Differential metabolic host response to pathogens associated with community-acquired pneumonia

Hartog, I. den; Karu N.; Zwep, L.B.; Voorn, P.G.; Garde, E.M.W. van de; Hankemeier, T.; Hasselt, C.J.G. van

Metabolism Open 18 (2023) 100239

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.sciencedirect.com/journal/metabolism-open

Differential metabolic host response to pathogens associated with community-acquired pneumonia

Ilona den Hartog [a,b], Naama Karu [b], Laura B. Zwep [a], G. Paul Voorn [c], Ewoudt M.W. van de Garde [d,e], Thomas Hankemeier [b], J.G. Coen van Hasselt [a,*]

[a] Division of Systems Pharmacology & Pharmacy, Leiden Academic Centre for Drug Research, Leiden University, Leiden, the Netherlands
[b] Metabolomics and Analytics Centre, Leiden Academic Centre for Drug Research, Leiden University, Leiden, the Netherlands
[c] Department of Medical Microbiology and Immunology, St. Antonius Hospital, Nieuwegein, the Netherlands
[d] Division of Pharmacoepidemiology and Clinical Pharmacology, Department of Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands
[e] Department of Clinical Pharmacy, St. Antonius Hospital, Nieuwegein, the Netherlands

ARTICLE INFO

Keywords:
Metabolomics
Immune response
Lipids
Amines
Carnitines
Biomarkers
Community-acquired pneumonia

ABSTRACT

Background: Metabolic changes induced by the host immune response to pathogens found in patients with community-acquired pneumonia (CAP) may provide insight into its pathogenesis. In this study, we characterized differences in the host metabolic response to common CAP-associated pathogens.
Method: Targeted metabolomic profiling was performed on serum samples obtained from hospitalized CAP patients (n = 119) at admission. We quantified 347 unique metabolites across multiple biochemical classes, including amines, acylcarnitines, and signaling lipids. We evaluated if unique associations between metabolite levels and specific CAP-associated pathogens could be identified.
Results: Several acylcarnitines were found to be elevated in *C. burnetii* and herpes simplex virus and lowered in *M. pneumoniae* as compared to other pathogens. Phenylalanine and kynurenine were found elevated in *L. pneumophila* as compared to other pathogens. S-methylcysteine was elevated in patients with *M. pneumoniae*, and these patients also showed lowered cortisol levels in comparison to almost all other pathogens. For the herpes simplex virus, we observed a unique elevation of eicosanoids and several amines. Many lysophosphatidylcholines showed an altered profile in *C. burnetii* versus S. *pneumoniae, L. pneumophila,* and respiratory syncytial virus. Finally, phosphatidylcholines were negatively affected by the influenza virus in comparison to *S. pneumoniae*.
Conclusions: In this exploratory analysis, metabolites from different biochemical classes were found to be altered in serum samples from patients with different CAP-associated pathogens, which may be used for hypothesis generation in studies on differences in pathogen host response and pathogenesis of CAP.

1. Introduction

Community-acquired pneumonia (CAP) is a common infection of the lower respiratory tract, caused by bacterial or viral pathogens [1]. Causative pathogens that are commonly found in CAP patients include *Streptococcus pneumoniae*, respiratory viruses, *Haemophilus influenzae*, and, to a lesser extent *Mycoplasma pneumoniae* and *Legionella pneumophila* [1–3]. CAP is associated with high mortality and morbidity, especially in the elderly [4,5], and with distinct differences in clinical outcomes between different CAP-associated pathogens [6,7].

There is an increasingly recognized role of the patient-associated host metabolic response to infection and its association with overall innate immune system activation and clinical outcomes [8–10]. Previous studies have found distinct effects of this host metabolic response in COVID-19 and sepsis [11–14]. We previously evaluated the discriminatory power of host-associated metabolites to support the microbial diagnosis of CAP and identified metabolic biomarkers to support the diagnosis of atypical bacterial pathogens [15].

In this study, we aimed to further characterize the differential metabolic host response associated with distinct CAP-associated pathogens, which may support understanding the role of metabolic changes in the pathogenesis associated with specific pathogens. To this end, we quantified 347 unique metabolites in 119 serum samples taken at

* Corresponding author.
E-mail address: coen.vanhasselt@lacdr.leidenuniv.nl (J.G.C. van Hasselt).

https://doi.org/10.1016/j.metop.2023.100239
Received 3 February 2023; Accepted 20 March 2023
Available online 23 March 2023

Abbreviations

AHR	aryl-hydrocarbon receptor
aLEA	alpha-linolenoyl ethanolamide
ANOVA	analysis of variance
BH4	tetrahydrobiopterin
C. burnetii	*Coxiella burnetii*
C. psittaci	*Chlamydophila psittaci*
CAP	community-acquired pneumonia
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
COX	cyclooxygenase
CRP	C-reactive protein
DEA	docosatetraenoyl ethanolamide
DGLEA	dihomo-gamma-linolenoyl ethanolamide
EA	ethanolamine
FC	fold change
FDR	false discovery rate
Gln	glutamine
Glu	glutamic acid
HPA axis	hypothalamic–pituitary–adrenal axis
HSV	herpes simplex virus
IDO	indoleamine-2,3-dioxygenase
L. pneumophilia	*Legionella pneumophilia*
LCAC	long-chain acylcarnitine
LOX	lipoxygenase
LPC	lysophosphatidylcholine
LPE	lysophosphatidylethanolamine
LPI	lysophosphatidylinositol
LPS	lysophosphatidylserine
M. pneumoniae	*Mycoplasma pneumoniae*
MCAC	medium-chain acylcarnitine
NMDA	N-methyl-D-aspartate
NOS	nitric oxide synthase
NSAID	non-steroidal anti-inflammatory drugs
PC	phosphatidylcholine
PCA	principal component analysis
PGE2	prostaglandin E2
PGF2a	prostaglandin F2 alpha
Phe	phenylalanine
PLA2	phospholipase A2
POEA	palmitoleoyl ethanolamide
PSI	Pneumonia Severity Index
PUFA	polyunsaturated fatty acids
q	p-adjusted significance
ROS	reactive oxygen species
RS virus	respiratory syncytial virus
S. pneumoniae	*Streptococcus pneumoniae*
SCAC	short-chain acylcarnitine
SDMA	symmetric dimethylarginine
TDO	tryptophan-2,3-dioxygenase
TriHOME	9,10,13-TriHOME
Trp	tryptophan
TXB2	thromboxane B2
Tyr	tyrosine

hospital admission from patients with CAP and studied their association with the pathogens found in these patients.

2. Materials and methods

2.1. Study population

We analyzed previously collected serum samples obtained from 119 hospitalized patients with CAP, obtained as part of two previously conducted clinical studies executed between October 2004 and September 2010 and collected within 24 h after hospital admission [2, 16]. For all patients, microbial disease etiology was confirmed using conventional diagnostic methods such as culturing, PCR, and urinary antigen tests. Inflammatory markers such as CRP and leukocyte counts were also available. Most patients (80%) were diagnosed with bacterial infections and the sample size per pathogen varied between 3 and 48 samples. Pathogen groups, a group of patients in which the same pathogen was found, including only one patient were excluded. The patient distribution per pathogen showed no significant differences in sex and pneumonia severity index (PSI) score. However, the mean age differed significantly between pathogen groups ($p < 0.05$). All patients that participated in the previous clinical studies provided informed consent for the secondary use of their materials. Data were handled in an anonymized way. An overview of the patient characteristics is provided in

Table 1
Patient characteristics per CAP-associated pathogen.

Patient characteristic	*S. pneumoniae* (n = 48)	*L. pneumophila* (n = 18)	*C. burnetii* (n = 17)	*C. psittaci* (n = 7)	*M. pneumoniae* (n = 5)	Influenza virus (n = 11)	HS virus (n = 6)	RS virus (n = 4)	Parainfluenza virus (n = 3)
Age (years) *									
Mean (SD)	62.2 (18.9)	62.9 (12.5)	47.4 (10.0)	62.6 (14.5)	38.8 (10.4)	67.0 (15.9)	71.3 (18.6)	69.3 (25.9)	83.3 (5.77)
Sex									
Male	22 (45.8%)	13 (72.2%)	14 (82.4%)	5 (71.4%)	2 (40.0%)	9 (81.8%)	5 (83.3%)	2 (50.0%)	2 (66.7%)
PSI score									
<50	9 (18.8%)	0 (0%)	5 (29.4%)	1 (14.3%)	3 (60.0%)	1 (9.1%)	0 (0%)	1 (25.0%)	0 (0%)
51–70	7 (14.6%)	3 (16.7%)	9 (52.9%)	0 (0%)	1 (20.0%)	3 (27.3%)	1 (16.7%)	0 (0%)	0 (0%)
71–90	5 (10.4%)	6 (33.3%)	1 (5.9%)	2 (28.6%)	1 (20.0%)	1 (9.1%)	1 (16.7%)	1 (25.0%)	0 (0%)
91–130	23 (47.9%)	7 (38.9%)	1 (5.9%)	4 (57.1%)	0 (0%)	6 (54.5%)	2 (33.3%)	1 (25.0%)	2 (66.7%)
131>	4 (8.3%)	2 (11.1%)	1 (5.9%)	0 (0%)	0 (0%)	0 (0%)	2 (33.3%)	1 (25.0%)	1 (33.3%)
Duration of symptoms before admission *									
Mean (SD)	4.06 (3.03)	5.11 (1.23)	5.06 (1.71)	4.14 (2.04)	13.4 (16.1)	4.45 (2.34)	5.17 (4.45)	6.50 (5.07)	4.00 (2.65)
CRP upon admission									
Mean (SD)	265 (166)	306 (90.8)	233 (115)	272 (110)	145 (86.0)	207 (87.9)	271 (121)	289 (135)	220 (142)
Leukocyte count upon admission*									
Mean (SD)	16.9 (5.99)	13.0 (4.24)	9.49 (1.56)	10.7 (5.03)	10.5 (3.29)	10.1 (4.93)	15.3 (4.89)	14.2 (7.94)	13.7 (9.30)

* Significant difference between pathogens (p-value <0.05). Abbreviations: HS: herpes simplex; RS: respiratory syncytial; PSI: pneumonia severity index; CRP: C-reactive protein; SD: standard deviation.

Table 1 and additional information is available in Table A1.

2.2. Bioanalytical procedures

Metabolomic profiling was performed by the Biomedical Metabolomics Facility of Leiden University (BMFL), Leiden, The Netherlands. Details of the bio-analytical procedures are described elsewhere [15]. Briefly, the serum samples were quantitatively analyzed using five liquid- and one gas-chromatography methods coupled to mass spectrometry detectors, with a total coverage of 596 metabolite targets from 25 biochemical metabolite classes such as organic acids, amino acids, biogenic amines, acylcarnitines, and lipids. Following data integration and quality control steps, 347 unique metabolites were reported and underwent statistical analysis.

2.3. Data analysis

The metabolite levels were log-transformed and standardized prior to statistical analysis. Biological and biochemical relevant sums and ratios of metabolites (Table A.2) were calculated from the raw metabolite data and were added as variables to the dataset, undergoing the same analysis together with metabolites.

Exploratory data analysis was performed using principal component analysis (PCA). To identify differences in metabolite levels between pathogens, we performed analysis of variance (ANOVA) with a false discovery rate (FDR) multiple testing correction. P-adjusted significant metabolites ($q < 0.1$) were tested with Tukey's posthoc test to identify which pathogen groups the metabolite was able to distinguish between. In addition to the significance threshold, we applied a cutoff of >20% fold change between the medians of the raw metabolite levels of each group.

All data analysis was performed using R. The scripts used for the data analysis are available on GitHub at http://github.com/vanhasseltlab/MetabolicHostResponseToCAP.

3. Results and discussion

3.1. Metabolic markers differentiate between pathogens

The metabolomics analysis of the patient serum samples yielded 347 quantifiable unique metabolites from different biochemical metabolite classes. No visible separation between samples obtained from patients infected by the various pathogens was apparent using PCA (Fig. A.1). Systematic analysis of individual metabolites for a specific pathogenic species yielded 64 unique metabolites that were significantly altered (ANOVA, $q < 0.1$ and FC > 20%) as compared to other pathogenic species (Figs. 1–5). All significant results are shown together in Fig. A.3 and Table A.3. Large variation in group size for specific pathogenic species (n = 3–48) and large within pathogen species group variation was present (Fig. A.2), limiting the ability to identify associations between metabolites and specific pathogenic species. Nonetheless, although often not statistically significant, trends between groups of metabolites and specific pathogenic species were apparent, which is why we have maintained these in visualization for each metabolic class (Figs. 1–4). The discriminating metabolites belong to different biochemical classes. The results are reported by biochemical class to allow focused biochemical discussion. Fig. 5 provides a comprehensive qualitative overview of the main associations between pathogen species and metabolites within their biochemical context.

3.1.1. Carnitine and acylcarnitines

We identified significant differences in carnitines between serum samples of CAP patients that carried the pathogens *C. burnetii*, herpes simples virus (HSV), or *M. pneumoniae* compared to CAP patients with other pathogens (Fig. 1). The most consistent and statistically significant differences were found in patients with *C. burnetii* compared to patients with *L. pneumophila* or *S. pneumoniae* infections. Patients with C. *burnetii* showed 60–80% elevated long-chain acylcarnitines (LCAC), leading to a 2-fold lower ratio of free carnitine/total acylcarnitines. Changes in acylcarnitines can be related to mitochondrial energy production shifts between glycolysis and beta-oxidation of long-chain fatty acids [17,18]. Naturally, such shifts depend on the glucose stores and the availability of carnitine as a limiting factor.

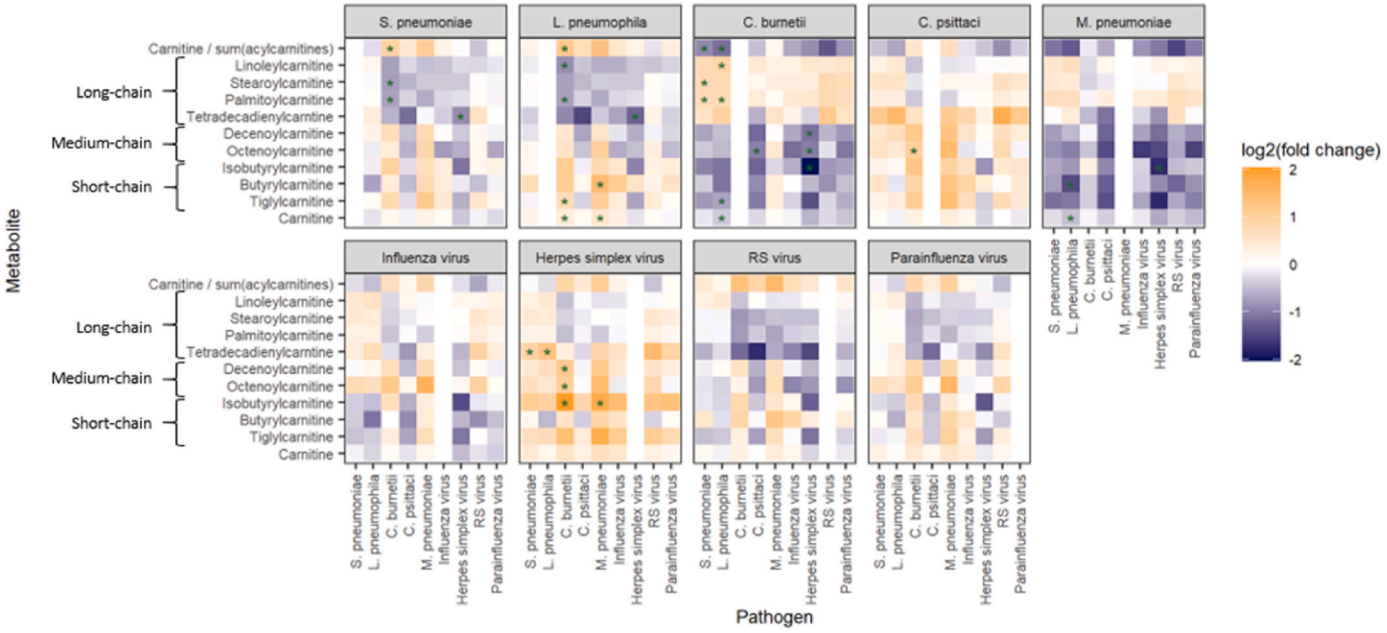

Fig. 1. Fold changes of carnitine and acylcarnitines as median values per pathogen group compared to CAP patients with other pathogens. Significant differences ($q < 0.1$ and FC > 20%) are marked with a green star. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

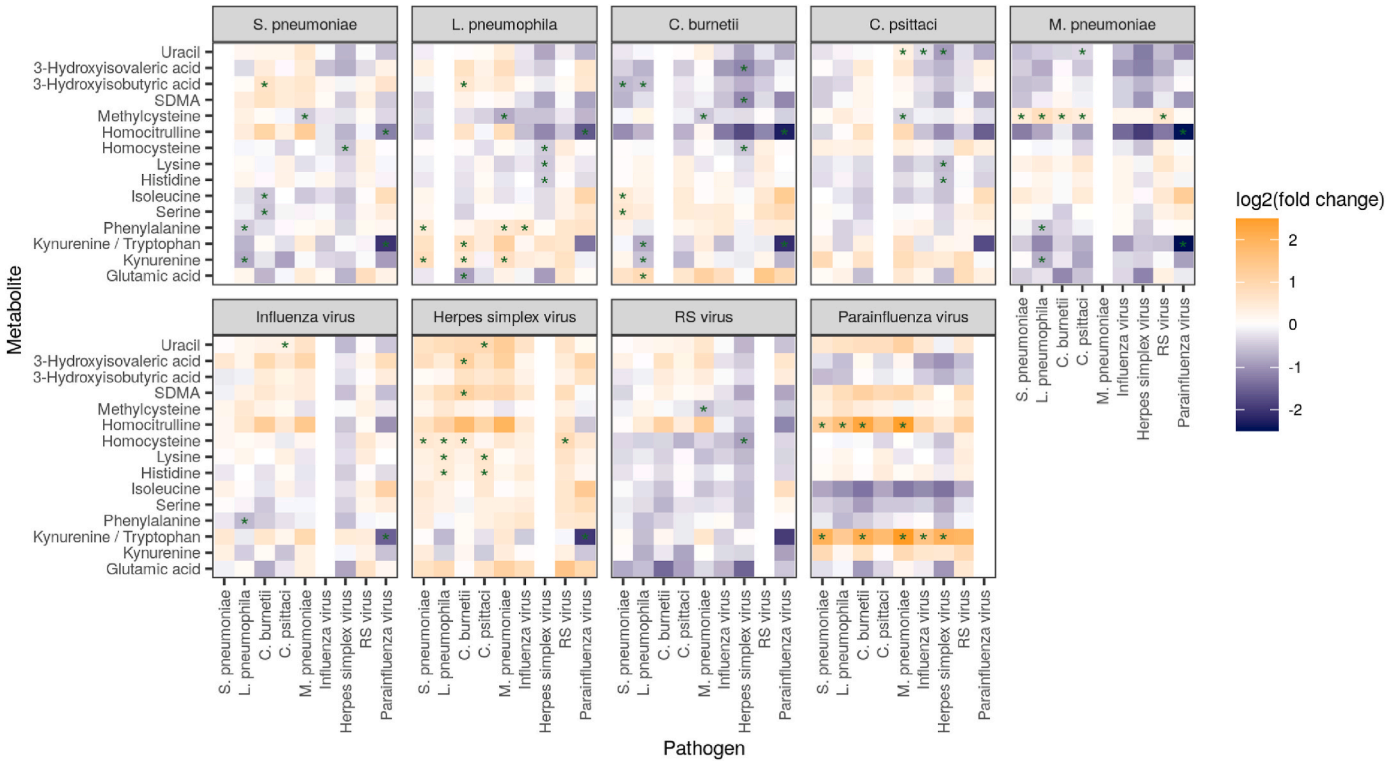

Fig. 2. Fold changes of organic acids, amino acids, and derivatives as median values per pathogen group compared to CAP patients with other pathogens. Significant differences ($q < 0.1$ and $FC > 20\%$) are marked with a green star. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

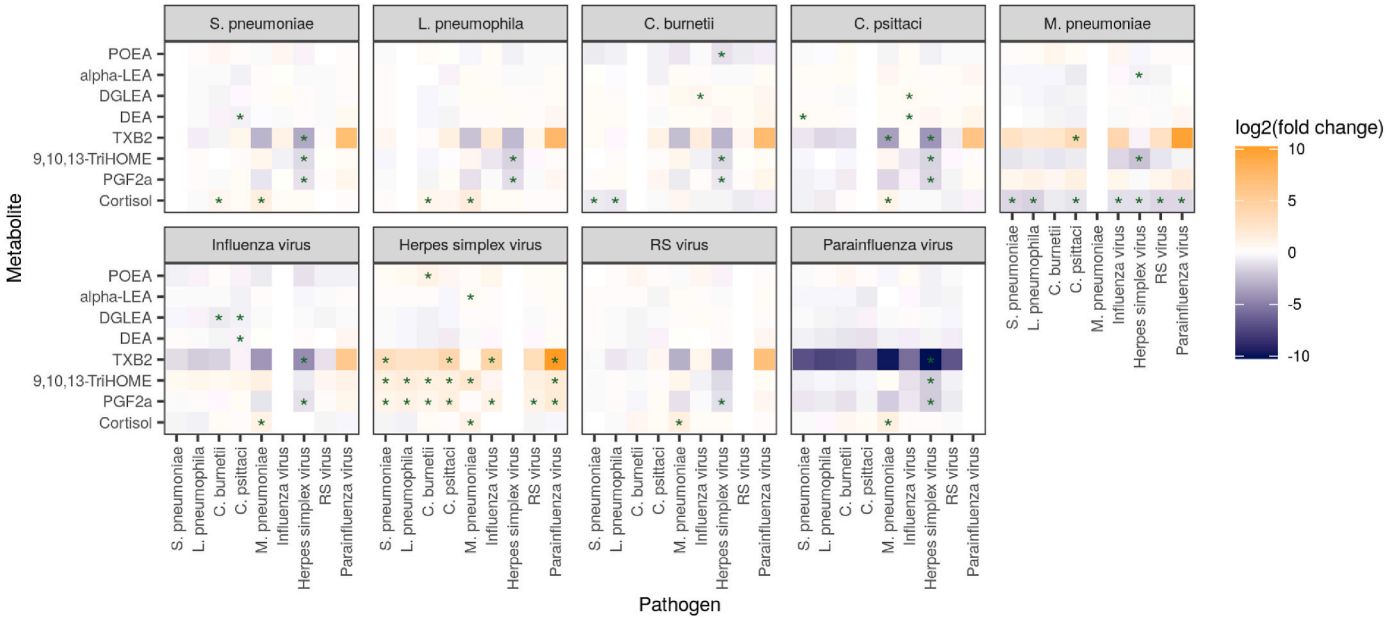

Fig. 3. Fold changes of oxylipin, endocannabinoid, and cortisol as median values per pathogen group compared to CAP patients with other pathogens. Significant differences ($q < 0.1$ and $FC > 20\%$) are marked with a green star. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

We observed increased levels of carnitine and short-chain acylcarnitines (SCAC) for *L. pneumophila* vs. *C. burnetii* and *M. pneumophila*. Elevated carnitine and short-chain acylcarnitines suggest successful fatty acid beta-oxidation. Conversely, we also saw elevated mid-chain acylcarnitines (MCAC), most pronounced in patients with herpes simplex virus versus *C. burnetii*. MCAC could indicate incomplete beta-oxidation of long-chain fatty acids and may be related to mitochondrial dysfunction in the diseased state [19]. In contrast, LCAC were elevated mainly in patients with *C. burnetii* infection, suggesting a low rate of beta-oxidation rather than an incomplete process. It is unknown how well the levels of circulating acylcarnitines reflect the cellular levels, however, accumulation of long-chain acylcarnitines in lung cells

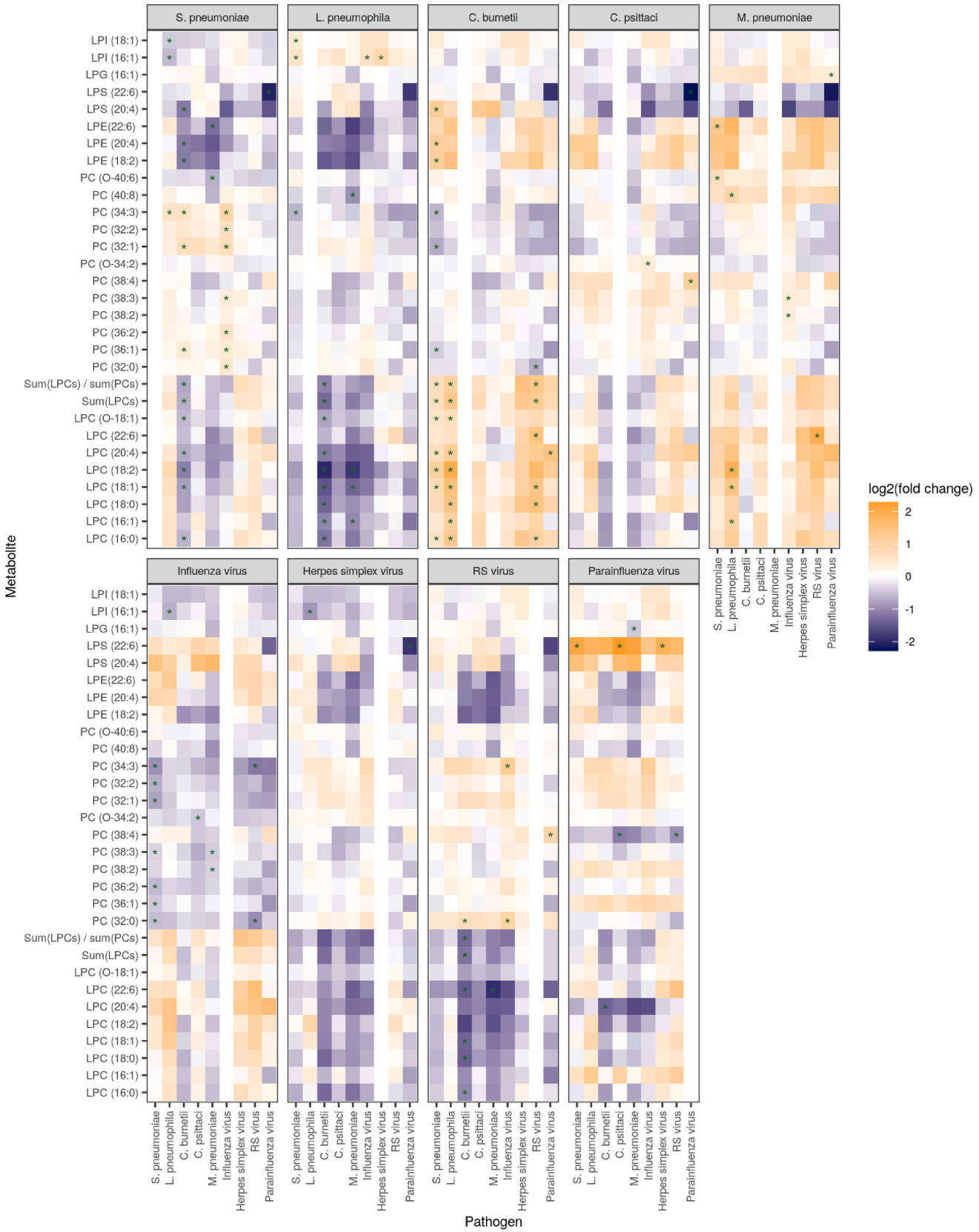

Fig. 4. Fold changes of phospholipids including (lyso)phosphatidylcholines median values per pathogen group as median values per pathogen group compared to CAP patients with other pathogens. Significant differences ($q < 0.1$ and $FC > 20\%$) are marked with a green star. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

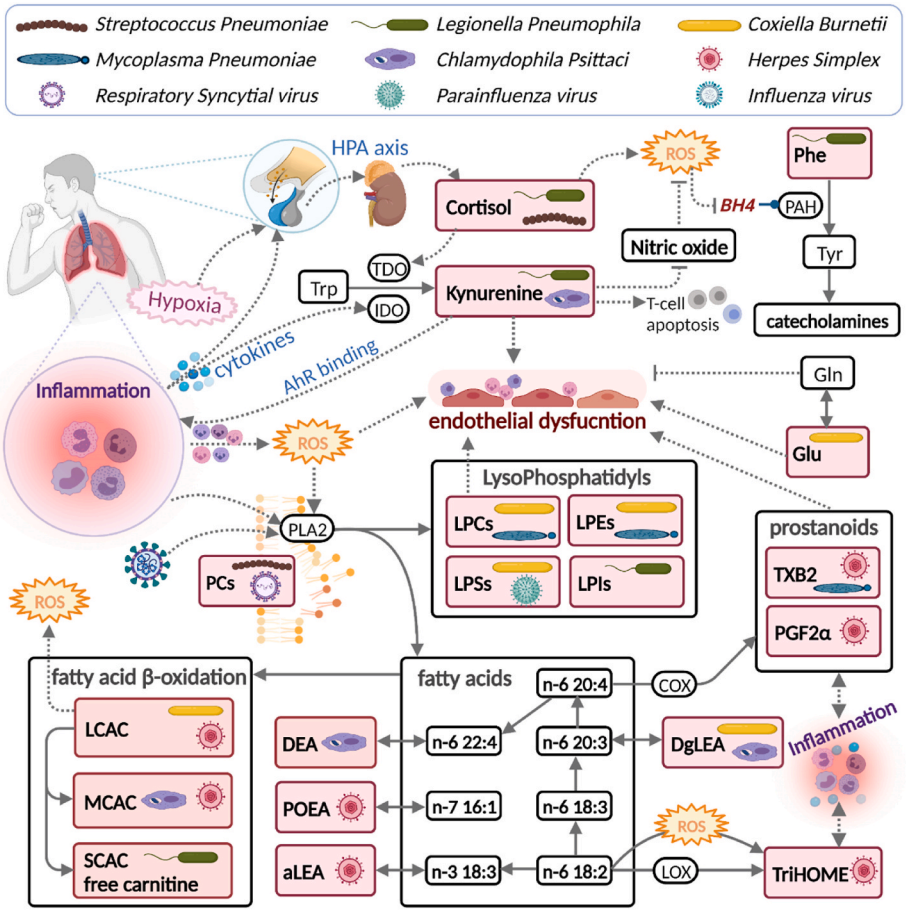

Fig. 5. Overview of differential metabolic host response associations for community-acquired pneumonia-associated pathogens inferred from the current analysis. A pathogen symbol indicates that this patient group had the highest levels of metabolite(s) in the red box. Metabolites that were either not detected or detected without significant results are in white boxes. Enzymes are in ellipses, full lines are part of a metabolic pathway or transformation, and broken lines indicate an inducing or inhibiting effect. Abbreviations: see the abbreviation list. This figure was created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

indicated a defective beta-oxidation process, increasing oxidative stress and potentially leading to epithelial cell death [20]. Moreover, LCAC accumulation in the air-fluid interface of the lung can reduce lung function due to the inhibition of pulmonary surfactant [21].

It has been reported that impaired utilization of acylcarnitines for energy production can be caused by various viruses, including influenza and respiratory syncytial virus [20], although the latter two did not show any significant results in our study.

Overall, for acylcarnitines high within-pathogen group variance was present (Fig. A.2), which limited identifying further metabolite-pathogen associations. Acylcarnitine levels are associated with food consumption, certain medications such as valproic acid, and carnitine supplementation [22–24].

3.1.2. Organic acids, amino acids, and related compounds

Significant differences in serum amino acids and derivatives were found in all groups of patients infected by CAP-associated pathogens (Fig. 2). Several significantly different metabolites were only found in infections by specific pathogens. For example, patients with *L. pneumophila* had elevated kynurenine (1.5–1.7 fold) and phenylalanine (1.4–1.6 fold), while *M. pneumoniae* showed elevated S-methylcysteine (1.3–1.7 fold). Also, glutamate was elevated in *C. burnetii* compared to *L. pneumophila* (1.8 fold). Most of the stronger findings can be related to inflammation and oxidative stress and were associated with negative health outcomes in studies of various infections [25].

Elevated kynurenine was observed in patients with CAP and sepsis [26,27], and severe COVID-19 [12,25,28,29]. Kynurenine increases due to enhanced IDO (Indoleamine-2,3-dioxygenase) conversion of tryptophan during the inflammatory response. IDO is expressed in the lungs and blood cells, and its activity and expression are induced by pro-inflammatory cytokines, viral proteins, and lipopolysaccharides [30–32]. Kynurenine acts as an immune suppressor via binding to the aryl-hydrocarbon receptor (AHR) [33], and also inhibits nitric oxide synthase (NOS), thereby increasing oxidative stress. Kynurenine also contributes to vasodilation and hypotension in sepsis [14,34] and increases endothelial activation which leads to impaired microvascular reactivity [26,35].

Elevated phenylalanine was associated with immune activation and negative outcome in patients with COVID-19 [11,12,25], post-trauma, or sepsis [13,14]. A possible explanation for elevated phenylalanine in the inflammatory environment is ROS-induced oxidation of the cofactor tetrahydrobiopterin (BH4) which is essential for the conversion of phenylalanine to tyrosine and the downstream catecholamines by the enzyme phenylalanine 4-hydroxylase [13,36].

Glutamate, a neurotransmitter in the CNS, also modulates the activity of immune cells via the NMDA receptor, and excessive production was related to damaged endothelial cells [37–39].

We also found elevated uracil in patients with influenza and herpes simplex virus compared to patients with *C. psittaci* (1.2–1.9 fold). Patients with *S. pneumoniae* and *L. pneumophila* showed higher 3-hydroxyisobutyric acid (1.5–1.6 fold) and lower isoleucine (1.4 fold lower) compared to *C. burnetii*.

3.1.3. Signaling lipids

The signaling lipids covered in the applied platforms included oxylipins, endocannabinoids, their precursor fatty acids, and also cortisol. Metabolites that showed significant results were included in Fig. 3.

Although we did not find significant differences in fatty acids in this study, the oxylipin profile of patients with herpes simplex virus was unique in comparison to other infections, showing significantly higher (all FC > 1.8) prostaglandin F2 alpha (PGF2a), 9,10,13-TriHOME (TriHOME), and thromboxane B2 (TXB2). Oxylipins are immune

modulators produced by the peroxidation of C20 or C22 polyunsaturated fatty acids (PUFA). The omega-6 PUFA oxylipins exhibit mainly pro-inflammatory action during the acute phase of inflammation. Some of these oxylipins also aid in the transition from innate to adaptive immune response. The arachidonic acid-produced PGF2-alpha is a direct metabolite of PGE2 and its increase aligns with the existing evidence that prostaglandin synthesis reactivates the herpes virus and increases its spread [40]. Although fluctuating along the course of inflammation, low levels of prostanoids (PGE2 and TXB2) are characteristic of sepsis non-survivors [41,42] and COVID-19 [43,44]. TriHOMEs were reported to be dysregulated in respiratory diseases such as asthma and COPD [45,46].

Of the covered endocannabinoids, several fatty acyl ethanolamides (EAs) were significantly different between patients with various infections. Docosatetraenoyl ethanolamide (DEA), which is the ethanolamide of the omega-6 adrenic acid (22:4) docosatetraenoic acid, was elevated in patients with *C. psittaci* compared to patients with *S. pneumoniae* or influenza virus (1.3–1.4 fold). Patients with the influenza virus showed the lowest levels of both DEA and dihomo-gamma-linolenoyl ethanolamide (DGLEA), the ethanolamide of the omega-6 (20:3) dihomo-gamma-linolenic acid. In comparison to other pathogens, patients with herpes simplex virus showed trends of elevated palmitoleoyl ethanolamide (POEA) and alpha-linolenoyl ethanolamide (aLEA), which are the ethanolamides of the omega-7 (16:1) palmitoleic acid, and the omega-3 (18:3) alpha-linolenic acid, respectively. A 2-fold significant elevation of POEA was measured in comparison to patients with *C. burnetii* and a 1.4-fold aLEA elevation in comparison to *M. pneumoniae*. Endocannabinoids do not only act as CNS modulators but are also peripheral immune mediators. Their pro- or anti-inflammatory activity varies and depends on their acyl group, the type of cells (immune; endothelial, etc.), and the receptors they bind to. In times of increased demand, for example during an acute immune response, and depending on diet and fat reserves, increased levels of endocannabinoids may reflect accelerated catabolism of cellular lipids, to serve as precursors for free fatty acids and oxylipins. It is also possible to see a shift towards the synthesis of fatty acyl EAs from fatty acids that occurs without medication [47], or due to treatment with corticosteroids and NSAIDs, that inhibit PLA2 and COX enzymes [48,49]. The last significant signaling lipid, cortisol, was lower in patients with *M. pneumoniae* compared to all other pathogens (2–3.3 fold lower), and lower for *C. burnetii* patients in comparison to *S. pneumoniae* and *L. pneumophila* patients (1.6 fold lower, Fig. A.3). Reduced excretion of cortisol indicates lower activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress and innate immune activation following any infection. Cortisol stimulates gluconeogenesis and reduces inflammation, therefore its levels also affect (directly or indirectly) other metabolites measured in this study. In multiple studies on infections of the lower respiratory system, higher cortisol levels at hospital admission were predictive of mortality [50], indicating its relation to acute inflammation and disease severity.

3.1.4. Phospholipids and derivatives

Phospholipids form cell membranes and are hydrolyzed into lysophospholipids and long-chain fatty acids by various phospholipase A2 (PLA2) enzymes. The host's innate immune response to infection, as well as the pathogen itself, can induce the activity of PLA2 [51]. Patients with *S. pneumoniae* infection showed the lowest levels of any lysophospholipids (LPCs) compared to other pathogens, alongside the highest levels of intact phosphatidylcholines (PCs), suggesting reduced activity of PLA2. Such a profile was also characteristic of sepsis [50,52], possibly due to the varying degree of expression and activities of different PLA2 enzymes. Many LPCs and the total LPC/PC ratio were significantly higher in *C. burnetii* compared to *S. pneumoniae*, *L. pneumophila*, and RS virus (1.5–4 fold, Fig. 4). Bacteria can use host lipids as building blocks for bacterial membrane formation and as an energy source. Also, host lipids are crucial for the entry of bacteria, viruses or toxins into cells. For example, *C. burnetii* has been found to use phospholipase A to integrate lipids into its membrane [53]. *L. pneumophila* increased its virulence by secretion of PLA2 that destructed alveolar surfactant phospholipids [54]. Conversely, many intact PCs showed significantly lower levels in patients with the influenza virus in comparison to those with *S. pneumoniae*. The M protein of viruses like the influenza virus can interact with lipids and phosphatidylcholine vesicles allowing membrane fusion [55–58].

Regarding lysophosphatidyls with serine or inositol functional groups (LPS and LPI), patients with parainfluenza virus showed 3–5 fold higher LPS (22:6) compared to *S. pneumoniae*, *C. psittaci,* and herpes simplex virus. Patients with L. *pneumophila* infection showed 30–90% higher LPIs compared to *S. pneumoniae*, influenza, and herpes simplex virus.

3.2. Summary of metabolic findings per pathogen

The consolidation of the obtained results associates specific bacterial and viral CAP infections with metabolic perturbations related to inflammation, oxidative stress, lipids utilization, and energy production (Fig. 5). Patients with *S. pneumoniae* infection showed high levels of the stress hormone cortisol that can be excreted as an HPA axis response to pro-inflammatory cytokines and hypoxia. Moreover, patients with *S. pneumoniae* infections consistently showed the highest PCs in comparison to all other pathogen groups, and very low lysophospholipids, suggesting lower activation of PLA2 enzymes, as described before in studies on sepsis [14,50]. The lowest levels of LPCs were found in patients with *L. pneumophilia* infection, who also showed the highest levels of cortisol, kynurenine, and phenylalanine, all characteristic of acute inflammatory response. Possibly indicating signs of oxidative stress and potentially also mitochondrial dysfunction [59], these patients had increased production of energy via beta-oxidation of fatty acids, evidenced by the elevated end-products of free carnitine and short-chain acylcarnitines. In comparison, patients with *C. burnetii* infection showed inhibition of fatty acid beta-oxidation, suggested by high levels of long-chain acylcarnitines, which are associated with increased ROS. These patients also had elevated glutamate accompanied by the highest levels of lysophospholipids, all can be linked to either inflammation, oxidative stress, or endothelial dysfunction [38,39,50,57,60]. Patients with *C. psittaci* infection possibly suffered from metabolic defects causing incomplete beta-oxidation of long-chain fatty acids, suggested by the elevated mid-chain fatty acids. This interrupted metabolism may be related to their relatively high levels of omega-6 fatty acyl ethanolamides, and high kynurenine, indicating acute inflammation. Despite the small sample size, patients with herpes simplex infection showed unique disruption in lipid metabolism. This included accumulation of long- and medium-chain acylcarnitines, elevated non-omega-6 fatty acyl ethanolamides, and very high levels of certain oxylipins, that possibly support the spread of the infection.

3.3. Study limitations and future perspectives

In this study, the sample size of patient groups with different CAP-associated pathogenic species was unbalanced and, in several cases, low (Table 1). Furthermore, a large within-group variance of metabolite levels was observed, which may impact our findings, especially in small sample size pathogen groups. Finally, the mean age of patients was unbalanced across the cohort, possibly acting as an additional confounder, since many aspects of metabolism change with age [61]. Overall, these factors limit the power to detect statistically significant metabolite-pathogen associations and require careful consideration of the relationships identified and further validation in a larger and more balanced study cohort. Nonetheless, several of the identified relationships are in line with expectations and other literature reports, supporting the biological relevance of our findings.

Our study only considered metabolite levels at a single time point,

even though patients are admitted to the clinic at different stages of their infection. The use of longitudinal metabolite levels measured in patients thus represents an important next step to help to correct for such differences, and, to elucidate the relationship between changes in metabolite levels and biochemical or cellular biomarkers for immunological and/or inflammatory response markers, e.g. C-reactive protein levels, cytokines, and leukocyte cell counts [62].

Importantly, we have made available the full raw dataset underlying this study, which could be of interest for further bioinformatics analyses of the immune-metabolic responses reported [63].

4. Conclusion

In this study, we identified metabolites associated with the host response to specific CAP-associated pathogens. Our findings may support further elucidation of the pathogenesis of specific pathogens. We identified 64 metabolites that were significantly different between several pathogens. Specific bacterial and viral pathogens could be associated with metabolic perturbations that can be related to inflammation, oxidative stress, lipids utilization, and energy production. Further research should be conducted to validate our results and to further unravel the mechanisms of the metabolic host response to different pathogens causing CAP.

Funding

This work is part of the research program 'Metabolomic fingerprint biomarkers to guide antibiotic therapy and reduce resistance' with project number 541001007, which is financed by ZonMW, the Netherlands Organization for Health Research and Development associated with the Dutch Research Council (NWO). The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Author contributions

Ilona den Hartog: conceptualization, methodology, investigation, formal analysis, visualization, data curation, writing - original draft. **Naama Karu**: methodology, formal analysis, visualization, writing – original draft. **Laura B. Zwep**: methodology, formal analysis, visualization, writing – review, and editing. **G. Paul Voorn**: writing – review, and editing. **Ewoudt M.W. van de Garde**: conceptualization, supervision, writing – review, and editing. **Thomas Hankemeier**: supervision, writing – review, and editing. **J.G.C. van Hasselt**: conceptualization, methodology, formal analysis, visualization, supervision, writing – review, and editing.

Declaration of competing interest

None to declare.

Acknowledgments

We thank Marinda van der Kuijl and Maik Kok for the critical review of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2023.100239.

References

[1] Torres A, Cilloniz C, Niederman MS, Menéndez R, Chalmers JD, Wunderink RG, et al. Pneumonia. Nat Rev Dis Prim 2021 Apr 8;7(1):1–28. 2021 7:1.
[2] Meijvis SCA, Hardeman H, Remmelts HHF, Heijligenberg R, Rijkers GT, Van Velzen-Blad H, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. Lancet 2011;377(9782):2023–30.
[3] Endeman H, Schelfhout V, Paul Voorn G, van Velzen-Blad H, Grutters JC, Biesma DH. Clinical features predicting failure of pathogen identification in patients with community acquired pneumonia. Scand J Infect Dis 2008 Jan 8;40 (9):715–20.
[4] Prina E, Ranzani OT, Torres A. Community-acquired pneumonia. Lancet 2015 Sep 12;386(9998):1097–108.
[5] Wunderink RG, Waterer GW. Community-acquired pneumoniaSolomon CG, editor. N Engl J Med 2014 Feb 6;370(6):543–51.
[6] Falguera M, Carratalà J, Bielsa S, García-Vidal C, Ruiz-González A, Chica I, et al. Predictive factors, microbiology and outcome of patients with parapneumonic effusion. Eur Respir J 2011 Nov 1;38(5):1173–9.
[7] Sureja BR, Govani KJ, Sureja JB, Sureja RB. An assessment of clinical profile & outcome in patients with community acquired pneumonia (CAP). National Journal of Community Medicine 2021 Aug 31;12(8):221–4.
[8] Tounta V, Liu Y, Cheyne A, Larrouy-Maumus G. Metabolomics in infectious diseases and drug discovery. Mol Omics 2021 Jun 14;17(3):376–93.
[9] Saleh MAA, Van Hasselt CJG, Van De Garde EMW. Host-response biomarkers for the diagnosis of bacterial respiratory tract infections. Clin Chem Lab Med 2018;57 (4).
[10] Kok M, Maton L, van der Peet M, Hankemeier T, van Hasselt JGC. Unraveling antimicrobial resistance using metabolomics. Drug Discov Today 2022 Jun 1;27 (6):1774–83.
[11] Páez-Franco JC, Torres-Ruiz J, Sosa-Hernández VA, Cervantes-Díaz R, Romero-Ramírez S, Pérez-Fragoso A, et al. Metabolomics analysis reveals a modified amino acid metabolism that correlates with altered oxygen homeostasis in COVID-19 patients. Sci Rep 2021;11(1):1–12.
[12] Overmyer KA, Shishkova E, Miller IJ, Balnis J, Bernstein MN, Peters-Clarke TM, et al. Large-scale multi-omic analysis of COVID-19 severity. Cell Syst 2021;12(1): 23–40. e7.
[13] Ploder M, Neurauter G, Spittler A, Schroecksnadel K, Roth E, Fuchs D. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. Amino Acids 2008;35(2):303–7.
[14] Wang J, Sun Y, Teng S, Li K. Prediction of sepsis mortality using metabolite biomarkers in the blood: a meta-analysis of death-related pathways and prospective validation. BMC Med 2020;18(1):83.
[15] Hartog I den, Zwep LB, Vestjens SMT, Harms AC, Voorn GP, Lange DW de, et al. Metabolomic profiling of microbial disease etiology in community-acquired pneumonia. PLoS One 2021;16(6).
[16] Endeman H, Schelfhout V, Paul Voorn G, van Velzen-Blad H, Grutters JC, Biesma DH. Clinical features predicting failure of pathogen identification in patients with community acquired pneumonia. Scand J Infect Dis 2008 Jan 8;40 (9):715–20.
[17] Marcovina SM, Sirtori C, Peracino A, Gheorghiade M, Borum P, Remuzzi G, et al. Translating the basic knowledge of mitochondrial functions to metabolic therapy: role of L-carnitine, vol. 161. Translational Research; 2013.
[18] Hoppel C. The role of carnitine in normal and altered fatty acid metabolism, vol. 41. American Journal of Kidney Diseases; 2003.
[19] Langley RJ, Tipper JL, Bruse S, Baron RM, Tsalik EL, Huntley J, et al. Integrative "omic" analysis of experimental bacteremia identifies a metabolic signature that distinguishes human sepsis from systemic inflammatory response syndromes. Am J Respir Crit Care Med 2014 Aug 15;190(4):445–55.
[20] Ayres JS. A metabolic handbook for the COVID-19 pandemic. Nature Metabolism 2020 Jun 30;2(7):572–85. 2020 2:7.
[21] Otsubo C, Bharathi S, Uppala R, Ilkayeva OR, Wang D, McHugh K, et al. Long-chain acylcarnitines reduce lung function by inhibiting pulmonary surfactant. J Biol Chem 2015 Sep 25;290(39):23897–904.
[22] Rousseau M, Guénard F, Garneau V, Allam-Ndoul B, Lemieux S, Pérusse L, et al. Associations between dietary protein sources, plasma BCAA and short-chain acylcarnitine levels in adults. Nutrients 2019;11(11):1–173. Page 173. 2019 Jan 15.
[23] Moreno FA, Macey H, Schreiber B. Carnitine levels in valproic acid-treated psychiatric patients: a cross-sectional study. J Clin Psychiatry 2005 May 15;66(5): 2314.
[24] Mynatt RL. Carnitine and type 2 diabetes. Diabetes Metab Res Rev 2009 Sep 1;25 (S1):S45–9.
[25] Karu N, Kindt A, van Gammeren AJ, Ermens AAM, Harms AC, Portengen L, Vermeulen RC, Dik WA, Langerak AW, van der Velden VH, Hankemeier T. Severe COVID-19 is characterised by perturbations in plasma amines correlated with immune response markers, and linked to inflammation and oxidative stress. Metabolites 2022 Jul 2;12(7):618.
[26] Darcy CJ, Davis JS, Woodberry T, McNeil YR, Stephens DP, Yeo TW, et al. An observational cohort study of the kynurenine to tryptophan ratio in sepsis: association with impaired immune and microvascular function. PLoS One 2011;6 (6):e21185.
[27] Suzuki Y, Suda T, Yokomura K, Suzuki M, Fujie M, Furuhashi K, et al. Serum activity of indoleamine 2,3-dioxygenase predicts prognosis of community-acquired pneumonia. J Infect 2011;63(3).
[28] Fraser DD, Slessarev M, Martin CM, Daley M, Patel MA, Miller MR, et al. Metabolomics profiling of critically ill coronavirus disease 2019 patients: identification of diagnostic and prognostic biomarkers. Crit Care Explor 2020;2 (10):e0272. e0272.
[29] Lawler NG, Gray N, Kimhofer T, Boughton B, Gay M, Yang R, et al. Systemic perturbations in amine and kynurenine metabolism associated with acute SARS-

CoV-2 infection and inflammatory cytokine responses. J Proteome Res 2021;20(5):2796–811.
[30] Takikawa O. Biochemical and medical aspects of the indoleamine 2, 3-dioxygenase-initiated L-tryptophan metabolism. Biochem Biophys Res Commun 2005;338(1):12–9.
[31] Orabona C, Pallotta MT, Grohmann U. Different partners, opposite outcomes: a new perspective of the immunobiology of indoleamine 2, 3-dioxygenase. Mol Med 2012;18(5):834–42.
[32] Jones SP, Franco NF, Varney B, Sundaram G, Brown DA, de Bie J, et al. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: implications for inflammatory and neurodegenerative disease. PLoS One 2015;10(6). e0131389–e0131389.
[33] Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol 2010;185(6):3190–8.
[34] Changsirivathanathamrong D, Wang Y, Rajbhandari D, Maghzal GJ, Mak WM, Woolfe C, et al. Tryptophan metabolism to kynurenine is a potential novel contributor to hypotension in human sepsis. Crit Care Med 2011;39(12):2678–83.
[35] Leisman DE, Deutschman CS, Legrand M. Facing COVID-19 in the ICU: vascular dysfunction, thrombosis, and dysregulated inflammation. Intensive Care Med 2020;1–4.
[36] Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. Curr Drug Metabol 2008;9(7):622–7.
[37] Pacheco R, Gallart T, Lluis C, Franco R. Role of glutamate on T-cell mediated immunity. J Neuroimmunol 2007 Apr 1;185(1–2):9–19.
[38] Nassar T, Bdeir K, Yarovoi S, Fanne RA, Murciano JC, Idell S, et al. tPA regulates pulmonary vascular activity through NMDA receptors. Am J Physiol Lung Cell Mol Physiol 2011 Sep;301(3):307–14.
[39] Collard CD, Park KA, Montalto MC, Alapati S, Buras JA, Stahl GL, et al. Neutrophil-derived glutamate regulates vascular endothelial barrier function. J Biol Chem 2002 Apr 26;277(17):14801–11.
[40] Baker DA, Thomas J, Epstein J, Possilico D, Stone ML. The effect of prostaglandins on the multiplication and cell-to-cell spread of herpes simplex virus type 2 in vitro. Am J Obstet Gynecol 1982;144(3).
[41] Dalli J, Colas RA, Quintana C, Barragan-Bradford D, Hurwitz S, Levy BD, et al. Human sepsis eicosanoid and pro-resolving lipid mediator temporal profiles: correlations with survival and clinical outcomes. Crit Care Med 2017 Jan 1;45(1):58.
[42] Bruegel M, Ludwig U, Kleinhempel A, Petros S, Kortz L, Ceglarek U, et al. Sepsis-associated changes of the arachidonic acid metabolism and their diagnostic potential in septic patients. Crit Care Med 2012 May;40(5):1478–86.
[43] Schwarz B, Sharma L, Roberts L, Peng X, Bermejo S, Leighton I, et al. Severe SARS-CoV-2 infection in humans is defined by a shift in the serum lipidome resulting in dysregulation of eicosanoid immune mediators. J Immunol 2021 Jan 15;206(2):329.
[44] Karu N, Kindt A, Lamont L, van Gammeren AJ, Ermens AAM, Harms AC, Portengen L, Vermeulen RC, Dik WA, Langerak AW, van der Velden VH. Plasma Oxylipins and Their Precursors Are Strongly Associated with COVID-19 Severity and with Immune Response Markers. Metabolites 2022 Jul 2;12(7):619.
[45] Lundström SL, Levänen B, Nording M, Klepczynska-Nyström A, Sköld M, Haeggström JZ, et al. Asthmatics exhibit altered oxylipin profiles compared to healthy individuals after subway air exposure. PLoS One 2011 Aug 29;6(8):e23864.
[46] Balgoma D, Yang M, Sjödin M, Snowden S, Karimi R, Levänen B, et al. Linoleic acid-derived lipid mediators increase in a female-dominated subphenotype of COPD. Eur Respir J 2016 Jun 1;47(6):1645–56.
[47] Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. Nat Rev Immunol 2015 Jul 3;15(8):511–23. 2015 15:8.
[48] Malcher-Lopes R, Franco A, Tasker JG. Glucocorticoids shift arachidonic acid metabolism toward endocannabinoid synthesis: a non-genomic anti-inflammatory switch. Eur J Pharmacol 2008 Apr 7;583(2–3):322–39.
[49] Chen JS, Alfajaro MM, Chow RD, Wei J, Filler RB, Eisenbarth SC, et al. Nonsteroidal anti-inflammatory drugs dampen the cytokine and antibody response to SARS-CoV-2 infection. J Virol 2021 Mar 10;95(7).
[50] Nickler M, Ottiger M, Steuer C, Huber A, Anderson JB, Müller B, et al. Systematic review regarding metabolic profiling for improved pathophysiological understanding of disease and outcome prediction in respiratory infections, vol. 16. Respiratory Research. BioMed Central; 2015. p. 125.
[51] Hurley BP, McCormick BA. Multiple roles of phospholipase A2 during lung infection and inflammation. Infect Immun 2008 Jun;76(6):2259–72.
[52] Mecatti GC, Fernandes Messias MC, Sant'Anna Paiola RM, Figueiredo Angolini CF, da Silva Cunha IB, Eberlin MN, et al. Lipidomic profiling of plasma and erythrocytes from septic patients reveals potential biomarker candidates. Biomark Insights 2018 Mar 26;13:1177271918765137.
[53] Vromman F, Subtil A. Exploitation of host lipids by bacteria. Curr Opin Microbiol 2014 Feb 1;17(1):38–45.
[54] Flieger A, Gong S, Faigle M, Mayer HA, Kehrer U, Mußotter J, et al. Phospholipase A secreted by Legionella pneumophila destroys alveolar surfactant phospholipids. FEMS Microbiol Lett 2000 Jul 1;188(2):129–33.
[55] Gregoriades A. Interaction of influenza M protein with viral lipid and phosphatidylcholine vesicles. J Virol 1980 Nov;36(2):470–9.
[56] Kawasaki K, Ohnishi S ichi. Membrane fusion of influenza virus with phosphatidylcholine liposomes containing viral receptors. Biochem Biophys Res Commun 1992 Jul 15;186(1):378–84.
[57] Vijay R, Hua X, Meyerholz DK, Miki Y, Yamamoto K, Gelb M, et al. Critical role of phospholipase A2 group IID in age-related susceptibility to severe acute respiratory syndrome–CoV infection. J Exp Med 2015 Oct 19;212(11):1851.
[58] Müller C, Hardt M, Schwudke D, Neuman BW, Pleschka S, Ziebuhr J. Inhibition of cytosolic phospholipase A2α impairs an early step of coronavirus replication in cell culture. J Virol 2018 Feb 15;92(4):1463–80.
[59] Houten SM, Wanders RJA. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis 2010 Oct 1;33(5):469–77.
[60] Newsholme P. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? J Nutr 2001 Sep 1;131(9):2515S–22S.
[61] Lawton KA, Berger A, Mitchell M, Eric Milgram K, Evans AM, Guo L, et al. Analysis of the adult human plasma metabolome. Pharmacogenomics 2008;9(4):383–97.
[62] Aulin LBS, de Lange DW, Saleh MAA, van der Graaf PH, Völler S, van Hasselt JGC. Biomarker-guided individualization of antibiotic therapy. Clin Pharmacol Ther 2021 Aug 1;110(2):346–60.
[63] Maas P, den Hartog I, Kindt A, Boman S, Hankemeier T, van Hasselt C. The Immunometabolic Atlas: a tool for design and interpretation of metabolomics studies in immunology. PLoS One 2022 May 1;17(5):e0268408.