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Prognostic value of IFN-γ, sCD163, CCL2 and CXCL10 involved in acute exacerbation of idiopathic pulmonary fibrosis

Xianhua Gui[a,1], Xiaohua Qiu[a,1], Yaqiong Tian[a,1], Miaomiao Xie[a], Hui Li[a], Yujuan Gao[a], Yi Zhuang[a], Mengshu Cao[a], Hui Ding[b], Jingjing Ding[a,*], Yingwei Zhang[a,*], Hourong Cai[a,*]

[a] Department of Respiratory Medicine, The Affiliated Drum Tower Hospital of Nanjing University Medical School, No. 321 Zhongshan Road, Nanjing 210008, Jiangsu, PR China

[b] Department of Respiratory Medicine, Yixing People Hospital, Affiliated Jiangsu University, No. 75 Tongzhenguan Road, Yixing 214200, Jiangsu, PR China

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ABSTRACT

Objective: Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) is of concern because of its propensity for rapid deterioration and high mortality. Its aetiology and mechanism are still unclear. The aims of this study were to clarify the pathophysiology differences between AE-IPF and stable IPF (S-IPF) by comparing the serum levels of various cytokines and chemokines in the two groups and to identify those involvement in the occurrence of acute exacerbation and associated with mortality.

Methods: The study included 28 patients with AE-IPF, 32 patients with S-IPF, and 18 healthy control subjects. We measured the serum cytokine and chemokine levels in all cases by multiplex assay. Serum levels of cytokines and chemokines were compared between AE-IPF and S-IPF subjects. Logistic regression analysis was applied to identify the ability of these variables to predict acute exacerbation. Kaplan-Meier curves were used to analyse survival and Cox proportional hazard regression was used to identify predictors of survival.

Results: Levels of several cytokines and chemokines were significantly higher in both patient groups with IPF (with the exception of interleukin-2 [IL-2], chemokine cc-motif ligand 3, and RANTES [regulation upon activation normal T-cell express sequence]) than in healthy controls. Serum IL-1β ($p = 0.008$) and interferon (IFN)-γ ($p = 0.007$) levels tended to be higher in patients with AE-IPF than in those with S-IPF. The concentration of chemokine cc-motif ligand (CCL) 2 was significantly higher in bronchoalveolar lavage fluid than in serum ($p = 0.001$). Higher C-reactive protein, lactate dehydrogenase, percent forced vital capacity, percent diffusing capacity of the lung for carbon monoxide, and IFN-γ values in the patients with IPF were correlated with acute exacerbation status, with respective odds ratios of 1.241 ($p = 0.011$), 1.050 ($p = 0.004$), 1.043 ($p = 0.001$), 0.927 ($p = 0.014$), and 0.929 ($p = 0.020$). Acute exacerbation status was associated with an increased risk of mortality (hazard ratio 0.107, 95% confidence interval 0.036–0.314; $p < 0.001$). Univariate Cox regression demonstrated an association of IFN-γ, CCL2, C-X-C motif chemokine 10 (CXCL10) and sCD163 levels with an increased mortality risk ($p = 0.015$, $p = 0.002$, $p = 0.001$, and $p = 0.030$, respectively).

Conclusions: Our data demonstrate that serum levels of some pro-inflammatory cytokines and macrophage chemokines are upregulated during acute exacerbations of IPF and that these exacerbations are associated with the serum IFN-γ level. Chemokines and protein such as sCD163, CCL2, and CXCL10 are associated with activation of macrophages and may have a serious impact on overall survival in patients with IPF.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is characterised by progressive deterioration of dyspnoea and ultimately respiratory failure [1,2] and has a median survival time of 2–5 years from diagnosis [3]. Recently, it has been recognised that accelerated progression of respiratory symptom and acute deterioration of pulmonary function may be an important cause of mortality [4]. This process is known as acute exacerbation (AE) of IPF. Patients with AE-IPF experience sudden aggravation of dyspnoea and are found to have new ground-glass opacities on chest imaging and diffuse alveolar damage [4]. The incidence of AE-IPF has been reported to be 14% at 1 year and 20% at 3 years,

* Corresponding authors.
E-mail addresses: dingflower@hotmail.com (J. Ding), yingwei16@aliyun.com (Y. Zhang), caihourong2013@163.com (H. Cai).
[1] These authors contributed equally to this work.

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with > 90% of patients needing treatment in the intensive care unit and 50% dying within a short time after diagnosis [5,6]. AE is considered to be an important cause of mortality in patients with IPF. Therefore, there is an urgent need for circulating biomarkers of disease activity in IPF for more accurate evaluation of the risk of AE and prediction of survival.

The aetiology and mechanisms of AE-IPF are poorly understood. However, there is an increasing amount of evidence suggesting the importance of diffuse alveolar damage and enhanced epithelial injury during AE in patients with IPF [7,8]. It is well known that the cytokine and chemokine networks have multiple interactions and play a key role in diffuse alveolar damage. For instance, C-X-C motif chemokine 8 (CXCL8) was found to be a potent neutrophil attractant in lung fluid from patients with acute respiratory distress syndrome (ARDS) and has been reported to cause inflammation that accelerates alveolar damage [9]. An elevated serum IL-6 level reflects a high degree of local lung tissue damage and predicts the severity of ARDS [10]. Furthermore, tumor necrosis factor (TNF)-α and IL-1β are present in the bronchoalveolar lavage fluid (BALF) of patients with ARDS, promote polymorphonuclear degranulation, and release lysosomes that accelerate progression of diffuse alveolar damage [11]. Moreover, several studies have demonstrated the important role of cytokines and chemokines in AE-IPF. Papiris et al. reported that high levels of IL-6 and CXCL8 in patients with AE-IPF are associated with a worse outcome [12]. In addition, upregulation of pro-inflammatory and anti-inflammatory M2 macrophage cytokines has a strong influence on the risk of an AE [13]. These findings suggest that serum cytokine and chemokine levels could vary according to the status of the disease, including AE-IPF and S-IPF. Although several studies have demonstrated the role of a limited number of cytokines and chemokines in AE-IPF, the precise differences in levels of other cytokines/chemokines between these two subsets of patients remain unclear. Therefore, we investigated the characteristics of cytokine and chemokines in IPF with or without AE and assessed their probable role as indicators of the risk of AE-IPF and predictors of IPF-related mortality.

2. Materials and methods

2.1. Study subjects

The criteria used for enrolment of patients in this study are the same as those already published [1,4]. The inclusion criterion for IPF was detection of a pattern of usual interstitial pneumonia on high-resolution computed tomography (HRCT). Patients with other known causes of interstitial lung disease, such as domestic or occupational environmental exposure, connective tissue disease with autoimmune features, and drug toxicity, were included. The patients with S-IPF were required to have had no severe symptoms of dyspnoea or rapid deterioration on imaging for at least 3 months beforehand. AE-IPF was diagnosed according to the revised diagnostic criteria described by Collard et al. in 2016 [4]. Briefly, the criteria for a diagnosis of AE-IPF were as follows: 1) previous or concurrent diagnosis of IPF; 2) acute worsening or development of dyspnoea typically of less than one month in duration; 3) high-resolution computed tomography findings of new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with usual interstitial pneumonia; and 4) deterioration not fully explained by cardiac failure or fluid overload. Patients were excluded if they had complications, such as a malignant lung tumor, abnormal renal or liver function, or progression that was clearly associated with another disease. Clinical data were obtained from the medical records at admission and during follow-up. Blood samples were obtained from 60 adult Chinese patients with IPF (28 with AE-IPF, 32 with S-IPF) who were admitted to Nanjing Drum Tower Hospital between January 2016 and January 2018. Eighteen healthy subjects from the Center of Physical Examination were included as a control group. All subjects provided informed consent before participating in the study and gave permission for use of their serum for research purposes. This study was approved by the Ethics Committee at Nanjing Drum Tower Hospital and conducted in accordance with the principles of the Declaration of Helsinki (1989).

2.2. Measurement of cytokine and chemokine levels

The serum cytokine and chemokine levels were measured at WayenBiotechnology (Shanghai , CHINA) using a Luminex-100 Multiplex Bio-Assay Analyzer (Luminex Corporation, Austin, TX) according to the manufacturer's instructions. The calculations were performed using Bio-Plex Manager software version 5.0 (Bio-Rad Laboratories, Hercules, CA, USA). IL-1β, IL-2, IL-6, CXCL 8, interferon (IFN)-γ, chemokine cc-motif ligand (CCL) 2, TNF-α, CXCL10, and RANTES (regulation upon activation normal T-cell express sequence) levels were measured by Bio-Assay Analyzer and Luminex assays from Millipore Corporation (Billerica, MA, USA) and IL-1α, sCD163, CCL4, and CCL3 levels were measured by Multiplex Bio-Assay Analyzer and Luminex assays from R&D Systems (Minneapolis, MN, USA).

2.3. Bronchoscopy and bronchoalveolar lavage

Bronchoscopy and bronchoalveolar lavage (BAL) were performed under local anaesthesia. The bronchoscope was wedged in a middle lobe or lingual bronchus, with at least 100 ml of preheated sterile saline instilled in five aliquots of 20 ml each. The BAL samples obtained by bronchoscopy were placed on ice and then centrifuged at 1500 rpm for 10 min. Next, the BALF supernatant was collected and frozen at −80 °C.

2.4. Statistical analysis

Descriptive statistics for the clinical characteristics are presented as the median and interquartile range. Continuous variables were compared between the AE-IPF, S-IPF, and control groups using the Mann-Whitney *U* test. Spearman correlation analysis was used to evaluate the associations between cytokines and clinical parameters. Logistic regression analysis was used to identify cytokines and chemokines with a meaningful value for AE. Univariate and multivariate Cox regression models were created to identify predictors of time to death in patients with IPF. Survival time was calculated as the time from initial diagnosis until death or the censoring time. The results are summarised as hazard ratios, indicating the relative risk of death. The statistical analysis was performed using SPSS for Windows version 19 (IBM Corp., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics of the study population

The demographic and clinical characteristics of the 60 patients and 18 control subjects are shown in Table 1. There was no significant difference in the sex or age distribution or in the proportion of patients with a positive smoking history between the AE-IPF and S-IPF groups. Patients with AE-IPF had worse forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide (DLCO) and markedly higher C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels than those with S-IPF (Table 1).

3.2. Serum cytokine/chemokine levels and their correlation with study variables at baseline

The serum IL-1α, IL-1β, IL-6, CXCL8, IFN-γ, TNF-α, sCD163, CXCL10, CCL2, and CCL4 levels were elevated in patients with AE-IPF and in those with S-IPF (except for IFN-γ) when compared with the controls. However, serum IL-1β and IFN-γ levels were significantly higher in patients with AE-IPF than in those with S-IPF (2.7 [2.3, 3.5] versus 1.9 [1.6, 2.8] pg/ml, $p = 0.008$) and (8.3 [6.3, 11.5] versus 6.0

Table 1
Clinical characteristics of the study objects.

Clinical characteristics	Controls n = 18	AE-IPF patients n = 28	S-IPF patients n = 32	p value AE-IPF vs S-IPF
Age (years)	59.5 (50.8–65.0)	68.5 (66.0–72.3)	65.5 (55.5–72.8)	0.112
Male	88% (16)	89% (25)	96% (31)	0.244
Smoker (%)	0 (0)	61% (17)	43% (14)	0.181
Corticosteroids (treat in hospital)	0 (0)	89% (25)	9% (3)	< 0.001***
ICU (treat in hospital)	0 (0)	54% (15)	3% (1)	< 0.001***
FVC % predicted	NA	47.9 (43.5–57.8)	67.9 (58.9–74.8)	0.004**
DLCO % predicted	NA	34.1 (22.6–42.6)	49.0 (40.4–67.4)	0.016*
LDH (U/L)	NA	382.0 (326.3–500.0)	227.0 (199.8–250.5)	< 0.001***
CRP (mg/L)	NA	40.7 (16.8–86.5)	4.8 (2.1–14.2)	< 0.001***

CRP, C-reactive protein; LDH, lactate dehydrogenase; DLco, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity.
* $p < 0.05$ (Mann-Whitney *U* test).
** $p < 0.01$ (Mann-Whitney *U* test).
*** $p < 0.001$ (Mann-Whitney *U* test).

Table 2
Serum cytokines and chemokines of study population.

Variables	Control (n = 18), median (IQR)	AE-IPF patients (n = 28), median (IQR)	S-IPF patients (n = 32), median (IQR)	p value AE-IPF vs S-IPF
IL-1α (pg/ml)	1.7 (0.9–2.4)	8.3 (5.1–11.3)***	6.5 (5.3–9.1)###	0.135
IL-1β (pg/ml)	1.6 (1.4–2.1)	2.7 (2.3–3.5)***	1.9 (1.6–2.8)###	0.008
IL-2 (pg/ml)	NA	5.5 (2.1–8.9)	4.1 (0.5–10.7)	0.631
IL-6 (pg/ml)	1.8 (0.9–2.4)	3.9 (2.4–8.3)***	2.9 (2.1–3.9)###	0.067
CXCL8 (pg/ml)	20.1 (18.9–27.2)	63.1 (37.5–254.9)***	51.1 (28.6–143.0)###	0.314
IFN γ (pg/ml)	5.2 (3.1–6.5)	8.3 (6.3–11.5)**	6.0 (4.2–8.2)	0.007
TNF α (pg/ml)	26.2 (22.1–28.6)	42.5 (36.7–56.6)***	37.9 (30.8–51.1)###	0.118
sCD163 (ng/ml)	492.8 (353.9-726.9)	870.0 (523.9–1280.3)**	954.3 (557.8-1192.3)##	0.923
CXCL10 (ng/ml)	0.7 (4.1–8.2)	10.7 (0.8–40.1)***	1.2 (0.9–1.8)###	0.767
CCL2 (pg/ml)	22.8 (14.5–60.1)	62.6 (35.7–106.6)*	51.9 (30.1–78.5)#	0.197
CCL3 (pg/ml)	NA	619.5 (522.2–1078.7)	522.2 (316.4–700.9)	0.05
CCL4 (pg/ml)	346.7 (266.8–436.6)	745.1 (603.5–925.2)***	624.9 (508.6–753.6)###	0.054
RANTES (ng/ml)	7.6 (6.6–23.3)	11.5 (87.8–16.8)	9.8 (82.2–12.9)	0.415

The statistical comparisons of the patients with AE-IPF/S-IPF and the control subjects were performed using the Mann-Whitney *U* test. IQR, interquartile range; IL-1α, interleukin-1 alpha; IL-1β: interleukin-1β; IL-2, interleukin-2; IL-6, interleukin-6; CXCL8, C-X-C motif chemokine 8; IFN-γ, interferon-γ; TNF-α: tumor necrosis factor-α; sCD163, soluble CD163; CXCL10, C-X-C motif chemokine 10; CCL2, chemokine cc-motif ligand 2; CCL3, chemokine cc-motif ligand 3; CCL4, chemokine cc-motif ligand 4; RANTES, regulation upon activation normal T cell express sequence. AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis; IPF, idiopathic pulmonary fibrosis.
* $p < 0.05$ (for control subjects and patients with AE-IPF).
** $p < 0.01$ (for control subjects and patients with AE-IPF).
*** $p < 0.001$ (for control subjects and patients with AE-IPF).
# $p < 0.05$ (for control subjects and patients with S-IPF).
$p < 0.01$ (for control subjects and patients with S-IPF).
$p < 0.001$ (for control subjects and patients with S-IPF).

[4.2, 8.2] pg/ml, respectively, $p = 0.007$). The remaining variables tended to be higher in the AE-IPF group than in the S-IPF group (Table 2, Fig. 1). Correlation analyses of the clinical variables showed that the serum IL-1α, IL-1β, IL-6, CXCL8, IFN-γ, TNF α, sCD163, CXCL10, CCL2, and CCL4 levels in IPF had no association with lung function (FVC% or DLCO%). The serum IL-1β and IFN-γ levels correlated positively with the LDH level. There was also a positive correlation between the IL-6 and CRP levels. There was no correlation between any of the other study variables and the inflammatory markers (CRP and LDH; Table 3).

3.3. Cytokine and chemokine levels in BALF

The levels of several cytokines and chemokines in BALF, including IL-1α, CXCL8, TNF-α, CXCL10, CCL2, and CCL4, were tested in 4 of the 28 patients with AE-IPF and 4 of the 32 patients with S-IPF. Compared with the serum level, the level of CCL2 in BALF was significantly elevated (7305.5 [1140.2, 11,878.4] vs 62.9 [36.1, 86.5] pg/ml; $p = 0.001$). There were no between-group differences in the other variables (Table 4).

3.4. Meaningful value of cytokines and chemokines for AE in patients with IPF

On logistic regression, higher serum IFN-γ, CRP, and LDH levels and worse lung function (FVC%, DLCO%) were significantly associated with a higher risk of AE in IPF, with respective odds ratios of 1.241 (95% confidence interval [CI] 1.051–1.465, $p = 0.011$), 1.050 (95% CI 1.016–1.086, $p = 0.004$), 1.043 (95% CI 1.017–1.070, $p = 0.001$), 0.927 (95% CI 0.873–0.985, $p = 0.014$), and 0.929 (95% CI 0.874–0.988, $p = 0.020$). An increase in the serum IFN-γ level by 1 pg/ml increased the odds of AE by 24% (Table 5).

3.5. Prediction of survival in patients with IPF

Kaplan-Meier curves were generated for the two groups of patients. Survival of patients with AE-IPF was significantly worse than that of those with S-IPF. AE status was significantly associated with a higher risk of mortality (hazard ratio 0.10, 95% CI 0.036–0.314, $p < 0.001$; Fig. 2). Univariate Cox regression was used to evaluate the mortality risk in patients with IPF. Elevated INF-γ, sCD163, CXCL10, and CCL2

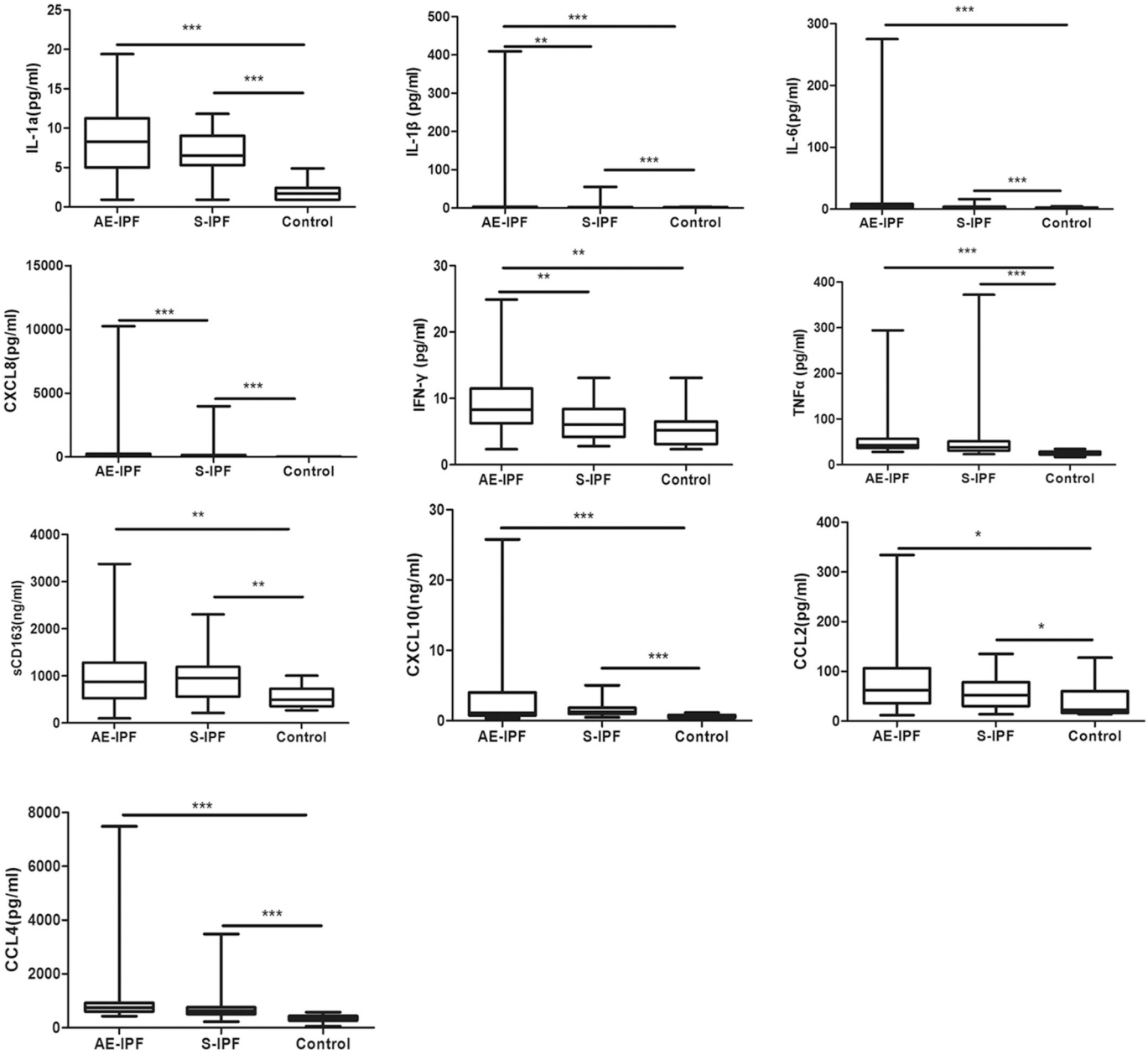

Fig. 1. Serum cytokine and chemokine levels in normal controls, patients with stable IPF and AE-IPF.
The data are presented as box plots that show the median and interquartile range (IQR) as a line. Serum IL-1β and IFN-γ levels were elevated significantly in AE-IPF patients (n = 28) when compared with S-IPF patients (n = 32) (2.7 [2.3, 3.5] versus 1.9 [1.6, 2.8] pg/ml, p = 0.008) and (8.3 [6.3, 11.5] versus 6.0 [4.2, 8.2] pg/ml, p = 0.007). Serum IL-1β in S-IPF patients was also increased significantly compared with normal controls (n = 18) (1.9 [1.6, 2.8] versus 1.6 [1.4, 2.1] pg/ml, p < 0.001).The remaining variables tended to be higher in the AE-IPF group than in the S-IPF group. AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis; IPF, idiopathic pulmonary fibrosis *p < 0.05, **p < 0.01, ***p < 0.001 (Mann-Whitney *U* test).

levels were markedly worse prognostic factors with respective hazard ratios of 1.096 (95% CI 1.018–1.180, p = 0.015), 1.000 (95% CI 1.000–1.001, p = 0.030), 1.124 (95% CI 1.050–1.203, p = 0.001), and 1.008 (95% CI 1.003–1.014, p = 0.002). Patient age, FVC, CRP, and LDH were also associated with the prognosis, with respective hazard ratios of 1.056 (95% CI 1.010–1.110, p = 0.031), 0.948 (95% CI 0.900–0.998, p = 0.043), 1.015 (95% CI 1.008–1.022, p < 0.001), and 1.008 (95% CI 1.005–1.012, p < 0.001). Subsequently, all of the variables that had been found to be important covariates were included in the multivariate analysis; however, no obvious associations with patient survival were found (Table 6).

4. Discussion

Acute exacerbation is a main contributor to mortality in patients with IPF. Many factors have been identified as triggers for AE, including mechanical procedures, laboratory variables, and secondary pulmonary hypertension [14–16], but the underlying mechanism remains unclear. In this study, we assessed the relationship between baseline clinical, laboratory, and serum variables in patients with IPF. All cytokine and chemokine levels included in the study were markedly increased in the patients with IPF, except for IL-2, CCL3, and RANTES, when compared with the controls. Serum levels of IL-1β and IFN-γ were significantly increased in the patients with AE-IPF, while the other cytokine and chemokine in this study were not significantly different from those in

Table 3
Correlation between serum cytokines and chemokines levels and clinical variables.

Variables	CRP (mg/l)	LDH (U/L)	FVC%	DLCO%
IL-1α	0.626	0.328	0.943	0.93
IL-1β	0.104	0.012*	0.701	0.976
IL-6	0.029*	0.227	0.859	0.253
CXCL8	0.534	0.116	0.915	0.366
IFN γ	0.187	0.029*	0.363	0.654
TNF α	0.352	0.262	0.723	0.066
sCD163	0.515	0.705	0.686	0.242
CXCL10	0.357	0.869	0.586	0.926
CCL2	0.224	0.072	0.483	0.563
CCL4	0.77	0.089	0.703	0.344

The statistical comparisons of the cytokines/chemokines and clinical variables were performed using Spearman's rank correlation coefficient.
* $p < 0.05$.

the patients with S-IPF. CRP and LDH levels were also higher in patients with AE. Elevated serum IFN-γ, CRP, LDH, and FVC% had significant value when evaluating AE status. The findings of this study suggest that AE in IPF was not incidental but triggered by endogenous factors, including inflammation and activation of macrophages.

It is well known that an AE of IPF typically manifests histopathologically as diffuse alveolar damage [17]. Multiple factors are involved in the pathogenesis of IPF, including injury of alveolar epithelial cells, alveolar inflammation, and cytokines, and rapid changes in these factors can lead to AE [4]. Upregulation of pro-inflammatory cytokines, such as IL-6, has been suggested to play an important role in AE-IPF [12]. It has been shown that IL-1β is a pro-inflammatory and profibrogenic cytokine that promotes the transformation and proliferation of fibroblasts and also inhibits degradation of the extracellular matrix and increases interaction with other pro-fibrotic cytokines [18–20]. In this study, we found higher levels of IL-1β during AE, indicating more severe inflammation than that present in patients with S-IPF.

As a Th1 cytokine, IFN-γ can promote progression of pulmonary fibrosis by regulating inflammation and cell apoptosis. Chen et al., found a significantly elevated IFN-γ level in the BALF of IFN-γ (−/−) mice models of pulmonary fibrosis showed less inflammation of the pulmonary parenchyma [21]. In addition, IFN-γ enhanced Fas/FasL-mediated apoptosis of alveolar epithelial cells, which play a key role in pulmonary fibrosis [22]. However, IFN-γ was able to inhibit fibroblasts proliferation and production of collagen. There has been an encouraging early report on the usefulness of IFN-γ in the treatment of patients with IPF, but its results are yet to be confirmed by a large-scale randomised controlled trial [23]. In the present study, we found higher levels of IFN-γ during AE, indicating more severe inflammation than that during stable IPF. Moreover, an elevated IFN-γ level was associated with an acute acceleration. However, none of our observations regarding the relationship between IFN-γ and patient survival rate was statistically significant, likely because of our relatively small study population.

Table 5
Variables associated with acute exacerbation status of IPF.

Variable	Odds ratio	95% CI	p value
Age (years)	1.062	0.993–1.136	0.078
Male	3.720	0.364–37.993	0.268
Smoker	1.558	0.749–3.241	0.236
CRP	1.050	1.016–1.086	0.004**
LDH	1.043	1.017–1.070	0.001**
FVC % predicted	0.927	0.873–0.985	0.014*
DLCO % predicted	0.929	0.874–0.988	0.020*
IL-1α	1.163	1.000–1.352	0.051
IL-1β	1.019	0.977–1.063	0.383
IL-2	1.032	0.913–1.166	0.613
IL-6	1.046	0.906–1.207	0.541
CXCL8	1.000	1.000–1.001	0.306
IFN γ	1.241	1.051–1.465	0.011*
TNFα	1.001	0.993–1.009	0.760
sCD163	1.000	0.999–1.001	0.534
CXCL10	1.248	0.947–1.644	0.116
CCL2	1.011	1.000–1.023	0.060
CCL3	1.000	1.000–1.001	0.272
CCL4	1.000	1.000–1.001	0.230
RANTES	1.016	0.949–1.087	0.651

The prognostic values of cytokines and chemokines for acute exacerbation of IPF (n = 60) were evaluated by logistic regression analysis. The data are shown as the OR with the 95% CI. OR, odds ratio; CI, confidence interval.
* $p < 0.05$.
** $p < 0.01$.

The serum CXCL10 level was also higher in the AE-IPF and S-IPF patient groups than in the control subjects. CXCL10 is a cytokine that is induced in monocytes and macrophages by IFN-γ and mediates recruitment of activated lymphocytes into the lungs [24]. CXCL10 levels in both serum and BALF have been reported to be markedly elevated in patients with idiopathic nonspecific interstitial pneumonia and a tendency towards elevation in patients with IPF; furthermore, the CXCL10 level in BALF was correlated with the number of lymphocytes in IPF patients [25]. The results of univariate Cox regression analysis in our study indicate that CXCL10 could play a role in the high mortality associated with IPF, which could be attributable to excessive accumulation of lymphocytes in the lung.

CCL2 as a proinflammatory chemokine is responsible for homing and migration of lymphocytes and for recruitment of mononuclear macrophages via binding to the CCR2 receptor, and is produced by a variety of cells, including alveolar macrophages, monocytes, and epidermal cells [26]. It is well known that macrophages play an important role in fibrosis by secreting a variety of cytokines and growth factors, regulating infiltration of inflammatory cells, removing damaged tissues, and collecting and activating myofibroblasts, which mediate pro-fibrotic and anti-fibrotic processes in the tissue. CCL2 has been detected to be markedly elevated in serum [27], BALF [28] and alveolar epithelium [19] in patients with IPF. Moreover, an elevated CCL2 level in BALF might be a predictor of a poor outcome in these patients [29]. Studies using murine models of pulmonary fibrosis have shown that

Table 4
Comparison of cytokines and chemokines in serum and BALF.

Variables	IPF patients (n = 8) at BAlF, median (IQR)	IPF patients (n = 8) in serum, median (IQR)	p value
IL-1α (pg/ml)	10.4 (8.6–56.2)	7.8 (2.4–10.8)	0.141
CXCL8 (pg/ml)	893.1 (210.3–3322.5)	87.7 (35.9–230.9)	0.059
TNF α (pg/ml)	21.3 (2.5–92.2)	43.1 (33.5–51.1)	0.294
CXCL10 (ng/ml)	0.8 (0.5–1.6)	1.1 (0.9–4.1)	0.203
CCL2 (pg/ml)	7305.5 (1140.2–11,878.4)	62.9 (36.1–86.5)	0.001**
CCL4 (ng/ml)	374.6 (340.2–776.8)	677.8 (562.5–924.4)	0.051***

AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis; IPF, idiopathic pulmonary fibrosis.
** $p < 0.01$.
*** $p < 0.001$, Mann-Whitney *U* test.

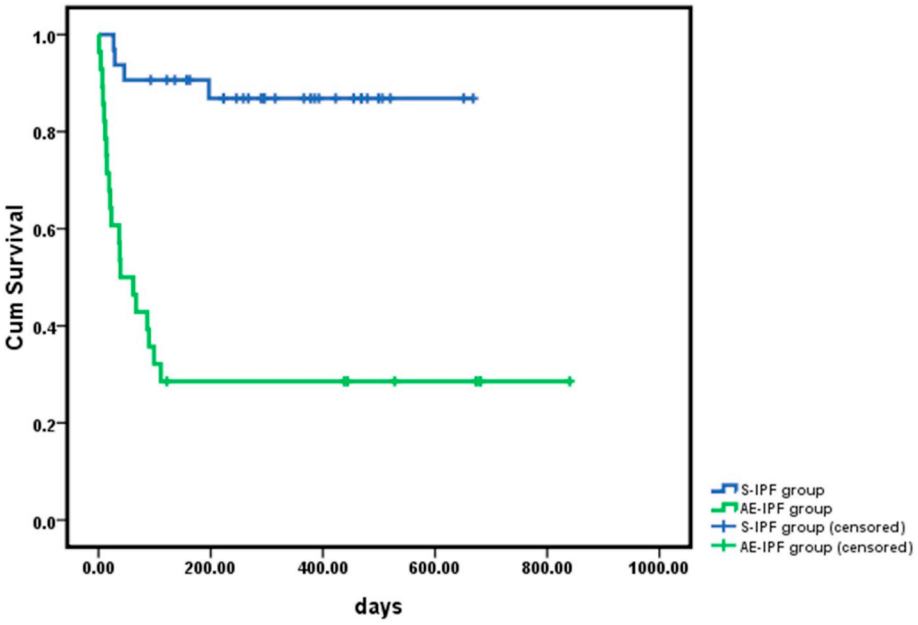

Fig. 2. Kaplan-Meier survival curves in patients with AE-IPF and S-IPF.
Kaplan-Meier survival curves showed that mortality was significantly higher in patients with AE-IPF than in those with S-IPF by log-rank test ($p < 0.001$). AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis; IPF, idiopathic pulmonary fibrosis.

Table 6
Associations of variables with survival status.

Clinical variables	Univariate model			Multivariate model		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Age (years)	1.056	1.010–1.110	0.031*	1.175	0.960–1.437	0.118
Male	3.533	1.047–11.922	0.052			
Smoker	0.850	0.380–1.890	0.680			
CRP	1.015	1.008–1.022	< 0.001***	1.044	1.000–1.090	0.051
LDH	1.008	1.005–1.012	< 0.001***	0.995	0.983–1.007	0.392
FVC % predicted	0.948	0.900–0.998	0.043*	0.907	0.818–1.006	0.061
DLCO % predicted	0.971	0.931–1.014	0.186			
IL-1α	1.119	1.013–1.236	0.051			
IL-1β	1.002	0.997–1.008	0.341			
IL-2	1.003	0.964–1.044	0.865			
IL-6	1.004	0.999–1.010	0.140			
CXCL8	1.000	1.000–1.000	0.252			
IFN γ	1.096	1.018–1.180	0.015*	1.445	0.899–2.323	0.128
sCD163	1.000	1.000–1.001	0.030*	0.997	0.994–1.001	0.107
TNFα	1.000	0.994–1.006	0.969			
CXCL10	1.124	1.050–1.203	0.001**	1.000	0.999–1.001	0.646
CCL2	1.008	1.003–1.014	0.002**	0.969	0.922–1.019	0.220
CCL3	1.000	1.000–1.000	0.313			
CCL4	1.000	1.000–1.000	0.278			
RANTES	1.022	0.976–1.070	0.349			

Predictive value of cytokines and chemokines for mortality of IPF (n = 60) were evaluated by univariate Cox regression. Important covariates found in univariate Cox regression were evaluated in the multivariate analysis. The data are shown as the HR with the 95% CI. HR, hazard ratio; CI, confidence interval.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

CCL2 contributes to fibrosis via an interplay between IL-13 and CCL2 that promotes proliferation of fibroblasts [30]. Moreover, decreased production of matrix metalloproteinase (MMP)-2 and MMP-9 was associated with alleviation of pulmonary fibrosis in CCR2-deficient mice [31,32]. In the present study, we found a difference in serum CCL2 levels between patients with IPF and the controls. We also found higher levels of MCP-1 in BALF than in serum, indicating that recruitment of alveolar macrophages could play an important role in IPF, which is consistent with the previous research.

Alveolar macrophages are also widely recognised to have a major role in the pathogenesis of lung fibrosis [33,34]. Although it is generally believed that alveolar macrophages in fibrotic lungs are alternatively activated M2 cells, Schupp et al. reported a distinct type of macrophage activation with features of M1 as well as M2 in AE of IPF [13].

CD163 is a transmembrane protein expressed on the cell membrane of monocytes and macrophages [35]. Soluble CD163 (sCDl63), in addition to being expressed on the cell membrane, is also found in serum and tissue fluid [36]. Macrophages can be polarised to two heterogeneous populations, i.e., classically activated (M1) and alternatively

activated (M2) [37]. The CD163 molecule is known to be a marker of the activation state of M2 macrophages. These macrophages secrete a variety of mediators, such as IL4, IL-10, IL-13, transforming growth factor-β, and IL-10, and promote the migration, accumulation, and transformation of fibroblasts, leading to pulmonary fibrosis [38,39]. In the present study, we found higher levels of sCD163 in patients with IPF; a trend towards an increase was also observed in patients with AE-IPF, indicating polarisation of M2 macrophages, which play an important role in pulmonary fibrosis.

Overall, our present findings indicate that inflammatory cytokines and chemokines may play a significant role in AE of IPF. However, we are aware that our study has some limitations. Our ability to perform serial analysis of cytokines and chemokines was limited because of the small study population, which is understandable in view of the rarity of IPF. Furthermore, there was a predominance of male subjects, so it is uncertain whether or not our results can be generalised to the entire population of patients with IPF. A longitudinal study in a larger IPF population would be helpful for further research.

In summary, we found that serum IL-1β and IFN-γ levels were elevated in patients with AE-IPF and that a high serum IFN-γ level was associated with AE. Acute exacerbation of IPF was closely related to an increased mortality risk. The levels of chemokines associated with activation of macrophages, i.e., sCD163, CCL2, and CXCL10, were markedly increased in patients with IPF, particularly in those with AE-IPF. Although this finding failed to reach statistical significance because of our limited study population, we believe that the chemokines associated with activation of macrophages may increase the mortality risk in patients with IPF.

Conflicts of interest

The authors have declared that no conflict of interest exists.

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