Pneumonia-specific plasma metabolite profiles among patients hospitalised with infection in Southeast Asia

Taylor D. Coston[1], Lu Xia[2], Shelton W. Wright[3], Viriya Hantrakun[4], Parinya Chamnan[5], Gumphol Wongsuvan[4], Rungnapa Phunpang[6], Adul Dulsuk[6], Ekkachai Thiansukhon[5], Ali Shojaie[7], Narisara Chantratita[6], Direk Limmathurotsakul[4,8,10], Sina A. Gharib[1,10] and T. Eoin West[1,9,10]

[1]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Washington, Seattle, WA, USA. [2]Department of Statistics and Probability, Michigan State University, East Lansing, MI, USA. [3]Division of Pediatric Critical Care Medicine, Department of Pediatrics, University of Washington, Seattle, WA, USA. [4]Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. [5]Department of Social Medicine, Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand. [6]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. [7]Department of Biostatistics, University of Washington, Seattle, WA, USA. [8]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. [9]Department of Global Health, University of Washington, Seattle, WA, USA. [10]These authors contributed equally.

Corresponding author: Taylor D. Coston (costontd@uw.edu)

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Among adults hospitalised with infection in rural Thailand, community-acquired pneumonia was associated with a distinct plasma metabolic profile compared with other infectious presentations and a four-metabolite signature predicted 28-day mortality https://bit.ly/3YJEMuM

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Abstract

Background Community-acquired pneumonia (CAP) is a major public health threat globally but is understudied in regions with the highest burden. The host immune response during infection may differ based on the site of infection. We hypothesised that analysis of the plasma metabolome in patients hospitalised with suspected infection could identify host response pathways specific to CAP.

Methods We analysed the plasma metabolomes of adults admitted to a tertiary care hospital in northeastern Thailand with suspected community-acquired infection. Multivariable linear regression was performed for differential metabolite analyses and the global test was used for pathway analysis comparing patients with CAP *versus* non-CAP infections and uninfected controls. The least absolute shrinkage and selection operator (LASSO) was used to identify a parsimonious metabolite prognostic signature that was tested on an internal validation set to predict mortality.

Results 841 metabolites from 107 CAP patients and 152 non-CAP infected patients were analysed. 52 metabolites were differentially abundant between the CAP and non-CAP groups. CAP was characterised by increased metabolites involved in polyamine metabolism and decreased metabolites involved in lipid pathways. 13 pathways were differentially enriched between the CAP and non-CAP groups, consistent with individual metabolite analyses. 40 metabolites and four pathways were associated with CAP-specific mortality. A four-metabolite signature predicted 28-day mortality in CAP (area under the curve 0.79, 95% CI 0.62–0.97).

Conclusion In a rural tropical setting, CAP induced a distinct metabolomic state compared to non-CAP presentations of infection that may reflect the activation of select host immune responses.

Introduction

Community-acquired pneumonia (CAP) poses a significant global threat and remains understudied in regions of the world with the highest burden, including Southeast Asia [1, 2]. Reducing this health burden requires research in diverse at-risk populations due to global heterogeneity in host factors and aetiologic pathogens [3]. Progress in developing host-directed therapies for CAP has been hampered by a limited understanding of the underlying host response mechanisms driving CAP pathogenesis and adverse outcomes [4].

Immunometabolism offers a promising framework for unravelling the intricate dynamics of host immune responses and metabolic pathways during CAP [5]. Metabolites serve as mediators of cellular function and signalling pathways, orchestrating immune cell activation, proliferation and effector functions in response to infectious stimuli [6]. Emerging evidence suggests that metabolic reprogramming plays a central role in inflammatory responses, with dysregulated metabolism implicated in numerous infectious and inflammatory conditions [7, 8]. While many features of the host response to infection are likely shared across clinical presentations, CAP may induce a distinct metabolic state characterised by the selective activation of host immune responses tailored to combat respiratory pathogens [9]. Plasma metabolomic signatures have been identified that discriminate severe CAP from nonsevere CAP, bacterial *versus* viral aetiology, and pneumonia from uninfected critical illness, but few studies have investigated CAP-specific signatures compared with other infections and none have done so in a low- or middle-income country [10–16]. Unravelling the metabolic signatures associated with CAP compared with other infectious presentations could provide valuable insights into disease pathogenesis and identify novel biomarkers for diagnosis, prognosis and therapeutic intervention.

Therefore, in this study, we analysed the plasma metabolome of patients hospitalised with suspected infection, including CAP and non-CAP infections, from a prospective cohort study in northeastern Thailand. By comparing metabolomic profiles between those with CAP and other infections, as well as uninfected controls, we aimed to delineate CAP-specific elements of the host metabolic response and identify a parsimonious metabolite signature predictive of mortality in CAP.

Methods

Study design and population

Metabolomic analyses were performed on plasma obtained from patients enrolled in a prospective cohort study conducted in northeastern Thailand that has been described previously [17–19]. In brief, this study prospectively enrolled patients ⩾18 years old who were admitted to the general medical wards or the medical intensive care units at a tertiary care facility in Ubon Ratchathani, Thailand, from March 2013 to January 2017 with a primary diagnosis of infection made by the attending physician, who were within 24 h of admission to the study hospital and had at least three Surviving Sepsis Campaign criteria for sepsis documented in their medical record [18]. Patients were excluded if diagnosed with hospital-acquired infections, had a previous hospitalisation within the past 30 days, or were transferred from other hospitals with a total duration of hospitalisation >72 h. Plasma samples were obtained from all participants at the time of enrolment and metabolomics was performed on a subset [17]. The current study analysed plasma from a random sampling of patients with CAP and patients with non-CAP infections (*e.g.*, genitourinary infection and intra-abdominal infection), and nonhospitalised "control" individuals (without active infections) who were recruited from the outpatient clinic at Udon Thani Hospital, Udon Thani, Thailand. All infected patients were either bacteraemic or culture-negative. Aetiologies of bacteraemia were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pneumoniae* and *Burkholderia pseudomallei* – all common causes of bacteraemic CAP in northeastern Thailand. Cultures other than blood were recorded in the Ubon sepsis cohort study only for *B. pseudomallei*; patients with *B. pseudomallei*-positive cultures other than blood were excluded. Additional patient selection details are described in the supplementary material.

CAP and non-CAP classifications

Among hospitalised patients with infection, CAP was defined by the presence of all three of the following criteria: 1) clinician admission diagnosis of pneumonia, 2) respiratory symptoms (cough, sputum production or shortness of breath) and 3) discharge diagnosis of either pneumonia or melioidosis (*B. pseudomallei* infection). Non-CAP community-acquired infection was defined by both of the following criteria: 1) no admission diagnosis of pneumonia and 2) no discharge diagnosis of pneumonia. Discharge diagnosis of melioidosis was included in the CAP criteria because it is common practice to record a melioidosis pneumonia patient as only "melioidosis" in the medical record.

Plasma metabolomics

Sample preparation and ultrahigh performance liquid chromatography–tandem mass spectroscopy were performed by Metabolon, Inc. (Morrisville, NC, USA), as described in the supplementary material.

Statistical analysis

Metabolomic analyses were performed in two batches and merged as described in the supplementary material. Differential metabolites between CAP or non-CAP *versus* noninfected control groups were identified *via* linear regression adjusted for age, sex, body mass index (BMI) and diabetes. Next, differentially abundant metabolites between the CAP *versus* non-CAP infected groups were identified *via*

linear regression adjusted for age, sex, chronic lung disease, chronic kidney disease and infectious aetiology. Since illness severity may be in the causal pathway between CAP and altered metabolic pathways, the initial model did not contain the modified SOFA (Sequential Organ Failure Assessment) score (a measure of organ dysfunction) and results are reported in the supplementary material. The analysis was repeated by adding modified SOFA score to the model to assess a direct relationship between CAP and altered metabolic pathways independent of severity of illness. The global test, a functional class scoring method that tests for differential pathways, was used for pathway analysis with the R package "globaltest" (version 5.50.0) [20].

To investigate whether metabolites were associated with mortality in a CAP-specific manner, differentially abundant metabolites were identified comparing nonsurvivors *versus* survivors in the CAP group and the non-CAP infected group separately. Finally, a parsimonious metabolite signature to predict 28-day mortality in the CAP group was developed in a derivation set and tested in an internal validation set. This signature was tested against mortality prediction scores in CAP, namely the modified SOFA score and the CURB-65 score (confusion, urea >19 $mg \cdot dL^{-1}$, respiratory rate ⩾30, systolic blood pressure <90 mmHg or diastolic blood pressure ⩾60 mmHg, and age ⩾65 years) [21, 22]. For differentially abundant metabolite and pathway analyses, adjusted p-values were obtained using the Benjamini–Hochberg procedure for false discovery rate control and significance was denoted at <0.05. Data were analysed in an R statistical environment (R Core Team, 2022). Additional methodological details and sensitivity analyses are described in the supplementary material.

Human subjects

This study was approved by the Mahidol University Faculty of Tropical Medicine Ethics Committee (MUTM 2012-024-01, MUTM 2018-046-01 and MUTM 2024-022-01), the Sunpasitthiprasong Hospital Ethics Committee (039/2556), the Udon Thani Hospital Ethics Committee, the University of Washington Institutional Review Board (42988) and the University of Oxford Tropical Research Ethics Committee (OXTREC172-12). Informed consent was obtained from all study participants or their representatives.

Role of funders

The funders had no role in the study design, data collection and analysis, decision to publish, nor preparation of the manuscript.

Results

Study population

A total of 309 patients were included for analysis, including 107 with CAP, 152 with non-CAP infection and 50 uninfected controls. Demographics and clinical variables are provided in table 1. The proportions of infectious aetiologies were similar between the CAP and non-CAP groups, except for *S. pneumoniae*, which was only diagnosed in the CAP group (table 1). Compared to non-CAP infected patients, CAP patients were on average older (64 *versus* 56 years), had more chronic lung disease (11% *versus* 3%) and had higher modified SOFA score (6 *versus* 4). A higher proportion of patients with CAP died by 28 days following hospital admission (55 out of 107; 51%) compared to those with non-CAP infection (45 out of 152, 30%).

Differentially abundant metabolites

841 metabolites were analysed. We first compared the plasma metabolomes of patients with CAP *versus* uninfected controls and, separately, patients with non-CAP infection *versus* uninfected controls. In multivariable analyses adjusted for age, sex, BMI and diabetes, 667 metabolites were differentially abundant between CAP patients *versus* controls and 653 metabolites were differentially abundant between non-CAP patients *versus* controls (figure 1a and b). Of these differentially abundant metabolites, the majority were common to both comparisons, but 98 metabolites (14%) were specific to the infectious presentation (figure 1c). To further investigate CAP-specific metabolic signatures independent of illness severity, we subsequently compared the metabolomes of patients with CAP to patients with non-CAP infection adjusted for age, sex, chronic lung disease, chronic kidney disease, infectious aetiology and modified SOFA score. 52 metabolites were significantly differentially abundant (13 increased in CAP and 39 decreased in CAP) (figure 2a and b, table 2). The metabolites that were increased to the greatest degree in the CAP group compared with the non-CAP group accounting for illness severity belonged to the carbohydrate, estrogenic steroid and polyamine pathways. The metabolites that were decreased to the greatest degree in the CAP group compared with the non-CAP group belonged predominantly to the lipid super-pathway, including the monoacylglycerol pathway and the sphingomyelins and ceramide pathway.

TABLE 1 Demographic and clinical variables

Variable	Uninfected controls (n=50)	CAP (n=107)	Non-CAP infected (n=152)	p-value[#]
Demographics				
Age in years, median (IQR)	50.5 (45–64)	64 (46–74)	56 (46–66)	0.02
Sex, female, n (%)	20 (40.0)	48 (44.9)	62 (40.8)	0.51
BMI, median (IQR)	24.4 (22.9–27.9)	20.3 (18.7–23.5)	22.3 (19.5–24.7)	0.02
Comorbidities, n (%)				
Diabetes	25 (50.0)	39 (36.4)	55 (36.2)	0.97
Heart disease	–	8 (7.5)	7 (4.6)	0.42
Chronic kidney disease	–	11 (10.3)	25 (16.4)	0.2
Chronic lung disease	–	12 (11.2)	5 (3.3)	0.019
Current smoking	–	3 (2.8)	1 (0.7)	0.31
HIV	–	0 (0)	1 (0.7)	10
Infection aetiology, n (%)				
Klebsiella pneumoniae	–	7 (6.5)	12 (7.9)	0.92
Escherichia coli	–	14 (13.1)	22 (14.5)	0.95
Burkholderia pseudomallei	–	44 (41.1)	59 (38.8)	0.93
Staphylococcus aureus	–	8 (7.5)	18 (11.8)	0.52
Streptococcus pneumoniae	–	12 (11.2)	0 (0)	<0.001
Culture-negative	–	22 (20.6)	41 (27.0)	0.50
Modified SOFA score, median (IQR)	–	6 (3–9)	4 (2–7)	0.003
Symptom duration, n (%)				0.85
⩽2 days	–	42 (39.3)	55 (36.2)	
3–7 days	–	13 (12.1)	20 (13.2)	
>7 days	–	51 (47.7)	77 (50.7)	
Transferred from outside hospital, n (%)	–	91 (85.0)	119 (78.3)	0.12
Batch number, n (%)				0.001
Batch 1	50 (100)	53 (49.5)	105 (69.1)	
Batch 2	0 (0)	54 (50.5)	47 (30.9)	
Death by day 28, n (%)	–	55 (51.4)	45 (29.6)	<0.001

[#]: p-values obtained comparing CAP *versus* non-CAP infected using Wilcoxon rank-sum test for continuous variables and the Chi square or Fisher exact test for categorical variables. –: Data that were not collected in the uninfected control group; BMI: body mass index; CAP: community-acquired pneumonia; IQR: interquartile range; SOFA: Sequential Organ Failure Assessment.

Differentially enriched pathways

Having assessed individual metabolites, we next determined which pathways were differentially enriched between CAP and non-CAP patients independent of illness severity. 13 out of a total of 67 pathways were differentially enriched among patients with CAP compared to patients with non-CAP infection adjusted for age, sex, chronic lung disease, chronic kidney disease, infectious aetiology and modified SOFA score (table 3). These significant pathways spanned six super-pathways, namely amino acid, carbohydrate, cofactors and vitamins, nucleotide, xenobiotic, and lipid metabolism. The plasmalogen and tryptophan pathways were among the most significantly differentially enriched between CAP and non-CAP infected patients.

Metabolites and pathways associated with death specific to CAP

Among CAP patients, 55/107 (51%) died by day 28 and, among non-CAP infected patients, 45/152 (30%) died by day 28 (table 1). When the metabolomes of CAP nonsurvivors were compared with CAP survivors, 257 metabolites were differentially abundant adjusted for age, sex, chronic lung disease, chronic kidney disease and infectious aetiology (figure 3a). When non-CAP nonsurvivors were compared with non-CAP survivors, 448 metabolites were differentially abundant adjusted for the same covariables (figure 3b). Of these differentially abundant metabolites, 40 (8%) were unique to CAP, 231 (47%) unique to non-CAP infection, and 217 (45%) shared among CAP and non-CAP infection (figure 3c, Tables S4G-S4I). The most significantly increased metabolites unique to CAP nonsurvivors were from the cofactor and vitamin super-pathway including 1-methylnicotinamide, a metabolite in the nicotine adenine dinucleotide (NAD^+) pathway (table 4). The most significantly decreased metabolites unique to CAP nonsurvivors belonged to fatty acid metabolism pathways (table 4).

In pathway analysis, 51 out of a total of 67 pathways were differentially enriched in CAP nonsurvivors compared with CAP survivors. Of these pathways, four were unique to CAP, namely acetylated peptides, androgenic steroids, benzoate metabolism and dipeptide (supplementary table S7A). 55 pathways were

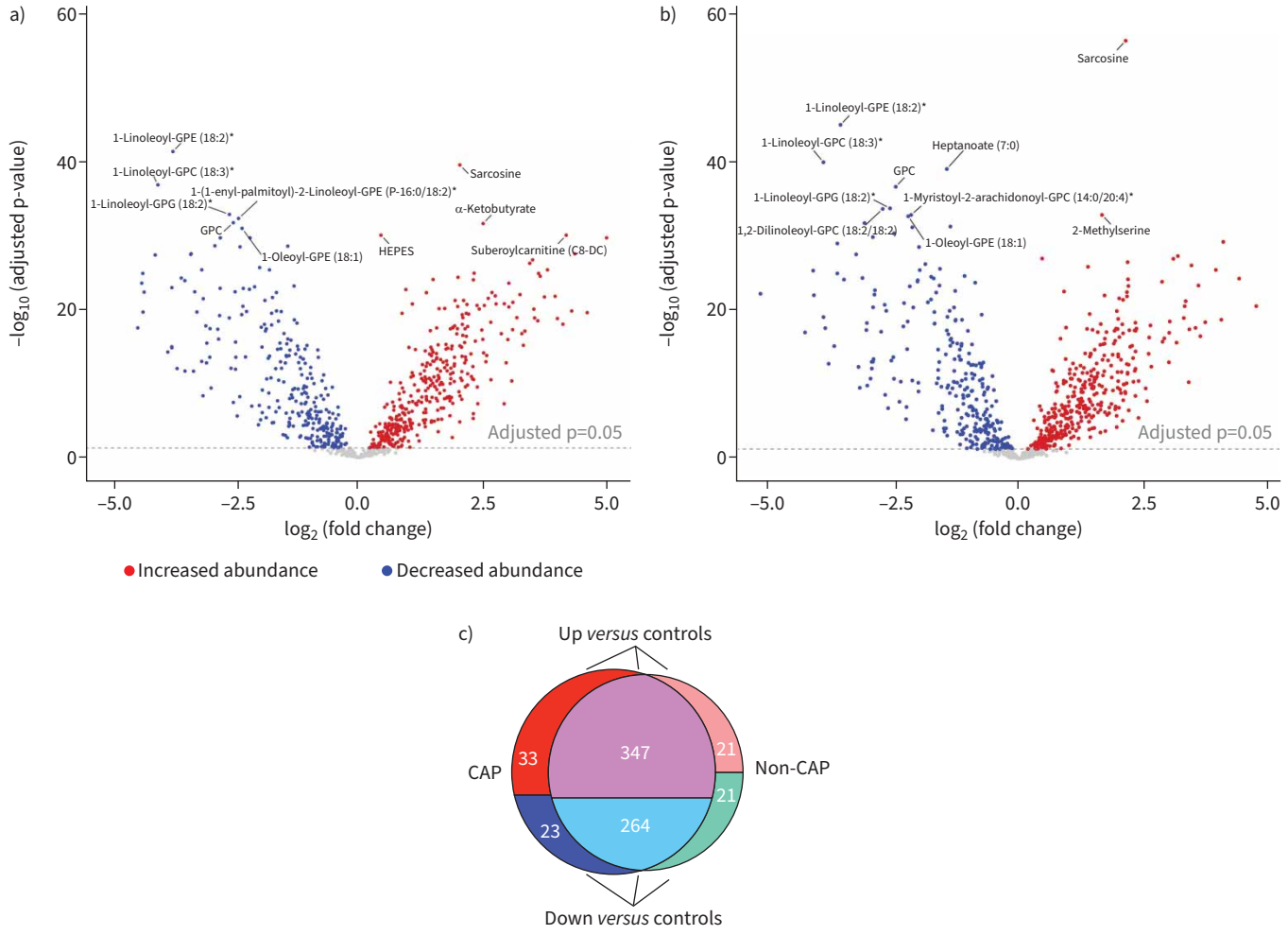

FIGURE 1 Differentially abundant metabolites comparing a) community-acquired pneumonia (CAP) (n=107) *versus* uninfected controls (n=50) and b) non-CAP infection (n=152) *versus* uninfected controls (n=50). c) Venn diagram of the differentially increased and decreased metabolites identified in these two comparisons. Models are adjusted for age, sex, body mass index, and diabetes. *: Significant metabolites with adjusted p-value<0.05 are highlighted. Red: increased abundance; blue: decreased abundance. GPC: glycerophosphorylcholine; GPE: glycerophosphorylethanolamine; GPG: glycerophosphorylglycerol.

differentially enriched in non-CAP nonsurvivors compared with non-CAP survivors. Of these, eight were unique to non-CAP infection including fatty acid metabolism (supplementary table S7B).

Sensitivity analyses

Two sensitivity analyses were performed to assess robustness of differentially abundant metabolite and pathway analyses (detailed in the supplementary methods), as follows: 1) comparing batch normalisation with bridge normalisation for merging the two metabolomic datasets and 2) selection of a subset of patients with bacteraemia that more closely reflects the prevalence of bacterial aetiologies in the region [18, 23, 24]. Results were broadly consistent with the primary analysis (supplementary tables S5 and S6), although sensitivity analysis 2 yielded fewer significant metabolites and pathways given the reduced sample size compared with the primary analysis.

Prognostic metabolomic signature in CAP

A parsimonious metabolite signature to predict 28-day mortality in CAP may have prognostic utility and could facilitate prioritisation of metabolites for further study. CAP patients (n=107) were randomly split into a derivation set (70% of CAP patients, n=75) and validation set (30% of CAP patients, n=32). Repeated data splitting with LASSO (least absolute shrinkage and selection operator) generated a list of top metabolites ranked by selection frequency in the derivation set (figure 4a). Metabolites were chosen for internal validation if selection frequency was >50%, which resulted in selection of four metabolites,

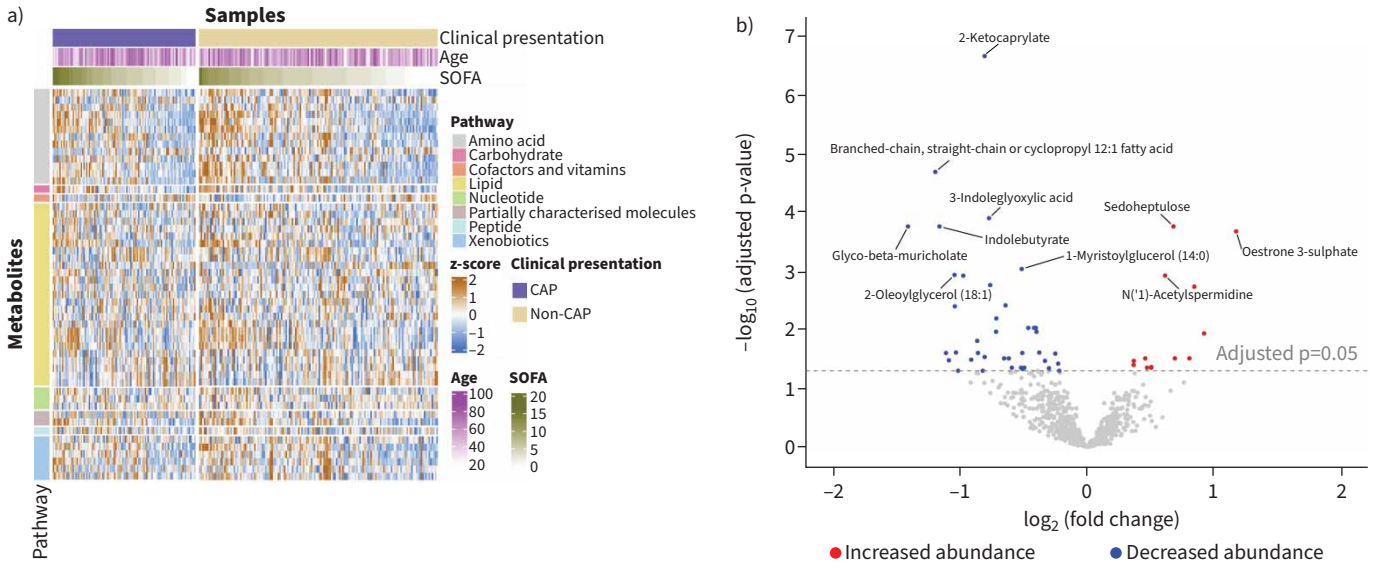

FIGURE 2 Differentially abundant metabolites comparing community-acquired pneumonia (CAP) (n=107) *versus* non-CAP infected patients (n=152). a) Heatmap showing CAP *versus* non-CAP adjusted for age, sex, chronic lung disease, chronic kidney disease, infectious aetiology and modified SOFA (Sequential Organ Failure Assessment) score. b) Volcano plot showing the same comparison.

namely 1-methylnicotinamide, 1,5-anhydroglucitol, heptanoate (7:0) and carboxyethyl-γ-aminobutyric acid (carboxy-ethyl GABA). This four-metabolite panel achieved an area under the curve (AUC) of 0.92 (95% CI 0.85–0.98) to predict 28-day mortality in the derivation set (supplementary figure S2A) and AUC of 0.79 (95% CI 0.62–0.97) in the internal validation set (figure 4b), which was comparable to the modified SOFA score (AUC 0.62, 95% CI 0.42–82, p=0.15) and CURB-65 score (AUC 0.57, 95% CI 0.37–0.77, p=0.07) in the internal validation set (figure 4b). However, adding the four metabolites to modified SOFA

TABLE 2 Top 15 significant differentially abundant metabolites comparing community-acquired pneumonia (CAP) patients with non-CAP infected patients in adjusted analysis

Metabolite	p-value[#]	Adjusted p-value[¶]	Super-pathway	Pathway
Increased in CAP *versus* non-CAP				
Sedoheptulose	1.23×10^{-6}	1.73×10^{-4}	Carbohydrate	Carbohydrate
Oestrone 3-sulphate	1.74×10^{-6}	2.10×10^{-4}	Lipid	Estrogenic steroids
N(1)-Acetylspermidine	1.42×10^{-5}	1.19×10^{-3}	Amino acid	Polyamine metabolism
N1,N12-Diacetylspermine	2.62×10^{-5}	1.84×10^{-3}	Amino acid	Polyamine metabolism
Decreased in CAP *versus* non-CAP				
2-Ketocaprylate	2.55×10^{-10}	2.14×10^{-7}	Amino acid	Leucine, isoleucine and valine metabolism
Branched-chain, straight-chain or cyclopropyl 12:1 fatty acid	4.81×10^{-8}	2.02×10^{-5}	Partially characterised molecules	Partially characterised molecules
3-Indoleglyoxylic acid	4.45×10^{-7}	1.25×10^{-4}	Xenobiotics	Food component/plant
Indolebutyrate	8.92×10^{-7}	1.73×10^{-4}	Amino acid	Tryptophan metabolism
Glyco-beta-muricholate	1.23×10^{-6}	1.73×10^{-4}	Lipid	Primary bile acid metabolism
1-Myristoylglycerol (14:0)	8.73×10^{-6}	9.17×10^{-4}	Lipid	Monoacylglycerol
2-Oleoylglycerol (18:1)	1.24×10^{-5}	1.16×10^{-3}	Lipid	Monoacylglycerol
2-Aminophenol sulphate	1.56×10^{-5}	1.20×10^{-3}	Xenobiotics	Food component/plant
Indoleacetoylcarnitine	3.30×10^{-5}	2.13×10^{-3}	Amino acid	Tryptophan metabolism
Carotene diol (3)	6.37×10^{-5}	3.83×10^{-3}	Cofactors and vitamins	Vitamin A metabolism
Glycohyocholate	7.09×10^{-5}	3.98×10^{-3}	Lipid	Secondary bile acid metabolism

[#]: p-values obtained *via* linear regression adjusted for age, sex, chronic lung disease, chronic kidney disease, infectious aetiology and modified Sequential Organ Failure Assessment score. [¶]: Adjusted p-values obtained using the Benjamini–Hochberg procedure. 37 additional significant differentially abundant metabolites are shown in supplementary table S4C.

TABLE 3 All 13 significantly differentially enriched pathways in patients with community-acquired pneumonia (CAP) compared with non-CAP infected patients in adjusted pathway analysis

Pathway	Super-pathway	Adjusted p-value[#]	Total number of metabolites
Plasmalogen	Lipid	4.67×10^{-4}	11
Tryptophan metabolism	Amino acid	4.67×10^{-4}	22
Food component/plant	Xenobiotic	2.09×10^{-3}	31
Monoacylglycerol	Lipid	2.73×10^{-3}	9
Polyamine metabolism	Amino acid	2.73×10^{-3}	9
Purine metabolism	Nucleotide	4.44×10^{-3}	14
Primary bile acid metabolism	Lipid	7.45×10^{-3}	10
Leucine, isoleucine and valine metabolism	Amino acid	1.42×10^{-2}	28
Carbohydrate	Carbohydrate	1.42×10^{-2}	26
Vitamin A metabolism	Cofactors and vitamins	1.89×10^{-2}	5
Tyrosine metabolism	Amino acid	2.44×10^{-2}	21
Pyrimidine metabolism	Nucleotide	2.67×10^{-2}	21
Ascorbate and aldarate metabolism	Cofactors and vitamins	3.22×10^{-2}	6

[#]: p-values for significantly enriched pathways obtained using the global test adjusted for age, sex, chronic kidney disease, chronic lung disease, infectious aetiology and modified Sequential Organ Failure Assessment score. Benjamini–Hochberg adjusted p-values, super-pathways and total numbers of metabolites in each pathway are shown.

score significantly improved mortality discrimination compared with modified SOFA score alone (AUC (95% CI), 0.80 (0.62–0.97) *versus* 0.62 (0.42–0.82); integrated discrimination improvement (IDI) p=0.001; supplementary table S8, figure 4c) in the internal validation set. Similarly, the addition of the four metabolites to CURB-65 significantly improved mortality discrimination compared with CURB-65 alone (AUC (95% CI), 0.77 (0.58–0.96) *versus* 0.57 (0.37–0.77); IDI p<0.001, supplementary table S8, figure 4c) in the internal validation set.

Discussion

To the best of our knowledge, this study is one of the largest and most comprehensive to date comparing the host metabolic response in CAP to other infectious presentations. We report that while CAP and non-CAP infections elicit many shared metabolic signals, distinct subsets of metabolites and pathways distinguish between these two clinical presentations even after adjusting for severity of illness. Furthermore, we identified metabolites and metabolic pathways associated with mortality in patients with CAP but not in patients with non-CAP infections. Finally, we report a novel four-metabolite signature for predicting CAP-associated 28-day mortality.

Consistent with other metabolic studies of patients hospitalised with infection, we found that the host metabolome undergoes striking changes during infection as evidenced by the metabolites that were differentially abundant between infected patients and uninfected controls. [17, 25–27] The metabolites associated with 28-day mortality in both the CAP and non-CAP groups may represent conserved elements of the host response to infection or, alternatively, a stress response shared by severe illness. The novel analyses in our study, however, add unique insights into metabolic alterations specific to CAP compared with non-CAP infection, as metabolites from a wide range of pathways were differentially abundant in a CAP-specific manner. While studies only comparing the metabolome of CAP patients to uninfected controls can yield valuable information regarding pathways activated during infection, our study delineated metabolic alterations that may underly CAP pathophysiology specifically. Additional strengths of our study include the prospective nature of the severe infection cohort and the early time-point at which plasma was collected from hospitalised patients. Finally, this is the first study to apply plasma metabolomic analyses to CAP in a resource-limited setting, where the CAP burden is highest yet least well-studied [1, 2].

Plasmalogen metabolism was the most significantly differentially altered pathway between CAP and non-CAP patients independent of illness severity, with most metabolites from this pathway decreased in CAP. Plasmalogens are key constituents of lung surfactant, regulating macrophage phagocytosis and acting as antioxidants through the preferential oxidation of their vinyl-ether bonds [28, 29]. The plasmalogen depletion in CAP patients observed in this study may be attributed to oxidation of these vinyl-ether bonds by reactive oxygen species, which are produced by the host to combat respiratory pathogens, thus protecting the lung from oxidative stress and tissue damage [30]. Corroborating this finding, a similar pattern was observed in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and sepsis-induced

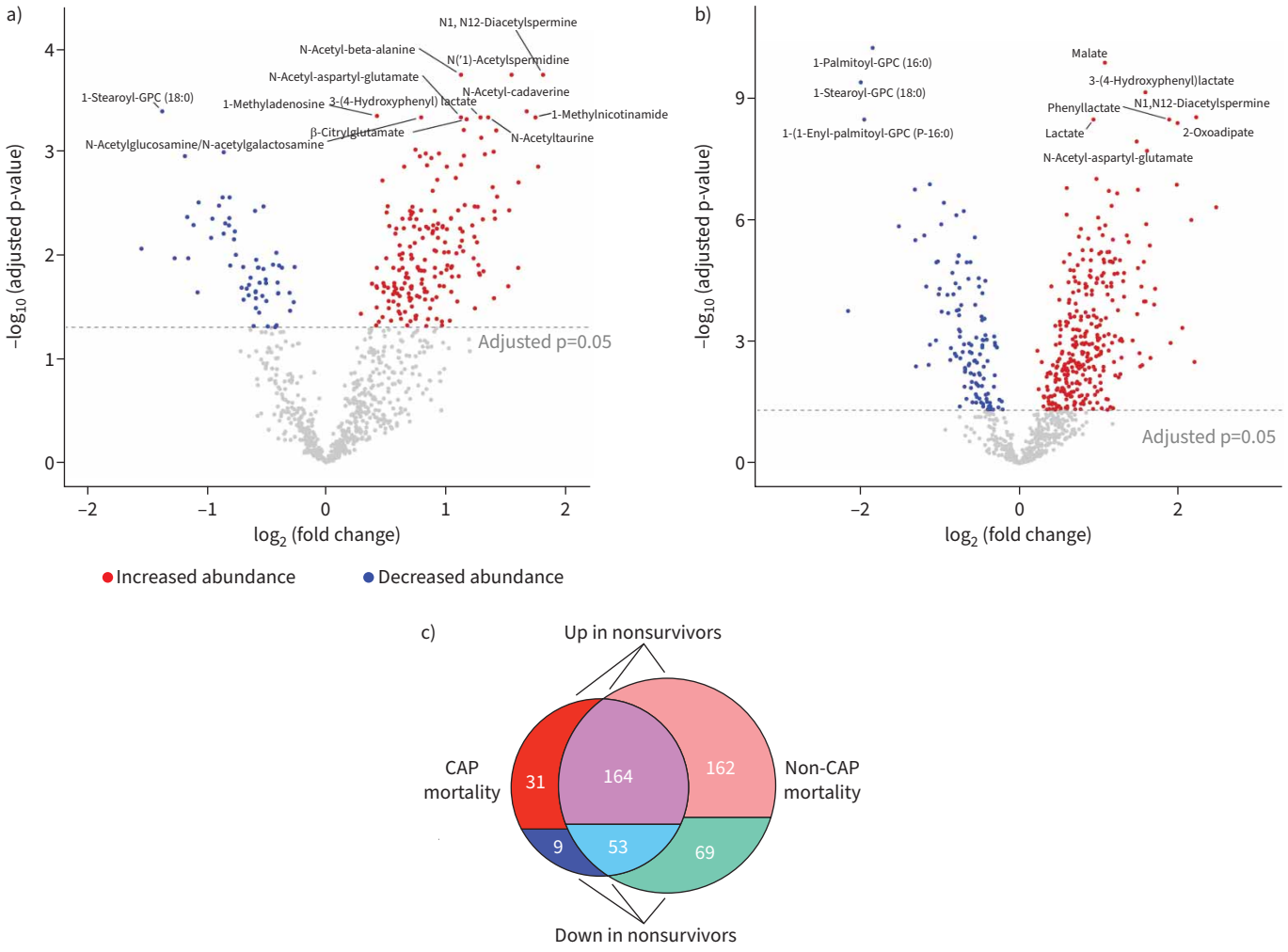

FIGURE 3 Differentially abundant metabolites comparing a) community-acquired pneumonia (CAP) nonsurvivors (n=55) *versus* CAP survivors (n=52) and b) non-CAP nonsurvivors (n=45) *versus* non-CAP survivors (n=107). c) Venn diagram of the differentially increased and decreased metabolites identified in these two comparisons. Models are adjusted for age, sex, chronic lung disease, chronic kidney disease and infectious aetiology. GPC: glycerophosphorylcholine.

acute respiratory distress syndrome [31]. In a recent CAP lipidomic study, plasmalogens were decreased comparing CAP patients with uninfected controls [26]. Our study is the first to further demonstrate this finding could be specific to, or at least most robust in, CAP compared with non-CAP infections.

An intriguing finding was that altered nicotinamide adenine dinucleotide (NAD^+) pathways were associated with 28-day mortality in CAP, distinct from non-CAP infection. Higher levels of 1-methylnicotinamide (MNA) and nicotinamide riboside (NR) were both strongly associated with 28-day mortality in CAP, but MNA was not associated with mortality in the non-CAP group. Elevation of both MNA and NR suggests impairment of the NAD^+ salvage pathway; NAD^+ depletion in the setting of concomitant failed *de novo* and salvage pathways has been associated with several inflammatory diseases and recently garnered interest in pathogenesis of pneumonia and acute lung injury [32, 33]. In preclinical models of pneumonia, nicotinamide supplementation alleviated lung injury by reducing the release of proinflammatory mediators [34]. Additionally, NAD^+ repletion improved ischaemia-reperfusion injury in the lung and, in a mouse model of SARS-CoV-2 pneumonia, partially rescued disturbed NAD^+ gene expression leading to improved survival [35, 36]. Nicotinamide phosphoribosyltransferase, the rate-limiting enzyme of the NAD^+ salvage pathway, induces NF-κB signalling in the lung endothelium and was shown to be a major contributor to ventilator-induced lung injury [37]. Additional research is needed to clarify the role of the NAD^+ pathway in the CAP host response, as existing therapeutics targeting the pathway might be repurposed for host-directed CAP treatment [38].

TABLE 4 Top 15 significant differentially abundant metabolites associated with community-acquired pneumonia (CAP)-specific mortality in adjusted analysis

Metabolite	p-value[#]	Adjusted p-value[¶]	Super-pathway	Pathway
Increased in CAP nonsurvivors				
1-Methylnicotinamide	6.04×10^{-6}	4.76×10^{-4}	Cofactors and vitamins	Vitamin B2, B3, B5 and B6 metabolism
2-Aminophenol sulphate	1.97×10^{-4}	3.46×10^{-3}	Xenobiotics	Food component/plant
S-Carboxyethylcysteine	2.36×10^{-4}	3.78×10^{-3}	Amino acid	Methionine, cysteine, SAM and taurine metabolism
3-Aminoisobutyrate	2.50×10^{-4}	3.82×10^{-3}	Nucleotide	Pyrimidine metabolism
Arabonate/xylonate	5.05×10^{-4}	5.38×10^{-3}	Carbohydrate	Carbohydrate
4-Hydroxyphenylacetate	5.52×10^{-4}	5.60×10^{-3}	Amino acid	Phenylalanine metabolism
2,3-Dihydroxyisovalerate	6.40×10^{-4}	5.98×10^{-3}	Xenobiotics	Food component/plant
1-Methylurate	8.62×10^{-4}	7.32×10^{-3}	Xenobiotics	Xanthine metabolism
Fructosyllysine	1.39×10^{-3}	1.00×10^{-2}	Amino acid	lysine metabolism
3-Hydroxypyridine sulphate	2.03×10^{-3}	1.29×10^{-2}	Xenobiotics	Chemical
Heptenedioate (C7:1-DC)	2.31×10^{-3}	1.34×10^{-2}	Peptide	Acetylated peptides
Decreased in CAP nonsurvivors				
3-CMPF	7.93×10^{-4}	6.88×10^{-3}	Lipid	Fatty acid metabolism (dicarboxylate, amino, synthesis, acyl glutamine)
Hydroxy-CMPF	1.39×10^{-3}	1.00×10^{-2}	Lipid	Fatty acid metabolism (dicarboxylate, amino, synthesis, acyl glutamine)
3-Beta-hydroxy-5-cholestenoate	1.96×10^{-3}	1.27×10^{-2}	Lipid	Sterol and mevalonate metabolism
Heptanoate (7:0)	2.11×10^{-3}	1.31×10^{-2}	Lipid	Medium-chain fatty acid

[#]: p-values obtained *via* linear regression adjusted for age, sex, chronic lung disease, chronic kidney disease and infectious aetiology. [¶]: Adjusted p-values obtained using the Benjamini–Hochberg procedure. 25 additional significant differentially abundant metabolites are shown in supplementary table S4G. CMPF: carboxy-4-methyl-5-propyl-2-furanpropanoate; SAM: S-adenosylmethionine.

We found that four metabolites (MNA, 1,5-anhydroglucitol (1,5-AG), heptanoate (7:0) and carboxyethyl-GABA) predicted 28-day mortality in CAP. This four-metabolite model augmented both the CURB-65 and modified SOFA scores for mortality prediction and this limited metabolic panel predicted mortality with accuracy at least as good as these clinical risk scores. This finding indicates the utility of integrating molecular and clinical factors for CAP outcome prognostication. Heptanoate (7:0) is a medium-chain fatty acid anion that was found to be protective in Mendelian randomisation studies of sepsis through unclear mechanisms. The authors speculated that higher levels of heptanoate (7:0) could correct mitochondrial respiratory impairment that occurs during severe infection [39, 40]. In our study, CAP survivors had higher plasma concentrations of heptanoate (7:0), providing additional evidence that it may be a protective factor in severe infection. 1,5-AG and MNA have not previously been associated with CAP mortality. As discussed, higher MNA levels in CAP nonsurvivors may reflect dysregulation of the NAD^+ pathway. 1,5-AG is a marker of short-term glycaemic control with a shorter timeframe than haemoglobin A1c, reflecting glucose fluctuations over several days to 2 weeks [41]. It is possible that high levels reflect impaired glucose control, which is a known risk factor for CAP mortality [42, 43]. Given the intricate relationship between NAD^+ and glucose metabolism, it is interesting that two metabolites in these pathways had the highest selection frequency in LASSO for mortality prediction, raising the possibility of a unifying metabolic derangement contributing to detrimental CAP host responses [44].

Our study does have important limitations. The recruitment of hospitalised patients from a single referral centre in northeastern Thailand may limit generalisability, although these individuals were referred from 38 hospitals across the region. Differences in metabolite abundance between groups could be due to factors other than the host immune response including patient characteristics, diet, treatments, sedating medications and some metabolites originating from the infecting pathogen rather than from the host. We attempted to address this by including relevant covariables in the models and by excluding drug metabolites. Though the CAP-specific mortality analysis results are intriguing, they must be interpreted with caution as subgroup analyses can lead to spurious associations. Interaction analysis was not performed given the substantial loss of power inherent to high-dimensional interaction analyses. The proportion of infectious aetiologies in both the CAP and non-CAP groups do not reflect true epidemiology of the region, as the parent metabolomic study was designed to over-represent patients with melioidosis. A sensitivity analysis with a more balanced representation of infectious aetiologies was performed to address this limitation. Finally, given the uniqueness of these data in a resource-limited setting, a suitable cohort was not available for external validation.

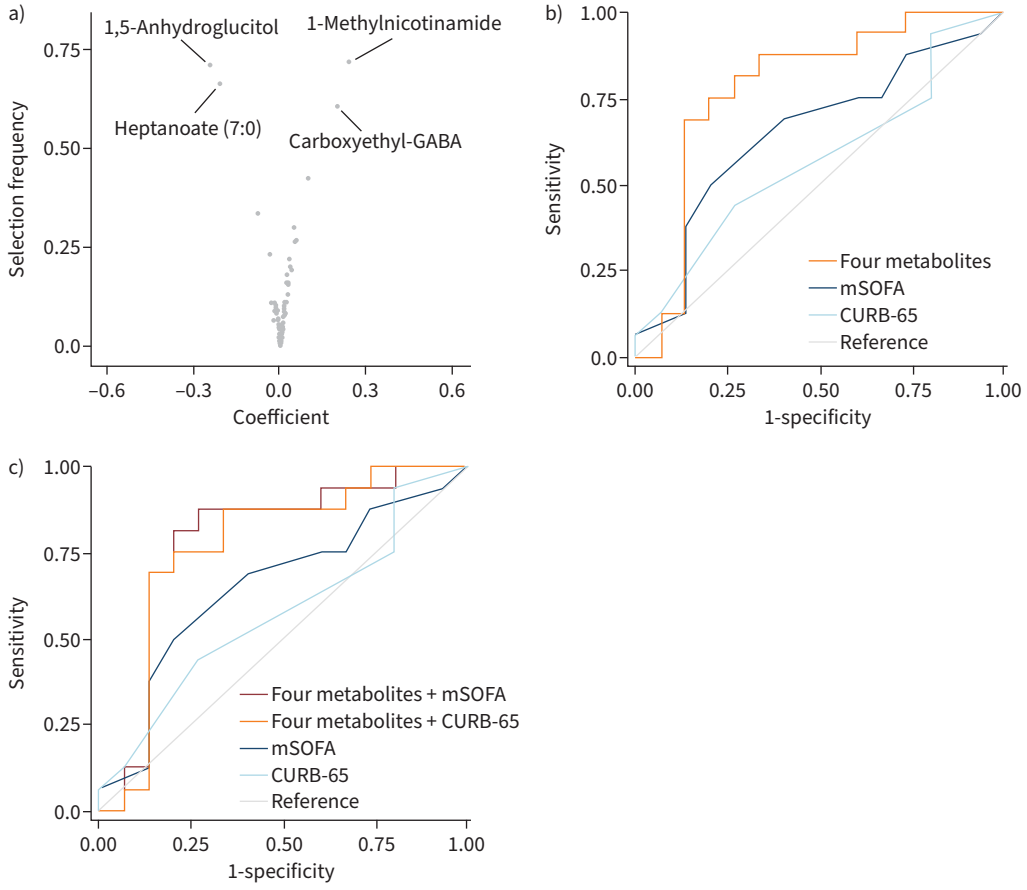

FIGURE 4 a) Average selection frequency (*y*-axis) and coefficient estimates (*x*-axis) in the repeated sample splitting with LASSO (least absolute shrinkage and selection operator) for identifying fatal community-acquired pneumonia (CAP) cases in the derivation set (n=75). b) Receiver operating characteristic curve for identifying fatal CAP cases in the validation set (n=32) set using the four-metabolite signature (1-methylnicotinamide, 1,5-anhydroglucitol, heptanoate (7:0) and carboxyethyl-γ-aminobutyric acid (GABA)). Receiver operating characteristic curves are shown for CURB-65 (confusion, urea >19 mg·dL^{-1}, respiratory rate ⩾30, systolic blood pressure <90 mmHg or diastolic blood pressure ⩾60 mmHg, and age ⩾65 years), modified Sequential Organ Failure Assessment (mSOFA) score and the four-metabolite model (area under the receiver operating curve (AUC) 0.57, 0.62 and 0.79, respectively). c) Receiver operating characteristic curves for CURB-65, mSOFA score, CURB-65 plus the four-metabolite model and mSOFA score plus the four-metabolite model (AUC 0.57, 0.62, 0.77 and 0.80, respectively).

Overall, our study significantly expands upon previous studies of the metabolomic response to CAP. It is the first study to compare the metabolomes of CAP *versus* non-CAP infection directly and the first metabolomic study of CAP in a resource-limited setting. Our data demonstrate profound metabolic alterations in CAP compared with controls and identify changes that were CAP-specific including several associated with mortality. Future mechanistic studies are needed to verify the role of identified metabolites in the CAP host response. Addressing these knowledge gaps will enhance our understanding of CAP pathophysiology and inform the development of host-directed therapies.

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Data availability: Deidentified demographic information and metabolomic data will be deposited in Metabolomics Workbench.

Provenance: Submitted article, peer reviewed.

Ethics statement: This study was approved by the Mahidol University Faculty of Tropical Medicine Ethics Committee (MUTM 2012-024-01, MUTM 2018-046-01 and MUTM 2024-022-01), the Sunpasitthiprasong Hospital Ethics Committee (039/2556), the Udon Thani Hospital Ethics Committee, the University of Washington Institutional Review Board (42988) and the University of Oxford Tropical Research Ethics Committee (OXTREC172-12). Informed consent was obtained from all study participants or their representatives.

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