

# Risk Factors for Bile Aspiration and its Impact on Clinical Outcomes

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**INTRODUCTION:** Bile reflux may cause for lung allograft rejection, yet there are no studies that determine (i) the relationship between gastric and lung bile concentrations, (ii) whether bile is present in lungs of nontransplant patients, (iii) the relationship between gastric dysmotility and lung bile, (iv) the impact of reflux therapies on lung bile, and (v) whether lung bile worsens outcomes in nontransplant patients. This study will address these gaps in the literature.

**METHODS:** We prospectively recruited lung transplant (LTX) patients and nontransplant patients with respiratory symptoms (RP) and collected paired gastric and lung samples. Bile concentration and composition of samples was assessed using liquid chromatography–mass spectrometry. Bile results were compared with clinical parameters, including the presence of esophagitis, gastric dysmotility, and/or pathologic gastroesophageal reflux.

**RESULTS:** Seventy patients (48 RP and 22 LTX) were recruited. Overall, 100% of gastric and 98% of bronchoalveolar lavage samples contained bile. The mean gastric bile concentrations in RP and LTX patients were  $280 \pm 703$  nmol/L and  $1,004 \pm 1721$  nmol/L, respectively ( $P = 0.02$ ). There was no difference in lung bile concentrations between RP ( $9 \pm 30$  nmol/L) and LTX ( $11 \pm 15$  nmol/L,  $P = 0.7$ ). Patients with delayed gastric emptying had higher lung bile concentrations ( $15.5 \pm 18.8$  nmol/L) than patients with normal gastric emptying ( $4.8 \pm 5.7$  nmol/L,  $P = 0.05$ ) independently of reflux burden. Proton pump inhibitor use increased the proportion of unconjugated gastric bile acids. High lung bile concentrations were associated with an increased risk of hospitalization and longer hospital stays in RP patients ( $P < 0.05$ ).

**DISCUSSION:** Lung bile is almost universally present in symptomatic patients, and higher concentrations are associated with poorer respiratory outcomes.

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## INTRODUCTION

Diagnosis of extraesophageal reflux disease is difficult because the gold standard tests to diagnose gastroesophageal reflux only measure esophageal reflux burden rather than the impact of reflux events on the lung. Studies that have tried to correlate esophageal reflux exposure with respiratory symptoms have shown conflicting results (1–8). Therefore, new biomarkers of reflux-related lung disease are needed.

The search for biomarkers has focused on the identification of gastric-specific substrates that are found in the lung as evidence of gastric aspiration. One of the first proposed biomarkers was pepsin which is produced in the stomach and if present in bronchoalveolar lavage (BAL) fluid obtained during bronchoscopy, may suggest

aspiration of refluxed gastric contents. Studies in both adults and children suggest that pepsin is found in the lungs of 44%–100% of patients, and the sensitivity of pepsin compared with esophageal reflux burden by pH impedance (pH-MII) was only 45%–71%, suggesting new biomarkers are needed (2,3,9,10).

One potential alternative biomarker is bile. In the adult lung transplant population, bile was found in 38% of transplanted lungs and was associated with pathologic full column reflux events by pH probe and impaired lung allograft survival (11,12). However, whether bile is a valid biomarker in children or in nontransplant patients is not known. Furthermore, data are lacking, in adults and children on (i) the relationship between the

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presence of gastric bile and subsequent presence of lung bile, (ii) the impact of acid suppression on bile composition in the gastric or lung samples, (iii) the impact of gastric dysmotility and gastroesophageal reflux on the presence of lung bile, and (iv) the impact of lung bile on clinical prognosis in nontransplant patients. It was the goal of this study to overcome the limitations of the current literature to advance our understanding of bile as a potential biomarker and the associated implications for prognosis in both patients with respiratory symptoms and pediatric lung transplant (LTX) patients.

## METHODS

This is a prospective cross-sectional study of 2 groups of patients who were undergoing endoscopy and bronchoscopy: patients with respiratory symptoms (RP) and LTX patients recruited between 2010 and 2017 at Boston Children's Hospital. While RP patients were undergoing testing for evaluation of symptoms, LTX patients were undergoing surveillance procedures. The performance of these combined procedures (esophagogastroduodenoscopy/bronchoscopy/laryngoscopy) under a single anesthesia with all specialists present (otolaryngology/pulmonology/gastroenterology) has been endorsed by all large pediatric hospitals nationwide as essential and standard care for the evaluation of children with extraesophageal symptoms (13). To obtain samples for bile analysis, a bronchoscopy was first performed during which 1 cc/kg (maximum: 30 cc) of normal saline was instilled into the right middle lobe or, if clinically indicated, the lobe with more severe disease based on chest x-ray or clinical findings. Bronchoscopy fluid was saved at  $-80^{\circ}$  until ready for analysis using mass spectroscopy for the evaluation of bile acids (14). After the bronchoscopy, gastric fluid was collected at the time of endoscopy by inserting the endoscope into the stomach and suctioning gastric fluid from the fundus immediately at the beginning of the scope before the scope was passed into the duodenum and before any other suctioning was performed. Bile analysis to measure total bile acid concentration and qualitative distribution of 15 different unconjugated and conjugated bile acids was performed using a validated liquid chromatography–mass spectrometry method as previously described (14); concentrations and composition of bile were reported. Bile concentrations and composition were compared with (i) results of endoscopy (the presence of microscopic esophagitis), (ii) results of bronchoscopy (percentage of neutrophils, bacterial culture growth, and, in LTX patients, the presence of chronic lung allograft dysfunction [CLAD]), and (iii) Gastroesophageal Reflux Symptom Assessment Scale (GSAS) obtained at the time of procedures.

Subgroups of patients underwent, if clinically indicated, pH-MII testing, nuclear medicine gastric emptying scans, and videofluoroscopic swallow studies (VFSSs). Gastric and/or lung bile concentrations were compared between patient groups with different testing results. An abnormal pH-MII study was defined as abnormal if there was either an abnormal acid exposure (defined as reflux index  $> 6\%$ ) or an abnormal number of events by impedance (defined as  $\geq 73$  total episodes per 24-hour study). Gastric emptying scans were considered delayed if there was greater than 60% residual if a 1-hour study was performed (as is the case in children  $< 5$  years old) or greater than 10% residual at 4 h if a 4-hour study was performed (for children  $> 5$  years old); the decision to perform a 1-hour vs a 4-hour study was based on age (15,16). VFSS results were categorized as normal (no evidence of aspiration or penetration), isolated penetration, or aspiration.

Recognizing that bile composition could be affected by other clinical factors, we then determined the impact of proton pump inhibitors (PPIs) on bile composition; we anticipated that PPIs would increase the unconjugated bile acid pools due to bacterial overgrowth. PPI status was defined as either “on PPI” or “off PPI,” where “on PPI” was defined as PPIs taken within 48 hours of endoscopy/bronchoscopy and “off PPI” was defined as no PPI exposure within at least 48 hours of endoscopy/bronchoscopy. We also determined the impact of macrolides which are motilin agonists, on bile pools; we anticipated less total bile in patients taking macrolides. Patients were considered “on macrolides” if they had taken them within 4 weeks of the procedures and “off macrolides” if they had not taken them within 4 weeks of the procedure.

To determine the potential impact of bile on clinical outcomes, 2 different outcomes were chosen. For RP patients, hospitalization frequency and length and type were assessed in the 6 months preceding the bronchoscopy to best assess the impact of bile on clinical outcomes; a prebronchoscopy/endoscopy history was chosen as the primary outcome over the postbronchoscopy period to ensure that the hospitalizations reflected the impact of bile and not changes in management resulting from the endoscopy or bronchoscopy. However, to further validate the 6-month pre-endoscopy results, we also looked post hoc at the 6-month postendoscopy results as well. Hospitalizations were categorized as total hospitalizations (all cause) or respiratory hospitalizations. Emergency department visits were also assessed in the 6 months after the bronchoscopy. Recognizing that postbronchoscopy outcomes in patients may also be of clinical relevance, we also determined, in RP patients, the impact of lung bile on post-bronchoscopy hospital and emergency department outcomes.

For LTX patients, CLAD was chosen as the most clinically meaningful outcome because hospitalization/emergency department data for LTX patients are skewed based on comorbidities and complications related to transplantation.

Continuous variables were compared using *t* tests, and categorical variables were compared using  $\chi^2$  tests across different group of patients. Logistic regression was performed to determine predictors of higher lung bile concentration, which is defined as lung bile concentrations in the top quartile of values (top 75%). Generalized linear models with log link function and negative binomial distribution were used to examine the associations between number of hospitalizations and its potential risk factors. Given that this is the first study to correlate gastric and lung bile concentrations, we powered our study based on a desired correlation  $r = 0.4$ . Using a 2-sided, 5% significance level test ( $\alpha = 0.05$ ) with 90% power ( $\beta = 0.1$ ), we estimated that we would need at least 62 patients included in this study.

This study was approved by the Boston Children's Hospital IRB (IRB#: 06-10-0439), and an informed consent document was signed by each family and/or patient.

## RESULTS

Seventy patients were recruited for participation; 48 patients had RP and 22 patients were LTX. Demographic data are listed in Table 1. Esophagogastroduodenoscopy performed resulted in the following diagnoses: 4 patients were diagnosed as having eosinophilic esophagitis, 14 were diagnosed with reflux esophagitis, and 30 patients had normal biopsies. The bronchoscopy/laryngoscopy performed resulted in the following diagnoses: 4 patients were diagnosed with a laryngeal cleft, 9 were diagnosed

**Table 1. Patient characteristics**

	Respiratory patients n = 48	Lung transplant patients n = 22	P value
Age (yr)	7.8 ± 5.8	14.4 ± 4.8	<0.0001
Symptom necessitating evaluation			
Cough	28/48		
Recurrent infections	11/48		
Oropharyngeal dysphagia	9/48		
Comorbidities			
Cardiac	7/48	12/22	0.0001
Neurologic	8/48	4/22	0.8
Development	8/48	2/22	0.4
Genetic	5/48	10/22	0.001
Otolaryngology	31/48	7/22	0.01
Number of patients with 3+ comorbidities	8/48	3/22	0.7
Mean FEV1 (N = 17 in each group)	89 ± 21	49 ± 27	<0.0001
Mean (SD) time from lung transplant to sample collection (mo)		8.1 ± 17.4	
% Of gastric fluid containing bile	100	100	1.0
% Of bronchoscopy fluid containing bile	98	100	0.9
Mean (SD) bile concentration in gastric fluid (nmol/L)	280 ± 703	1,004 ± 1721	0.02
Mean (SD) bile concentration in bronchoscopy fluid (nmol/L)	9 ± 30	11 ± 16	0.7
Reflux esophagitis	12/48	2/22	0.1
Number with abnormal gastric emptying scan	4/9	11/17	0.3
Number with abnormal pH-MII	13/28	7/16	0.8
PPI use in preceding mo	25/48	22/22	<0.0001
Antibiotic use in the preceding mo	9/48	21/22	<0.0001
Inhaled steroid use at the time of testing	28/48	3/22	<0.0001
Abnormal VFSS	11/30	11/18	0.6
pH-MII, pH impedance; PPI, proton pump inhibitor; VFSS, videofluoroscopic swallow study			

with isolated oropharyngeal dysphagia, and 35 did not have a definitive diagnosis based on airway examination. Despite the range of diagnoses, bile was identified in 100% of gastric samples and in 98% of pediatric lung samples.

### Gastric bile

There was a wide range of gastric bile concentrations but no single factor which predicted bile concentrations including gastric emptying, medications, or underlying disease. As listed in Table 1, gastric bile was greater in patients after lung transplantation than pediatric patients with respiratory symptoms. No patients had erosive esophagitis. There were also no differences in the mean bile concentrations of patients with and without microscopic esophagitis ( $P > 0.4$ ).

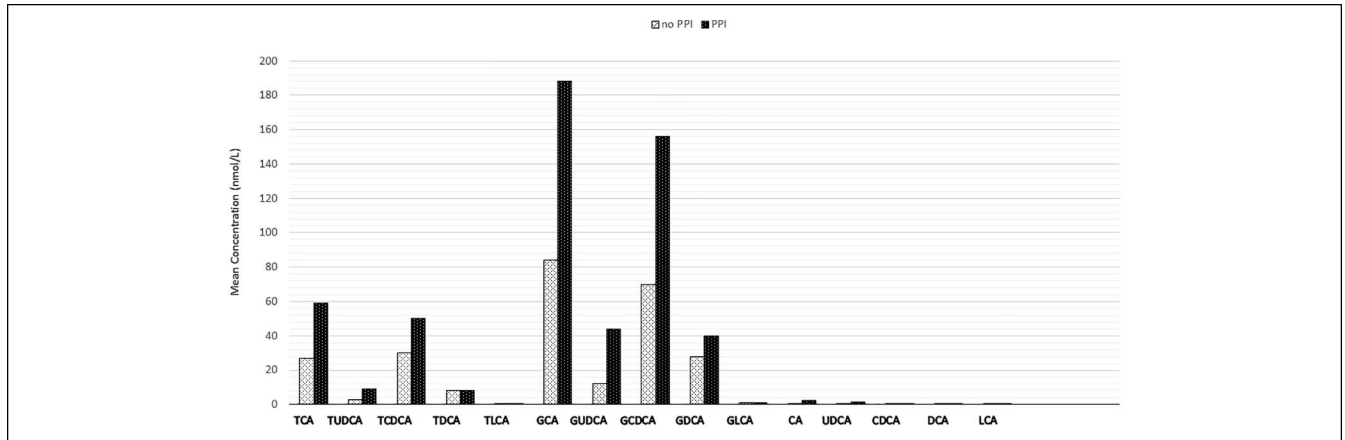
The distribution of bile acids seen in gastric fluid of patients on and off PPIs is shown in Figure 1. Sixty seven percent of patients (47/70) were taking PPIs. Although there were no significant differences in the composition of individual bile acids, the

proportion of unconjugated bile acids relative to total bile acids was higher in PPI-treated patients ( $P = 0.05$ ; Table 2).

Because macrolides are used to promote motility, we then looked at the relationship between macrolide antibiotic use and gastric bile metabolites as listed in Table 3. Twenty five percent of patients (25/70) were taking macrolides. As with PPIs, macrolide use was associated with an increase in the proportion of unconjugated bile acids relative to conjugated bile acids.

### Lung bile

There was no significant correlation between total lung bile concentrations and gastric bile concentrations ( $r(2) = 0.047$ ,  $P = 0.6$ ). Although there were more bile acids in the lungs of patients who were taking PPIs ( $13 \pm 31$  nmol/L) compared with those who were not ( $5 \pm 7$  nmol/L), this was not statistically significant ( $P = 0.1$ ). Despite the potential benefit to gastric motility, there was no difference in the mean bile concentrations of patients who were ( $9 \pm 13$  nmol/L) and were not ( $11 \pm 30$  nmol/L) taking macrolides ( $P = 0.7$ ).



**Figure 1.** Differences in gastric bile acid composition in patients on and off PPI. (N = 70,  $P > 0.2$  for all comparisons). PPI, proton pump inhibitor.

There was no difference in concentration of lung bile in patients who did ( $16 \pm 40$  nmol/L) and did not grow an organism during bronchoscopy ( $7 \pm 11$  nmol/L) from the BAL washing ( $P = 0.2$ ). There was no significant correlation between the percentage of neutrophils on bronchoscopy and the concentration of bile acids ( $r(2) = 0.177$ ,  $P = 0.2$ ).

The mean amount of bile acids in patients with normal swallow function, isolated penetration, and aspiration is shown in Figure 2. There were no significant differences in mean values when comparing the 3 groups for the swallow study closest to the bronchoscopy. We then categorized patients into those with any history of aspiration on any previous swallow study. Based on these results, patients with any abnormalities on the swallow study (either a history of aspiration or penetration) had significantly higher lung bile concentrations ( $12.4 \pm 16.4$ ) than patients who had a normal VFSS ( $4.5 \pm 5.1$ ,  $P = 0.02$ ).

In the LTX patients, patients with CLAD had higher lung bile concentrations than patients without CLAD as shown in Figure 3; higher concentrations of conjugated bile acids, in particular, were associated with CLAD.

#### Gastroesophageal reflux, gastric dysmotility, and lung bile

A subgroup of 45 patients had pH-MII studies at the time of endoscopy/bile sampling. There was no difference in the lung bile acid concentrations in the patients with abnormal ( $7 \pm 11$  nmol/L) vs normal ( $14 \pm 42$  nmol/L) pH-MII testing. There was no significant correlation between any reflux parameters (total number of reflux episodes, amount of acid vs nonacid reflux episodes, and percentage of full column reflux, pH < 4) and the amount of total bile in the lungs ( $P > 0.2$ ). There was no difference in the mean lung bile concentrations in patients with ( $4.6 \pm 9.4$  nmol/L) and without ( $11.2 \pm 29.2$  nmol/L,  $P = 0.4$ ) microscopic esophagitis. We then compared the lung bile concentrations between patients with any positive reflux test (either pH-MII or endoscopically); there were no significant differences in total lung bile concentrations between patients with normal ( $11.6 \pm 31.1$  nmol/L) vs abnormal ( $6.6 \pm 11.5$  nmol/L,  $P = 0.4$ ) reflux testing. When looking at symptoms measured by GSAS, there was no significant correlation between the GSAS total or reflux subscores and gastric or lung bile concentrations ( $P > 0.4$ ).

A subset of 26 patients had gastric emptying scans. Abnormal gastric emptying scans were significantly associated with higher lung bile concentrations ( $16 \pm 19$ ) than patients with normal

gastric emptying ( $5 \pm 6$ ,  $P = 0.05$ ). There was no difference in the mean gastric bile concentrations between patients with normal gastric emptying ( $999 \pm 1,678$ ) and patients with abnormal gastric emptying ( $651 \pm 1,444$ ,  $P = 0.6$ ).

Recognizing the complexities of diagnosing gastroesophageal reflux in children, we then looked at the relationship between a history of emesis and lung bile; patients with a history of emesis had a mean lung bile concentration of  $9 \pm 13$  nmol/L, and patients without emesis had a mean lung bile concentration of  $11 \pm 32$  nmol/L ( $P = 0.8$ ).

#### Hospitalizations and emergency department visits

Mean number of total hospitalizations, respiratory hospitalizations, and emergency department visits in the 6 months preceding and 6 months after bile sampling are shown in Figure 4.

**Table 2.** Differences in bile composition in patients who were and were not taking PPIs

	No PPI (n = 23)	PPI (n = 47)	P value
Gastric samples			
Total bile concentration (nmol/L)	262 ± 531	562 ± 1,273	0.3
Total unconjugated bile concentration (nmol/L)	0.3 ± 0.4	5.3 ± 18	0.08
Total conjugated bile concentration (nmol/L)	262 ± 530	557 ± 1,271	0.3
Percentage of total bile acids that are unconjugated (%)	1.0 ± 2.0	6.5 ± 17.0	0.05
Lung samples			
Total bile concentration (nmol/L)	4.5 ± 7.4	12.6 ± 31.4	0.1
Total unconjugated bile concentration (nmol/L)	0.8 ± 2.1	1.3 ± 3.1	0.4
Total conjugated bile concentration (nmol/L)	3.7 ± 6.1	11.3 ± 31.0	0.1
Percentage of total bile acids that are unconjugated (%)	9.1 ± 20.8	10.8 ± 18.9	0.7

PPI, proton pump inhibitor.

**Table 3.** Differences in bile composition in patients who were and were not taking macrolides

	No macrolide (N = 45)	Macrolide (N = 25)	P value
<b>Gastric samples</b>			
Total bile concentration (nmol/L)	375 ± 917	679 ± 1,435	0.3
Total unconjugated bile concentration (nmol/L)	1.0 ± 4.2	10 ± 27	0.01
Total conjugated bile concentration (nmol/L)	374 ± 914	668 ± 1,439	0.3
Percent unconjugated bile (%)	1.4 ± 3.5	13.4 ± 25	0.002
<b>Lung samples</b>			
Total bile concentration (nmol/L)	11 ± 30	9 ± 13	0.7
Total unconjugated bile concentration (nmol/L)	1.1 ± 3.0	1.2 ± 2.5	0.7
Total conjugated bile concentration (nmol/L)	9 ± 30	7 ± 11	0.7
Percent unconjugated bile (%)	1.4 ± 3.5	13.4 ± 25	0.5

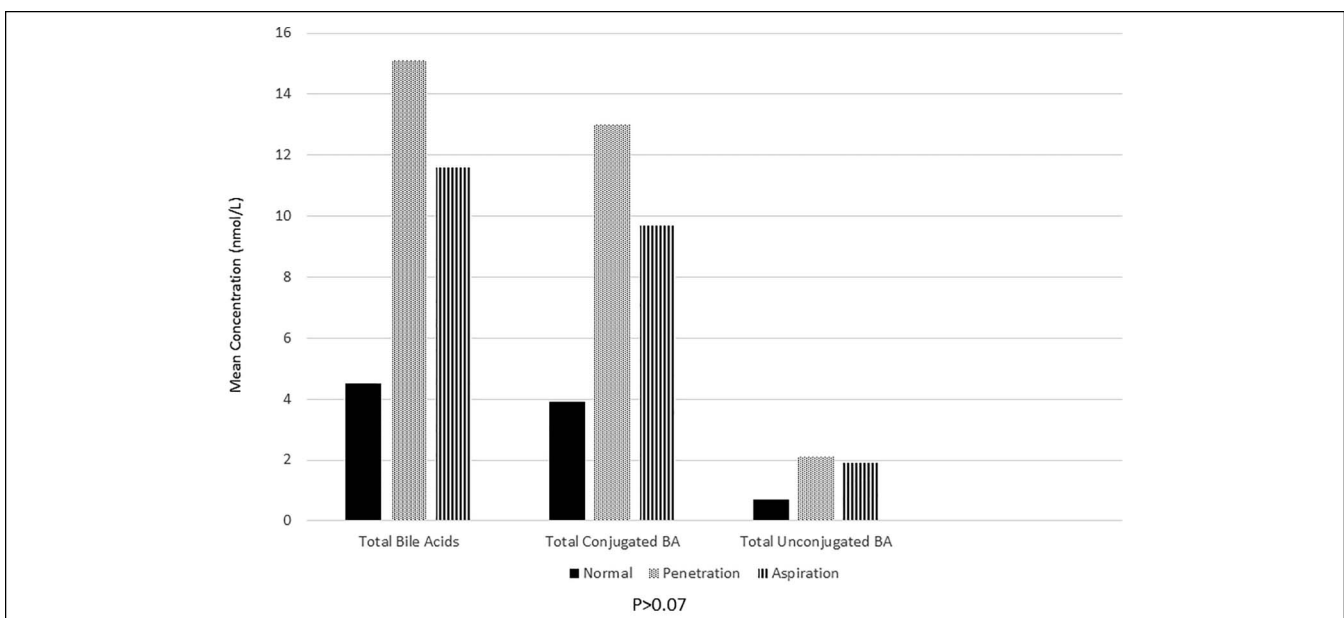
Hospitalizations and emergency department visits were higher in patients with the highest quartile of lung bile concentrations in the 6 months before endoscopy/bronchoscopy with similar trends seen in the 6 months after endoscopy/bronchoscopy (Figure 4). In addition, in the 6 months preceding endoscopy/bronchoscopy, the total number of hospital days was higher in the high bile group ( $4.0 \pm 6.3$  days) compared with the low bile group ( $0.9 \pm 3.5$  days,  $P = 0.05$ ). In the 6 months after endoscopy/bronchoscopy, the total number of hospital days was higher in the high bile group ( $2.0 \pm 5.0$  days) compared with the low bile group, but this difference was not significant ( $1.6 \pm 6.5$  days,  $P = 0.8$ ).

When performing negative binomial regression to determine predictors of total hospitalizations, only high lung bile remained a significant predictor of outcome (rate ratio: 4.87, CI: 1.07–22.13).

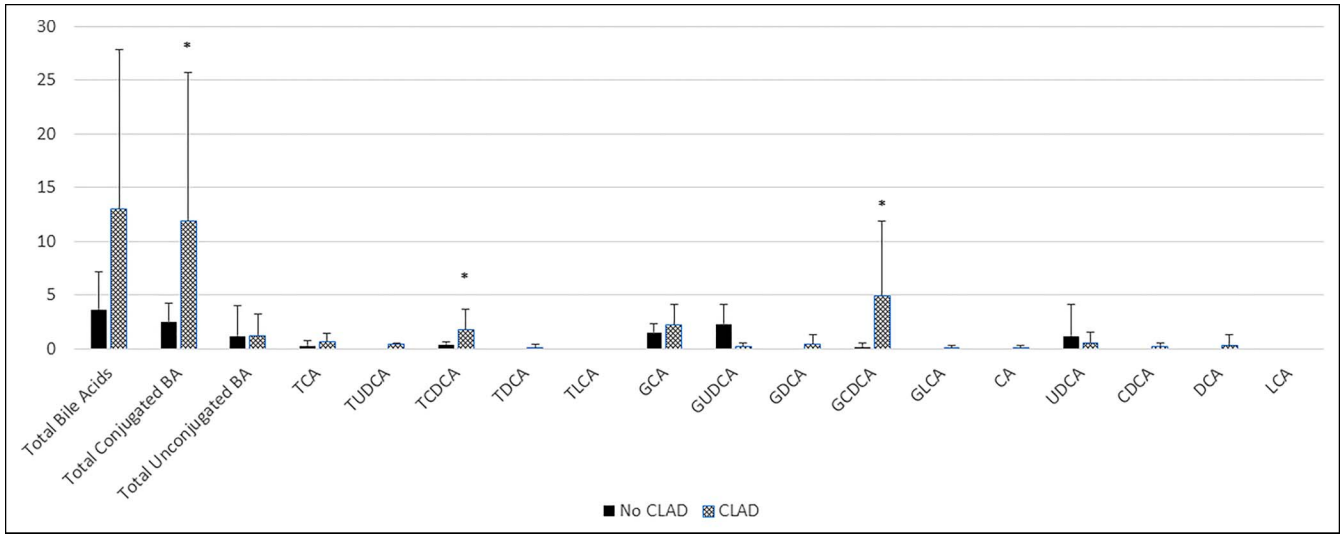
## DISCUSSION

Bile has been proposed in the lung transplant population as a marker of aspiration and a harbinger of more severe lung disease. Although we also show that bile (and particularly conjugated bile acids) is associated with CLAD, we now show that bile is also a predictor of more severe disease in nontransplant patients as well. We also show for the first time that bile is almost universally found in the lungs of children, so its presence to diagnose gastroesophageal reflux disease or aspiration is not adequate, thus undoing assumptions made previously (17). While we absolutely expected to see bile in the lungs of transplant patients given their high rates of esophageal and gastric dysmotility, we did not expect to see the frequency or quantity of bile in the lungs of patients outside the transplant realm.

Although bile presence is ubiquitous in the patients included in this study, the relative amount of bile may be of clinical importance and this has been shown in the literature. For example, recent studies in patients with cystic fibrosis show that higher lung bile acid concentrations are associated with increased inflammatory cytokine production which may portend a worse clinical prognosis (18,19). In these studies, the median BAL bile concentrations of patients with increased inflammatory profiles were approximately 60 nmol/L. In our study, the top quartile range of bile concentrations causing increased hospitalization risk was 8.2–204 nmol/L, a range that encompasses the values seen in patients with cystic fibrosis with increased inflammatory profiles. These elevations in inflammatory profiles may have clinical significance. In our study, we show similar findings in non-CF patients, that is, patients with increased lung bile have higher rates of hospitalization and emergency department visits. We also show, in LTX patients, that higher bile acids are associated with increased allograft rejection.

**Figure 2.** Relationship between specific swallow study results' bile acid (BA) concentrations (N = 48,  $P > 0.07$  for each group).

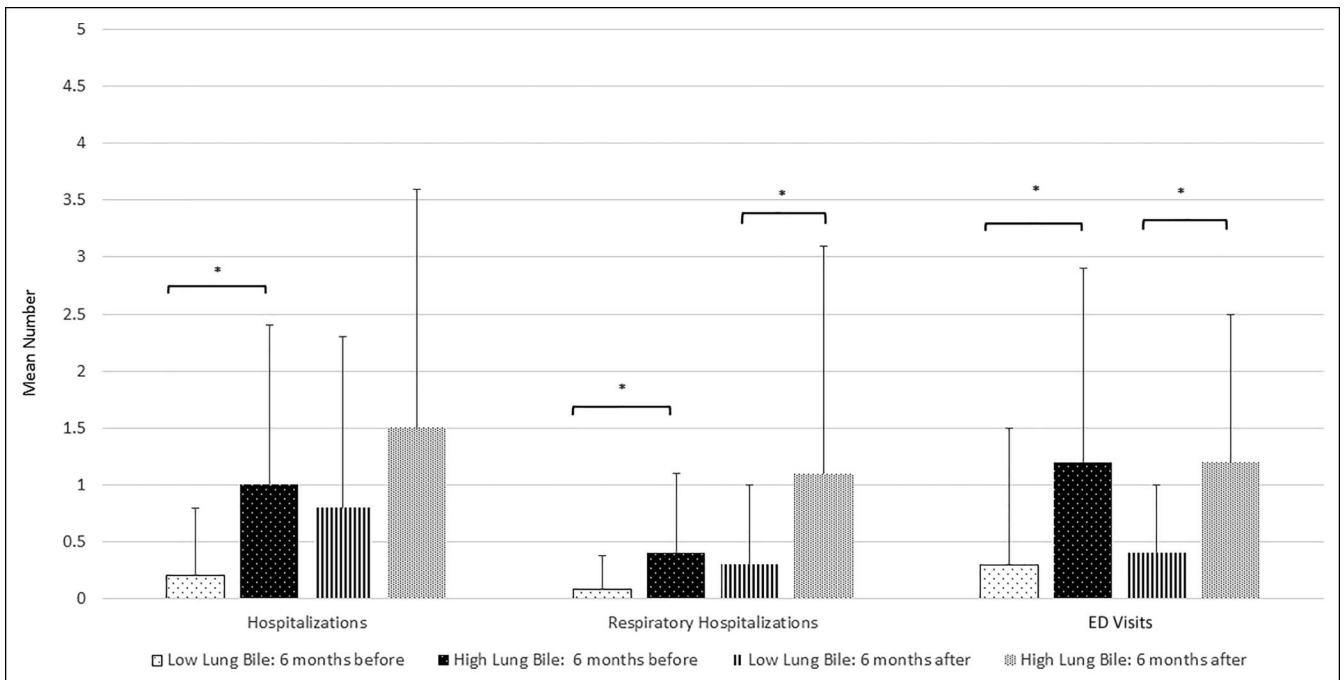




**Figure 3.** Bile acid profiles seen in patients with and without CLAD (N = 22, \*P < 0.05). BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; CLAD, chronic lung allograft dysfunction; DCA, deoxycholic acid; GCA, glychocholic acid; GCDCA, glychochenodeoxycholic acid; GDCA, glychodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glychoursodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, tauroolithocholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

To explain these higher rates of lung bile, previous literature has suggested gastroesophageal reflux as a cause for elevations. We show, for the first time using pH-MII, that gastroesophageal reflux burden does not correlate with lung bile concentrations; lung bile concentrations are not related to the amount of reflux, the type of refluxate (acid/nonacid), or the degree of full column reflux. In addition, the presence of neither reflux esophagitis by endoscopy nor reflux symptoms by a validated questionnaire predicted lung bile concentrations. Based on previous studies

from our group and others in LTX patients which showed that the CLAD correlated with gastric dysmotility, we hypothesized that delayed gastric emptying might predispose patients to higher lung bile concentrations (20,21). In fact, we did find that lung bile concentrations are higher in children with delayed gastric emptying, thus providing a possible mechanism behind the observed worse lung transplant outcomes or even providing a mechanism why patients may have lung disease that progressed to transplantation (20). Surprisingly, we did not find objective differences



**Figure 4.** Hospitalization utilization in the 6 months before and after endoscopy/bronchoscopy in patients with high and low lung bile concentrations (N = 48, \*P < 0.05). ED, emergency department.

in the gastric bile pools of patients with and without dysmotility nor did we find that these patients had increased reflux burden, suggesting that more studies are needed to understand the lung bile–dysmotility relationship.

Recognizing that there are 2 potential therapies for gastroesophageal reflux, we sought to determine their impact on bile acid pools. We first hypothesized that PPIs would result in bacterial overgrowth such that there would be a shift in the composition of bile toward unconjugated bile acids. We did, in fact, see this shift in the composition of gastric bile in patients taking acid suppression with a higher percentage of unconjugated bile acids in treated patients. Our results are supported in the literature in a single study of 30 adults taking PPIs; the authors found that gastric fluid containing bacteria had a shift in bile acids such that the ratio of conjugated to unconjugated shifted from 4:1 in patients without bacterial overgrowth to 1:3 in patients with bacterial overgrowth (22). Interestingly, in patients taking PPIs, there were higher levels of bile acids associated with the development of Barrett's esophagus (e.g., taurocholic acid and glycocholic acid). Additional studies are clearly needed to determine whether PPIs effectively change the bile pool to reduce inflammation and how PPI efficacy changes when bacterial overgrowth (with resultant deconjugation) is present (10,23,24).

We further hypothesized that macrolides, which are used to improve motility, may have an impact on bile acid pools in the stomach and potentially in the lung by improving gastric emptying. We did not find that macrolides reduce gastric or lung bile concentrations. We did, unexpectedly, find an increase in the proportion of unconjugated bile acids; we expected, given the antibiotic properties of macrolides, that there would be a reduction in gastric bacteria and therefore less bile deconjugation. Instead, we found that macrolides affected the bile acid pool in the same manner as PPIs. However, when looking at the data more closely, 18 of the 19 patients taking macrolides were also taking PPIs, so teasing out the macrolide effect alone is impossible. However, we know from prior microbiome analyses that the combination of macrolides and PPIs has a synergistic effect in reducing microbial diversity which may be affecting the bile acid pool, but further studies are clearly needed (25).

Neither PPIs nor macrolides reduce lung bile concentrations, and recognizing that lung bile negatively affects clinical outcomes, new therapies to reduce lung bile are needed. Bile acid sequestrants have been trialed to treat recalcitrant gastroesophageal reflux symptoms (26), and although they do improve typical symptoms of gastroesophageal reflux, their role in improving pulmonary outcomes is not known. The next critical study would be to perform bile acid sequestrant trials in high-risk patients with respiratory symptoms undergoing serial bronchoscopies with bile acid measurements that can be paired with clinically meaningful outcomes.

There are several limitations to this study. Gastric and lung fluid are sampled during simultaneous endoscopy and bronchoscopy; these procedures require general anesthesia. It is possible that, during the anesthesia process, there could have been seeding of the lungs or a change in motility such that the bile composition of the stomach may vary. We recognize this limitation, but we also acknowledge that our lung and gastric sampling methods are the only possible methods to ethically obtain these samples in pediatric studies and are similar in methodology to adult studies (3,10). We also clearly found that lung bile correlated with clinical outcomes such as hospitalization suggesting

that our findings are valid and not simply an anesthesia artifact. We also recognize that, without performing bronchoscopies in healthy children, we may miss subjects who do not have lung bile and if we did have these patients, bile may have a greater sensitivity for diagnosing extraesophageal reflux disease. However, again, because it would be unethical to sample gastric and lung fluid in healthy children, we compared children with respiratory symptoms with a pediatric lung transplant population, the latter of which is at greatest risk for bile-related lung disease based on the adult literature. While we acknowledge this limitation, we also recognize that we are not alone in this issue as all of the previous adult studies lack control populations as well.

In conclusion, bile is present universally in the gastric fluid of pediatric patients and almost universally in the lungs of pediatric patients with respiratory symptoms. Standard therapies with PPIs and macrolides may shift the bile acid pool toward unconjugated bile acids but do not reduce bile acid concentrations in the stomach or the lungs. Lung bile acids negatively affect prognosis and represent a potential target for therapies in children with respiratory disease.

#### CONFLICTS OF INTEREST

**Guarantor of the article:** Rachel Rosen, MD MPH

**Specific author contributions:** R.R.: study design, sample collection, data analysis, and manuscript drafting including writing and approval of the final manuscript. M.L., M.K., C.D., and A.C.: sample collection and processing, data entry, and approval of the final manuscript. D.F., D.B., and G.V.: sample collection, patient classification, and approval of the final manuscript. M.N.R. and K.S.: sample analysis and approval of the final manuscript. E.L.: data analysis and approval of the final manuscript.

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**Potential competing interests:** None to report.

## Study Highlights

#### WHAT IS KNOWN

- ✓ Bile is commonly found in the lungs of lung transplant patients.
- ✓ Lung bile may correlate with lung allograft rejection.
- ✓ There are no studies of lung bile outside of transplant populations.

#### WHAT IS NEW HERE

- ✓ Lung bile is found almost universally in the lungs of patients with respiratory symptoms.
- ✓ Delayed gastric emptying rather than gastroesophageal reflux burden correlates with lung bile.
- ✓ Higher lung bile concentrations correlate with increased risk of hospitalization.

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