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Dysregulation of Immunity in Pulmonary Fibrosis is Associated with Increased Myeloid-specific Triggering Receptor-1 and Transforming Growth Factor-beta1 Expression

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ABSTRACT

Fibrosing pneumonia (FP) is classified into usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP), each having its own etiology and prognosis. Both types of FP are progressive and chronic conditions with distinct etiologies. Cytokines and inflammatory mediators play critical roles in the pathogenesis of FP. Among them, the role of transforming growth factor beta-1 (TGF-β1) and modulators triggering fibrosis are not well understood. In this study, the expression of triggering receptor expressed on myeloid cells-1 (TREM-1) as a stimulator for the production of TGF-β1 and also $CD4^+CD25^+Foxp3^+$ regulatory cells were investigted in FP patients.

Sixteen UIP, 14 NSIP and 4 pulmonary fibrosis following *Mycobacterium tuberculosis* (TB) infection patients, were compared with 12 healthy controls. The frequency of blood $CD14^+TGF\text{-}\beta1^+$ and $CD14^+TREM1^+$-gated monocytes and $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg), as well as the plasma levels of TGF-β1 and IL-10 were measured.

Fibrosis patients compared to healthy controls had a greater frequency of $CD14^+TGF\text{-}\beta1^+$ [15.9 (0.2-88.2) vs. 0.6 (0.2-11.0)] and $CD14^+TREM1^+$ [21.1 (2.3-91.2) vs. 10.3 (3.1-28.6)]-gated monocytes, and $CD4^+CD25^+Foxp3^+$ [1.2 (0.3-3.6) vs. 0.2 (0.1-0.4)]-gated lymphocytes. Plasma TGF-β1 were also significantly increased in patients with fibrosis compared to healthy controls [9316.2 (±5554.4) vs. 3787.5 (±2255.6)].

These results confirm the importance of TGF-β1 and TREM1 in pulmonary fibrosis. It seems that this reciprocal cycle in healthy people is modulated by the production of IL-10 by Treg cells,

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thus limiting fibrosis, as observed in patients following TB infection. Further investigations are recommended to evaluate possible immunomodulatory mechanisms defects in pulmonary fibrosis.

Keywords: Interleukin-10; Idiopathic pulmonary fibrosis; Flow cytometry; Triggering receptor expressed on myeloid cells-1; Transforming growth factor beta; T-lymphocytes; Regulatory

INTRODUCTION

Injury accompanied by endothelial and epithelial damage is associated with platelet activation, and fibrin-rich clot formation leads to inflammation caused by the release of cytokines, chemokines, and growth factors. As a result, angiogenesis and the recruitment of (myo)-fibroblasts, epithelial to mesenchymal transformation (EMT), and fibrocytes related to repair end in apoptosis and phagocytosis with wound contraction related to reepithelialization and regeneration. The presence of persistent (known/unknown) irritants leads to the disrupted resolution of inflammation in pulmonary fibrosis[1] as a chronic, progressive and devastating interstitial lung disease.

Triggering receptor expressed on myeloid cells-1 (TREM-1) activation following its engagement with toll-like and nod-like receptors induces proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α) and inhibits antiinflammatory factors (e.g., IL-10).[2] As a result, the prolongation of the survival of macrophages in infectious and noninfectious inflammatory conditions leads to excessive inflammatory responses.[3] The expression of TREM-1 in alveolar macrophages[4] and its association with the poor prognosis of non-small-cell lung cancer (NSCLC) has been proven.[5] Elevated serum levels of TREM-1 in diffuse cutaneous systemic sclerosis and its association with the severity of pulmonary fibrosis were described.[6] However, the role of TREM-1 and its association with core modulators of fibrosis, such as TGF-β1 and regulatory T (Treg) cells, on pulmonary fibrosis patients is not well documented.

The most common type of idiopathic interstitial pneumonia (IIP) is idiopathic pulmonary fibrosis (IPF), which accounts for about 55% of IIP cases.[7] IPF, as a disease of older adults (aged>50 years) with unknown etiology, mostly occurring in men, is associated with the usual interstitial pneumonia (UIP) pathological pattern. On the other hand, nonspecific interstitial pneumonia (NSIP), comprising 25% of IIP cases, is more common in a younger age and shows a slight female predominance.[8] NSIP can be idiopathic or associated with connective tissue disorders (CTD), HIV infection, and toxins. Despite the mentioned differences, both types are associated with similar pulmonary symptoms and physical weakness. Given the longer survival (>9 years) reported in patients with NSIP compared to UIP (2-5 years), NSIP patients have a better prognosis.[9]

Currently, the mechanisms involved in the development of fibrosis in UIP and NSIP are not well understood. The bleomycin-induced murine pulmonary fibrosis model has demonstrated a positive correlation between TGF-β1 and TREM1 expression in a dose- and time-dependent manner through the activator protein-1 (AP-1) signaling pathway.[10] The current study investigated the surface expression of TGF-β1 and TREM-1 and their correlation with Tregs and IL-10 in patients with UIP and NSIP as well as patients with pulmonary fibrosis following *Mycobacterium tuberculosis* (TB) to provide insights into the regulatory mechanisms of pulmonary fibrosis and the potential therapeutic targets for its treatment.

MATERIALS AND METHODS

Subjects

All procedures performed were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran) (IR.SBMU.MSP.REC.1398.568). Written informed consent was obtained from all participants or their families. A total of 34 patients with mild-to-moderate pulmonary fibrosis (16 UIP, 14 NSIP, and 4 TB) at Masih Daneshvari Hospital, Tehran, Iran, between April 2019 and January 2020 were enrolled according to the IPF nontraumatic diagnostic criteria formulated by ATS/ERS/JRS/ALAT in 2011.[11] The inclusion criteria for mild to moderate patients, who were not at the exacerbation episode, included forced vital capacity (FVC) of 50 to 75%, diffusing capacity of the lungs for carbon monoxide (DLCO) of 36 to 55%, and a 6-minute-

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walk test of at least 150 meters.[12,13] Patients with connective tissue disorders, active malignancies, active infections, diabetes, hypertension, and medication or occupational exposures causing pulmonary fibrosis were excluded. At the same time, 12 age- and sex-matched healthy individuals with the same inclusion criteria were included in the healthy control group.

Patients with pulmonary fibrosis following chronic infection with TB have also been recruited in this study, and due to the low incidence of TB, only 4 were enrolled. There were two reasons why the inclusion of patients with TB was important: first, they bear fibrosis, and second, in the pathogenesis of the disease, common cytokines play a critical role in the development of fibrosis but not progressively.

Sample Preparation

From the antecubital vein of all participants, 5-6 mL whole blood was obtained (2 tubes containing 1 μL of 10% potassium EDTA per 100 μL, greiner bio-one, United States). After centrifugation of 4 mL whole blood at 1500*g* at 4°C for 10 min, obtained plasma was stored at -80°C until use. 1 to 2 mL of whole blood containing EDTA as anticoagulant was used for flow cytometric assays.

Flow Cytometry Analysis

To determine the immunophenotyping of $CD14^+TREM1^+$ and $CD14^+TGF\text{-}\beta1^+$ cells, surface staining was performed using anti-CD14-FITC (Clone MφP9, BD Biosciences, San Diego, CA, USA), anti-TREM1 conjugated with R-phycoerythrin (PE) (Clone #02, Sino Biologicals, Beijing, China) and anti-TGF-β1 PE (Clone TW4-9E7, BD Biosciences, San Diego, CA, USA) for 20 min at 4°C. Cells were washed twice using cold PBS after 15 min of incubation with lysis buffer (NH4Cl, NaHCO3, EDTA solution). Immunophenotyping of Treg cells was performed using anti-CD4-FITC (Clone MEM-241, Exbio, Vestec, Czechia) and anti-Hu-CD_{25}-APC (Clone MEM-181, Exbio, Vestec, Czechia) for 20 min at room temperature. Intracellular staining was followed by washing the cells and incubation with fixation and permeabilization with EXCELLYSE XPerm solutions (Exbio, Vestec, Czech Republic) for 10 and 20 min at room temperature, respectively. The cells were subsequently washed with cold PBS, and intracellular staining was performed using anti-FoxP3 PE (Clone 3G3, Exbio, Vestec, Czech Republic) for 20 min 4°C. One thousand events were evaluated using BD FACSCalibur (BD Biosciences) and analyzed using FlowJo Software version 10 (BD Company, USA).

Enzyme-linked Immune-sorbent Assay (ELISA)

Plasma IL-10 and TGF-β1 levels were measured using ELISA kits (both from R & D Systems, USA) according to the manufacturer's instructions.

Statistical Analysis

Analysis was performed using SPSS version 16.0 and GraphPad Prism version 8.0.2. Nonparametric chi-squared and Mann-Whitney U Test (median) were used for the variables without normal distribution. A one-way analysis of variance (ANOVA), expressed in mean±SD, was was used for normally distributed variables. Correlation analysis was performed using Spearman correlation test. p values<0.05 were considered statistically significant.

RESULTS

Patient Characterization

Among 46 participants with a mean age of 61.91±12.50 years, 20 were female, and 26 were male. Age- and sex-matched patients with UIP and NSIP had no statistically significant difference in clinical symptoms and spirometry results (Table 1).

Increased Frequency of $CD14^+TGF\text{-}\beta1^+$ and $CD14^+TREM1^+$ Monocytes in Blood of Pulmonary Fibrosis Patients

Table 2 shows that the white blood cell (WBC) count in patients with pulmonary fibrosis (11150±3187) was significantly compared with the control group (5463±1042). This difference between NSIP (12065±3416) and UIP patients (9320±1737) was not significant (p=0.061).

Although not statistically significant, monocytes were increased in patients compared to healthy controls (p=0.151) (Table 2). There was a significant increase in CD14+TGF-β1+ monocytes in patients with fibrosis compared to healthy controls (p<0.0001) (Table 2). No statistically significant difference was reported between the UIP and NSIP groups (p=0.710). Moreover, $CD14^+TGF\text{-}\beta1^+$-gated monocytes in patients with TB were significantly fewer than in patients with NSIP (p=0.045). Similarly, $CD14^+TREM1^+$-gated monocytes increased significantly in patients with UIP and NSIP

Table 1. Demographics, clinical symptoms and spirometry.

Characteristics	Groups	UIP (n=16)	NSIP (n=14)	HC (n=12)	TB (n=4)	*p*
Demographics	**Age (years)**	69.0±2.28	53.64±3.15	66.17±2.72	49.75±5.95	0.298
	Sex (M/F)	11/5	7/7	4/8	4/0	0.175
Symptoms	**Crackle (n)**	9	6	-	-	0.715
	Headache (n)	0	1	-	-	0.467
	Weight Loss (n)	3	4	-	-	0.675
	Weakness (n)	2	2	-	-	1.000
	Respiratoty Distress (n)	6	3	-	-	0.440
	productive cough (n)	6	8	-	-	0.464
	Chest Pain (n)	1	3	-	-	0.315
	Shortness of Breath (n)	15	12	-	-	0.586
	Cough (n)	12	13	-	-	0.336
	Drowsiness (n)	2	0	-	-	0.485
	Edema (n)	2	0	-	-	0.485
	Back Pain (n)	1	0	-	-	1.000
	Tachypnea (n)	0	1	-	-	0.467
	Koilonychia (n)	7	6	-	-	1.000
Spirometry	**FVC (%)**	74.4	69.9	-	-	0.625
	FEV1(%)	81.5	76.6	-	-	0.623
	TLCO (%)	47.4	63.3	-	-	0.139
	TLC (%)	66.5	65.9	-	-	0.932

HC, healthy controls; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; TB, *Mycobacterium tuberculosis*; n, number; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second, TLCO; transfer capacity of carbon monoxide; TLC, total lung capacity.

Table 2. Distribution of Immunophenotyped Cells.

	HC	Fibrosis (UIP & NSIP)	UIP	NSIP	TB	*p*
WBC ($\times 10^9$/L)	5463±1042	11150±3187	9320±1737	12065±3416	8805±3193	**<0.0001***
Monocytes (C)	551.8±208.0	664.7±229	652.6±230.7	680.8±235.9	784.5±277.2	0.151
Monocytes (%)	5.5 (2.7-45.9)	6.7 (2.3-11.4)	6.1 (2.7-10.3)	7.2 (2.3-11.4)	7.2 (5.2-11.8)	0.256
CD_{14}^+, TGF-β1$^+$(C)	3.0 (1.0-89.0)	80 (2-689)	80.0 (2.0-442.0)	94.0 (5.0-689.0)	9.5 (3.0-15.0)	**<0.0001***
CD_{14}^+, TGF-β1$^+$(%)	0.6 (0.2-11.0)	15.9 (0.2-88.2)	12.9 (0.19-88.2)	19.7 (0.8-81.7)	1.2 (0.4-2.1)	**<0.0001***
CD_{14}^+, TREM1$^+$ (C)	69.0(12.0-215.0)	160.5 (8.0-636.0)	137.0 (18.0-451.0)	185.0 (8.0-636.0)	33.0 (30.0-73.0)	**0.02***
CD_{14}^+, TREM1$^+$ (%)	10.3 (3.1-28.6)	21.1 (2.3-91.2)	21.1 (2.6-91.2)	23.5 (2.3-83.2)	5.8 (3.8-6.8)	**0.02***
Lymphocytes (C)	2993.0±1117.9	2201.7±647.2	2167.9±614.9	2248.0±713.1	1957.8±855.0	**0.008***
Lymphocytes (%)	29.4±9.2	22.0±6.5	21.7±6.2	22.5±7.1	19.6±3.1	**0.006***
CD_4^+, CD_{25}^+, Foxp3$^+$ (C)	3.0 (1.0-5.0)	5.0 (2.0-16.0)	5.0 (2.0-9.0)	6.0 (3.0-16.0)	4.5 (4.0-5.0)	**<0.0001***
CD_4^+, CD_{25}^+, Foxp3$^+$ (%)	0.2 (0.1-0.4)	1.2 (0.3-3.6)	1.1 (0.3-3.6)	1.3 (0.5-1.6)	1.1 (0.9-1.5)	**<0.0001***

WBC and Monocytes (C) were presented as Mean±SD and other parameters were presented as Median (IQR). The *p* values were calculated for fibrosing pneumonia patients and healthy controls.

*Statistically significant.

WBC, white blood cells; HC; healthy controls; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; TB, *Mycobacterium tuberculosis*; C, count; CD, cluster of differentiation; TREM-1, triggering receptor expressed on myeloid cells-1; TGF-β1, transforming growth factor- β1; Foxp3, forkhead box P3;

(*p*=0.03 and *p*=0.04, respectively). No statistically significant difference was found between the UIP and NSIP groups (*p*=0.889). $CD14^+$, $TREM1^+$ gated monocyte counts in patients with TB were lower compared to patients with UIP and NSIP (*p*=0.073 and *p*=0.090, respectively).

There was a significant decrease in patients lymphocytes compared to healthy controls (*p*=0.008), but no statistically significant difference between UIP and NSIP patients was found (*p*=0.791) (Table 2). $CD4^+CD25^+Foxp3^+$-gated lymphocytes showed a significant increase in patients as compared to healthy controls (*p*<0.0001) (Table 2).

A significant positive correlation was reported in the frequency of $CD14^+TREM1^+$ cells with the frequency of $CD14^+TGF\text{-}\beta1^+$ cells in healthy controls (r=0.725; *p*=0.010) and in patients (r=0.700; *p*<0.0001) (Figure 1). This significant positive correlation was also observed in the UIP (r=0.721 and *p*=0.002) and NSIP (r=0.832 and *p*=0.001) groups (Figure 1).

Discordance of Plasma TGF-β1 and IL-10 in Patients with Pulmonary Fibrosis

Plasma TGF-β1 analysis showed significant increase in FP patients compared to healthy controls (*p*=0.002) (Table 3). No significant difference was observed among NSIP and UIP in terms of TGF-β1 plasma levels (*p*=0.987). A significant positive correlation was reported between the $CD14^+TGF\text{-}\beta1^+$ monocytes count and plasma TGF-β1 levels in patients with UIP (r=0.515; *p*=0.044) (Figure 1).

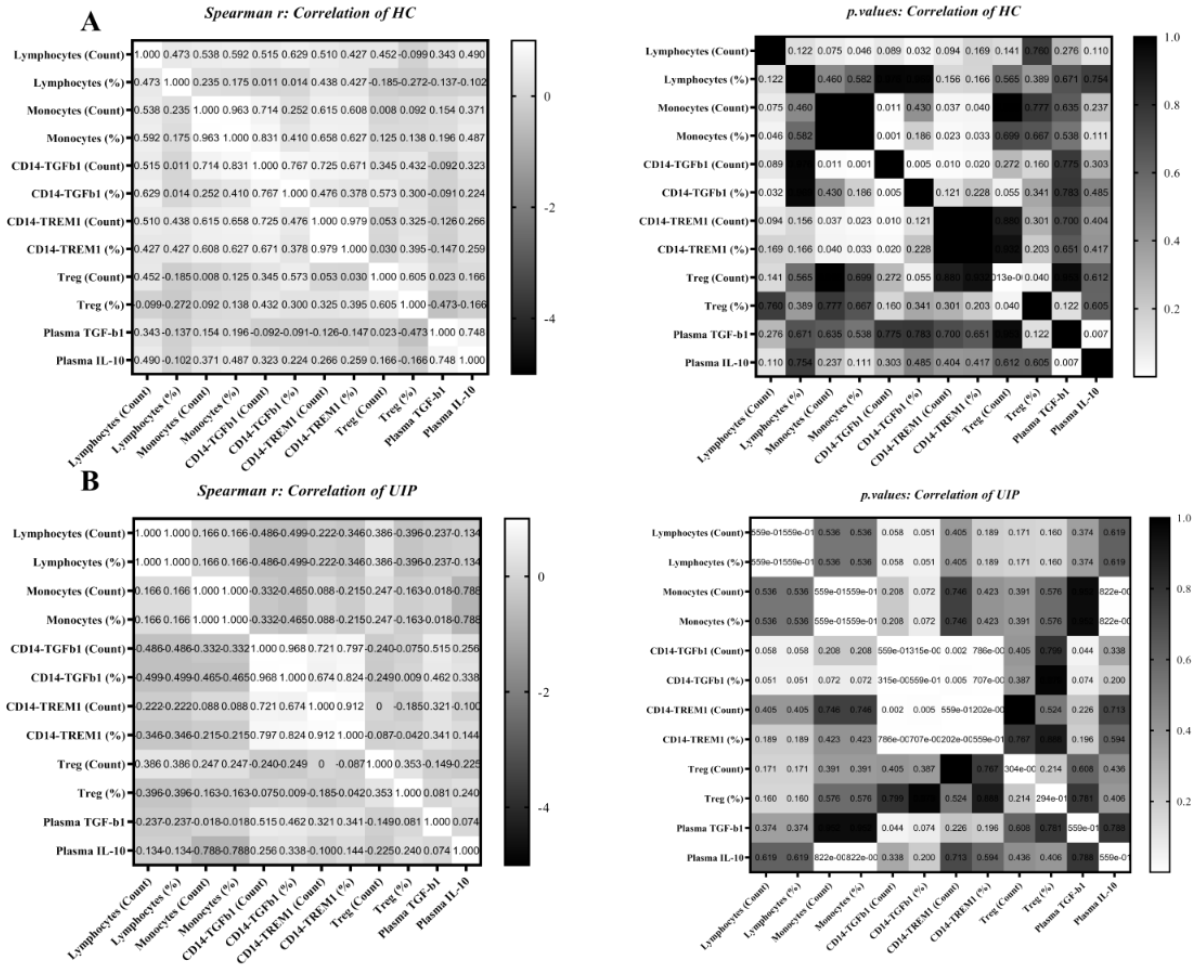
Plasma IL-10 levels did not show any significant changes in PF patients compared to healthy controls (*p*=0.989) (Table 3). IL-10 levels showed a positive correlation with plasma TGF-β1 levels in healthy controls (r=0.748; *p*=0.07) (Figure 1). In addition, there was a significant negative correlation between IL-10 levels and the number of monocytes in patients with UIP (r=−0.788; *p*<0.0001) (Figure 1).

Table 3. Plasma Cytokine Levels.

	HC	Fibrosis (UIP & NSIP)	UIP	NSIP	TB	*p*
TGF-β1 (pg/mL)	3787.5 (±2255.6)	9316.2 (±5554.4)	9302.2 (±6012.6)	9332.4 (±5206.7)	9967.8 (±3882.9)	**0.002***
IL-10 (pg/mL)	26.9 (11.3-282.3)	30.2 (2.3-825.9)	33.0 (2.3-825.9)	22.3 (7.4-72.8)	52.4 (19.4-102.4)	**0.989**

Plasma TGF-β1 is presented as mean (±SD), and IL-10 is shown as median (IQR).

HC; Healthy Control, UIP; Usual interstitial pneumonia, NSIP; Nonspecific interstitial pneumonia, TB; Mycobacterium Tuberculosis, TGF-β1; Transforming Growth Factor-beta1, IL-10; Interleukin-10, pg/mL; pictogram per milliliter. *Statistically significant.



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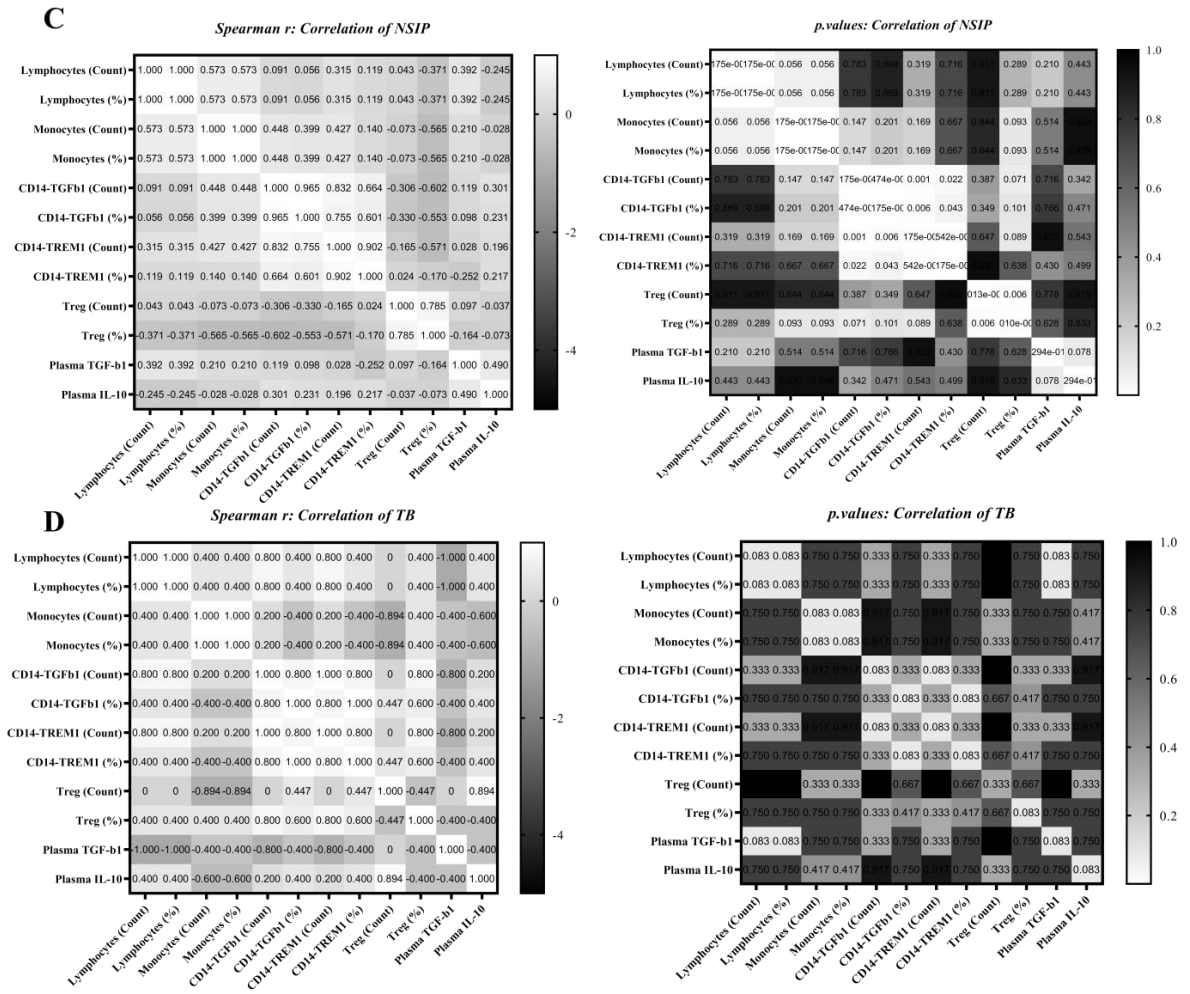

Figure 1. Correlation analysis; A) healthy controls; B) pulmonary fibrosis (both UIP and NSIP) patients; C) UIP; D) NSIP; E) TB. HC, healthy controls; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; TB, Mycobacterium tuberculosis; CD, cluster of differentiation; TREM-1, triggering receptor expressed on myeloid cells-1; TGF-β1, transforming growth factor-β1; Foxp3, forkhead box P3; IL-10, interleukin-10.

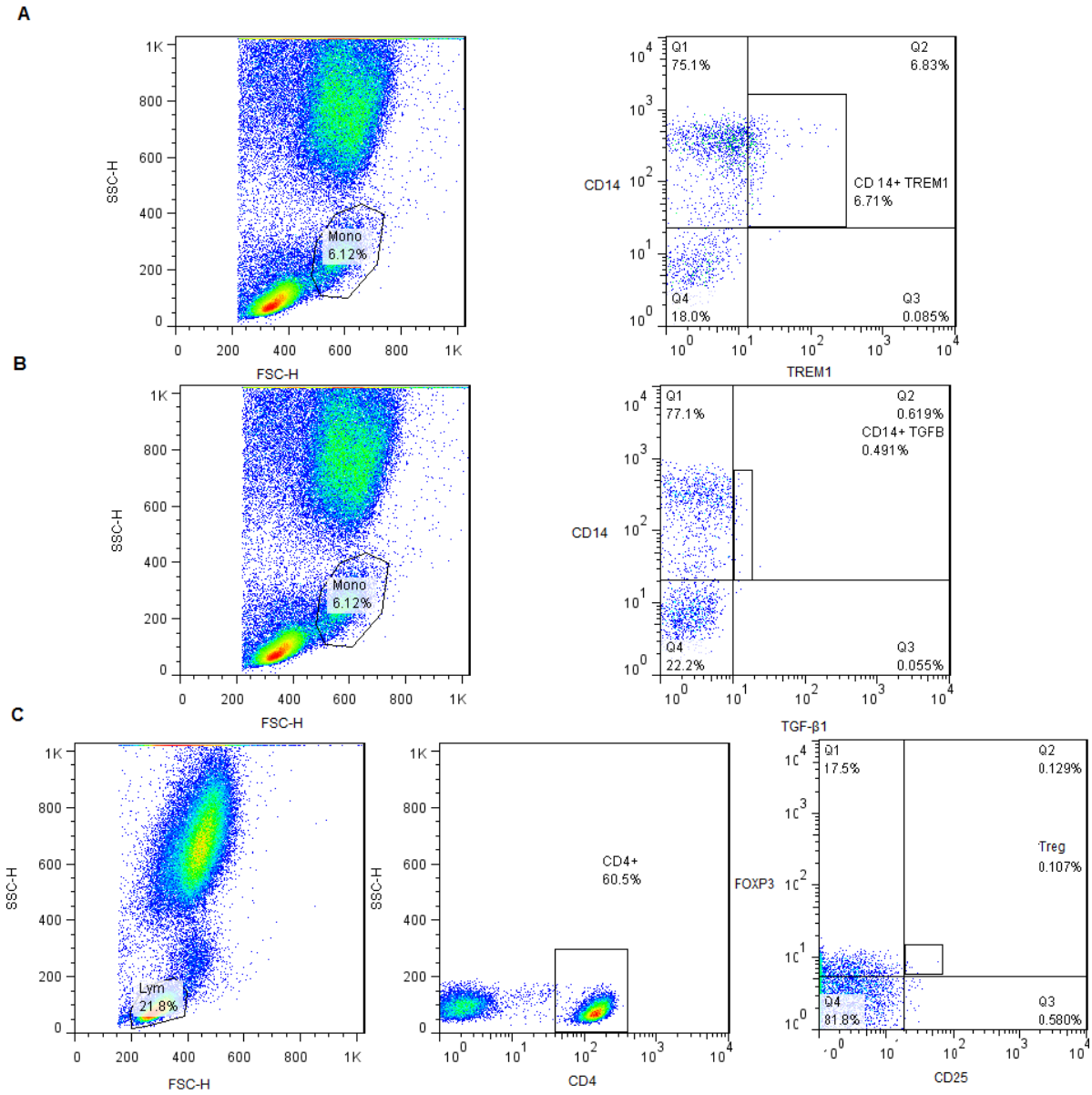

Figure 2. Gating strategy for identifying $CD14^{+}TGF\text{-}\beta1^{+}$ and $CD14^{+}TREM1^{+}$ monocytes and regulatory T cells. A) Representative flow cytometric dot plots showing the expression of TREM1 on $CD14^{+}$-gated monocytes in a sample of healthy controls; B) Representative flow cytometric dot plots showing the expression of TGF-β1 on $CD14^{+}$ gated monocytes in a sample of healthy controls; C) Representative flow cytometric dot plots showing expression of $CD25^{+}FOXP3^{+}$ on $CD4^{+}$ gated T cells in a healthy control subject. Data from (A, B, and C) are from duplicate samples of the same individual.

CD, cluster of differentiation; TREM-1, triggering receptor expressed on myeloid cells-1; TGF-β1, transforming growth factor-β1; Foxp3, forkhead box P3.

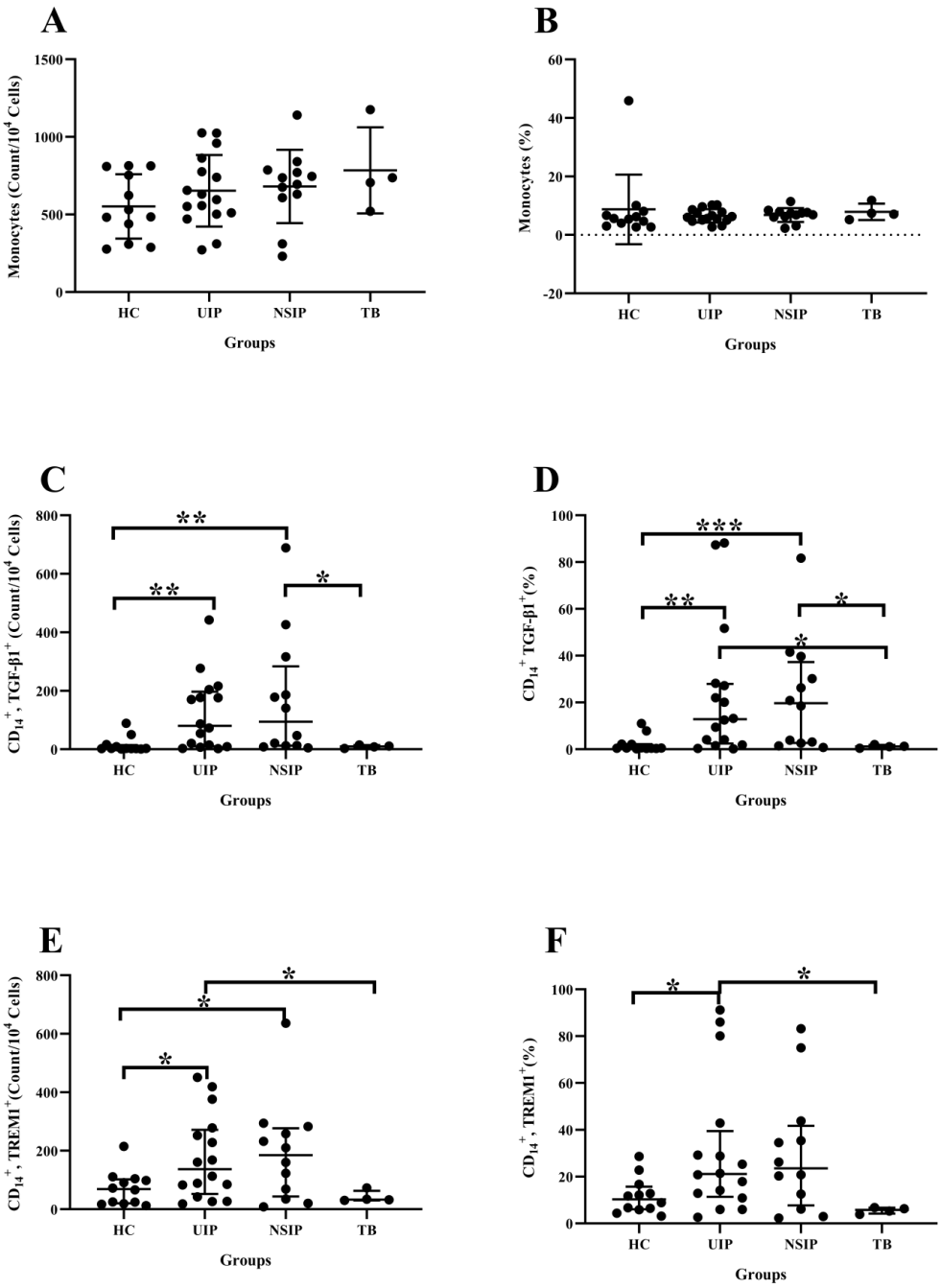

Figure 3. Monocyte immunophenotyping; A and B) Monocyte counts indicated no significant differences among groups. *p<0.05, **p<0.01, ***p<0.001.

C, D) CD14+ TGF-β1+ gated monocytes showed a significant increase in UIP compared to HC and TB. Also, CD14+ TGF-β1+ gated monocytes showed a significant increase in NSIP compared to HC and TB. E, F) $CD14^+$ $TREM\text{-}1^+$ gated monocytes showed a significant increase in UIP compared to HC and TB.

HC, healthy controls; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; TB, *Mycobacterium tuberculosis*; CD, cluster of differentiation; TREM-1, triggering Receptor expressed on myeloid cells-1; TGF-β1, transforming growth factor- β 1.

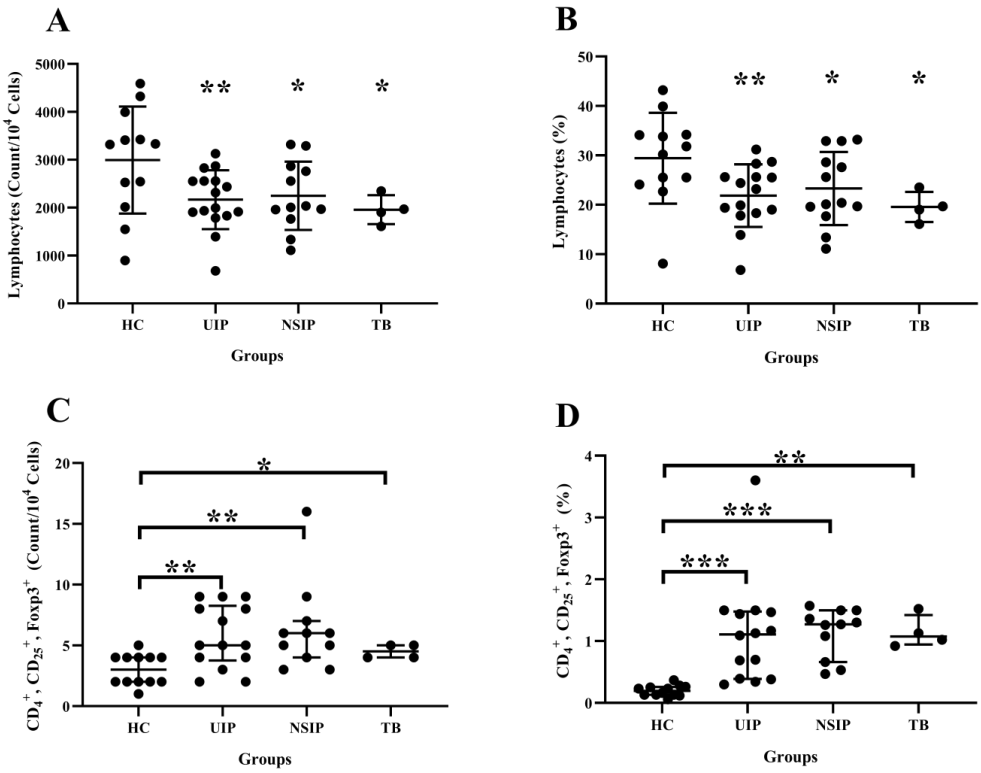

Figure 4. Lymphocytes immunophenotyping. A, B) There was a significant decrease in UIP, NSIP, and TB groups lymphocytes as compared to HC. C, D) There was a significant increase in UIP, NSIP, and TB groups $CD4^+$, $CD25^+$, and $Foxp3^+$-gated lymphocytes as compared to HC. *p<0.05, **p<0.01, ***p<0.001.

HC, healthy control; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; TB, *Mycobacterium tuberculosis*; CD; cluster of differentiation; Foxp3, forkhead box P3.

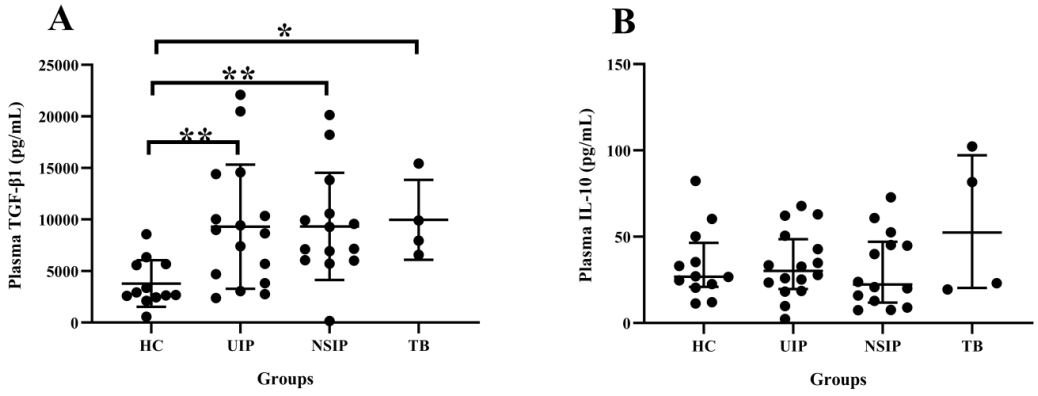

Figure 5. Plasma TGF-β1 and IL-10. A) Plasma TGF-β1 was elevated in UIP, NSIP, and TB compared to HC. B) Plasma IL-10 was reported not to have any significant changes in UIP, NSIP, and TB compared to HC. *p<0.05, **p<0.01, ***p<0.001.

HC; Healthy Control, UIP; Usual interstitial pneumonia, NSIP; Nonspecific interstitial pneumonia, TB; Mycobacterium Tuberculosis, TGF-β1; Transforming Growth Factor-beta1, IL-10; Interleukin-10, pg/mL; pictogram per milliliter.

DISCUSSION

IPF is considered to be an aging disease based on epidemiological and biological evidence. The association of age with IPF has been reported.[14] Valid cohort studies have consistently found a mean patient age of about 65 years, and IPF is rarely reported in young adults.[15] Other studies in the field of disease biology also confirm the main role of age-related changes at the cellular and clinical levels in the development of the disease.[16] For this reason, the selection of healthy controls was considered in line with the age and gender distribution of the patients participating in the study (Table 1.). UIP and NSIP patients showed no significant difference in clinical symptoms or spirometric parameters (Table 1). These findings are in agreement with previous studies, which show no difference in the clinical results of the NSIP and UIP.[17]

There was a significant increase in both NSIP and UIP WBCs compared to healthy controls (p<0.0001) (Table 2). Similarly, increased WBC as an increase in the absolute neutrophil count was shown along with any significant changes in absolute lymphocyte count and absolute monocyte count.[18] Similarly, no significant changes in monocyte or lymphocyte frequency (Table 2, Figure 3A and B) were shown in pulmonary fibrosis patients compared to healthy controls (p=0.008) (Table 2, Figure 3A and B). In this regard, increased neutrophils, lymphopenia, and increased neutrophil-to-lymphocyte ratio in IPF were shown, which were associated with decreased FVC.[19] Furthermore, both the levels of monocytes and neutrophils correlated with IPF mortality. Thus, neutrophilia and lymphopenia are suggested as sensitive indicators of IPF progression.

Neutrophils and mononuclear macrophages selectively express TREM-1, a cell membrane surface receptor related to the IgG superfamily.[20] In this study, $CD14^+TREM1^+$ monocyte frequency was increased in patients with UIP and NSIP (p=0.03 and 0.043, respectively). Similarly, increased TREM1 on BALF $CD14^+$ cells in patients with sarcoidosis has been reported.[21] These findings of inflammatory events following the activation of this receptor on macrophages and monocytes by activating hematopoietic stem cells (HSCs) are in concordance with fibrosis progression.[22]

TREM-1 activation increased the expression of TGF-β family genes by 96.7 times.[23] The results of this study also indicated a positive correlation of CD_{14}^+ $TREM1^+$ with CD_{14}^+ $TGF\text{-}\beta1^+$ monocytes (Figure 1), along with a significant increase in $CD14^+TGF\text{-}\beta1^+$ monocytes and plasma TGF-β1 in patients (p<0.0001 and p=0.002, respectively) (Tables 2 and 3, and Figures 3 C and D). In this regard, similar to the results of this study, Peng et al. showed a positive correlation between TGF-β1 and TREM-1 overexpression in a mouse model of bleomycin-induced pulmonary fibrosis.[10] They also showed that the increase in TREM1 induced by TGF-β1 was dose- and time-dependent. Thus, this reciprocal synergistic effect of TGF-β1 and TREM1 may explain the disease's progressive nature. Interestingly, there was a positive correlation of $CD14^+TGF\text{-}\beta1^+$ monocytes and plasma TGF-β1 in patients with UIP (r=0.515; p=0.044) (Figure 1). It may indicate the possible role of $CD14^+$ monocytes as a possible source of TGF-β1 required for the development of pulmonary fibrosis in patients with UIP. It seems that this finding deserves further investigation to clarify differences in the mechanism of pulmonary fibrosis in patients with UIP and NSIP.

In addition to the important profibrotic role of TGF-β, it plays a key role in the survival, activity, and function of Treg cells.[24,25] Increased TGF-β1 and increased $CD4^+CD25^+Foxp3^+$ lymphocytes were reported in patients with pulmonary fibrosis (p=0.0001) (Table 2. Figure 4). Previously, increased Tregs and their positive correlation with the severity of the disease has been shown in IPF.[26, 27] Interestingly, decreased number of Tregs, along with a decrease in their suppressive activity, correlated with the severity of the disease.[28,29] In addition, TGF-β1-producing Tregs in murine lung fibrosis had an immunoregulatory effect by inducing the production of IL-10.[30] This is while patients' plasma IL-10 levels were not shown to be increased (Table 3) or correlated (unlike healthy controls) with TGF-β1 (Figure 1.). Treg cell's protective role depends on both TGF-β and IL-10, which is impaired in pulmonary fibrosis.[29,30]

In summary, this study confirms an interplay between TREM1 and TGF-β1 in pulmonary fibrosis, which was proposed in a previous study in a mouse model of pulmonary fibrosis.[10] Also, the defect in the protective role of the increased Treg cells in these patients was noticed with the lack of increase in plasma IL-10. The results emphasize the necessity of treatment using TREM-1 and TGF-β1 inhibitors while considering the necessary strategies to increase the short half-life of IL-10 to take advantage of the therapeutic effects of this cytokine (in the context of increased TGF-β1).

Although this study had several strengths, there were some limitations. These include the small number of participants, the limited range of monocyte and lymphocyte subsets and mediators investigated, and the lack of a validation cohort. In mitigation, the study is almost as large as several other studies attempting to compare UIP and NSIP. In addition, methodologically, it would have been optimal to have used anti-CD3 and anti-CD127 antibodies to identify the Treg population. Future studies are required in a larger multicenter cohort to validate these results.

In conclusion, current data suggest an interplay of TREM1 and TGF-β1 in pulmonary fibrosis. However, it needs to be extended extensively by multicenter studies for possible roles in disease progression and exacerbation episodes.

STATEMENT OF ETHICS

The study was approved by the ethical committee of the Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.MSP.REC.1398.568).

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CONFLICT OF INTEREST

The authors declare that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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