

altos se asociaron a peor SaO₂% y a un mayor grosor íntima-media. La asociación del SAHS con aterosclerosis subclínica por ecografía ha sido reportada recientemente en una amplia cohorte de pacientes menores de 68 años². También, estudios previos^{3,5} han encontrado que los pacientes con SAHS tienen niveles más altos de leptina en comparación con sujetos sin SAHS; la diferencia con nuestros resultados podría estar explicada por el pequeño tamaño de nuestra muestra, sin descartar el posible papel de la variación circadiana de los niveles de la hormona. Niveles altos de leptina también se han asociado a mayores alteraciones en los parámetros polisomnográficos⁶, y uno de los mecanismos que podría mediar este fenómeno es la hipoxia intermitente, la cual tiene efecto estimulante en la liberación de la hormona por el tejido adiposo⁷. La leptina está asociada a un aumento de la actividad simpática, elevación de la presión arterial y de la frecuencia cardíaca, y también participa en la actividad inflamatoria y en el estrés oxidativo³, procesos que en su conjunto están implicados en la patogénesis de la aterosclerosis. Estos fenómenos han de mediar una mayor presencia de signos de aterosclerosis subclínica en relación con la leptinemia en estos pacientes. Se necesitan más estudios que refuercen esta hipótesis.

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Bibliografía

1. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol*. 2013;177:1006–14.
2. Zhao YY, Javaheri S, Wang R, Guo N, Koo BB, Stein JH, et al. Associations between sleep apnea and subclinical carotid atherosclerosis: The multi-ethnic study of atherosclerosis. *Stroke*. 2019;50:3340–6.
3. Phillips BG, Kato M, Narkiewicz K, Choe I, Somers VK. Increases in leptin levels, sympathetic drive, and weight gain in obstructive sleep apnea. *Am J Physiol Heart Circ Physiol*. 2000;279:H234–7.
4. Durán-Cantolla J, González-Mangado M. Documento de Consenso Nacional sobre el Síndrome de Apneas-Hipopneas del Sueño. *Arch Bronconeumol*. 2005;41 Supl 4:7–9.
5. Ip MS, Lam KS, Ho C, Tsang KW, Lam W. Serum leptin and vascular risk factors in obstructive sleep apnea. *Chest*. 2000;118:580–6.
6. Ozturk L, Unal M, Tamer L, Celikoglu F. The association of the severity of obstructive sleep apnea with plasma leptin levels. *Arch Otolaryngol Head Neck Surg*. 2003;129:538–40.
7. Grosfeld A, Zilberfarb V, Turban S, André J, Guerre-Millo M, Issad T. Hypoxia increases leptin expression in human PAZ6 adipose cells. *Diabetologia*. 2002;45:527–30.

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Pro-fibrotic Factors as Potential Biomarkers of Anti-fibrotic Drug Therapy in Patients With Idiopathic Pulmonary Fibrosis



Factores profibróticos como potenciales biomarcadores de la terapia antifibrótica en pacientes con fibrosis pulmonar idiopática

Dear Editor:

Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive fatal fibrotic interstitial lung disease of unknown etiology characterized by progressive deposition of extracellular matrix and destruction of alveolar architecture.¹ It results in respiratory failure and patients have a poor prognosis, the median survival being about 3–5 years following diagnosis.² Lung transplantation is the only curative treatment for IPF. However, notable advances have been made in its pharmacological therapy during the last 5 years with the development of new agents designed to inhibit the processes that drive fibrosis.³ Promising results have recently been made public from phase I and II clinical trials with the novel targets of the autotaxin-lysophosphatidic acid (ATX/LPA) pathway,^{4,5} and the transforming growth factor- β (TGF- β) pathway^{6,7} that are hypothesized to be central in the development of IPF.^{8,9} Thus, the ATX inhibitor GLPG1690 was generally well tolerated and exhibited a safety profile similar to placebo, demonstrating favorable effects on mean change from baseline in forced vital capacity (FVC) at week 12 compared to placebo (25 mL vs –70 mL).⁵ Two phase III trials are currently ongoing.¹⁰ In the case of TGF- β suppression,

GSK3008348 an α v β 6 integrin inhibitor administered as an inhaled solution, was also well tolerated with no reports of serious adverse events or clinically significant abnormalities that were attributable to study treatment.⁶ Due to the increasing interest triggered by the new agents, reliable serum biomarkers would help to improve both diagnostic approach and monitoring of drug effects. Thus, serum levels of surfactant protein A (SP-A), a pulmonary collectin, have been shown to be useful in predicting prognosis or monitoring disease activity.^{11,12} Even changes in serum SP-A levels could reflect the outcomes of anti-fibrotic drug therapy with pirfenidone or nintedanib.¹³ The usefulness of serum biomarkers in patients undergoing anti-fibrotic drug therapy based on (ATX/LPA) and TGF- β pathways is not well characterized. The purpose of this study is to assess whether the pro-fibrotic compounds ATX, LPA and TGF- β , together with SP-A, could serve as potential biomarkers to monitor the therapeutic response of these novel IPF treatments.

We here report the results of a single-center study consisted of the measurement of serum levels of pro-fibrotic markers in 29 IPF confirmed patients recruited from the Interstitial Lung Diseases Unit at the Pneumology Service at University Hospital of Malaga ‘Virgen de la Victoria’ during 56 months. Thirty healthy individuals were included as comparison group and paired with sex, age, and smoking status with IPF patients. The local ethics committee approved this study. Prior to enrolment, participants were informed about the study, and provided written informed consent. A multidisciplinary group of Pneumologists, Radiologists and Pathologists established and discussed the diagnostics of IPF cases following the European Respiratory Society/American Thoracic Society/Japanese Respiratory Society/Latin American Thoracic Society criteria.¹

Table 1
Baseline Characteristics of the Patients in Terms of Demographics, Smoking Status, Pulmonary Function and Comorbidities.

Characteristics	IPF ^a (n = 29)	Healthy Individuals (n = 30)	P Value
Demographics			
Age, years	63.3 ± 2.7	58.3 ± 3.5	NS
Time since diagnosis, months	38.9 ± 6.7		
Male	19 (65.5)	18 (60)	NS
Caucasian	29 (100)	30 (100)	NS
Smoking status			
Active or former smokers	21 (72.4)	20 (66.7)	NS
Pack/year	37.6 ± 4.1	34.23 ± 6.2	NS
Pulmonary function			
FVC, % predicted	62.6 ± 15	101.8 ± 20	<.001
FEV1, % predicted	74 ± 4	99 ± 5	<.001
TLC, % predicted	65 ± 3	NE	
DLCO, % predicted	44.4 ± 15.2	NE	
Comorbidities			
Arterial hypertension	11 (37.9)	1 (3.3)	
Cardiomyopathy	7 (24.1)		
Diabetes mellitus	8 (27.6)		
Dyslipidemia	10 (34.5)	1 (3.3)	
Prior diagnosis of COPD	2 (6.9)		
PH secondary to IPF	3 (10.3)		
SAHS	3 (10.3)		

Continuous variables are expressed as the mean (± SD). Categorical data are expressed as n (%). Abbreviations: IPF, idiopathic pulmonary fibrosis; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; TLC, total lung capacity; DLCO, diffusing capacity for carbon monoxide; COPD, chronic obstructive pulmonary disease; PH, pulmonary hypertension; SAHS, sleep apnea-hypopnea syndrome; NS, non-significant; NE, not evaluated.

^a IPF diagnosis: 27 patients were diagnosed by radiologic definite usual interstitial pneumonia pattern, 2 patients by histopathologic and radiologic pattern.

Table 2
Serum Levels Markers and ROC Curves Analysis to Distinguish IPF From Healthy Participants.

Parameter ^a	IPF (n = 29)	Healthy Individuals (n = 30)	P Value	Cut-off Value	Sensitivity (%)	Specificity (%)	AUC	P Value
ATX (ng/mL)	1152.1 ± 556.0	594.3 ± 326.8	.037	869.48	72.22	71.42	0.70	<.001
LPA (nM)	380.9 ± 171.2	122.9 ± 48.2	<.001	190.25	88.88	93.33	0.96	<.001
TGF-β1 (ng/mL)	17.5 ± 4.7	10.5 ± 1.2	<.001	12.43	77.77	100	0.94	<.001
SP-A (ng/mL)	20.5 ± 0.3	14.9 ± 0.6	<.001	19.22	88.9	66.7	0.74	<.001

ATX: autotaxin; LPA: lysophosphatidic acid; TGF-β1: transforming growth factor-β1; SP-A: surfactant protein A; AUC: area under the curve.

^a The values were detectable in all samples analyzed. ELISA kits, sensitivity limits: Autotaxin K-5600 (3.21 ng/mL, Intra-Assay: CV 3%, Inter-Assay: CV 9%); LPA K-2800S (24 nM, Intra-Assay: 6.5%–8.7%, Inter-Assay: 8.9%–15.33%); SP-A NBP2-76692 (9.375 pg/mL, Intra-Assay: 5.03%, Inter-Assay: 4.83%); TGF-β1 SEA124Hu (6.3 pg/mL, Intra-Assay: CV < 10%, Inter-Assay: CV < 12%).

The serum levels markers in IPF patients were compared with the levels found in healthy volunteers. The study included a single visit of participants. After providing the informed consent, a pulmonary function test was performed and a whole blood sample was drawn from a vein (venipuncture), and collected after fasted for 8 h in tubes containing EDTA (50 mM). For plasma preparation, blood was centrifuged at 1200 × g for 10 min at 4 °C and the supernatant was stored in siliconized tubes at –80 °C until use. The IPF treatments at the time of venipuncture were recorded. Thus, 11 (37.9%) patients were treated with N-acetylcysteine as monotherapy, 3 (10.3%) with systemic corticosteroids and 1 (3.4%) with monoclonal antibodies. In 8 (27.6%), these drugs were taken in combination, together with immunosuppressive drugs (azathioprine or cyclophosphamide) in 3 (10.3%) patients. Levels of ATX, LPA, SP-A and TGF-β1 in serum were measured by sandwich-type enzyme-linked immunosorbent assays, using commercially available ELISA kits (Autotaxin K-5600, LPA K-2800S, Echelon®, SP-A NBP2-76692, Novus Biologicals; TGF-β1 SEA124Hu, Cloud-Clone Corp) according to the manufacturer's instructions. To compare quantitative measures (mean ± SD), *t*-test or Mann-Whitney *U* test were used, while chi-square test was used for qualitative (%). The receiver-operating characteristic (ROC) curves and Youden index were applied to determine the optimal cut-off of serum markers to distinguish IPF from healthy participants. A significance of 5% (*P* < .05) was required to consider a difference to be statistically

significant. Statistical analysis was performed using SPSS software (version 24.0; IBM SPSS Statistics, Chicago, IL).

These two populations showed similar demographics and smoking status (Table 1) whereas the functional parameters (% predicted) FVC and FEV1 (forced expiratory volume in 1 second) in IPF patients were statistically significantly lower than those in comparison group (Table 1, *P* < .001). However, the serum levels of the pro-fibrotic markers in IPF patients were all statistically significantly higher than those in healthy participants. The IPF patients showed a clear increase of 66.66% of TGF-β1 plasma levels (+7.05 ng/mL), two-fold more abundant the values of ATX (+557.77 ng/mL), and three-fold the LPA values (+257.91 nM) (Table 2). Similarly, a cohort study found two-fold more abundant lysophosphatidylcholine (a LPA precursor) in IPF patients compared to healthy participants, being proposed as biomarker.¹⁴ According to the ROC curve analyses, the sensitivities and specificities estimated of each biomarker are shown in Table 2. The serum biomarkers values in IPF patients were all higher than the optimal cut-off values of ATX, LPA and TGF-β1 (869.48 ng/mL, 190.25 nM and 12.43 ng/mL, respectively); whereas the serum biomarkers values in healthy participants were lower (Table 2). The areas under the ROC curves (AUC) for IPF patients in comparison with control participants were all ≥ 0.70 (*P* < .001), showing LPA and TGF-β1 better AUC profiles to distinguish IPF from healthy individuals (>0.90). Our study also demonstrated a statistically significant increase of SP-A serum

levels in IPF patients of 37.58% compared to control ($P < .001$), being higher than the optimal cut-off values found (Table 2). The SP-A AUC (0.74) was similar to ATX AUC, lower than LPA and TGF- β 1 AUC profile, which showed the largest AUC for distinguishing IPF from healthy participants (0.96 and 0.94, respectively). Limitations: first, our work is a single-center study, which limits its external validity. Second, the small sample size does not allow studying the effect of different variables (tobacco with a high percentage of smokers, age or time since diagnosis) on the levels of these biomarkers. In conclusion, changes in these serum biomarkers may serve as useful tools for monitoring the novel IPF anti-fibrotic drugs, providing novel insights for further investigations to verify the clinical relevance of these serum biomarkers, mainly LPA and TGF- β , in the context of anti-fibrotic therapies for IPF.

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Bibliografía

- Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med*. 2011;183:788–824.
- Nathan SD, Shlobin OA, Weir N, Ahmad S, Kaldjob JM, Battle E, et al. Long-term course and prognosis of idiopathic pulmonary fibrosis in the new millennium. *Chest*. 2011;140:221–9.
- Saito S, Alkhatib A, Kolls JK, Kondoh Y, Lasky JA. Pharmacotherapy and adjunctive treatment for idiopathic pulmonary fibrosis (IPF). *J Thorac Dis*. 2019;11:S1740–54.
- van der Aar E, Desrivot J, Dupont S, Heckmann B, Fieuw A, Stutvoet S, et al. Safety, pharmacokinetics, and pharmacodynamics of the autotaxin inhibitor GLPG1690 in healthy subjects: phase 1 randomized trials. *J Clin Pharmacol*. 2019;59:1366–78.
- Maheer TM, van der Aar EM, Van de Steen O, Allamassey L, Desrivot J, Dupont S, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of GLPG1690, a novel autotaxin inhibitor, to treat idiopathic pulmonary fibrosis (FLORA): a phase 2a randomised placebo-controlled trial. *Lancet Respir Med*. 2018;6:627–35.
- Maden CH, Fairman D, Chalker M, Costa MJ, Fahy WA, Garman N, et al. Safety, tolerability and pharmacokinetics of GSK3008348, a novel integrin alphavbeta6 inhibitor, in healthy participants. *Eur J Clin Pharmacol*. 2018;74:701–9.
- Maheer TM, Simpson JK, Porter JC, Wilson FJ, Chan R, Eames R, et al. A positron emission tomography imaging study to confirm target engagement in the lungs of patients with idiopathic pulmonary fibrosis following a single dose of a novel inhaled alphavbeta6 integrin inhibitor. *Respir Res*. 2020;21:75.
- Koli K, Myllarniemi M, Keski-Oja J, Kinnula VL. Transforming growth factor-beta activation in the lung: focus on fibrosis and reactive oxygen species. *Antioxid Redox Signal*. 2008;10:333–42.
- Tager AM. Autotaxin emerges as a therapeutic target for idiopathic pulmonary fibrosis: limiting fibrosis by limiting lysophosphatidic acid synthesis. *Am J Respir Cell Mol Biol*. 2012;47:563–5.
- Maheer TM, Kreuter M, Lederer DJ, Brown KK, Wuyts W, Verbruggen N, et al. Rationale, design and objectives of two phase III, randomised, placebo-controlled studies of GLPG1690, a novel autotaxin inhibitor, in idiopathic pulmonary fibrosis (ISABELA 1 and 2). *BMJ Open Respir Res*. 2019;6:e000422.
- Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med*. 2012;18:441–6.
- Chiba H, Otsuka M, Takahashi H. Significance of molecular biomarkers in idiopathic pulmonary fibrosis: a mini review. *Respir Investig*. 2018;56:384–91.
- Yoshikawa T, Otsuka M, Chiba H, Ikeda K, Mori Y, Umeda Y, et al. Surfactant protein A as a biomarker of outcomes of anti-fibrotic drug therapy in patients with idiopathic pulmonary fibrosis. *BMC Pulmon Med*. 2020;20:27.
- Rindlisbacher B, Schmid C, Geiser T, Bovet C, Funke-Chambour M. Serum metabolic profiling identified a distinct metabolic signature in patients with idiopathic pulmonary fibrosis – a potential biomarker role for LysoPC. *Respir Res*. 2018;19:7.

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