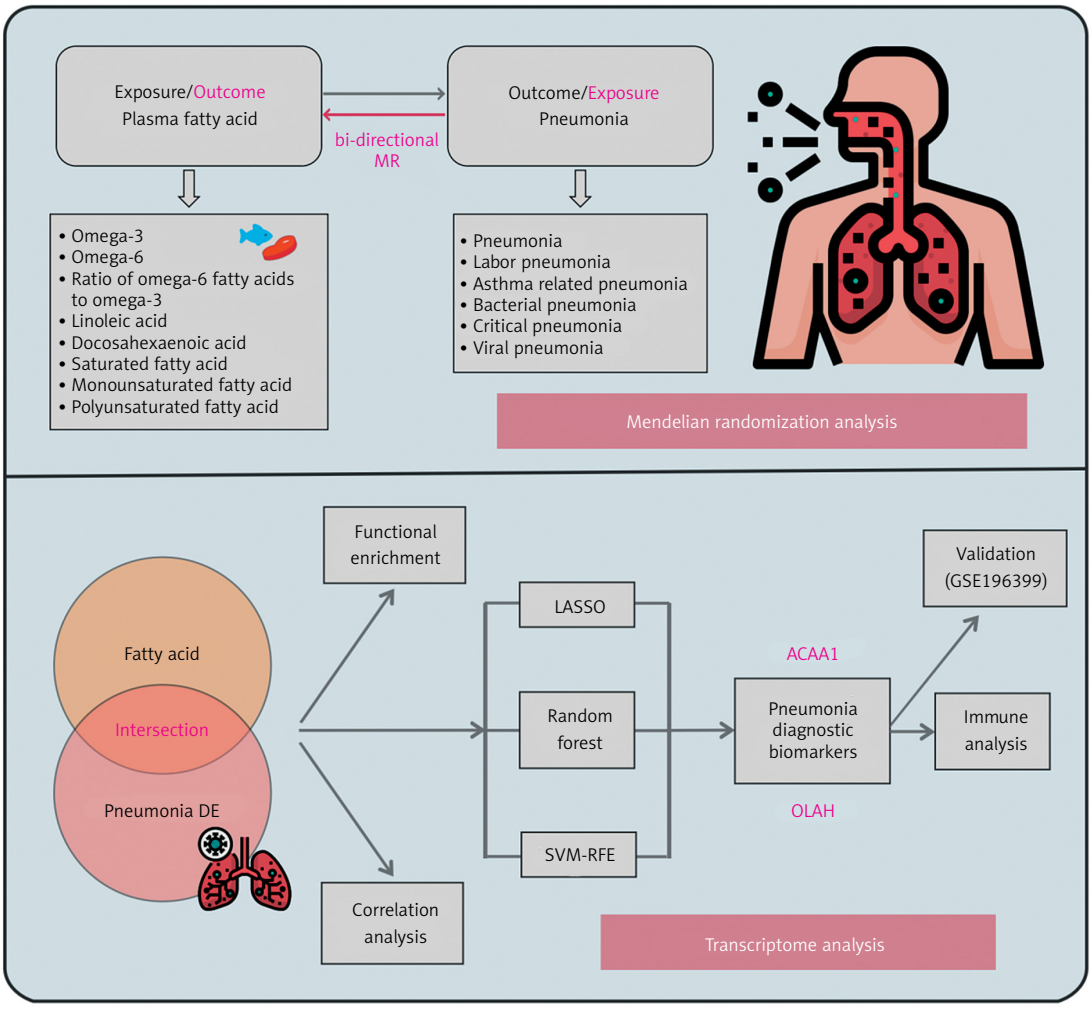

Figure 1. Study workflow

cytokine modulation, reducing pro-inflammatory interleukin-6 while elevating anti-inflammatory interleukin-10 [14–16]. Parallel studies revealed that butyrate-induced omega-3 metabolites exert antiviral effects via interferon-λ induction in viral pneumonia models [17]. Despite these mechanistic insights, translational challenges persist in distinguishing observational associations from causal relationships, particularly regarding pneumonia-specific lipid signatures and targeted therapeutic strategies.

To address these gaps, this study employed bidirectional two-sample Mendelian randomization (MR) to evaluate the potential causal associations between eight plasma fatty acids and six distinct pneumonia phenotypes. The workflow of this study is illustrated in Figure 1. Building on MR-derived causal evidence, we further applied three complementary machine-learning algorithms to transcriptomic data, identifying diagnostic biomarkers of fatty acid metabolism. This integrated methodology synergistically combined genetic causality analysis with multi-algorithm biomarker identification, establishing a unified framework in pneumonia research that bridged mechanistic discovery and clinical translation.

Material and methods

Study design

We first applied a two-sample MR framework to evaluate the causal effect of circulating plasma fatty acids on pneumonia risk. To address potential reverse causation, we then performed a reciprocal MR analysis [18], treating pneumonia liability as the exposure and plasma fatty acid levels as the outcome. In every MR analysis, we ensured that instrumental variables (IVs) satisfied the three core assumptions: strong association with the exposure, independence from confounders, and influence on the outcome exclusively through the exposure pathway [19]. To minimize bias from population stratification, all summary statistics were drawn from cohorts of European ancestry (Figure 2).

Data source

Genome-wide association study (GWAS) summary statistics for eight plasma fatty acid (FA) traits – omega-3, omega-6, omega-6/omega-3 ratio, linoleic acid (LA), docosahexaenoic acid (DHA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA – were obtained from the OpenGWAS platform. These data derive from 115,006 European ancestry participants in the UK Biobank. Pneumonia-related GWAS data were accessed via the IEU OpenGWAS repository and included overall pneumonia, severe pneumonia, lobar pneumonia, asthma-related pneumonia, viral pneumonia, and bacterial pneumonia. All summary statistics were drawn from cohorts of European ancestry, with detailed population descriptors provided in Table I.

Selection of instrumental variants

The criteria for selecting IVs were as follows: SNP selection: SNPs were used as IVs, with a genome-wide significance threshold of $p < 5e\text{-}08$. However, for some pneumonitis-related traits, the number of SNPs meeting this threshold was limited. To increase the number of available SNPs, we relaxed the threshold to $p < 5e\text{-}06$, and for lobar pneumonia, a threshold of $p < 5e\text{-}05$ was applied.

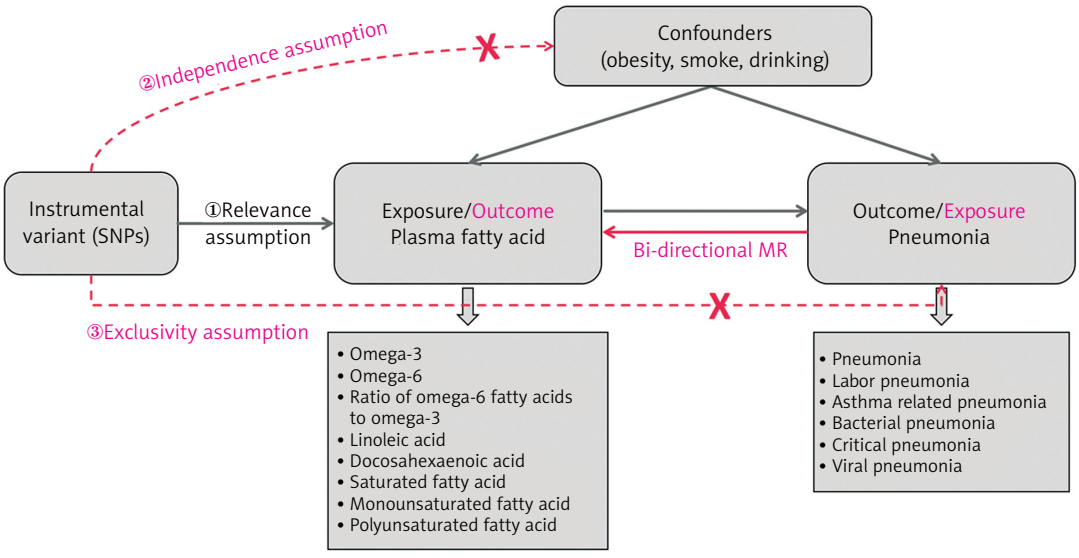

Figure 2. Flowchart of Mendelian randomization study design

Table I. Detailed information of the genome-wide association studies in our analysis

Phenotype	PMID (ID)	Sample size (case/control)	Population
Omega-3 fatty acid levels	35213538 (ebi-a-GCST90092931)	115006 /	European
Omega-6 fatty acid levels	35213538 (ebi-a-GCST90092933)	115006 /	European
Ratio of omega-6 fatty acids to omega-3 fatty acids	35213538 (ebi-a-GCST90092934)	115006 /	European
Linoleic acid levels	35213538 (ebi-a-GCST90092880)	115006 /	European
Docosahexaenoic acid levels	35213538 (ebi-a-GCST90092816)	115006 /	European
Saturated fatty acid levels	35213538 (ebi-a-GCST90092980)	115006 /	European
Monounsaturated fatty acid levels	35213538 (ebi-a-GCST90092928)	115006 /	European
Polyunsaturated fatty acid levels	35213538 (ebi-a-GCST90092939)	115006 /	European
Pneumonia	34594039 (ebi-a-GCST90018901)	480299 (16887/463412)	European
Asthma related pneumonia	/ (ukb-d-ASTHMA_PNEUMONIA)	361194 (5900/355294)	European
Bacterial pneumonia	/ (finn-b-J10_PNEUMOBACT)	196855 (7987/188868)	European
Critical pneumonia	/ (ieu-b-4978)	431365 (2758/428607)	European
Viral pneumonia	/ (finn-b-J10_VIRALPNEUMO)	189568 (700/188868)	European
Lobar pneumonia	/ (ukb-b-6576)	463010 (2359/460651)	European

Clumping: SNPs were clumped to remove the effect of linkage disequilibrium ($r^2 < 0.001$, with a region length of 10,000 kb). F-statistic: the F-statistic was used to assess the strength of IVs. SNPs with an F-statistic > 10 were retained, indicating strong instrument strength. Weak instruments were excluded. Allelic consistency: SNPs associated with both exposure and outcome were aligned in terms of allelic direction. Palindromic SNPs and SNPs with inconsistent allelic directions were removed. MR-PRESSO outliers: Outlier SNPs identified by the MR-PRESSO test were excluded from the analysis.

Mendelian randomization analysis

The primary causal estimates were obtained using the inverse variance weighted (IVW) method under a random effects model, supplemented by MR-Egger regression, weighted median, weighted mode, and simple mode analyses to assess robustness. Prior to MR, any SNPs associated with known confounders (BMI, waist measures, smoking, alcohol) were excluded. For reverse MR, the same procedure was followed with pneumonia as the exposure and individual fatty acid traits as outcomes.

Sensitivity analysis

To assess the robustness of the identified associations, we conducted sensitivity analyses using three methods: the heterogeneity test, the horizontal pleiotropy test, and the leave-one-out test. Cochran's Q test was used to assess SNP heterogeneity. If heterogeneity was detected ($p < 0.05$), a random-effects IVW model was applied. The presence of horizontal pleiotropy was evaluated using MR-Egger regression, with statistical significance of the intercept ($p < 0.05$) indicating pleiotropy. Additionally, MR-PRESSO was used to identify and remove outliers. A "leave-one-out" test was performed to evaluate the impact of individual SNPs on the causal associations by sequentially excluding each SNP.

Transcriptomic data acquisition and differential expression

We retrieved two gene expression datasets from the Gene Expression Omnibus (GEO):

GSE40012 (training set: 16 pneumonia, 18 controls) and GSE196399 (validation set: 56 pneumonia, 21 controls). A curated list of 308 fatty acid metabolism-related genes was compiled from the MSigDB Hallmark, KEGG, and Reactome collections (version 2024.1). Differential expression between pneumonia and control samples was determined using the limma package in R, with thresholds of $|\log_2$ fold change$| > 1$ and adjusted $p < 0.05$ (Benjamini–Hochberg correction).

Functional enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted in R with the clusterProfiler package. We controlled for multiple testing using the Benjamini-Hochberg method and considered pathways with adjusted $p < 0.05$ to be significantly enriched.

Feature selection and predictive modeling

To identify key fatty acid-related genes predictive of pneumonia, we applied three machine learning approaches: least absolute shrinkage and selection operator (LASSO) regression for initial feature retention, support vector machine recursive feature elimination (SVM-RFE) with ten-fold cross-validation for iterative refinement, and random forest for ranking variable importance. The intersection of genes selected by all three methods constituted the final feature set used in model construction.

Immune infiltration analysis

Immune cell proportions were estimated using the CIBERSORT algorithm implemented in the IOBR R package, and single sample gene set enrichment analysis (ssGSEA) of immune-related functions was performed via the GSVA package. Differences between pneumonia and control groups were tested using the Wilcoxon rank sum test, with $p < 0.05$ denoting significance.

Statistical analysis

All analyses were performed in R version 4.4.1, using the TwoSampleMR (0.6.8) [20], MRPRESSO (1.0) [21], limma [22], clusterProfiler [23], and IOBR [24] packages. Statistical significance was defined as two-sided $p < 0.05$ throughout.

Results

Causal relationship between fatty acids and pneumonia

Following rigorous selection and harmonization of genetic IVs, along with MR-PRESSO-mediated removal of pleiotropic outliers, we employed IVW MR to estimate the causal effects of eight plasma fatty acids on pneumonia risk. We observed that higher omega-6 levels were associated with increased risk of severe pneumonia (OR = 1.28, 95% CI: 1.01–1.61, $p = 0.038$), whereas greater LA levels conferred protection against bacterial pneumonia (OR = 0.85, 95% CI: 0.73–0.99, $p = 0.047$). Similarly, elevated DHA was inversely related to overall pneumonia incidence (OR = 0.83, 95% CI: 0.74–0.93, $p = 0.002$). No statistically significant causal associations were detected for the remaining fatty acids (Figure 3). Consistent direction and magnitude across MR-Egger, weighted median, weighted mode, and simple mode analyses reinforced these findings (Supplementary Table SI), and the complete lists of SNP instruments appear in Supplementary Tables SII–SVII with corresponding scatter and forest plots in Supplementary Figures S1, S2.

Sensitivity analysis

To verify robustness, we assessed heterogeneity and pleiotropy across instruments. For DHA, Cochran's Q test indicated no heterogeneity ($p > 0.05$), whereas omega-6 and LA instruments displayed modest heterogeneity ($p < 0.05$) but no evidence of horizontal pleiotropy by MR-Egger intercept (Table II). All instruments demonstrated adequate strength ($F > 30$; Supplementary Tables SVIII–SX). Applying a random effects IVW model for heterogeneous traits did not materially alter effect estimates ($p < 0.05$), and MR-PRESSO detected no additional outliers. Symmetrical funnel plots (Supplementary Figure S3) and stable leave-one-out analyses (Supplementary Figure S4) further support the reliability of these causal inferences (Supplementary Table SXI).

Reverse causality between fatty acids and pneumonia

In reverse MR, we tested whether genetic liability to pneumonia subtypes influences fatty acid levels. Notably, higher genetic risk of viral pneumonia was causally associated with marginally lower omega-3 levels (OR = 0.98, 95% CI: 0.97–0.99, $p = 0.006$) and with an elevated omega-6/omega-3 ratio (OR = 1.02, 95% CI: 1.01–1.04, $p = 0.005$) (Figure 4). A random effects IVW framework accounted for heterogeneity ($p < 0.05$), and sensitivity plots (Supplementary Figures S5–S8) confirmed absence of bias or influential SNPs.

Identification of differential genes associated with fatty acid metabolism

Analyzing GSE40012, we identified 549 genes differentially expressed between pneumonia pa-

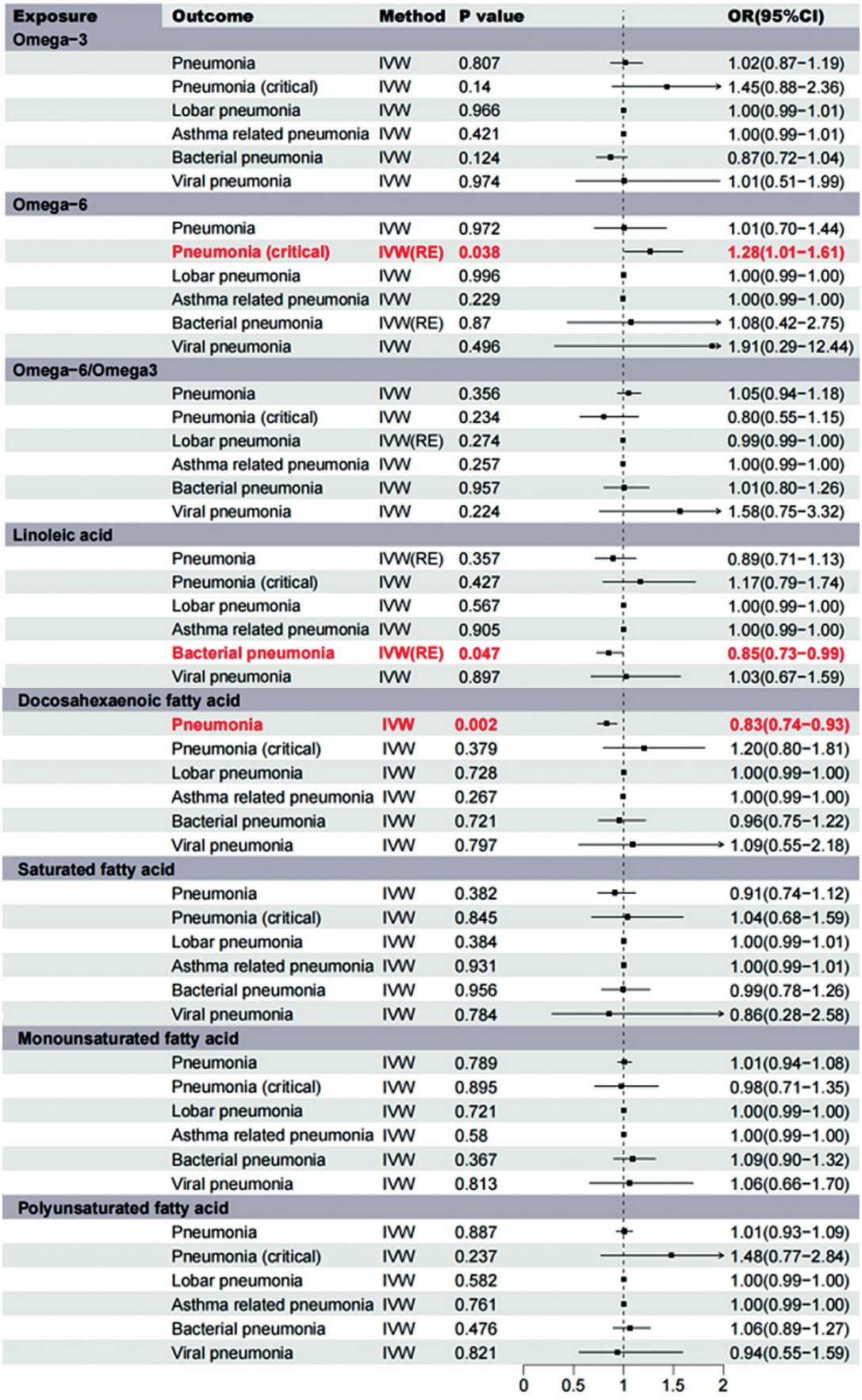

Figure 3. Univariable Mendelian randomization results for the effects of plasma fatty acid on pneumonia

Table II. Sensitivity analysis of causal relationship between plasma fatty acid and pneumonia subtypes

Exposure	Outcome	Heterogeneity				Pleiotropy
		MR-Egger statistics Q	MR-Egger *p*-value	IVW statistics Q	IVW *p*-value	*p*-value
Docosahexaenoic acid levels	Pneumonia	11.74	0.698	14.78	0.541	0.102
Omega-6 fatty acid levels	Critical pneumonia	62.52	0.022	63.00	0.025	0.573
Linoleic acid levels	Bacterial pneumonia	48.62	0.030	49.33	0.034	0.499

tients and controls ($|\log_2 FC| > 1$, $p < 0.05$), including 251 up-regulated and 298 down-regulated genes (Figure 5 A). Intersection with our curated set of 308 fatty acid metabolism genes yielded 16 overlapping targets (Figure 5 B). Correlation analysis revealed strong positive co-expression among most of these 16 genes (Figure 5 C). GO enrichment highlighted their roles in fatty acid and unsaturated fatty acid metabolism, carboxylic acid biosynthesis, and localization to peroxisomes, outer mitochondrial membranes, and rough endoplasmic reticulum (Figure 5 D, F).

Identification of key fatty acid metabolism genes in pneumonia

In order to exclude unimportant genes and identify key genes associated with pneumonia, we employed three machine learning algorithms for gene selection. Starting with 16 fatty acid metabolism-related differential genes, we applied LASSO regression, which selected 9 genes (Figure 6 A, B), SVM-RFE selected 5 genes (Figure 6 C), and the random forest algorithm identified 7 genes (Figure 6 D, E). Only ACAA1 and OLAH were consistently selected by all three methods (Figure 6 F). In the training cohort (GSE40012), ROC area under the curve values (AUCs) were 0.969 for ACAA1 and 0.979 for OLAH (Figure 6 G); in validation (GSE196399), AUCs were 0.781 and 0.965, respectively (Figure 6 H). PCA based on these two genes demonstrated clear separation of cases versus controls (Figure 6 I), and expression differences were highly significant in both datasets (Figure 6 J, K).

Immune cell infiltration and immune-related function in pneumonia

To characterize immune microenvironment alterations in pneumonia, we first applied CIBERSORT to quantify immune cell proportions in pneumonia versus healthy lung tissues (Figure 7 A, B). Compared to controls, pneumonia tissues showed significant increases in neutrophils, monocytes, M0 macrophages, and γδ T cells, accompanied by reduced regulatory T cells (Tregs), resting NK cells, and CD8+ T cells. Next, to explore broader functional implications, we performed single-sample gene set enrichment analysis (ssGSEA), which revealed distinct immune-related functional signatures and infiltration patterns between pneumonia patients and controls (Figure 7 C). Notably, ssGSEA further demonstrated that elevated expression of ACAA1 and OLAH correlated positively with macrophage infiltration but inversely with cytotoxic immune functions (Figure 7 D, E), suggesting that these fatty acid metabolism-related genes may drive macrophage polarization while suppressing adaptive immunity during pneumonia pathogenesis.

Discussion

In this study, we investigated the causal relationships between plasma fatty acids and pneumonia, with a specific emphasis on identifying key genes involved in fatty acid metabolism and their potential roles in disease pathogenesis. Our MR analysis revealed that LA and DHA are causally associated with a reduced risk of pneumonia, suggesting their protective effects. These findings support and extend earlier observational studies, which have reported anti-inflammatory and immunomodulatory effects of omega-3 and omega-6 fatty acids in various inflammatory conditions, including pneumonia [25, 26]. For instance, a large prospective cohort study demonstrated that every 1-gram increase in LA intake was associated with a 4% reduction in pneumonia risk [27], which is consistent with our MR-derived protective estimate for LA.

Our study further confirms the anti-inflammatory role of omega-3 fatty acids, particularly DHA, which are known to reduce the severity of inflammatory diseases by modulating immune responses [14]. While previous observational studies have suggested a beneficial association, our MR analysis strengthens the causal interpretation, overcoming potential confounding and reverse causation. On the other hand, omega-6 fatty acids are generally considered pro-inflammatory and have been associated with adverse outcomes in certain pneumonia subtypes [28, 29]. Our results support this view, as genetically predicted higher omega-6 levels were associated with increased risk of severe pneumonia, thereby aligning with earlier evidence and reinforcing the dualistic role of fatty acids in inflammation.

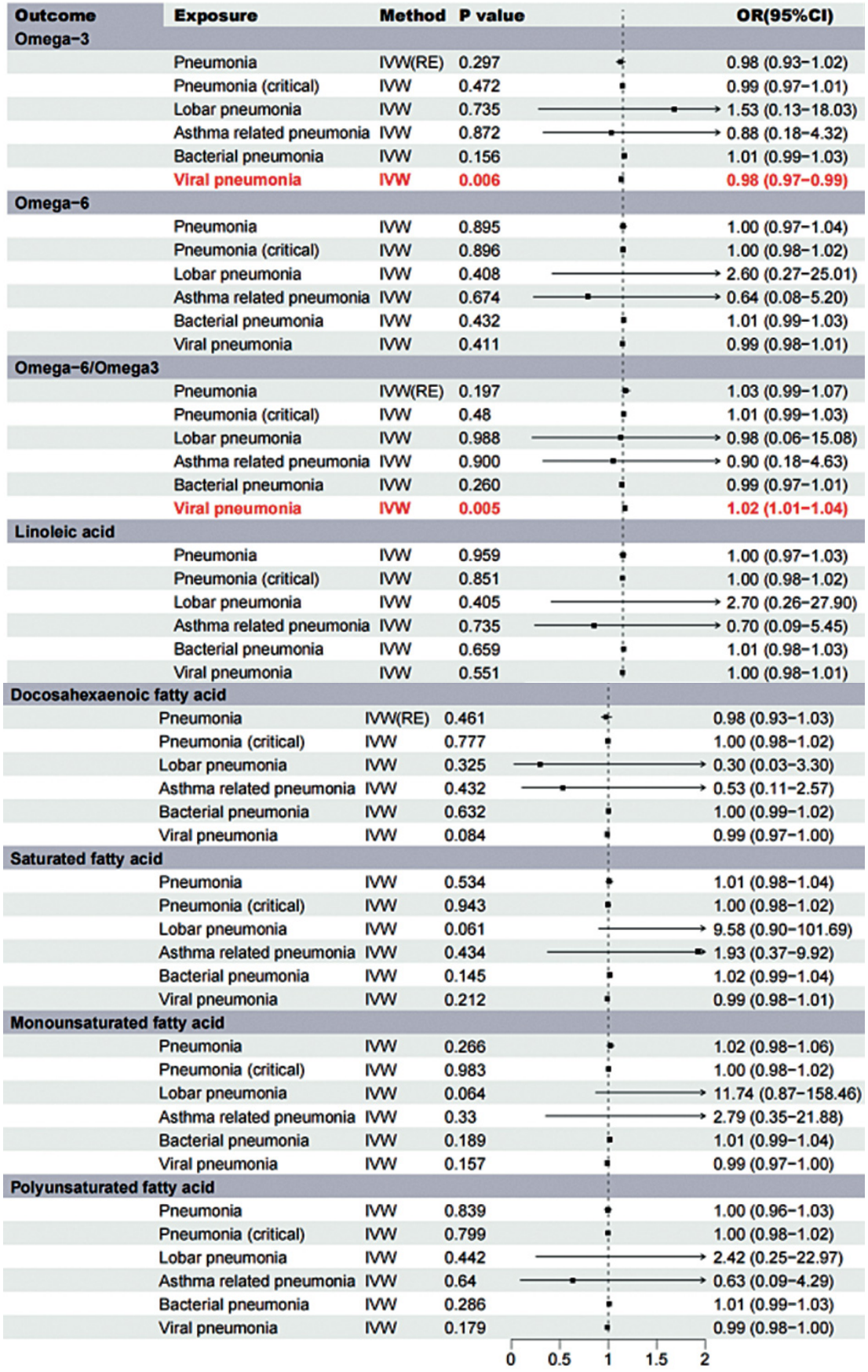

Figure 4. Reverse Mendelian randomization estimates for the effects of pneumonia on plasma fatty acid

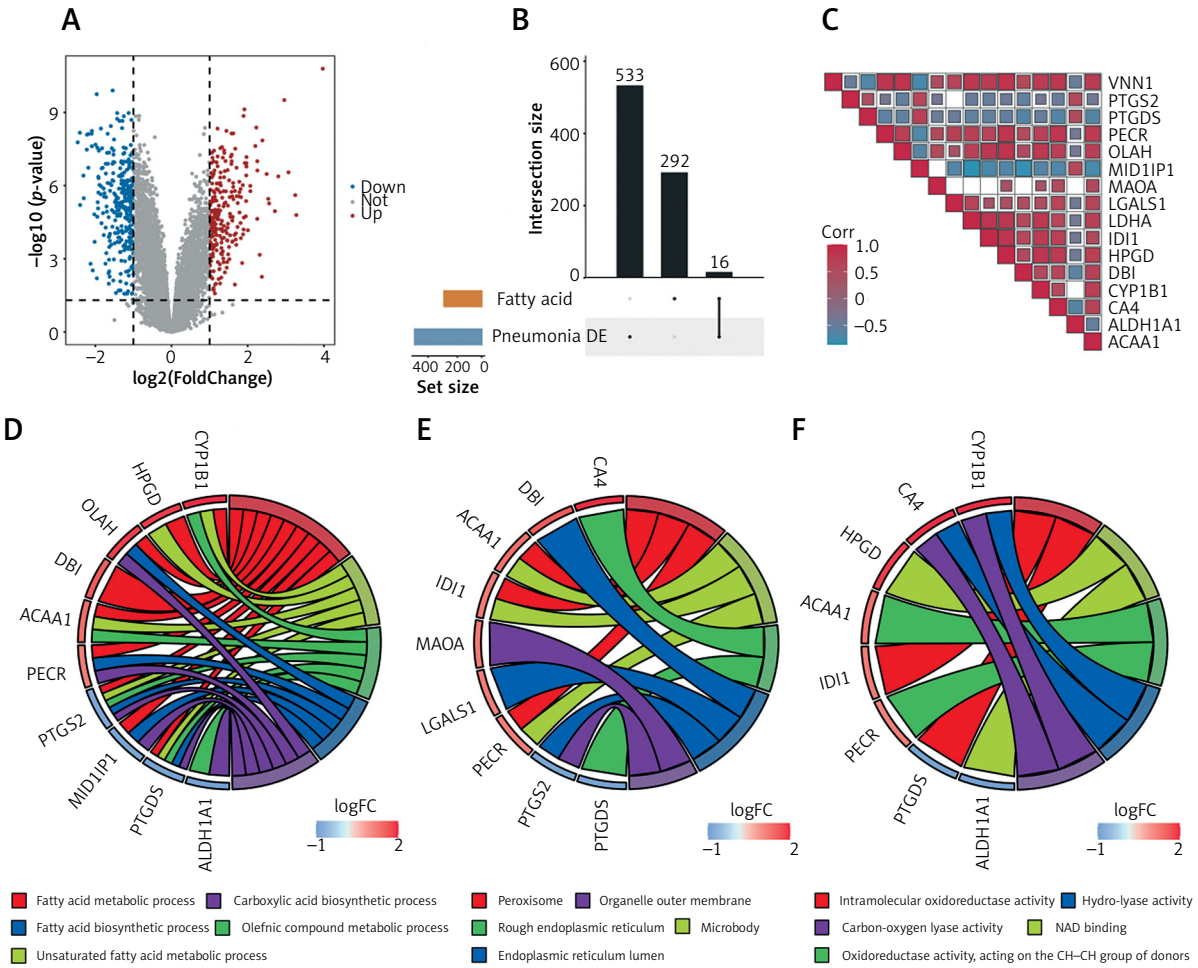

Figure 5. Differential gene expression and functional enrichment analysis of fatty acid metabolism-related genes in pneumonia. **A** – Volcano plot depicting differential gene expression in pneumonia. **B** – Intersection of fatty acid metabolism-related genes and pneumonia differential genes. **C** – Correlation heatmap of differential genes associated with fatty acid metabolism. **D–F** – GO enrichment map of 16 fatty acid metabolism differential genes enriched in BP, CC and MF

BP – biological process, CC – cellular component, MF – molecular function.

In animal models, omega-3 supplementation has been shown to mitigate pneumonia severity by suppressing microglial activation and inflammatory cytokine release through HMGB1 and TLR4/NF-κB signaling pathways [14, 30]. Furthermore, omega-3 and its derivatives have been shown to alleviate intestinal inflammation and modulate systemic immune responses in diseases such as ulcerative colitis [31, 32]. Short-chain fatty acids, another important class of fatty acids, exert anti-inflammatory effects by lowering colonic pH and limiting the growth of pathogenic bacteria [33]. These consistent findings across different disease models underscore the importance of fatty acid metabolism in modulating immune function and inflammatory responses.

We further explored potential molecular mediators linking fatty acids to pneumonia by applying machine learning algorithms. Through this approach, we identified ACAA1 and OLAH as key genes involved in fatty acid metabolism. ACAA1 plays a crucial role in mitochondrial β-oxidation, while OLAH is involved in fatty acid biosynthesis and homeostasis [34, 35]. Previous studies have linked ACAA1 variants with asthma and other inflammatory airway diseases, suggesting its involvement in immune regulation [36]. The identification of these genes complements and expands previous research by offering specific molecular targets through which fatty acids may exert their protective effects in pneumonia.

Infiltration analysis of immune cells revealed significant differences between pneumonia patients and healthy individuals. In line with previous reports, healthy controls exhibited higher infiltration of Tregs, resting NK cells, and $CD8^+$ T cells, whereas pneumonia patients showed increased infiltration of neutrophils, monocytes, M0 macrophages, and γδ T cells. These findings are consistent with the established role of neutro-

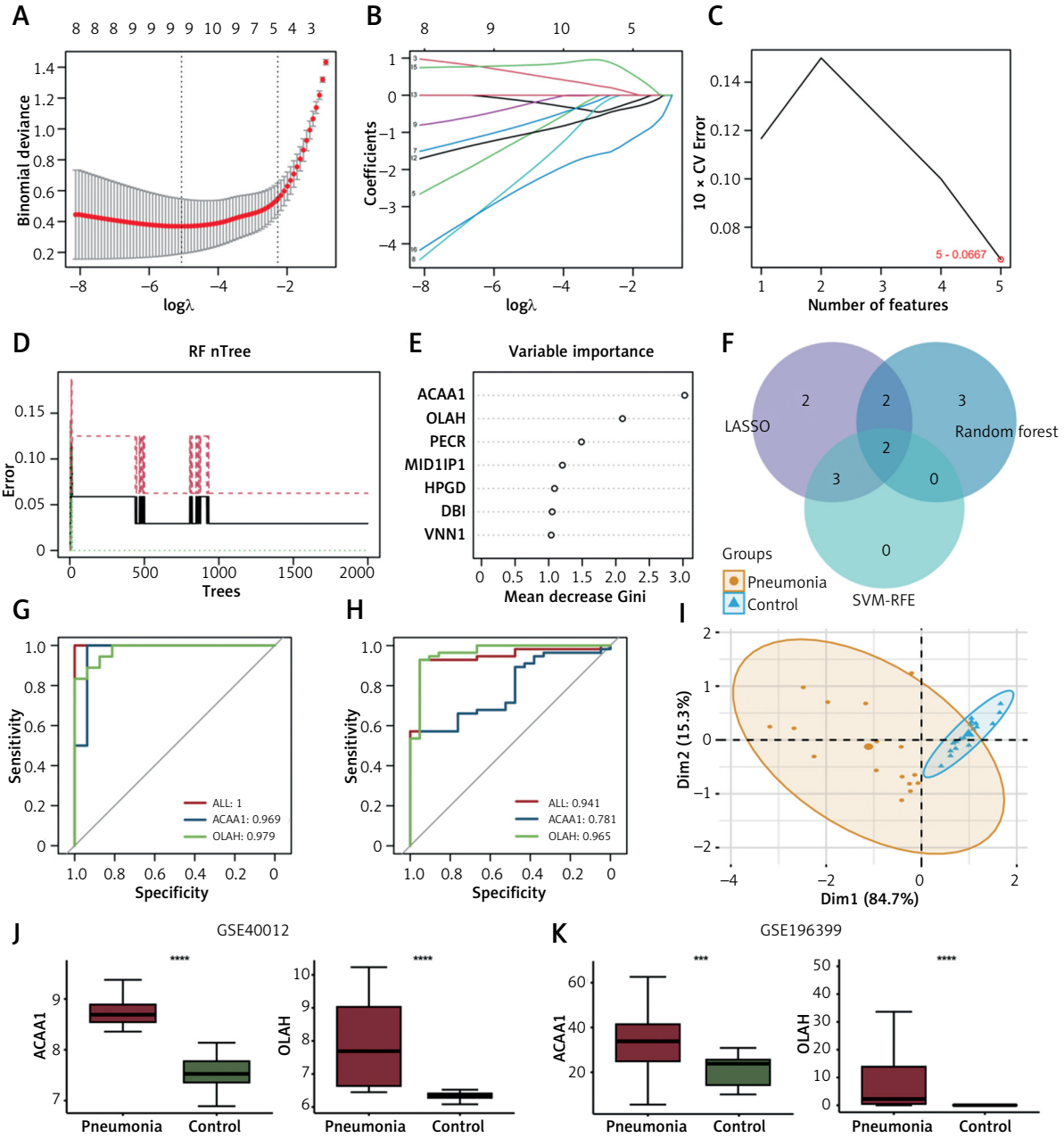

Figure 6. Identification and validation of pneumonia diagnostic markers. (**A**) LASSO regression and (**B**) 10-fold cross validation diagram. (**C**) Error rate curve based on SVM-RFE algorithm with 10-fold cross validation. (**D**) Relationship between random forest error rate and number of classification trees and (**E**) gene importance. (**F**) Genes shared by three machine learning models. (**G**) ROC curves of each signature gene in the training set (GSE40012) and (**H**) validation set (GSE196399) of the diagnostic markers. (**I**) PCA plot of training set (GSE40012). (**J**) Expression of signature genes in the training set (GSE40012) and (**K**) validation set (GSE196399) in pneumonia and normal samples

phils and macrophages in pneumonia pathogenesis [37, 38]. In *Streptococcus pneumoniae*-induced pneumonia, for example, neutrophil accumulation is a hallmark of the early immune response, contributing to bacterial clearance but also to lung tissue damage and systemic spread [39, 40]. Macrophages act as first responders to pathogens and contribute to both host defense and inflammation by releasing cytokines and orchestrating immune responses [41–44]. The elevated levels of γδ T cells in pneumonia samples also suggest a role in early immune activation, consistent with their known function in bridging innate and adaptive immunity [45].

In addition to lipid mediators, various acute-phase plasma proteins, including C-reactive protein, procalcitonin, surfactant proteins, and complement components, are key players in the pathophysiology of pneumonia [46–49]. These proteins are closely correlated with disease se-

verity and outcomes [50]. Notably, their interplay with fatty-acid-driven cytokine modulation implies that integrating lipid and protein biomarkers into combined panels could significantly enhance the accuracy of pneumonia diagnosis and improve prognostication [51, 52].

While our MR framework reduces confounding and reverse causation, it is limited by reliance on peripheral blood-derived GWAS and transcriptomic data, which lack direct validation in lung tissue. Future studies should combine clinical phenotyping with analyses of lung biopsy or bronchoalve-

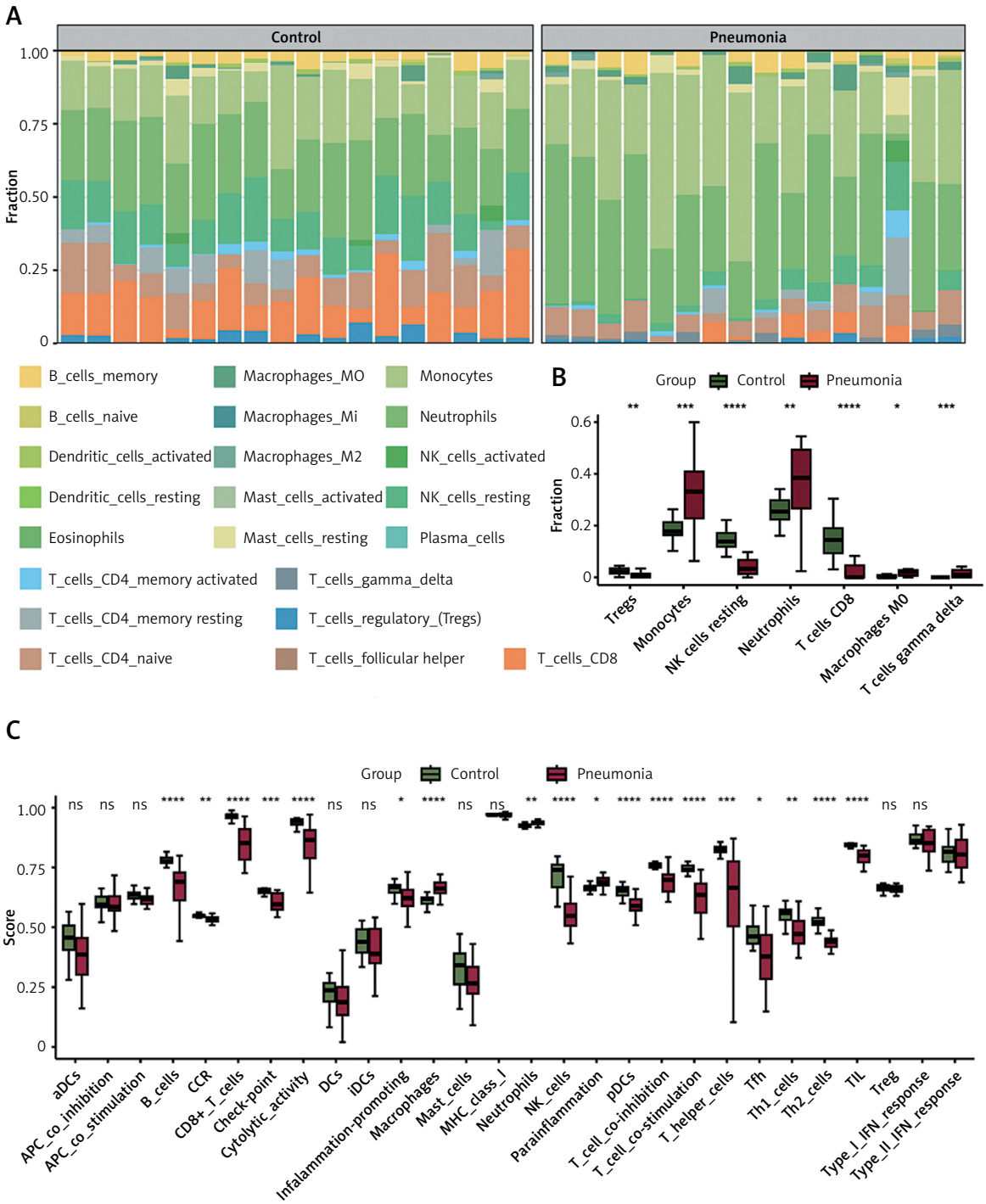

Figure 7. Analysis of immune cell infiltration in patients with pneumonia and normal controls. (**A**) Distribution and (**B**) differences in levels of immune cell infiltration between normal samples and pneumonia patients. (**C**) Differences in immune cell infiltration between normal and pneumonia patients using ssGSEA

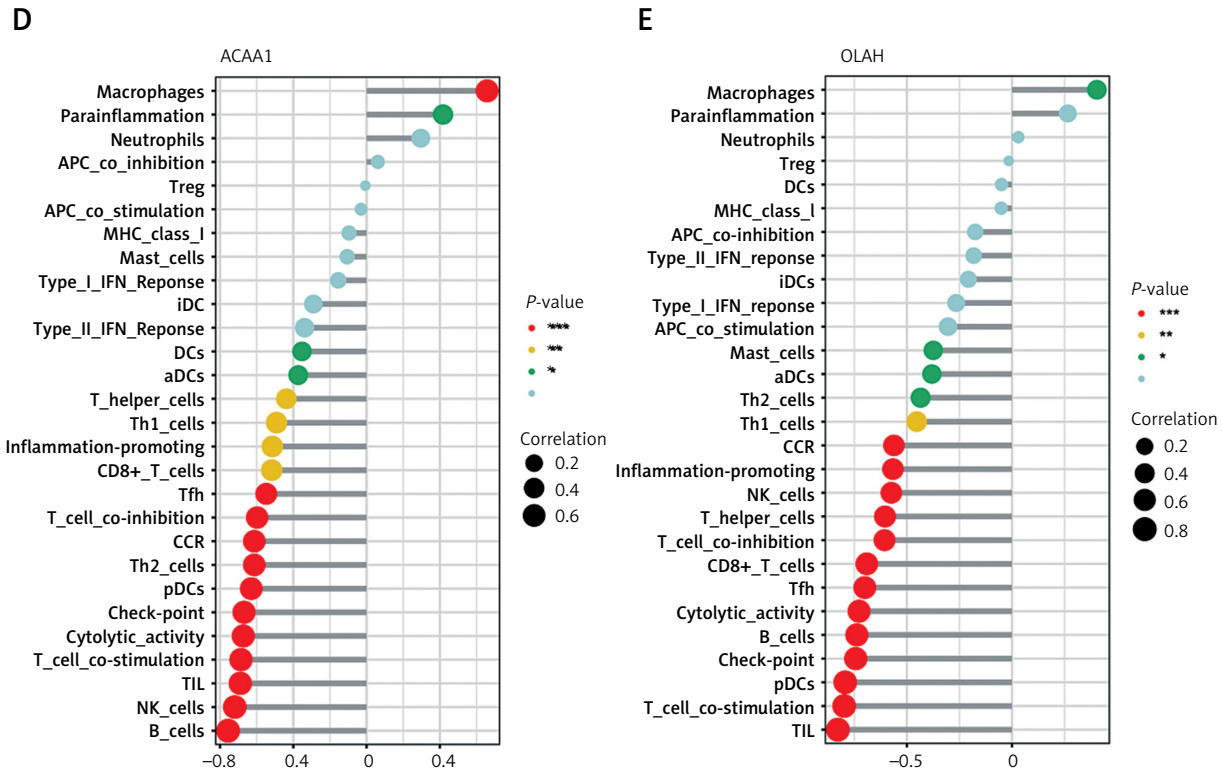

Figure 7. Cont. (**D**) Association between ACAA1, (**E**) OLAH and immune cell infiltration levels. The circle size indicates the correlation strength, with larger circles representing stronger correlations. The color reflects the *p*-value significance: red for $p < 0.001$, yellow for $p < 0.01$, and green for $p < 0.05$

olar lavage specimens to validate these findings. Additionally, functional studies of ACAA1 and OLAH in animal models will be critical to translate these genetic insights into targeted therapies.

In conclusion, our bidirectional MR study establishes that genetically higher levels of LA and DHA reduce pneumonia risk, while elevated omega-6 increases susceptibility to severe disease. Integrating machine learning driven transcriptomic analysis, we identified ACAA1 and OLAH as novel biomarkers linking fatty acid metabolism to pneumonia pathogenesis. Immune cell deconvolution further reveals a shift toward neutrophil and macrophage driven inflammation alongside reduced regulatory and cytotoxic T-cell subsets in patients. Together, these findings deepen our understanding of lipid-immune crosstalk in pneumonia and highlight promising targets for future diagnostic and therapeutic strategies.

Funding

No external funding.

Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Torres A, Cilloniz C, Niederman MS, et al. Pneumonia. Nat Rev Dis Primers 2021; 7: 25.
2. Neill S, Dean N. Aspiration pneumonia and pneumonitis: a spectrum of infectious/noninfectious diseases affecting the lung, Curr Opin Infect Dis 2019; 32: 152-7.
3. Zhang H, Zhan D, Chen D, et al. Next-generation sequencing diagnosis of severe pneumonia from fulminant psittacosis with multiple organ failure: a case report and literature review, Ann Transl Med 2020; 8: 401.
4. Savla SR, Prabhavalkar KS, Bhatt LK. Cytokine storm associated coagulation complications in COVID-19 patients: pathogenesis and management. Expert Rev Anti Infect Ther 2021; 19: 1397-413.
5. Kusumawardhani NY, Putra ICS, Kamarullah W, et al. Cardiovascular disease in post-acute COVID-19 syndrome: a comprehensive review of pathophysiology and diagnosis approach. Rev Cardiovasc Med 2023; 24: 28.
6. Cilloniz C, Dela Cruz CS, Dy-Agra G, Pagcatipunan RS Jr. World Pneumonia Day 2024: fighting pneumonia and antimicrobial resistance. Am J Respir Crit Care Med 2024; 210: 1283-5.
7. Garcia C, Andersen CJ, Blesso CN. The role of lipids in the regulation of immune responses. Nutrients 2023; 15: 3899.
8. Torres RM, Cyster J. Lipid mediators in the regulation of innate and adaptive immunity. Immunol Rev 2023; 317: 4-7.
9. Bogie JFJ, Haidar M, Kooij G, Hendriks JJA. Fatty acid metabolism in the progression and resolution of CNS disorders. Adv Drug Deliv Rev 2020; 159: 198-213.
10. Kotlyarov S, Kotlyarova A. Anti-inflammatory function of fatty acids and involvement of their metabolites in the

resolution of inflammation in chronic obstructive pulmonary disease. Int J Mol Sci 2021; 22: 12803.
11. Guo B, Xue M, Zhang T, et al. Correlation between immune-related Tryptophan-Kynurenine pathway and severity of severe pneumonia and inflammation-related polyunsaturated fatty acids. Immun Inflamm Dis 2023; 11: e1088.
12. Krupa A, Kowalska I. The kynurenine pathway-new linkage between innate and adaptive immunity in autoimmune endocrinopathies. Int J Mol Sci 2021; 22: 9879.
13. Siska PJ, Jiao J, Matos C, et al. Kynurenine induces T cell fat catabolism and has limited suppressive effects in vivo. EBioMedicine 2021; 74: 103734.
14. Hinojosa CA, Gonzalez-Juarbe N, Rahman MM, Fernandes G, Orihuela CJ, Restrepo MI. Omega-3 fatty acids in contrast to omega-6 protect against pneumococcal pneumonia. Microb Pathog 2020; 141: 103979.
15. Zhao H, Dennery PA, Yao H. Metabolic reprogramming in the pathogenesis of chronic lung diseases, including BPD, COPD, and pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2018; 314: L544.
16. Kocherlakota C, Nagaraju B, Arjun N, Srinath A, Kothapalli KSD, Brenna JT. Inhalation of nebulized omega-3 fatty acids mitigate LPS-induced acute lung inflammation in rats: implications for treatment of COPD and COVID-19. Prostaglandins Leukot Essent Fatty Acids 2022; 179: 102426.
17. Hagihara M, Yamashita M, Ariyoshi T, et al. Clostridium butyricum-induced ω-3 fatty acid 18-HEPE elicits anti-influenza virus pneumonia effects through interferon-λ upregulation. Cell Rep 2022; 41: 111755.
18. Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. Nat Rev Methods Primers 2022; 2: 6.
19. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. Stat Methods Med Res 2017; 26: 2333-55.
20. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. Int J Epidemiol 2016; 45: 1717-26.
21. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018; 50: 693-8.
22. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.
23. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation (Camb) 2021; 2: 100141.
24. Zeng D, Ye Z, Shen R, et al. IOBR: multi-omics immuno-oncology biological research to decode tumor microenvironment and signatures. Front Immunol 2021; 12: 687975.
25. Radzikowska U, Rinaldi AO, Çelebi Sözener Z, et al. The Influence of dietary fatty acids on immune responses. Nutrients 2019; 11: 2990.
26. Djuricic I, Calder PC. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021. Nutrients 2021; 13: 2421.
27. Merchant AT, Curhan GC, Rimm EB, Willett WC, Fawzi WW. Intake of n-6 and n-3 fatty acids and fish and risk of community-acquired pneumonia in US men. Am J Clin Nutr 2005; 82: 668-74.
28. Poggioli R, Hirani K, Jogani VG, Ricordi C. Modulation of inflammation and immunity by omega-3 fatty acids: a possible role for prevention and to halt disease progression in autoimmune, viral, and age-related disorders. Eur Rev Med Pharmacol Sci 2023; 27: 7380-400.
29. Lemoine C, Brigham E, Woo H, et al. Relationship between omega-3 and omega-6 fatty acid intake and chronic obstructive pulmonary disease morbidity. Ann Am Thorac Soc 2020; 17: 378-81.
30. Chen X, Wu S, Chen C, et al. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation 2017; 14: 143.
31. Marton LT, Goulart RA, Carvalho ACA, Barbalho SM. Omega fatty acids and inflammatory bowel diseases: an overview. Int J Mol Sci 2019; 20: 4851.
32. Reifen R, Karlinsky A, Stark AH, Berkovich Z, Nyska A. α-Linolenic acid (ALA) is an anti-inflammatory agent in inflammatory bowel disease. J Nutr Biochem 2015; 26: 1632-40.
33. Shin Y, Han S, Kwon J, et al. Roles of short-chain fatty acids in inflammatory bowel disease. Nutrients 2023; 15: 4466.
34. Huang X, Li Y, Zhuang P, et al. Habitual fish oil supplementation and risk of incident inflammatory bowel diseases: a prospective population-based study. Front Nutr 2022; 9: 905162.
35. Palanki R, Yamagata H, Mitchell MJ. OLAH connects fatty acid metabolism to the severity of respiratory viral disease. Cell 2024; 187: 4549-51.
36. Sordillo JE, Sharma S, Poon A, et al. Effects of endotoxin exposure on childhood asthma risk are modified by a genetic polymorphism in ACAA1. BMC Med Genet 2011; 12: 158.
37. Pechous RD. With friends like these: the complex role of neutrophils in the progression of severe pneumonia. Front Cell Infect Microbiol 2017; 7: 160.
38. Zhou A, Chen K, Gao Y, et al. Bioengineered neutrophil extinguisher targets cascade immune pathways of macrophages for alleviating cytokine storm in pneumonia. ACS Nano 2023; 17: 16461-77.
39. Domon H, Terao Y. The role of neutrophils and neutrophil elastase in pneumococcal pneumonia. Front Cell Infect Microbiol 2021; 11: 615959.
40. Palmer CS, Kimmey JM. Neutrophil recruitment in pneumococcal pneumonia. Front Cell Infect Microbiol 2022; 12: 894644.
41. Guillon A, Arafa EI, Barker KA, et al. Pneumonia recovery reprograms the alveolar macrophage pool. JCI Insight. 2020; 5: e133042.
42. Miyagi M, Uchida K, Takano S, et al. Macrophage-derived inflammatory cytokines regulate growth factors and pain-related molecules in mice with intervertebral disc injury. J Orthop Res 2018; 36: 2274-9.
43. Turner S, Khan MA, Putrino D, Woodcock A, Kell DB, Pretorius E. Long COVID: pathophysiological factors and abnormalities of coagulation. Trends Endocrinol Metab 2023; 34: 321-44.
44. Wei H, Zhao H, Li R, Yang F, Wu Y. Rhinovirus impairs the immune response of alveolar macrophages to facilitate Streptococcus pneumonia infection. Pathog Dis 2020; 78: ftaa020.
45. Hu Y, Hu Q, Li Y, et al. γδ T cells: origin and fate, subsets, diseases and immunotherapy. Signal Transduct Target Ther 2023; 8: 434.
46. Güleç T, Yılmaz S, Ak R, Tatlıparmak AC, Karcıoğlu Ö. Can we recognize severe community-acquired pneumonia without pneumonia severity index? Use of modified qSOFA with procalcitonin. Heliyon 2023; 9: e19937.

47. Saleh NY, Ibrahem RAL, Saleh AAH, Soliman SES, Mahmoud AAS. Surfactant protein D: a predictor for severity of community-acquired pneumonia in children. Pediatr Res 2022; 91: 665-71.
48. Bhattacharya S, Munshi C. Biological significance of C-reactive protein, the ancient acute phase functionary. Front Immunol 2023; 14: 1238411.
49. Xu Z, Hou XF, Feng CM, et al. The association between serum complement C3a and severity in patients with community-acquired pneumonia. Front Immunol 2023; 14: 1034233.
50. Zinellu A, Mangoni AA. Serum complement C3 and C4 and COVID-19 severity and mortality: a systematic review and meta-analysis with meta-regression. Front Immunol 2021; 12: 696085.
51. Ozbay S, Ayan M, Ozsoy O, Akman C, Karcioglu O. Diagnostic and prognostic roles of procalcitonin and other tools in community-acquired pneumonia: a narrative review. Diagnostics (Basel) 2023; 13: 1869.
52. Elmore A, Almuntashiri A, Wang X, Almuntashiri S, Zhang D. Circulating surfactant protein D: a biomarker for acute lung injury? Biomedicines 2023; 11: 2517.