

REVIEW ARTICLE

## The metabolomics of airway diseases, including COPD, asthma and cystic fibrosis

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### Abstract

Chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis (CF) are characterized by airway obstruction and an inflammatory process. Reaching early diagnosis and discrimination of subtypes of these respiratory diseases are quite a challenging task than other chronic illnesses. Metabolomics is the study of metabolic pathways and the measurement of unique biochemical molecules generated in a living system. In the last decade, metabolomics has already proved to be useful for the characterization of several pathological conditions and offers promises as a clinical tool. In this article, we review the current state of the metabolomics of COPD, asthma and CF with a focus on the different methods and instrumentation being used for the discovery of biomarkers in research and translation into clinic as diagnostic aids for the choice of patient-specific therapies.

### Keywords

Asthma, chronic obstructive pulmonary disease, cystic fibrosis, mass spectrometry, metabolomics, NMR spectroscopy

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### Introduction

Small airways are usually defined as airways with an internal diameter <2 mm (Burgel et al., 2009). Obstructions of these airways are not easily visualized by imaging techniques and their histopathological analysis is best analyzed in surgical lung biopsies because bronchoscopic transbronchial biopsies usually contain only a few small airways. The difficulties of sampling these airways in human subjects are responsible for the limited recognition of the important pathophysiological roles of small airways. The diagnosis of small airways disease has to rely on integration of multiple data including clinical context and medical history, computerized tomography (CT) scan pattern and pulmonary function tests. Moreover, histological findings will improve diagnostic accuracy, which requires bronchoscopic or surgical biopsy (invasive diagnostic method) that cannot be performed in all patients (Hogg, 2004). Hence, these limitations have led to the proposal of a non-invasive diagnostic method that may eliminate the requirement for biopsy in patients, particularly when bronchoscopic or surgical lung biopsy is not possible. Biomarker discovery using metabolomics tools is a promising approach for diagnosing pulmonary diseases. In this review, a brief explanation about metabolomics and its approaches, the advantages and

disadvantages of each method and the importance of biological specimens (as valuable sources of biomarkers) are presented. Then, a number of metabolomics studies using diverse biological specimens for various types of small and large airway diseases including, chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis (which are also characterized by both central and peripheral airways involvement; Burgel et al., 2007; Tiddens et al., 2010) are described and discussed in the other part of article.

### Metabolomics

Metabolomics is the comprehensive assessment of endogenous metabolites and attempts to systematically identify and quantify metabolites from a biological specimen in a global and targeted manner (Clayton et al., 2006; Nicholson & Lindon, 2008). These small molecules (<1000 Da), including peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids and inorganic species act as small-molecule biomarkers that represent the functional phenotype in a cell, tissue or organism (Arakaki et al., 2008).

The main metabolic fingerprinting approaches used for disease diagnostics are Raman and Infrared spectroscopy, Nuclear magnetic resonance (NMR) and mass spectrometry (MS; Ellis et al., 2007). Metabolomics methods are mostly focused on the information-rich analytical techniques of NMR and MS. NMR-based metabolomics has several advantages over other methods, e.g. MS. NMR requires little pre-treatment of samples; it also is rapid (10–15 min), non-destructive and non-invasive; and provides highly reproducible results (Fathi et al., 2013a,b, 2014a,b; Godet et al., 2001; Lacey

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et al., 1999; Lindon, 2003; Raftery, 2004). The main disadvantage of NMR is its relatively insensitive and therefore is limited to the highly concentrated metabolites. While MS offers a significantly lower limit of detection, disadvantages of MS methods include the potential biases introduced by sample preparation and differential ionization effects which can affect the detection and quantitation of metabolites (Wolak et al., 2009). The intrinsic high sensitivity of MS detection makes it an important approach for measuring metabolites in complex biofluids. MS methods coupled with prior separation modalities such as gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) provide a substantial amounts of chemical information for metabolomics studies (Soga, 2007; van der Greef & Smilde, 2005; Villas-Bôas et al., 2005; Wilson et al., 2005b; Zhang et al., 2007). Recently, ultrahigh pressure liquid chromatography (UPLC) method has been developed for this purpose which significantly improved the chromatographic resolution, and reduced the limit of detection by 3- to 5-fold (Wilson et al., 2005a). Although NMR, GC–MS, LC–MS and UPLC–MS are most often used for large-scale analysis, metabolomic detection is not limited to these techniques. Other alternative methods include thin-layer chromatography, high-pressure liquid chromatography (HPLC) with Ultra violet–visible (UV-Vis) absorbance, photodiode array or electrochemical detectors, Fourier transform infrared spectroscopy (FT-IR), Matrix-assisted laser desorption/ionization–Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR-MS) and a variety of other enzymatic assays (Zhang et al., 2012).

### Biological specimens

Metabolomics has been employed to investigate several body fluids such as urine, plasma, serum, cerebrospinal fluid, bile, seminal fluid, amniotic fluid, synovial fluid, gut aspirate, exhaled breath condensate (EBC), bronchoalveolar lavage fluid (BALF), saliva, sweat, intact tissue and its extracts as well as *in vivo* cells and their extracts (Bala et al., 2006; Beckonert et al., 2007; Bollard et al., 2005; Gowda et al., 2006; Griffin & Kauppinen, 2007; Ho et al., 2013; Ibrahim et al., 2013; Mena-Bravo & Luque de Castro, 2014). Urine and blood are the most commonly used biofluids for metabolomics based studies, as both samples contain thousands of detectable metabolites and can be obtained via non-invasive and minimally invasive methods, respectively.

### Urine

Urine is an excellent biological fluid for various medical studies. The relatively low concentrations of proteins and cells, high concentrations of metabolites, and its ease of collection in patients of all ages are obvious advantages of urine analysis (Forsythe & Wishart, 2009). In addition, urine requires minimal sample pre-treatment for NMR analysis. However, the high salt content of urine is more challenging for MS measurements which typically require sample preparation.

### Blood

Blood keeps a normal homeostasis in the body by constant regulatory mechanisms and so metabolic profiling of blood

provides a global view of the instantaneous metabolic status. MS analysis of serum is normally performed using extracts, and in the case of GC/MS, derivatization procedures are required (Major et al., 2006).

### EBC

Exhaled breath condensate is a simple, non-invasive and useful tool to study the biochemical and inflammatory molecules in the airway lining fluid (Kharitonov & Barnes, 2001). It is obtained by cooling exhaled air from spontaneous breathing and can be easily repeated (Horvath et al., 2005). The major advantage of EBC is to analyze both volatile and non-volatile metabolites (Maniscalco et al., 2006). The analysis of EBC currently has important limitations as the influence of age, sex, circadian rhythm and infection remains unknown (Accordino et al., 2008). Also various factors including smoking, alcohol consumption, equipment, exercise, mode and rate of breathing, nasal contamination, environmental temperature, humidity, exogenous contamination, ammonia and sulfur-containing compounds from the oral cavity affect EBC and lead to undesirable variability (Czebe et al., 2008; Kullmann et al., 2008; Slupsky et al., 2009).

### BALF

Bronchoalveolar lavage fluid (BALF) is performed by flushing the lungs with fixed volumes of physiologic solutions. BALF is a physiologically relevant sample for studying respiratory diseases (Wolak et al., 2009). However, it has the important limitation of being invasive, requiring the introduction of exogenous fluid into alveolar space (Sofia et al., 2011).

### Intact tissue

The rich metabolic profile of tissue is useful tool for the detection of biomarkers. However, it is an invasive method (Griffin & Kauppinen, 2007).

### Sweat

Sweat sampling is a non-invasive method. It avoids infections risk to patients who need daily analysis. Sweat is almost devoid of impurities. Compared to urine, sweat sample pre-treatment is less complex; therefore, sweat is the preferred method to use for drug control analysis in athletes. The major disadvantages of sweat as clinical sample are the low volume of secreted sweat, variability in secretion among individuals for analysis, sample evaporation, lack of appropriate sampling devices and errors in the results due to the presence of pilocarpine (stimulating sweat in human body). The main limitation is normalization of the sampled volume (Mena-Bravo & Luque de Castro, 2014).

### Metabolomics applications in the most common obstructive lung diseases

Asthma and COPD are heterogeneous diseases with various symptoms. Both diseases are characterized by airway obstruction and early diagnosis of them is quite a challenging task for the clinician (especially asthma in children). In case of cystic fibrosis (CF), a recent diagnosis of this disease is

based on genetic tests which show limitations. Therefore, research has focused on a simple, non-invasive test for early detection of these patients. Metabolomics has been applied to define metabolites related to prognosis or diagnosis of diseases and could provide earlier disease detection and greater pathophysiological understanding of diseases. Herein, we imply some published metabolomics studies for several airway diseases including, COPD, asthma and cystic fibrosis.

### Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by incompletely reversible airflow limitation that results from small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). COPD is one of the leading causes of mortality and morbidity worldwide. It is the fourth leading cause of death worldwide and is expected to be the third leading cause by 2030 (Mathers & Loncar, 2006). The most important environmental causative factor for COPD is cigarette smoking. COPD is a heterogeneous disease with features that are not captured by the measurement of forced expiratory volume in 1 s (FEV<sub>1</sub>; Vestbo et al., 2008). In addition to the effects seen in the lungs, a significant proportion of individuals with COPD develop cachexia, systemic inflammation and muscle dysfunction (Debigare et al., 2001; Mador & Bozkanat, 2001). The early diagnosis of COPD is quite a challenging task for the clinician, and considerable efforts have been made to find sensitive and specific predictive markers. Thus, the discovery of novel biomarkers that can identify disease phenotypes and detect early stage of disease by new techniques such as ‘‘omics’’ is of prime interest. For this aim, some investigators used metabolomic methods that were summarized below (some important metabolites were shown in Table 1).

Izquierdo-García et al. (2010) used mice in their study and analyzed metabolites by NMR. A total number of 112 mice underwent tobacco smoke for 6 months (they chose two models mice, chronic and acute model). The levels of glutathione (GSH), glycerophosphocholine, phosphorylcholine, taurine and an unidentified metabolite (probably isoleucine) in lung tissues helped to discriminate between control and chronic exposed samples.

EBC and saliva (because salivary contamination is of particular concern in EBC samples, so saliva was also obtained) metabolites were investigated in COPD patients using NMR spectroscopy. In the case of EBC, pyruvate concentration decreased intensely in patients compared to the control group. It was found that the levels of succinate was low in the control group ( $n = 12$ ) but absent in the COPD patients ( $n = 12$ ). Glutamine was present in the healthy control. Choline and phosphorylcholine were absent in the COPD patients in compared to healthy controls. Saliva samples of control group and COPD patients were positioned differently from EBC and from each other. This study has also revealed that despite the presence of some common metabolites (leucine, lactate, propionate, acetate, etc.) saliva spectra clearly differ from EBC, due to the lack of the carbohydrate signals in the EBC spectra. Therefore, NMR spectra of EBC did not show the presence of saliva signals (de Laurentiis et al., 2008).

Bertini et al. (2013) reported about exhaled breath condensate metabolomics in COPD patients using NMR. The study included 37 patients and 25 healthy subjects. The patients displayed significantly lower levels of acetone, valine and lysine, and significantly higher levels of lactate, acetate, propionate, serine, proline and tyrosine. Samples of saliva were obtained shortly before the EBC sampling to detect possible salivary contaminations. By comparing the spectra from EBC and saliva in the same subject, they excluded any significant salivary contamination of EBC.

There is evidence of abnormal muscle bioenergetics in COPD patients. For this reason, Rodríguez et al. (2011) studied the effects of exercise on plasma metabolic profile in COPD patients. They collected 18 COPD patients and 12 healthy individuals. Metabolome analysis of plasma was undertaken using NMR in two groups of pre- and post-training. The COPD patients showed lower plasma levels of some amino acids (valine, alanine and isoleucine) than healthy controls before training. It should be mentioned that these metabolites were positively associated with body composition. In healthy subjects, exercise generated a significant decrease in plasma levels of amino acids (glutamine, tyrosine, alanine, valine and isoleucine) and other substrates such as creatine, creatinine, citrate and glucose. Exercise significantly increased plasma levels of lactate, succinate and pyruvate. However, COPD patients only showed a significant post-training fall in plasma lactate. This study showed that plasma metabolic profiling contributes to the phenotypic characterization of COPD patients.

Another investigation on serum metabolomic profiles of COPD (in three stages: II, III and IV) and emphysema patients using <sup>1</sup>H NMR followed by LC-MS/MS confirmed metabolite changes detected by <sup>1</sup>H NMR (Ubhi et al., 2012b). In this study, a decrease in levels of lipoproteins [very low density lipoprotein (VLDL), low density lipoprotein (LDL) and chylomicrons] and N,N-dimethylglycine as well as an increase in levels of phenylalanine, glutamine, methylhistidine and ketone bodies in COPD patients was reported. In severely affected patients (stage IV), increased degradation of methylhistidine (a muscle protein) and utilization of branched-chain amino acids (BCAAs) as an alternative source of energy for muscle and adipose tissue were indicated. However, in emphysema patients BCAAs, aspartate, asparagine, glycine, creatine, N,N-dimethylglycine, glycerol, lipids (high density lipoprotein (HDL), VLDL, and LDL), O- and N-acetylglycoproteins and 3-hydroxyisobutyrate were significantly decreased whereas 3-methylhistidine, phenylalanine, glutamine and 3-hydroxybutyrate were elevated. In this study, MS results were in agreement with NMR results.

Paige et al. (2011) analyzed plasma metabolites in healthy non-smokers, smokers without emphysema and smokers with emphysema by Ultra High Performance Liquid Chromatography/quadrupole-time-of-flight mass spectrometry (UPLC-QTOF). They found predictive 12 biomarkers which were reported based on retention time. Also, they created predictive models with a supervised learning set, and these predictive models were found to be highly accurate in identifying the subjects with the emphysematous phenotype of COPD with excellent sensitivity and specificity.

Table 1. Summary of recent metabolomic studies in the field of COPD.

Author and year	Study population	Biological specimens	Technological platform used	Metabolites name (increment and decrement of metabolites in the patients compared to the control group)
Izquierdo-García et al. (2010)	Mice	Lung tissue	NMR	Taurine ↑ Glutathione ↑ Phosphoryl choline ↑ Glycero-phosphocholine ↑
de Laurentiis et al. (2008)	Human	EBC	NMR	Pyruvate ↓ Succinate ↓ Glutamine ↓ Choline and phosphorylcholine ↓
Bertini et al. (2013)	Human	EBC	NMR	Acetone ↓ Valine ↓ Lysine ↓ Lactate ↑ Acetate ↑ Serine ↑ Proline ↑ Tyrosine ↑
Rodríguez et al. (2011)	Human	Plasma	NMR	Valine ↓ Alanine ↓ Isoleucine ↓ Lactate ↓
Ubhi et al. (2012b)	Human	Serum	NMR and LC–MS/MS	VLDL/LDL/Chylomicrons ↓ N,N-dimethylglycine ↓ Methylhistidine ↑ Phenylalanine ↑ Glutamine ↑ Hydroxybutyrate ↑ BCAAs ↓
Paige et al. (2011)	Human	Plasma	UPLC-QTOF	Name of metabolites was not reported
McClay et al. (2010)	Human	Plasma Urine	NMR NMR	– Trigonelline ↓ Hippurate ↓ Formate ↓
Ubhi et al. (2012a)	Human	Serum	NMR and LC–MS/MS	Glutamine ↑ Aspartic acid ↑ Arginine ↑ Amino adipic acid ↓ BCAAs ↓ β-aminoisobutyric acid ↑ 3-methylhistidine ↑ 1-methylhistidine ↑ Sarcosine (in emphysema) ↓ Serine (in emphysema) ↑ Tryptophan (in emphysema) ↓

EBC: Exhaled breath condensate; BCAAs: Branched-chain amino acids; NMR: Nuclear magnetic resonance; UPLC–QTOF: Ultra High Performance Liquid Chromatography/quadrupole–time-of-flight mass spectrometry; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein.

Another metabolomics study was performed by McClay et al. (2010) using NMR. Plasma and urine were collected from 197 COPD patients and 195 healthy controls (90 cigarette smokers and 105 non-smokers). Their results showed that the urinary metabolites, e.g. trigonelline, hippurate and formate, are associated with lung function. The levels of all three metabolites were elevated in individuals with better lung function. No significant associations were found with plasma metabolites. Urinary hippurate and formate are often related to gut microflora. This could suggest that the microbiome varies between individuals with different lung function.

Ubhi et al. (2012a) distinguished three different cohorts of patients with COPD including severe COPD subjects from controls, patients with and without emphysema and patients with and without cachexia. They collected 30 smoker control subjects, 30 COPD, 38 with emphysema, 21 without

emphysema, 30 with cachexia, 30 without cachexia patients and 12 pancreatic cancer (7 with cachexia and 5 non-cachexia). Metabolomic study was performed by <sup>1</sup>H NMR and then, LC–MS/MS was used to confirm metabolite changes detected by <sup>1</sup>H NMR. They found that levels of glutamine, aspartic acid and arginine increased and amino adipic acid decreased in patients with severe COPD, emphysema, cachexia and pancreatic cancer. Concentrations of serine, sarcosine, tryptophan, BCAAs and 3-methylhistidine altered in severe COPD but not patients with pancreatic cancer. Common amino acids profiles across the cachexia models were that of β-aminoisobutyric acid, 1-methylhistidine and asparagine, while increased concentrations of γ-aminobutyric acid (GABA) were specific to the pancreatic cancer with cachexia. Their results displayed that targeted metabolomic amino acid profiles may assist in the diagnosis of COPD and discrimination of its subtypes.



## Asthma

Asthma is one of the most common chronic illnesses, especially in children. It is characterized by shortness of breath due to reversible airway obstruction and abnormal airway reactivity to various stimuli (Saude et al., 2009). Asthma is a heterogeneous syndrome with many clinical classifications based on patient symptoms, lung function and response to therapy. Unfortunately for clinicians, reaching the diagnosis of asthma and its management are more difficult than other chronic illnesses (Centers for Disease Control and Prevention, 2011; Knuffman et al., 2009). Although accurate invasive airway measurements are possible (such as bronchoscopy), this method remains expensive and also unavailable in routine clinical settings. Thus, research has focused on a simple, non-invasive test for detection of asthmatic patients. For this purpose, investigators used some techniques such as metabolomics. For instance, Saude et al. (2011) in 2010 investigated urine metabolomics by NMR. A total of 135 children (4–16 years) were recruited in two groups of asthmatic patients, stable ( $n=73$ ) and unstable asthma ( $n=20$ ), and healthy controls ( $n=42$ ). Their results showed that 23 and 28 metabolites were used to separate stable asthmatic versus healthy controls and stable asthmatic versus acute asthmatic patients, respectively. The best model to separate these three groups was 30 metabolites (some important metabolites were shown in Table 2).

In the study of Carraro et al. (2007), EBC was collected from 25 patients with asthma (17 with persistent asthma treated with inhaled corticosteroids, 8 with intermittent asthma inhaled corticosteroid naive) and 11 healthy subjects. Metabolomic profile in EBC was performed by NMR. They identified some profiles indicative of acetylated and oxidized compounds that significantly distinguished children with asthma from healthy children.

In another study, EBC metabolomics in asthma patients was investigated by Gahleitner et al. (2013). In this study, acceptable for children, healthy and asthmatic individuals were distinguished based on the eight volatile organic compounds at elevated levels in the breath of asthmatic children by GC–MS (these metabolites were shown in Table 2).

Another study on asthma was performed by Mattarucchi et al. (2012). They analyzed urine of 41 children with asthma and 12 healthy individuals by LC–MS. Their results displayed a decrease in excretion of methyl-imidazole acetic acid, urocanic acid and a metabolite similar to the structure of an isolusine–proline (Ile–Pro) fragment in the asthmatics. These metabolites could recognize asthmatic patients from control group.

Ibrahim et al. (2013) utilized NMR for analyzing EBC metabolomic profile to identify asthmatics from controls and to observe whether these profiles could be used to distinguish asthmatic phenotypes (subgroups were sputum eosinophilia, neutrophilia, asthma control and inhaled corticosteroid use). In this investigation, 82 asthmatics and 35 healthy controls were recruited. Results showed that five regions of NMR spectra were different between asthmatic patients and healthy volunteers [area under the receiver operating curve (AUROC) was 0.84]. Additional regions were able to discriminate

between sputum neutrophilia and use of inhaled corticosteroids, but could not distinguish between eosinophilia and asthma control.

Jung et al. (2013) investigated serum metabolomic profile on asthma patients. Serum samples of 39 asthmatics and 26 age-matched controls were analyzed by NMR. Levels of histidine, glutamine and methionine increased in the sera of patients with asthma whereas levels of acetate, methanol, formate, arginine, glucose, choline and O-phosphocholine decreased.

Ho et al. (2013) used GC–MS and LC–MS to analyze BALF samples from 32 mice with asthma (12 ovalbumin-challenged, 12 asthma model and 8 asthma treated with dexamethasone) and 12 native mice. Ovalbumin was used for treatment to produce allergic asthma. The metabolites that were detected in the BALF of mice with asthma as compared with allergic asthma or control were two groups (carbohydrates and lipids) that were characterized by increased levels of lactate, malate and creatinine and by decreased levels of mannose, galactose and arabinose. In the case of lipids, it was observed that phosphatidylcholines, diglycerides, triglycerides, cholesterol, cholic acid and cortol levels decreased and choline and hexadecenoylcholine levels increased. They investigated the effects of corticosteroids in lung metabolism resulting from asthma with administering dexamethasone. Dexamethasone was effective in suppressing airway inflammation and reversed many carbohydrate, lipid and sterol changes in experimental asthmatic lungs but ineffective in increased lactate, malate and creatinine.

Cap et al. (2004) measured leukotriene (LT) concentrations in EBC on asthmatic adults and children and in healthy age matched controls by GC–MS. Their results showed that the concentrations of LTD<sub>4</sub> and LTE<sub>4</sub> in EBC increased in both groups of patients with asthma. Also, they showed concentrations of LTs in EBC in controls were increased with age. Contamination of saliva in EBC was determined by the colorimetric detection of  $\alpha$ -amylase. No amylase activity was detected in any study sample, excluding significant salivary contamination.

Also, Dallinga et al. (2010) investigated metabolites profile in EBC for asthmatic children using GC–MS. This study included 63 asthmatic patients (in the age range of 5–16 years) and 57 healthy subject controls. Some metabolites were able to discriminate between patients and healthy subjects that were shown in Table 2. Patients had higher concentrations of metabolite than control subjects.

## Cystic fibrosis

Mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein cause CF. It promotes alterations in the transport of chloride and sodium in epithelial secreting cells, producing more viscid mucus (respiratory tract secretions), chronic respiratory tract infection, dysregulated and heightened inflammatory responses and progressive lung tissue destruction (Fahy & Dickey, 2010). CF is also known to be associated with abnormalities of lipid absorption and plasma lipid constituents. These abnormalities are primarily secondary to dysfunctional pancreatic and hepatobiliary secretory defects (Freedman et al., 2004).

Table 2. Summary of recent metabolomic studies in the field of asthma.

Author and year	Study population	Biological specimens	Technological platform used	Metabolites name (increment and decrement of metabolites in the patients compared to the control group)
Carraro et al. (2007)	Human	EBC	NMR	Acetylated and oxidized compounds ↑
Saude et al. (2011)	Human	Urine	NMR	Acetone ↑ Phenylalanine ↑ 2-Oxoglutarate ↓ Hydroxybutyrate ↑ Alanine ↑ 1-Methylnicotinamide ↑ O-Acetylcarnitine ↑
Gahleitner et al. (2013)	Human	EBC	GC–MS	1-(Methylsulfanyl)propane ↑ Ethylbenzene ↑ 1,4-Dichlorobenzene ↑ 4-Isopropenyl-1-methylcyclohexene ↑ 2-Octenal ↑ Octadecyne ↑ 1-Isopropyl-3-methylbenzene ↑ 1,7-Dimethylnaphtalene ↑
Mattarucchi et al. (2012)	Human	Urine	LC–MS	Urocanic acid ↓ Methyl-imidazoleacetic acid ↓ Fragment like isoleucyl–proline ↓ Metabolites were not identified
Ibrahim et al. (2013)	Human	EBC	NMR	VLDL/LDL ↑
Jung et al. (2013)	Human	Serum	NMR	Lactate ↑ Succinate ↑ Methionine ↑ Acetylglycoproteins ↑ Glutamine and Glutamate ↑ Alanine ↓ Histidine ↑ Formate ↓ Methanol ↓ Acetate ↓ Choline and O-phosphocholine ↓ Arginine ↓ Glucose ↓
Ho et al. (2013)	Mice	BALF	GC–MS LC–MS	Lactate ↑ Malate ↑ Creatinine ↑ Mannose ↓ Galactose ↓ Arabinose ↓ Phosphatidylcholines ↓ Diglycerides ↓ Triglycerides ↓ Cholesterol ↓ Cortol ↓ Cholic acid and hexadecenoylcholine ↑
Cap et al. (2004)	Human	EBC	GC–MS	Leukotriene B4, D4, E4 ↑
Dallinga et al. (2010)	Human	EBC	GC–MS	Branched hydrocarbon (C13H28) & (C11H24) ↑ Carbon disulphide (CS2) ↑ 1-Penten-2-on ↑ Butanoic acid ↑ 3-(1-methylethyl)-benzene ↑ Unsaturated hydrocarbon (C15H26) ↑ Benzoic acid ↑ <i>p</i> -Xylene ↑

EBC: Exhaled breath condensate; BALF: Bronchoalveolar lavage fluid; NMR: Nuclear magnetic resonance; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein; GC–MS: Gas chromatography–mass spectrometry; LC–MS: Liquid chromatography–mass spectrometry.

A recent diagnosis of CF is based on genetic tests which currently show limitations inherent to genotyping technology. The choice of mutations should be tested and clinical context in which the test is developed should be taken into account, i.e. each parameter can influence the interpretation of the genetic information. Hence, metabolomics biomarkers have demonstrated their usefulness in clinical, thus supporting the hypothesis that the metabolites may be useful to identify

diseases and the genetic tests, in this case, are used as a complementary test in the diagnostics this disease (Castellani et al., 2008).

Wolak et al. (2009) investigated metabolite profiles of BALF from 11 children (7.5–14 years) with CF with varying levels of inflammation by NMR. They found that the metabolite profiles from subjects with high airway inflammation displayed signals from numerous metabolites whereas

the spectra from subjects with low levels of inflammation were very sparse. Markers of inflammation such as alanine, valine, taurine and lactate were discriminant between patients with high versus low airway inflammation. Each of these metabolites was undetectable in the low-inflammation samples, but easily quantifiable in the high-inflammation samples.

Montuschi et al. (2012) studied metabolomic profiling in patients with stable and unstable CF by NMR. EBC and saliva (for determination of salivary contamination) were collected from 29 patients with stable and 24 with unstable CF and 31 healthy individuals (9–24 years). They showed that in EBC, acetate is higher in healthy controls than in patients with CF whereas ethanol, 2-propanol and acetone are higher in patients with CF than in healthy subjects. Among CF patients, ethanol and 2-propanol are higher in those with stable CF whereas acetate and methanol are higher in patients with unstable CF. Salivary and EBC NMR spectra are completely different, indicating no significant salivary contamination of EBC (Montuschi et al., 2012).

Another study was performed by Yang et al. (2012) using LC-MS/MS. They assessed regulatory lipid mediators (oxylipins) in sputum from 16 patients with CF lung disease (20–69 years). They used three different extraction protocols, the best results for the extraction were obtained using whole sputum (both supernatant and pellet parts). The results revealed that a broad range of both pro-inflammatory and anti-inflammatory oxylipins increased including, some of prostaglandins (PGE<sub>2</sub>, D<sub>2</sub>), thromboxanes (TXB<sub>2</sub>), leukotrienes (LTB<sub>4</sub>, hydroxy and carboxy LTB<sub>4</sub>), hydroxyeicosatetraenoic acids (HETEs), resolvine E<sub>1</sub> (anti-inflammatory lipid mediator) and lipoxine A<sub>4</sub>. They have also displayed that there is a negative correlation between FEV<sub>1</sub> and the LTB<sub>4</sub>, as well as one of the epoxides of linoleic acid and TXB<sub>2</sub> were positively correlated with FEV<sub>1</sub>.

In another study, sputum metabolomics was investigated in CF patients ( $n=7$ ) as compared to healthy control ( $n=9$ ) using NMR. Chlorinated and brominated tyrosine residues were detected in sputum samples from patients, but were not detected in the control group. They have demonstrated that neutrophils produce chlorinated tyrosine residues, while eosinophils produce predominantly 3-bromotyrosine and 3,5-dibromotyrosine. NMR spectroscopic analysis has been shown to be a rapid approach for measuring metabolites in sputum samples from patients with CF, and may lend itself readily to metabolomic analysis of disease phenotypes (Saude et al., 2004).

In study by Joseloff et al. (2014), serum metabolites profile was investigated in children with CF. The study included 31 patients with CF and 31 non-CF patients with other pulmonary diseases. They used ultrahigh performance liquid chromatography/tandem mass spectrometry (UHLC/MS/MS<sup>2</sup>) for basic and acidic species and GC-MS which is more suitable for non-polar metabolites. They showed that 92 from 459 metabolites were significantly different between CF and non-CF. These included lower levels of 3-hydroxybutyrate (ketone bodies), medium chain carnitines (involvement in fatty acid  $\beta$ -oxidation), 2-hydroxybutyrate (involvement in amino acid metabolism), tauroolithocholate 3-sulfate, glycocholenate sulfate, taurocholenate sulfate (secondary bile acids), bilirubin

and oxoproline (a metabolite of glutathione) and higher levels of di-carboxylic acids (alternate energy source by  $\omega$ -oxidation when  $\beta$ -oxidation is impaired) in serum of CF patients as compared with non-CF. Their results showed alterations in cellular energy metabolism potentially reflecting mitochondrial dysfunction and abnormalities in bile acid processing in CF. They have also displayed that bacterial metabolites, increased indole acetate and 4-hydroxyphenylacetate. While a decrease in 3-indoxyl sulfate and indole propionate were identified and differed between the groups indicating intestinal dysbiosis in CF.

Robroeks et al. (2010) recruited 48 children with CF and 57 controls and analyzed their EBC by GC-MS. They report that it was possible to identify patients 100% correctly by means of 22 compounds in exhaled breath. The discrimination between CF and controls was mainly based on C5–C16 hydrocarbons and *N*-methyl-2-methylpropylamine.

In another study (Wetmore et al., 2010), primary human airway epithelial cell were obtained following lung transplantation from 28 CF and 28 non-CF donors who were recruited from three different cohorts of patients. Samples were analyzed by UHPLC/MS/MS and GC-MS. It was reported that the most significant differences between the CF and non-CF cells were in nucleotide metabolism (decreased adenosine, inosine, hypoxanthine, guanosine and cytidine), tryptophan metabolism (increased kynurenine, anthranilate and 1-methylnicotinamide and decreased nicotinamide), organic osmolytes (low level of sorbitol and glycerophosphorylcholine) and energy metabolism (glycolysis: decreased glucose, glucose 6-phosphate, fructose 6-phosphate and lactate; pentose phosphate pathway: diminished ribulose-5-phosphate; tricarboxylic acid cycle: decreased malate; and sorbitol pathway: reduced sorbitol and fructose). They have also showed that GSH and ophthalmate (Glu-2-aminobutyrate-gly), a metabolite related to the synthesis of GSH, in CF cells decreased. Their results may indicate that glucose metabolism was suppressed in the CF cells. In addition, adenosine, guanosine and their derivatives have been identified as compounds that can act as activators of CFTR (Table 3).

### The major metabolites and metabolic pathways in COPD, asthma and CF patients

The shared metabolites among three pulmonary diseases have been shown in Table 4. Herein, it has been described the role of some these metabolites in the metabolic pathways of these diseases.

Carboxyhemoglobin (COHb) increases because of tobacco smoking (through carbon monoxide inhalation). Since COHb releases carbon monoxide slowly, less Hb will be accessible to transport oxygen from the lungs to other parts of the body (Crocker et al., 2013). As a result, COPD patients require more energy to breathe. This finding is consistent with some theories that suggested the major limitation to exercise performance in COPD patients is inadequate energy supply to the respiratory and locomotor muscles (Aliverti & Macklem, 2008). Therefore, when caloric intake is not enough to obtain energy needs, body resorts to breaking down fat and muscle tissue. Since BCAAs are utilized in the

Table 3. Summary of recent metabolomic studies in the field of CF.

Author and year	Study population	Biological specimens	Technological platform used	Metabolites name (increment and decrement of metabolites in the patients compared to the control group)
Wolak et al. (2009)	Human	BALF	NMR	Lactate ↑ Taurine ↑ Valine ↑ Alanine ↑
Montuschi et al. (2012)	Human	EBC	NMR	Acetone (Stable CF) ↑ Ethanol (Stable CF) ↑ 2-propanol (Stable CF) ↑ Acetate (Unstable CF) ↑ Methanol (Unstable CF) ↑
Yang et al. (2012)	Human	Sputum	LC-MS/MS	Prostaglandins ↑ Thromboxanes ↑ Hydroxy eicosatetraenoic acids ↑ Leukotrienes ↑ Lipoxine ↑ Resolvine E1 ↑
Saude et al. (2004)	Human	Sputum	NMR	Chloro and Bromotyrosine ↑
Joseloff et al. (2014)	Human	Serum	UHLC/MS/MS <sup>2</sup> GC-MS	Hydroxybutyrate ↓ Secondary bile acids ↓ Bilirubin ↓ Carnitine ↓ Di-carboxylic acids ↑ Indoleacetate ↑ Hydroxyphenylacetate ↑ Indolepropionate ↓ 3-indoxyl sulfate ↓ Oxoproline ↓
Robroeks et al. (2010)	Human	EBC	GC-TOF-MS	C5–C16 Hydrocarbons ↑ N-methyl-2-methylpropylamine ↑
Wetmore et al. (2010)	Human	Lung tissue	UHPLC/MS/MS GC-MS	Adenosine ↓ Inosine ↓ Hypoxanthine ↓ Guanosine ↓ Cytidine ↓ Kynurenine ↑ Anthranilate ↑ 1-methylnicotinamide ↑ Nicotinamide ↓ Sorbitol ↓ Glycerophosphorylcholine ↓ Glucose and G-6-p ↓ Fructose and F-6-p ↓ Ribulose-5-phosphate ↓ Lactate ↓ Malate ↓ Ophthalmate ↓ Glutathione ↓

EBC: Exhaled breath condensate; BALF: Bronchoalveolar lavage fluid; NMR: Nuclear magnetic resonance; UHLC/MS/MS<sup>2</sup>: Ultrahigh performance liquid chromatography/tandem mass spectrometry. G-6-p: Glucose 6-phosphate; F-6-p: Fructose 6-p.

synthesis of muscle proteins, their reduced levels appear consistent with muscle wasting and weight loss that are known to occur in advanced COPD (Agustí et al., 2002). Hence, it shows increased utilization of ketone bodies (3-hydroxybutyrate) and BCAAs as alternative energy sources in these patients. COPD patients also showed increased concentrations of serum methyl histidine, an amino acid formed during crosslinking of muscle protein synthesis, suggesting increased muscle actin and myosin protein degradation (Young & Munro, 1978). In fasting and starving states (Tom & Nair, 2006), concentrations of BCAAs increase due to elevated protein degradation. The muscle protein degradation rate diminishes after several weeks. Thus, BCAAs levels reduce as ketone-body production increases. Metabolomic data for COPD patients showed elevated ketone

bodies and reduced BCAAs. Also, it has been shown decreased mitochondrial function, consistent with recent reports in the early stages of COPD where skeletal muscle mitochondria show an electron transport chain blockade and excessive production of Reactive oxygen species (ROS) and stress oxidative (Puente-Maestu et al., 2009). It seems that measurable changes in the concentration of the GSH, present in lung tissue, can be used to develop biomarkers of early and chronic COPD. GSH protects cells against oxidants which are present in the epithelial lining fluid. Oxidant stress induces GSH synthesis in bronchial cells from COPD patients (Izquierdo-García et al., 2010).

The other important aspect of COPD pathogenesis is inflammation. Although COPD is known as an inflammatory lung disease, but many evidences show that COPD is associated



Table 4. Common metabolites among COPD, asthma and CF.

Metabolites	COPD	Asthma	CF
Choline and phosphocholine	Lung tissue ↑	BALF ↑	–
	EBC ↓	Serum ↓	
Glycerophosphorylcholine Phosphatidylcholines	Lung tissue ↑	–	Lung tissue ↓
	–	BALF ↓	–
Taurine	Lung tissue ↑	–	BALF ↑
Glutathione	Lung tissue ↑	–	Lung tissue ↓
Alanine	Plasma ↓	Urine ↑	BALF ↑
		Serum ↓	
Phenylalanine	Serum ↑	Urine ↑	–
Glutamine	Serum ↑	Serum ↑	–
	EBC ↓		
Arginine	Serum ↑	Serum ↓	–
BCAAs	EBC, blood ↓	–	–
Acetone	EBC ↓	Urine ↑	EBC ↑
Hydroxybutyrate	Serum ↑	Urine ↑	Serum ↓
Acetate	EBC ↑	Serum ↓	EBC ↑
Lactate	Plasma ↑	BALF ↑	BALF ↑
	EBC ↓		Lung tissue ↓
VLDL/LDL	Serum ↓	Serum ↑	–
Malate	–	BALF ↑	Lung tissue ↓
Methanol	–	Serum ↓	EBC ↑
Glucose	–	Serum ↓	Lung tissue ↓
methylnicotinamide	–	Urine ↑	Lung tissue ↑
Leukotriene	–	EBC ↑	Sputum ↑

EBC: Exhaled breath condensate; BALF: Bronchoalveolar lavage fluid; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein; BCAAs: Branched-chain amino acids.

with systemic inflammation (Wouters et al., 2009). The metabolomics studies showed some evidences in favor of lung local inflammation in COPD such as increased acetate levels in EBC. However, some other markers showed inconsistent result. As mentioned, alanine, valine, taurine and lactate are markers of inflammation and they did not change in parallel pattern in COPD that could be related to heterogeneity of COPD based on inflammation pattern.

In asthma, the evidence for systemic inflammation is rarer than in COPD (Wouters et al., 2009) and airway (local) inflammation showed by exhaled nitric oxide (NO) that known as a surrogate marker of airway inflammation (Erzurum & Gaston, 2012). Metabolomic pattern of asthma showed increased arginine (Arg) methylation. It regulates cytokine overexpression in allergic diseases and asthma (Boisvert & Richard, 2004; Lara et al., 2008). The results disclose that asthma patients have higher arginase activity and fractional exhaled nitric oxide (FE<sub>NO</sub>) than control subjects, which confirms that asthma is a disease characterized by elevated Arg catabolism (Lara et al., 2008). Arginase is strongly induced by Th2 (T helper) cytokines, such as IL-4 and IL-13, which are increased in asthma and related to the atopic phenotype (Corraliza et al., 1995; Modolell et al., 1995). Some metabolites (choline, arginine, acetone and protectin D1) are related to asthma severity and airflow limitation (Kim et al., 2014). 1-Methylnicotinamide is a derivative of nicotinamide and is reported as protective against asthma exacerbation (Bekier et al., 1974). Leukotrienes, LTD<sub>4</sub>, LTE<sub>4</sub> and LTB<sub>4</sub> are important inflammatory mediators that increase in EBC in subject with asthma and could be as potential marker for assessing disease activity, severity and treatment monitoring (Cap et al., 2004). Choline can suppress inflammation and oxidative stress when given in substantial amounts in asthma (Mehta et al., 2009, 2010). Increased choline levels may

possibly be an adaptive response in experimental asthmatic lungs to control the severity of inflammation, as a consequence of depleting other beneficial lipid metabolites. Phosphatidylcholine (PC), pulmonary surfactant, significantly diminished in subjects with asthma that it can lead to reduced lung functions in these patients (Baritussio, 2004).

In asthmatic patients, levels of glucose and lactate in serum are low and high, respectively, due to hypoxia-induced change (Waddell et al., 1967). Lipids are other important metabolites that play important role in this disease (Fogarty & Britton, 2000). High Levels of VLDL/LDL products are measured in asthmatic patients. When glucose levels are low, breakdown these elevated lipids may lead to increase acetone in this patients. There are evidences that VLDL and their derivatives activate the release of histamine, which is an important mediator of vascular permeability and smooth muscle construction in the airways (Gonen et al., 1987).

Cystic fibrosis is a recessive genetic disease characterized by dehydration of the airway surface liquid and impaired mucociliary clearance. As a result, individuals with the disease have difficulty clearing pathogens (mostly *Pseudomonas aeruginosa*) from the lung and experience chronic pulmonary infections and inflammation (Flume et al., 2010). Metabolomics evaluations give important information about disease activity through monitoring of inflammation and infection. Metabolomics markers of inflammation are increased levels of lactate and taurine in BAL, and LTB<sub>4</sub> in sputum (Wolak et al., 2009; Yang et al., 2012).

Some evidence of lung infection is related to *P. aeruginosa* colonization that express a secreted cytotoxin with enzymatic phospholipase activity that is able to release free unsaturated fatty acids from host cells (Saliba et al., 2005; Sato et al., 2005). In addition, *P. aeruginosa* expresses a number of fatty acid-metabolizing enzymes such as dioxygenases, and

lipoxygenase and so on (MacEachran et al., 2007; Vance et al., 2004) that may contribute to the virulence and air way persistence of the organism (Twomey et al., 2011). 2-Propanol, an enzyme-mediated product of reduction of acetone, was shown in EBC from CF patients infected with *P. aeruginosa*. The elevated 2-propanol levels might be due to bacterial metabolism or increased lipolysis and lipid peroxidation (Wang et al., 2006). Hydroxyphenylacetic and indole acetate are bacterial metabolite markers in serum in patients with CF (Joseloff et al., 2014). Therefore, metabolomics evaluation could help physicians for detecting inflammation and infection in CF patient and then management and control of it.

Other metabolites showing significant differences between CF and non-CF patients were GSH levels and its derivatives that significantly decreased in CF cells (Wetmore et al., 2010). CFTR cell surface expression and channel activation increase via GSH and their derivatives in CF epithelial cells (Servetnyk et al., 2006; Zaman et al., 2006). CFTR in addition to being able to transport GSH into the epithelial lining, may have other roles such as decreasing mucus viscosity by breaking disulfide bonds and playing important role in the inflammatory response to infection (Ballatori et al., 2009; Hudson, 2001, 2004). Inability to raise GSH levels in response to infection is the cause of poor response rate to infection in CF patients (Ballatori et al., 2009). Metabolism glucose in glycolysis, pentose phosphate pathway and sorbitol pathway has been reduced in CF cells. It may aggravate oxidative stress. The important source in cells for production of nicotinamide adenine dinucleotide phosphate (NADPH) is pentose phosphate pathway. On the other hand, NADPH is the most equivalent for reduction of two main antioxidants GSH and thioredoxin. Therefore, the pentose phosphate pathway may augment cell sensitivity to oxidative stress via reduced metabolic flux (Wetmore et al., 2010).

$\beta$ -oxidation of fatty acids (a key pathway for energy generation in the mitochondria) decreases in CF patients. The production of ketone bodies (3-hydroxybutyrate and acetoacetate) is used as a marker for the rate of  $\beta$ -oxidation that decreases in CF patients. The elevated di-carboxylic acid levels (from  $\omega$ -oxidation) as an alternate energy source support the opinion that mitochondrial energy production by  $\beta$ -oxidation may be impaired CF patients. The decreased metabolic activities of fatty acid  $\beta$ -oxidation catabolism can indicate mitochondrial dysfunction in subject with CF. Hence, alteration of mitochondrial activity may inter-relate with inflammation and oxidative stress (Joseloff et al., 2014).

## Conclusion

Asthma and COPD are the most prevalent lung diseases and one of the important causes of morbidity and mortality in the worldwide. In addition, these diseases are very variable and they cannot be precisely defined by clinical course due to their heterogeneous patho-physiological phenotypes. The complexity and the heterogeneity of asthma and COPD pose many problems in the development of highly reliable tests for their diagnosis and management. Metabolomics is new tools that can be used for earlier disease detection, therapy monitoring and ultimately for understanding the pathogenesis

of some diseases and reaching the goal of personalized medicine. In this context, some metabolomics studies that have been focused on the identification of metabolites associated with several respiratory diseases including COPD, asthma and cystic fibrosis were summarized. It has been displayed that metabolomics could discriminate these patients with healthy controls and may assist in discrimination of their subtypes using targeted metabolomics or detection of their severity. In addition, current review has explained some major metabolic pathways in these diseases based on metabolites biomarkers including, altered cellular energy metabolism (alteration in  $\beta$ -oxidation of fatty acids, glycolysis, pentose phosphate pathway and so on), modified mitochondrial function and increased inflammation and oxidative stress. These early studies are promising, however, validation studies are critically needed to confirm the identity and generality of the putative biomarkers.

## Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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