RESEARCH

Gamma Interferon and prednisone decreasing-dose therapy in patients with Idiopathic Pulmonary Fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a fatal disease to which no treatment has demonstrated to be a modifier of the disease pattern. The aim of this study was to obtain evidences of the efficacy and safety of a 6 month-course gamma interferon (IFNγ) treatment and prednisone decreasing-dose in patients with IPF. A pilot, open label, non-controlled clinical trial was carried out. Twelve patients with histophatologically-confirmed IPF were treated with 1 000 000 IU of recombinant human intramuscularl IFNγ, three times per week. Oral daily prednisone was concomitantly administered, once a day for a year, whose dose was gradually reduced from 60 to 20 mg. Clinical, functional, and imagenological evaluations were done before and at 3, 6 and 12 months after treatment. Most of the patients had clinical improvement; dyspnea, dry cough and crepitations were notably reduced. Forced vital capacity increased by over 12% in three patients while only one reduced such magnitude. Alterations in arterial gases were less frequent in the last evaluations. Fibrotic lesions were reduced in around half of the treated patients. Two of them died, but just one due to the disease. Considering withdrawals as failures, a 75% of patients was considered as responders (improvement + stable) at the end of IFNγ treatment (month 6), while 58.3% of response was obtained after follow-up (month 12). Treatment with IFNγ was well tolerated, since mild to moderate flu-like adverse reactions predominated. Two severe, unexpected events (deaths) occurred but no related to treatment. These results suggest that in IPF a rapid clinical response could be obtained with a therapeutic schedule with the well-tolerated IFNγ combined with prednisone decreasing-dose. Further, extensive controlled studies are encouraged.

Keywords: Idiopathic Pulmonary Fibrosis, Gamma interferon, prednisone, dyspnea

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RESUMEN

Terapia con interferón gamma y dosis decreciente de prednisona en pacientes con fibrosis pulmonar idiopática. La Fibrosis Pulmonar Idiopática (FPI) es una enfermedad letal para la cual no existen tratamientos que modifique el curso de la enfermedad. El objetivo del presente estudio fue obtener evidencias de la eficacia y seguridad de un ciclo de 6 meses de tratamiento con interferón (IFNγ) y dosis decrecientes de prednisona en pacientes con FPI. Se llevó a cabo un ensayo clínico piloto, abierto, no controlado. Doce pacientes con diagnóstico histopatológico confirmado de FPI se trataron con 1 000 000 UI de IFNγ humano recombinante por vía intramuscular, 3 veces por semana. Concomitantemente se administró prednisona oral, diaria durante un año, reduciendo gradualmente la dosis de 60 a 20 mg. Se realizaron evaluaciones clínicas, funcionales e imagenológicas antes del tratamiento y en los meses 3, 6 y 12. La mayoría de los pacientes presentó mejoría clínica; la disnea, la tos seca y las crepitaciones se redujeron notablemente. La Capacidad Vital Forzada aumentó más de 12% en 3 pacientes, mientras que uno solo redujo esta magnitud. Las alteraciones hemogasométricas fueron menos frecuentes en las últimas evaluaciones. Las lesiones fibróticas se redujeron en aproximadamente la mitad de los pacientes. Fallecieron 2 de ellos, pero solo uno a causa de la enfermedad. Al final del tratamiento con IFNγ (mes 6), el 75% de los pacientes fueron considerados respondedores (mejoría + estabilización), por intención de tratar, respuesta que se redujo a un 58.3%.posterior al seguimiento (mes 12). El tratamiento con IFNγ fue bien tolerado, ocurriendo reacciones típicas pseudogripales leves a moderadas. Los dos eventos serios inesperados (muertes) presentados no se asociaron al tratamiento. Estos resultados sugieren que en esta enfermedad puede obtenerse una respuesta clínica rápida al utilizar un tratamiento con IFNγ y dosis decrecientes de prednisona. Ello alienta la realización de estudios controlados extensos.

Palabras clave: Fibrosis Pulmonar Idiopática, Interferón gamma, prednisona, disnea

*I*ntroduction

Idiopathic pulmonary fibrosis (IPF) is a progressive lung inflammation with a fibrotic response and represent the most common and lethal idiopathic interstitial pneumonia [1]. Half of the patients die 4-5 years after the diagnosis and only 20% survive 10 yrs [2]. The recommended combined treatment with corticosteroids and immunosuppressive drugs neither improve the course of the disease, nor stop fibrosis progression. Clinical trials are non-controlled and demonstrate low survival, limited objective response and high toxicity [1, 3].

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Enough evidences demonstrate the potential role of gamma interferon (IFNγ) to control the disease, since its immumodulating and antifibrotic properties. A deficit in IFNγ production compared to Th2 cytokines has been observed in these patients [4]. Gamma IFN inhibits in a dose-dependent manner fibroblasts proliferation in the lung reduces collagen synthesis and inhibits the potent fibrogenic agent transforming growth factor (TGF), among other antifibrotic actions [5-7].

The first report about the use of IFNγ in IPF demonstrated a considerable clinical improvement in these patients treated for a year compared to those receiving placebo [8]. Afterwards, a phase III study was carried out, but no significant advantages in progression-free survival, pulmonary functionality or quality of life was observed. Nevertheless, patients with an initial less deteriorated pulmonary function impairment showed better survival [9]. Other authors indicate that IFNγ can slow or arrest the loss of lung function, increases longevity and makes possible lung transplantation [10]. Long-term treatment with this cytokine may improve survival and outcome in patients with mild-to-moderate IPF [11]. However, the members of the recent INSPIRE trial declared that they cannot recommend a one-year treatment with gamma-1b interferon since the drug did not improve survival in this disease [12].

The present clinical study was done to obtain evidences of the efficacy and safety of a 6 month-course with Cuban interferon gamma (IFNγ) treatment and prednisone decreasing-dose in patients with idiopathic pulmonary fibrosis, according to clinical, functional and imagenological evaluations.

Materials and methods

A pilot, open label, non-controlled clinical trial was carried out at the "Benéfico Jurídico" Hospital, Havana, which is the national reference unit for respiratory diseases as pulmonary fibrosis, and where most of the patients with unfavorable evolution are remitted. Another participant institution was the "Hermanos Ameijeiras" Hospital, Havana. The clinical protocol was approved by the Ethics Committee of the corresponding sites and by the Cuban Regulatory Authority. The study complied with the Declaration of Helsinki.

Patients

Study population was constituted by Cuban patients, 18-70 years old with histologically-verified IPF, with or without previous treatment with corticosteroids, who gave their written informed consent to participate. The main histopathological features were a prevalence residual fibrosis and subpleural and periacinary fibrotic lesions, with only minor cellular infiltration in the lung biopsy samples. Findings on chest high-resolution computerized tomography (HRCT) were considered typical for IPF if they showed absence of bilateral patchy infiltrates and peripheral and basilar predominance of lesions. Dyspnea and dry cough were considered as the main symptoms and crepitations the main sign. Patients with a history of exposure to organic or inorganic agents or drugs known to cause pulmonary fibrosis, and those with connective-tissue diseases or other chronic lung diseases causing pulmonary fibrosis were excluded. Patients with end-stage IPF, as identified on the basis of a forced vital capacity (FVC) of less than 40% of the predicted normal value were also excluded. Other criteria for exclusion were to have other chronic disease, be pregnant or nursing, a Karnofsky's index < 50%, severe psychiatric dysfunction, multiple sclerosis or any other autoimmune disorder, well-known hypersensitivity to interferon, diabetes, and moderate to severe hypertension. Immunosuppressive agents had to be suspended at least 3 months before entry. Patients were withdrawn from the trial if they voluntarily abandoned, had serious adverse reactions, required invasive treatment or if any exclusion criteria arose.

Study design and treatment

All patients received 1 x 10^6 IU of human recombinant IFNγ (produced in *E. coli*, specific activity: 1 x 10^7 IU/mg of proteins; Heberon Gamma R®, Heber Biotec, Havana), intramuscularly, 3 times per week during 6 months. All of them also received a schedule of decreasing doses of oral prednisone (daily): 60 mg the first and second months, 50 mg the third, 40 mg the fourth, 30 mg the fifth, 20 mg the sixth, 10 mg until the ninth month and 5 mg up to complete 12 months.

Subjects were hospitalized during the first 15 days of treatment to check the initial evolution and for a better assessment of immediate adverse events. Antipyretic medication was given orally at the same time as the first IFN injections, in order to mitigate the expected IFN-dependent flu-like syndrome. Afterwards, patients were followed up to 18 months as out-patients.

Evaluation

To define response to treatment, evaluations were carried out at entry (month 0), during treatment (month 3), after treatment (month 6) and after follow-up (month 12). Nevertheless, clinical and safety data were recorded monthly during the IFNγ treatment. Efficacy evaluation integrated clinical, pulmonary function tests, arterial gasometry and radiological outcome. Improvement was defined as a decrease of the symptoms and signs, increase in FVC more than 12%, PO_2 between 50 and 60 mm Hg and reduction or stabilization of the fibrotic area. Stable disease included maintenance of the symptoms and signs, variations in FVC less than 12%, PO_2 values similar to initials and stable fibrotic lesions. Worsening of the symptoms and signs, decrease in FVC more than 12%, PO_2 between 35 and 50 mm Hg or progression of the fibrotic lesions was considered as no response.

The reduction or disappearance of the respiratory symptoms was the first criterion to consider clinical improvement. Dyspnea perception was quantified by using a simple scale, based on exertion level, modified from that of the British Medical Research Council (BMRC) [13]. Subjects were asked if and when they got short of breath. Scores were assigned from 0 to 4, with the higher values indicating increasing dyspnea. Score 0: no dyspnea at all; 1: dyspnea while climbing hills or stairs; 2: dyspnea while walking at a rapid pace on ground level; 3: dyspnea while walking at own pace on ground level; 4: dyspnea at rest. A complete examination of the respiratory tract was performed.

1. King TE Jr, Schwarz MI. Section M - Infiltrative and Interstitial Lung Diseases, Chapter 53 - Approach to Diagnosis and Management of the Idiopathic Interstitial Pneumonias. In Mason: Murray & Nadel's Textbook of Respiratory Medicine. 4th ed. Copyright© 2005 Saunders, An Imprint of Elsevier.

2. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. Am J Respir Crit Care Med 2000; 161:646-64.

3. Selman M, Navarro C, Gaxiola M. Fibrosis pulmonar idiopática: en busca de un tratamiento eficaz. Arch Bronconeumol 2005;41:15-20.

4. Zhang K, Phan SH. Cytokines and pulmonary fibrosis. Biol Signals 1996;5: 232-9.

5. Sempowski GD, Derdak S, Phipps RP. Interleukin 4 and interferon gamma discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. J Cell Physiol 1996;167:290-6.

6. Tredget EE, Wang R, Shen Q, Scott PG, Ghahary A. Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma *in vitro* and *in vivo*. J Interferon Cytokine Res 2000; 20:143-51.

7. Williams TJ, Wilson JW. Challenges in pulmonary fibrosis: 7- Novel therapies and lung transplantation. Thorax 2008; 63:277-84.

8. Ziesche R, Hofbauer E, Wittmann K, Petkov V, Block LH. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. N Engl J Med 1999;341:1264-9.

9. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, *et al*. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med 2004; 350:125-33.

10. Nathan SD, Barnett SD, Moran B, Helman DL, Nicholson K, Ahmad S, *et al*. Interferon gamma-1b as therapy for idiopathic pulmonary fibrosis. An intrapatient analysis. Respiration 2004;71:77-82.

11. Antoniou KM, Nicholson AG, Dimadi M, Malagari K, Latsi P, Rapti A, *et al*. Long-term clinical effects of interferon gamma-1b and colchicine in idiopathic pulmonary fibrosis. Eur Respir J 2006;28:496-504.

12. King TE Jr, Albera C, Bradford WZ, Costabel U, Hormel P, Lancaster L, *et al*. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. Lancet 2009;374:222-8.

13. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. Chest 1988; 93:580-6.

For respiratory function testing, values from the American Thoracic Society were applied [2]. Valuation of the severity was done according to FVC (% of the predicted value). Normal: 80-100%; Mild: 70-79%; Moderate: 60-69%; Moderately severe: 50-59%; Severe: 40-35%; Very severe: <35%. Gasometrical alterations were detected through the BMS 3 HK2 Blood Microsystem. Thorax tomographies and radiographies were obtained using validated equipment.

Safety and tolerability were monitored by means of a rigorous adverse events control. Additionally, blood samples were taken for routine hematological and biochemical determinations.

Statistics

Data were double entered and validated on Microsoft Access and then imported into SPSS version 13.0 for further analysis. Continuous variables were expressed as mean ± standard deviation (SD) or median ± interquartile range (QR) and minimum and maximum values. With these variables an analysis of normality (Shapiro Wilk's test) and homogeneity of variance (Levene's test) were carried out. Categorical variables were given as absolute values and percentages. Paired analysis *versus* before treatment was performed by Paired t test or Wilcoxon's test (according to normality) for continuous variables. The Fisher's exact test was applied to associate response to treatment with baseline characteristics. The level of significance chosen was 0.05. All the analyses of the overall response and clinical outcome were carried out under an "intention-to-treat" basis, where missing data were considered as failures.

Results

Twelve patients were enrolled from February 2001 to December 2003. That was the entire IPF population that could be included by the participant institutions at this period. Patients were follow-up continued up to September 2007. Most of them (91.7 %) were included at the "Benéfico Jurídico" Hospital. Half of patients were men, whereas white skin color predominated (58.3%). median age was 56 years and the mean body mass index (BMI) was 31.4. The onset of IPF symptoms was approximately 28 months, and a Karnosfky's index of 60% prevailed. Two patients (16.7%) were current smokers and 7 (58.3%) were former smokers. Patients with a history of cardiac antecedents were common, mainly arterial hypertension (41.7%). Twenty five percent of patients had antecedents of occupational and environmental exposure to inorganic agents, one of them with exposure to acid, irritants and lead. Most of the patients (58.3%) were taking prednisone at entry. Diagnosis was confirmed throughout bronchoscopy with transbronchial biopsy in 10 patients, by video-assisted surgical lung biopsy in one patient and by clinical, functional tests and HRCT in the other, a 66 year-old patient (Table 1).

All of the included patients received recombinant IFNγ. Only two patients did not fulfill the period of treatment with this cytokine, one of them because died of Mesotelioma after the first month, and the other voluntarily abandoned at the same time. Another patient died of IPF progression during the follow-up, exactly 3 months after IFNγ treatment (Figure 1).

Table 1. Characteristics of the population study at entry

Characteristic		Patients (N = 12)
	Male gender	6 (50%)
	White	7 (58.3%)
	Age (yrs), median ± QR	56 ± 7 (39-66)
	BMI (kg/m²), mean ± SD	31.4 + 9.9(13.5-49.8)
	Onset of symptoms (mo), median + QR	28 + 18 (9-72)
	50-60	7 (58.3%)
Karnofsky's index	70-80	4 (33.3%)
	Missing	1 (8.3%)
	Current smokers	2 (16.7%)
Smoking status	Ex-smokers	7 (58.3%)
	Non-smokers	3 (25%)
Pathological antecedents	Arterial hypertension	5 (41.7%)
	Ischemic cardiopathy	1 (8.3%)
	Arrhythmia	1 (8.3%)
	Diabetes mellitus	1 (8.3%)
	Psoriactic dermatitis	1 (8.3%)
	Bronchitis	1 (8.3%)
	COPD	1 (8.3%)
	Thyroidectomy	1 (8.3%)
	Hepatitis C	1 (8.3%)
	Duodenal ulcer	1 (8.3%)
Exposure to inorganic agents	Acids	1 (8.3%)
	Irritants	1 (8.3%)
	Lead	1 (8.3%)
	Feed	1 (8.3%)
Current treatment	Prednisone	7 (58.3%)
	Imuran	2 (16.7%)
	Theophylline	1 (8.3%)
	Levothyroxine	1 (8.3%)
	Glibenclamide	1 (8.3%)
	Captopril	1 (8.3%)
	Furosemide	1 (8.3%)

BMI: Body mass index; COPD: Chronic Obstructive Pulmonary Disease.

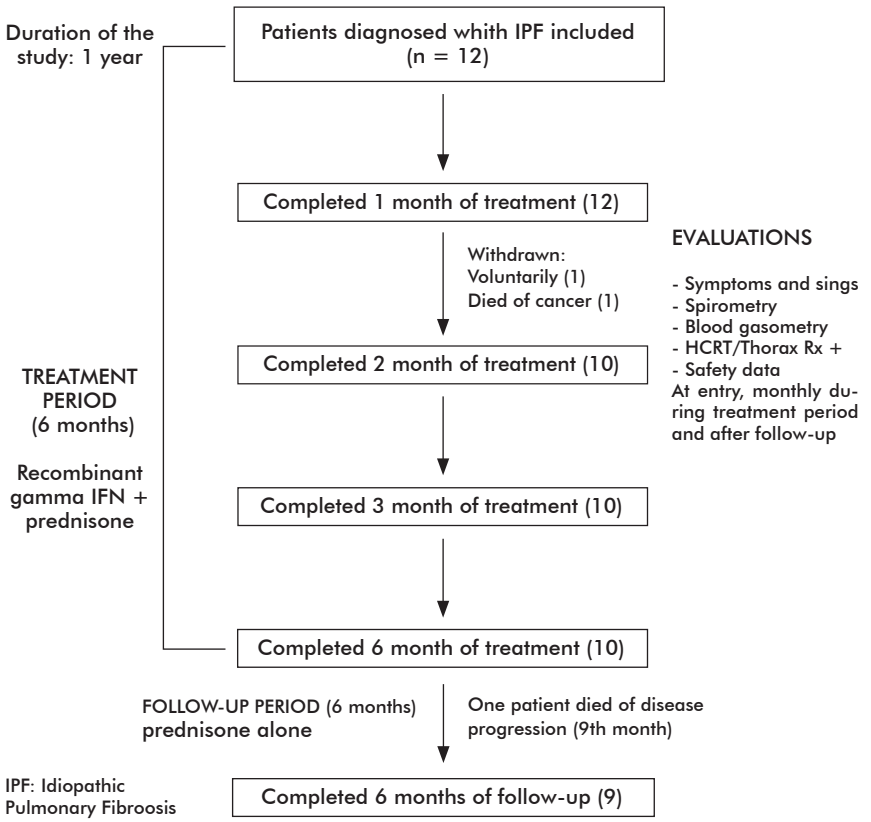

Figure 1. Algorithm of the study.

During the study, a reduction in the number of patients with dry cough, dyspnoea or crepitations was observed (Table 2). Particularly, the dyspnoea disappeared in 5 patients, with a mean time of 6 months. At the end of the study, less than half of the evaluated patients presented this symptom. Dyspnoea score was also significantly reduced, where the mean value zero was reached at the end of the follow-up. One of the patients reduced from 4 to 0 this score, other 2 had a reduction 3 to 1, and none of them increased it. Two thirds of the patients improved clinically from the third month of treatment, by intention to treat. Progression took place in one patient after follow-up. In the functional respiratory test, FVC values increased above 12% in 3 patients (> 20% in 2 of them), but it was reduced the same magnitude just in one, according to the response criteria. Before treatment, according to FVC, more than 80% of the patients were within the categories moderate or moderately severe. After 6 months of treatment these categories decreased up to 60%, but after the follow-up (12 months) more than 75% of the evaluated patients were within these categories, none normal. Two patients passed to the normal category, one of them from moderately severe and the other from mild. However, other 2 turned severe, one from moderately severe and the other from moderate. Nevertheless, mean FVC changes were non-relevant.

Most of the evaluated patients changed to the normality by gasometrical tests, as showed in table 2. Before treatment, 66.7% of them presented alterations, mainly hypoxemia and respiratory alkalosis, but that were reduced to 28.6% in the last evaluation. PO_2 increased relevantly from 77 to 93 mmHg; one patient improved from 33.3 to 86.0 mmHg but other worsened 63.5 to 95.0 mmHg. On the other hand, at the end of the study, close to the half of the evaluated patients had a reduction of the lesions either HCRT as Thorax Rx. Interestingly, in one patient a complete resolution of these lesions was observed after treatment (Figure 2). The rest of the patients remained stable, without new lesions. Fibrosis (diffuse) was initially located in parahilar and vertex regions, but it was confined to basal region as the treatment advanced. An initial reticulonodular and reticular pattern prevailed, but it was mostly reticular at the final evaluations. Lesions of nodular appearance were not observed at any time.

To a better characterization of the overall response, since the progressive nature of this disease, stable patients were also considered as responders. By intention to treat analysis, at the end of gamma IFN treatment (month 6), 4 patients (33.3%) were considered as improved and other 5 (41.7%) were stable, summing 75% of responders. At the end of the follow-up (month 12), two patients (16.7%) were evaluated as improved, 5 (41.7%) stable, for 58.3% as responders. Following per-protocol analysis or patients that could be evaluated at each time, the response increased to 90% (40% improved) at month 6 and more than 70% at month 12 (22.2% improved). Two of the responders at month 6 presented relapses during follow-up, one of them died because of worsening of the symptoms, reason why is not noted in per-protocol analysis. That non-responder patient at month 6 remained in the same status at the end of the study (Table 2).

Table 2. Clinical, functional, imagenological and overall outcome during the trial

Evaluation		Month 0	Month 3	Month 6	Month 12
Symptoms and signs					
Dyspnea		12/12 (100%)	9/10 (90%)	7/10 (70%)	4/9 (44.46%)
Dyspnea score[a]		2.0 ± 1.0 (N = 12)	1.0 ± 1.0 (N = 10)	1.0 ± 1.0 (N =10)*	0.0 ± 1.0 (N = 9)*
Dry cough		11/12 (91.7%)	4/10 (40%)	5/10 (50%)	2/9 (22.2%)
Crepitations		12/12 (100%)	6/10 (60%)	8/12 (66.6%)	4/9 (44.4%)
Evaluation (intention-to-treat)	Improvement		8/12 (66.6%)	2/12 (16.7%)	8/12 (66.6%)
	Stabilization		2/12 (16.7%)	2/12 (16.7%)	0
	Progression		2/12 (16.7%)	2/12 (16.7%)	4/12 (33.3%)
Respiratory function					
FVC (% predited)[b]		59.3 ± 8.4 (N = 12)	61.8 ± 12.6 (N = 10)	62.0 ± 9.7 (N = 9)	60.4 + 9.7 (N = 9)
Normal		0	1/10 (10%)	2/10 (20%)	0
Mild		1/12 (8.3%)	1/10 (10%)	1/10 (10%)	1/9 (11.1%)
Moderate		5/12 (41.7%)	4/10 (40%)	1/10 (10%)	4/9 (44.4%)
Moderately severe		5/12 (41.7%)	3/10 (30%)	5/10 (50%)	3/9 (33.3%)
Severe		1/12 (8.3%)	1/10 (8.3%)	1/10 (10%)	1/9 (11.1%)
Blood gasometry					
Normal		4/12 (33.3%)	4/9 (40%)	5/8 (62.5%)	5/7 (71.4%)
Hypoxemia		4/12 (33.3%)	2/9 (8.3%)	1/8 (12.5%)	1/7 (14.3%)
Respiratory alkalosis		3/12 (25%)	3/9 (33.3%)	2/8 (25%)	2/7 (28.7%)
Respiratory acidosis		0	1/9 (11.1%)	0	0
Hypercapnia		1/12 (8.3%)	0	0	0
Hypocapnia		1/12 (8.3%)	1/10 (8.3%)	1/10 (10%)	1/9 (11.1%)
HCRT/Thorax Rx					
Reduction of fibrosis		0	1/10 (10%)	3/10 (30%)	4/9 (44.4%)
Stabilization		12/12 (100%)	9/10 (90%)	7/10 (70%)	5/9 (55.6%)
Progression		0	0	0	0
Overall response					
Intention-to-treat	Improvement		2/12 (16.7%)	4/12 (33.3%)	2/12 (16.7%)
	Stable disease		7/12 (58.3%)	5/12 (41.7%)	5/12 (41.7%)
	Progression		3/12 (25%)	3/12 (25%)	5/12 (41.7%)
Per protocol	Improvement		2/10 (20%)	4/10 (40%)	2/9 (22.2%)
	Stable disease		7/10 (70%)	5/10 (50%)	5/9 (55.6%)
	Progression		1/10 (10%)	1/10 (10%)	2/9 (22.2%)

Categorical variables are expressed as absolute values and percentages. Dyspnea score is expressed as median ± QR. Forced vital capacity (FVC) is expressed as mean ± SD.
*$p < 0.05$ vs month 0 by Wilcoxon's test.
[a]Dyspnea score: British Medical Research Council (BMRC) modified.
0: no dyspnea at all.
1: dyspnea while climbing hills or stairs.
2: dyspnea while walking at a rapid pace on ground level.
3: dyspnea while walking at own pace on ground level.
4: dyspnea at rest.
[b]Valuation of the severity according to FVC (American Thoracic Society):
Normal: 80% - 100%
Mild: 79% - 70%
Moderate: 69% - 60%
Moderately severe: 59% - 50%
Severe: 49% - 35%
Very severe: < 35%

The mean time to obtain improvement was of 4.5 ± 1.7 months, with a range of 3-6 months. For those non-responder patients, by intention to treat, IPF progressed (including all withdrawals) by 6.2 ± 5.4 months. The mean estimated probability to obtain improvement at month 6 was 0.360 ± 0.123, while the probability to obtain improvement + stable was 0.718 ± 0.116. At the end of the follow-up, both probabili-

ties were superior to 0.57. No significant correlation between any baseline characteristics and the response was detected, although a tendency to a better response in non-white, female and younger patients could be observed, as well as in patients with less than 24 months of evolution of the disease and without toxic habits. Two patients died, but only one due to the disease. The other ten patients remained alive, some of them with 7-9 yrs of survival (Table 3).

Eight adverse reactions were presented during the study. At least one reaction occurred in 66.7%; flu-like events prevailed. Most of the events were classified as mild (62.1%), none of them severe (Table 4). Only two deaths were recorded during the study, both commented, the other ten patients remained alive. Half the patients normalized their globular sedimentation rate whereas normal whole leukocyte counts were reached in the five patients with initial decreased values. These were the most important laboratory findings.

Discussion

The results demonstrate that IFNγ treatment combined with prednisone decreasing-dose can be highly beneficial in patients with IPF. Keeping into the mind the progressive natural course of this illness, we also considered those patients with stable disease as responders, since the progression could be arrested. All the patients evaluated as stable experienced clinical (symptoms and signs) or gasometrical improvement or both, even one of them had reduction of fibrosis by HRCT; however, none of them increased FVC above 12%. Disappearance of dyspnoea in most of the patients is remarkable, since this symptom affects the quality of life more than others. Regarding imagenological evaluations, it results notorious that no patient had progression of the fibrotic lesions, while four patients had a reduction of these, one of them with complete resolution seen from the 3rd month. This last aspect clearly evidences the antifibrotic effect of IFNγ.

By intention-to-treat analysis, a 75% of patients was considered as responders at the end of IFNγ treatment (month 6), 4 of them as improvement. At the end of the follow-up (month 12) responders were reduced to 58.3%, 2 of them improved. These levels of response are better to others, more prolonged IFN γ schedules from comparable studies which other less rigorous response criteria are applied. Nevertheless, the evaluation of the parameters Total Lung Capacity (TLC) and Carbon Monoxide diffusing capacity (DL_{CO}) as well as the use of some validated Respiratory Questionnaire has to be necessarily implemented in our future studies.

The criteria for overall response here used are in coincidence with those agreements in the IPF International Consensus Statement, that took place almost simultaneously with the beginning of this trial [2]. Only small differences in PO_2 and FVC exist, for FVC the variations were higher in our study (12% *vs* 10%).

Two relapses were present during the follow-up time, -one of them died-, manifested by clinical and functional worsening, which indicate that the period of treatment with IFNγ was not sufficient. This period was based in a previous study with a similar commercial IFNγ, where the better response was obtained between 3-6 months [8]. Therefore, we consider that in order to obtain a more sustained response, this treatment has to be extended at least by a year for further studies. Additionally, a more frequent (daily) administration at the first months could be taken into consideration. A more prolonged period could prevent a new crisis in this highly recidivated disease. The same approach has been applied in Cuban pediatric patients with Juvenile Rheumatoid Arthritis treated with this cytokine [14]. It is impossible to associate relapses with the reduction in prednisone dose since both patients had been receiving high ineffective prednisone doses for 6 months prior to their entry.

Mortality in this study could be considered low since only two patients (16.7%) died, one of them because of the direct disease but the other by an apparent

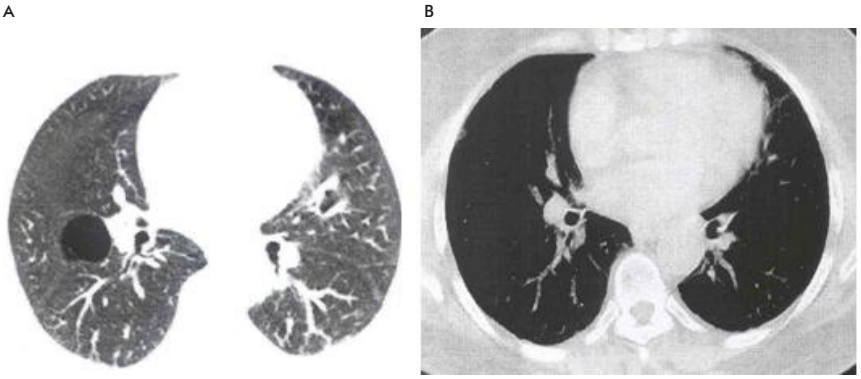

Figure 2. Imagenological improvement with IFN gamma treatment (HRCT of one patients is shown). A) Diffuse bibasal fibrotic lesions before IFN gamma treatment. B) Disappearance of these lesions 6 months after treatment. Other lesions as emphysematous bullous in basal right region and bronchiectasis remained in the same conditions.

Table 3. Current status of the treated patients (updated August 2009)

Patient	Inclusion date	Status	Time of survival
HA-01	24/10/2000	Alive	9 yrs
BJ-01	12/02/2001	Alive	8 $^1/_2$ yrs
BJ-02	18/09/2001	Alive	8 yrs
BJ-03	1/02/2002	Alive	7 $^1/_2$ yrs
BJ-04	31/01/2002	Alive	7 $^1/_2$ yrs
BJ-05	06/02/2002	Alive	7 $^1/_2$ yrs
BJ-06	22/04/2002	Alive	7 $^1/_2$ yrs
BJ-07	29/01/2003	Alive	6 $^1/_2$ yrs
BJ-08	11/03/2003	Alive	6 $^1/_2$ yrs
BJ-09	04/02/2003	Died of cancer (mesotelioma)	1 $^1/_2$ yrs
BJ-10	13/05/2003	Died of IPF progresion	9 mos
BJ-11	16/06/2003	Alive	6 yrs

Table 4. Adverse reactions during IFN gamma treatment

Adverse reaction	Patients (N =12)
Any adverse reaction	12 (100%)
Myalgias	10 (83.3%)
Weight loss	3 (25%)
Chills	2 (16.7%)
ALT increase	1 (8.3%)
Arthralgias	1 (8.3%)
Digestive bleeding	1 (8.3%)
Pain at injection site	1 (8.3%)

*Data are expressed as absolute values and percentages.

14. Coto C, Varela G, Hernández V, del Rosario M, López-Saura P. Use of recombinant interferon gamma in pediatric patients with advanced juvenile chronic arthritis. Biotherapy 1998;11:15-20.

IPF non-related cancer. Despite the median, two years of onset of the disease, all the patients that concluded the trial are still alive.

Gamma IFN is a key Th1 cytokine produced by T and NK cells with well-know antiviral, anti-proliferative, immunomodulating and anti-fibrotic effects. Although for many years IFNγ has been considered as a pro-inflammatory cytokine, sometimes associated with the pathogenesis of inflammatory and autoimmune diseases, more and more evidences of their anti-inflammatory action appears nowadays as supposing a dual effect. It unregulated several pro-inflammatory parameters such as IL-12, tumor necrosis factor α (TNF-α), IFN-inducible protein 10 (IP-10), among others, but it also induces anti-inflammatory molecules as Interleukin 1 receptor antagonist (IL-1Ra) or IL-18 binding protein (IL-18BP), modulates the production of pro-inflammatory cytokines, and induces suppressive pathways of the inflammation [15].

The potent anti-fibrotic effect of IFNγ is very relevant in this type of diseases. It acts directly on the fibroblasts proliferation or reducing the collagen synthesis and chemiotaxis [5, 7], increases the activity of the collagenase [16], and also inhibits TGF-β [6], involved directly in severe lung fibrosis progression [17]. Furthermore, IFNγ contributes to tissue repairment and their remodeling [18]. These actions were also evident in the results with the systemic or aerosolized use of this product in patients with pulmonary drug-resistant tuberculosis [19-21], and suggests that this cytokine can have future indications in other pulmonary diseases where fibrosis is present.

On the other hand, patients treated with IFNγ present changes in fibrosis, angiogenesis, proliferation, immunomodulating and antimicrobial biomarkers that could affect IPF through multiple mechanisms [22].

The rationality to use IFNγ in IPF is reaffirmed with some demonstrations that inflammation doesn't play a crucial role in the pathogenesis of this disease. Inflammation doesn't constitute a prominent histopathological finding and the epithelial damage in absence of permanent inflammation is sufficient to stimulate the development of fibrosis. In addition, the inflammatory response to fibrogenic damage in the lungs doesn't necessarily have to be related to the fibrotic response. The clinical measurements of inflammation fail to correlate with the disease outcome, and the potent anti-inflammatory therapy neither is successful [23, 24]. Some authors postulated that IPF can constitute a disorder of epithelial but not inflammatory cells [17].

The observation that "early" IPF looks as "late", but there is less from the first, has re-evaluated the paradigm that IPF is a consequence of an uncontrollable lung inflammation. Therefore, new therapies are addressed to regulate the fibroblasts more than inflammatory response [25]. For that reason, the most appropriates schedules of IFNγ treatment in this illness has to be assayed.

Treatment with IFNγ was well tolerated. Flu-like symptoms such as myalgias and fever are among those expected for interferons since their first clinical applications [26]. Weight loss, ALT increase and pain at the injection site which appeared in one case each have been also reported. Digestive bleeding is an adverse reaction probably more related to the use of corticosteroids, in this case prednisone [1,3]. Both deaths presented cannot be related to IFNγ treatment.

Although efficacy of recombinant IFNγ in IPF seems to be contradictory as reported [8-12], a differential feature with our study constitutes the scheme used for oral corticosteroids. In these reports, much lower doses were applied, even therapeutically ineffective for this aggressive affectation. In this trial, for ethical reasons and also to avoid an eventual sudden crisis the initial prednisone dose was 60 mg daily, a common dose in medical practice, which was gradually reduced. During IFNγ treatment, the dose of prednisone (minimum 20 mg daily) was above other reports, without significant toxicity added. This combination apparently has better results in terms of efficacy in advanced disease, which needs to be confirmed in a larger future study.

The obtained results justify more extensive, controlled clinical trials to confirm the rationality to use IFNγ as adjuvant to decreasing-dose prednisone therapy in patients with idiopathic pulmonary fibrosis. This combination could reduce treatment duration, toxicities and possible relapses. In some cases it could prevent recessional surgery.

Appendix

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15. Muhl H, Pfeilschifter J. Anti-inflammatory properties of pro-inflammatory interferon-gamma. Int Immunopharmacol 2003;3:1247-55.

16. Ghahary A, Shen YJ, Nedelac B, Scott PG, Tredget EE. Interferon γ and α-2b differentially regulate the expression of collagenase and TIMP-1 mRNA in human hypertrophic and normal dermal fibroblasts. Wound Repair Regent 1995;3:13-21.

17. Xu YD, Hua J, Mui A, O'Connor R, Grotendorst G, Khalil N. Release of biologically active TGF-beta1 by alveolar epithelial cells results in pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2003;285:L527-39.

18. Pilette Ch, Fort J, Riffet H, Cales P. Effets anti-fibrossants des interferons. Gastroenterol Clin Biol 1997;21:466-71.

19. Condos R, Rom WN, Schulger NW: Treatment of multidrug-resistant tuberculosis with interferon γ via aerosol. Lancet1997;349:1513-5.

20. Koh WJ, Kwon OJ, Suh GY, Chung MP, Kim H, Lee NY, *et al*. Six-month therapy with aerosolized interferon-gamma for refractory multidrug-resistant pulmonary tuberculosis. J Korean Med Sci 2004;19:167-71.

21. Suárez-Méndez R, García-García I, Fernández-Olivera N, Valdés-Quintana M, Milanés-Virelles MT, Carbonell D, *et al*. Adjuvant interferon gamma in patients with drug - resistant pulmonary tuberculosis: a pilot study. BMC Infect Dis 2004;4:44.

22. Strieter RM, Starko KM, Enelow RI, Noth I, Valentine VG; Idiopathic Pulmonary Fibrosis Biomarkers Study Group. Effects of interferon-

gamma 1b on biomarker expression in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2004;170:133-40.

23. Selman M, King TE, Pardo A; American Thoracic Society; European Respiratory Society; American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001;134:136-51.

24. Green FH. Overview of pulmonary fibrosis. Chest 2002;122:334S-9S.

25. Noble PW, Homer RJ. Idiopathic pulmonary fibrosis: new insights into pathogenesis. Clin Chest Med 2004;25:749-58.

26. Vial T, Descotes J. Clinical toxicity of the interferons. Drug Saf 1994;10:115-50.

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