Docosatetraenoyl LPA is elevated in exhaled breath condensate in idiopathic pulmonary fibrosis

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

**Citation**
Montesi, Sydney B, Susan K Mathai, Laura N Brenner, Irina A Gorshkova, Evgeny V Berdyshhev, Andrew M Tager, and Barry S Shea. 2014. “Docosatetraenoyl LPA is elevated in exhaled breath condensate in idiopathic pulmonary fibrosis.” BMC Pulmonary Medicine 14 (1): 5. doi:10.1186/1471-2466-14-5. http://dx.doi.org/10.1186/1471-2466-14-5.

**Published Version**
doi:10.1186/1471-2466-14-5

**Citable link**
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11879673

**Terms of Use**
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Docosatetraenoyl LPA is elevated in exhaled breath condensate in idiopathic pulmonary fibrosis

Sydney B Montesi1,2, Susan K Mathai3, Laura N Brenner1, Irina A Gorshkova4, Evgeny V Berdyshev4, Andrew M Tager1,2 and Barry S Shea1,2*

Abstract

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal disease with no effective medical therapies. Recent research has focused on identifying the biological processes essential to the development and progression of fibrosis, and on the mediators driving these processes. Lysophosphatidic acid (LPA), a biologically active lysophospholipid, is one such mediator. LPA has been found to be elevated in bronchoalveolar lavage (BAL) fluid of IPF patients, and through interaction with its cell surface receptors, it has been shown to drive multiple biological processes implicated in the development of IPF. Accordingly, the first clinical trial of an LPA receptor antagonist in IPF has recently been initiated. In addition to being a therapeutic target, LPA also has potential to be a biomarker for IPF. There is increasing interest in exhaled breath condensate (EBC) analysis as a non-invasive method for biomarker detection in lung diseases, but to what extent LPA is present in EBC is not known.

Methods: In this study, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess for the presence of LPA in the EBC and plasma from 11 IPF subjects and 11 controls.

Results: A total of 9 different LPA species were detectable in EBC. Of these, docosatetraenoyl (22:4) LPA was significantly elevated in the EBC of IPF subjects when compared to controls (9.18 pM vs. 0.34 pM; p = 0.001). A total of 13 different LPA species were detectable in the plasma, but in contrast to the EBC, there were no statistically significant differences in plasma LPA species between IPF subjects and controls.

Conclusions: These results demonstrate that multiple LPA species are detectable in EBC, and that 22:4 LPA levels are elevated in the EBC of IPF patients. Further research is needed to determine the significance of this elevation of 22:4 LPA in IPF EBC, as well as its potential to serve as a biomarker for disease severity and/or progression.

Keywords: Idiopathic pulmonary fibrosis, Exhaled breath condensate, Lysophosphatidic acid

Background

Idiopathic pulmonary fibrosis is a progressive and ultimately fatal disease in which normal lung is replaced by fibrous scar tissue. The cause of the disease is unknown; however, exposure to refluxed gastric acid, occupational exposures, and viral infections have been postulated as inciting insults [1-3]. The average duration from diagnosis to time of death is 2–3 years [4]. Diagnosis is made either by pathology consistent with usual interstitial pneumonia or radiographic findings showing areas of fibrosis and honeycombing in the absence of an alternate diagnosis [5]. Once the diagnosis of IPF is made limited options exist for treatment except for lung transplantation.

Recent advances have occurred in our understanding of the mechanisms involved in IPF pathogenesis. Specifically, aberrant wound healing responses to tissue injury, such as epithelial cell apoptosis, increased vascular permeability, extravascular coagulation, and fibroblast migration and activation, have all been implicated in the development of lung fibrosis [6,7]. Research efforts have
focused on identifying molecular pathways central to the progression from normal to fibrotic lung, as a better understanding of such pathways may provide potential targets for pharmacologic therapy and biomarkers to aid in diagnosis or prognosis [7]. One such area of interest involves the role of lysophosphatidic acid (LPA) in the development and progression of pulmonary fibrosis.

LPA is a biologically active lysophospholipid that has been shown to mediate numerous biological processes thought to contribute to tissue fibrosis [7]. Structurally, LPA consists of glycerol-phosphate with a single fatty acid esterified at the sn–1 or sn–2 position. There are numerous LPA species present in biological fluids, identified by the length and degree of saturation of the fatty acid moiety [8]. The majority of extracellular LPA is produced from lysophosphatidylcholine (LPC) by the enzyme autotaxin (also known as lysosphospholipase D) [9,10]. LPA's activity is mediated by interaction with specific G protein-coupled receptors, six of which have been definitively identified (LPA1-6) [7,11,12]. The role of LPA and its receptors has been investigated in the development of fibrosis in multiple organ systems, including the lung, liver, kidneys, skin and peritoneum [13-17]. In the setting of lung injury, LPA has been shown to contribute to epithelial cell death, increased vascular permeability, and fibroblast migration and persistence via interaction with the LPA1 receptor, and genetic deficiency or pharmacologic inhibition of LPA1 confers protection against bleomycin-induced lung fibrosis in mice [13,18,19]. Furthermore, LPA is elevated in the BAL fluid of IPF patients and contributes to fibroblast migration into the injured airspaces in this disease [13]. Based on the apparent importance of the LPA-LPA1 pathway for the development of lung fibrosis, a Phase II clinical trial of an oral LPA1 antagonist for the treatment of IPF has recently been initiated (ClinicalTrials.gov identifier: NCT01766817). Recent evidence indicates that the LPA2 receptor can also mediate profibrotic effects of LPA, such as activation of latent transforming growth factor-β (TGF-β), and genetic deficiency of this receptor also results in protection against the development of lung fibrosis in mice [13,20,21].

Given its potentially important and central role in the development of pulmonary fibrosis, LPA is not only a therapeutic target but also a potential biomarker in IPF. While elevated LPA levels have been detected in the BAL from IPF patients [13], the extent to which LPA is present and detectable in exhaled breath condensate (EBC) is not known. EBC has become an area of interest for potential biomarker analysis in respiratory diseases [22]. Collection of EBC can be performed in a low-cost and non-invasive manner. For the detection of certain biologic molecules, correlation has been demonstrated between EBC and BAL results, though further research is needed [23]. In addition to volatile gases, EBC contains nonvolatile particles representing airway and alveolar lining fluid contents [24]. The ability to analyze components from the lining of the respiratory epithelium offers great potential for biomarker discovery. EBC has been studied in different respiratory diseases, including asthma and COPD [25,26]. However, few studies have analyzed EBC in the setting of interstitial lung disease, specifically IPF [27,28]. If LPA were detectable in EBC, it may provide information about the disease and/or the disease course. In this study we sought to assess for the presence of LPA in plasma and EBC and determine if differences exist in the amount of LPA in subjects with IPF versus controls.

**Methods**

**Study subjects**

Subjects with IPF were identified from those being cared for in the Massachusetts General Hospital (MGH) outpatient pulmonary clinic or inpatient pulmonary consult service. For inclusion in this study, subjects had to meet criteria for a diagnosis of IPF based on the recent joint consensus statement of the American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), and Latin American Thoracic Association (ALAT) [5]. Controls were recruited through the Partners Healthcare System Research Study Volunteer Program (RSVP). Controls were non-smoking individuals at least 50 years of age without a history of chronic lung disease. Study approval was obtained through the Partners Institutional Review Board, and informed consent was obtained on all subjects. Eleven IPF subjects and eleven controls were included in this study. EBC was obtained on all subjects, and plasma was obtained on all 11 IPF patients and 10 of the controls.

**Exhaled breath condensate (EBC) collection**

EBC was collected using the handheld RTube™ exhaled breath condensate collector (Respiratory Research, Inc.), according to the manufacturer’s instructions, and following the ATS/ERS methodological recommendations for EBC collection [29]. Collection was performed during 10 minutes of tidal breathing, with a nose clip in place, using a cooling chamber pre-cooled to −20°C. EBC samples were placed in aliquots and immediately frozen and stored at −80°C until analysis.

**Plasma collection**

Blood was obtained via venipuncture into tubes containing CTAD (citrate-theophylline-adenosine-dipyrindamole) additive, in order to potently inhibit platelet activation, as activated platelets are known to release abundant amounts of LPA [30]. Within 30 minutes of collection, whole blood was centrifuged at 1500 × g for 15 minutes to obtain plasma, which was then placed in aliquots and immediately frozen and stored at −80°C until analysis.
Lipid extraction
EBC samples were subjected to lipid extraction using the modified Bligh and Dyer method as described [31,32]. Briefly, lipid extraction was initiated by adding 2 ml methanol and 1 ml chloroform to 0.5 ml EBC, followed by the addition of 2 pmol C17-LPA (internal standard; Avanti Polar Lipids, Alabaster, AL, USA). Extraction was allowed for 30 minutes with the samples kept on ice. Then, phase separation was achieved by adding 1 ml chloroform and 1.3 ml 0.1 N HCl with vigorous vortexing. The chloroform phase was collected, the solvent was evaporated under a stream of nitrogen gas, and residues were dissolved in methanol and transferred into autosampler vials for LC-MS/MS analysis.

Measurement of LPA species by liquid chromatography-tandem mass spectrometry (LC-MS/MS)
LPA levels were determined using electrospray ionization liquid chromatography tandem mass spectrometry (ESI-LC/MS/MS) with an AB Sciex 5500 QTRAP hybrid triple quadrupole/ion trap mass spectrometer coupled with an Agilent 1200 liquid chromatography system. Lipids were separated on Ascentis Express C8 (75 × 2.1 mm, 2.7 μm) column using methanol:water:HCOOH, 60:40:0.5, v/v with 5 mM NH₄COOH as solvent A and acetonitrile:water:HCOOH, 80:20:0.5:0.5, v/v with 5 mM NH₄COOH as solvent B. LPA molecular species were analyzed in negative ionization mode with declustering potential and collision energy optimized for each LPA molecular species. Individual saturated and unsaturated LPA molecular species (16:0-, 17:0-, 18:0-, 18:1-, and 20:4-LPA, all obtained from Avanti Polar Lipids, Inc., Alabaster, AL) were used as reference compounds. 17:0-LPA was used as the internal standard, and LPA quantitation was performed by creating standard curves with variable amounts of each available LPA molecular species versus fixed amount of the internal standard (17:0-LPA). Total lipid extract from fetal bovine serum was used as a source of otherwise unavailable LPA molecular species to determine their chromatographic behavior and parameters of ionization and collision-induced decomposition, and the quantitation of these LPA molecular species was achieved via the use of the best possible approximation from the standard curves obtained with available individual LPA standards. The identification of LPA molecular species was achieved via monitoring for selected transitions from molecular to product (m/z 153) ions specific for each LPA molecular species, and by the analyte retention time identified by the available LPA standards and by comparing with LPA extracted from bovine serum.

Statistical analyses
Statistical analysis was performed using Prism 6.0 (GraphPad Software, Inc.). Differences in LPA levels between IPF patients and controls were analyzed for statistical significance using a two-tailed Student’s t-tests or Mann Whitney tests for parametric and nonparametric data, respectively. To adjust for multiple comparisons, we used the Bonferroni method to calculate the accepted α (Type I) error rate for each individual comparison performed, keeping the family-wise error rate at 0.05. Therefore, for EBC LPA levels, in which 9 different LPA species measured were measured, p values ≤ 0.0055 (0.05/9) were considered statistically significant. For plasma LPA levels, in which 13 different LPA species were measured, p values ≤ 0.0038 (0.05/13) were considered statistically significant.

Results
Patient characteristics
Relevant demographic and clinical data for IPF subjects (n = 11) and controls without lung disease (n = 11) on whom EBC and plasma LPA measurements were performed are summarized in Table 1. Of the 11 IPF subjects, 6 were diagnosed by surgical lung biopsy, and 5 were diagnosed by clinical and radiographic criteria alone. The mean age was 67.7 (+/− 8.5) years in the IPF group and 68.2 (+/− 7.1) years in the control group. The male to female ratio was 10:1 in the IPF group and 9:2 in the control group. There were no current smokers in either group; however, both groups contained former smokers. Spirometry data were available on 8/11 subjects and DLCO (diffusion capacity of carbon monoxide) data on 7/11 IPF subjects as shown in Table 1. Spirometry was not obtained on control subjects. Of the available pulmonary function results, the majority of testing (5/8) was performed on the day of EBC and plasma collection. All pulmonary function testing was performed within 15 days of sample collection. Supplemental oxygen was needed for 7/11 of IPF subjects. None of the IPF subjects were taking inhaled corticosteroids at the time of data collection.

Levels of LPA in exhaled breath condensate and plasma
Nine different LPA species were detected in the EBC from IPF subjects and controls (Table 2). Of these, docosatetraenoyl (22:4) LPA exhibited a statistically significant

Table 1 Subject characteristics

|                | IPF (n = 11) | Controls (n = 11) |
|----------------|-------------|------------------|
| Age (yrs)      | 67.7 (+/− 8.5) | 68.2 (+/− 7.1)   |
| Sex (M/F)      | 10/1        | 9/2              |
| Smoking (current/ever/never) | 0/8/3 | 0/9/2 |
| FVC (% predicted) | 60.5 (+/− 15.1) | N/A             |
| TLC (% predicted) | 61.5 (+/− 8.1)  | N/A             |
| DLCO [Hb] (% predicted) | 43.7 (+/− 20.8) | N/A             |

Data are presented as mean +/− SD as appropriate. N/A refers to data that was not available or collected.
difference between the two groups, with levels being significantly higher in IPF patients compared to controls (9.18 vs. 0.34 pM; p = 0.001). Furthermore, there was minimal overlap between EBC 22:4 LPA levels in IPF patients and controls. It was detected at levels > 1.5 pM in 9/11 IPF patients but was undetectable in all but three of the controls, and in only one control was the level > 0.4 pM (Figure 1). For the remaining eight LPA species, no statistically significant differences were detected between the two groups; however, there were trends towards increased levels of 18:2 LPA and 20:3 LPA in EBC of IPF patients (p = 0.13 and p = 0.055, respectively). There was no significant difference in the total amount of LPA in EBC between the two groups (664.69 +/− 83.03 vs. 766.15 +/− 137.35 pM, p = 0.73). Thirteen different LPA species were detected in plasma from IPF subjects and controls (Table 3). None of these 13 species showed statistically significant differences between the two groups, nor was there a statistically significant difference in the total amount of LPA between the two groups (mean control LPA 77.90 +/− 22.31 nM and mean IPF 64.51 +/− 12.82 nM, p = 0.10).

**Docosatetraenoyl (22:4) LPA and subject characteristics**

The average EBC 22:4 LPA level in IPF patients was 9.18 +/− 5.19 pM. There was no correlation between 22:4 LPA levels and disease severity, as determined by percent predicted FVC or DLCO (data not shown). One subject had an EBC 22:4 LPA level of 60 pM, which far exceeded the standard deviation of the mean. This subject was a 46 year-old man with biopsy-proven usual interstitial pneumonia (UIP) who required hospital admission for worsening respiratory status in the setting of a suspected IPF exacerbation. Spirometry performed during the patient’s hospitalization and within 10 days of EBC collection demonstrated a severe restrictive deficit, with a FVC of 33% predicted. He subsequently developed respiratory failure and eventually underwent lung transplantation within one month of sample collection. Pathologic review of the explanted lung revealed UIP in the accelerated phase.
None of the other ten IPF patients in this study were in the midst of IPF exacerbations during sample collection.

Discussion

LPA has emerged as an important pro-fibrotic mediator in multiple organ systems, particularly the lungs, and the first clinical trial of an LPA receptor antagonist has recently been initiated in IPF patients (ClinicalTrials.gov identifier: NCT01766817). In this study, we analyzed the exhaled breath condensate (EBC) and plasma from eleven IPF patients and eleven controls without lung disease for the presence of lysophosphatidic acid (LPA), using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We demonstrated that at least nine LPA species are detectable in EBC, and that one of these species, docosatetraenoyl (22:4) LPA, is significantly elevated in the EBC of IPF patients compared to controls. Thirteen LPA species were detectable in plasma; however, none of these differed significantly between the two groups.

Multiple species of LPA exist in biological fluids and are identified according to the composition of their fatty acid side chain. While all LPA species are thought to signal through LPA receptors, there are data indicating that the different species may have differing affinities for the various receptors [33]. Very little is known about 22:4 LPA specifically, and it is unclear whether or not its signaling profile differs significantly from that of other LPA species. Notably, unsaturated LPA species appear to have higher affinity for most LPA receptors than do saturated species [34]. In particular, long chain, polyunsaturated LPA species (like 22:4 LPA) have been shown to be the most potent activators of certain biological processes, such as platelet activation [35]. Therefore, it is possible that 22:4 LPA may have more potent pro-fibrotic effects compared to other LPA species, and that the increase in 22:4 LPA in the EBC of IPF patients may be playing a role in driving the disease process. It should be noted, however, that the amount of 22:4 LPA in EBC was only a small fraction of total LPA, which may argue against a significant pathophysiological role for this particular LPA species in IPF. The increase in 22:4 LPA may instead indicate the generation of LPA from a specific source, such as lung epithelial cells, which are known to contain high levels of polyunsaturated phospholipids [36].

In addition to being a therapeutic target, LPA may also serve as a useful biomarker for IPF. Elevations in LPA have been detected in the bronchoalveolar lavage (BAL) fluid from mice after intratracheal bleomycin administration and from humans with known IPF [13]. 22:4 LPA was not specifically measured in this previous report of IPF patients, but it is detectable in BAL fluid, and it and other long-chain, polyunsaturated LPA species have been found to be elevated in BAL fluid in a mouse model of asthma and in human allergic airway inflammation [37,38]. Our data suggest that EBC 22:4 LPA levels may be a useful biomarker for IPF diagnosis and/or prognosis. From a diagnostic standpoint, our data demonstrate minimal overlap between EBC 22:4 LPA levels in IPF patients and controls. To be of true value in the diagnosis of IPF, EBC 22:4 LPA levels would have to be able to differentiate between IPF and other forms of chronic interstitial lung diseases, most notably nonspecific interstitial pneumonia (NSIP) and chronic hypersensitivity pneumonitis (HP). As such comparisons were not performed in this study, further research would be needed to fully evaluate the potential role of EBC 22:4 LPA levels as a diagnostic biomarker in IPF.

It is notable that the EBC 22:4 LPA level in one patient was far outside the standard deviation of the mean, and that this patient was in the midst of an IPF exacerbation at the time of sample collection. This observation raises the hypothesis that EBC 22:4 LPA levels may be a useful biomarker of disease activity and/or acute exacerbations in IPF. Analysis of our data failed to reveal an association between EBC 22:4 LPA levels and disease severity or outcomes (decline in pulmonary function or mortality), although this study was likely underpowered to detect any such associations. Further study of EBC LPA levels in IPF, specifically in patients with rapidly progressive disease and those suffering from acute exacerbations, may shed light on the potential role of EBC 22:4 LPA levels as a prognostic biomarker in this disease.

While BAL has long been considered the optimal means of sampling the alveolar surfaces for analysis, it is invasive and not without risk, especially in subgroups of patients with advanced respiratory disease, such as those with pulmonary fibrosis. In comparison, EBC provides a method for non-invasive sampling of the lower respiratory tract. There are concerns regarding the accuracy with which EBC reflects the distal lung microenvironment, however, as there is risk of contamination with oral and gastrointestinal secretions, as well as an unknown (and potentially variable) dilution factor due to condensed water vapor [24]. Recommendations regarding optimized EBC collection have been made to minimize contamination and variations in solute dilution [24,29]. In our current study, it is reassuring that the two most abundant LPA species detected in EBC (16:0 and 18:0) were also the two most abundant species measured in BAL fluid from control subjects in the IPF (unpublished data) and asthma studies referenced above, suggesting that our EBC samples accurately reflect the distal lung compartments [38]. Furthermore, the total LPA levels in our EBC samples are similar to those seen in BAL fluid, with respect to both the mean values and the standard deviations, suggesting that the dilution
of the staff in the Massachusetts General Hospital Clinical Research Program HL108975 (to A.M.T.). The authors thank S. Engelstad, J. Oakley, and the rest U.S. National Institutes of Health grants K08-HL105656 (to B.S.S) and R01-

Acknowledgements

This study builds on previous work showing that LPA is increased in BAL fluid in IPF patients [13], and advances the current field of pulmonary research by showing that LPA can be extracted from EBC. Additional research is needed to determine any relationships between LPA species detectable in EBC and disease severity or progression in IPF.

Conclusions

LPA is detectable in exhaled breath condensate (EBC), and 22:4 LPA levels are elevated in the EBC of IPF patients compared to controls.

Abbreviations

IPF: Idiopathic pulmonary fibrosis; LPA: Lysophosphatidic acid; EBC: Exhaled breath condensate; BAL: Bronchoalveolar lavage; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; FVC: Forced vital capacity; DLCO: Diffusion capacity of carbon monoxide.

Competing interests

AMT has applied for the following patent: U.S. Patent Application, 12/450,051, filed September 9, 2009, “Lysophosphatidic Acid Receptor Targeting for the Treatment of Lung Disease”.

Authors’ contributions

SBM assisted in data collection, performed statistical analyses, and prepared the manuscript. SMK and LNB assisted in subject enrollment and performed clinical data collection and analyses. IG and EVB performed lipid extraction and measurement of LPA species by LC-MS/MS. BSS and AMT performed clinical data collection and analyses. IG and EVB performed lipid extraction and measurement of LPA species by LC-MS/MS. BSS and AMT designed and oversaw all aspects of this study. All authors read and approved of the final manuscript.

Acknowledgements

The authors gratefully acknowledge that these studies were supported by U.S. National Institutes of Health grants K08-HL105656 (to B.S.S) and R01-HL108975 (to A.M.T.). The authors thank S. Engelstad, J. Oakley, and the rest of the staff in the Massachusetts General Hospital Clinical Research Program for their assistance with this study.

Author details

1Pulmonary and Critical Care Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. 2Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. 3Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Denver, Aurora, CO, USA. 4Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA.

Received: 6 January 2014 Accepted: 14 January 2014 Published: 27 January 2014

References

1. Selman M: Idiopathic Pulmonary Fibrosis: Prevailing and Evolving Hypotheses about Its Pathogenesis and Implications for Therapy. Ann Intern Med 2001, 134:136–151.
2. Gross TJ, Hunnignhake GW: Idiopathic pulmonary fibrosis. N Engl J Med 2003, 345:517–525.
3. Wyers WA, Agostini C, Antoniou KM, Boursou D, Chambers RC, Cottin V, Egan JJ, Lambrecht BN, Lories R, Parfrey H, Prasse A, Robalo-Cordeiro C, Verbeken E, Verschakelen JA, Wells AU, Verleden GM: The pathogenesis of pulmonary fibrosis: a moving target. Eur Respir J 2013, 41:1207–1218.
4. Levy B, Collard HR, King TE: Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011, 183:431–440.
5. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier J-F, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancoojea J, Boursou D, Canvalho C, Costabel U, Elinea M, Hansell DM, Johkoh T, Kim DS, King TE, Kondoh Y, Myers J, Müller NL, Nicholson AG, Richeldi L, Selman M, Duddin RF, et al: An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011, 183:788–824.
6. Coward WR, Saini G, Jenkins G: The pathogenesis of idiopathic pulmonary fibrosis. Thor Adv Respir Dis 2010, 4:367–388.
7. Shea BS, Tager AM: Role of the lysophospholipid mediators lysophosphatidic acid and sphingosine 1-phosphate in lung fibrosis. Proc Am Thorac Soc 2012, 9:102–110.
8. Baker DL, Urmston ES, Desiderio DM, Tigi GI: Quantitative analysis of lysophosphatidic acid in human blood fractions. Am J Acad Sci 2000, 905:267–269.
9. van Meeteren LA, Ruurs P, Stortelers C, Bouwman P, van Rooijen MA, Pradère JP, Pettit TR, Wakeham MJ, Saudinier-Bäche J-S, Mummery CL, Mooilenaar WH, Jonkers J: Autotaxin, a secreted lysophosphatidase D, is essential for blood vessel formation during development. Mol Cell Biol 2006, 26:5015–5022.
10. Albers HMHG, Dong A, van Meeteren LA, Egan DA, Sunkara M, van Tilburg EW, Schuurman K, van Tellingen O, Smyth SS, Mooilenaar WH, Ovaa H: Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. Proc Natl Acad Sci U S A 2010, 107:2757–7262.
11. Mooilenaar WH, van Meeteren LA, Giepmans BN: The ins and outs of lysophosphatidic acid signaling. Bioessays 2004, 26:780–881.
12. Davenport AP, Alexander SPH, Sharmal JLM, Pawson AJ, Benson HE, Monaghan AE, Liew WC, Mamparnha CP, Bonner TJ, Neubig RR, Pin JP, Spedding M, Harmar A: International Union of Basic and Clinical Pharmacology. LXXXVIII. G protein-coupled receptor list: recommendations for new pairings with cognate ligands. Pharmacol Rev 2013, 65:967–986.
13. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah RA, Kim ND, Hart WR, Pardo A, Blackwell TS, Xu Y, Chun J, Luster AD: The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med 2008, 14:45–54.
14. Castelino EV, Seiders J, Bain G, Brooks SF, King CD, Swaney JS, Lorrain DS, Chun J, Luster AD, Tager AM: Amelioration of dermal fibrosis by genetic deletion or pharmacologic antagonism of lysophosphatidic acid receptor 1 in a mouse model of scleroderma. Arthritis Rheum 2011, 63:1405–1415.
15. Watanabe N, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Tomiya T, Tejima K, Nishikawa T, Arai M, Yanase M, Aoki I, Arai H, Omata M, Fujiwara K, Yatomi Y: Plasma lysophosphatidic acid level and serum autotaxin activity are increased in liver injury in rats in relation to its severity. Life Sci 2007, 81:1009–1015.
16. Sakai N, Chun J, Duffield JS, Wada T, Luster AD, Tager AM: LPA1-induced cytoskeleton reorganization drives fibrosis through CTGF-dependent fibroblast proliferation. FASEB J 2013, 27:1830–1846.
17. Pradère JP, Klein J, Gres S, Guigné C, Neau E, Valet P, Callie D, Chun J, Bascandis J-H, Saudinier-Bäche J-S, Schansstra JP: LPA1 receptor activation promotes renal interstitial fibrosis. Am J Physiol Nephrol 2007, 183:3110–3118.
18. Funke M, Zhao Z, Xu Y, Chun J, Luster AD: The lysophosphatidic acid receptor LPA1 promotes epithelial cell apoptosis after lung injury. Am J Respir Cell Mol Biol 2012, 46:355–364.
19. Swaney JS, Chapman C, Correa LD, Stettbiers KJ, Bundey RA, Prodanovich PC, Fagan P, Bocci E, Santini AM, Hutchinson JH, Seiders TJ, Pan TRA, Pratt J, Evans JR, Lorrain DS: A novel, orally active LPA1 receptor antagonist.
inhibits lung fibrosis in the mouse bleomycin model. Br J Pharmacol 2010, 160:1699–1713.

20. Huang LS, Fu P, Patel P, Harthith A, Sun T, Zhao Y, Garcia JGN, Chun J, Natarajan V: Lysophosphatidic Acid Receptor 2 Deficiency Confers Protection Against Bleomycin-Induced Lung Injury and Fibrosis in Mice. Am J Respir Cell Mol Biol 2013, 49:912–922.

21. Xu MY, Porte J, Knox AJ, Weinreb PH, Maher TM, Violette SM, McNalty RJ, Stepphard D, Jenkins G: Lysophosphatidic acid induces alphabeta6 integrin-mediated TGF-beta activation via the LPA2 receptor and the small G protein alphaq. Am J Pathol 2009, 174:1264–1279.

22. Kazani SS, Israel EE: Utility of exhaled breath condensates across respiratory diseases. Am J Respir Crit Care Med 2012, 185:791–792.

23. Antczak A, Pietrowski W, Marczak J, Ciebiada M, Gorski P, BARNES P: Correlation between eicosanoids in bronchoalveolar lavage fluid and in exhaled breath condensate. Dis Markers 2011, 30:213–220.

24. Effros RMR, Casaburi RR, Porszasz JJ, Morales EME, Rehan VV: Exhaled breath condensates: analyzing the expiratory plume. Am J Respir Crit Care Med 2012, 185:803–804.

25. Kazani S, Planaguma A, Ono E, Bonini M; Zahid M, Marigowda G, Wechsler ME, Levy BD, Israel E: Exhaled breath condensate eicosanoids levels associate with asthma and its severity. J Allergy Clin Immunol 2013, 132:547–553.

26. Antczak A, Ciebiada M, Petras T, Pietrowski WJ, Kurmanowska Z, Gorski P: Exhaled eicosanoids and biomarkers of oxidative stress in exacerbation of chronic obstructive pulmonary disease. Arch Med Sci 2012, 8:277–285.

27. Chow SS, Thomas PSP, Malouf MM, Yates DHD: Exhaled breath condensate (EBC) biomarkers in pulmonary fibrosis. J Breath Res 2012, 6:016004-016014.

28. Piaathakis KK, Mermigkis DD, Papatheodorou GG, Loukides SS, Panagou PP, Polychronopoulos W, Siafakas N, Bouros DD: Exhaled markers of oxidative stress in idiopathic pulmonary fibrosis. Eur J Clin Invest 2006, 36:363–367.

29. Horvath L, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJC, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jöbsis Q, Beurden WJC, Jalink K, Fahrenfort I, Moolenaar WH: The bioactive phospholipid lysophosphatidic acid is released from activated platelets. Biochem J 1993, 291(Pt 3):677–680.

30. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911–917.

31. Bandodh K, Aoki J, Taira A, Tsujimoto M, Arai H, Inoue K: Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. Structure-activity relationship of cloned LPA receptors. FEBS Lett 2000, 478:159–165.

32. Cite this article as: Montesi et al. Docosatetraenoyl LPA is elevated in exhaled breath condensate in idiopathic pulmonary fibrosis. BMC Pulmonary Medicine 2014 14:5.

33. Tigyi G: Aiming drug discovery at lysophosphatidic acid targets. Br J Pharmacol 2010, 161:241–270.

34. Georas SN, Berdisthein E, Hubbard W, Gershkova IA, Usatuyk PV, Saatian B, Myers AC, Williams MA, Xia Q, Liu M, Natarajan V: Lysophosphatidic acid is detectable in human bronchoalveolar lavage fluids at baseline and increased after segmental allergen challenge. Clin Exp Allergy 2007, 37:311–322.