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The diagnostic value of gastroesophageal reflux disease (GERD) symptoms and detection of pepsin and bile acids in bronchoalveolar lavage fluid and exhaled breath condensate for identifying lung transplantation patients with GERD-induced aspiration

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Abstract

Background—Gastroesophageal reflux disease (GERD) is thought to lead to aspiration and bronchiolitis obliterans syndrome after lung transplantation. Unfortunately, the identification of patients with GERD who aspirate still lacks clear diagnostic indicators. The authors hypothesized that symptoms of GERD and detection of pepsin and bile acids in the bronchoalveolar lavage fluid (BAL) and exhaled breath condensate (EBC) are effective for identifying lung transplantation patients with GERD-induced aspiration.

Methods—From November 2009 to November 2010, 85 lung transplantation patients undergoing surveillance bronchoscopy were prospectively enrolled. For these patients, self-reported symptoms of GERD were correlated with levels of pepsin and bile acids in BAL and EBC and with GERD status assessed by 24-h pH monitoring. The sensitivity and specificity of pepsin and bile acids in BAL and EBC also were compared with the presence of GERD in 24-h pH monitoring.

Results—The typical symptoms of GERD (heartburn and regurgitation) had modest sensitivity and specificity for detecting GERD and aspiration. The atypical symptoms of GERD (aspiration and bronchitis) showed better identification of aspiration as measured by detection of pepsin and bile acids in BAL. The sensitivity and specificity of pepsin in BAL compared with GERD by 24-h pH monitoring were respectively 60 and 45 %, whereas the sensitivity and specificity of bile acids in BAL were 67 and 80 %.

Conclusions—These data indicate that the measurement of pepsin and bile acids in BAL can provide additional data for identifying lung transplantation patients at risk for GERD-induced aspiration compared with symptoms or 24-h pH monitoring alone. These results support a diagnostic role for detecting markers of aspiration in BAL, but this must be validated in larger studies.

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Keywords

Gastroesophageal reflux disease; Pepsin; Aspiration

Lung transplantation has become an increasingly attractive treatment strategy for patients with end-stage lung diseases [1], and survival rates after lung transplantation have improved considerably [2]. However, the 5-year survival rates still are low, at approximately 50 %, far lower than for other organ transplantations including liver, kidney, and heart, which have 5-year survival rates higher than 70 % [3–5]. Much of the mortality risk is attributed to chronic rejection caused by bronchiolitis obliterans syndrome (BOS). One of the main culprits in BOS is thought to be gastroesophageal reflux disease (GERD), which is approximately twice as prevalent among patients with endstage lung disease or after lung transplantation [6].

Recent findings indicate that lung transplantation patients may be at risk for GERD-related aspiration, contributing to a process of chronic inflammation and hastening the fibrosing process. Evidence from our institution has demonstrated that normal individuals do not aspirate gastric refluxate, that lung transplantation patients do aspirate, and that laparoscopic antireflux surgery (LARS) can minimize GERD-related aspiration as measured by pepsin recovered in the bronchoalveolar lavage fluid (BAL) [7].

Direct proof of aspiration requires the recovery of substances from the lungs that are produced in the gastrointestinal tract, such as pepsin and bile acids. Unfortunately, data on the diagnostic role of GERD symptoms and the detection of markers denoting aspiration in the lung transplantation population are lacking. Moreover, no suitable noninvasive test exists for detecting aspiration in these patients, although some studies have suggested that pepsin and bile acids can be recovered noninvasively in the exhaled breath condensate (EBC).

Currently, lung transplantation patients with GERD are evaluated for LARS by ambulatory esophageal pH monitoring. The measurement of pepsin or bile acids in BAL does not guide clinical decisions for managing GERD in lung transplantation patients due to a lack of consensus on appropriate cutoff levels, a lack of standardization of assay techniques, and an inability to determine the frequency of aspiration events.

We hypothesized that the detection of pepsin and bile acids in BAL and EBC identifies lung transplantation patients with GERD-induced aspiration who would eventually benefit from LARS and that a large proportion of these patients would not be detected using 24-h pH monitoring. In addition, we examined which symptoms correlated best with the presence of pepsin and bile acids in BAL and EBC as opposed to 24-h pH monitoring.

Materials and methods

Patients

From November 2009 to November 2010, 85 lung transplantation patients were prospectively enrolled in this study. Of these 85 patients, 13 had undergone LARS. The study population consisted of adults (≥ 18 years old) who had single- or double-lung

transplantation for any cause, combined heart-lung transplantation, retransplantation, or subsequent transplantation of the contralateral lung performed at Loyola University Medical Center, with no time limit after transplantation. The Institutional Review Board of the Loyola University Medical Center approved this study, and written informed consents were obtained from all the patients.

EBC and BAL sample collection

In this study, EBC and BAL samples were collected at routine follow-up visits for lung transplantation patients. The EBC collection was performed per protocol [8] in the Bronchoscopy Suite using a condenser (RTube; Respiratory Research Inc., Austin, TX, USA). Immediately thereafter, bronchoscopy with BAL was performed according to a standardized protocol [9]. The EBC samples and cell-free BAL fluid were centrifuged, aliquoted, and stored at -80°C .

Levels of bile acids were measured by spectrophotometric enzymatic assay (Sigma Diagnostic Inc., St. Louis, MO, USA), and pepsin levels were measured by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody to porcine pepsin as described by the manufacturer (Biodesign International, Saco, ME, USA). Any detectable bile acid or pepsin was considered abnormal. The lowest levels of detection by spectrophotometry using the spectrophotometric enzymatic assay and the ELISA technique are $0.2\text{ }\mu\text{mol/L}$ for bile acids and 1 ng/mL for pepsin [10].

Assessment of GERD status and symptoms of GERD

Assessment of GERD status was performed by pH monitoring using a previously described technique [11]. Briefly, proton pump inhibitors (PPIs) were stopped for 14 days and histamine H_2 -receptor antagonists were stopped for 3 days before pH monitoring. A pH catheter (sleuth system with BioVIEW software; Sandhill Scientific Inc., Denver, CO, USA) was placed with the distal pH sensor positioned 5 cm superior to the manometrically determined upper border of the lower esophageal sphincter (LES). The DeMeester score was calculated for the distal pH recordings, and a score higher than 14.7 was considered diagnostic of GERD [12].

Symptoms of GERD were self-reported by the patients at each follow-up appointment in the Bronchoscopy Suite using the validated 21-point symptoms questionnaire used in the laboratory of Drs. Pellegrini and Oelschlager [13]. Briefly, symptoms were graded on a frequency scale of 0 (never), 1 (once per month), 2 (once per week), 3 (once per day), and 4 (several times per day). A positive test was defined as one showing a patient with symptoms at least once per day (≥ 3 on the 4-point frequency scale).

The questionnaire asked 21 symptom questions: 10 gastrointestinal questions focused on heartburn, regurgitation, abdominal pain, belching, dysphagia with both liquids and solids, bloating, nausea, chest pain, odynophagia, and globus; and 11 extraesophageal questions focused on coughing, hoarseness, wheezing, laryngitis, aspiration, choking, dyspnea, sore throat, asthma, bronchitis, and pneumonia. Because some patients completed multiple questionnaires, sensitivity and specificity were calculated using each questionnaire as a

separate observation, using the most recent questionnaire, or using the average response, with results compared to assess for bias.

Statistical analyses

Sensitivity was calculated as the number of diseased subjects with positive test results divided by total number of diseased subjects. Specificity was calculated as the number of disease-free subjects with negative test results divided by the total number of disease-free subjects. The positive likelihood ratio (+LR) was defined as sensitivity/1-specificity. Potential bias introduced by subjects with multiple questionnaires was assessed by the chi-square test or Fisher's exact test if the cell counts were 5 or lower. Receiver operator characteristic (ROC) curve analysis was used to define cutoff values for positive and negative test results for pepsin and bile acids in EBC and BAL.

The sensitivity and specificity of each possible value were plotted, and the values of pepsin and bile acids in BAL and EBC that maximized (sensitivity + specificity) were used as cutoffs to define positive and negative test results for subsequent analyses. The area under the curve (AUC) was calculated for each ROC curve, and the AUCs for different tests were compared using the method described by Hanley and McNeil [14]. Statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA). The significance for all tests was set at a *p* value of 0.05 or lower.

Results

The average age of the study cohort was 54.4 ± 12.9 years, and 49 % of the participants were women. Table 1 shows the demographics of the study participants. From the 85 patients enrolled in the study, we collected 132 symptoms questionnaires (115 BAL and 114 EBC samples), with some patients contributing multiple samples or questionnaires (13 % completed 2 questionnaires, 15 % completed 3 questionnaires, and 3 % completed 4 questionnaires).

Overall, 32 patients (38 %) were tested by 24-h pH monitoring, with 50 % showing positive results. Of the patients with measurements, 9 (38 %) had positive measurements for bile acids in BAL, 18 (43 %) had positive measurements for pepsin in BAL, 25 (60 %) had positive measurements for either bile acids or pepsin, and 2 (6 %) had positive measurements for both bile acids and pepsin.

The results from the symptom questionnaire are displayed in Table 2. The results shown are for each questionnaire treated as a separate observation. No statistically significant differences were found when the average questionnaire response or the most recent questionnaire response was used to assess sensitivity and specificity (data not shown).

The typical symptoms of GERD (heartburn and regurgitation) had modest sensitivity and specificity in detecting GERD as measured by 24-h pH monitoring, which is evident by positive likelihood ratios (+LR) of 1.53 for heartburn and 1.90 for regurgitation. Heartburn and regurgitation displayed lower +LR when pepsin and bile acids in BAL were used as the standards.

The atypical symptoms of GERD (aspiration and bronchitis) showed a higher +LR in detecting GERD when BAL was used as the standard. The highest +LR was with the use of aspiration to detect pepsin in BAL (3.38), and the second highest +LR was with the use of bronchitis to detect bile acids in BAL (3.09).

Figure 1 shows the ROC curves for pepsin and bile acids in BAL and EBC, with 24-h pH monitoring as the standard. The ROC curve for bile acids in EBC could not be calculated because bile acids could not be detected in EBC. The curves showed that pepsin in BAL (AUC 0.46; 95 % confidence interval [CI] 0.38–0.62) and EBC (AUC 0.51; 95 % CI 0.39–0.64) did not differ from the 45° line of no discrimination, indicating that these tests are not accurate at predicting GERD status measured by 24-h pH monitoring. Bile acids in BAL (AUC 0.69; 95 % CI 0.48–0.90) displayed moderate accuracy for predicting GERD status measured by 24-h pH monitoring. Comparisons of the AUCs for bile acids in BAL versus pepsin in BAL ($p = 0.15$), bile acids in BAL versus pepsin in EBC ($p = 0.08$), and pepsin in BAL versus pepsin in EBC ($p = 0.60$) did not show any statistically significant differences.

The sensitivity and specificity of pepsin and bile acids in BAL and EBC compared with GERD by 24-h pH monitoring as the standard are shown in Table 3. The cutoff values for pepsin and bile acids were determined using the ROC curve analysis method described earlier. The sensitivity and specificity of pepsin in BAL were respectively 0.60 and 0.45, whereas the sensitivity and specificity of pepsin in EBC were respectively 0.22 and 0.79. The sensitivity and specificity of pepsin in BAL were respectively 0.67 and 0.80, whereas the specificity and sensitivity of bile acids in EBC could not be calculated because the amounts measured were below the limits of detection. Bile acids in BAL showed higher sensitivity (0.67) and specificity (0.80) than pepsin in either BAL or EBC. The presence of either bile acids or pepsin in BAL had a sensitivity of 0.64 and a specificity of 0.45 using 24-h pH monitoring as the standard. When either bile acids or pepsin in BAL was used as the standard, 24-h pH monitoring had a sensitivity of 0.56 and a specificity of 0.53.

Discussion

This study demonstrated that the typical symptoms of GERD (heartburn and regurgitation) had modest sensitivity and specificity for detecting GERD as measured by 24-h pH monitoring, whereas the atypical symptoms of GERD (aspiration and bronchitis) showed better test properties in detecting pepsin and bile acids in BAL. The level of bile acids in EBC could not be detected by our assay, and thus we were unable to assess its utility in this study.

The ROC curves in this study showed that pepsin in BAL and EBC were not accurate predictors of GERD status measured by 24-h pH monitoring. Bile acids in BAL displayed moderate accuracy for predicting GERD status measured by 24-h pH monitoring. Our results showed that pepsin and bile acids in BAL would be useful additions to items of symptoms questionnaires in detecting lung transplantation patients at risk for aspiration-induced BOS.

The symptoms questionnaire showed low to moderate sensitivities and specificities for GERD status measured by 24-h pH monitoring. One explanation for this phenomenon is the subjects' use of PPIs and histamine H₂-receptor antagonists. Although PPIs were stopped for 14 days and histamine H₂-receptor antagonists were stopped for 3 days before pH monitoring, the subjects may have been less likely to recall GERD-related symptoms than if they had never taken medication. Nonetheless, it is evident that symptoms alone are not sufficient for assessing a patient's risk for reflux and aspiration.

The most useful items on the symptoms questionnaire were aspiration for detection of pepsin in BAL and bronchitis for detection of bile acids in BAL. These findings are in line with expectations because pepsin and bile acids in BAL are intended to identify patients who aspirate and are at risk for BOS.

Currently, no standard exists for identifying lung transplantation patients at risk for aspiration. The standard for measurement of GERD is 24-h esophageal pH monitoring, yet it is not without its limitations. The sensitivity of 24-h esophageal pH monitoring for reflux is only 50–80 %. Furthermore, it often is not well tolerated. In addition, it is expensive and invasive [15]. Patients without GERD according to pH monitoring may nonetheless have aspiration-related airway disease, implying that pH monitoring misses a significant amount of aspiration events [13]. Identification of transplantation patients with aspiration is of paramount importance because it is highly associated with BOS, which decreases 5-year survival rates by 20–40 % [16] and because LARS is an effective intervention for the prevention of GERD.

Measurement of bile acids and pepsin in BAL has several advantages over 24-h esophageal pH monitoring. Surveillance BAL after lung transplantation has become routine in some centers, making it a more convenient test than 24-h esophageal pH monitoring. Although BAL did not prove sensitive or specific compared with 24-h pH monitoring as the standard measure of GERD, this may reflect inaccuracies in the 24-h pH monitoring rather than in the BAL results. The 24-h pH test used in this study does not measure proximal acid exposure or non-acid reflux, which have been demonstrated to increase the risk for BOS. Additionally, day-to-day variation in reflux may be missed by a 24-h study.

The use of pepsin and bile acids in EBC to identify patients with aspiration was complicated by difficulties detecting those compounds by our assay. The sensitivity and specificity of bile acids in EBC could not be determined due to the small number of samples with detectable bile acids in EBC. Although it is a noninvasive and well-tolerated test, EBC appears to have no diagnostic role in identifying lung transplantation patients with aspiration.

Our study had several limitations. A large proportion of the study population did not undergo 24-h pH testing, which increased variability and could have created a selection bias. Some patients contributed multiple BAL samples, EBC samples, or symptom questionnaires, which raises the question of bias introduced by correlated data. However, analysis of only the initial sample or questionnaire for each patient produced comparable sensitivities and specificities, indicating that correlated data did not distort our results. We were unable to

stratify by indication for lung transplantation due to an insufficient number of subjects, and thus could not determine whether the underlying disease process affected the results.

Conclusions

The study data indicate the following:

1. Typical symptoms of GERD do not predict the presence or absence of pepsin or bile acids in BAL, whereas symptoms such as aspiration and bronchitis have greater predictive value.
2. The measurement of pepsin and bile acids in BAL can provide additional data for identifying lung transplantation patients at risk for GERD-induced aspiration.
3. Pepsin and bile acids in BAL identify patients who aspirate and would otherwise be missed by measuring 24-h esophageal pH.
4. Because EBC is fraught with low sensitivity, its value is questionable.

In summary, it is likely that a combination of symptoms, 24-h esophageal pH monitoring, and measurement of pepsin and bile acids in BAL is optimal for identifying lung transplantation patients who are candidates for LARS, which is crucial to improving outcomes by reducing the risk for BOS. Further studies are needed to form a consensus on what constitutes aspiration and which metrics should be used to determine its frequency and severity.

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Fig. 1.

Receiver operator characteristic (ROC) curves for pepsin and bile acids in bronchoalveolar lavage fluid (*BAL*) and exhaled breath condensate (*EBC*) compared with gastroesophageal reflux disease (GERD) detected by 24-h pH monitoring. **A** ROC curve for pepsin in *BAL* (area under the curve [AUC] 0.46; 95 % confidence interval [CI] 0.38–0.62). **B** ROC curve for pepsin in *EBC* (AUC 0.51; 95 % CI 0.39–0.64). **C** ROC curve for bile acids in *BAL* (AUC 0.69; 95 % CI 0.48–0.90)

Table 1

Demographics

	Cohort	GERD (+)	GERD (–)	<i>p</i> value*
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
No. of patients	85	16	16	
Age (years)	54.4 ± 12.9	55.5 ± 12.5	58.8 ± 11.7	0.45
Females	42 (49)	6 (38)	6 (38)	1.00
Transplant indication				0.49
AATD	8 (9)	1 (6)	1 (6)	
BOOP	1 (1)	0	0	
CF	12 (14)	2 (13)	1 (6)	
COPD	30 (35)	8 (50)	7 (44)	
IPF	22 (26)	4 (25)	7 (44)	
Jo-1 syndrome	1 (1)	0	0	
LAM	1 (1)	0	0	
PAH	1 (1)	0	0	
PF from pneumoconiosis	3 (4)	1 (6)	0	
PVOD	1 (1)	0	0	
Sarcoidosis	4 (5)	0	0	
Scleroderma	1 (1)	0	0	
BAL samples collected	115	23	20	
EBC samples collected	114	23	19	

AATD Alpha-1 antitrypsin, BOOP bronchiolitis obliterans organizing pneumonia, CF cystic fibrosis, COPD chronic obstructive pulmonary disease, IPF idiopathic pulmonary fibrosis, LAM lymphangioleiomyomatosis, PAH pulmonary artery hypertension, PF pulmonary fibrosis, PVOD pulmonary veno-occlusive disease, BAL bronchoalveolar lavage fluid, EBC exhaled breath condensate

* *p* Value was calculated for comparison of GERD (+) to GERD (–)

Table 2

Sensitivity and specificity of symptoms self-reported by the patients using a validated questionnaire

Survey item	GERD by 24-h pH			Pepsin in BAL			Bile acids in BAL		
	Sensitivity	Specificity	+LR	Sensitivity	Specificity	+LR	Sensitivity	Specificity	+LR
Heartburn	0.80	0.48	1.53	0.51	0.45	0.93	0.57	0.56	1.29
Regurgitation	0.60	0.68	1.90	0.36	0.70	1.21	0.40	0.65	1.14
Abdominal pain	0.28	0.62	0.74	0.38	0.42	0.66	0.46	0.52	0.96
Belching	0.88	0.29	1.23	0.67	0.28	0.92	0.76	0.26	1.03
Dysphagia	0.28	0.77	1.23	0.23	0.74	0.90	0.27	0.81	1.42
Odynophagia	0.16	0.90	1.60	0.09	0.89	0.84	0.14	0.90	1.35
Bloating	0.46	0.73	1.68	0.43	0.45	0.73	0.56	0.57	1.30
Nausea	0.36	0.62	0.95	0.33	0.63	0.90	0.42	0.74	1.59
Chest pain	0.44	0.78	1.98	0.20	0.67	0.60	0.29	0.71	0.98
Lump in throat	0.36	0.78	1.61	0.23	0.79	1.09	0.22	0.83	1.30
Coughing	0.96	0.29	1.34	0.77	0.25	1.02	0.86	0.31	1.25
Hoarseness	0.36	0.76	1.51	0.30	0.64	0.83	0.24	0.69	0.79
Wheezing	0.44	0.71	1.54	0.41	0.64	1.14	0.43	0.69	1.40
Laryngitis	0.12	1.00	–	0.05	0.97	1.77	0.03	0.98	1.14
Aspiration	0.14	0.82	0.81	0.19	0.94	3.38	0.09	0.84	0.56
Choking	0.09	0.94	1.64	0.12	0.88	1.00	0.08	0.79	0.40
SOB	0.43	0.47	0.81	0.50	0.58	1.20	0.47	0.62	1.23
Sore throat	0.32	0.81	1.70	0.21	0.73	0.79	0.21	0.74	0.78
Asthma	0.14	1.00	–	0.06	0.93	0.86	0.09	0.94	1.46
Bronchitis	0.15	1.00	–	0.06	0.92	0.85	0.09	0.97	3.09
Pneumonia	0.11	0.88	0.84	0.11	0.92	1.48	0.06	0.94	1.00

GERD Gastroesophageal reflux disease, BAL bronchoalveolar lavage fluid, SOB shortness of breath, +LR positive likelihood ratio

Note: +LR could not be calculated in tests with specificities of 1.00 due to a divisor of zero

Table 3

Sensitivity and specificity of pepsin and bile acids in BAL and EBC compared with GERD detected by 24-h pH monitoring

	Sensitivity	Specificity
24-h pH monitoring as standard		
Pepsin in BAL	0.60	0.45
Pepsin in EBC	0.22	0.79
Bile acids in BAL	0.67	0.80
Bile acids in EBC	ND	ND
Pepsin or bile acids in BAL	0.64	0.45
Pepsin or bile acids in BAL as standard		
GERD by 24-h pH	0.56	0.53

BAL Bronchoalveolar lavage fluid, *EBC* exhaled breath condensate, *GERD* gastroesophageal reflux disease, *ND* none detected