Presence of large scavenger receptor A-positive cells in systemic circulation: A potential prognostic indicator for idiopathic pulmonary fibrosis, connective tissue disease-associated interstitial lung disease, acute exacerbation and risk factor for acute exacerbation

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Abstract

Background: Acute Exacerbation (AE) of Idiopathic Pulmonary Fibrosis (IPF) and Connective Tissue Disease (CTD)-associated Inflammatory Lung Disease (ILD) are considered systemic disorders that may lead to multiple organ injury. Presumably, large scavenger receptor A-positive (SRA$^+$) cells in systemic circulation play a central role in multiple organ injury. We investigated the utility of large SRA$^+$ cells as a risk factor for AE and prognostic factor for IPF, CTD-associated ILD, and AE.

Methods: A total of 159 subjects with IPF or CTD-associated ILD and 71 with AE were examined. Fresh peripheral blood samples were examined with immune cytochemical method. Patients with IPF or CTD-associated ILD were classified into group A (large SRA$^+$ cells were not detected before the onset of an AE), group B (large SRA$^+$ cells were detected before the onset of an AE), and patients with AE were classified into group C (large SRA$^+$ cells were not detected at the onset or after the onset of an AE) and group D (large SRA$^+$ cells were detected at the onset or after the onset of an AE), and were followed up for 1 year.

Findings: Sixteen of 114 Group A patients and 23 of 45 of Group B developed AE. One of 15 Group C patients and 47 of 56 Group D died. The AE rate and the mortality rate were significantly higher in Group B and D than Group A and C. Kaplan-Meier curves for AE and survival of Group B and D were significantly worse than those of Group A and C.

Interpretation: Our data indicated that the appearance of large SRA$^+$ cells in systemic circulation might be a useful risk factor for AE, and prognostic factor for IPF, CTD-associated ILD, and AE.

Introduction

Many patients with Idiopathic Pulmonary Fibrosis (IPF) may experience sudden worsening of respiratory symptoms [1]. This frequently occurs without an identifiable cause, and is termed Acute Exacerbation (AE) of IPF [2]. Currently, AE have now also been reported in patients with Connective Tissue Disease (CTD)-associated interstitial lung disease (ILD) and other ILDs [3,4]. Respiratory failure from AE is associated with high in-hospital mortality [5-7]. For this reason, sensitive biomarkers and prognostic factors are necessary to predict the onset of AE as well as the need for early interventions. Several physiologic candidate risk factors and prognostic factors have been reported [8]. Elevated serum level of Krebs von den Lungen-6 (KL-6) at baseline has been associated with increased risk for AE-IPF [9], but results were conflicting [10].

In a previous report, we reported that 1) AE of IPF and CTD-associated ILD as systemic disorders that lead to multiple organ injury, 2) large scavenger receptor A positive (SRA$^+$) cells, tumor necrosis factor (TNF)-α positive cells and neutrophils in systemic circulation played a central role in multiple organ injury, and 3) large SRA$^+$ cells had been detected in many patients before the onset of an AE, conversely, TNF-α$^+$ cells had been detected after the onset of an AE [11].

The aim of this study was to investigate whether the presence of large SRA$^+$ cells in systemic circulation is as a risk factor for AE and whether it can serve as a prognostic factor for IPF, CTD-associated ILD, and AE thereof.

Methods

Patients with IPF and CTD-associated ILD (during April 2009 and October 2015) and those with AE (during April 2005 and October 2015) were examined. The Japanese Red Cross Nagaoka Hospital ethical committee approved the study (No. 406).

IPF and CTD-associated ILD group

A total of 159 consecutive patients with IPF or CTD-associated ILD were chosen for the present study (Table 1). All patients were classified

Table 1. Baseline characteristics of the study population with idiopathic pulmonary fibrosis and collagen disease associated-inflammatory lung disease, and acute exacerbation rate.

Variable	Group A (n=114)	Group B (n=45)	p
Age	65.2 ± 9.9	67.7 ± 9.6	0.139
Sex (M) n, (%)	57(50%)	21(47%)	0.42
KL-6	1663 ± 1388	1241 ± 585	0.008
Coll. Dis. n, (%)	43(37%)	16(35%)	0.474
Steroid n, (%)	82(72%)	24(53%)	0.04
Pulse n, (%)	9(8%)	8(18%)	0.088
Pulse + CMP n, (%)	9(8%)	12(26%)	0.003
AE n, (%)	16(14%)	23(51%)	<0.001

n: number, Coll. Dis.: Collagen disease, Pulse: Steroid pulse therapy, CPM: cyclophosphamide, AE: Acute exacerbation

into two groups: Group A patients (114 cases) without large SRA+ cells in cytological preparations before the onset of an AE, and Group B (45 cases) with one or more large SRA+ cells in cytological preparations identified at one or more-time points before the onset of an AE. Patients in Group A were followed for 1 year from hospitalization. Large SRA+ cells were detected during the clinical course in many of group B patients. So, patients in Group B were followed for 1 year from the detection of large SRA+ cells to assess the utility of the presence of large SRA+ cells as a risk factor for AE.

Acute exacerbation group

A total of 71 consecutive patients with AE of IPF or CTD-associated ILD were chosen for the present study (Table 2). All patients were classified into Group C (large SRA+ cells were not detected in cytological preparations at the onset or after the onset of an AE: 15 cases) or Group D (large SRA+ cells were detected at the onset or after the onset of an AE: 56 cases), and were followed for 1 year from the onset of an AE.

Patients whose immediate causes of death were considered to be severe bronchopneumonia, aspiration pneumonia, lung cancer, heart failure or bacterial sepsis were excluded from the analysis.

Cytology

Blood samples of 159 Group A and B patients obtained before the onset, at the onset and after the onset of an AE were examined, but those of 32 Group C and D patients obtained before the onset of an AE (14 patients who developed AE during 2005 to 2008 and 18 patients whose diagnosis at admission was AE) were not examined. The rest of fresh peripheral blood samples obtained for the examination of differential white blood counts during the hospitalization were examined (median times [range], 4 [1-18]). Peripheral blood was collected into tubes containing EDTA (Vacutainer plastic, EDTA 2K; Becton Dickinson, Franklin Lakes, NJ, USA). Red blood cells were lysed with lysing reagent (826 mg NH_4CL + 3.7 mg EDTA-4Na + 100 mg $KHCO_3$ in 100 ml H_2O). Nucleated cells were suspended in isotonic sodium chloride solution. Suspensions containing about 5×10^6 nucleated cells were smeared on glass slides using Auto smear CF-12 (Sakura Seiki, Tokyo, Japan). Cells that did not adhere to glass slides were gently washed off with 95% ethanol solution. Approximately 1.0×10^6 nucleated cells were evenly smeared in one 1.2×1.2 cm^2 area. Smear preparations were fixed in 95% ethanol solution and stained using the Papanicolaou method.

Immunocytochemistry

Papanicolaou-stained smears were used for examination, which was performed with the simple stain MAX-PO method (NICHIREI Co., Tokyo, Japan) and with diaminobenzidine as the chromogen using mouse monoclonal anti-human SRA antibody (CD204, a macrophage SRA marker, 1:200; Trans Genic Inc., Kumamoto, Japan). An antigen retrieval method using citrate buffer and microwave heating was employed. As a negative control, the primary antibody was substituted by phosphate-buffered saline. In these controls, no positive stain was observed.

KL-6: We measured KL-6 levels (median times [range], 2 [1–5]) using the KL-6 electrochemiluminescence immunoassay kit (EIDIA Co., Ltd., Tokyo, Japan) with <500 U/ml as the reference value. The highest level of KL-6 during the hospitalization of each patient was presented.

Diagnoses: We chose the patients who met the diagnostic criteria of IPF or AE-IPF [12,13]. The diagnosis of AE of CTD-associated ILD was made using the same criteria as IPF. SRA+ cells in peripheral blood that were over twice as large as monocytes were defined as large SRA+ cells, and SRA+ cells smaller than large SRA+ cells were defined as small SRA+ cells.

Statistical analysis

All continuous variables were reported as mean ± SD. Continuous variables were compared by a t-test or Fisher's exact test. Discrete variables were analyzed by using the chi-square test. Survival analyses were performed by using the Kaplan-Meier method and log-rank tests. In all analyses, a p-value less than 0.05 was considered to be statistically significant. The software SPSS statistics version 17.0 (SPSS Japan Inc., Tokyo, Japan) was used for data management and statistical analyses.

Results

Study population

IPF and CTD-associated ILD group: Baseline characteristics are shown in Table 1. Ninety-three of 152 cases satisfied the diagnostic criteria for IPF and 59 patients presented CTD-associated ILD. Percent vital capacity were measured 1 or 2 times during admission in 81 patients (Group A: 54, B:27). We chose lower value, and there was no significant difference between group A (68.0 ±13.2) and B (65.1 ± 15.9).

Acute exacerbation group: Of 71 cases, 14 patients developed AE during 2005 to 2008. Thirty-nine patients were from the Group A and B. Nine of 16 cases from Group A were classified into Group C, and 7 of the 16 cases and all 23 from Group B were classified into Group D. The diagnosis at admission was AE in 18 cases. Baseline characteristics are shown in Table 2.

Cytological findings

Small SRA+ cells were detected in all cases. Only one to five large SRA+ cells were observed in some of the smears of patients in Group B

Table 2. Baseline characteristics of the study population with acute exacerbation and the mortality rate.

Variable	Group A (n=15)	Group B (n=56)	p
Age	70.5 ± 8.5	69.4±10.0	0.679
Sex (M) n, (%)	6(40%)	30(54%)	0.397
KL-6	2657 ± 2245	1633 ± 1004	0.105
Coll. Dis. n, (%)	3(20%)	13(23%)	1
Steroid n, (%)	5(33%)	7(13%)	0.113
Pulse n, (%)	4(27%)	18(32%)	0.763
Pulse + CPM n, (%)	6(40%)	31(55%)	0.386
Death n, (%)	1(7%)	47(84%)	<0.001

n: number, Coll. Dis.: Collagen disease, Pulse: Steroid pulse therapy, CPM: cyclophosphamide

before the onset of an AE (Figure 1). Large SRA+ cells were detected before the onset or at the onset of an AE in 49 of 56 Group D patients (87.5 %), and after the onset of an AE in 7 group D patients from Group A (12.5 %).

Risk factor

Sixteen of 114 (14 %) patients in Group A and 23 of 45 (51 %) in Group B developed AE. The AE rate was significantly higher in Group B than Group A (Table 1, *p*<0.001). The Kaplan-Meier curve of Group B showed a significantly worse AE than in Group A. (Figure 2, *p*<0.001).
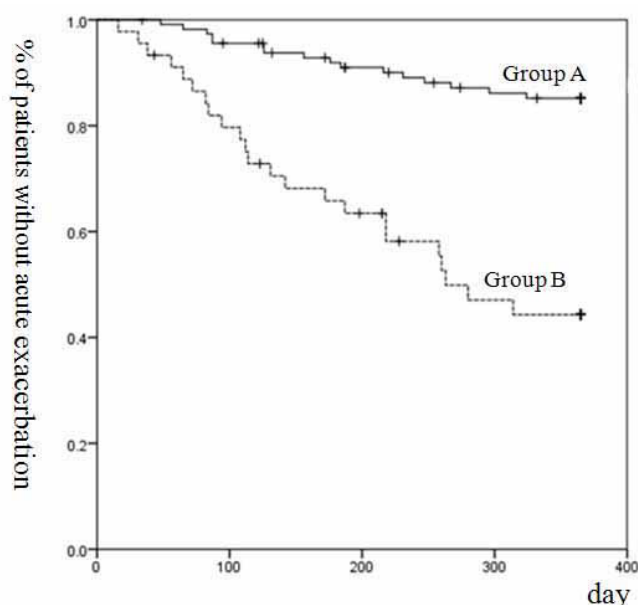
Mortality

One of 15 (7%) patients in Group C and 47 of 56 (84%) in Group D died of respiratory failure. The mortality rate of was significantly higher
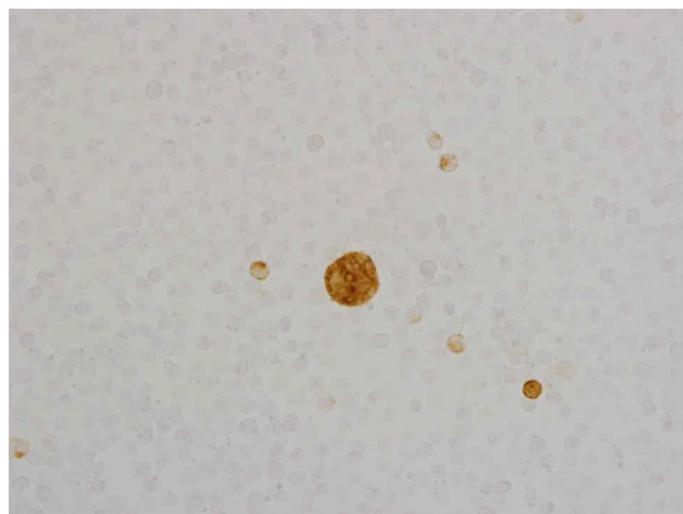

Figure 1. Scavenger receptor A positive cells in peripheral blood.
Footnote: Large and small scavenger receptor A-positive cells.

Figure 2. Kaplan-Meier analysis for the onset of an acute exacerbation of IPF and CTD associated ILD.
Footnote: Kaplan–Meier survival curve for acute exacerbation of group B was significantly worse than that of Group A. (*p* <0.001, log-rank test). IPF, idiopathic pulmonary fibrosis; CTD, connective tissue disease; ILD, inflammatory lung disease
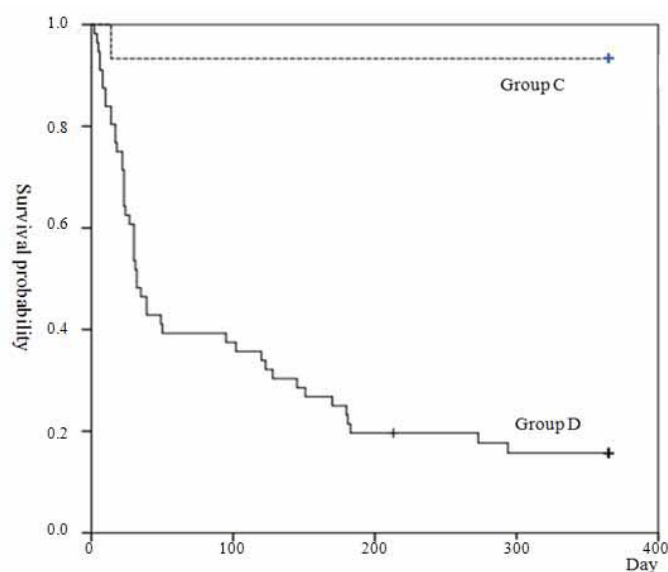
Figure 3. Kaplan-Meier survival curve for acute exacerbation groups.
Footnote: Kaplan–Meier survival curves of group D showed significantly worse survival than Group C (p<0.001, log-rank test).

in Group D than in Group C (Table 2, *p*<0.001). Kaplan-Meier survival curves of Group D showed significantly worse survival than in Group C (Figure 3, *p*<0.001).

Discussion

The present our data indicated that the appearance of large SRA+ cells in systemic circulation might be a useful risk factor for AE, and prognostic factor for IPF, CTD-associated ILD, and AE.

Forty-eight of 71 (67.6 %) patients with AE (Group C: 1 and Group D: 47) died. Our present results support those of previous reports indicating that respiratory failure from AE of IPF or CTD-associated ILD is associated with high in-hospital mortality [5-7]. Furthermore, the AE rate was significantly higher in Group B than Group A, and the Kaplan-Meier curve of Group B showed a significantly worse AE than in Group A. All 23 patients in Group B who developed AE were classified into Group D. The mortality rate of was significantly higher in Group D than in Group C. Kaplan-Meier survival curves of Group D showed significantly worse survival than in Group C. From these data, the presence of large SRA+ cells seemed to be a useful indicator of the poor prognosis of IPF, CTD-associated ILD, and AE.

The AE rate was significantly higher in Group B than in Group A. The Kaplan-Meier curve for AE of Group B was significantly worse than that of Group A. Our present data indicated that the appearance of large SRA+ cells might be a useful risk factor for AE.

Diffuse alveolar damage is frequently observed in patients who have died from an AE of IPF [14-16], but pathological findings of systemic organs were not described in these reports. Autopsy was performed in 14 of 47 Group D patients and we reported that 1) Multiple organ injury (including diffuse alveolar damage) was confirmed in all cases, 2) Large SRA+ cells and TNF-α+ cells in systemic circulation were considered to play central roles in multiple organ injury. 3) large SRA+ cells were detected before the onset of an AE and TNF-α+ cells were detected after the onset of an AE in all cases [11]. In the present study, large SRA+ cells were detected before the onset or at the onset of an AE in 45 Group B patients, and after the onset of an AE in 7 group D

patients from Group A. These finding indicate that during the clinical course, the disease continued to progress, even after the onset of an AE, eventually leading to multiple organ injury.

Large SRA$^+$ cells were detected in multiple organ dysfunction syndrome cases (including ARDS cases) [16], and the coincidence of the appearance of large SRA$^+$ cells and increase of small SRA$^+$ cells might be useful to predict poor outcome in patients with systemic inflammatory response syndrome or multiple organ dysfunction syndrome [17]. We speculated that large SRA$^+$ cells differentiated from monocytes in systemic circulation. SRA-negative monocytes become positive for SRA by the stimulation of macrophage colony-stimulating factor [18,19]. Endothelial cells, fibroblasts, etc. have an ability to produce one or other colony stimulating factors [20], and are activated locally by injury of cells and tissues to induce production and secretion of cytokines [21]. ARDS develops secondary to a wide range of primary insults [22]. We could find definite primary insults responsible for the differentiation of large SRA$^+$ cells in each our multiple organ dysfunction syndrome case [16]. However, we could not find any evidence of extra-pulmonary primary insults responsible for the differentiation of large SRA$^+$ cells in IPF and CTD-associated ILD cases [11]. It seemed important to find definite primary insult in IPF and CTD-associated ILD cases for the better understanding of AE.

We acknowledge certain limitations of this study. First, we consider that the cytological method used in this study for the examination of peripheral blood was originally developed by us. Thus, we could not find any papers discussing the presence of large SRA$^+$ cells in the peripheral blood of patients with IPF, CTD-associated ILD, or AE. We could not compare our results with those of other reports. Second, as this was a single-center study, the patient population and standards of care may vary from those in other centers. Thus, the reported results may not be broadly representative. However, the cytological method is used worldwide and the presence of large SRA$^+$ cells in systemic circulation may be an attractive marker, as blood is readily available, and its measurement can be easily standardized. Future studies are necessary to confirm the clinical utility of the presence of large SRA$^+$ cells in circulation as a risk and prognostic factor for patients with IPF, CTD-associated ILD, or AE.

Acknowledgement

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