Acute/subacute interstitial pneumonia in dermatomyositis (DM-A/SIP) is a disease associated with a poor prognosis that resists treatment with glucocorticosteroids (GC) and progresses rapidly in a period of weeks to months to death [1,2]. Recently, early intervention with a combination of GC and a calcineurin inhibitor such as cyclosporine A (CSA) and tacrolimus and this combination plus intravenous pulse cyclophosphamide therapy (IVCY) was reported to improve the prognosis of DM-A/SIP [3–6]. However, there are still many patients who cannot be saved even with these regimens. Early additional immunosuppressant therapy is necessary for such patients, but an accurate prognosis or evaluation of the severity of the disease is difficult before the beginning of treatment.

Clinical amyopathic DM (C-ADM), autoantibodies such as anti-aminoacyl tRNA synthetase (ARS) and anti-melanoma
differentiation-associated gene 5 (MDA5) antibodies (Ab), high-resolution computed tomography findings such as ground-glass attenuation and reticular opacity, and a decrease in the diffusing capacity of respiratory function tests have been reported as findings related to the severity and prognosis of DM-IP [6–12]. Recently, among serum biomarkers, KL-6 and ferritin have been reported to be useful prognostic factors for DM-A/SIP [9,13,14]. However, prognostic factors of DM-A/SIP are difficult to analyse because, in addition to these factors, the disease type of IP, time until the beginning of treatment, and therapeutic strategy are also known to affect the outcome.

We retrospectively investigated the relations of blood gas analysis results and serum markers such as KL-6 and ferritin before the beginning of treatment with outcome in DM-A/SIP patients who underwent early CSA/GC combination therapy and 2-hour postdose blood concentration (C2) monitoring. We also evaluated the relations of prospective prognostic factors with outcome in DM-A/SIP.

Materials and Methods

Study design

Participants in this retrospective study comprised 41 patients with DM-A/SIP admitted to Osaka Medical College Hospital during the period from March 2004 to April 2012. DM was diagnosed according to the criteria of Bohan and Peter [15,16]. Clinical amyopathic DM was diagnosed according to the criteria proposed by Southemier and Gerami et al. [17,18]. IP was evaluated with chest radiography and chest HRCT. A/SIP was defined as IP with a respiratory condition, laboratory findings, arterial gas findings, and HRCT images rapidly exacerbating in a period of days to 3 months after disease onset [3,19]. After excluding 1 patient with ANCA-related vasculitis, 1 with scleroderma, 6 who had received high-dose GC therapy, steroid pulse therapy, or immunosuppressant therapy before admission to our hospital, and 1 in whom CSA could not be used due to an adverse event (liver dysfunction), 32 patients were analysed. The patients were divided into those who were still alive and those who had died by 24 weeks after the beginning of CSA/GC combination therapy. Those who died were divided into those who died due to DM-A/SIP and those who died due to causes other than deterioration of IP, such as infection. Clinical and laboratory findings were compared between the survivors and those who had died due to DM-A/SIP, prognostic factors were extracted, and their relations with survival rate were evaluated.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Osaka Medical College and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

Treatment

The administration of CSA and prednisolone (PDN) was initiated simultaneously in all patients a mean of 4.5±2.5 days after admission. PDN and CSA were orally administered at 0.75–1.0 and 3.5–4.5 mg/kg/day, respectively. CSA was administered once a day before breakfast by adjusting the C2, which is correlated with the immunosuppressant effect of CSA, at 1,500 ng/ml or above [20,21]. In this study, the addition of steroid pulse therapy, IVCY, and intravenous immunoglobulin (IVIG) was permitted depending on the disease state.

Clinical findings and laboratory parameters

Patient background, period from the appearance of respiratory symptoms to the beginning of treatment, and contents of treatment were evaluated. The laboratory test items evaluated were creatine kinase, lactic acid dehydrogenase, the creatine kinase/lactic acid dehydrogenase ratio, aldolase, creatinine, C-reactive protein, ferritin, KL-6, antinuclear antibodies, anti-MDA5 and anti-ARS Ab: anti-OJ, anti-EJ, anti-PL-7, anti-PL-12 and anti-Jo-1 Ab. Anti-MDA5 Ab was determined by enzyme-linked immunosorbent assay using recombinant MDA5 antigen (Ori-gene, USA) as described previously [22]. Anti-ARS Ab was determined using a commercially available line blot test kit (Myositis Profile Euroline Blot test kit, Euroimmun, Lu¨beck, Germany). On blood gas analysis, PaO2, PaCO2, and the alveolar-arterial oxygen gradient (P[A-a]O2) were evaluated. The P[A-a]O2 was calculated approximately using the following formula:

\[ P[A-a]O_2 = 713 \times \frac{FiO_2}{FiO_2 \times PaCO_2} - 0.8 - PaO_2 \]

Statistical analysis

Statistical analyses were performed with Fisher’s exact test or the Mann-Whitney U-test for the comparison of baseline clinical and laboratory findings. Data are presented as the mean ± s.e.m. and were analysed with the statistical program JMP for Windows, version 9.0 (SAS Institute Inc., Cary, NC, USA). To determine the most suitable cut-off level, we used receiver operating characteristic (ROC) curve analysis. To identify prognostic factors, multivariate analyses with a multiple logistic regression model were conducted including the variables with significant differences on univariate analysis. Age- and sex-adjusted Cox regression analysis was performed to establish the prognostic factors. The Kaplan-Meier method was used to assess survival curves and the log-rank test to evaluate the significance of differences between the two groups. A P value of <0.05 was considered to indicate significance.

Results

Outcomes of patients with DM-A/SIP treated by CSA/GC combination therapy

Figure 1 shows the outcomes at 24 weeks after the beginning of early CSA/PDN combination therapy. Of the 32 DM-A/SIP patients, 25 survived and 7 died. Of these 7 patients, 5 died due to exacerbation of DM-A/SIP within 8 weeks after the beginning of treatment. The remaining 2 died due to sepsis and cytomegalovirus infection.

Comparison of clinical and laboratory findings between survivors and dead

Table 1 shows the clinical findings and contents of treatment in the 5 patients who died of DM-A/SIP and the 25 survivors. No significant differences were observed in age, sex, number of C-ADM, or time from the appearance of respiratory symptoms to the beginning of treatment between the surviving patients and those who died. In the 5 patients who died, the mean survival period after the beginning of treatment was 6.2±2.5 weeks, indicating early death (P<0.001). Concerning the contents of treatment, there was no difference in the dose of PDN. Although the dose of CSA was significantly higher in those who died compared with the survivors, no difference was observed in the C2 of CSA between
the two groups. Also, steroid pulse therapy, IVCY, and IVIG were used significantly more frequently in those who died.

Table 2 shows the laboratory findings from the two groups. Among the serum biomarkers, the ferritin level was significantly higher in those who died than in the survivors ($P<0.001$). No difference was observed in the creatine kinase, lactic acid dehydrogenase, the creatine kinase/lactic acid dehydrogenase ratio, aldolase, creatinine, C-reactive protein values between the two groups. Although there was no difference in the positive rate of ANA between the two groups, all patients positive for anti-ARS Ab survived. No significant differences were noted in PaO$_2$ or PaCO$_2$, but the difference was not significant ($P=0.085$). No difference was observed in the KL-6 level as well in those who died than in the survivors, but the difference was not significant ($P=0.053$).

Table 3 shows the hazard ratios of the prognostic factors. Ferritin and P[A-a]O$_2$ were suggested as independent prognostic factors of poor outcome in DM-A/SIP patients treated with early CSA/PSL combination therapy (table 3).

**Table 1. Clinical characteristics of patients.**

|                | Dead   | Survivors | $P$ value |
|----------------|--------|-----------|-----------|
| No.            | 5      | 25        | -         |
| Age, yrs       | 65.6±10.3 | 57.2±9.5 | 0.080     |
| Female, n (%)  | 3 (60.0) | 21 (84.0) | 0.254     |
| C-ADM, n (%)   | 5 (100) | 17 (68.0) | 0.286     |
| From onset to treatment, months | 2.5 (1.0–3.5) | 2.5 (1.0–5.5) | 0.832     |
| PDN, mg/kg/day | 1.0 (0.8–1.1) | 1.0 (0.8–1.1) | 0.075     |
| CSA, mg/kg/day | 4.0 (3.5–4.5) | 4.0 (3.5–4.5) | 0.046     |
| CSA trough, ng/ml | 191.4 (154.1–456.0) | 175.4 (95.0–445.7) | 0.175     |
| CSA C2, ng/ml  | 1488 (1262–2003) | 1953 (814–2873) | 0.984     |
| Steroid pulse, n (%) | 4 (80.0) | 3 (12.0) | 0.006     |
| IVCY, n (%)    | 4 (80.0) | 10 (40.0) | 0.042     |
| IVIG, n (%)    | 3 (60.0) | 0 (0.0) | 0.003     |
| Survival time, weeks | 7 (2–8) | 24 (24–24) | <0.001    |

**Table 2. Pre-treatment laboratory findings of patients.**

|                | Dead   | Survivors | $P$ value |
|----------------|--------|-----------|-----------|
| Ferritin, ng/ml | 1611 (1013–2376) | 133 (28–1102) | <0.001    |
| KL-6, U/ml     | 1329 (512–3789) | 1064 (206–2620) | 0.085     |
| ANA, n (%)     | 3 (60.0) | 16 (69.6) | 1.000     |
| Cytoplasmic staining, n(%) | 0 (0.0) | 8 (50.0) | -         |
| Anti-ARS antibodies, n (%) | 0 (0.0) | 11 (68.8) | -         |
| Anti-MDA5 antibodies, n (%) | 2 (100) | 2 (18.1) | -         |
| PaO$_2$, Torr | 652.5 (51.8–101.7) | 70.2 (50.5–97.6) | 0.985     |
| PaCO$_2$, Torr | 375.2 (30.0–40.0) | 38.1 (32.1–67.6) | 0.285     |
| PaO$_2$/FiO$_2$ ratio | 281 (245–348) | 325 (186–464) | 0.062     |
| P[A-a]O$_2$, Torr | 83.5 (46.0–432.5) | 28.5 (4.8–61.3) | 0.001     |

**Table 3. Hazard ratios (per unit) of prognostic factors in acute/subacute interstitial pneumonia in dermatomyositis.**

| Prognostic factor | Hazard ratio | 95% CI | $P$ value |
|-------------------|--------------|--------|-----------|
| Ferritin          | 1.003        | 1.001–1.005 | 0.002 |
| P[A-a]O$_2$       | 1.045        | 1.017–1.085 | 0.017 |

Age- and sex-adjusted Cox regression analysis was performed to establish the prognostic factors. doi:10.1371/journal.pone.0089610.t003
Cut-off values of ferritin and $P[A-a]O_2$ and survival rate

To determine cut-off points effective for the prognosis of DM-A/SIP, ROC curve analysis was carried out on ferritin and the $P[A-a]O_2$. The values that maximised the area under the ROC curve were 595.7 ng/ml for ferritin (sensitivity: 87.5%, specificity: 100%) and 41.9 Torr for $P[A-a]O_2$ (sensitivity: 91.7%, specificity: 100%). From these results, a ferritin level of $\geq 600$ ng/ml and a $P[A-a]O_2$ of $\geq 45$ Torr were determined as cut-off values for a poor prognosis. The patients were then divided into 2 groups on the basis of these cut-off values, and Kaplan-Meier survival curves were plotted (Figures 2 and 3). The survival rate after 24 weeks was significantly lower in patients with both rather than 1 or none of these factors.

Survival rate by number of poor prognostic factors

Table 4 shows the number of these prognostic factors observed in each of the survivors and those who died. The number was significantly higher in those who died than in the survivors ($P<0.001$). Figure 4 shows Kaplan-Meier survival curves by the number of prognostic factors. Whereas all patients who had both factors died within 8 weeks, no patient with neither or only 1 of the factors died, with a clear difference in the survival rate ($P<0.001$).

Discussion

We evaluated prognostic factors in DM-A/SIP patients treated using early CSA/PDN combination therapy. Similar to the findings of previous reports, the ferritin level at the beginning of therapy was higher in those who died of DM-A/SIP than in those who survived, and the outcome was poor at ferritin levels of $\geq 600$ ng/ml [9,13]. Also, the $P[A-a]O_2$ was increased more in those who died than in the survivors, and the prognosis was poor at a $P[A-a]O_2$ of $\geq 45$ Torr. A ferritin level of $\geq 600$ ng/ml and $P[A-a]O_2$ of $\geq 45$ at the beginning of treatment were independent prognostic factors in DM-A/SIP patients, and the outcome was significantly poorer in patients with both rather than 1 or none of these factors.

The production of ferritin is increased by the activation of reticulendothelial cells, particularly macrophages. An elevation of the ferritin level in DM-A/SIP patients is considered to indicate the activation of alveolar macrophages and reflects inflammation of and damage to the lungs [9,13]. Ferritin has been reported as a prognostic factor in DM-A/SIP patients. Gono et al. reported that the serum ferritin level was markedly increased in DM-A/SIP patients and that the survival rate was significantly lower in patients with a ferritin level of $\geq 1,500$ ng/ml [9]. Moreover, they reported that the serum ferritin level was increased in DM-A/SIP patients positive for anti-MDA5 Ab and was related to the prognosis and disease activity [8]. In our study also, the outcome was poor when the ferritin level at the beginning of treatment was elevated to $\geq 600$ ng/ml. The ferritin level that exacerbated the prognosis differed compared with that in previous reports, but this discrepancy is considered to have been due to the limited number of subjects, time of ferritin measurement, patients’ background, and treatment contents.

The $P[A-a]O_2$ is a simple index of the pulmonary diffusion capacity and is known to increase in IP patients due to interstitial proliferation [23]. In this study, the $P[A-a]O_2$ as well as ferritin level at the beginning of treatment were found to be prognostic factors of DM-A/SIP with early CSA/PDN combination therapy. The $P[A-a]O_2$ was reportedly increased in anti-MDA5 antibody-positive patients who died due to rapidly progressing DM-IP compared with survivors [9]. In the acute exacerbation of DAD and interstitial pneumonia, pulmonary diffusion is severely impaired by fibrosis of the alveolar matrix or by inflammatory cell infiltration. On respiratory function testing, the degree of gas transfer reduction is reflected by the decrease in the diffusing
The blood KL-6 level, which increases due to the excessive expression of pulmonary alveolar type II epithelial cells and an increase in alveolar vascular permeability, is a biomarker used for the diagnosis and evaluation of IP. In patients with IP including DM, the prognosis was reported to be exacerbated when the KL-6 level was $\geq 1,000$ U/l [14]. However, regarding DM-IP in particular, there have been no reports that the pre-treatment KL-6 level is related to disease severity or outcome. There was no difference in KL-6 level between those who died due to DM-A/SIP and the survivors in this study. Araiz et al. reported that the KL-6 level 2–4 weeks after the beginning of treatment was markedly elevated in those who died due to DM-IP compared with those who survived [27]. The clinical course of DM-A/SIP is rapidly progressive and this biomarker may not reflect the disease activity in rapid course of interstitial pneumonia.

This study was carried out retrospectively in a small number of patients. In addition, autoantibodies and lung high-resolution computed tomography findings were not evaluated in all enrolled patients or in a large number of patients. Prognostic factors associated with the disease must still be extracted by evaluating a larger number of patients.

**Conclusion**

We showed that increase of the serum ferritin level and P[A-a]O2 are prognostic factors in DM-A/SIP patients and that the outcome is exacerbated when these factors are present. Serum ferritin and P[A-a]O2 are easy and readily-available screening tools. This study provides a simple and low-cost approach to risk-stratify DM-A/SIP patients upon admission thereby assisting clinicians in evaluating which patients may benefit from more aggressive initial immunosuppressive therapy.

**Author Contributions**

Conceived and designed the experiments: KI TK TT. Performed the experiments: KI. Analyzed the data: KI KH TI. Contributed reagents/materials/analysis tools: KI TS SY YK SM TH. Wrote the paper: KI.

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**Table 4. Number of prognostic factors and survival rate in the study patients.**

| No. of prognostic factors | Dead (n = 5) | Survivors (n = 16) | Survival rate (%) |
|---------------------------|-------------|-------------------|------------------|
| 0                         | 0           | 13                | 100              |
| 1                         | 0           | 3                 | 100              |
| 2                         | 5           | 0                 | 0                |

The number of subjects P < 0.001.
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