

Quantifying Plasma Levels of Transforming Growth Factor β_1 in Idiopathic Pulmonary Fibrosis

María Molina-Molina,^a Sergio Lario,^b Patricio Luburich,^c José Ramírez,^d María Teresa Carrión,^a and Antoni Xaubet^a

^aSección de Neumología, Hospital Clínic, IDIBAPS, Universidad de Barcelona, Barcelona, Spain.

^bUnidad de Trasplante Renal, Hospital Clínic, IDIBAPS, Universidad de Barcelona, Barcelona, Spain.

^cServicio de Radiodiagnóstico, Hospital Clínic, IDIBAPS, Universidad de Barcelona, Barcelona, Spain.

^dServicio de Anatomía Patológica, Hospital Clínic, IDIBAPS, Universidad de Barcelona, Barcelona, Spain.

OBJECTIVE: Transforming growth factor β_1 (TGF- β_1) is one of the key profibrotic mediators in the pathogenesis of idiopathic pulmonary fibrosis (IPF). The purpose of this study was to investigate the prognostic value of quantifying TGF- β_1 levels in patients with IPF.

PATIENTS AND METHODS: We conducted a prospective study of 29 IPF patients and 27 healthy controls. Enzyme-linked immunosorbent assays were used to quantify TGF- β_1 levels.

RESULTS: Mean (SD) TGF- β_1 levels were significantly higher in the IPF patients than in the control subjects (11.1 [7.5] ng/mL vs 4 [2.4] ng/mL; $P < .01$). Weak inverse correlations were observed between TGF- β_1 levels and both forced vital capacity and total lung capacity. Thirteen IPF patients were evaluated at 8 (1.2) months (range, 5-9 months). The mean TGF- β_1 level was 18.2 (15) ng/mL and there were no significant differences with respect to the initial measurement of 11.1 (7.5) ng/mL.

No correlation was observed between changes in respiratory function and changes in TGF- β_1 levels.

CONCLUSIONS: Although plasma levels of TGF- β_1 were high in the patients with IPF, they do not appear to be a useful prognostic marker of disease activity or therapeutic response.

Determinación en plasma del factor transformador del crecimiento β_1 en la fibrosis pulmonar idiopática

OBJETIVO: El factor transformador del crecimiento β_1 (TGF- β_1) es uno de los mediadores fibrogénicos con más relevancia en la patogenia de la fibrosis pulmonar idiopática (FPI). El objetivo del estudio ha sido investigar el valor pronóstico de la determinación en plasma del TGF- β_1 en la FPI.

PACIENTES Y MÉTODOS: Se ha realizado un estudio prospectivo en el que se incluyó a 29 pacientes con FPI y 27 controles sanos. La determinación del TGF- β_1 se realizó mediante enzimoanálisis.

RESULTADOS: La concentración de TGF- β_1 fue significativamente mayor en los pacientes con FPI que en los controles (media \pm desviación estándar: 11,1 \pm 7,5 frente a 4 \pm 2,4 ng/ml; $p < 0,01$). Se observó una débil relación inversa de la concentración del TGF- β_1 con los valores de la capacidad vital forzada y de la capacidad pulmonar total. Se evaluó a 13 pacientes con FPI a los 8 \pm 1,2 meses (rango: 5-9 meses). La concentración de TGF- β_1 fue de 18,2 \pm 15 ng/ml, sin diferencias significativas respecto a la primera determinación (11,1 \pm 7,5 ng/ml). No se observó ninguna relación entre los cambios evolutivos en la exploración funcional respiratoria y los cambios en la concentración de TGF- β_1 .

CONCLUSIONES: Aunque la concentración plasmática de TGF- β_1 está elevada en los pacientes con FPI, este parámetro no parece ser útil como marcador del pronóstico de la enfermedad ni de la respuesta terapéutica.

Key words: Idiopathic pulmonary fibrosis. Interstitial pulmonary diseases. Transforming growth factor β_1 . Serum markers.

Palabras clave: Fibrosis pulmonar idiopática. Enfermedades pulmonares intersticiales. Factor transformador del crecimiento β_1 . Marcadores séricos.

This study was funded by grants from SEPAR: Fundación Respira 2000, FIS 00/371, and Red Respira V-2003-REDC 11 D-O.

Correspondence: Dra. M. Molina-Molina.
Servicio de Neumología. Hospital Clínic.
Villarroel, 170. 08036 Barcelona, España.
E-mail: mariamolinalina@hotmail.com

Manuscript received August 24, 2005. Accepted for publication November 22, 2005.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a diffuse interstitial lung disease of unknown etiology and poor prognosis: mean survival is 3 years from the time of diagnosis. The underlying pathogenetic mechanism is usual interstitial pneumonia.¹ The current pathophysiological hypothesis is that repeated episodes of lung injury favor apoptosis of alveolar epithelial cells,

fibroblast proliferation, and myofibroblast formation, leading to excessive collagen deposition and abnormal lung architecture.^{2,4} The process is mediated by several tissue growth factors and T helper 2 cytokines.^{2,6} Transforming growth factor β_1 (TGF- β_1) is one of the key profibrotic mediators in the pathogenesis of IPF. TGF- β_1 is a 25-kD homodimeric protein that is secreted by numerous cells including macrophages, alveolar epithelial cells, and fibroblasts.⁷⁻⁹ Its role in altered epithelial differentiation and fibroblast proliferation has been widely demonstrated in experimental pulmonary fibrosis.^{7,10,11} It is chemotactic for fibroblasts, induces protein synthesis in the extracellular matrix, and inhibits collagen degradation.^{7,8}

We hypothesized that the elevated expression of TGF- β_1 protein in the lung tissue of IPF patients would give rise to elevated plasma levels of TGF- β_1 . The purpose of this study was to investigate the prognostic value of quantifying these levels in patients with IPF.

Patients and Methods

Patients

Our study group included 29 IPF patients (10 women and 19 men) with a mean (SD) age of 67 (7.1) years. Diagnosis was established in accordance with the consensus statement issued by the American Thoracic Society and the European Respiratory Society,¹² and the recommendations of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).¹³ Twenty-one patients were diagnosed on the basis of symptoms while 8 were diagnosed by surgical lung biopsy. Eight of the patients were smokers and 9 were ex-smokers.

Control Group

The control group consisted of 27 healthy volunteers (14 men and 13 women) with a mean (SD) age of 42 (10.6) years. None had a history of respiratory or other disease involving fibrosis (liver or kidney disease, systemic autoimmune disease, diabetes, myelofibrosis, or essential thrombocytopenia). Ten were smokers and 17 were nonsmokers.

Methods

The study was approved by the ethics committee at the hospital where it was conducted. Ten milliliters of venous blood was collected and centrifuged at 500 g for 30 minutes for quantification of plasma TGF- β_1 levels. The supernatant was divided into aliquots and frozen at -70°C until use. Plasma levels of TGF- β_1 were determined by a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minnesota, USA).

Statistical Analysis

Statistical analysis was performed with the software package SPSS, version 10.0 (Chicago, Illinois, US). Data were expressed as means (SD). The Student *t* test was used to compare the mean TGF- β_1 values for the control group and the study group; equal variances were assumed for the 2 groups and verified using the Levene test. Pearson's correlation coefficient was used to establish relationships between variables. Statistical significance was set at a value of *P* less than .05.

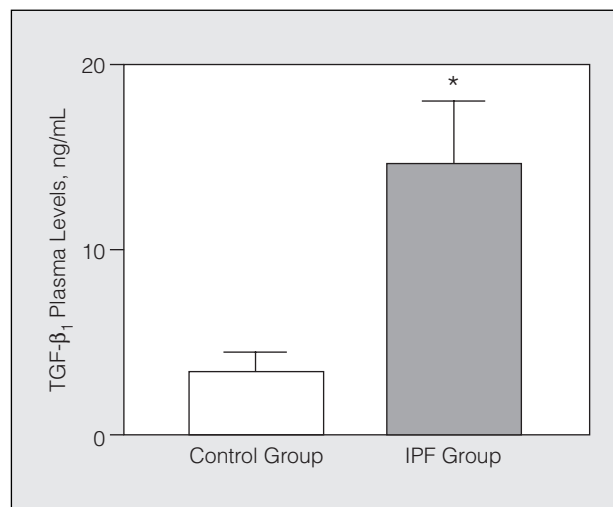


Figure. Plasma levels of transforming growth factor β_1 (TGF- β_1) in the control group and the idiopathic pulmonary fibrosis (IPF) group. Mean [SD] TGF- β_1 levels were significantly higher in the IPF patients than in the control subjects (11.1 [7.5] ng/mL vs 4 [2.4] ng/mL; *P* < .01). The Student *t* test was used to compute the differences between the mean values for paired data (*P* < .01).

Results

Table I shows the lung function test results for the IPF group. The mean duration of symptoms was 11.6 (7.1) months (range, 3-30 months) at the time of TGF- β_1 quantification. Eight of the patients were receiving treatment (glucocorticoids in isolation or combined with azathioprine).

Mean TGF- β_1 levels were significantly higher in the IPF group than in the control group (11.1 [7.5] ng/mL vs 4 [2.4] ng/mL; *P* < .01) (Figure). Weak inverse correlations were observed between plasma TGF- β_1 levels and both total lung capacity (*r* = -0.47; *P* < .01) and forced vital capacity (*r* = -0.48; *P* < .01) but not with any of the other lung function parameters. There was no significant difference in TGF- β_1 levels between smokers and nonsmokers or between patients receiving treatment and patients not receiving treatment. Age was not correlated with TGF- β_1 levels in either the control or study group.

Thirteen IPF patients were followed at a mean of 8 (1.2) months (range, 5-9 months) after TGF- β_1

TABLE 1
Lung Function Measurements for Patients With Idiopathic Pulmonary Fibrosis at Time of Initial Quantification of Transforming Growth Factor β_1 *

	Absolute Value	Reference Values, %
FVC, L	2.6 (0.8)	73.2 (18)
TLC, L	43.3 (12.5)	7.3 (16)
DLCO, mL/min/mm Hg	11.4 (3.1)	49.3 (13)
PAO ₂ -PaO ₂ , mm Hg	37.2 (12)	

*Data are expressed as means (SD). PAO₂-PaO₂ indicates alveolar-arterial oxygen gradient; DLCO, carbon monoxide diffusing capacity; FVC, forced vital capacity; and TLC, total lung capacity.

TABLE 2
Lung Function Changes During Follow-Up of Patients With Idiopathic Pulmonary Fibrosis*

Variable	Value
Number of patients	13
Follow-up time, months	8 (1.2)
FVC, %†	-0.4 (1.1)
DLCO, %†	-1.28 (1.4)

*Data are expressed as means (SD). DLCO indicates carbon monoxide diffusing capacity; FVC, forced vital capacity; and TLC, total lung capacity.
†Values are expressed as a percentage of the change in the absolute value with respect to the initial value, divided by the number of follow-up months.

quantification. All the patients underwent lung function testing and had plasma TGF- β_1 levels measured for a second time. Overall, lung function had deteriorated. We decided to assess lung function changes in relation to follow-up time in order to determine with greater accuracy the changes that had occurred during this period. To do this, we divided lung function changes by follow-up time (Table 2). No significant differences were found between the initial and follow-up mean plasma TGF- β_1 levels for the group of 13 patients (10.9 [7.9] ng/mL vs 18.2 [15] ng/mL). Neither was any correlation observed between TGF- β_1 levels and lung function, or between changes in TGF- β_1 levels and lung function changes during the follow-up period.

Discussion

Our study demonstrates that plasma TGF- β_1 levels are elevated in IPF patients. Our findings also show, however, that TGF- β_1 levels are not useful for evaluating disease activity or progress as no correlation was found between changes in TGF- β_1 levels and changes in lung function parameters.

Disease activity and therapeutic response in IPF patients are currently assessed by high-resolution computed tomography and lung function tests.^{12,13} A number of studies have been conducted in recent years to evaluate the usefulness of serum markers for optimizing the diagnosis and management of IPF¹⁴⁻²² and for evaluating therapeutic response.²³ Our study sought to investigate whether the quantification of TGF- β_1 levels in IPF patients was of prognostic value. TGF- β_1 is one of the key profibrotic mediators in the pathogenesis of fibrosing pulmonary diseases.^{7,8,10,11} Salez and colleagues,²⁴ for example, found elevated levels of TGF- β_1 in the bronchoalveolar lavage fluid of IPF patients. TGF- β_1 gene polymorphisms have also been linked to the progress of IPF and the development of pulmonary fibrosis in patients who have undergone lung transplantation to treat such diseases as cystic fibrosis, IPF, bronchiectasis, and emphysema.²⁵⁻²⁸ Anscher and colleagues²⁹ found a significant correlation between plasma TGF- β_1 levels and the development of idiopathic interstitial pneumonitis in autologous bone marrow transplant patients. Only a single study, however, has investigated the usefulness of plasma TGF- β_1 levels as a prognostic marker of IPF.¹⁴ In that study, Yong and colleagues measured plasma TGF- β_1

levels in 38 IPF patients and 6 healthy controls. Their results, like ours, showed significantly higher plasma TGF- β_1 levels in the IPF group although these fell significantly in 6 patients treated with glucocorticoids and colchicine for 3 months. In our study, however, glucocorticoid and immunosuppressant treatment had no effect on plasma levels of TGF- β_1 . Yong and colleagues did not analyze the correlation between plasma TGF- β_1 levels and lung function changes, but we did find a weak correlation between both forced vital capacity and total lung capacity and initial plasma TGF- β_1 levels. No correlation between lung function changes and changes in TGF- β_1 levels during the follow-up period were found, however. The discrepancies between the 2 studies and the variability in terms of the relationship between lung function changes and TGF- β_1 levels are probably partly due to the small number of patients evaluated at follow-up. The variability might also be due to the interaction of other mediators involved in IPF with TGF- β_1 ,^{2-5,14} which suggests that serum levels of a single profibrotic mediator are unlikely to be of use for prognostic and disease activity evaluation.

Other serum markers that have been analyzed in patients with IPF include surfactant proteins A and D, the lung epithelial marker KL-6, lactate dehydrogenase, interleukin 8, the adhesion molecule E-selectin, the soluble apoptosis marker Fas, and the monocyte chemoattractant protein-1.¹⁵⁻²² The studies that have analyzed these markers have shown that they are all elevated in IPF patients. Although surfactant proteins A and D have been seen to be linked to patient survival, and KL-6 is used to distinguish between diffuse interstitial diseases and other lung diseases, there is no evidence that the quantification of their levels in serum provides efficient prognostic or activity markers for IPF.^{15-17,20-22}

One possible limitation of our study is the age difference between the controls and the IPF patients. While it is not known whether age influences plasma levels of TGF- β_1 , we observed no relationship between age and TGF- β_1 levels.

In conclusion, our findings indicate that, if used in conjunction with other analytical data, plasma TGF- β_1 levels could be useful for diagnosing IPF but not for predicting disease activity or therapeutic response. More studies involving a greater number of patients and a longer follow-up period, however, are required to corroborate our findings.

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