

Lipid-laden macrophages in induced sputum are a marker of oropharyngeal reflux and possible gastric aspiration

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ABSTRACT: The diagnostic properties of a "lipid index" of macrophages in induced sputum as a noninvasive marker of aspiration of acidic gastric contents were evaluated.

In a cross-sectional study, 33 subjects (17 with symptoms suggestive of gastro-oesophageal reflux) with normal chest radiographs and no symptoms of aspiration or sinus disease, underwent dual-channel 24-h ambulatory oesophageal pH recording and sputum induction. Oropharyngeal reflux, defined as at least one episode of a fall in pH to <4 at the upper oesophageal electrode, was considered indicative of aspiration of acidic gastric contents ("gold standard"). An index for the presence of intracellular lipid in sputum macrophages, detected by oil red O stain, was obtained. The sensitivity, specificity and predictive values of this "lipid index" were calculated.

The "lipid index" could be calculated in 29 of 33 samples with high interobserver repeatability (intraclass correlation coefficient 0.96). Twenty subjects showed oropharyngeal reflux and nine did not. The median "lipid index" in subjects with oropharyngeal reflux (24.5) was significantly greater than that in those without reflux (1.0) ($p < 0.001$). A "lipid index" of 7.0 had a sensitivity of 90%, a specificity of 89%, a positive predictive value of 95% and a negative predictive value of 80%.

A "lipid index" of 7.0 in the macrophages of induced sputum is a good marker of oropharyngeal reflux.

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Reflux of acidic gastric contents causing parenchymal and interstitial lung disease is thought to be common [1, 2], particularly in elderly debilitated patients [3] and children [4, 5]. Gastro-oesophageal reflux (GOR) may be associated with pulmonary microaspiration [6] and may also contribute to the pathogenesis of cough [7, 8] and asthma [9]. There is presently no noninvasive test for the detection of aspiration of gastric juice into the airway or the assessment of the success of antireflux treatment. Demonstration of lipid in alveolar macrophages may be an indicator of aspiration of acidic gastric contents [10], and cytological examination of bronchoalveolar lavage fluid for lipid-laden macrophages has been suggested as a useful diagnostic test for aspiration [11–15]. Determination of cell counts in induced sputum separated from saliva is a reliable noninvasive method of assessing airway inflammation [16], and the use of an index of macrophages in sputum staining positive for intracellular lipid has been reported [17]. In previous studies there have been two difficulties, the lack of a "gold standard" for the diagnosis of aspiration and the inclusion of patients with a variety of chronic respiratory diseases [18]. Therefore, the present study investigated the diagnostic value of an index of sputum lipid-laden macrophages in identifying acid aspiration, using dual-channel 24-h pH monitoring, in subjects without any chronic respiratory disorder other than asthma.

Subjects and methods

Subjects

Thirty-three adult subjects who consented to undergo dual-channel 24-h ambulatory pH recording were recruited from the patients and staff of the Firestone Chest and Allergy and the Gastro-intestinal Motility Disorders Clinics of St Joseph's Hospital (table 1). Seventeen subjects had symptoms suggestive of GOR and 16 did not. None had a history suggestive of aspiration, had a lipid storage disease or fat embolism, or were receiving nutritional supplementation with fat emulsions. All had normal chest radiographs. No patient had experienced any occupational dust exposure or known exposure to moulds. None were taking nasal medications. Seventeen subjects had mild asthma (10 atopics, mean \pm SD forced expiratory volume in one second (FEV₁) 82.9 \pm 13.8% of the predicted value, geometric mean \pm SD provocative concentration of methacholine causing a 20% fall in FEV₁ 1.7 \pm 2.8 mg·mL⁻¹). Five of them were on inhaled corticosteroids (median dose 500 μ g equivalent of beclomethasone, range 50–800 μ g). None of the subjects had any other respiratory problem. The study was approved by the Ethics Committee of St Joseph's Hospital and all subjects gave written informed consent.

Table 1. – Subject characteristics, oesophageal pH and induced sputum indices

	Oropharyngeal reflux present	Oropharyngeal reflux absent
Subjects n	20	9
Males n	9	2
Age yrs	40±16	34±12
GOR symptoms n*	10	3
Confirmed GOR diagnosis n	14	2
Asthma n	9	8
FEV ₁ % pred	90.2±15.9	94.4±9.5
Lower oesophagus pH monitor		
Cumulative time at pH <4%	5.9±6.2	2.9±3.1
Reflux episodes n	84.5±95.6	74.1±80.4
Upper oesophagus pH monitor		
Cumulative time at pH <4%	0.5±0.9	0
Reflux episodes n	14.8±12.5	0
Sputum lipid index	35.1±29.1 24.5 (4–100)	4.7±10.3 1.0 (0–28)
Sputum cell count		
Total 10 ⁶ cells·g ⁻¹	3.0±1.6	5.7±5.8
Neutrophils %	35.8±20.2	38.6±23.9
Eosinophils %	1.5±2.9	1.7±2.9
Macrophages % [#]	60.0±19.0	57.0±22.0
Lymphocytes %	1.3±0.7	1.2±0.8

Data are presented as mean±SD or median (range). *: four excluded patients showed symptoms of gastro-oesophageal reflux (GOR); #: total number not the number staining positive for lipids. FEV₁: forced expiratory volume in one second; % pred: percentage of the predicted value.

Methods

The present investigation was a cross-sectional single-centre, observational study. All subjects underwent oesophageal motility and pH studies and sputum examination and completed a GOR symptom questionnaire within one week of each other. Symptoms of GOR were assessed using a validated questionnaire [19]. Oesophageal measurements were made after stopping any antireflux medications for ≥5 days. An ambulatory dual-channel digitrapper (Medtronic-Synectics, Stockholm, Sweden) was used to perform the 24-h pH testing. The lower pH electrode was positioned 5 cm above, and the upper pH electrode 25 cm above, the manometrically determined lower oesophageal sphincter. The following measurements were made using both electrodes: cumulative time (expressed as a percentage of the total recording time) during which the pH was <4.0; and the number of episodes during which the pH fell <4.0. GOR was defined as a cumulative time of >4% of the recording time with a pH of <4.0 at the lower pH electrode. Oropharyngeal reflux was defined as at least one episode of a fall in pH to <4.0 at the upper pH electrode, not associated with eating or drinking. This was considered to be an indicator of possible aspiration of acidic gastric contents.

Sputum was induced with hypertonic saline, and then separated from saliva and processed within 2 h; cell counts were determined as previously described [16]. Cytospins were prepared and stained with oil red O (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada) to detect cytoplasmic lipid droplets. One hundred consecutive macrophages were evaluated using a five-point scale similar to that reported by CORWIN and IRWIN [11] (0: absence of staining; 1: one or a few lipid droplets; 2: many distinct droplets; 3: many droplets with visible nucleus; and 4: many droplets completely covering the cytoplasm and

obscuring the nucleus). The "lipid index" was the sum of the scores for 100 consecutive macrophages and thus had a possible range of 0–400. Assessments were carried out by two independent observers (high interobserver agreement with an intraclass correlation coefficient of 0.96), who were blind to the clinical details of the subjects.

Analysis

All data were analysed using the statistical package SPSS for Windows®, release 10.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). The sensitivity, specificity and predictive values of the lipid index in the detection oropharyngeal reflux were calculated from the receiver operating characteristic curve, constructed for different cut-off values of the index. Between-group comparisons were made using the Mann-Whitney U-test. Correlations between variables were examined using Spearman's coefficient of rank correlation.

Results

The lipid index could not be calculated in four of the 33 sputum samples because of poor quality of staining; these four subjects were excluded from subsequent analysis. The demographic, pH and sputum data (table 1) of the 29 included subjects were evaluated. There were 20 subjects with at least one episode of reflux to the oropharynx. The lipid index in these subjects was significantly greater ($p<0.001$) than that in those without reflux, and there was a significant correlation between the lipid-index and the number of reflux episodes ($r_s=0.5$, $p<0.01$). The receiver operating characteristic curve (fig. 1) showed a lipid index of 7.0 to the maximum area under the curve (upper left corner), with a sensitivity of 90%, a specificity of

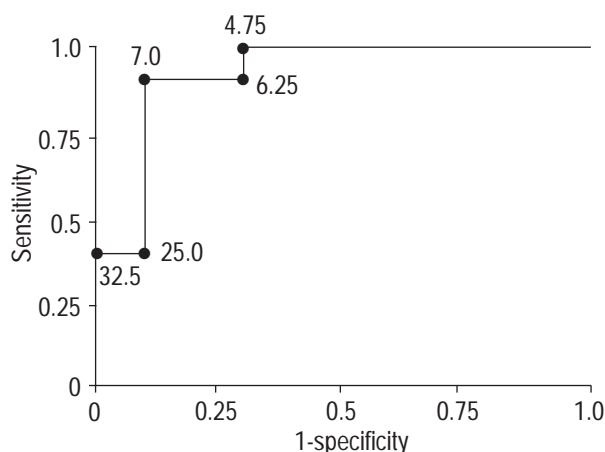


Fig. 1. – Receiver operating characteristic curve for the lipid index of macrophages in induced sputum for the detection of oropharyngeal reflux measured by dual-channel 24-h ambulatory oesophageal pH recording. Numbers corresponding to dots represent lipid index of macrophages.

89%, a positive predictive value of 95% and a negative predictive value of 80% (table 2). The only difference between the asthmatic and nonasthmatic subjects was in sputum eosinophil count (mean \pm SD 3.6–4.0 and 0.2 \pm 1.2%) respectively, $p < 0.001$). There were no significant differences in lipid index (median interquartile range) 16 (33) in asthmatics and 15.5 (25) in nonasthmatics. The total and neutrophil differential cell counts in sputum, the 24-h pH scores and the number of reflux episodes in both the lower and upper oesophagus were also similar in asthmatic and nonasthmatic subjects.

Discussion

In the present pilot study, it has been shown that a lipid-laden macrophage index of 7.0 in induced sputum from subjects with a normal chest radiograph and no clinical history to suggest pulmonary aspiration is a sensitive (90%) and specific (89%) indicator of oropharyngeal reflux, with high positive (95%) and negative (80%) predictive values.

The presence of lipid in alveolar macrophages is a consequence of aspiration of gastric contents or exogenous causes of lipoid pneumonia [20]. In the present study, the latter possibility was excluded by including only subjects with normal chest radiographs. The staining method and semiquantitative index used are the same as previously reported by CORWIN and IRWIN [11]. From the available literature, it is difficult to determine the measurement properties of this index because there is uncertainty regarding the gold standard for aspiration [18]. Most studies have used relatively crude indicators such as response to therapy, clinically witnessed aspiration, barium oesophagography or conventional single-channel 24-h pH monitoring to detect aspiration [11–13, 15, 21]. Some studies have used symptoms alone [17], which are insensitive in the diagnosis of oropharyngeal reflux and GOR. In the present study, dual-channel oesophageal pH recording was used to detect oropharyngeal reflux.

A number of previous studies have attempted to detect aspiration by examining sputum [15, 17], tracheobronchial

Table 2. – Evaluation of the sputum lipid index diagnostic test*

	Oropharyngeal reflux present	Oropharyngeal reflux absent	Total
Lipid index >7	18	1	19
Lipid index <7	2	8	10
Total	20	9	29

*: n=29. Prevalence (pre-test likelihood): 69%; Sensitivity: 90%; Specificity: 89%; Positive predictive value (post-test likelihood of oropharyngeal reflux): 95%; Negative predictive value (post-test likelihood of oropharyngeal reflux despite negative test): 80%; Likelihood ratio of a positive test (95% confidence interval CI): 8.1 (2.0–45.4); Likelihood ratio of a negative test (95% CI): 0.1 (0.03–0.36).

washings [14] or bronchoalveolar lavage fluid [11–13, 21] for the presence of lipid in macrophages. In those studies that included healthy controls [22], lipid-laden macrophages were seldom found and therefore the lipid index was low (mean \pm SD 1.31.5). A lipid index of 85–100 in bronchoalveolar lavage fluid has been suggested to be a sensitive indicator of parenchymal disease associated with clinically evident macroaspiration [11, 21]. The significance of lower values is less clear. The lower lipid indexes observed with induced sputum, as compared to previous studies using bronchoalveolar lavage, may be related to a number of factors. First, none of the present subjects had a clinical history of aspiration and all had normal chest radiographs; therefore micro- rather than macroaspiration was being identified. Secondly, the previous studies sampled focal areas of overt disease characterized by an abnormality on the chest radiograph where it would be expected that the lipid index would be high. In contrast, sputum induction, which samples both lungs diffusely, was used. The authors believe that the present results from induced sputum are valid because the index correlated with the number of episodes of reflux to the oropharynx. The index may be useful clinically for the identification of oropharyngeal reflux, and presumably microaspiration, associated with gastro-oesophageal reflux in patients with respiratory symptoms.

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