Screening for *Helicobacter pylori* in Idiopathic Pulmonary Fibrosis Lung Biopsies

Michael Kreuter^{a, e} Detlef Kirsten^{f, h} Thomas Bahmer^{f, h} Roland Penzel^c
Martin Claussen^{f, h} Svenja Ehlers-Tenenbaum^a Thomas Muley^{b, e}
Karin Palmowski^a Monika Eichinger^d Marta Leider^h Felix J.F. Herth^{a, e}
Klaus F. Rabe^{f, h} Iris Bittmann^h Arne Warth^{c, e}

^aCentre for Interstitial and Rare Lung Diseases, Department of Pneumology and Respiratory Critical Care Medicine and ^bTranslational Research Unit, Thoraxklinik, ^cInstitute of Pathology and ^dDiagnostic and Interventional Radiology, University of Heidelberg, and ^eTranslational Lung Research Centre, German Centre for Lung Research, Heidelberg, ^fOutpatient Ambulance for Interstitial Lung Diseases and LungenClinic, and ^gAirway Research Centre North, German Centre for Lung Research, Grosshansdorf, and ^hInstitute of Pathology, Diakoniekrankenhaus, Rotenburg (Wümme), Germany

For editorial comment see p. 1

Key Words

Gastro-oesophageal reflux disease · *Helicobacter pylori* · Idiopathic pulmonary fibrosis · Lung biopsy · Polymerase chain reaction

Abstract

Background: Increasing evidence suggests a role of gastro-oesophageal reflux (GER) in idiopathic pulmonary fibrosis (IPF) pathogenesis. Recently, an association between serum *Helicobacter pylori* (HP) antibody positivity and more severe disease was described, but HP has not been directly analysed in lung tissue so far. **Objective:** To investigate the presence of HP in the lung tissue of IPF patients. **Methods:** Two tertiary interstitial lung disease care centre databases were screened for available lung biopsy material from IPF patients. Clinical and radiological data, including presence of GER and antiacid medication, were evaluated. HP-specific PCR was carried out on the IPF lung biopsy specimens. **Re-**

sults: A total of 39 IPF patients were included, of whom 85% were male. The patients' median age was 66 years, their vital capacity was 79% predicted, and their diffusing capacity for carbon monoxide was 53% predicted. In all, 82% of the lung biopsies were surgical and 18% transbronchial. Comorbidities were GER disease in 23% (n = 9), sleep apnoea in 13% (n = 5) and hiatal hernia in 38% of the cases (n = 15). Proton pump inhibitors were prescribed at the time of biopsy in 21% of the cases (n = 9). After a median follow-up of 25 months (range 6–69), there were 1 death, 1 lung transplantation and 8 acute exacerbations without relevant differences between the GER and non-GER subgroups. HP DNA was not detected in any of the lung tissue samples. **Conclusion:** The fact that no HP DNA was detected in the lung tissues calls into question the proposed relevance of HP to the direct pathogenesis of IPF.

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I. Bittmann and A. Warth contributed equally to this work.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease and is the most common idiopathic interstitial lung disease (ILD) [1]. IPF is associated with progressive scarring of the lung interstitium, resulting in irreversible loss of lung function, and has the worst prognosis of all ILDs, with a median survival of only 2–3 years [1, 2]. Although, by definition, IPF is an idiopathic disease (i.e. its aetiology is unknown), some potential risk factors have been described. Besides smoking, environmental exposures, microbial agents and diabetes mellitus, gastro-oesophageal reflux (GER) has also been proposed, presumably through chronic microaspiration of gastric contents into the lung [1, 3, 4].

In 1976, Mays et al. [5] first hypothesized a relationship between GER and pulmonary fibrosis. Following this, further reports have discussed on the potential role of GER in the aetiology of IPF as well as in other ILDs [6–9]. A retrospective analysis by Lee et al. [10] showed that antacid therapy was an independent predictor of longer survival in this cohort of IPF patients and concluded that this finding supports the hypothesis that GER disease (GERD), as well as resultant chronic microaspiration, may play an important role in the pathobiology of IPF. More recently, the same group [11] reported outcomes on the routine use of antacid therapy and IPF disease progression using prospectively collected data from the placebo arms of the three IPFnet randomized clinical trials [12–14]. Here, they demonstrated that patients receiving medical treatment for GERD had a significantly reduced decline in pulmonary functional parameters compared with those not undergoing antacid therapy [11].

Despite these data, there is an ongoing debate regarding the true impact of GERD on IPF, as there are several limitations concerning this potential association, e.g. that the above-mentioned medical treatment strategies only have an antacid effect and do not influence reflux per se. In addition, despite the high prevalence of GERD in the general population (estimated at about 20%), only a small proportion of individuals develop IPF. This suggests that other aspects influencing reflux disease have to be considered, such as predispositions in susceptible individuals, the role of comorbidities or other factors [3, 4]. In 2007, Ibrahim [15] hypothesized that IPF might develop only in patients with reflux disease whose gastric juice contains *Helicobacter pylori* (HP). HP is a Gram-negative bacterium which produces cytotoxins that can cause mucosal damage [16]. Activation of pro-inflammatory

cytokines by HP could serve as a possible pathogenetic mechanism resulting in inflammatory changes of the interstitium, potentially influencing the development of interstitial fibrosis [17].

To understand this possible relationship, we conducted a retrospective study. Here, we investigated the hypothesis that HP-containing microaspiration is involved in the pathogenesis of IPF, using a PCR-based screening study of HP DNA in lung biopsy specimens of IPF patients from the time of the biopsy.

Materials and Methods

Patients

We retrospectively reviewed the databases of two tertiary care ILD centres (Thoraxklinik, Heidelberg, and LungClinic, Grosshansdorf, Germany) to identify patients with fibrotic lung disease who underwent lung biopsies for further workup in cases where IPF was suspected. Only patients with completely available clinicopathological data, such as gender, age, smoking status, relevant medication, time of ILD diagnosis, comorbidities, high-resolution CT scans and outcome data, were included. Patients included in this study predominantly underwent surgical lung biopsy; patients unable to undergo surgical lung biopsy due to functional limitations [18], comorbidity or patient decline and having transbronchial biopsy with sufficient material for histopathological evaluation were also eligible.

IPF was diagnosed in a multidisciplinary team meeting according to the current ATS/ERS/JRS/ALAT statements [1]. This retrospective study was performed in a pre-specified two-step analysis to exclude centre bias: after a screening for HP resulted negative in one centre (Thoraxklinik; centre 1), a second centre (LungClinic; centre 2) performed their screening with other primers as described below. Patient CT scans were also re-evaluated for the presence of hiatal hernia. The patients were followed up for acute exacerbations and outcome (death or lung transplantation).

PCR and Controls

Extraction of genomic DNA was performed by proteinase K digestion and fully automated purification using a QIASymphony SP device (Qiagen, Hilden, Germany) in centre 1, and manually using the QIAamp DNA FFPE Tissue Kit (Qiagen) in centre 2. HP-specific PCR was carried out with the following primers: HP 16S rRNA (forward) 5'-CTG GAG AGA CTA AGC CCT CC-3' and HP 16S rRNA (reverse) 5'-ATT ACT GAC GCT GAT TGT GC-3' (centre 1), or HP 23S rRNA (forward) 5'-AGA TGG GAG CTG TCT CAA CCA G-3' and HP 23S rRNA (reverse) 5'-TCC TGC GCA TGA TAT TCC C-3' (centre 2). HP-positive gastric biopsies of patients outside the reported IPF cohort served as positive controls.

Statistical Analysis

Risk factors associated with pulmonary microaspiration were defined as a history of GERD/presence of typical reflux symptoms, intake of proton pump inhibitors (PPIs) or hiatal hernia on CT; in a risk factor model for GERD, PPI intake, hiatal hernia, treatment with corticosteroids, sleep apnoea and diabetes mellitus were eval-

uated. All parameters were assessed in an explorative or descriptive manner, providing medians and ranges. All p values were two-sided if not stated otherwise. Comparisons between patient groups were analysed by t test using log-rank testing. Changes in pulmonary function test values were analysed using the Wilcoxon signed-rank test, with $p < 0.05$ considered to be statistically significant.

Statement of Ethics

The study was approved by the Ethics Committee of the Medical School of the University of Heidelberg (IRB approval No. S-318/2013). All patient records were anonymized and de-identified prior to analysis.

Results

Patient Characteristics

A total of 39 IPF patients (26 from centre 1, diagnosed between February 2008 and September 2013; 13 from centre 2, diagnosed between July 2008 and November 2013; table 1) who underwent lung biopsy were identified and included in the retrospective analysis. The cohort had a median age of 66 years; predominantly, the patients were male (85%) and current or ex-smokers (67%). Pulmonary function tests demonstrated mild-to-moderate disease, with a median vital capacity of 79% predicted and a median single-breath diffusing capacity of the lung for carbon monoxide of 53%. Relevant comorbidities with regard to this study were: GERD (23%), hiatal hernia diagnosed by CT (38%) and sleep apnoea (13%). At the time of diagnosis, 23% of the patients had received a PPI and 16% corticosteroids. In none of the patients had HP been detected before, especially not HP-positive gastritis, and none had received prior HP eradication therapy. Surgical lung biopsy was performed in 82% of the patients, and transbronchial biopsy in 18%. The median follow-up time was 25 months (range 6–69). Death occurred in 1 patient (after 69 months), and 1 patient received lung transplantation 20 months after the first diagnosis. Eight patients experienced an acute exacerbation of their underlying IPF according to the definition and criteria of Collard et al. [19].

Correlation of Pulmonary Function with Risk Factors for Pulmonary Microaspiration

As described above, risk factors associated with pulmonary microaspiration were defined as a history of GERD/presence of typical reflux symptoms, intake of PPIs or hiatal hernia on CT. There was no correlation between a history of GERD/typical reflux symptoms, intake of PPIs or the presence of hiatal hernia and pulmonary function.

Table 1. Patient characteristics (n = 39)

Age, years	66 (49–77)
Gender, %	
Male	85
Female	15
Smoking status	
Former or current smoker, %	67
Pack years	15 (1–120)
Non-smoker, %	33
Baseline lung function	
VC, l	2.8 (1.68–3.3)
VC, % predicted	79 (45–109)
FEV ₁ , l	2.4 (1.4–4.2)
FEV ₁ , % predicted	81 (48–126)
FEV ₁ /VC, %	83 (68–94)
TLC, l	4.9 (3.2–7.4)
TLC, % predicted	74 (51–109)
DLCO _{sb} , %	53 (21–81)
HRCT pattern	
UIP	44 (17)
Possible UIP	51 (20)
Unclassifiable	5 (2)
Biopsy, %	
Surgical lung biopsy	82
Transbronchial biopsy	18
Final diagnosis after MDT discussion	
IPF	100 (39)
Relevant comorbidities	
GERD	23 (9)
Previous eradication for HP	0 (0)
Hiatal hernia (CT diagnosis)	38 (15)
Combined fibrosis with emphysema	15 (6)
Diabetes mellitus	20 (8)
Sleep apnoea	13 (5)
Relevant medical treatments	
PPIs	23 (9)
Corticosteroids	16 (7)
Prior eradication for HP (combined PPI and antibiotics)	0 (0)
Follow-up time, months	25 (6–69)
Deaths, n	1
Lung transplantation rate, n	1
Acute ILD exacerbations, n	8

Values are medians (ranges) or % (n) unless specified otherwise. VC = Vital capacity; FEV₁ = forced expiratory volume in 1 s; TLC = total lung capacity; DLCO_{sb} = single-breath diffusing capacity of the lung for carbon monoxide; HRCT = high-resolution CT; UIP = usual interstitial pneumonia; MDT = multidisciplinary team.

Fig. 1. Representative example of a negative PCR amplification for HP with DNA from a PCR control amplification using DNA derived from 5 HP-positive gastric biopsies (**a**) and an IPF lung biopsy (**b**). L = No template control; A/B = reactions with case DNA.

Correlation of GERD with Clinical Characteristics

As described above, in a risk factor model for GERD PPI intake, hiatal hernia, treatment with corticosteroids, sleep apnoea and diabetes mellitus were evaluated. PPI intake ($p < 0.0001$) and hiatal hernia ($p = 0.003$) correlated with the presence of GERD, but all other factors did not. In a correlation analysis between smokers and non-smokers and the presence of GERD, ex-smokers had a lower prevalence of GERD than smokers. A correlation analysis between acute exacerbation of the underlying IPF and GERD revealed that the presence of GERD at the time of first diagnosis was not a risk factor for acute exacerbation in this cohort.

Detection of HP by PCR

HP DNAs were not detected by PCR in any of the lung biopsies, whereas the specificity and sensitivity of the approach were evident in the HP-positive gastric biopsies (fig. 1).

Discussion

This retrospective analysis is the first study of the potential relationship between the presence of HP in IPF lung biopsy specimens using PCR and outcomes. In this cohort of 39 IPF patients, PPI treatment and hiatal hernia on CT correlated strongly with the presence of GERD. This cohort therefore contained a subset of patients considered at significant risk for microaspiration [3]. However, the PCR analysis did not detect HP DNA in any of the IPF tissues.

Our findings raise a number of questions regarding the possible relationship between GER, microaspiration and

the role of HP in IPF. It might be suggested that microaspiration of GER had not occurred at the time of biopsy or that the microaspirate did not contain HP. While there is a strong association between GER and IPF as measured using 24-hour oesophageal pH monitoring with reflux extending into the proximal oesophagus (which is considered a high risk for microaspiration), direct evidence demonstrating that microaspiration leads to IPF has not been provided yet, although the presence of pepsin has been suggested [3–6, 20]. If there had been microaspiration at the time of diagnosis, it is unlikely that components of the aspirate would not have included HP based on seroprevalence data of up to 90% in males >65 years [21]. Furthermore, Bennett et al. [22] showed that the seroprevalence of HP in the general population and in IPF patients was similar at 40–51.4% (mean age 65.7–66.8 years).

The possibility that GER with HP microaspiration is a later event in IPF and associated with disease progression should also be considered, since in the study by Bennett et al. [22], HP seroprevalence was associated with more advanced disease and reduced lung function in patients with IPF, while the patients in our cohort had less advanced disease. In contrast to our results, Hogaboam [23; pers. commun./unpubl. results] reported HP in IPF lung biopsy specimens using immunohistochemistry. Interestingly, the author also showed HP-positive staining in lung specimens from severe IPF patients as compared to no/weaker HP positivity in patients with less advanced IPF. Another recent study identified HP VacA, an exotoxin of HP, in human lung tissue of patients with ILDs by immunohistochemistry [24]. Yet, while VacA had been detected in a significant subset of lung tissues of patients with connective tissue disease-ILD and some with

non-specific interstitial pneumonia, only 1 in 21 patients with IPF was positive, suggesting that HP infection of the lung is a rare event in IPF [24].

Recent data have demonstrated that IPF may inherit features of an airway-derived disease [25]. In this regard, chronic microaspiration may be involved in chronic inflammatory injuries of the airway epithelium, which is similar to the finding in airway-centred interstitial fibrosis, where in a significant subset of patients GERD induces a reticular pattern with peribronchovascular infiltrates [26]. Our findings, however, disagree with the hypothesis that pro-inflammatory cytokines from HP contribute to this inflammatory state [15, 17].

The discrepancy between HP seroprevalence as shown in the report by Bennett et al. [22] and our negative results is in line with findings in other chronic lung diseases [16, 27–29]. This leads to the hypothesis that HP microaspiration may not be directly involved in the induction or progression of fibrotic lung disease in IPF, but that gastric infection with HP initiates a systemic immune response that has an impact on the pulmonary system in susceptible patients. Similarly, the assumption that HP infection stimulates an immune response was recently discussed for COPD; there, HP infection was associated with reduced lung function, which was hypothesized to be most likely due to the effect of the bacterium on lung growth earlier in life and which was also associated with systemic inflammation [30]. This is also supported by reports on the manipulation of T-cell-mediated adaptive immunity and specific factors produced by HP which can block T-cell activation, proliferation and effector functions (reviewed in Salama et al. [31]).

Our study has several limitations. First of all, there may be other explanations of why HP DNA was not detected

in any of the IPF tissues, including possible technical reasons. In this regard, an important question raised by our results is whether HP is eliminated/dies shortly after microaspiration into the lungs, and whether this led to the negative findings of our study, even if HP has been isolated from tracheal secretions [32] and been detected by PCR in the upper and lower respiratory tract in other diseases, albeit inconsistently [29, 33]. Also, the small number of patients at risk of GER/microaspiration has to be considered, and it cannot be discounted that our cohort of patients at risk of microaspiration was not infected with HP. The major limitation of the current study, however, might be the absence of a control group with demonstration of positivity for HP in their lung specimens, even if this has recently been shown by others [23, 24, 29]. Furthermore, data on the seroprevalence of HP are missing due to the retrospective nature of our analyses. Another limitation is the step-up approach adopted to avoid centre bias in identifying HP using PCR, which may have reduced the identification of HP-positive biopsies.

In conclusion, the lack of findings of HP DNA in lung tissue in our study calls into question the proposed relevance of direct HP involvement to the pathogenesis of IPF; however, since only patients with mild-to-moderate IPF were included, a role of HP in more advanced IPF cannot be excluded and requires further investigation, as does the hypothesis of a systemic immune response initiated by HP affecting lung tissue.

Financial Disclosure and Conflicts of Interest

The trial was funded by unrestricted grants from InterMune and the German Centre for Lung Research.

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