

GASTRO-OESOPHAGEAL REFLUX AND GASTRIC ASPIRATION IN IDIOPATHIC PULMONARY FIBROSIS PATIENTS

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ABSTRACT (Words Count 200)

Objective:

To characterize gastro-oesophageal reflux (GOR) in idiopathic pulmonary fibrosis (IPF).

Methods:

Forty consecutive IPF patients underwent pulmonary high-resolution computed tomography (HRCT) scan and impedance-pH monitoring off anti-secretory therapy. The presence of pulmonary fibrosis was assessed using validated HRCT-scores. Reflux features included distal oesophageal acid exposure, number of acid/weakly-acidic reflux episodes and their proximal migration. Forty consecutive patients with interstitial lung disease other than IPF (non-IPF patients) and 50 healthy volunteers (HVs) were also enrolled.

Results:

IPF patients had significantly higher ($p < 0.01$) oesophageal acid exposure (9.25[4.7-15.4] vs. 3.3[1.4-7.4] vs. 0.7[0-2.4.2]), number of acid (45[23-55] vs. 32[19-44] vs. 18[10-31]), weakly-acidic (34[19-43] vs. 21[11-33]) vs. 18[15-28]) and proximal reflux events (51[26.5-65.5] vs. 20[9.5-34.5] vs. 9[5-20]) compared to non-IPF patients and HVs. Pulmonary fibrosis HRCT-scores correlated well with reflux episodes in both distal ($r^2=0.567$) and proximal ($r^2=0.6323$) oesophagus. Patients with IPF had more bile acids and pepsin ($p < 0.03$) in broncho-alveolar lavage (BAL) (62%/67%) and saliva (61%/68%) than non-IPF patients (25%/25% in BAL, 33%/36% in saliva) and controls (0%/0% in BAL/saliva).

Conclusions:

Acid GOR is common in IPF, but weakly-acidic GOR may also occur. Patients with IPF have risk or definite pulmonary aspiration of gastric contents. Outcome studies with intense anti-reflux therapy are needed.

Key Words: idiopathic pulmonary fibrosis, impedance pH-metry, acid and nonacid reflux, gastro-oesophageal reflux

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic lung disease with a median survival ranging from 2 to 3 years from diagnosis (1). To date, no cure is available and treatment strategies show little effect. The mechanisms leading to IPF remain unknown. Gastro-oesophageal reflux disease (GORD) has been previously associated with a number of interstitial lung diseases (2-6).

Evidences from both animal (7) and human (8) studies support the concept that pulmonary fibrosis can occur after repeated tracheobronchial aspiration of small amounts of gastric contents over long periods of time (i.e. chronic microaspiration). Moreover, recent investigations have demonstrated disease stabilization or delay of disease progression after medical or surgical treatment of GOR (9-11). In particular, Lee et al. showed that the use of GR medications is associated with decreased radiologic fibrosis and is an independent predictor of longer survival time in patients with IPF (11).

Until recently, diagnosis and therapy of GORD has focused on gastric acid as the main monitoring parameter and treatment target. Recent studies highlighted that lung injury is independent of acidity and factors other than acid (i.e. foods, pepsin, bile acids) may be involved in its pathogenesis (12,13). In particular, Mertens et al. showed that exposure of bronchial epithelial cells to gastric juice from patients “on” anti-secretory therapy is able to induce high IL-8 production, the most relevant cytokine for the acute-phase response of inflammation (13). However, in IPF patients, GER has been previously assessed by means of 24-h pH-metry that permits detection of acid reflux only. The recent use of impedance–pH monitoring allows us to detect both acid and weakly acidic GER and to assess the extent of reflux into proximal oesophagus (14,15).

The aim of our study was to further explore the association of GOR and pulmonary fibrosis by prospectively assessing the prevalence of all kinds of reflux in a carefully selected, well-defined population of IPF patients and compare the findings to those of patients with ILD other than IPF and healthy volunteers.

PATIENTS AND METHODS

Subjects

Between November 2007 and October 2011, consecutive patients with a definite diagnosis of IPF were prospectively enrolled in the study. The diagnosis of IPF was based on the absence of an identifiable etiology for ILD and a histopathologic/radiologic pattern of usual interstitial pneumonia on surgical lung biopsy and high-resolution computed tomography (HRCT) scans (1). Forty consecutive patients with ILD other than IPF, referred for routine visits (independently of reported symptoms and primary disease type or stage) were also enrolled for comparison (participation rate of 92%). They were found to have ascertained diseases associated with the development of pulmonary fibrosis: sarcoidosis (10 patients), systemic lupus erythematosus (6 patients), mixed connective tissue disease (14 patients) and bronchiolitis obliterans organizing pneumonia (10 patients), which were diagnosed according to characteristic histopathologic findings on surgical lung biopsy or appropriate autoimmune markers and clinical presentations (16-19).

Fifty healthy volunteers (HVs) without any disease or history of surgery were also enrolled for comparison.

The study protocol was approved by the local Ethics Committee and performed according to the Declaration of Helsinki. All participants gave written informed consent prior to being enrolled in the study.

Study Protocol

All patients completed a structured questionnaire including: patient demographics, history of pulmonary and reflux symptoms, medications and tobacco use. Then, they underwent oesophageal and pulmonary investigations within 1 month of the date of diagnosis (defined as the date of initial visit) and saliva samples were collected for measuring bile acids and pepsin. Moreover, in patients who underwent bronchoscopy, broncho-alveolar lavage (BAL) samples using standardized

procedures were also collected for measuring bile acids and pepsin. All patients were asked to discontinue any medication that would influence oesophageal motility (i.e. nitrates, calcium antagonists, domperidone, benzodiazepines, metoclopramide, etc.) and acid suppressive therapy at least 30 days before study start.

OESOPHAGEAL INVESTIGATIONS.

Manometry Testing. Oesophageal manometry was performed by means of multichannel intraluminal impedance-oesophageal manometry (MII-EM) using a Koenigsberg 9-channel probe (Sandhill EFT; Sandhill Scientific Inc.; Highlands Ranch, CO). Testing methodology was previously reported (20).

We measured lower oesophageal sphincter (LOS) pressure and relaxation, peak contraction amplitude, duration of contraction, coordination and propagation of velocity after swallows. Manometric patterns were reported according to international criteria (21).

Impedance-pH monitoring. The equipment used (Sleuth[®], Sandhill Scientific, Inc.; Highland Ranch, CO) and testing methodology were previously described (22). Studies were performed off-PPI treatment.

Data were downloaded and analysed using dedicated software (BioView Analysis) and subsequently reviewed manually by an experienced investigator blinded to the basal condition of the overall patients and HVs (ES). GOR episodes were classified as acid (nadir pH<4), weakly acidic (nadir pH 4-7) and weakly alkaline (nadir pH >7) following established criteria (23).

Number and type of reflux episodes, acid exposure (reflux time [min] and reflux percent time), proximal extent (reflux reaching 15-cm above the LOS) were calculated.

Total distal oesophageal acid exposure <4.2% over 24 hours was considered normal (24,25). A number of reflux episodes lower than 54 was considered normal (26)..

PULMONARY INVESTIGATIONS.

Pulmonary involvement was systematically investigated, during initial evaluation of IPF in all patients, by chest radiographs, HRCT scan of the lungs, and pulmonary function test (PFT).

Pulmonary function tests (PFTs). Forced vital capacity (FVC) and forced expiratory volume (FEV) curves were measured in a constant-volume plethysmograph (Sensor Medics 28000; Yorba Linda, CA). A 10-s single-breath DL_{CO} test (Morgan; Kent, UK) was carried out. Pulmonary function was considered abnormal if volumes were less than 80% of predicted values and/or when DL_{CO} was less than 75% of predicted value (27,28).

Pulmonary HRCT. High resolution scanning (CT Lightspeed; GE, Milwaukee, WI) of the lungs was performed using standardized protocols to identify radiographic abnormalities related to IPF (1). The testing methodology was previously described (3).

HRCT scans were evaluated by two experienced radiologists, independently and in random order without knowledge of patients' status. The overall interobserver agreement in assigning the fibrosis score was excellent (K=0.88). Any discrepancy in the assessment was further discussed and a consensus was reached. A semi-quantitative analysis of the severity of fibrosis on HRCT was calculated by estimating the percentage of lung affected by fibrosis (i.e., reticular abnormality and/or honeycombing) to the nearest 5 percent in three zones for each lung, as previously described by Best et al. (29). These numbers were averaged to obtain a net radiologic fibrosis score.

MONITORING OF BILE ACIDS/PEPSINOGEN IN BRONCHOALVEOLAR LAVAGE (BAL) AND SALIVA.

Bile acids assay. BAL and saliva/sputum samples were analyzed for the presence of total bile acids using a commercial assay (Bioquant, San Diego, USA). The lowest level of accurate detection was 0.2 mmol/l (30).

Pepsin Test. BAL and saliva/sputum samples were analyzed for the presence of pepsin using a commercial enzyme-linked immunoadsorbent assay (Peptest, RD Biomed Limited, Hull, United Kingdom) (31).

Saliva. Bile acids and pepsin presence were measured in saliva samples collected under fasting condition in 38 IPF patients at the time of the first visit. Bile acids and pepsin were also measured in saliva from 36 patients with ILD other than IPF [sarcoidosis (8 patients), systemic lupus erythematosus (5 patients), mixed connective tissue disease (13 patients) and bronchiolitis obliterans organizing pneumonia (10 patients)] and from 50 healthy volunteers at the same time.

Bronchoalveolar lavage. BAL was performed in a single sub-segment of the right middle lobe or lingula, with at least 100 mL of sterile saline instilled. The BAL fluid was recovered by gentle manual suction, kept on ice and processed within 1 h of collection. Bile acid and pepsin were measured in BAL samples of 21 IPF patients obtained at the time of diagnosis during bronchoscopy. They were also measured in BAL of 20 patients with ILD other than IPF [10 sarcoidosis and 10 bronchiolitis obliterans organizing pneumonia] and in 16 patients undergoing bronchoscopy for other diseases (non-ILD patients: 10 lung cancer, 6 chronic obstructive pulmonary disease [COPD]).

Statistical Analysis

Differences in proportions were compared using the chi-square or Fisher's exact test. As reflux parameters were not normally distributed, results are reported as median and percentiles (median [25th-75th perc]; 95th perc). Differences between groups were assessed using Kruskal-Wallis and/or Mann-Whitney tests. The correlation between the severity of pulmonary fibrosis and reflux parameters was calculated using Spearman correlation. The interobserver variability in grading fibrosis on HRCT was assessed by kappa statistics with linear weighting. For statistical significance alpha was set at 0.05.

RESULTS

Patients characteristics

Forty patients with a definite diagnosis of IPF and 40 with pulmonary fibrosis other than IPF (non-IPF) were consecutively enrolled in the study. Detailed demographic and clinical characteristics of all patients and HVs are shown in **Table 1**. Patients with IPF had similar Dlco% (48% vs. 50%, $p=0.1202$), FEV% (55% vs. 63%, $p=0.2235$), FVC% (58% vs. 62%, $p=0.0833$) and radiologic fibrosis score (20% vs. 18%, $p=0.0999$) compared to non-IPF patients. Moreover, IPF patients tended to have a smoking history higher compared to non-IPF patients, but statistical significance was not reached ($p=0.1759$). All HVs had normal pulmonary (PFTs, chest radiographs and HRCT) and oesophageal (manometry and impedance-pH) investigations.

Oesophageal manometric evaluation

Patients with IPF had similar lower LOS resting pressure compared to non-IPF patients (14.1 vs. 16.7; $p=0.0999$). Mean contraction amplitude in the distal and proximal oesophagus were slightly higher in IPF compared to non-IPF patients (92.4 vs. 84 and 90.6 vs. 79.9, $p=0.5285$ and $p=0.2582$ respectively). No differences were found also in terms of mean UOS basal pressure and prevalence of hiatal hernia between patients with IPF and non-IPF ones (60.1 vs. 62.4 and 55% vs. 38%, $p=0.9386$ and $p=0.1782$ respectively). Moreover, IPF and non-IPF patients differed from HVs only in terms of mean LOS basal pressure and prevalence of hiatal hernia (**Table 2**).

As to the oesophageal motility patterns, no differences were found between IPF and non-IPF patients in terms of prevalence of normal peristalsis ($p=1$), distal oesophageal spasm ($p=0.737$), nutcracker oesophagus ($p=1$), ineffective oesophageal motility ($p=1$) and abnormally low LOS pressure ($p=0.6001$).

Oesophageal 24-h impedance-pH monitoring

pH-METRY DATA (**Figure 1**). Of 40 IPF patients, 33 (83%) had an abnormal distal acid exposure, compared with 17/40 (43%) of non-IPF ones ($p < 0.0001$). Total, upright and recumbent % time with $\text{pH} < 4$ was significantly higher in IPF compared to non-IPF patients and HVs (9.25 [4.7-15.4;25.6] vs. 3.3 [1.4-7.4;17.3] vs. 0.7 [0-2.4.1;4.2]; $p < 0.0001$).

IMPEDANCE DATA (**Figure 2**). In IPF patients the total (both acid and weakly acidic) number of reflux episodes was higher (76 [43-96;117]) than that of non-IPF patients (47 [30.5-72;104]; $p < 0.0070$) and HVs (32 [20-45;55]; $p < 0.0001$). This was found also considering acid and weakly acidic reflux episodes separately ($p < 0.03$)

More reflux episodes reached the proximal oesophagus 51 [26.5-65.5;95] in IPF patients than non-IPF ones (20.5 [9.5-34.5;62]; $p < 0.0001$) and HVs (9 [5-20;32]; $p < 0.0001$), as shown in **Figure 3**. Also the percentage of total reflux episodes reaching the proximal measuring site was higher in IPF (66%) than non-IPF patients (42%; $p < 0.0001$) and HVs (31%; $p < 0.0001$).

Pulmonary aspiration of gastroduodenal contents

BILE ACIDS AND PEPSIN IN SALIVA. Saliva samples were collected in 38 IPF patients, 36 patients with ILD other than IPF and in 50 HVs. IPF patients had more bile acids and pepsin in saliva than non-IPF ones and HVs. Bile acids were present in saliva of 23/38 (61%) IPF patients compared to 0/50 (0%; $p < 0.0001$) of HVs and 12/36 (33%; $p < 0.0223$) of non-IPF patients. Pepsin was present in saliva of 26/38 (68%) patients compared to 0/50 (0%; $p < 0.0001$) of HVs and 13/36 (36%; 0.0099) of non-IPF patients. The concentration of bile acids in saliva was significantly higher in patients with IPF [3.70 $\mu\text{mol/l}$ (2.90–6.35)] compared to non-IPF patients [1.5 $\mu\text{mol/l}$ (1.2–1.8)] ($p < 0.01$). All IPF and non-IPF patients with bile acids or pepsin in saliva (92% and 85%, respectively) had an abnormal impedance-pH testing (i.e. abnormal AET or abnormal number of reflux episodes).

BILE ACIDS AND PEPSIN IN BRONCHOALVEOLAR LAVAGE. BAL samples were collected in 21 IPF patients, 20 patients with ILD other than IPF and in 16 patients undergoing bronchoscopy for other diseases. Patients with IPF had more bile acids and pepsin in BAL than the non-IPF ones and non-ILD patients. Bile acids were detected in BAL of 13/21 (62%) IPF patients compared to 0/16 (0%; $p<0.0001$) of non-ILD patients and in 5/20 (25%; 0.0278) of non-IPF patients. Pepsin was present in BAL of 14/21 (67%) IPF patients compared to 0/16 (0%; $p<0.0001$) of non-ILD patients and 5/20 (25%; $p<0.0122$) of non-IPF patients. The concentration of bile acids in BAL was higher in IPF patients [0.90 $\mu\text{mol/l}$ (0.80–1.0)] than in non-IPF ones [0.50 $\mu\text{mol/l}$ (0.40–0.60)] ($p<0.01$). All IPF and non-IPF patients with bile acids or pepsin in BAL (93% and 80%, respectively) had an abnormal impedance-pH testing. Among the IPF patients, 3 patients were discrepant in terms of presence of pepsin and bile acids in BAL. Among the non-IPF patients, none was discrepant in relation to the presence of pepsin and bile acids in BAL.

Correlation between HRCT score and impedance-pH findings (oesophageal acid exposure time and reflux episodes) with the presence/concentration of pepsin and bile acids in saliva and BAL

In IPF patients there was a good correlation between degree of pulmonary fibrosis (HRCT score) and total number of reflux episodes in both distal ($r^2=0.567$, $p<0.001$) and proximal ($r^2=0.6323$, $p<0.001$) oesophagus. In contrast, in non-IPF patients a not significant correlation was found between degree of pulmonary fibrosis and total number of reflux episodes in both distal ($r^2=0.0955$, $p=0.052$) and proximal ($r^2=0.0224$, $p=0.356$) oesophagus.

Detailed data on the correlation between HRCT score and impedance-pH parameters with presence/concentration of gastric contents are summarized in **Table 3**. A good correlation was found between HRCT score and presence of pepsin and bile acids in saliva ($r^2=0.5260$, $p<0.001$ and $r^2=0.4269$, $p<0.001$, respectively) as well as in BAL ($r^2=0.6033$, $p<0.001$ and $r^2=0.4605$, $p=0.001$,

respectively). A poor but significant correlation was observed between HRCT score and the concentration of bile acids in saliva ($r^2=0.2591$, $p=0.013$) and in BAL ($r^2=0.3843$, $p=0.024$).

DISCUSSION

Recent data have emphasized the role of GOR in the pathogenesis and potential management of IPF patients (9-13), but, although GOR is recognized to be increased in IPF patients, its prevalence, characteristics and association with pulmonary aspiration of gastric contents have been poorly defined. In the current study, we investigated oesophageal motility, acid and weakly acidic reflux, proximal migration of refluxate, markers of gastric aspiration and their correlation with GOR and radiologic fibrosis, as well as the correlation between GOR and lung fibrosis in patients with IPF. We compared the results with those obtained from patients with ILD other than IPF and normal subjects. To our knowledge, this study is the first investigating the association of weakly-acidic GOR with pulmonary findings in patients with IPF. We observed a higher frequency of GOR episodes (both acid and weakly-acidic) and reflux episodes reaching the proximal oesophagus in patients with IPF compared to non-IPF patients, despite a similar oesophageal peristalsis profile at manometry testing. Moreover, we noted that the majority of IPF patients have risk of gastric aspiration (increased bile acids and pepsin in saliva) or definite gastric aspiration (bile acids or pepsin in BAL). Finally, we found a good correlation between the degree of pulmonary fibrosis and the severity of GOR, as well as between the degree of pulmonary fibrosis and the presence/concentration of gastric contents in the lungs. These findings with the not significant correlation between the degree of pulmonary fibrosis and the severity of GOR in non-IPF patients suggest that patients with IPF have more severe GOR, potentially leading to more extensive lung damage and fibrosis progression.

To date, the mechanisms determining IPF are not clear. Current concepts implicate epithelial–fibroblast interactions as a result of repeated insults to the lung parenchyma by an unknown noxious stimulus. This prolonged stimulus would determine the development of pulmonary fibrosis over a long period of time (1). Recent studies have suggested chronic microaspiration of gastric contents into the lungs as the trigger mechanism able to induce pulmonary parenchymal lesions, thus leading to the hypothesis that GOR therapy could improve

symptoms and PFT parameters (3-6,8-11). This possibility has been recently emphasized, and at least in part confirmed, by the retrospective study of Lee et al., who observed that the use of GOR medication was associated with lower HRCT fibrosis scores and was an independent predictor of longer survival time in 96 out of 204 IPF patients (11). Moreover, the same Authors in a subsequent investigation showed that BAL pepsin was elevated in a subgroup of patients with acute exacerbation of IPF further confirming the major role of micro-aspiration in IPF patients (32). However, all the above-mentioned studies have been performed by means of traditional pH-metry, which limited these investigations to measuring only acid reflux without any information on other chemical types (i.e. weakly acidic reflux) and number of reflux episodes, as well as the risk of proximal migration of the refluxate. Using the current state-of-the-art method to assess GOR (i.e. combined impedance–pH monitoring), we observed that IPF patients had a very severe degree of overall reflux disease, compared not only to non-IPF patients but also to those with other respiratory disorders with an established GOR association, such as asthma, cough and laryngitis, as reported in medical literature (33). Moreover, we showed that IPF patients had higher acid and weakly-acidic reflux episodes in both distal and proximal oesophagus, thus favouring the risk of microaspiration into the lungs compared to non-IPF patients. Finding an increased number of weakly acidic reflux episodes is of paramount importance since this represents a possible explanation of why medical acid suppression alone might fail in preventing reflux and reflux-associated progression of ILD. Moreover, this finding supports the data from the study of Lee et al. reporting that IPF patients who underwent Nissen Fundoplication in order to block all reflux had an additional benefit in terms of life survival time (11). The good correlation we found between the degree of pulmonary fibrosis (HRCT score) and the number of both distal and proximal reflux episodes in IPF patients, as well as the presence/concentration of pepsin and bile acids in BAL, reinforces the potential role of GOR in the development and/or progression of pulmonary fibrosis. In particular, it is worth of noting that the not significant correlation we observed between the degree of pulmonary fibrosis and the number of both distal and proximal reflux episodes in non-IPF patients suggests that abnormal

GOR in IPF subjects may not simply be the result of the underlying fibrosis of the lungs but has the potential to represent a factor unrelated to pulmonary stiffness.

The relevance of both types of reflux was also corroborated by the fact that we documented abnormal levels of pepsin and bile acids in saliva and BAL fluid of a great number of our IPF patients. These data witness the increased risk of pulmonary aspiration of gastric contents (increased bile acids and pepsin in saliva) or the definite evidence of gastric fluid regurgitation and aspiration into the airways (bile acids and pepsin in BAL). To date, although it is generally accepted that the pulmonary aspiration of gastric material may occur in IPF patients, the evidence of this phenomenon is scarce, and mainly based on studies performed in patients with other diseases or conditions (34-36). Our study is the first evaluating bile acids and pepsin in BAL of IPF patients who concomitantly underwent impedance-pH to correlate reflux findings with specific biomarkers in human fluids. Bile acids were found in BAL of 62% IPF compared to 25% non-IPF patients and 0% non-ILD patients, while pepsin was present in 67% IPF compared to 25% non-IPF patients and 0% non-ILD patients. These data emphasize the role of aspiration of gastric contents in IPF patients and confirm the high specificity of pepsin and bile salts for diagnosing GOR-associated pulmonary aspiration, as previously reported (36). Our results also document that 61% of IPF patients had bile acids in their saliva compared to 33% non-IPF patients and 0% HVs, while pepsin was present in 68% IPF compared to 36% non-IPF patients and 0% HVs. These data allow us to estimate the high risk of pulmonary aspiration of material from the stomach in our IPF patients.

Despite the vast majority of our IPF patients had abnormal GOR, only 48% (n=19/40) complained of typical symptoms of GOR, a finding in accordance with previous reports (5,37). Therefore, abnormal acid or weakly acidic GOR was often clinically silent. These findings suggest that all ILD patients with or without apparent reflux symptoms should be carefully evaluated and, eventually, should undergo oesophageal impedance-pH to detect asymptomatic GOR. However, we have to stress that further outcome data are necessary to support testing or treatment of IPF patients for abnormal GOR.

As to the limitations of our study, it has been hypothesized that the increased respiratory workload in patients with ILD could contribute to GOR by increasing the trans-diaphragmatic pressure gradient (38), thus suggesting that stiffened lungs due to fibrosis may cause associated reflux. Unfortunately, our current methodology (i.e., impedance manometry) did not allow us to assess this parameter. Moreover, we did not find any difference concerning the mean LOS pressure and the prevalence of abnormal motility patterns between the two groups of IPF and non-IPF patients. This finding seems to suggest that the degree of oesophageal motor disturbances does not seem to be associated with the development of pulmonary fibrosis in IPF patients. Therefore, although causality has not been demonstrated, a strong association between IPF and GOR was observed and the good correlation between degree of pulmonary fibrosis (HRCT score) and reflux disease (number of reflux episodes), as well as the presence/concentration of pepsin and bile acids in BAL calls for further investigations in this direction.

In conclusion, current data indicate that IPF patients have greater GOR compared to non-IPF ones. Acid is predominant, but also weakly acidic reflux is increased in them. IPF patients have a high risk of pulmonary aspiration of gastric contents even in the absence of typical reflux symptoms and despite evidence of normal oesophageal peristalsis. The increased frequency of weakly acidic reflux implies that therapies aimed at reducing overall and not only acid reflux should be included in studies aimed at assessing whether or not the development of IPF can be prevented by blocking every type of GOR. Although outcome studies are mandatory to confirm the prominent role of GOR in IPF patients, our data suggest that abnormal GOR should be searched and treated adequately to prevent micro-aspiration of gastric contents and its potential deleterious effect in the induction, progression, and/or exacerbation of pulmonary fibrosis in patients with IPF.

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B: Authors contribution:

- Edoardo Savarino, MD, PhD: design of the study, data collection and analysis, writing of the manuscript, approving final version
- Roberto Carbone: design of the study, data collection, writing of the manuscript, approving final version
- Elisa Marabotto, MD: data collection and analysis, writing of the manuscript, approving final version
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Table 1. Demographic and clinical characteristics of IPF patients, those with ILD other than IPF and HVs. IPF= idiopathic pulmonary fibrosis; ILD= interstitial lung disease; GOR = gastro-oesophageal reflux; HVs = healthy volunteers. Data are expressed as median (range).

<i>Demographic and clinical parameters</i>	IPF	ILD Other Than IPF	HVs	<i>P value</i>
Patients, n	40	40	50	
Female patients, n	18	21	27	0.676
Age, years	65 (42-82)	62 (38-80)	58 (34-76)	0.277
Body Mass Index, kg/m ²	24 (17-31)	25 (17-37)	24 (18-34)	0.072
Previous Tobacco Use	55%	35%	30%	0.082
Alcohol Use	48%	45%	44%	1
Surgical Lung biopsies	100%	30%	N/A	<0.001
Pulmonary Symptoms (i.e. cough and dyspnea)	88%	85%	N/A	1
Long Term Oxygen Use	35%	30%	N/A	0.8116
DLCO %	48 (31-62)	50 (31-62)	98 (81-110)	<0.001
FVC %	55 (37-78)	63 (37-79)	102 (87-110)	<0.001
FEV %	58 (38-72)	62 (40-74)	108 (98-131)	<0.001
Radiologic Fibrosis Score*	20%	18%	N/A	0.0999
Esophageal Symptoms (i.e. heartburn/regurgitation)	48%	43%	N/A	0.8224
GOR Medication Use (i.e. proton pump inhibitor or H2 blocker)	40%	43%	N/A	1
Drugs potentially influencing esophageal motility use (i.e. nitrates, Ca antagonists, domperidone, benzodiazepines, metoclopramide, etc.)	20%	30%	N/A	0.4391

Table 2. Manometric parameters of IPF patients, those with ILD other than IPF and HVs. IPF= idiopathic pulmonary fibrosis; ILD= interstitial lung disease; LOS= lower oesophageal sphincter; UOS= upper oesophageal sphincter; HVs = healthy volunteers. Data are expressed as mean (range)

<i>Manometric parameters</i>	IPF	ILD Other Than IPF	HVs	<i>P value</i>
Mean basal LOS pressure	14.1 (4-32.5)	16.7 (4-37.2)	22 (12-42)	<0.001
LOS relaxation	100%	100%	100%	1
Mean proximal contraction amplitude	92.4 (40-190)	84 (35-170)	94.7 (35-170)	0.441
Mean distal contraction amplitude	90.6 (10-210)	79.9 (10-210)	85.36 (30-190)	0.439
Manometric hiatal hernia	55%	38%	14%	<0.001
Mean basal UOS pressure	60.1 (38-100)	62.4 (35-110)	64 (35-110)	0.787

Table 3. Correlation between HRCT score and impedance-pH findings (acid exposure time and impedance-detected reflux episodes) with the presence/concentration of pepsin and bile acids in saliva and BAL in IPF patients. HRCT = high-resolution computed tomography; BAL = broncho-alveolar lavage

	HRCT score	Acid Exposure Time	Total Number of Reflux Episodes
Presence of Pepsin in Saliva	r ² =0.5260 (p<0,001)	r ² =0.2193 (p=0,003)	r ² =0,4007, (p<0,001)
Presence of Bile Acids in Saliva	r ² =0.4269 (p<0,001)	r ² =0.1493 (p=0,017)	r ² =0,5264, (p=0,001)
Concentration of Bile Acids in Saliva	r ² =0.2591 (p=0,013)	r ² =0,0129 (p=0,606)	r ² =0,0712 (p=0,218)
Presence of Pepsin in BAL	r ² =0.6033 (p<0,001)	r ² =0,2344 (p=0,026)	r ² =0,2845 (p=0,013)
Presence of Bile Acids in BAL	r ² =0.4605 (p=0,001)	r ² =0,3660 (p=0,004)	r ² =0,4566 (p=0,001)
Concentration of Bile Acids in BAL	r ² =0.3843 (p=0,024)	r ² =0,0427 (p=0,498)	r ² =0,3558 (p=0,031)

Figure Legends

Figure 1. Median values of oesophageal acid exposure time (AET) in healthy volunteers (n=50), patients with IPF (n=40) and non-IPF patients (n=40). Bars indicate median values. AET= Acid Exposure Time; IPF= idiopathic pulmonary fibrosis

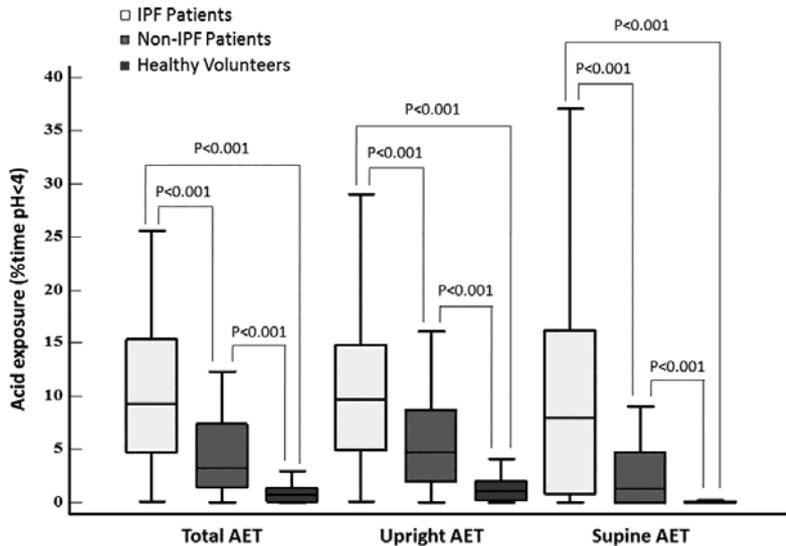


Figure 2. Number and types of gastro-oesophageal reflux in healthy volunteers (n=50), patients with IPF (n=40) and non-IPF patients (n=40). Bars indicate median values. IPF= idiopathic pulmonary fibrosis

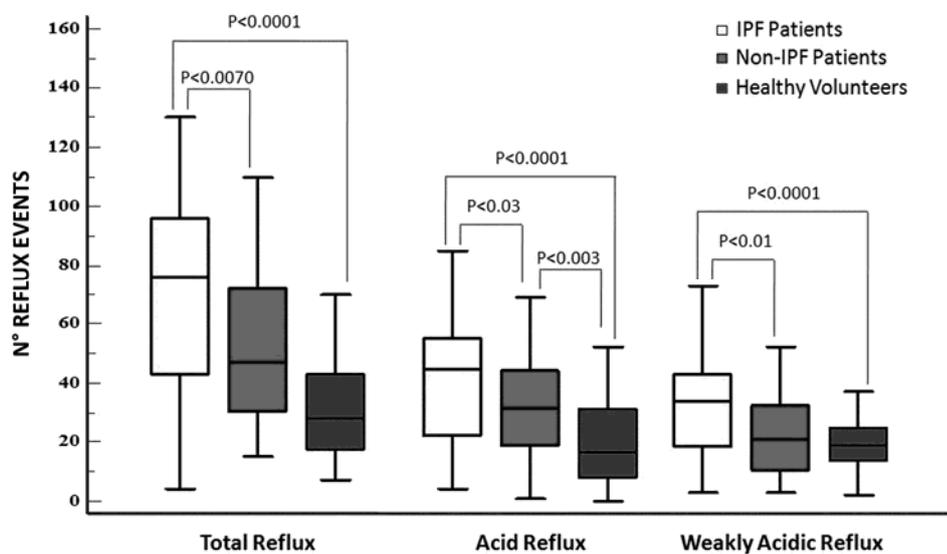


Figure 3. Median number of reflux episodes reaching the proximal oesophagus in healthy volunteers (n=50), patients with IPF (n=40) and non-IPF patients (n=40). Bars indicate median values. IPF= idiopathic pulmonary fibrosis

