Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Diagnostic Microbiology and Infectious Disease 85 (2016) 249–254

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Lipid metabolites as potential diagnostic and prognostic biomarkers for acute community acquired pneumonia

Kelvin K.W. To [a,b,c,d,1], Kim-Chung Lee [d,1], Samson S.Y. Wong [a,b,c,d], Kong-Hung Sze [d], Yi-Hong Ke [d], Yin-Ming Lui [d], Bone S.F. Tang [e], Iris W.S. Li [d], Susanna K.P. Lau [a,b,c,d], Ivan F.N. Hung [a,b,c,f], Chun-Yiu Law [g], Ching-Wan Lam [g], Kwok-Yung Yuen [a,b,c,d,*]

[a] State Key Laboratory for Emerging Infectious Diseases, The University of Hong Kong, Hong Kong SAR, China
[b] Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong SAR, China
[c] Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong SAR, China
[d] Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China
[e] Department of Pathology, Hong Kong Sanatorium Hospital, Hong Kong SAR, China
[f] Department of Medicine, The University of Hong Kong, Hong Kong SAR, China
[g] Department of Pathology, The University of Hong Kong Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form 2 March 2016
Accepted 11 March 2016
Available online 14 March 2016

Keywords:
Biomarkers
Glycolipids
Phospholipids
Pneumonia
Sphingolipids

ABSTRACT

Early diagnosis of acute community-acquired pneumonia (CAP) is important in patient triage and treatment decisions. To identify biomarkers that distinguish patients with CAP from non-CAP controls, we conducted an untargeted global metabolome analysis for plasma samples from 142 patients with CAP (CAP cases) and 97 without CAP (non-CAP controls). Thirteen lipid metabolites could discriminate between CAP cases and non-CAP controls with area-under-the-receiver-operating-characteristic curve of >0.8 ($P \leq 10^{-9}$). The levels of glycosphingolipids, sphingomyelins, lysophosphatidylcholines and L-palmitoylcarnitine were higher, while the levels of lysophosphatidylethanolamines were lower in the CAP cases than those in non-CAP controls. All 13 metabolites could distinguish CAP cases from the non-infection, extrapulmonary infection and non-CAP respiratory tract infection subgroups. The levels of trihexosylceramide (d18:1/16:0) were higher, while the levels of lysophosphatidylethanolamines were lower, in the fatal than those of non-fatal CAP cases. Our findings suggest that lipid metabolites are potential diagnostic and prognostic biomarkers for CAP.

1. Introduction

Acute community-acquired pneumonia (CAP) is one of the most common causes of hospitalization and death (Musher and Thorner, 2014). In particular, emerging viral infections including the pandemic and avian influenza viruses, and the SARS and MERS coronaviruses, are associated with mortality rates ranging from 10–60% (Chan, et al., 2015; Cheng, et al., 2007; Cheng, et al., 2012; To, et al., 2013a). Early recognition and treatment of CAP improve outcome (Li, et al., 2010; Musher and Thorner, 2014). The diagnosis of CAP depends on compatible symptoms and signs, together with new pulmonary infiltrates on chest imaging (Musher and Thorner, 2014). However, distinguishing CAP from other pulmonary pathologies can be difficult (Wunderink and Waterer, 2014). Some patients with CAP may not have obvious respiratory symptoms. Non-CAP causes of pulmonary infiltrates on chest radiograph can mimic those due to CAP. Therefore, alternative modalities of diagnosing CAP are important.

Biomarkers in body fluids have been increasingly used in determining the diagnosis and predicting the prognosis in lung diseases (Upadhyay and Niederman, 2013; Wheelock, et al., 2013). Procalcitonin is a sensitive biomarker for bacterial pneumonia, but the level is much lower in pneumonia caused by viruses or atypical bacteria (Muller, et al., 2010; Upadhyay and Niederman, 2013). Adenosine deaminase has been used as a biomarker for tuberculosis pleural effusion (Chen, et al., 2004). Prognostic markers include C-reactive protein, procalcitonin, midregional proadrenomedullin, midregional pro-atrial natriuretic peptide, copeptin, endothelin-1 precursor, kallistatin, and cytokines (Christ-Crain and Opal, 2010; Lin, et al., 2013).

Metabolomics study, which is a global strategy to detect low-molecular weight components (Wheelock, et al., 2013), has been increasingly used to identify biomarkers associated with infections (Lam, et al., 2014; Lam, et al., 2015; Langley, et al., 2013; Lau, et al., 2015; Tam, 2013). Using metabolomic analysis, we have previously identified 5 metabolites which could distinguish patients with bacteremia from those without bacteremia (To, et al., 2015). In a small study involving 11 children with severe pneumonia, plasma and urine metabolites were found to be significantly different from healthy community controls (Laiakis, et al., 2010). Plasma metabolites were able to

* Corresponding author. Tel.: +852-22554892; fax: +852-28551241.
E-mail address: kyyuen@hku.hk (K.-Y. Yuen).
[1] These authors contribute equally.

http://dx.doi.org/10.1016/j.diagmicrobio.2016.03.012
0732-8893/

discriminate between patients with tuberculosis and healthy controls (Frediani, et al., 2014). Urine metabolites were found to be different between patients with pneumococcal pneumonia and those with other causes of pneumonia (Slupsky, et al., 2009). Metabolic profiles may also predict the prognosis of patients with CAP (Seymour, et al., 2013).

In this study, we used a metabolomic approach to identify biomarkers that can differentiate between patients with CAP and those without CAP. Furthermore, we determined whether these biomarkers can predict the prognosis of patients with CAP.

2. Materials and methods

2.1. Study design and definitions

This study included patients admitted to Queen Mary Hospital in Hong Kong between 2007 and 2013. Archived plasma samples collected at admission were retrieved for metabolomics study. CAP cases were hospitalized adult patients 18 years or above with radiological evidence of consolidation within 48 hours of admission and at least one of the following symptoms (cough with or without sputum production, dyspnea, tachypnea, pleuritic chest pain) plus one auscultatory finding or one sign of infection (core body temperature >38 °C, shivers, leukocyte count >10,000 cells/μL or <4000 cells/μL (Muller, et al., 2010). Non-CAP controls were hospitalized adult patients not fulfilling the inclusion criteria for cases, and these included 3 subgroups: the non-infection subgroup consisted of patients without any clinical evidence of infection; the extrapulmonary infection subgroup consisted of patients with infection outside the respiratory tract; and the non-CAP respiratory tract infection subgroup consisted of patients with respiratory tract infections but without radiological evidence of pneumonia. Patients were excluded if they were aged <18 years or had radiological evidence of consolidation occurring >2 days after hospital admission, their clinical records were not accessible for review, or if archived plasma samples were not available. Clinical and microbiological data were retrieved from the Clinical Management System. Pneumonia Severity Index was used to assess the clinical severity (Fine, et al., 1997). Fisher's exact test and Student's *t* test were used in the comparison of categorical and continuous variables, respectively. This study was approved by the Institutional Review Board (HKU/HA HKW IRB; reference number UW 11-360).

2.2. Untargeted plasma metabolomics profiling and identification of metabolites

Untargeted plasma metabolomic profiling and identification of metabolites were performed as we described previously with modifications (To, et al., 2015). Ultra-high-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry (UHPLC-ESI-Q-TOF-MS) analysis was used to identify the metabolites in the plasma (To, et al., 2013b; To, et al., 2015; Woo, et al., 2014). Details of the sample preparation, UHPLC-ESI-Q-TOF-MS and identification of metabolites are provided in the supplementary information.

The metabolomic data was analyzed and reported according to the Metabolomics Standards Initiative (http://msi-workgroups.sourceforge.net/). To identify molecular features (MFs) that could discriminate CAP cases with non-CAP controls, both multivariate and univariate analyses were performed. For multivariate analyses, principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA) were performed for unsupervised and supervised analyses, respectively. For univariate analysis, volcano plots were constructed and analyzed using Student's t-test. Significant MFs with variable importance in projection (VIP) score of >1 in PLS-DA and volcano plot analysis of fold-change >1.5 and *P* values <0.05 were included for univariate receiver operating characteristic (ROC) curve analysis. Significant MFs with area-under-the-ROC curve (AUC) ≥0.8 were identified and box-whisker plots were generated. Heatmap based on significant metabolites were constructed using hierarchical clustering analysis (HCA). The identities of significant MFs were characterized.

3. Results

3.1. Baseline characteristics of patients

A total of 239 patients were included in this study, including 142 CAP cases and 97 non-CAP controls. The mean age was 76.7 years. There were no statistically significant differences in terms of age, gender and in-hospital mortality during hospitalization between CAP cases and non-CAP controls (Table 1). There were no differences in the underlying diseases except that chronic lung disease was more common among the CAP cases (28.9% vs 15.5%; $P = 0.019$). Among the CAP cases, the most commonly identified respiratory pathogens were *Haemophilus influenzae* (n = 10) and *Streptococcus pneumoniae* (n = 8) (Table S1), and blood culture was positive in 5 patients, including 3 patients with *S. pneumoniae*, 1 patient with *Escherichia coli* and 1 patient with *Corynebacterium* species. Among the 97 non-CAP controls, 49 did not have infection, 26 had extrapulmonary infections, and 22 had respiratory tract infections without evidence of pneumonia (Table S2).

3.2. Global metabolome changes associated with CAP

Global metabolome was determined using UHPLC-ESI-Q-TOF-MS. A total of 2960 and 1796 MFs were found in positive and negative modes, respectively. Unsupervised PCA without bias in positive mode showed that 36.5% of the total variance was represented by the first 2 principal components (Supplementary Fig. S1A). PCA in negative mode showed that 30.4% of the total variance was represented by the first 2 principal components (Supplementary Fig. S1B). PCA score plots of all MFs identified showed that samples within the same group were closely clustered in both positive and negative modes.

Supervised PLS-DA score plots after data training were constructed to select MFs that can discriminate CAP cases from non-CAP controls. The score plot in positive mode showed an accuracy of 97.5%, a multiple correlation coefficient (R2) of 90.4% and a cross-validated R2 (Q2) of 83.9% (Supplementary Fig. S1C), while the score plot in negative mode showed an accuracy of 96.3%, a R2 of 86.9% and a Q2 of 79.3%

Table 1
Demographics, underlying diseases, and outcome.

	CAP Cases (n = 142)	Non-CAP controls (n = 97)	*P* value[a]
Demographics			
Female	41 (28.9)	40 (41.2)	N.S.
Mean age (standard deviation)	78.0 (15.6)	74.9 (14.3)	N.S.
Underlying diseases			
Heart disease	45 (31.7)	29 (29.9)	N.S.
Lung disease	41 (28.9)	15 (15.5)	0.019
Renal disease	12 (8.5)	16 (16.5)	N.S.
Cirrhosis	1 (0.7)	4 (4.1)	N.S.
Neurological conditions	52 (36.6)	29 (29.9)	N.S.
Diabetes mellitus	36 (25.4)	26 (26.8)	N.S.
Solid organ malignancy	19 (13.4)	14 (14.4)	N.S.
Hematological malignancy	2 (1.4)	3 (3.1)	N.S.
Autoimmune disease	4 (2.8)	7 (7.2)	N.S.
Pneumonia Severity Index risk class			
I	3 (2.1)	N.A.	N.A
II	13 (9.2)	N.A.	N.A
III	25 (17.6)	N.A.	N.A
IV	67 (47.2)	N.A.	N.A
V	34 (23.9)	N.A.	N.A
Outcome			
In-hospital mortality	16 (11.3)	6 (6.2)	N.S.

Data are no. (%) unless otherwise indicated.
N.A. = no applicable; N.S. = non-significant
[a] *P* values were calculated using Fisher's exact test for categorical variables and Student's test for continuous variable.

(Supplementary Fig. S1D). The parameters indicated optimal fitness and prediction performance of the PLS-DA models in both ionization modes.

More detailed analysis of the MFs using volcano plot analysis showed that 368 MFs in the positive mode and 448 MFs in the negative mode were significantly different between the CAP cases and non-CAP controls (Supplementary Fig. S1E and F). Among these MFs, 274 MFs in positive mode and 180 MFs in negative mode had VIP scores >1. MFs were manually inspected, and those that were not related to interference were included in univariate ROC curve analysis.

The univariate ROC curve analysis showed that 13 metabolites had AUC ≥0.8. The metabolites were identified as acylcarnitine, sphingolipids (glycosphingolipid and sphingomyelin) and glycerophospholipids (lysophosphatidylcholine [LysoPC] and lysophosphatidylethanolamine [LysoPE]) (Supplementary Table S3, Supplementary Fig. S2). CAP cases had higher levels of acylcarnitine, glycosphingolipids, LysoPC and sphingomyelins, but lower levels of LysoPE, than non-CAP controls (Fig. 1). HCA of the 13 differential metabolites separated the CAP cases and non-CAP controls into 2 major clusters (Supplementary Fig. S3). Univariate ROC curve analysis showed that LysoPC(18:1[9Z]) had the largest AUC (0.905), and highest sensitivity (86.6%) and specificity (92.3%) (Table 2). The 13 metabolites were further compared between CAP cases and each of the control subgroups (non-infection subgroup, extrapulmonary infection subgroup and non-CAP respiratory tract infection subgroup). All 13 metabolites were significantly different between CAP cases and all these subgroups (Supplementary Fig. S4, Table S4).

The 13 metabolites were also compared between different subgroups of the CAP cases. The levels of trihexosylceramide(d18:1/16:0) were higher in the 16 fatal cases than those of the 126 non-fatal cases, while the levels of LysoPE(22:6[p4Z,7Z,10Z,13Z,16Z,19Z]/0:0), LysoPE(18:2[9Z,12Z]/0:0), LysoPE(20:4[8Z,11Z,14Z,17Z]/0:0) and LysoPE(18:1[9Z]/0:0) were lower in the fatal than those of non-fatal cases (Table 3). LysoPC(18:1[9Z]), LysoPE(22:6[p4Z,7Z,10Z,13Z,16Z,19Z]/0:0), LysoPE(18:2[9Z,12Z]/0:0) and LysoPE(20:4[8Z,11Z,14Z,17Z]/0:0) were also significantly lower in CAP patients with PSI class 5 than those with PSI class 4 or below. The levels of these 13 metabolites were not significantly different between CAP patients with bacterial and viral/mycoplasma pneumonia ($P > 0.1$ for all 13 metabolites) (Supplementary Fig. S5).

To assess whether the 13 metabolites can be biomarkers for pneumonia caused by classical pathogens that are commonly identified CAP in other case series, we have compared CAP cases with these classical pathogens (excluding *Enterobacteriaceae* and glucose non-fermenters) with non-CAP controls. All 13 metabolites remained significantly different between non-CAP controls and patients with CAP due to these classical pathogens (Table S5). LysoPC(18:1[9Z]) had the highest AUC (0.982), with a sensitivity of 94.4% and a specificity of 97.9%.

4. Discussion

In this study, we have used an untargeted global metabolomics approach to identify metabolites which have high sensitivities and high specificities in discriminating CAP cases from non-CAP controls. The levels of 5 sphingolipids (3 glycosphingolipids and 2 sphingomyelins), 1 glycerophospholipid (a LysoPC) and 1 acylcarnitine (L-palmitoylcarnitine) were higher, while the levels of 6 glycerophospholipids (all LysoPEs)

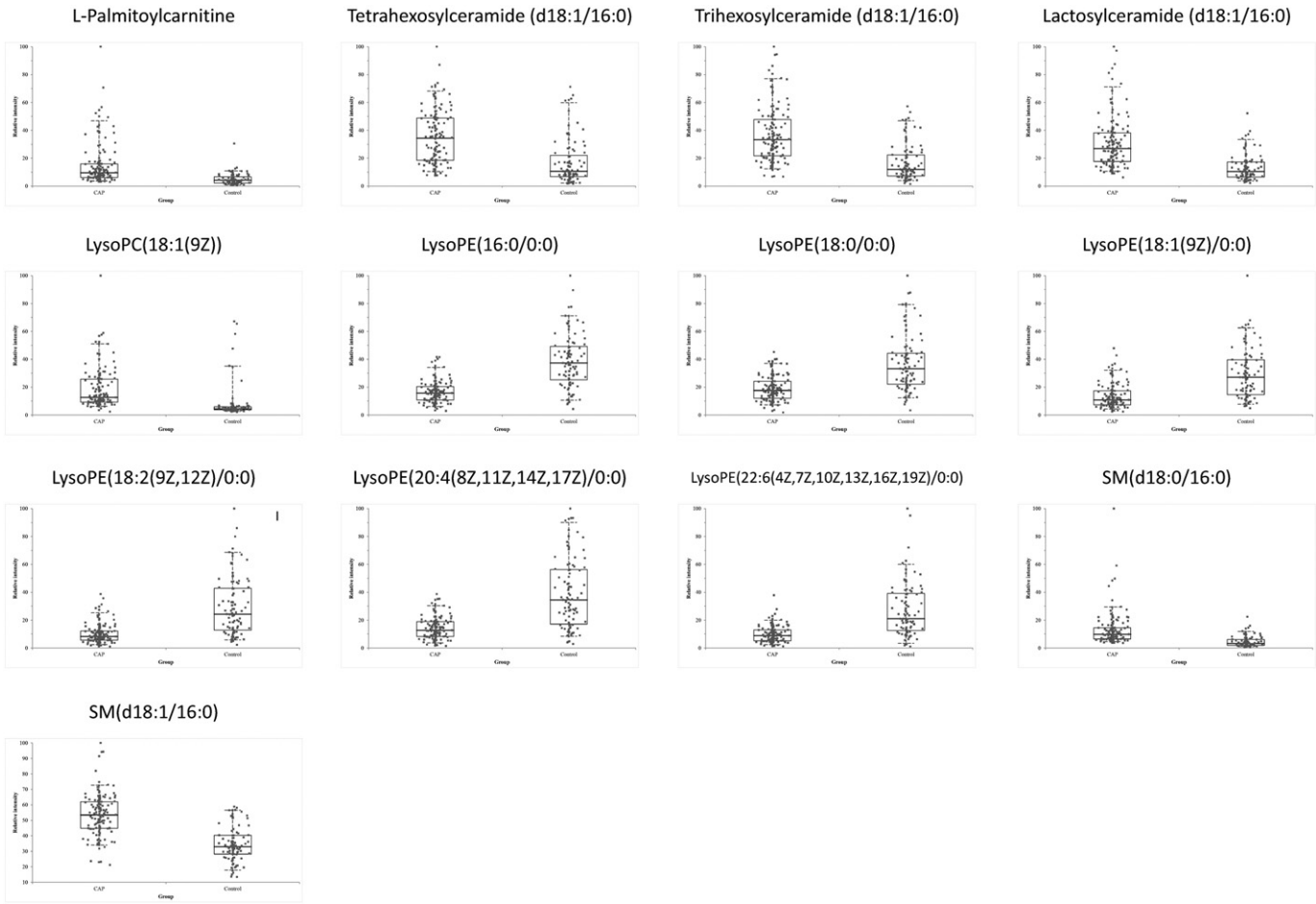

Fig. 1. Box-whisker plots of the 13 metabolites that distinguish between CAP cases and non-CAP controls. The horizontal line represents the median; the bottom and the top of the box represent the 25th and the 75th percentiles; whiskers represent 5% and 95% percentiles.

Table 2
The AUC, sensitivity and specificity for ROC curves calculated at optimal cutoff as well as VIP score, *P* value and fold-change for the 13 metabolites that distinguishes CAP cases from non-CAP controls.

Metabolite Class	Metabolites	AUC	95% CI	Sensitivity (%)	Specificity (%)	VIP Score[a]	Fold-change[b]	*P* value[c]
Acylcarnitine	L-palmitoylcarnitine	0.834	0.783–0.886	69.1	83.1	1.62	+2.98	8.31E-10
Glycosphingolipid	Tetrahexosylceramide (d18:1/16:0)	0.802	0.742–0.861	71.1	79.6	1.52	+2.06	5.46E-13
	Trihexosylceramide (d18:1/16:0)	0.828	0.773–0.882	70.1	82.4	1.62	+2.18	5.44E-16
	Lactosylceramide (d18:1/16:0)	0.847	0.797–0.897	78.4	75.4	1.68	+2.32	6.35E-16
Lysophosphatidylcholine	LysoPC(18:1[9Z])	0.905	0.854–0.955	86.6	92.3	1.71	+2.42	4.18E-09
Lysophosphatidylethanolamine	LysoPE(16:0/0:0)	0.86	0.807–0.913	75.3	89.4	1.69	−2.34	1.81E-27
	LysoPE(18:0/0:0)	0.802	0.743–0.860	74.2	73.9	1.39	−1.95	5.41E-18
	LysoPE(18:1[9Z]/0:0)	0.802	0.745–0.859	73.2	73.9	1.48	−2.20	1.61E-17
	LysoPE(18:2[9Z,12Z]/0:0)	0.829	0.775–0.884	76.3	78.2	1.58	−2.89	1.60E-21
	LysoPE(20:4[8Z,11Z,14Z,17Z]/0:0)	0.824	0.766–0.882	70.1	87.3	1.55	−2.74	4.86E-23
	LysoPE(22:6[4Z,7Z,10Z,13Z,16Z,19Z]/0:0)	0.831	0.774–0.887	72.2	78.9	1.48	−2.78	1.88E-21
Sphingomyelins	SM(d18:0/16:0)	0.861	0.812–0.911	74.2	81.7	1.76	+2.78	8.69E-11
	SM(d18:1/16:0)	0.861	0.814–0.908	78.4	82.4	1.69	+1.54	7.97E-24

AUC = area-under-the-receiver-operating-characteristic curve; CI = confidence interval; LysoPC = lysophosphatidylcholine; LysoPE = lysophosphatidylethanolamine; VIP = variable importance in projection.
[a] VIP score in PLS-DA analyses.
[b] Fold change of CAP cases relative to non-CAP controls. (+) indicates increased level while (-) indicates decreased level in CAP cases when compared to non-CAP controls
[c] Student's *t* test.

were lower in the CAP cases than those in non-CAP controls. The changes in plasma lipid metabolite levels in CAP patients may be related to several reasons. Firstly, lipids make up 90% of surfactant, and disturbance in surfactant during pneumonia may cause changes in lipid metabolism (Wheelock, et al., 2013). Secondly, lipids are important inflammatory mediators during infection, and changes in lipid metabolites are observed during conditions such as sepsis, bacteremia, and viral infection (Cui, et al., 2013; Langley, et al., 2013; To, et al., 2015). Thirdly, lipids are important in the structure and function of bacteria, and lipid metabolites are produced by bacteria (Kondakova, et al., 2015; Lau, et al., 2015). Therefore, in future studies for biomarker discovery, it would be worthwhile to optimize the extraction and detection steps for finding lipid metabolites.

In this study, 7 of the 13 lipid metabolites that discriminated CAP cases from non-CAP controls were glycerophospholipids (1 LysoPC or 6 LysoPEs), which are components of the pulmonary surfactant and are important in modulating inflammation (Makide, et al., 2009). LysoPC(18:1[9Z]) had the highest sensitivity and specificity. The level of LysoPC(18:1[9Z]) was higher in CAP cases than in non-CAP controls, while the levels of LysoPEs were lower in CAP cases than in non-CAP controls. Our findings differ from patients with dengue virus infection, in which both serum LysoPC and LysoPE were reduced (Cui, et al., 2013). The high level of LysoPC in CAP patients can be related to specific host changes in pneumonia. High plasma levels of LysoPC in CAP patients may be caused by the release of LysoPC from surfactant during lung injury (Arbibe, et al., 1998). LysoPC may also contribute to direct toxicity to the lung (Grossmann, et al., 1999; Vlaar, et al., 2010; Zhou, et al., 2014). However, a previous study showed that the plasma levels of LysoPC were lower among fatal than non-fatal patients, and low levels of LysoPC was postulated to be associated with dysregulated inflammatory response (Cho, et al., 2015). In our study, the plasma levels of LysoPC were significantly lower in high-risk CAP cases, and were also lower in fatal CAP cases than those of non-fatal CAP cases, although not reaching statistical significance (Table 3). The lack of statistical significance may be related to the relative small number of fatal cases in our cohort. Further studies are needed to assess whether LysoPC is a favorable prognostic factor.

On the other hand, the plasma levels of all LysoPEs were lower in CAP cases than those of non-CAP controls. In a mouse peritonitis model, LysoPE has been shown to increase the level of the anti-inflammatory cytokine interleukin-10, and reduces plasma leakage (Hung, et al., 2011). The levels of 4 LysoPEs were significantly lower in the fatal CAP cases than the non-fatal CAP cases. Therefore, a higher LysoPE level in CAP may improve survival via the amelioration of excessive inflammation.

In this study, the levels of 5 sphingolipids were higher in the CAP cases than in the non-CAP controls. Sphingolipids are associated with many pulmonary diseases (Uhlig and Gulbins, 2008). Sphingolipids are critical in the structure and function of the plasma membrane, and are involved in many cellular processes. Lactosylceramide, trihexosylceramide and tetrahexosylceramide are metabolites from ceramide (Fig. 2), and ceramide are important in regulating immune cell

Table 3
Comparison of the levels of the 13 metabolites between fatal (n = 16) and non-fatal (n = 126) CAP cases, and between patients in the high risk class (n = 34) and those in low risk class (n = 108).

Metabolites	Fold differences between fatal and non-fatal CAP cases[a]	*P* value	Fold differences between high risk and low risk CAP cases[a,b]	*P* value
L-palmitoylcarnitine	+1.17	0.524	+1.38	0.0736
Tetrahexosylceramide (d18:1/16:0)	+1.22	0.136	+1.14	0.211
Trihexosylceramide (d18:1/16:0)	+1.35	0.0188	+1.09	0.425
Lactosylceramide (d18:1/16:0)	+1.26	0.114	−1.01	0.942
LysoPC(18:1[9Z])	−1.30	0.246	−1.47	0.0234
LysoPE(16:0/0:0)	−1.29	0.0634	−1.12	0.225
LysoPE(18:0/0:0)	−1.20	0.177	−1.13	0.198
LysoPE(18:1[9Z]/0:0)	−1.52	0.0396	+1.19	0.16
LysoPE(18:2[9Z,12Z]/0:0)	−1.68	0.0190	−1.43	0.0154
LysoPE(20:4[8Z,11Z,14Z,17Z]/0:0)	−1.42	0.0370	−1.47	0.00116
LysoPE(22:6[4Z,7Z,10Z,13Z,16Z,19Z]/0:0)	−1.47	0.0357	−1.63	0.000253
SM(d18:0/16:0)	+1.32	0.195	+1.06	0.743
SM(d18:1/16:0)	+1.05	0.443	−1.02	0.668

[a] "+" indicates the level is higher in fatal cases than that of non-fatal cases. "−" indicates that the level is lower in fatal cases than that of non-fatal cases
[b] High risk = Pneumonia Severity Index class V; Low risk = Pneumonia Severity Index class 4 or below.

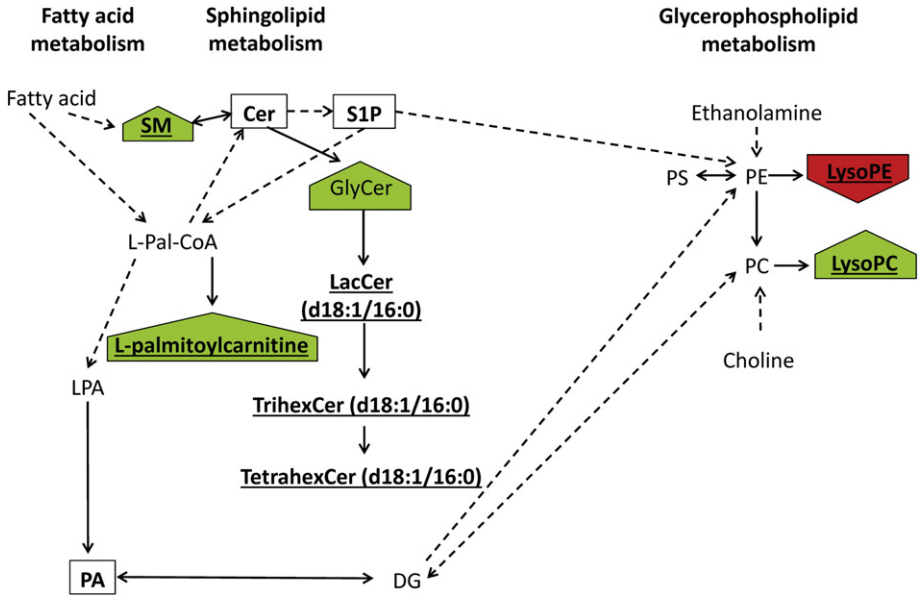

Fig. 2. Simplified metabolic pathways associated with the biomarkers identified in this study. Metabolites that are identified as significant biomarkers in this study are underlined. Metabolites in rectangular boxes are important in linking different pathways. Solid arrows indicate direct conversion from one compound to another, while dashed arrows indicate conversions requiring multiple steps. Direction of boxed block arrows indicates increased or decreased levels of metabolites in CAP cases as compared to non-CAP controls. CoA = coenzyme A; DG = diacylglycerol; GlcCer = glucosylceramide; LacCer = lactosylceramide; L-Pal-CoA = L-palmitoyl-coenzyme A; LPA = lysophosphatidic acid; PA = phosphatidic acid; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PS = phosphatidylserine; S1P = sphingosine-1-phosphate; SM = sphingomyelin; TrihexCer = trihexosylceramide; TetrahexCer = tetrahexosylceramide.

trafficking and the vascular and epithelial integrity (Maceyka and Spiegel, 2014). For example, lactosylceramide is a mediator of inflammatory response triggered by *Pneumocystis* (Evans, et al., 2012). *In vitro*, influenza virus infection induces elevated levels of hexosylceramides(Tanner, et al., 2014). Inhibition of glycosphingolipid synthesis has been associated with reduced pulmonary inflammation in mice challenged with lipopolysaccharide (LPS) (Dechecchi, et al., 2011). Sphingomyelin is a structural component of cell membranes (Maceyka and Spiegel, 2014). In mice, sphingomyelin synthase 2 (SMS2), which catalyzes the synthesis of sphingomyelin, is activated during LPS-induced lung injury, and inhibition of SMS2 attenuated lung injury and endothelial barrier disruption (Anjum, et al., 2012). This mice study suggested that sphingomyelin is important in lung injury, and the higher level of sphingomyelins in our CAP cases is compatible with this hypothesis. However, other studies suggest that high levels of sphingomyelin may be protective. Stringer *et al* showed that patients with acute lung injury had lower levels of sphingomyelin when compared to healthy controls, although the absolute difference is small (Stringer, et al., 2011).

The levels of L-palmitoylcarnitine were significantly higher in CAP cases than non-CAP controls. L-palmitoylcarnitine facilitates the transport of long-chain fatty acids from the cytoplasm into the mitochondria, where fatty acid beta-oxidation takes place. During acute lung injury, fatty acid metabolism is disturbed (Guo, et al., 2014). A previous study also showed that high levels of L-palmitoylcarnitine were associated with poorer prognosis in patients with chronic heart failure (Ueland, et al., 2013). In our study, there is no significant difference in the proportion of patients with heart disease.

These metabolic pathways are interconnected (Fig. 5). Fatty acid beta-oxidation is important in providing the substrate for the generation of sphingolipids and glycerophospholipids via ceramide and phosphatidic acid, respectively (Ecker and Liebisch, 2014; Maceyka and Spiegel, 2014). Ceramide is converted to glucosylceramide, which is then converted to lactosylceramide. Phosphatidic acid is converted to diacylglyerol, which is then converted to phosphatidylcholine and phosphatidylethanolamine. The sphingolipid pathway is also connected to the glycerophospholipid pathway via sphingosine 1-phosphate (Kihara, 2014; Maceyka and Spiegel, 2014).

There are several limitations in this study. Firstly, we have only tested the metabolome profile of the first available plasma samples of each patient. Serial monitoring of these metabolites may help to assess the disease progress. Secondly, we did not analyze the relationship between the specific pathogens and each metabolite because of the limited number of patients infected by each pathogen. Thirdly, a higher proportion of CAP cases had underlying chronic lung disease than non-CAP controls. Previous studies showed that the levels of plasma or serum metabolites are different between patients with chronic lung diseases and healthy controls. When compared with healthy controls, patients with chronic obstructive pulmonary disease had higher levels of glycerophophocholine but lower levels of lipoprotein and amino acids, while patients with asthma had higher levels of taurine, lathosterol, bile acids, nicotinamide and adenosine-5-phosphate than healthy controls (Comhair, et al., 2015; Wang, et al., 2013). Further studies are required to determine whether the levels of the metabolites identified in this study are different between patients with chronic underlying diseases and healthy controls. Fourthly, in our cohort, there were no patients with non-infective etiologies which can lead to pulmonary infiltrates, such as interstitial lung disease. Further studies should include patients with non-infective pulmonary disease without evidence of infection.

The detection of low-molecular-weight molecules is increasingly important in the diagnosis of infections. Using a global metabolomics approach, we have identified 13 lipid metabolites which may be used as rapid diagnostic or prognostic biomarkers for CAP. It would be important to verify these biomarkers in larger cohorts and in different populations. Furthermore, the role of these lipid metabolites in the pathogenesis of CAP deserves further studies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.diagmicrobio.2016.03.012.

Acknowledgments

We would like to thank Akhee S. Jahan for her help in the processing of the metabolomic data. This study was supported by the Research Fund for the Control of Infectious Diseases (Commissioned Study), Food and Bureau, Hong Kong SAR Government (Ref. No. HK-09-01-

06); the University Development Fund and Strategic Research Fund, The University of Hong Kong; and the Providence Foundation Limited in memory of the late Dr Lui Hac Minh.

References

Anjum F, Joshi K, Grinkina N, Gowda S, Cutaia M, Wadgaonkar R. Role of sphingomyelin synthesis in pulmonary endothelial cell cytoskeletal activation and endotoxin-induced lung injury. Am J Respir Cell Mol Biol 2012;47:94–103.

Arbibe L, Koumanov K, Vial D, Rougeot C, Faure G, Havet N, et al. Generation of lyso-phospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A2 and inhibited by a direct surfactant protein A-phospholipase A2 protein interaction. J Clin Invest 1998;102:1152–60.

Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev 2015;28:465–522.

Chen ML, Yu WC, Lam CW, Au KM, Kong FY, Chan AY. Diagnostic value of pleural fluid adenosine deaminase activity in tuberculous pleurisy. Clin Chim Acta 2004;341:101–7.

Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 2007;20:660–94.

Cheng VC, To KK, Tse H, Hung IF, Yuen KY. Two years after pandemic influenza A/2009/H1N1: what have we learned? Clin Microbiol Rev 2012;25:223–63.

Cho WH, Yeo HJ, Yoon SH, Lee SE, Jeon DS, Kim YS, et al. Lysophosphatidylcholine as a prognostic marker in community-acquired pneumonia requiring hospitalization: a pilot study. Eur J Clin Microbiol Infect Dis 2015;34(2):309–15.

Christ-Crain M, Opal SM. Clinical review: the role of biomarkers in the diagnosis and management of community-acquired pneumonia. Crit Care 2010;14:203.

Comhair SA, McDunn J, Bennett C, Fettig J, Erzurum SC, Kalhan SC. Metabolomic Endotype of Asthma. J Immunol 2015;195:643–50.

Cui L, Lee YH, Kumar Y, Xu F, Lu K, Ooi EE, et al. Serum metabolome and lipidome changes in adult patients with primary dengue infection. PLoS Negl Trop Dis 2013;7:e2373.

Dechecchi MC, Nicolis E, Mazzi P, Cioffi F, Bezzerri V, Lampronti I, et al. Modulators of sphingolipid metabolism reduce lung inflammation. Am J Respir Cell Mol Biol 2011;45:825–33.

Ecker J, Liebisch G. Application of stable isotopes to investigate the metabolism of fatty acids, glycerophospholipid and sphingolipid species. Prog Lipid Res 2014;54:14–31.

Evans SE, Kottom TJ, Pagano RE, Limper AH. Primary alveolar epithelial cell surface membrane microdomain function is required for Pneumocystis beta-glucan-induced inflammatory responses. Innate Immun 2012;18:709–16.

Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997;336:243–50.

Frediani JK, Jones DP, Tukvadze N, Uppal K, Sanikidze E, Kipiani M, et al. Plasma metabolomics in human pulmonary tuberculosis disease: a pilot study. PLoS One 2014;9:e108854.

Grossmann G, Tashiro K, Kobayashi T, Suzuki Y, Matsumoto Y, Waseda Y, et al. Experimental neonatal respiratory failure induced by lysophosphatidylcholine: effect of surfactant treatment. J Appl Physiol 1999;86:633–40.

Guo Z, Zhao C, Wang Z. Gene expression profiles analysis identifies key genes for acute lung injury in patients with sepsis. Diagn Pathol 2014;9:176.

Hung ND, Kim MR, Sok DE. 2-Polyunsaturated acyl lysophosphatidylethanolamine attenuates inflammatory response in zymosan A-induced peritonitis in mice. Lipids 2011;46:893–906.

Kihara A. Sphingosine 1-phosphate is a key metabolite linking sphingolipids to glycerophospholipids. Biochim Biophys Acta 2014;1841:766–72.

Kondakova T, D'Heygere F, Feuilloley MJ, Orange N, Heipieper HJ, Duclairoir Poc C. Glycerophospholipid synthesis and functions in Pseudomonas. Chem Phys Lipids 2015;190:27–42.

Laiakis EC, Morris GA, Fornace AJ, Howie SR. Metabolomic analysis in severe childhood pneumonia in the Gambia, West Africa: findings from a pilot study. PLoS One 2010;5.

Lam CW, Law CY, To KK, Cheung SK, Lee KC, Sze KH, et al. NMR-based metabolomic urinalysis: a rapid screening test for urinary tract infection. Clin Chim Acta 2014;436:217–23.

Lam CW, Law CY, Sze KH, To KK. Quantitative metabolomics of urine for rapid etiological diagnosis of urinary tract infection: Evaluation of a microbial-mammalian co-metabolite as a diagnostic biomarker. Clin Chim Acta 2015;438:24–8.

Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med 2013;5:195ra195.

Lau SK, Lam CW, Curreem SO, Lee KC, Lau CC, Chow WN, et al. Identification of specific metabolites in culture supernatant of Mycobacterium tuberculosis using metabolomics: exploration of potential biomarkers. Emerg Microbes Infect 2015;4:e6.

Li IW, Hung IF, To KK, Chan KH, Wong SS, Chan JF, et al. The natural viral load profile of patients with pandemic 2009 influenza A(H1N1) and the effect of oseltamivir treatment. Chest 2010;137:759–68.

Lin WC, Lu SL, Lin CF, Chen CW, Chao L, Chao J, et al. Plasma kallistatin levels in patients with severe community-acquired pneumonia. Crit Care 2013;17:R27.

Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature 2014;510:58–67.

Makide K, Kitamura H, Sato Y, Okutani M, Aoki J. Emerging lysophospholipid mediators, lysophosphatidylserine, lysophosphatidylthreonine, lysophosphatidylethanolamine and lysophosphatidylglycerol. Prostaglandins Other Lipid Mediat 2009;89:135–9.

Muller F, Christ-Crain M, Bregenzer T, Krause M, Zimmerli W, Mueller B, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121–9.

Musher DM, Thorner AR. Community-acquired pneumonia. N Engl J Med 2014;371:1619–28.

Seymour CW, Yende S, Scott MJ, Pribis J, Mohney RP, Bell LN, et al. Metabolomics in pneumonia and sepsis: an analysis of the GenIMS cohort study. Intensive Care Med 2013;39:1423–34.

Slupsky CM, Rankin KN, Fu H, Chang D, Rowe BH, Charles PG, et al. Pneumococcal pneumonia: potential for diagnosis through a urinary metabolic profile. J Proteome Res 2009;8:5550–8.

Stringer KA, Serkova NJ, Karnovsky A, Guire K, Paine III R, Standiford TJ. Metabolic consequences of sepsis-induced acute lung injury revealed by plasma (1)H-nuclear magnetic resonance quantitative metabolomics and computational analysis. Am J Physiol Lung Cell Mol Physiol 2011;300:L4–L11.

Tam VC. Lipidomic profiling of bioactive lipids by mass spectrometry during microbial infections. Semin Immunol 2013;25:240–8.

Tanner LB, Chng C, Guan XL, Lei Z, Rozen SG, Wenk MR. Lipidomics identifies a requirement for peroxisomal function during influenza virus replication. J Lipid Res 2014;55:1357–65.

To KK, Chan JF, Chen H, Li L, Yuen KY. The emergence of influenza A H7N9 in human beings 16 years after influenza A H5N1: a tale of two cities. Lancet Infect Dis 2013a;13:809–21.

To KK, Fung AM, Teng JL, Curreem SO, Lee KC, Yuen KY, et al. Characterization of a Tsukamurella pseudo-outbreak by phenotypic tests, 16S rRNA sequencing, pulsed-field gel electrophoresis, and metabolic footprinting. J Clin Microbiol 2013b;51:334–8.

To KK, Lee KC, Wong SS, Lo KC, Lui YM, Jahan AS, et al. Lipid mediators of inflammation as novel plasma biomarkers to identify patients with bacteremia. J Infect 2015;70:433–44.

Ueland T, Svardal A, Oie E, Askevold ET, Nymoen SH, Bjorndal B, et al. Disturbed carnitine regulation in chronic heart failure–increased plasma levels of palmitoyl-carnitine are associated with poor prognosis. Int J Cardiol 2013;167:1892–9.

Uhlig S, Gulbins E. Sphingolipids in the lungs. Am J Respir Crit Care Med 2008;178:1100–14.

Upadhyay S, Niederman MS. Biomarkers: what is their benefit in the identification of infection, severity assessment, and management of community-acquired pneumonia? Infect Dis Clin North Am 2013;27:19–31.

Vlaar AP, Hofstra JJ, Kulik W, van Lenthe H, Nieuwland R, Schultz MJ, et al. Supernatant of stored platelets causes lung inflammation and coagulopathy in a novel in vivo transfusion model. Blood 2010;116:1360–8.

Wang L, Tang Y, Liu S, Mao S, Ling Y, Liu D, et al. Metabonomic profiling of serum and urine by (1)H NMR-based spectroscopy discriminates patients with chronic obstructive pulmonary disease and healthy individuals. PLoS One 2013;8:e65675.

Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. Eur Respir J 2013;42:802–25.

Woo PC, Lam CW, Tam EW, Lee KC, Yung KK, Leung CK, et al. The biosynthetic pathway for a thousand-year-old natural food colorant and citrinin in Penicillium marneffei. Sci Rep 2014;4:6728.

Wunderink RG, Waterer GW. Clinical practice. Community-acquired pneumonia. N Engl J Med 2014;370:543–51.

Zhou M, Osanai K, Kobayashi M, Oikawa T, Nakagawa K, Mizuno S, et al. Adenovector-mediated gene transfer of lysophosphatidylcholine acyltransferase 1 attenuates oleic acid-induced acute lung injury in rats. Crit Care Med 2014;42:e716–24.