ORIGINAL ARTICLE

Studies of hepatocyte growth factor in bronchoalveolar lavage fluid in chronic interstitial lung diseases

Grzegorz Przybylski[1], Joanna Chorostowska-Wynimko[2], Andrzej Dyczek[3], Ewelina Wędrowska[4], Marek Jankowski[4,5], Adam Szpechciński[2], Agata Giżycka[2], Joanna Golińska[4], Piotr Kopiński[3]

1 Department of Lung Diseases, Tumors and Tuberculosis, Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland
2 Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland
3 The Cardiology Outpatient Clinic, John Paul II Hospital, Kraków, Poland
4 Department of Gene Therapy, Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland
5 Department of Dermatology, Sexually Transmitted Diseases and Immunodermatology, Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

KEY WORDS

alveolar lymphocytes, hepatocyte growth factor, idiopathic pulmonary fibrosis, interstitial lung diseases, neutrophils

ABSTRACT

INTRODUCTION Previous studies have suggested that hepatocyte growth factor (HGF) inhibits lung fibrosis as an antagonist of transforming growth factor β (TGF-β).

OBJECTIVES We assessed HGF expression levels in the lower airways of patients with selected interstitial lung diseases.

PATIENTS AND METHODS HGF levels were examined by an enzyme-linked immunosorbent assay in bronchoalveolar lavage (BAL) fluid supernatants from patients with pulmonary sarcoidosis (PS, n = 52), idiopathic pulmonary fibrosis (IPF, n = 23), nonspecific interstitial pneumonia (NSIP, n = 14), extrinsic allergic alveolitis (EAA, n = 6), bronchiolitis obliterans organizing pneumonia (BOOP, n = 8), chronic eosinophilic pneumonia (EP, n = 6), and in control subjects (n = 13). Intracellular HGF expression in BAL cells was evaluated by flow cytometry.

RESULTS HGF concentrations were elevated in BAL fluid from nonsmokers with IPF (261 ±204 pg/ml, $P < 0.02$), smokers with IPF (220 ±13 pg/ml, $P < 0.001$), and smokers with PS (172 ±33 pg/ml, $P < 0.02$), as compared with controls (148 ±17 pg/ml for nonsmokers; 137 ±9 pg/ml for smokers). HGF levels were positively correlated with TGF-β concentrations in BAL fluid ($r = 0.3$; $P = 0.02$) and negatively—with vital capacity ($r = -0.2$; $P = 0.02$). BAL neutrophils, and, for the first time, BAL lymphocytes, were identified as intracellular HGF-positive cells.

CONCLUSIONS Our results do not support evidence for strong antifibrotic HGF activity. The highest HGF concentrations were observed in BAL fluid from patients with IPF, and they were also positively correlated with TGF-β levels. Thus, although the local protective mechanisms such as the HGF expression are upregulated in chronic interstitial lung diseases, they are not enough to prevent lung fibrosis.

Correspondence to:
Grzegorz Przybylski, MD, PhD, Oddział Kliniczny Chorób Płuc, Nowotworów i Gruźlicy, Collegium Medicum, Uniwersytet Mikołaja Kopernika, Kujawsko-Pomorskie Centrum Pulmonologii, ul. Seminaryjna 1, 85-326 Bydgoszcz, Poland, phone: +48-52-325-67-80, fax: +48-52-585-34-87, e-mail: gprzybylski@cm.umk.pl
Received: December 11, 2014.
Revision accepted: February 19, 2015.
Published online: February 20, 2015.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2015; 125 (4): 260-271
Copyright by Medycyna Praktyczna, Kraków 2015

INTRODUCTION Hepatocyte growth factor (HGF), also known as scatter factor, fibroblast-derived tumor cytotoxic factor, and fibroblast-derived epithelial morphogen, is a paracrine growth factor that is mostly secreted by mesenchymal cells such as fibroblasts and macrophages.[1] It is secreted as an inactive polypeptide, pro-HGF, which is then cleaved by serine proteases and converted into a biologically active heterodimer (ie, mature HGF), which consists of 2 peptide chains linked by a disulfide bond.[2,3] Its biological effect is mediated by the cell membrane receptor protooncogene, MET (HGF receptor). Consequently, HGF bioactivity is blocked by inhibitors of protein kinase C and protein tyrosine kinases.[4]

HGF synthesis is regulated by prostaglandins, cytokines, and hormones, while cellular secretion is stimulated by acute phase proteins (tumor

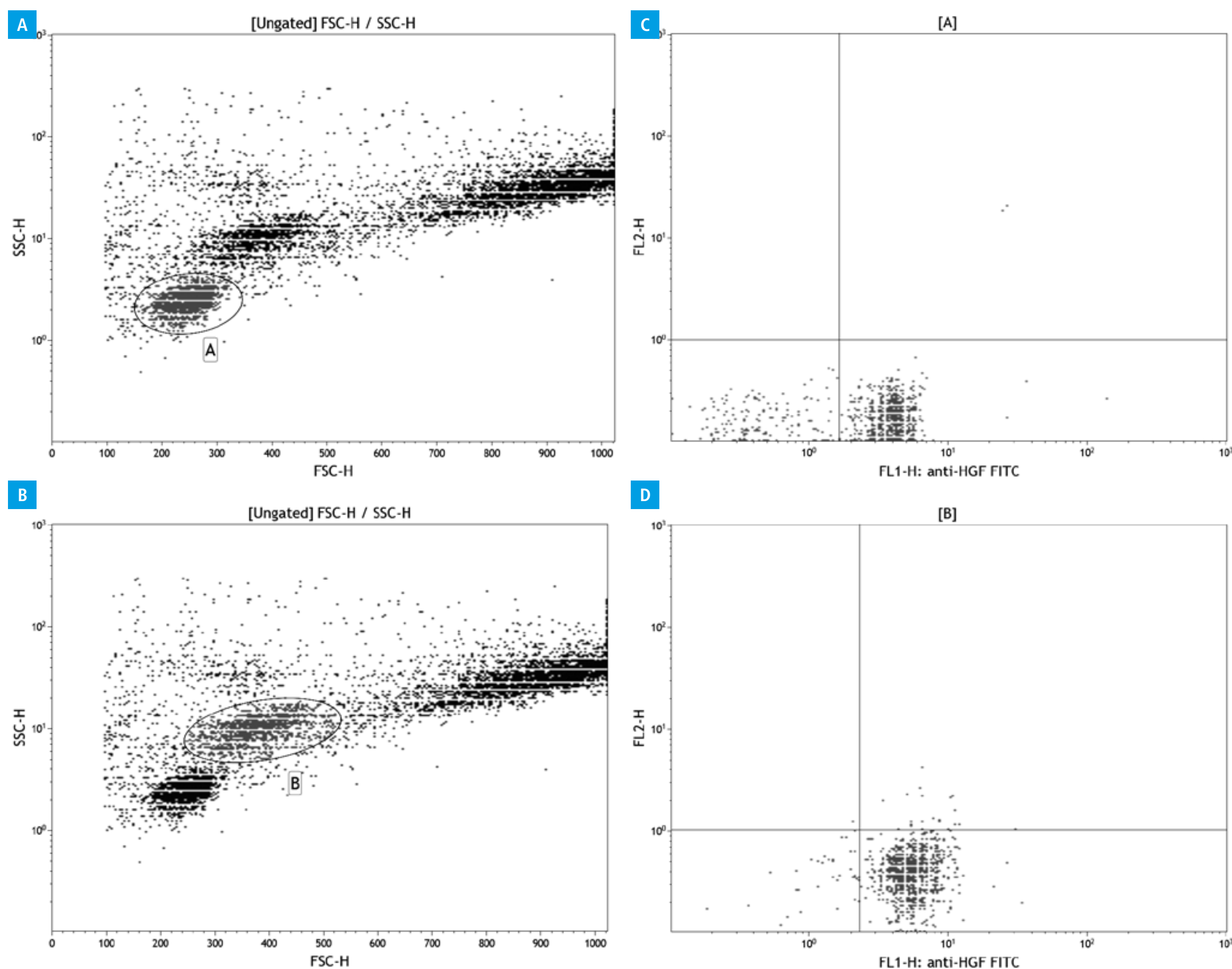

FIGURE 1 Intracellular hepatocyte growth factor (HGF) expression levels in alveolar lymphocytes (AL) and bronchoalveolar lavage (BAL) neutrophils. Flow cytometry sample of BAL cells collected from untreated nonsmoking patients with idiopathic pulmonary fibrosis (IPF); **A** – AL gating (gate A) according to fluorescein isothiocyanate (FSC) / side scatter (SSC) parameters (SSC in log scale); **B** – FL1/FL2 quadrant analysis of gate A in the sample stained intracellularly with mouse anti-HGF monoclonal antibody and secondary antimouse fluorescein isothiocyanate-conjugated serum; an average 77% of AL were positive with HGF; the markers are set according to the negative control sample (not shown); the nonspecific staining (2% of the gated AL) was attracted; **C** – BAL neutrophil gating (gate B, the same FSC/SSC parameters as in **A**); **D** – FL1/FL2 quadrant analysis of a neutrophil gate in the same staining as in **B**; an average of 98% of the BAL neutrophils were positive with HGF; the markers were set according to the negative control sample (not shown).
Abbreviations: FITC, fluorescein isothiocyanate, FL1(2)-H, fluorescence height in respective fluorescence channel

necrosis factor α, interleukin [IL] 1α, IL-1β, and IL-6, platelet-derived growth factor, and fibroblast growth factor β) as well as cyclic adenosine monophosphate.[5] Interestingly, interferon γ is active both as a stimulator of HGF secretion and an upregulator of MET expression by hepatocytes. It has been also demonstrated that, in response to a local injury, HGF activator is secreted and converted into an active form. On the other hand, 2 specific interrelated HGF activator inhibitors are present on the cell surface or in the cytoplasm.[2,6]

HGF is functional in a wide variety of tissues and cell types; it is a regenerative protein, protecting tissue repair in response to injury, especially epithelial and endothelial cells. HGF initiates angiogenesis, stimulates proliferation, differentiation, and motility of cells. It is also involved in the local regulation of fibrinolysis and coagulation.[1,7,8]

HGF is the key cytokine in pulmonary alveolar homeostasis and a potent mitogen for lower airway epithelial cells, which are mainly produced by lung fibroblasts, macrophages, and pneumocytes. It upregulates DNA synthesis in type II pneumocytes in vivo and inhibits their apoptosis.[6,9] Elevated HGF concentrations were found in serum and bronchoalveolar lavage (BAL) fluid from patients with sarcoidosis, interstitial lung disease (ILD) associated with rheumatoid arthritis, and acute lung injury. These findings were interpreted as evidence for HGF's involvement in lung repair.[10] Similar conclusions were drawn from the observation that serum HGF concentrations were increased in patients recovering from surgical lung resection.[11] In vitro, Faehling et al.[12] showed a similar inhibitory effect of HGF-rich BAL supernatants from patients with active sarcoidosis and of recombinant HGF on cultured

TABLE 1 Demographic and clinical data of patients and control subjects

Group	PS			IPF		
	NS	S	treated[a]	NS	S	treated[a]
n	24	21	7	10	8	5
M/F	10/14	13/8	2/5	6/4	6/2	4/1
age, y[b]	43.7 ±12.9 (25–75)	36.2 ±10.9 (23–64)	50.1 ±8.7 (38–66)	61.6 ±11.1 (40–75)	59.9 ±13.8 (33–75)	49 ±7.9 (44–57)[c]
VC predicted, %[b]	101.4 ±19.1 (58–133)	93.6 ±18.3 (50–114)	101.7 ±10.2 (86–110)	82.5 ±23.7 (44–93)	94.4 ±29.7 (44.1–113.9)	81 ±9.9 (44.1–113.9)
DL_{CO} predicted, %[b]	88.1 ±25.7 (55–141)	73.2 ±16.9 (57–102)	86 ±18.4 (62–113)	59.8 ±22.6 (33–63)	45.4 ±13.2 (21.8–55.7)	69.5 ±7.6 (21.8–55.7)
disease duration, mo	8 ±4.1 (3–15)	6.5 ±2.2 (4–7)	11.5 ±5.8 (8–14)	16 ±9.9 (11–26)	14 ±7.8 (11–22)	14.5 ±6.3 (10–18)
pack-years of smoking, y		18 ±9.1 (5–60)			42.5 ±15.7 (7–58)[c]	

Nonsmokers and smokers were compared with respective controls, and corticosteroid-treated patients with nonsmoking, corticosteroid-naive counterparts. Data are presented as median ± standard error of the mean (range).

a only nonsmoking patients were considered

b data are presented as mean ± standard deviation (range)

c $P < 0.05$ (Mann–Whitney test; exceptionally, t test for age was used)

Abbreviations: BOOP, bronchiolitis obliterans organizing pneumonia; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; EAA, exogenous allergic alveolitis; EP, eosinophilic pneumonia; F, female; IPF, idiopathic pulmonary fibrosis; M, male; NS, nonsmokers; NSIP, nonspecific interstitial pneumonia; PS, pulmonary sarcoidosis; S, smokers; VC, vital capacity

human fibroblasts. Consequently, the current literature provides rather scarce and mostly indirect evidence for the antifibrotic activity of HGF. On the basis of these findings, we attempted to assess the definite role of HGF as an antifibrotic factor in selected ILDs.

PATIENTS AND METHODS

Study population A total of 109 patients with ILDs including pulmonary sarcoidosis (PS, n = 52), idiopathic pulmonary fibrosis (IPF, n = 23), nonspecific interstitial pneumonia (NSIP, n = 14), extrinsic allergic alveolitis (EAA, n = 6), bronchiolitis obliterans organizing pneumonia (BOOP, n = 8), and eosinophilic pneumonia (EP, n = 6) were included into the study.

Newly-diagnosed PS was confirmed on the basis of a characteristic clinical presentation, patient-specific histology (noncaseating granulomas in biopsy), and high-resolution computer tomography (HRCT) findings.[14] PS patients were stratified according to conventional chest X-ray staging or, alternatively, according to a clinical phenotype into groups with Löfgren syndrome, chronic progressive sarcoidosis, and chronic stable sarcoidosis.[15] EAA was diagnosed on the basis of clinical data, including typical symptomatology following allergen exposure, the presence of specific antibodies in serum, typical HRCT findings, and lung function test results. In 5 patients (71%), the diagnosis was confirmed by lung biopsy.[16] In patients with IPF / usual interstitial pneumonia (IPF/UIP, n = 18, 72%) and NSIP (100%), the diagnosis was verified by a histological examination of the lung biopsy. In 6 patients with IPF/UIP, biopsy was not performed, and the diagnosis was established according to the joint criteria of the American Thoracic Society and the European Respiratory Society.[17] A diagnosis of BOOP (ie, organizing pneumonia) was made on the basis of a suggestive clinical presentation, radiological findings, and histological examination.[18]

Patients with chronic EP presented a distinctive clinical pattern including peripheral blood and BAL eosinophilia. BAL lymphocytes accounted for 25% of BAL leukocytes or more in all patients with EP, except 1 (a 37-year-old male; BAL eosinophilia, 17.6%). In all patients, the relative number of eosinophils in BAL fluid exceeded that of alveolar lymphocytes.[16]

The PS and IPF groups were stratified according to their smoking status, since the BAL immunocytological and biochemical results are strongly modified by tobacco consumption.[19] There were 21 smokers with PS (44% of 57 untreated subjects) and 9 with IPF (45% of 20). The nonsmoker subgroups of patients with PS (n = 9), IPF (n = 5), and NSIP (n = 5) who received ongoing oral corticosteroid therapy (prednisone, 15–60 mg, for at least 3 months before BAL) were analyzed separately. Otherwise, there was no history of previous systemic steroid or immune suppressive therapy. All of the corticosteroid-treated patients participating in the study were nonsmokers.

The control group consisted of 13 subjects: 7 nonsmokers and 6 smokers who were free from any lung pathology, with no symptoms of acute or chronic lung disorder, as well as normal lung function test results as well as chest X-ray and HRCT findings. They were not treated with corticosteroid or any medication known to be a potential

NSIP		EAA	BOOP	EP	Controls	
NS	treated[a]	NS	NS	NS	NS	S
9	5	6	8	6	7	6
2/7	0/5	3/3	6/2	3/3	2/5	4/2
59.2 ±7.0 (51–69)	51.0 ±9.9 (38–61)	48.1 ±12.2 (35–72)	59.6 ±5.2 (52–67)[c]	48.0 ±18.3 (22–77)	46.4 ±12.9 (26–66)	43.4 ±13.4 (25–67)
82.7 ±14.6 (65.0–104.0)	76.6 ±29.6 (48.0–120.0)	81 ±18.3 (71.0–105.0)	103 ±15.7 (78.1–122.0)	79.5 ±20.3 (50.8–102.0)		
62.3 ±20.5 (40–97)	64.2 ±11.1 (52–77)	61.1 ±17.4 (44–88)	72.9 ±9.7 (58–90)	74.9 ±25.2 (52–127)		
20.5 ±8.1 (16–26)	18.5 ±5.2 (18–24)	36.5 ±27.9 (6–432)	5.5 ±2.3 (1–11)	20.5 ±7.1 (3–15)		
						23.5 ±7.2 (10.5–48)

cause of an ILD. Three control subjects (2 nonsmokers and 1 smoker) were part of the control group in a previously published study.[13]

Bronchoalveolar lavage and cytology The BAL procedure was part of the routine diagnostic workup. Informed consent was obtained from all patients (Bioethics Committee of Nicolaus Copernicus University, approval no. KR116/2006). BAL was performed according to the European Respiratory Society guidelines, as described before.[20] In brief, premedication with midazolam and local upper airway anesthesia with lidocaine (2%) was followed by bronchofiberoscopy with Olympus BF-H190 (Olympus, Tokyo, Japan). The right lung's middle lobe (alternatively left lung lingual) was lavaged with 200 ml of sterile NaCl solution (0.9%), instilled sequentially with 4 50-ml aliquots. Then, BAL fluid fractions were retrieved by gentle suction, pooled, filtered, and immediately transported to the laboratory.

Fluid recovery was calculated as the percentage of instilled volume. The total cell count, cell viability (Trypan Blue exclusion test), and differential count of BAL inflammatory cells were calculated, as described previously.[21]

Assessment of hepatocyte growth factor and transforming growth factor β concentrations in bronchoalveolar lavage supernatants HGF concentrations in BAL supernatants was evaluated by an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine cat. nº DHG00, R&D Systems, Minneapolis, Minnesota, United States) according to the manufacturer recommendations. TGF-β concentrations in BAL supernatants was assessed with an ELISA kit (Quantikine cat. nº DB100B, R&D Systems). The optical density was measured at 450 nm using a spectrophotometric reader, ELx800 (Biotek Instruments, Inc., Winooski, Vermont, United States). The results were expressed as pg/ml.

Bronchoalveolar lavage immune cell typing and flow cytometry All of the BAL samples fulfilled the precise criteria for cytometric material acquisition and analysis.[21] Direct 3-color typing was applied. In brief, BAL samples containing 50 μl of cell suspension (2–10 × 10^6 cells/ml) were incubated with saturating amounts of mouse, fluorochrome-conjugated, monoclonal antibodies directed against human superficial CD4, CD8, and CD45 markers (cat. nº 345 768, 345 773, and 345 809, respectively, BD Biosciences, San Jose, California, United States) for 30 minutes in the dark, washed in phosphate buffered saline (PBS), and then resuspended in 300 μl of PBS with formaldehyde (1%). The internal control consisted of a sample stained with a negative isotype control (Tritest cat. nº 340 385, BD Biosciences).[21,22]

The intracellular HGF expression in alveolar lymphocytes (AL) was examined using indirect immunofluorescence staining. Cell suspensions (100 μl, 2–10 × 10^6 cells/ml) were subsequently incubated with FACS Lysing Solution (cat. nº 349 202, BD Biosciences) and FACS Permeabilizing Solution 2 (cat. nº 340 973, BD Biosciences), and washed in PBS with bovine serum albumin (BSA, 0.5%; cat. nº 554 657, BD Biosciences) and NaN_3 (0.1%). The cell pellets were then resuspended in PBS with BSA/NaN_3, incubated for 30 minutes with a saturating amount (usually 10 μl) of

mouse antihuman HGF monoclonal antibody (cat. nº MAB294, R&D Systems), and washed with PBS. For secondary incubation, polyclonal rabbit, fluorescein isothiocyanate (FITC)-conjugated, antimouse serum (cat. nº F0313, DAKO Cytomation, Glostrup, Denmark) was applied for 10 minutes in the dark. The cells were washed in PBS and resuspended in 300 μl of PBS with formaldehyde (1%). The internal control consisted of a sample that was proceeded identically, except it was stained with primary antibody, which was replaced with the respective volume of PBS.

Flow cytometric data were acquired within 24 hours after staining, using a standard 488-nm argon ion laser (FACSCalibur cytometer, BD Biosciences). Emitted light was detected through specific barrier filters for the emission range of the fluorochromes used in the study: standard fluorescence channel FL1 for FITC (CD4, HGF), FL2 for PE-phycoerythrin (CD8), and FL3 for PE-Cy5-phycoerythrin-cyanine 5 (CD45). In each sample, from 8000 to 12 000 cells were obtained. Alveolar macrophages, AL, and BAL granulocytes were gated according to cell granularity (side scatter) and the intensity of CD45 staining (**FIGURE 1**). The AL phenotype was yielded by a dot plot quadrant analysis of the respective fluorescence channels.[13,21]

Statistical analysis Patient age and lung function data were presented as mean ± standard deviation, while the concentrations of HGF and TGF-β in BAL supernatant, AL staining results, and cytology data—as a median ± standard error of the mean (owing to the nonparametric distribution of the values).[23] The Mann–Whitney test was used to compare the groups (untreated patients with ILD versus controls, independently for nonsmokers and smokers, corticosteroid-treated versus untreated). The correlations between 2 random variables were tested by the Spearman rank correlation coefficient. A P value of less than 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the study groups are presented in **TABLE 1**.

The group receiving corticosteroid therapy included only nonsmoking individuals because the subgroups of corticosteroid-treated smokers were too small. Cytoimmunological BAL data are presented in **TABLE 2**. There were significant differences in the results between nonsmokers and smokers, both in patients with ILDs and controls.

HGF concentrations in BAL supernatants were significantly increased in patients with IPF, both in nonsmokers (261 ±204 pg/ml [range, 116–2476] vs 148 ±17 pg/ml [68–217] in controls, $P < 0.02$) and smokers (220 ±13 pg/ml [174–273] vs 137 ±9 pg/ml [109–178] in controls, $P < 0.001$). The HGF concentration was also slightly increased in other ILD groups. Still, except for smoking patients with PS (172 ±33 pg/ml, $P < 0.02$, as compared with smoking controls), significant differences were not observed owing to a small number of subjects (**FIGURE 2**). There was no difference in airway HGF expression levels, if PS was stratified according to the clinical form of the disease, ie, into Löfgren syndrome, chronic progressive sarcoidosis, or chronic stable sarcoidosis (data not shown). Of note, all of the BAL samples were eligible for assessment using the highly sensitive ELISA.

Interestingly, there was a trend towards the reduction of HGF concentrations in corticosteroid-treated patients with PS, but not in those with IPF and NSIP (**FIGURE 3**). Additionally, the immunophenotypic analysis of BAL cells demonstrated that HGF was commonly expressed by AL in all of the examined ILD groups. **FIGURE 4** shows data for nonsmokers (PS, 71% ±7.7% [range, 33%–97%]; IPF, 90.5% ±5.0% [74%–96%]; and NSIP, 75.5% ±11.5% [64%–87%]) and smokers with PS (65% ±9.6% [22%–85%]), as compared with controls (35.5% ±33.5% [2%–69%] and 64% ±8.1% [44%–71%], respectively; median ± standard error of the mean for all, nonsignificant).

HGF expression levels in BAL neutrophils exceeded 95% of these cells in all subjects with ILD with relative neutrophil numbers of 5% or more (33% of the patients, 39 of 120). In subjects with sparse neutrophil pools (<5%), the distinct BAL neutrophil dot plot was not available for a reliable analysis owing to the scatter sharing with other cells, mainly macrophages. The same problem was found in controls, since this group usually revealed only some neutrophils in their BAL material (**TABLE 1**). Thus, we were not able to compare the HGF expression in BAL neutrophils of ILD patients with control subjects.

Interestingly, a strong positive correlation between HGF concentrations and a relative number of BAL neutrophils ($r = 0.32$; $P = 0.0002$) as well as eosinophils ($r = 0.31$; $P = 0.0002$) was demonstrated in ILDs. A significant positive correlation was also shown for HGF and TGF-β concentrations in BAL supernatants ($r = 0.29$; $P = 0.02$). The HGF concentration in BAL fluid was negatively correlated with predicted vital capacity in ILD patients ($r = -0.21$; $P = 0.02$).

DISCUSSION

ILDs are characterized by disturbed airway homeostasis caused by primary epitheliopathy, as observed in IPF,[24] or due to prolonged lower airway inflammation, as in other disorders.[25] Both phenomena result in tissue damage or irreversible lung fibrosis or both driven by fibroblast activation and proliferation, myofibroblast foci development, epithelial-myofibroblast transition (EMT), and increased apoptotic activity demonstrated by inflammatory and structural lung cells.[26,27] This process may lead to unfavorable outcomes, especially in IPF with fatal lung fibrosis as an inherent feature.

HGF has been considered a powerful lung protective factor that promotes epithelial and endothelial cell survival, fibroblast quiescence, and normal extracellular matrix turnover.[1] Its significant

TABLE 2 Cytoimmunological bronchoalveolar lavage data of patients and control subjects

Group	PS			IPF			NSIP		EAA	BOOP	EP	Controls	
	NS	S	treated[a]	NS	S	treated[a]	NS		NS	S	treated[a]	NS	S
BAL fluid recovery, %	50 ±3.1 (30–86)	65 ±3.9 (30–87)	55 ±7.5 (30–70)	45 ±4.9 (30–85)	66 ±9.1 (30–92.5)	45.5 ±3.1 (40–57)	60 ±3.2 (43–65)	48 ±4.4 (31–55)	46.5 ±3.4 (40–67)	55.5 ±7.7 (30–76)	40.5 ±2.7 (30–44)	48 ±4.0 (30–60)	51 ±4.1 (30–67)
BAL cell number, 10^3/ml	210 ±68.2 (71–1480)b	306 ±43.4 (105–1017)	88 ±23.5 (63–220) [b]	145 ±60.5 (58–913)	185 ±90.3 (78–576)	197 ±65.7 (75–334)	200 ±23.5 (146–262)	198 ±49.4 (34–280)	211 ±36.0 (68–362)[b]	287 ±220.2 (111–1980)[b]	229 ±133.0 (103–784)	104 ±31.0 (13–225)	248 ±107.5 (164–854)
macrophages, %	57.5 ±4.1 (12.7–85.9)[b]	76.4 ±3.2 (36.0–93.6)[c]	56.6 ±6.7 (38.4–90.0)	66.8 ±5.9 (25.4–89.8)	65.8 ±11.1 (9.0–86.6)[b]	63.9 ±4.6 (61.9–76.3)	72.8 ±6.3 (40.3–80.2)	84.7 ±6.7 (60.9–95.4)	56.4 ±11.1 (13.2–75.9)[b]	64.3 ±6.0 (46.2–96.0)	34.9 ±10.3 (23.9–79.3)[b]	76.6 ±5.5 (45.0–90.9)	91.3 ±1.4 (84.0–95.4)
lymphocytes, %	41.7 ±4.0 (12.3–85.5)[c]	21.8 ±2.9 (4.0–60.7)[d]	40.7 ±6.3 (9.8–60.2)	10.3 ±2.6 (5.0–32.5)	8.4 ±2.9 (4.0–22.0)	21.1 ±4.2 (11.2–28.0)	17.5 ±5.2 (2.5–44.1)	12.6 ±2.6 (3.5–17.3)	38.0 ±9.8 (10.5–75.4)[b]	27.2 ±6.8 (3.1–51.1)	7.7 ±2.6 (3.8–17.2)	20.0 ±3.9 (6.0–42.0)	6.4 ±1.4 (3.6–15.0)
neutrophils, %	0.8 ±0.6 (0–11.6)	1.0 ±0.5 (0.2–14.2)	1.2 ±1.4 (0.1–10.6)	6.8 ±5.8 (0.1–63.6)[b]	23.5 ±12.5 (1.9–85.0)[b]	8.2 ±1.9 (5,5–12.0)	5.9 ±6.4 (2.2–49.1)[b]	5.9 ±4.2 (1.1–21.6)	5.1 ±3.8 (1.5–26.4)[b]	1.1 ±5.8 (0.3–46.7)	0.4 ±0.4 (0.1–2.3)	1.0 ±1.4 (0–3.8)	0.8 ±0.6 (0–5.6)
eosinophils, %	0.6 ±0.2 (0.0–3.9)	0.6 ±0.3 (0–7.2) [b]	0.4 ±0.2 (0.1–1.3)	3.1 ±1.2 (1.1–14.6) [d]	2.0 ±2.7 (0.6–18.3)[c]	0.9 ±1.1 (0.6–3.7)	1.6 ±2.2 (1.1–14.9)[c]	0.3 ±0.3 (0–1.5)[b]	3.8 ±1.5 (0.1–9.4)	0.5 ±0.2 (0.3–1.6)[b]	47.1 ±9.6 (12.6–69.2)[d]	0.2 ±0.9 (0–3.0)	0.2 ±0.1 (0–1.0)
$CD4^+$, %	77 ±2.6 (38–97)[b]	65 ±4.9 (31–78)[b]	69 ±4.6 (44–87)	48 ±7.7 (18–78)	35 ±11.0 (13–45)	42 ±9.6 (30–51)	50 ±4.9 (21–79)	26 ±10.0 (11–63)	40 ±7.1 (11–68)	62.5 ±11.6 (46+79)	NA	57 ±2.8 (34–75)	38 ±2.9 (19–57)
$CD8^+$, %	13 ±1.5 (1.7–22) [c]	18 ±7.1 (6.0–43)[b]	21 ±3.3 (5.9–39)	37 ±7.1 (19–65)	52 ±16.4 (30–78)	55 ±5.6 (41–71)	35 ±4.2 (8.0–63)	58 ±9.1 (37+75)	41 ±4.7 (11–68)	31 ±9.6 (11–50)	NA	29 ±3.3 (21–57)	40 ±2.9 (30–59)
CD4/CD8	7.8 ±1.0 (1.2–23.8)[d]	4.0 ±2.5 (1.1–64.6)[b]	2.9 ±1.9 (1.3–15.2)	1.8 ±0.5 (0.3–5.5)	0.9 ±0.2 (0.9–1.8)	0.7 ±0.1 (0.5–0.9)	1.6 ±0.4 (0.6–3.3)[b]	0.6 ±0.3 (0.3–1.9)	1.1 ±0.5 (0.1–3.1)[b]	1.9 ±0.6 (0.8–5.4)	NA	2.9 ±0.3 (0.8–3.8)	0.8 ±0.1 (0.2–1.5)

Nonsmokers and smokers were compared with respective controls, and corticosteroid-treated patients with nonsmoking, corticosteroid-naive counterparts.

Data are presented as median ± standard error of the mean (range).

a only nonsmoking patients were considered, b $P < 0.05$, c $P < 0.01$, d $P < 0.001$ (Mann–Whitney test)

Abbreviations: BAL, bronchoalveolar lavage; NA, not available; others, see TABLE 1

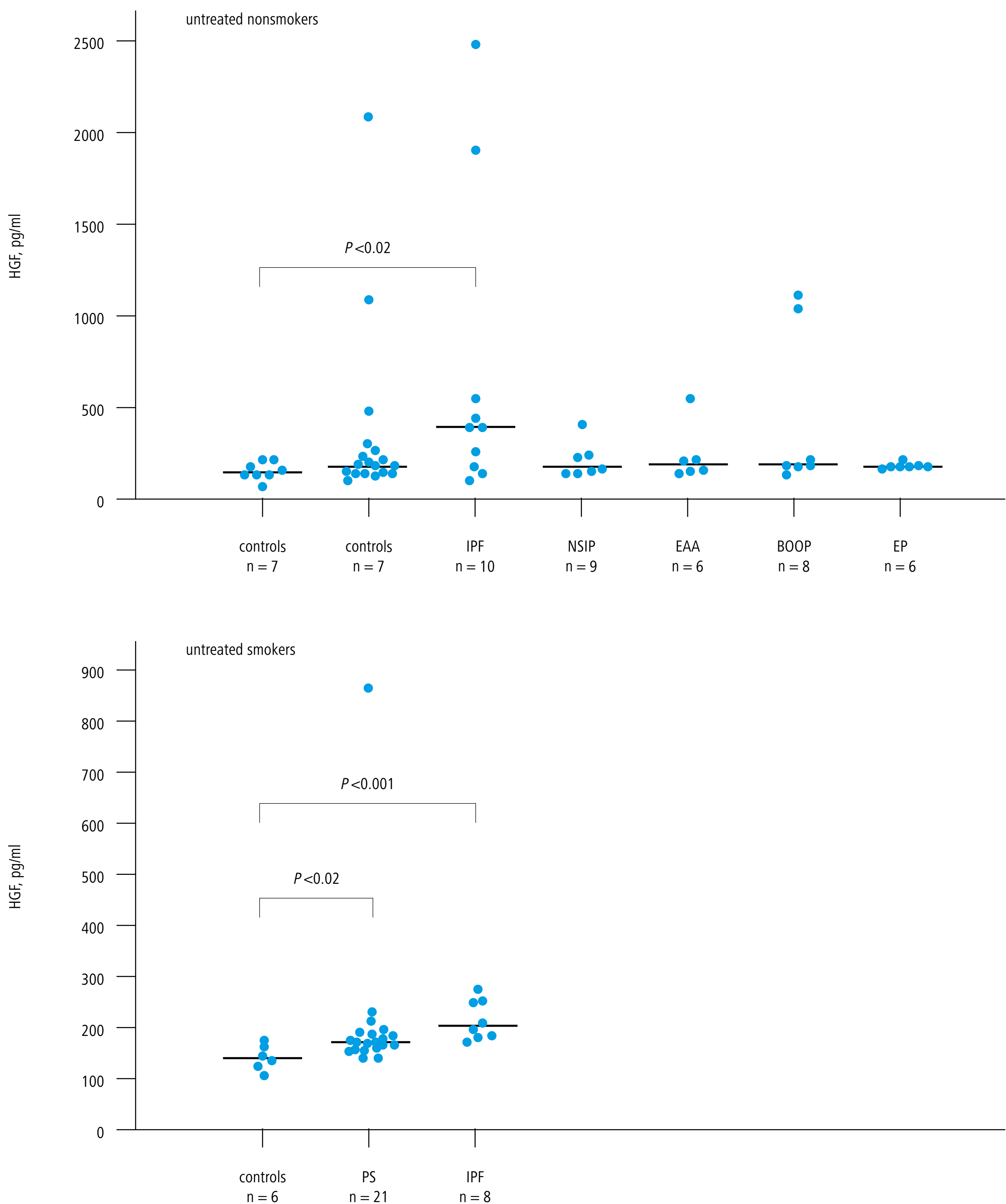

FIGURE 2 Hepatocyte growth factor (HGF) concentrations in bronchoalveolar lavage supernatants from healthy controls and steroid-naive patients; significant *P* values according to the Mann–Whitney test as compared with respective controls; the dots represent individual results, and the lines represent median values
Abbreviations: see TABLE 1

antifibrotic effects, resulting in the downregulation of fibroblast activity, the induction of myofibroblast apoptosis, and EMT inhibition, were demonstrated in vivo in animals. In the model of bleomycin-induced lung injury in mice, HGF administration reduced morphological changes, including fibrosis, presumably via TGF-β downregulation.[28,29] HGF was also shown to inhibit EMT in the murine model of cardiac fibrosis,[30] while Gazdhar et al.[28] demonstrated a significant antifibrotic effect of the *HGF* gene transfer to the lungs in rats exposed to bleomycin. Consequently, HGF has been regarded as a promising experimental therapeutic tool for lung fibrosis, mostly in gene therapy for IPF.[31]

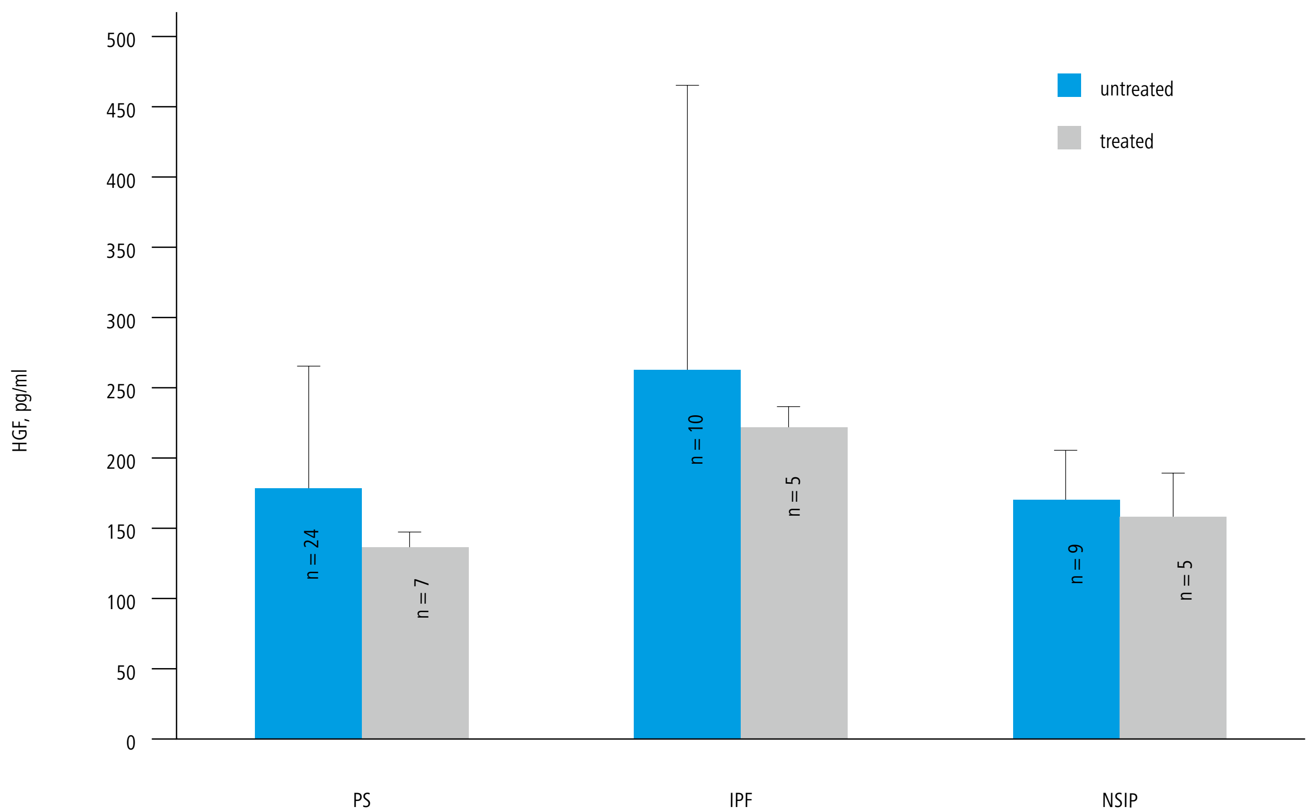

FIGURE 3 Hepatocyte growth factor (HGF) concentration in bronchoalveolar lavage supernatants from steroid-treated patients. Data presented as median ± standard error of the mean, juxtaposed with untreated counterparts. No significant changes were found in the Mann–Whitney test
Abbreviations: see **TABLE 1**

In this context, our results may seem quite surprising. While the HGF concentration was significantly higher in BAL from IPF patients than from healthy controls, it was strongly positively correlated to BAL TGF-β and negatively to vital capacity, the major lung function indicator of ventilatory restriction. Moreover, AL and BAL neutrophils were identified as important sources of HGF in the airways. Thus, we believe that increased HGF expression level in both BAL and respiratory cells might result from lung injury rather than from a protective role against lung fibrosis.

The above conclusion is supported by previously published data. Verghese et al.[32] clearly showed that excessive HGF levels in edema fluid were strongly associated with higher mortality in patients with acute lung injury. Similarly, in subjects with interstitial pneumonitis or bacterial pneumonia, increased HGF concentrations in serum and exhaled breath condensate were positively correlated with disease severity, while low HGF levels were predictive of clinical improvement.[33] In a group of 30 patients with sarcoidosis, Piotrowski et al.[34] observed a strong correlation between HGF levels and total BAL cell count, as well as with a total number of alveolar macrophages, but not with a radiological stage, lung function parameters, or disease duration.

We failed to demonstrate any significant increase in HGF concentrations in BAL fluid from subjects with ILDs that are typically characterized by full restitution such as PS, EAA, and BOOP. The low number of patients in the EAA (n = 7) and BOOP (n = 7) groups was obviously an important bias factor. However, data from the available literature are rather inconclusive as well. Faehling et al.[12] reported a distinct HGF BAL phenotype in clinically active patients, including those with Löfgren syndrome, versus patients with stable sarcoidosis. Meanwhile, in a very adequately powered study, Piotrowski et al.[34] did not observe any significant differences between healthy controls and PS subjects for HGF concentrations in BAL fluid or exhaled breath condensate and did not find any correlation between HGF expression levels and parameters of PS activity.

In our study, only the IPF group demonstrated a significantly higher HGF concentration in BAL supernatant, compared with healthy controls. In patients with other ILDs characterized by a lower risk of progressive pulmonary fibrosis or fatal outcome, HGF expression was not significantly upregulated. Meanwhile, the reduced expression of growth factors, including HGF, has for years been regarded as the hallmark of pulmonary fibrosis. As discussed in the Introduction section, this concept was based mainly on animal in vivo studies[28,29,35] or human fibroblasts cultured in vitro.[3,5,36] The clinical data from ILD patients are rather unequivocal. As early as in 1998, Hojo et al.[37] reported markedly higher HGF levels in serum and BAL fluid from patients with IPF. However, they studied a nonhomogenous group including both smokers and nonsmokers as well as corticosteroid-treated patients. Also, the methodologies that were available at that time did not allow for a reliable evaluation of HGF concentrations in as many as 43% of the samples (13 of 30). Recently, HGF expression was shown to be downregulated in IPF as compared with EAA in BAL fluid obtained from patients with ILD. An increase in HGF levels was also observed, as compared with controls, but the difference was statistically insignificant.[38]

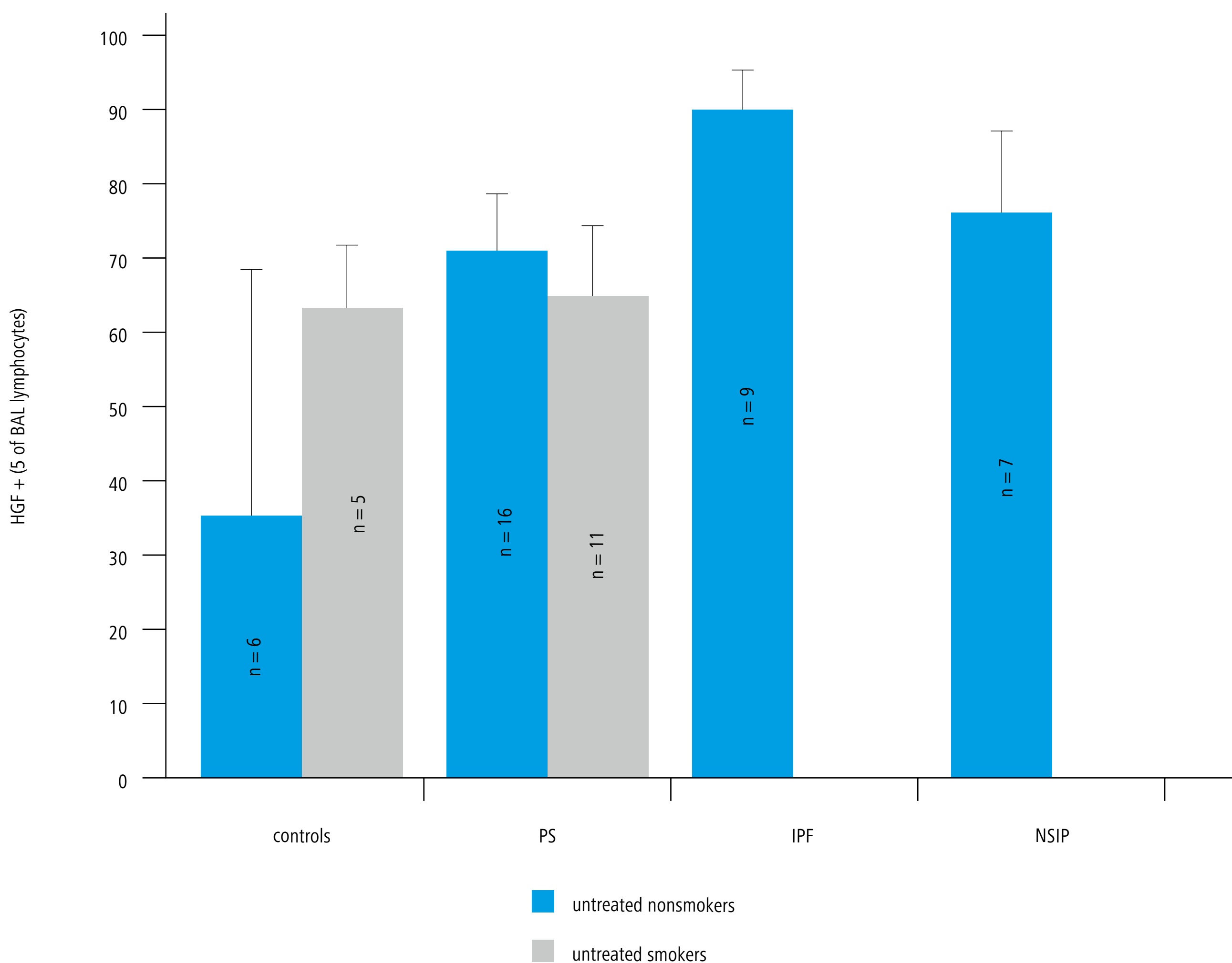

FIGURE 4 Hepatocyte growth factor (HGF) expression in alveolar lymphocytes from steroid-naive patients; data are presented as median ± standard error of the mean; no significant changes were observed in the Mann––Whitney test
Abbreviations: see **TABLE 1**

Considering the pathomechanism of the disease, the upregulated synthesis of HGF observed in IPF patients should not be surprising. It is well known that HGF acts via a MET receptor expressed, among others, by alveolar epithelial cells and pulmonary fibroblasts, and that it is downregulated during the acute phase of lung injury. Importantly, both MET mRNA and protein expression in alveolar epithelial cells are upregulated by interferon γ.[4] As we reported previously, interferon-γ airway secretion is suppressed in fibrotic lungs.[39] Therefore, the upregulated HGF production might not be paralleled by an amplified biological effect but may rather represent a rebound effect in response to the diminished MET receptor expression. Additionally, lung fibroblasts derived from IPF patients are characterized by considerably reduced pro-HGF conversions to active HGF forms. Hence, pulmonary fibroblasts might not be locally suppressed enough by HGF, despite the protein's high expression in the lower airways.[5] Similarly suggestive is the significant positive correlation between HGF and TGF-β. In our opinion, the chronic inflammatory and profibrotic cytokine network observed in lung fibrosis may affect the opposite activities of antifibrotic factors such as HGF. However, TGF-β is probably the most important agent responsible for pulmonary fibrosis.[25] In other words, the protective antifibrotic mechanisms in IPF do occur (as observed in our study), but they are insufficient.

In our opinion, the significant positive correlation between HGF levels in BAL fluid and vital capacity demonstrated in the present study indirectly but suggestively supports the hypothesis that HGF indicates fibrosis progression in ILD.

Another interesting finding reported in this study was the effect of smoking and steroid therapy on airway HGF production. In fact, it was significantly higher in IPF smokers than in healthy individuals, but no difference was observed in comparison with IPF nonsmokers. These observations are in line with the data published by Bonay et al.,[40] who did not find any differences in airway HGF expression levels between healthy nonsmokers and smokers. We observed a suppressive effect of corticosteroids in a relatively small group of IPF patients. Although not significant, it was consistent with the in vitro studies showing the remarkable downregulation of *HGF* gene expression levels in human cells including lung fibroblasts cultured with corticosteroids.[36]

The exact lung cellular source of HGF has not been definitely established. The HGF pool originating from the respiratory epithelium is commonly considered to be part of an important autocrine regulatory mechanism.[41] However, Sakai et al.[10] detected HGF mRNA in alveolar

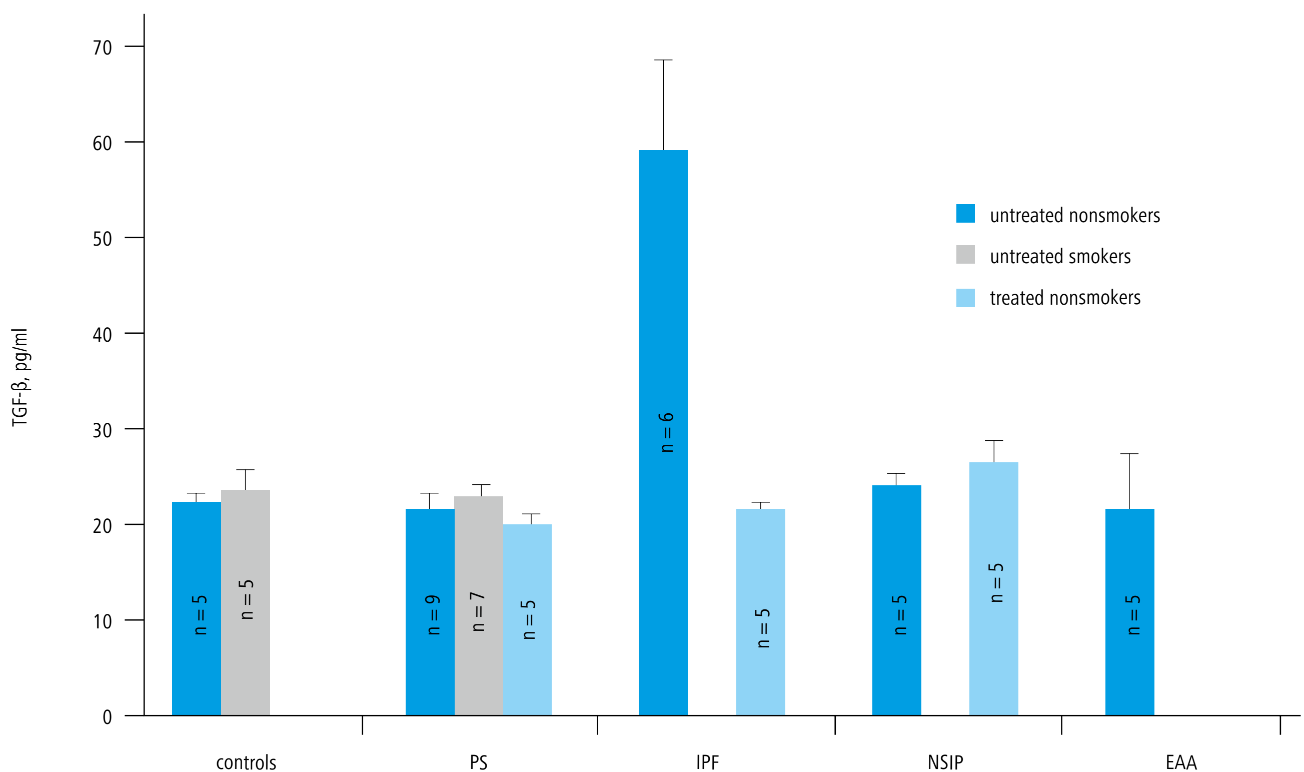

FIGURE 5 Tumor growth factor β (TGF-β) concentration in bronchoalveolar lavage (BAL) supernatants; data presented as median ± standard error of the mean; the number of examined BAL samples is shown on the bars; no significant changes were found in the Mann–Whitney test Abbreviations: see **TABLE 1**

macrophages as well, particularly in small monocyte-like cells. Here, we report consistent flow cytometry data indicating that AL and BAL neutrophils seem to be important sources of HGF in human lower airways. Since no correlation was observed between HGF + AL and HGF BAL concentrations, we believe that neutrophils should be regarded as the more essential population in this context. Our data show a significant relationship between HGF production, as assessed by its BAL level, and protein expression in neutrophils harvested by BAL (see the Results section and **FIGURE 5**). Grenier et al.[42] were the first to demonstrate that intracellular HGF is released by blood neutrophils upon activation. Later, Jaffré et al.[43] confirmed an upregulated HGF production by peripheral blood and alveolar neutrophils in acute respiratory failure, while Wislez et al.[44] documented HGF+ neutrophils in the lower airways of patients with bronchioalveolar carcinoma. Similarly, Crestani et al.[45] described HGF expression in neutrophils originating from the BAL fluid of IPF patients. In the present study, we report the intracellular expression of HGF by BAL neutrophils in patients with sarcoidosis, NSIP, and EAA. Thus, neutrophils are possibly a common HGF source of human lower airways. Considering the fact that BAL neutrophilia is an unfavorable diagnostic factor in lung fibrosis,[14,15] this finding undermines the putative positive prognostic role of HGF in ILD.

Likewise, the current study found a strong, positive link between HGF concentration in the lower airways, BAL eosinophilia, and TGF-β levels. Although our observations are consistent with the data published by Cui et al.,[35] who observed a remarkable surge of HGF and TGF-β in BAL fluid paralleled by eosinophil influx in a bleomycin-induced murine model, other authors demonstrated downregulated HGF expression in response to TGF-β instillation in an animal in vivo model.[36] We believe that both might reflect different phases of the homeostatic defensory mechanism in response to enhanced proinflammatory and profibrotic TGF-β activity. Indeed, we studied patients with chronic ILD with a relatively long disease duration at BAL examination. Meanwhile, Matsumoto et al.[36] who was the first to propose a key supportive role of HGF in the regenerative process following lung tissue damage, observed the onset of chronic injury in experimental animal models.[4,36]

In conclusion, our data do not support the antifibrotic activity of HGF implied by the negative correlation between its concentration in the lower airways and the clinical progression of lung fibrosis.[26] Quite the opposite, we demonstrated that, while HGF production is upregulated in IPF patients, it is also positively correlated with a major negative prognostic cellular biomarker, namely, BAL neutrophilia, and the key lung function parameter—vital capacity. It is possible that the positive correlation between BAL HGF and TGF-β concentrations in chronic ILDs just reflects the activation of homeostatic antifibrotic factors. However, these mechanisms might still not be effective enough to prevent lung fibrosis. Importantly, we were the first to show that, apart from macrophages and neutrophils, ALs might also be an important local source of HGF in the lungs of patients with ILDs.

Contribution statement GP collected and prepared clinical data from patients with sarcoidosis, IPF, and NSIP; performed the bronchoscopies; and helped draft the manuscript. JCW

analyzed clinical data and prepared the final manuscript. AD collected the control group data and performed statistical analysis of the ELISA results. MJ performed statistical analysis for all of the other results and drafted the manuscript. EW carried out cytological BAL examinations and ELISA assays and drafted the manuscript. AS and JG carried out flow cytometry and prepared the figures. AG performed BAL cell phenotyping and prepared the tables. PK was responsible for the study design and team coordination, analyzed flow cytometry data, and helped draft the manuscript. All authors edited and approved the final version of the manuscript.

REFERENCES

1 Panganiban R, Day RM. Hepatocyte growth factor in lung repair and pulmonary fibrosis. Acta Pharmacologica Sinica. 2011; 32: 12-20.

2 Nakamura T, Sakai K, Nakamura T, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. J Gastroen Hepatol. 2011; 26: 188-202.

3 Cohen M, Marchand-Adam S, Lecon-Malas V, et al. HGF synthesis in human lung fibroblasts is regulated by Oncostatin M. Am J Physiol Lung Cell Mol Physiol. 2006; 290: 1097-1103.

4 Nagahori T, Dohi M, Matsumoto K, et al. Interferon γ upregulates the c-Met/Hepatocyte growth factor receptor expression in alveolar epithelial cells. Am J Respir Cell Mol Biol. 1999; 21: 490-497.

5 Marchand-Adam S, Fabre A, Mailleux AA, et al. Defect of Pro-Hepatocyte Growth Factor Activation by Fibroblasts in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. 2006; 174: 58-66.

6 Ware LB, Matthay MA. Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair. Am J Physiol Lung Cell Mol Physiol. 2002; 282: 924-940.

7 Janda K, Krzanowski M, Dumnicka P, et al. Hepatocyte growth factor as a long-term predictor for total and cardiovascular mortality in patients on peritoneal dialysis. Pol Arch Med Wewn. 2013; 123: 453-459.

8 Wojta J, Nakamura T, Fabry A, et al. Hepatocyte growth factor stimulates expression of plasminogen activator inhibitor type 1 and tissue factor in HepG2 cells. Blood. 1994; 84: 151-157.

9 Yamada T, Hisanaga M, Nakajima Y, et al. Enhanced Expression of Hepatocyte Growth Factor by Pulmonary Ischemia-Reperfusion Injury in the Rat. Am J Respir Crit Care Med. 2000; 162: 707-715.

10 Sakai T, Satoh K, Matsushima K, et al. Hepatocyte growth factor in bronchoalveolar lavage fluids and cells in patients with inflammatory chest diseases of the lower respiratory tract: detection by RIA and in situ hybridization. Am J Respir Cell Mol Biol. 1997; 16: 388-397.

11 Dikmen E, Kara M, Kisa U, et al. Human hepatocyte growth factor levels in patients undergoing thoracic operations. Eur Respir J. 2006; 27: 73-76.

12 Faehling M, Hetzel M, Anders D, et al. Antifibrotic role of HGF in sarcoidosis. Lung. 2012; 190: 303-312.

13 Kopiński P, Balicka-Ślusarczyk B, Dyczek A, et al. Enhanced expression of Fas Ligand (FasL) in the lower airways of patients with fibrotic interstitial lung diseases (ILDs). Folia Histochem Cytobiol. 2011; 49: 636-645.

14 Drent M, Costabel U. Sarcoidosis. Eur Respir Mon. 2005; 32.

15 Ziegenhagen MW, Rothe ME, Schlaak M, Müller-Quernheim J. Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. Eur Respir J. 2003; 21: 407-413.

16 Meyer KC, Raghu G, Baughman RP, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. 2012; 185: 1004-1014.

17 American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med. 2002; 165: 277-304.

18 Cottin V, Cordier JF. Cryptogenic organizing pneumonia. Semin Respir Crit Care Med. 2012; 33: 462-475.

19 Osińska I, Wołosz D, Domagała-Kulawik J. Association between M1 and M2 macrophages in bronchoalveolar lavage fluid and tobacco smoking in patients with sarcoidosis. Pol Arch Med Wewn. 2014; 124: 359-364.

20 Klech H, Pohl W. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Eur Resp J. 1989; 2: 561-585.

21 Kopiński P, Szczeklik J, Lackowska B, et al. Flow cytometric characteristics of alveolar lymphocytes obtained by bronchoalveolar lavage (BAL) in the control group - proposal of normal value range of AL subsets in non-smokers. Central Eur J Immunol. 2004; 29: 63-72.

22 Szpechciński A, Kopiński P, Giedronowicz D, et al. Simple flow cytometric protocol of CD4+/CD8+ lymphocyte ratio assessment in bronchoalveolar lavage fluids from patients with interstitial lung diseases. Anal Quant Cytol Histol. 2011; 33: 289-296.

23 Merchant RK, Schwartz DA, Helmers RA, et al. Bronchoalveolar lavage cellularity. The distribution in normal volunteers. Am Rev Respir Dis. 1992; 146: 448-453.

24 Selman M, Thannicka VJ, Prado A. Idiopathic pulmonary fibrosis: pathogenesis and therapeutic approaches. Drugs. 2004; 64: 405-430.

25 Kelly M, Kolb M, Bonniaund P, Gauldie J. Re-evaluation of fibrogenic cytokines in lung fibrosis. Curr Pharm Des. 2003; 9: 39-49.

26 Uhal BD. Apoptosis in Lung Fibrosis and Repair. Chest. 2002; 122: 293-298.

27 Kopiński P, Sładek K, Szczeklik J, et al. Expression of insulin-like growth factor-I (IGF-I) in the lower airways immune cells. Evaluation of its possible role as an antiapoptotic agent. Fol Hist Cytobiol. 2006; 44: 249-258.

28 Gazdhar A, Fachinger P, van Leer C, et al. Gene transfer of hepatocyte growth factor by electroporation reduces bleomycin-induced lung fibrosis. Am J Physiol Lung Cell Mol Physiol. 2007; 292: 529-536.

29 Dohi M, Hasegawa T, Yamamto K, Marshall B. Hepatocyte growth factor attenuates collagen accumulation in a murine model of pulmonary fibrosis. Am J Respir Crit Care Med. 2000; 62: 2302-2307.

30 Okayama K, Azuma J, Dosaka N, et al. Hepatocyte growth factor reduces cardiac fibrosis by inhibiting endothelial-mesenchymal transition. Hypertension. 2012; 59: 958-965.

31 Xiong SD, Xiong WN, Xu YJ. Effect of intramuscular injection of hepatocyte growth factor plasmid DNA with electroporation on bleomycin-induced lung fibrosis in rats. Chin Med J. 2007; 120: 1432-1437.

32 Verghese GM, McCormick-Shannon K, Mason RJ, Matthay MA. Hepatocyte growth factor and keratinocyte growth factor in the pulmonary edema fluid of patients with acute lung injury. Biologic and Clinical Significance. Am J Respir Crit Care Med. 1998; 158: 386-394.

33 Maeda J, Ueki N, Hada T, Higashino K. Elevated serum hepatocyte growth factor/scatter factor levels in inflammatory lung disease. Am J Respir Crit Care Med. 1995; 152: 1587-1591.

34 Piotrowski WJ, Kurmanowska Z, Antczak A, et al. Hepatocyte growth factor in exhaled breath and BAL fluid in sarcoidosis. Pneumonol Alergol Pol. 2010; 78: 187-191.

35 Cui T, Kusunose M, Hamada A, et al. Relationship between the eosinophilia of bronchoalveolar lavage fluid (BALF) and the severity of pulmonary fibrosis induced by bleomycin in rats. Biol Pharm Bull. 2003; 26: 959-963.

36 Matsumoto K, Tajima H, Okazaki H, Nakamura T. Negative regulation of hepatocyte growth factor gene expression in human lung fibroblasts and leukemic cells by transforming growth factor-01 and glucocorticoids. J Biol Chem. 1999; 267: 24 917-24 920.

37 Hojo S, Fujita J, Yoshinouchi T, et al. Hepatocyte growth factor and neutrophil elastase in idiopathic pulmonary fibrosis. Respir Med. 1997; 91: 511-516.

38 Willems S, Verleden SE, Vanaudenaerde BM, et al. Multiplex protein profiling of bronchoalveolar lavage in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. Ann Thorac Med. 2013; 8: 38-45.

39 Kopiński P, Przybylski G, Jarzemska A, et al. [Interferon gamma (IFN-gamma) level in broncholaveolar lavage (BAL) fluid is positively correlated with CD4/CD8 ratio in selected interstitial lung diseases.] Pol Merkuriusz Lek. 2007; 23: 15-21. Polish.

40 Bonay M, Boutten A, Lecon-Malas V, et al. Hepatocyte and keratinocyte growth factors and their receptors in human lung emphysema. BMC Pulm Med. 2005; 5: 13.

41 Crestani B, Marchand-Adam S, Quesnel C, et al. Hepatocyte growth factor and lung fibrosis. Proc Am Thorac Soc. 2012; 9: 158-163.

42 Grenier A, Chollet-Martin S, Crestani B, et al. Presence of a mobilizable intracellular pool of hepatocyte growth factor in human polymorphonuclear neutrophils. Blood. 2002; 99: 2997-3004.

43 Jaffré S, Dehoux M, Paugam C, et al. Hepatocyte growth factor is produced by blood and alveolar neutrophils in acute respiratory failure. Am J Physiol Lung Cell Mol Physiol. 2002; 282: 310-315.

44 Wislez M, Rabbe N, Marchal J, et al. Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. Cancer Res. 2003; 63: 1405-1412.

45 Crestani B, Dehoux M, Hayem G, et al. Differential role of neutrophils and alveolar macrophages in hepatocyte growth factor production in pulmonary fibrosis. Lab Invest. 2002; 82: 1015-1022.

Badanie ekspresji czynnika wzrostu hepatocytów w popłuczynach oskrzelowo-pęcherzykowych w przewlekłych śródmiąższowych chorobach płuc

Grzegorz Przybylski[1], Joanna Chorostowska-Wynimko[2], Andrzej Dyczek[3], Ewelina Wędrowska[4], Marek Jankowski[4,5], Adam Szpechciński[2], Agata Giżycka[2], Joanna Golińska[4], Piotr Kopiński[3]

1 Oddział Kliniczny Chorób Płuc, Nowotworów i Gruźlicy, Uniwersytet Mikołaja Kopernika w Toruniu, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy, Bydgoszcz
2 Zakład Genetyki i Immunologii Klinicznej, Instytut Gruźlicy i Chorób Płuc, Warszawa
3 Przychodnia Kardiologiczna, Szpital Jana Pawła II, Kraków
4 Zakład Genoterapii, Uniwersytet Mikołaja Kopernika w Toruniu, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy, Bydgoszcz
5 Katedra i Klinika Dermatologii, Chorób Przenoszonych Drogą Płciową i Imunodermatologii, Uniwersytet Mikołaja Kopernika w Toruniu, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy, Bydgoszcz

SŁOWA KLUCZOWE

czynnik wzrostu hepatocytów, idiopatyczne włóknienie płuc, limfocyty pęcherzykowe, neutrofile, śródmiąższowe choroby płuc

STRESZCZENIE

WPROWADZENIE Dotychczasowe badania sugerują, że czynnik wzrostu hepatocytów (*hepatocyte growth factor* – HGF) hamuje włóknienie płuc, jako antagonista transformującego czynnika wzrostu *β* (*transforming growth factor β* – TGF-β).

CELE Oceniano ekspresję HGF w dolnych drogach oddechowych chorych z wybranymi śródmiąższowymi chorobami płuc.

PACJENCI I METODY Stężenie HGF badano za pomocą testów immunoenzymatycznych w nadsączach z popłuczyn oskrzelowo-pęcherzykowych (*bronchoalveolar lavage* – BAL) u chorych na sarkoidozę płucną (*pulmonary sarcoidosis* – PS; n = 52), idiopatycznym włóknieniem płuc (*idiopathic pulmonary fibrosis* – IPF; n = 23), nieswoistym śródmiąższowym zapaleniem płuc (*nonspecific interstitial pneumonia* – NSIP; n = 14), zewnątrzpochodnym alergicznym zapaleniem pęcherzyków płucnych (*extrinsic allergic alveolitis* – EAA; n = 6), idiopatycznym zarostowym zapaleniem oskrzelików z organizującym się zapaleniem płuc (*bronchiolitis obliterans organizing pneumonia* – BOOP; n = 8), przewlekłym eozynofilowym zapaleniem płuc (*eosinophilic pneumonia* – EP; n = 6), a także w grupie kontrolnej (n = 13). Wewnątrzkomórkową ekspresję HGF w komórkach BAL zbadano za pomocą cytometrii przepływowej.

WYNIKI Stężenie HGF było podwyższone w materiale BAL pochodzącym od niepalących chorych z IPF (261 ±204 pg/ml; p <0,02), palących chorych z IPF (220 ±13 pg/ml; p <0,001) i palących chorych z PS (172 ±33 pg/ml; p <0,02), w porównaniu z grupą kontrolną (148 ±17 pg/ml dla niepalących; 137 ±9 pg/ml dla palących). Stężenie HGF było dodatnio skorelowane z poziomem TGF-β w BAL (r = 0,3; p = 0,02), a ujemnie z czynnością płuc charakteryzowaną przez pojemność życiową (*r* = −0,2; p = 0,02). W neutrofilach oraz, po raz pierwszy, w limfocytach BAL wykazano wewnątrzkomórkową ekspresję HGF.

WNIOSKI Nasze wyniki nie dostarczają dowodów na silne przeciwzwłóknieniowe działanie HGF. Najwyższe stężenie cytokiny stwierdzono w płynie BAL chorych z IPF i było one dodatnio skorelowane ze stężeniem TGF-β. Tak więc miejscowe mechanizmy ochronne, jak ekspresja HGF, chociaż wzmożone w przewlekłych śródmiąższowych chorobach płuc, nie wystarczają do zapobieżenia włóknieniu płuc.

Adres do korespondencji:
dr med. Grzegorz Przybylski, Oddział Kliniczny Chorób Płuc, Nowotworów i Gruźlicy, Collegium Medicum, Uniwersytet Mikołaja Kopernika, Kujawsko-Pomorskie Centrum Pulmonologii, ul. Seminaryjna 1, 85-326 Bydgoszcz, tel.: 52-325-67-80, fax: 52-585-34-87, e-mail: gprzybylski@cm.umk.pl
Praca wpłynęła: 11.12.2014.
Przyjęta do druku: 19.02.2015.
Publikacja *online*: 20.02.2015.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2015; 125 (4): 260-271
Copyright by Medycyna Praktyczna, Kraków 2015