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Acid, non-acid GER and gastric aspiration in lung transplant patients with or without chronic rejection

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Short title:

GER & aspiration in lung transplantation

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ABSTRACT (word count : 200)

Acid gastroesophageal reflux (GER) and gastric aspiration have been labeled as risk factors for chronic rejection (BOS) after lung transplantation (LTx). We **aim**ed to further characterize GER (acid and non-acid) and the degree of gastric aspiration in LTx recipients with and without BOS.

Impedance-pH-recordings were used for GER detection. Pepsin and bile acids levels were measured in BALF.

48% of patients had increased GER of which 27% had exclusively increased non-acid reflux. CF patients had the highest prevalence of GER. Pepsin was found in BALF of all patients and bile acids in BALF of 50% of the patients. Patients with BOS did not have increased GER or elevated pepsin in BALF. However, 70% of the patients with BOS had bile in BALF compared to 31% of stable patients. PPI treatment reduced acid reflux but did not affect non-acid reflux. Moreover, pepsin and bile levels in BALF were not reduced by PPI.

Half of the LTx patients had increased reflux and non-acid reflux was common. Gastric aspiration occurred in most LTx patients. Pepsin was a more general marker and bile acids a more specific marker that might be associated with BOS. PPI treatment did not prevent non-acid reflux and gastric aspiration.

INTRODUCTION

Lung transplantation (LTx) has now become an effective therapeutic option for the treatment of different end-stage pulmonary disorders, improving the quality of life and extending survival [1]. A significant contributor to the limited long-term survival is the development of chronic allograft rejection [1]. Bronchiolitis obliterans syndrome or BOS, identified as a persistent drop in the FEV_1 after transplantation is the clinical correlate of chronic rejection and is caused by obliteration of the terminal bronchioles [2]. The pathophysiology of BOS is poorly understood but both immunological and non-immunological mechanisms are involved [3-5].

Gastroesophageal reflux (GER) has been implicated as a potential non-alloimmune cause of BOS. Standard esophageal pHmetry indicated an increased esophageal acid exposure in 70% of LTx patients [6;7]. Luminal gastric components such as pepsin and bile acids have been demonstrated in bronchial material of LTx recipients [8;9]. D'Ovidio et al. demonstrated increased bile acids levels in BALF (broncho-alveolar lavage fluid) in 22% of LTx patients, which were associated with reduced freedom from BOS [10]. Antireflux fundoplication surgery has been associated with improved allograft function [11-13]. These data suggest a possible causal link between GER and the development of BOS [7;12].

Although GER and gastric aspiration seem to occur in LTx recipients, understanding their role in the development of BOS requires additional information. Furthermore, it is necessary to establish the patient profile, on the basis of reflux indices and/or markers of gastric aspiration that might benefit from anti-reflux intervention.

So far, only acid GER (pH lower than 4) has been measured in LTx patients. However, less acidic reflux (pH >4) might also induce aspiration of harmful gastric components. Impedance-pH monitoring is currently considered the most sensitive method for GER detection and allows the recognition of acid and non-acid reflux [14]. Gastric aspiration can be analyzed by measuring pepsin or bile acids levels in BALF.

A significant proportion of LTx patients are empirically treated with proton pump inhibitors (PPI). It is unknown, whether this affects the prevalence of reflux and risk of gastric aspiration.

The **primary aim** of our study was to characterize the prevalence and type of GER (acid and non-acid) and the degree of gastric aspiration in LTx recipients with and without BOS. **The secondary aim** was to assess the prevalence and degree of acid, non-acid GER and gastric aspiration in a separate group of LTx patients, studied while being treated with PPI treatment.

METHODS

Design and patient recruitment

This study was performed as a cross-sectional study, recruiting patients unselectively from the LTx recipients (LTX between January 1996 and June 2005). Only patients with a survival and follow-up > 1 year were included. For each patient FEV_1 (L, % predicted and % personal best) was available at the day of the impedance-pH study. BOS, defined as an irreversible decline in FEV_1, was graded according to ISHLT criteria [15]. Acute rejection was excluded both clinically and by means of transbronchial biopsies. Infection was excluded on the basis of clinical presentation and sputum or BALF microbiology. Recipients with anastomotic complications or incomplete pulmonary function test data were excluded.

All patients received standard immunosuppressive therapy comprising cyclosporine or tracrolimus, azathioprine or mycophenolate and methylprednisolone.

Although one of the aims was to assess the impact of treatment with PPI on prevalence of GER, nearly all patients refused to undergo two separate pH-impedance recordings (one while 'off' PPI and one while 'on' PPI). As a result, patients were recruited in 2 different study cohorts. The first and largest study cohort consisted of patients evaluated with pH-impedance without or after discontinuing acid suppression treatment or prokinetic drugs for at least 2 weeks. In this first study cohort, the prevalence of GER and aspiration was compared between patients with and without BOS. The second study cohort, consisting of a smaller number of patients, was evaluated with pH-impedance while being treated with omeprazole 20 mg bid.

Impedance-pH monitoring

GER was assessed at the moment of inclusion using ambulatory 24hr esophageal impedance-pH monitoring. The impedance-pH recording was independently

analysed for GER using criteria described in a recent consensus report [16]. Several reflux indices were measured: acid exposure, number of acid and non-acid reflux events, volume exposure and proximal extent of reflux. Detailed methodology is available as an online repository

Detection of gastric aspiration (pepsin and bile acids in BALF)

For every patient that participated to this study, a BAL sample was obtained during the next scheduled routine bronchoscopy following the pH-impedance recording. BALF was performed by wedging the bronchoscope into a subsegmental bronchus of the right middle lobe or lingula, by instilling two aliquots of 50 ml and subsequently recovering the fluid by gentle manual suction. Pepsin and bile acids were determined in BALF. In the patients of the first study cohort, acid suppression and prokinetic treatment was stopped before the bronchoscopy while the patients in the second study cohort continued treatment with PPI.

Fourteen BALF samples of 'non-transplant' subjects requiring a bronchoscopy (11 lung cancer, 2 COPD, 1 lymphoma) were collected for comparative analysis. BALF was performed and analyzed identically.

Pepsin detection

Pepsin was measured using an ELISA. Samples were incubated with a primary polyclonal antibody to porcine pepsine (1:5000) and a secondary antibody (goat IgG) labeled with horseradish peroxidase (1:10000). Tetramethylbenzidine was added and color change was measured using a spectrophotometer. The specificity of the assay was verified using Western Blot and lowest level of accurate detection was 1 ng/L.

Bile acids

Bile acid determination was performed using a commercially available enzymatic assay (Bioquant, San Diego, USA). The lowest level of accurate detection allowed by this technique was 0.2 µmol/L.

Statistical analysis

Deviations from Gaussian distribution were tested using the Kolmogorov-Smirnov test. Comparisons between groups were done using one-way ANOVA and subsequent non-parametric testing. Categorical data were analyzed using Fischer's Exact test. Correlations were made using Pearson's test or Spearman's test, as appropriate. All results are expressed as median (25^{th}-75^{th} percentile) unless otherwise stated.

RESULTS

The prevalence of GER and gastric aspiration as well as the comparison between BOS and no BOS patients was analysed in the first study cohort. The results in the second study cohort are listed in a separate paragraph.

Patient characteristics

The first study cohort ('off PPI' patients) consisted of 45 LTx recipients (29 men, median age 52(19-69) years). The second study cohort ('on PPI' patients) consisted of a separate group of 18 patients (8 men, age 58(22-66) years). All 18 patients had adequate control of the gastric acid secretion (<50% of the recording time with gastric pH<4). The underlying diagnoses and patient characteristics are listed, according to BOS stage in Table 1. The mean time between the LTx and reflux assessment with impedance-pH monitoring was 36(14-45) months.

GER in patients studied 'off' PPI

Typical reflux symptoms (heartburn or regurgitation) were present in 18/45 patients (11 patients prior to LTX, 7 after LTx). Twenty-two patients (49%) had increased GER defined as having one or more abnormal reflux indices (increased acid exposure, volume exposure or number of reflux events). Sixteen patients had increased acid reflux, whereas 6 patients (27%) had only increased non-acid reflux. Median values of different reflux are displayed in Table 2.

Patients with CF had significantly more reflux episodes compared to patients with other diagnoses (Figure 2). The esophageal acid exposure and the proximal extent of reflux were also significantly increased in patients with CF compared to other LTx patients [8.3%(3.4-38.15) vs. 2.05%(1.0-5.05), p=0.001] and [(19(11-61) vs. 7(3-16),

p=0.005)], respectively. Other reflux indices were unaffected by the type of end-stage lung disease.

The type of transplant surgery had no effect on any of the reflux parameters.

Gastric aspiration in patients studied 'off' PPI

All LTx patients had detectable levels of pepsin in the BALF. The median pepsin concentration detected in BALF of LTx recipients was significantly higher than in 'non-transplant' patients (541(187-946) ng/ml versus 24(0-25) ng/ml, respectively, $p<0.0001$) (Figure 3). The pepsin concentration was slightly higher in CF patients when compared to other LTx patients but this was not significant (769(566-1190) ng/ml versus 488(166-918) ng/ml, respectively, p=NS). The BALF pepsin concentration was similar in patients that underwent SSLTx (601(285-1001) ng/ml), left SLTx (315(118-715) ng/ml), right SLTX (367(112-1517)) and HLTx (93(59-549) ng/ml). Pepsin levels were significantly correlated to the amount of neutrophils found in BALF ($r=0.3692$, $p=0.014$).

Twenty-two patients (49%) had detectable bile acids in BALF as opposed to none of the non-transplant controls. Six out of the seven CF patients (86%) had detectable bile acids in BALF. This was significantly higher when compared to non-CF patients (16/38; p= 0.04). The proportion of patients with bile acids in BALF was similar in patients that received SSLTx (15/30), SLTx (5/11) or HLTx (2/4).

GER and BOS in patients studied 'off' PPI

All of the 24hrs reflux parameters (acid exposure, bolus exposure, number of reflux events and proximal extent) were similar in patients with BOS $\geq$1, patients with BOS 0p and stable patients (BOS 0) (Table 3).

The number of patients having one or more abnormal reflux indices was not significantly different in the subgroups with different BOS stage (14/25 BOS 0; 2/9 BOS 0p and 6/11 BOS $\geq$ 1; p=NS).

Gastric aspiration and BOS in patients studied 'off' PPI

Pepsin levels in BALF were similar in patients with BOS $\geq$1, patients with BOS 0p and stable patients (BOS 0) (492(181-987) ng/ml, 435(171-795) ng/ml and 728(184-1282) ng/ml, respectively, p=NS).

There was no significant correlation between pepsin in BALF and the FEV_1, regardless of whether the FEV_1 was expressed as % predicted % personal best or in absolute volume.

The median level of bile acids in BALF was slightly higher in patients with BOS (0.5(0-0.8) µmol/L) compared to patients with BOS 0p (0.2(0-1.2) µmol/L) and patients with BOS 0 (0.1(0-0.32) µmol/L), although this did not reach statistical significance (p=0.1). However, significantly more patients with BOS (12/17) had detectable bile in the BALF compared to stable patients (5/16, p=0.03) (Figure 4).

There was no significant correlation between the concentration of bile acids in BALF and the FEV_1.

GER and gastric aspiration in patients studied 'on' PPI

Seven of 18 patients 'on' PPI had increased GER, of which 5/7 patients (71%) had increased weakly acidic reflux (number of episodes). Two patients had increased esophageal acid exposure, despite adequate control of gastric acid secretion.

The esophageal acid exposure and the number of acid reflux events was significantly reduced compared to the patients studied 'off' PPI ((0.5%(0.1-2.3) vs. 3.1%(1.0-6.4), p=0.001), (2(0-8) vs.16(7-26), p=0.002), respectively). PPI treatment was not

associated with reduced total number of reflux events, number of weakly acidic reflux, volume exposure or proximal extent of reflux (Table 2).

All 18 patients had detectable pepsin in BALF. Pepsin levels in patients ‘on’ PPI (658(146-1044) ng/ml) were similar to pepsin levels in patients studied ‘off’ PPI (541(187-946) ng/ml). Nine of 18 patients had bile in BALF. The proportion of patients ‘on PPI’ (9/18) with bile in BALF was similar compared to patients studied ‘off’ PPI (22/45).

Discussion

It has been suggested that non-alloimmune factors such as infections and GER contribute to the development of BOS after LTx [5;15;18]. In the current cross-sectional study we have investigated the presence of reflux and gastric aspiration in LTx recipients, both with and without BOS, using 24hrs impedance-pH recordings and detection of pepsin and bile acids BALF. Our main findings were: (1) 49% of the patients had increased GER (2) LTx patients with CF had the highest prevalence of GER (3) gastric aspiration occurred frequently after LTx as shown by the presence of pepsin in BALF of all patients and bile acids in BALF of 50% of patients (4) Patients with BOS did not have increased GER and did not have a particularly higher concentration of pepsin in BALF (5) 70% of BOS patients had detectable bile in the BALF compared to 31% of stable LTx patients (6) Non-acid reflux and degree of gastric aspiration was not lower in patients taking PPI treatment.

Using esophageal impedance-pH monitoring, the most sensitive method available for reflux detection [19], we found less GER over 24hr in our LTx patients than the previously reported prevalence of 69.8% to 78% [7;12;20]. The selection criteria for recruitment in our study were different: our patients were chronologically recruited for impedance-pH measurement, regardless of the presence of reflux symptoms, while in some other studies at least a subset of patients was recruited on the basis of reflux symptoms and data were collected retrospectively [7;12]. The later time after LTx of recording impedance-pH may also explain the lower prevalence of reflux observed in our population. In our study the patients were monitored for reflux much later than in previous reports [7;12;20]. Reflux rates might be higher early after transplantation due to surgery-induced anatomical changes that improve with time. This hypothesis requires experimental confirmation.

In the LTX patients with abnormal reflux indices, a small number of LTX patients was diagnosed with exclusively increased non-acid reflux. This population comprised 27% and 71% of the LTX patients with GER, studied 'off' PPI and 'on' PPI, respectively and would not have been diagnosed with pH monitoring alone.

We confirmed that CF patients have an increased prevalence of acid reflux [21]. In addition, we have shown that CF patients not only have acid but also non-acid reflux and more often proximal reflux when compared to other LTx recipients. They also had a trend towards higher pepsin levels in BALF and significantly more CF patients had bile acids in BALF, confirming that they are prone to gastric aspiration.

This is the first study which compared both markers of gastric aspiration in the same patients. All LTx recipients had increased levels of pepsin in BALF, suggesting that aspiration after LTx might be an ubiquitous event, even in those patients with normal GER indices. These data confirm earlier findings [9;22]. We have also detected bile acids in BALF in 49% of LTx patients and none of the non-transplant samples, which was similar to the results reported by D'Ovidio et al [8;10]. The slightly lower absolute levels of bile acids in BALF in our study may be due to a different BALF procedure and assay.

Our data thus suggest that the presence pepsin or bile acids provides us with different information: Pepsin is a general marker of aspiration of gastric content while bile acids are a more specific marker of gastric aspiration, which might be specifically related to the pathophysiology of aspiration-induced BOS.

In our cross-sectional study we could not find an increased prevalence of reflux (acid or non-acid) in patients with BOS compared to stable LTx recipients nor could we find a significant correlation between reflux and FEV_1. A few cross-sectional studies have previously looked at the effect of reflux on the pulmonary function and the

development of BOS after LTx. Davis et al. described an equally increased esophageal acid exposure both in patients with and without BOS [12]. Hadjiliadis et al described no difference in the prevalence of abnormal esophageal pH results between patients with or without allograft dysfunction although a significant negative correlation was found between acid exposure and FEV_1 [7]. In a prospective study in 48 patients, it was shown that abnormal pH testing at 3 months after LTx was associated with a significantly reduced time to development of BOS [10]. These results are different from our data and might be affected by timing of the reflux testing and type of diagnostic procedure. In most studies, pH-testing without impedance was performed at 3-6 months after LTx while in our study impedance-pH was performed more than 1 year after LTx. On the other hand, in the study by D'Ovidio et al., a slightly higher prevalence of abnormal pH-testing was found at 12 months than at 3 months after LTx. It was not reported if abnormal pH-testing at 12 months was also associated with a reduced freedom from BOS.

In the present study, we could not find an increased concentration of pepsin in BALF of patients with BOS compared to stable LTx patients. On the other hand, we did find an increased presence of bile acids in patients with BOS $\geq$ grade 1 , when compared to patients without BOS. These data are consistent with the data by D'Ovidio et al. who also demonstrated an association between the presence of bile acids in BALF and the onset of BOS [8]. Our results confirm that in addition to testing for GER, it may also be worthwhile to detect the presence of bile acid aspiration in order to determine those LTx patients that are prone to GER-induced BOS.

Previous studies suggested that LTx recipients with GER may benefit from anti-reflux treatment (either pharmacologically or surgically) [11-13;23;24]. It is a common practice after LTx to prescribe prophylactic therapy with PPI. Our results from the separate group of patients studied 'on PPI', showed that treatment significantly

reduced esophageal acid exposure and the number of acid reflux events although patients still had non-acid GER. Moreover, the levels of pepsin in BALF and the proportion of patients with detectable bile acids were similar in patients ‘on’ PPI compared to patients ‘off’ PPI. This suggests that PPI treatment, effectively reducing gastric acid secretion, does not prevent gastric aspiration. and will probably not protect against a GER-induced impairment of lung allograft.

We hypothesize that aspiration of gastric content in LTx patients might occur not only due to increased GER but also in the context of normal or even reduced number of reflux events (acid and non-acid). The contribution of reflux to the development of BOS might be more related to a particular component of the refluxate rather than its frequency or volume. This is supported by the finding of significant improvement of lung function and freedom from BOS occurring after surgical fundoplication, the most radical antireflux procedure [11;12;23]. Survival after early fundoplication was significantly better in this study than in those who had no evidence of acid GER, defined as having a normal pH-monitoring [11]. A possible explanation for this paradoxical observation might thus be that a number of patients with normal pH monitoring results might still be having subclinical aspiration triggering the development of BOS.

The main limitation of our study is its cross-sectional design. The main advantages are the use of impedance-pH and the combined measurement of 2 markers of aspiration in BALF. Further prospective studies using the same reflux indices and aspiration markers are needed to evaluate the effect of reflux on the long term evolution of the lung function and the development of BOS. This should also allow us to refine the specific indications for anti-reflux surgery in LTx recipients.

In conclusion, we found that using a state of the art diagnostic procedure, half of the LTX patients had increased GER, in part non-acid GER. Patients with BOS did not have particularly higher reflux indices. Gastric aspiration occurs frequently in LTx patients as shown by the presence of pepsin in BALF of all patients and bile acids in BALF of 50% of the patients. BALF pepsin is a general marker of aspiration of gastric content whereas bile acids in BALF is more specific and might be associated with the development of BOS. Treating patients with PPI does not protect patients from aspiration of gastric contents and probably will not prevent the development of BOS.

Online repository

Methodology of pH-impedance measurement]

[Esophageal impedance-pH was recorded with a 2.1 mm diameter catheter that comprised six electrode pairs to measure intraluminal impedance and 2 antimony pH sensors (Sandhill Scientific, Inc; Highlands Ranch, CO, USA). The catheter was passed transnasally and positioned to record pH in the stomach and pH and impedance in the esophageal body. Esophageal pH was measured at 5 cm and impedance at 3, 5, 7, 9, 15 and 17 cm proximal to the lower esophageal sphincter (LES) (Figure1). The impedance-pH catheter was connected to an ambulatory device containing the amplifiers (Sleuth, Sandhill Scientific, Inc; Highlands Ranch, CO, USA). The impedance amplifier delivered ultra-low current in a range of 1-2 kHz with resulting current flow variations in response to intraluminal impedance changes. The impedance and pH signals were digitized at 50Hz and stored in the data logger. Before the start of the recording, the pH electrodes were calibrated using pH 4.0 and pH 7.0 buffer solutions. Patients were asked to remain upright during the day, and lie down only at their usual bedtime. Event markers on the data logger recorded meal times and posture changes. Between meals, patients abstained from snacks, beverages with a pH < 5, and were asked to avoid lozenges and gum chewing. Before the study, patients were instructed to keep a careful diary and trained to use a dedicated event marker in the data logger, to record cough episodes and other events.

Total 24hr esophageal acid exposure was calculated as the percentage of time that the esophageal pH was below 4 and was considered increased if > 4.5% of the recording time.

Individual reflux events, detected by impedance, were counted and classified according to the corresponding pH change as *acid* and *non-acid.* A GERevent was defined as a sequential orally progressing drop in impedance to less than 50 % of the

baseline values starting distally (3 cm above LES) and propagating retrograde to at least the next 2 more proximal measuring segments. Reflux was classified as *acid* if pH fell below 4 for at least 4 seconds or, if pH was already below 4, as a decrease of at least 1 pH unit sustained for more than 4 seconds. *Non-acid reflux* was defined as a pH drop of at least 1 pH unit sustained for more than 4 seconds with the basal pH remaining between 4 and 7. The number of reflux events were regarded as increased if above the 95th percentile of normal data obtained in healthy subjects [17].

For each reflux episode detected by impedance, the volume exposure at 5 cm above LES was calculated as the time (sec) between the 50% drop in impedance until the 50% recovery of the impedance baseline. Total volume exposure/24hr was obtained by addition of volume exposure of all individual reflux events. The volume exposure was regarded as increased if above the 95th percentile of data obtained in healthy subjects [17].

The proximal extent of reflux was evaluated from the impedance tracings and expressed as total number of reflux episodes reaching 15 cm above the LES.

The gastric acid exposure was measured and was used to evaluate the effect of PPI on gastric acid secretion. The effect of the medication was considered adequate if the gastric pH was acid (pH < 4) during less than 50% of the time.

Figure Legends

Figure1: (A) Schematic representation of the impedance-pH catheter, positioned in the esophagus. The proximal pH electrode is positioned 5 cm above the LOS and the distal electrode in the stomach, allowing impedance measurements at 3-5-7-9-15-17 cm above the LOS.

Figure2: The total nr of reflux events in patients with different underlying diagnosis. Patients with cystic fibrosis (CF) had significantly (p=0.0052) more reflux episodes (67) compared to patients with COPD (32(19-44)), pulmonary fibrosis (38), pulmonary hypertension (19) and patients with other diagnosis (23 (9-43)).

Figure 3: The concentration of pepsin in the BALF was significantly higher in LTx recipients (532ng/ml (184-1190ng/m) compared to controls (23.83 ng/ml (0-25 ng/ml)).

Figure 4: The proportion of patients with bile acids in the BALF in relation to the BOS stage. Significantly more patients with BOS had detectable bile in the BALF compared to stable patients (p=0.03)

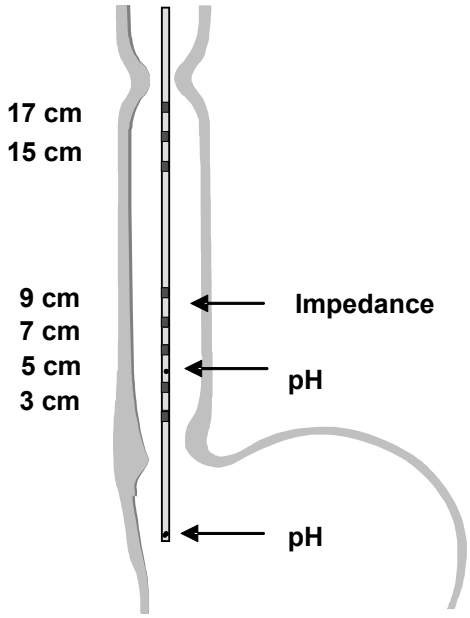
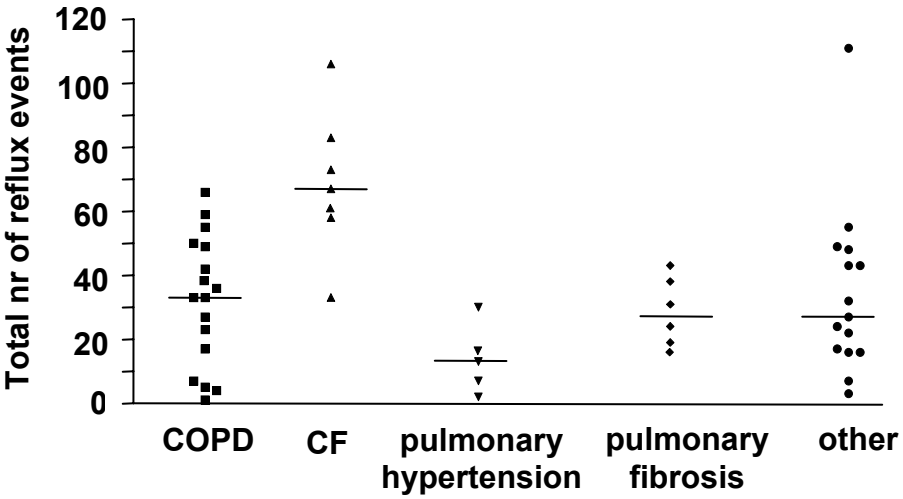


Figure 1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100
80
60
40
20
0
COPD
CF
pulmonary hypertension
pulmonary fibrosis
other

Figure 2

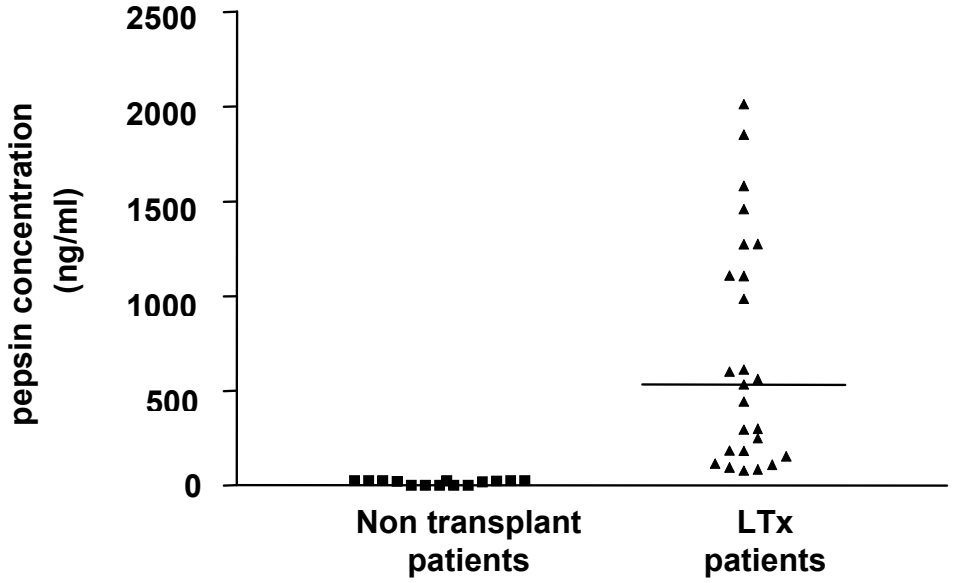
2500
2000
1500
1000
500
0
pepsin concentration (ng/ml)
Non transplant patients
LTx patients

Figure 3

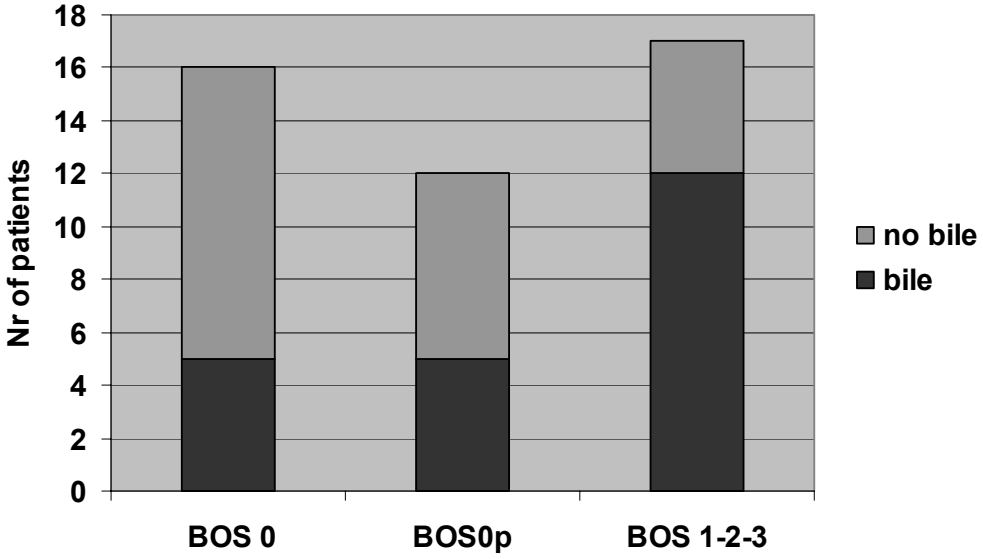
18
16
14
12
10
8
6
4
2
0
Nr of patients
BOS 0
BOS0p
BOS 1-2-3
no bile
bile

Figure 4

	Study cohort 1 ('off' PPI) **(n=45)**			Study cohort 2 ('on' PPI) **(n=18)**
	BOS 0 **(n= 25)**	BOS 0p **(n= 9)**	BOS ≥ 1 **(n=11)**	BOS 0 **(n=10)** BOS 0p **(n=2)** BOS ≥ 1 **(n=6)**
Male/Female	**19/6**	**3/6**	**7/4**	**10/8**
Age (mean ± SD)	**51±11**	**53±11**	**46±13**	**53±13**
CF/other *	**4/21**	**2/7**	**1/10**	**2/16**
SSLTx/SLTx/HLTx	**19/5/1**	**3/5/1**	**8/1/2**	**11/6/1**
Weeks after LTx (mean ± SD)	**114±69**	**130±54**	**227±124**	**112±60**
n° of patients with a history of AR≥2 episodes	**4**	**2**	**5**	**4**
n° of patients colonized at study day	**4**	**1**	**3**	**4**
CMV donor/receptor (1/2/3)	**7/9/9**	**5/2/2**	**4/3/4**	**13/2/3**

Table 1 Patient characteristics
* Other diagnoses in study cohort 1 include COPD (n=15), cystic fibrosis (CF) (n=7), pulmonary fibrosis (n=6), primary pulmonary hypertension (n=5), bronchiectasis (n=3), LAM (n=1), sarcoidosis (n=1), pulmonary fibrosis due to congenital dyskeratosis (n=1), hypersensitivity pneumonitis (n=2), α_1 antitrypsin deficiency emphysema (n=2) and Williams-Campbell syndrome (n=1). Other diagnoses in study cohort 2 include COPD (n=8), CF (n=2), pulmonary fibrosis (n=3), primary pulmonary hypertension (n=1), bronchiectasis (n=1), LAM (n=1), obliterative bronchiolitis (induced by a viral infection during childhood) (n=1) and histiocytosis X (n=1).
** CMV status of the LTx donor and receptor 1) D+/R+ or D-/R+2) D-/R- 3) D+/R-

	study population n = 45	**Patients studied "on" PPI** n = 18
Esophageal acid exposure (%)	3.1 (1.0-6.4)	0.5 (0.1-2.3)
Esophageal volume exposure (%)	0.7 (0.3-1.4)	0.75 (0.4-1.4)
Total number of reflux events (24 hrs)	33 (17-49)	34 (18-52)

Number of acid reflux events	16 (7-26)	2 (0-8)
Number of non-acid reflux events	15 (6-20)	26 (18-45)
Proximal extent of reflux (# of reflux events > 15 cm)	9 (3-19)	13 (2-17)

Table 2 GER indices . Results are expressed as median (25th-75th percentile).

	BOS 0 **n= 27**	BOS0p **n= 9**	BOS 1-2-3 **n=11**
Esophageal acid exposure (%)	**3.6 (1.7-8.7)**	**0.8 (0.5-3.9)**	**1.9 (0.8-18.7)**
Esophageal volume exposure (%)	**0.8 (0.54-1.7)**	**0.4 (0.3-1.5)**	**0.5(1.2-1.3)**
Total number of reflux events (24hrs)	**33 (29-48)**	**49 (36-71)**	**32 (9-45)**
Number of acid reflux events	**17 (13-24)**	**4 (0-10)**	**6 (2-20)**
Number of non-acid reflux events	**12 (6-19)**	**21 (11-29)**	**18 (6-33)**
Proximal extent of reflux at 15cm (# of reflux events > 15 cm)	**12 (7-21)**	**4 (1-13)**	**13 (6-25)**
Number of nocturnal reflux events	**4(2-6)**	**2(1-6)**	**5(3-9)**

Table 3 GER indices in patients with and without BOS . Results are expressed as median (25th-75th percentile).

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