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# Lipid-laden bronchoalveolar macrophages in asthma and chronic cough



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## KEYWORDS

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## Summary

**Background:** The presence of lipids in alveolar macrophages (AMs) may impair their phagocytic response, and determine airway inflammation and obstruction.

**Objective:** To determine the factors such as severity of asthma, chronic cough, airway inflammation and obesity that may influence the presence of lipids in lung macrophages.

**Methods:** Bronchoalveolar lavage fluid (BALF) was obtained from 38 asthmatics (21 severe and 17 mild/moderate), 16 subjects with chronic cough and 11 healthy control subjects. The presence of lipids in macrophages was detected using an Oil-red-O stain and an index of lipid-laden macrophages (LLMI) was obtained.

**Results:** LLMI scores were higher in healthy subjects (median 48 [IQR 10–61]) and the severe asthma group (37 [11.5–61]) compared to mild/moderate asthmatics (7 [0.5–37];  $p < 0.05$  each). Subjects reporting a history of gastro-oesophageal reflux disease (GORD) had higher LLMI values (41.5 [11.3–138] versus 13 [0–39.3],  $p = 0.02$ ). There was no significant correlation between LLMI and chronic cough, BAL cell differential counts, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC or body mass index (BMI).

**Conclusions:** The reduced LLMI in mild/moderate asthma may be related to lower incidence of GORD. However, this was not related to the degree of airflow obstruction, obesity or airway inflammation.

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### Abbreviations

ATS	American Thoracic Society
Ams	alveolar macrophages
BDP	beclomethasone dipropionate
BDR	bronchodilator reversibility
BMI	body mass index
BALF	bronchoalveolar lavage fluid
FEV <sub>1</sub>	forced expiratory volume in 1 s
FVC	forced vital capacity
IgE	immunoglobulin E
LLMI	lipid-laden macrophage index
LLAM	lipid-laden alveolar macrophage
PC <sub>20</sub> FEV <sub>1</sub>	provocative concentration of methacholine causing a 20% fall in pulmonary foam cell

## Introduction

Macrophages are found in most tissues including the lungs and airways where they play an important role in immune surveillance. They are responsible for the phagocytosis and endocytosis of dying cells and pathogens. Sputum macrophages from asthmatic subjects exhibit a phagocytic response that is inversely correlated with sputum eosinophilia and positively correlated with FEV<sub>1</sub> and PC<sub>20</sub>FEV<sub>1</sub> [1]. This suggests that decreased phagocytosis relates to airway inflammation and asthma severity. In contrast, sputum-derived macrophages from asthmatic patients with no sputum eosinophilia display impaired efferocytosis of apoptotic bronchial epithelial cells [2]. In addition, a reduced phagocytic response to *Staphylococcus aureus* [3] in macrophages from children with severe asthma has been related to the presence of oxidative stress as assessed by glutathione oxidation [4].

Alveolar macrophages (AMs) that become laden with lipid (predominantly cholesterol) are termed pulmonary foam cells or lipid-laden alveolar macrophages (LLAMs). These macrophages are more likely to undergo the process of programmed cell death and exhibit impaired phagocytosis [5], which may play an important pathogenic role in asthma. Furthermore, markers of lipid peroxidation measured in plasma are increased in asthma subjects [6] whilst erythrocytes and platelets exhibit alterations in membrane fatty acid composition [7].

A high fat content diet and the administration of a lipid emulsion in rats has been associated with the formation of LLAMs, leading to the view that the lungs may be a potential route of lipid excretion. However, it remains unclear whether such cells are derived from AMs ingesting lipids in the alveoli or from lipid-laden blood monocytes that migrate to the alveoli [8].

Lipid accumulation in alveolar macrophages appears to occur primarily through the phagocytosis of external lipoproteins. This process may be secondary to endogenous sources, such as lipid pneumonia; where the presence of bronchial obstruction, chronic lung infection or a lipid storage disorder results in the accumulation of LLAMs; or an

exogenous source, for example following aspiration or inhalation of lipid [9]. There are other factors which have been associated with an increase in LLAM formation. These include gastroesophageal reflux disease (GORD) [10,11], hypoxia [12] and iatrogenic causes, whereby drugs such as amiodarone, fluoxetine, and gentamicin containing a cationic amphiphilic structure can induce cellular phospholiposis through a dose-dependent process involving the inhibition of lysosomal phospholipase activity and accumulation of lamellar bodies [13,14]. In addition, macrophages have been shown to internalise and degrade surfactant lipids and surfactant protein A (SP-A) *in vitro*, suggesting a role for AMs in surfactant clearance [15]. Other factors such as obesity and cholesterol may also influence lipid-laden macrophage formation, but their role has not yet been established.

The aim of this study was to explore the relationship between the presence of LLAMs, chronic cough and asthma severity in a cohort of patients with mild/moderate and severe asthma. In addition, we determined whether obesity, the presence of GORD, lung inflammation or the degree of airflow obstruction influenced the accumulation of intracellular lipids.

## Methods

### Patients

Patients were recruited from our Asthma Clinic and through advertisement, and underwent a screening visit. Those with significant respiratory or inflammatory co-morbidities and those requiring treatment for a respiratory tract infection within the preceding four weeks were excluded. Healthy subjects had no smoking history and no history of respiratory or cardiac disease. All subjects underwent a bronchoscopic procedure with bronchoalveolar lavage.

Asthma was based on a physician diagnosis and patients either had a PC<sub>20</sub> methacholine concentration causing a 20% fall in FEV<sub>1</sub> of <8 mg/ml and/or an FEV<sub>1</sub> reversibility of ≥12% of baseline. Severe asthma was defined according to the American Thoracic Society (ATS) definition of refractory asthma [16]. Patients who did not fulfil the criteria for severe asthma were classified as non-severe asthmatics and they were taking ≤1000 mcg of inhaled beclomethasone dipropionate (BDP) or equivalent per day.

Patients with chronic cough were recruited from a specialist cough clinic at our hospital and all subjects reported a dry persistent cough for at least 8 weeks duration. All subjects had been investigated with a standard protocol to exclude a diagnosis of asthma or other respiratory disease.

Ethical approval was obtained from the West London 3 and the Brompton, Harefield and NHLI Research Ethics Committees (11/H0706/4 and 08/H0708/29).

### Fibreoptic bronchoscopy and bronchoalveolar lavage

Sixty-seven patients (21 severe asthma, 19 mild/moderate asthma, 16 patients with chronic cough, and 11 healthy subjects) underwent fibreoptic bronchoscopy with

bronchoalveolar lavage (BAL). These were performed under sedation according to standard clinical practice at the Royal Brompton Hospital. BAL was performed from a segment of the right middle lobe (RML). The bronchoscope was wedged and warmed aliquots of buffered normal saline were installed (50 ml  $\times$  4, total 200 ml) and immediately aspirated through the bronchoscope by gentle suction.

BAL fluid was centrifuged (500  $\times$  g for 10 min), washed with Hanks' balanced salt solution (HBSS) and the cell pellet re-suspended in culture media (RPMI with 0.5% fetal calf serum, antibiotics and L-glutamine) to a density of  $\sim 0.5 \times 10^6$  macrophages/ml. Cytospins were stained with REASTAIN Quick-Diff kit (Reagent International Oy Ltd, Finland) and a cell differential count was performed.

### Measurement of lipids in macrophages

We used the lipid-laden macrophage index (LLMI) to quantify lipid accumulation in AMs. Slides were air-dried and then stored at  $-80^\circ\text{C}$ . They were then stained with Oil-Red-O (Sigma–Aldrich Ltd, UK) to enable scoring and LLMI calculated by grading the amount of intracellular Oil-Red-O positive particles per 100 AMs (Fig. 1). Intracellular lipid was evaluated using a modified version of that proposed by Corwin and Irwin [17,18]. Cells were scored from 0 to 4 whereby 0 = no opacification, 1 = up to  $\frac{1}{4}$  opacified, 2 =  $\frac{1}{4}$  to  $\frac{1}{2}$  opacified, 3 =  $\frac{1}{2}$  to  $\frac{3}{4}$  opacified, and 4 = totally opacified (Fig. 1). The LLMI was calculated by the sum of the scores of 100 cells giving a potential range from 0 to 400. The person who measured the LLMI was not aware of clinical findings.

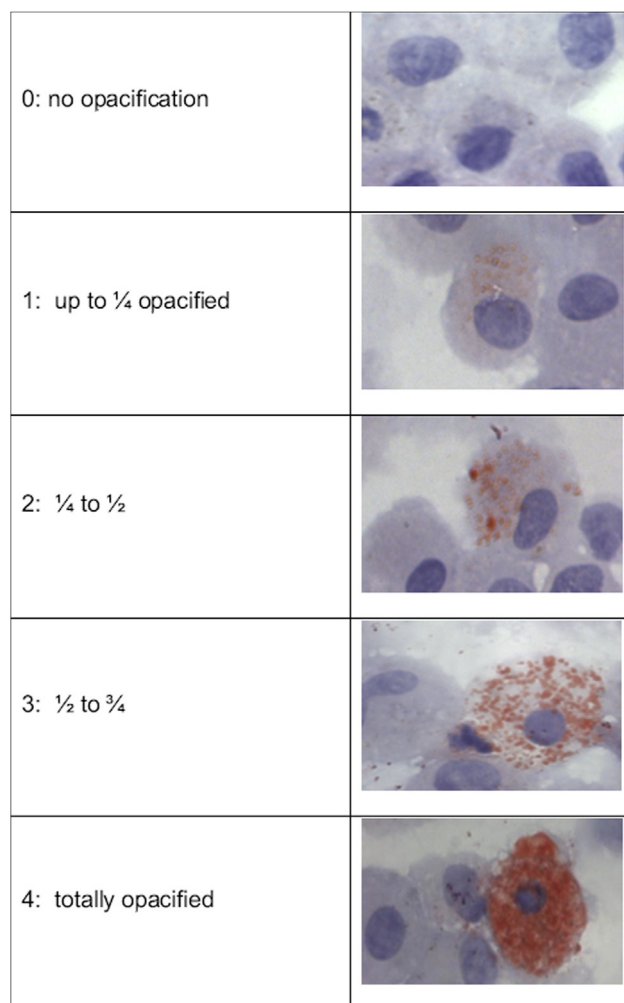
### Statistical analysis

Categorical variables were reported as percentages and comparisons done using the chi-square or Fisher's exact test. Numerical variables were reported as mean (SD) or median (IQR) and comparisons done using Mann–Whitney test or Kruskal Wallis test. Statistical significance was defined as  $p < 0.05$ . Logistic regression was used to assess the predictive factors for GORD.

## Results

### Patient characteristics

Two patients were excluded from subsequent analysis due to poor quality of the staining. Significant differences were seen between groups in terms of inhaled and oral corticosteroid use, FEV<sub>1</sub> % predicted and FEV<sub>1</sub>/FVC ratio (Table 1). The severe asthma group exhibited greater bronchodilator reversibility and lower PC<sub>20</sub>. Both asthmatic groups were more likely to be atopic than the chronic cough and healthy control group (81% and 87.5% compared to 33.3%, 27.3% respectively;  $p < 0.0005$ ). Sixteen patients (28.6%) reported a history of GORD of whom 14 were taking medication with proton pump inhibitors. A greater history of GORD and PPI use was noted in the severe asthma (40% and 42.1% respectively) and chronic cough (41.7% and 44.4%) groups



**Figure 1** Determination of lipid-laden macrophage index (LLMI). BAL cytopins were stained with Oil-Red-O. AMs were graded by the amount of lipid in the cytoplasm staining red. Cells were scored from 0 to 4: 0 = no opacification, 1 = up to  $\frac{1}{4}$  opacified, 2 =  $\frac{1}{4}$  to  $\frac{1}{2}$  opacified, 3 =  $\frac{1}{2}$  to  $\frac{3}{4}$  opacified, and 4 = totally opacified. The panels show examples of each score. LLMI was reported as the sum of the scores of 100 cells giving a potential score range of 0–400.

compared to the mild/moderate asthma (21.4% and 13.3%) and healthy control (none reported) groups.

BAL cell differential counts were similar between all the groups with an increase in eosinophils noted in the mild/moderate group ( $p < 0.05$ ). A higher neutrophil percentage in BALF was noted in the severe group (median 3.4%, [IQR 0.9–7.3]), but this did not reach statistical significance (Table 2).

### Lipid-laden macrophage index in different groups

A wide range was seen in the severe asthma (0–246) and chronic cough (0–297) groups. The distribution of LLMI by diagnoses can be seen in Fig. 2. The mild/moderate asthmatic group had the lowest LLMI (median, 7; IQR 0.5–37) with a significant difference noted versus the severe asthma (median, 37; IQR 11.5–61,  $p < 0.05$ ) and healthy

**Table 1** Demographics.

N	Healthy subjects	Chronic cough	Mild/moderate asthma	Severe asthma	p value
	11	16	17	21	
Age (years)	35.6 ± 13.9	43.1 ± 12.8	43.7 ± 14.6	43.8 ± 12.5	0.34
M/F	8/3	5/11	11/6	11/10	0.13
ICS (BDP equivalent [ $\mu$ g])	0	0 (0–500)	200 (0–600)	1600 (1600–4000)	<0.0001
Oral steroid (mg), (n)	0 (0)	0 (0)	0 (0)	10 (0–20), 14	
BMI	26.9 (25.6–32.4)	25.5 (23.9–27.4)	24.9 (21.1–30.3)	28.4 (24.6–33.5)	0.33
Smoking					
Current	0	1	0	0	0.45
Ex	1	1	0	3	
Never	10	13	17	18	
FEV <sub>1</sub> %	104.8 (98.2–108.0)	87.7 (83.6–100.4)	84.9 (69.8–88.9)	74.9 (60.6–84.0)	<0.0001
FEV <sub>1</sub> /FVC %	81.6 (75.6–86.1)	79.0 (75.3–84.4)	69.9 (67.7–74.2)	64.7 (54.5–72.9)	<0.0001
BDR (n)	1.4 ± 2.3 (6)	NA	13.9 ± 8.2 (14)	22.8 ± 13.8 (17)	0.0002
PC <sub>20</sub> (n)	>16 (11)	>16 (3)	2.3 ± 2.5 (14)	0.88 ± 0.6 (5)	<0.0001
Atopic (%) <sup>a</sup>	3 (27.3)	4 (33.3)	14 (87.5)	17 (81.0)	0.0005
IgE kU/l	73.5 (21–126.3)	58.5 (16–137.5)	151.0 (63–268.5)	184 (49.5–615.5)	0.06
GORD (%)	0 (0)	6 (50)	2 (14.3)	8 (42.1)	0.02
PPI (%)	0 (0)	4 (44.4)	2 (12.5)	8 (42.1)	0.03

Data are presented as mean ± SD or median (IQR). Between group comparisons for continuous variables were made using Kruskal–Wallis test and for categorical variables using  $\chi^2$  exact testing.

BMI, body mass index.

BDR, bronchodilator response.

BDP eq, beclomethasone dipropionate equivalent.

PPI, proton pump inhibitor.

<sup>a</sup> The data presented are for subjects with either a positive RAST or skin prick test.

groups (median, 48; IQR 10–61,  $p < 0.05$ ). No significant difference was noted between the other groups. A similar pattern was seen when the total number of cells (score out of 100) staining positive were analysed. The healthy (median 23; IQR 9–36) and severe (17.5; 5.5–48.5) were higher than the mild/moderate (7; 0.5–14) and chronic cough (7; 2–21) groups.

No significant associations were noted between BALF cell differential and the LLMI for the entire cohort or for individual groups. One chronic cough patient with an LLMI of 297 had a BAL neutrophilia of 69% although, in two other cases where the LLMI was greater than 200, there was no neutrophilia.

## GORD

Six of the top eight subjects with the highest LLMI scores, including the top four, reported a history of GORD. The

median LLMI score was higher in the GORD group (median 41.5 [IQR, 11.3–138] versus 12.5 [2.3–35.3]) compared to those who did not ( $p < 0.05$ ) (Fig. 3). Univariate logistic analysis (Table 3) showed a significant association between the presence of GORD and an LLMI score of  $\geq 60$ .

## Other associations

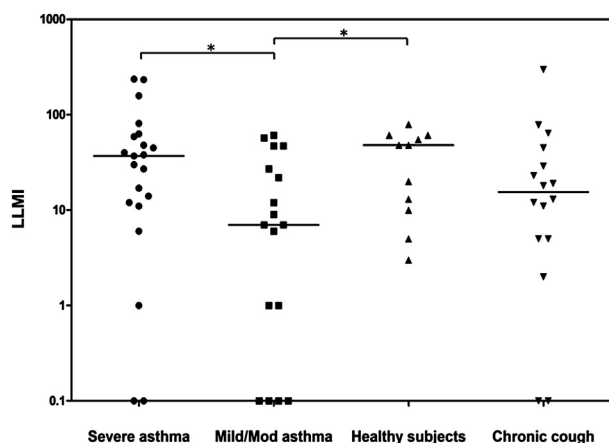
There was no association between FEV<sub>1</sub> and LLMI in individual groups or all subjects grouped together. There were no differences in LLMI according to eosinophilia, age, BMI, gender or atopy.

## Discussion

We have found that LLMI scores were lower in mild/moderate asthmatics compared to the severe asthmatics and healthy controls. The difference between the two asthma

**Table 2** Differential cell counts in bronchoalveolar lavage.

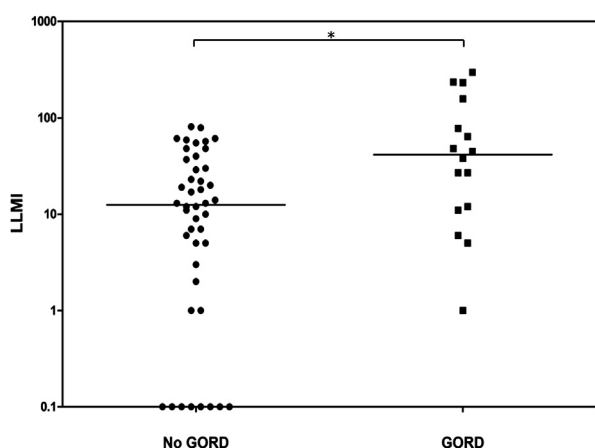
N	Healthy subjects	Chronic cough	Mild/moderate asthma	Severe asthma	p value
	11	15	16	18	
Neutrophils %	1.5 (0–1.8)	1.7 (0.9–5.2)	1.2 (0.5–2.6)	3.4 (0.9–7.3)	0.15
Eosinophils %	0.3 (0–0.8)	0.2 (0–1.0)	1.4 (0.3–6.4)	0.6 (0–3.8)	0.03
Macrophages %	90.2 (55.8–95.8)	90.4 (64.1–94.3)	87.0 (73.8–93.2)	87.4 (75.5–93.3)	0.98
Lymphocytes %	7.5 (2.0–39.8)	5.7 (4.0–24.7)	5.4 (2.1–12.0)	5.6 (2.0–12.9)	0.84



**Figure 2** Distribution of lipid-laden macrophage index (LLMI) in normal, patients with chronic cough and in mild-moderate and severe asthma patients. Median scores are shown. \* $p < 0.05$ .

cohorts may reflect the fact that GORD was reported more in the severe group (42.1% versus 14.3%). Another potential explanation relates to increased levels of oxidised or glycosylated low-density lipoproteins (LDL) seen in asthma [19,20]. This has been suggested as a modulator of inflammation and results in LLM formation via macrophage scavenger receptor recognition [21]. The use of oral corticosteroids (OCS) is another potential confounding factor. 14 (66.7%) of the severe asthma cohort were on maintenance oral corticosteroid therapy. However, subanalysis of the cohort according to OCS use showed no significant differences in LLMI scores.

Patients with chronic cough had a lower LLMI compared to the severe asthma group and healthy controls, although this did not reach statistical significance. LLMI has been evaluated as a marker of chronic aspiration in chronic cough. A study of 30 consecutive adults presenting to a tertiary referral centre with chronic cough found that there was no significant difference in LLMI scores when compared to 20 asymptomatic adults [24].



**Figure 3** Lipid-laden macrophage index (LLMI) (expressed on  $\log_{10}$  scale) in patients with and without a history of gastroesophageal reflux disease (GORD). Median scores are shown. \* $p < 0.05$ .

The higher scores seen in the healthy cohort are harder to explain given that no GORD was reported. This supports the view that lipid accumulation is a normal process including the ingestion of lipids endogenously present within the alveolar space, such as lung surfactant [15]. Another explanation may be related to micro-aspiration and the fact that no subjects in the healthy cohort were taking PPIs. LLMI scores were highly variable and there was considerable overlap between groups. Despite previous reports associating LLMI with BALF neutrophilia [22] and airflow obstruction [23], we did not find an association in this cohort.

There was a correlation between the presence of GORD and LLMI, with greater scores in the symptomatic group. The association between aspiration and LLMI has been previously evaluated in children with some studies reporting a positive correlation [18,25] and suggesting that particular cut-off values may be a useful diagnostic test [26]. However, other studies have failed to confirm that LLMI, measured in lavage fluid or sputum, is an indicator of GORD [18,27], highlighting a lack of specificity to detect reflux-related respiratory disease [28]. In adults, LLMI has been proposed as a marker for aspiration pneumonia [10]. In cystic fibrosis patients post lung transplantation, it may be a useful method of assessing response to Nissen fundoplication [29] and a score of  $>150$  was found to have a sensitivity of 82.3% and a specificity of 76.4% correlating with an abnormal 24-h pH test in these lung transplant recipients [30].

LLMI measured in macrophages obtained from sputum samples has been correlated to oropharyngeal reflux [11]. However, other studies have failed to confirm this [31] and the use of LLMI in induced sputum is limited in smokers; where the presence of inclusions from cigarette smoke within the cytoplasm, resulting from the phagocytosis of cigarette smoke particulates, increases the LLMI score [32].

Although this study has identified an association between GORD and LLMI, there are some limitations. We relied on self-reported GORD and did not use objective measures such as validated questionnaires or a 24-h pH probe. A study incorporating objective measures and selecting a more symptomatic cohort would be better placed to examine this potential association. LLMI calculation is time-consuming and a simpler semi-quantitative method of calculating alveolar lipid content has been suggested [23] which appear to produce similar results [33]. In addition, the repeatability of LLMI scores in individuals is not known. Further studies, incorporating repeat bronchoscopies, are required to establish whether this simpler method could replace the LLMI and provide information on individual variability. We have compared asthma and chronic cough patients to a cohort of healthy subjects. Furthermore, LLMI may fluctuate over time depending on factors such as hyperlipidaemia, BMI and micro-aspiration. LLMI should be correlated to the underlying disease process and co-morbidities that may influence the presence of lipid-laden macrophages.

This is the first time LLMI has been assessed in asthma. Although no correlation was seen with BALF neutrophils or with the degree of airways obstruction, there was a strong association with GORD.

**Table 3** Univariate Logistic Regression of predictive factors for presence of gastro-oesophageal reflux disease (GORD).

Predicting factors	Odds ratio	95% CI	p value
Age	1.01	0.97–1.05	NS
Gender	1.07	0.34–3.39	NS
FEV <sub>1</sub> (% pred)	0.99	0.96–1.02	NS
BAL eos (%)	1.31	0.28–6.02	NS
BAL neut (%)	0.78	0.28–2.91	NS
LLMI			
0–20	1.0		
21–59	2.64	0.64–10.91	0.18
≥60	8.7	1.79–42.3	0.007

CI = confidence interval, NS = non-significant.

## Contributions

Study conception and design – all authors. Data abstraction and statistical analysis – DG, AS, WB, CR, JP. The manuscript was written by DG and KFC. Manuscript revision and final approval – all authors.

## Conflicts of interest

DG, JZ, AS, WB, CR, JS, JPR, JH, AMG, PKB and KFC have no conflicts of interest to report with respect to this study.

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## References

- [1] Alexis NE, Soukup J, Nierkens S, Becker S. Association between airway hyperreactivity and bronchial macrophage dysfunction in individuals with mild asthma. *Am J Physiol Lung Cell Mol Physiol* 2001 Feb;280(2):L369–75.
- [2] Simpson JL, Gibson PG, Yang IA, Upham J, James A, Reynolds PN, et al. Impaired macrophage phagocytosis in non-eosinophilic asthma. *Clin Exp Allergy* 2013 Jan;43(1):29–35.
- [3] Fitzpatrick AM, Holguin F, Teague WG, Brown LA. Alveolar macrophage phagocytosis is impaired in children with poorly controlled asthma. *J Allergy Clin Immunol* 2008 Jun;121(6):1372–8, 1378.
- [4] Fitzpatrick AM, Teague WG, Burwell L, Brown MS, Brown LA. Glutathione oxidation is associated with airway macrophage functional impairment in children with severe asthma. *Pediatr Res* 2011 Feb;69(2):154–9.
- [5] Schrijvers DM, De Meyer GR, Kockx MM, Herman AG, Martinet W. Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005 Jun;25(6):1256–61.

- [6] Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 1996 Oct;154(4 Pt 1):1055–60.
- [7] De CJ, Hernandez-Hernandez A, Rodriguez MC, Sardina JL, Llanillo M, Sanchez-Yague J. Comparison of changes in erythrocyte and platelet phospholipid and fatty acid composition and protein oxidation in chronic obstructive pulmonary disease and asthma. *Platelets* 2007 Feb;18(1):43–51.
- [8] Shibuya K, Tajima M, Yamate J, Saitoh T, Sannai S. Genesis of pulmonary foam cells in rats with diet-induced hyper beta-lipoproteinaemia. *Int J Exp Pathol* 1991 Aug;72(4):423–35.
- [9] Hadda V, Khilnani GC. Lipoid pneumonia: an overview. *Expert Rev Respir Med* 2010 Dec;4(6):799–807.
- [10] Adams R, Ruffin R, Campbell D. The value of the lipid-laden macrophage index in the assessment of aspiration pneumonia. *Aust N Z J Med* 1997 Oct;27(5):550–3.
- [11] Parameswaran K, Anvari M, Efthimiadis A, Kamada D, Hargreave FE, Allen CJ. Lipid-laden macrophages in induced sputum are a marker of oropharyngeal reflux and possible gastric aspiration. *Eur Respir J* 2000 Dec;16(6):1119–22.
- [12] Bostrom P, Magnusson B, Svensson PA, Wiklund O, Boren J, Carlsson LM, et al. Hypoxia converts human macrophages into triglyceride-loaded foam cells. *Arterioscler Thromb Vasc Biol* 2006 Aug;26(8):1871–6.
- [13] Reasor MJ, Hastings KL, Ulrich RG. Drug-induced phospholipidosis: issues and future directions. *Expert Opin Drug Saf* 2006 Jul;5(4):567–83.
- [14] Jacobson W, Stewart S, Gresham GA, Goddard MJ. Effect of amiodarone on the lung shown by polarized light microscopy. *Arch Pathol Lab Med* 1997 Dec;121(12):1269–71.
- [15] Wright JR, Youmans DC. Degradation of surfactant lipids and surfactant protein A by alveolar macrophages in vitro. *Am J Physiol* 1995 May;268(5 Pt 1):L772–80.
- [16] Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2001;162:2341–51.
- [17] Corwin RW, Irwin RS. The lipid-laden alveolar macrophage as a marker of aspiration in parenchymal lung disease. *Am Rev Respir Dis* 1985 Sep;132(3):576–81.
- [18] Colombo JL, Hallberg TK. Recurrent aspiration in children: lipid-laden alveolar macrophage quantitation. *Pediatr Pulmonol* 1987 Mar;3(2):86–9.
- [19] Schunemann HJ, Muti P, Freudenheim JL, Armstrong D, Browne R, Klocke RA, et al. Oxidative stress and lung function. *Am J Epidemiol* 1997 Dec;146(11):939–48.
- [20] Sedgwick JB, Hwang YS, Gerbyshak HA, Kita H, Busse WW. Oxidized low-density lipoprotein activates migration and degranulation of human granulocytes. *Am J Respir Cell Mol Biol* 2003 Dec;29(6):702–9.
- [21] Brown BE, Nobecourt E, Zeng J, Jenkins AJ, Rye KA, Davies MJ. Apolipoprotein A-I glycation by glucose and reactive aldehydes alters phospholipid affinity but not cholesterol export from lipid-laden macrophages. *PLoS One* 2013 May;8(5):e65430.
- [22] Sacco O, Silvestri M, Sabatini F, Sale R, Moscato G, Pignatti P, et al. IL-8 and airway neutrophilia in children with gastro-oesophageal reflux and asthma-like symptoms. *Respir Med* 2006 Feb;100(2):307–15.
- [23] Kazachkov MY, Muhlebach MS, Livasy CA, Noah TL. Lipid-laden macrophage index and inflammation in bronchoalveolar lavage fluids in children. *Eur Respir J* 2001 Nov;18(5):790–5.
- [24] Carney IK, Gibson PG, Murree-Allen K, Saltos N, Olson LG, Hensley MJ. A systematic evaluation of mechanisms in chronic cough. *Am J Respir Crit Care Med* 1997 Jul;156(1):211–6.
- [25] Bauer ML, Lyrene RK. Chronic aspiration in children: evaluation of the lipid-laden macrophage index. *Pediatr Pulmonol* 1999 Aug;28(2):94–100.
- [26] Furuya ME, Moreno-Cordova V, Ramirez-Figueroa JL, Vargas MH, Ramon-Garcia G, Ramirez-San Juan DH. Cutoff

- value of lipid-laden alveolar macrophages for diagnosing aspiration in infants and children. *Pediatr Pulmonol* 2007 May; 42(5):452–7.
- [27] Kitz R, Boehles HJ, Rosewich M, Rose MA. Lipid-laden alveolar macrophages and pH monitoring in gastroesophageal reflux-related respiratory symptoms. *Pulm Med* 2012; 2012:673637.
- [28] Rosen R, Fritz J, Nurko A, Simon D, Nurko S. Lipid-laden macrophage index is not an indicator of gastroesophageal reflux-related respiratory disease in children. *Pediatrics* 2008 Apr;121(4):e879–84.
- [29] Hayes Jr D, Kirkby SS, McCoy K, Mansour HM, Khosravi M, Strawbridge H, Tobias JD. Reduction of lipid-laden macrophage index after laparoscopic Nissen fundoplication in cystic fibrosis patients after lung transplantation. *Clin Transplant* 2013 Sep; 27(1):121–5.
- [30] Hoskins G, McCowan C, Neville RG, Thomas GE, Smith B, Silverman S. Risk factors and costs associated with an asthma attack. *Thorax* 2000 Jan;55(1):19–24.
- [31] Koksall D, Ozkan B, Simsek C, Koksall AS, Agackyan Y, Sasmaz N. Lipid-laden alveolar macrophage index in sputum is not useful in the differential diagnosis of pulmonary symptoms secondary to gastroesophageal reflux. *Arch Med Res* 2005 Sep;36(5):485–9.
- [32] Wilson AM, Nair P, Hargreave FE, Efthimiadis AE, Anvari M, Allen CJ. Lipid and smoker's inclusions in sputum macrophages in patients with airway diseases. *Respir Med* 2011 Nov;105(11): 1691–5.
- [33] Ding Y, Simpson PM, Schellhase DE, Tryka AF, Ding L, Parham DM. Limited reliability of lipid-laden macrophage index restricts its use as a test for pulmonary aspiration: comparison with a simple semiquantitative assay. *Pediatr Dev Pathol* 2002 Nov;5(6):551–8.