asbestosis.[5] This suggests that a variety of chronic fibrosing processes of the lung can present a potential risk for AE.

Because a specific treatment for AE has not yet been established, the mortality rate in patients with AE of IPF remains high.[6] Furthermore, patients with secondary usual interstitial pneumonia (UIP) or a pathological pattern of NSIP, who generally have a more favourable prognosis than those with IPF, also show high mortality from AE.[2 3]

Recently, factors predicting the onset of AE have been described in patients with IPF. These include a high modified Medical Research Council score, a high body mass index, a decline in forced vital capacity (FVC) at 6 months from diagnosis[7]; a low FVC.[8] Factors affecting survival in patients with AE of IPF have also been described[9] and include high-resolution CT (HRCT) patterns (diffuse/multifocal/peripheral) of acute pulmonary infiltrates, a degree of CT involvement and a serum lactate dehydrogenase titre. Another report has found that the extents of ground-glass attenuation with traction bronchiectasis or bronchiolectasis and honeycombing on HRCT were the two independent prognostic factors in patients with AE of IPF.[10] CT findings directly associated with disease severity may predict patient survival; however, the exploration of more objective and easily applicable predictors of mortality would be of value for developing rational management strategies, including novel therapeutic approaches.[11]

In the present study, we retrospectively analysed 51 consecutive patients with AE of idiopathic chronic fibrosing interstitial pneumonia (CFIP) in order to identify novel in-hospital mortality predictors that are present on admission.

METHODS

Subjects

Consecutive patients with AE of idiopathic CFIP admitted to our department between January 2009 and May 2012 were retrospectively studied. During that interval, 56 patients were admitted for AE of CFIP. Of those, 19 patients were successfully managed to hospital discharge (survivors) and 37 patients died during hospitalisation (non-survivors). Of the latter, five patients who died from causes other than AE were excluded: two acute myocardial infarction (both of the patients had a medical history of old myocardial infarction), one rupture of aortic aneurysm after the graft replacement was performed 3 months ago and two advanced lung cancer (AE was improving or did not progressively worsen by administration of glucocorticoids). Eight patients with suspected AE who were diagnosed with lower respiratory tract infection by bacterial culture of specimens from the lower respiratory tract (4 patients), bacterial antigens in the urine (2 patients) and an elevated serum β-d-glucan (2 patients) had been excluded in advance.

During the study period, 218 patients with idiopathic CFIP had been under the management of our department and of them, 116 patients had been diagnosed with IPF. Of the 51 patients studied, 26 had been outpatients of our department and the remaining 25 were referred ones. The mean interval between diagnosis of CFIP and admission was 42.3 months, although the data excluded three patients who had not been previously diagnosed with CFIP. Eleven of the 51 patients had been treated with immunosuppressants: glucocorticoids, 8 patients and glucocorticoids and cyclosporine, 3 patients.

Diagnostic criteria

The following features based on the previously used criteria[12] were used to define an AE event: (1) aggravation of dyspnoea within 1 month, (2) decline of ≥10% in absolute FVC or a decline of ≥10 torr in Pao_2 or a decline of ≥5% in Spo_2, (3) new ground-glass opacities or consolidation on the chest radiograph or thin-section CT (TSCT), (4) negative respiratory culture and serological test results for respiratory pathogens and (5) no clinical evidence of pulmonary embolism, congestive heart failure or pneumothorax as a cause of acute decline.

Significant decline in oxygenation was confirmed by previous Pao_2 or Spo_2 in every patient. In 5 of 26 referred patients, previous chest radiographs had not been obtained; however, all of them evidently showed honeycombing on TSCT on admission. Extensive ground-glass opacity (GGO) was compatible with acute onset of respiratory distress. Cultures of the sputum or tracheobronchial aspirate for common bacteria, mycobacteria and fungi, urinary antigens for *Streptococcus pneumoniae* and *Legionella pneumophila* serotype 1, antigens for influenza A and B viruses by pharyngeal swab, antigenaemia for cytomegalovirus, serum antigen and antibody for *Aspergillus*, and a titre of serum β-d-glucan were examined in every patient studied and those disclosed negative results. Bronchoalveolar lavage was performed in 17 patients and all the bronchoalveolar lavage fluids were negative for routine microbiological culture. Of those, PCR detection for genomes of certain microorganisms (common bacteria, mycobacteria, *Aspergillus* and *Pneumocystis jirovecii*) was also performed in seven patients and gave negative results.

The diagnosis of idiopathic CFIP was first based on TSCT results: presence of a diffuse parenchymal lung disease with significant pulmonary fibrosis, defined as reticular opacities with peripheral and basal predominance, honeycombing, architectural distortion and/or traction bronchiectasis or bronchiolectasis.[13] Patients found to have other distinct diseases on the basis of clinical and/or radiographic findings, associated with the development of pulmonary fibrosis, such as connective tissue disease, drug-induced lung disease, pneumoconiosis, hypersensitivity pneumonitis, sarcoidosis, pulmonary histiocytosis and lymphangioleiomyomatosis, were

excluded. The diagnosis of IPF was made according to the 2011 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association (ATS/ERS/JRS/ALAT) statement on IPF.[14] For this study, we diagnosed patients with IPF when they displayed features that fit the criteria for UIP as assessed by HRCT or met the criteria for UIP after a combination of HRCT and surgical lung biopsy findings.

CT imaging

TSCT was performed for all patients, usually on the day of admission, using a Somatom Sensation 64 (Siemens Medical Solutions, Forchheim, Germany). Images were obtained with 1.5 mm collimation and 1.5 mm slice intervals from the pulmonary apex to the lung base, reconstructed with a high-spatial-resolution algorithm and a small field of view. The images were analysed at a window level of −700 HU and a window width of 1400 HU.

The extent of the TSCT findings characteristic of AE and CFIP were determined; a thoracic radiologist and a pulmonary physician, both of whom were experts in interstitial lung diseases, examined the CT images without knowledge of any of the clinical, functional and radiographic findings. GGO was defined as an area of slightly increased attenuation in which the vessels remained visible. Honeycombing was defined as an accumulation of cystic spaces with thickened walls. Emphysema was defined as well-demarcated areas of decreased attenuation in comparison with contiguous normal lung and marginated by a very thin (<1 mm) or no wall with upper zone predominance. The extent of GGO and honeycombing was scored to the nearest 10% at the six lung zones: right upper and middle lobes, left upper segment and lingula and bilateral lower lobes. The scores at the six lung zones were then averaged out to obtain a mean score.[15] The results by the two readers were well correlated (GGO: r=0.88, $p=2.27\times10^{-17}$ and honeycombing: r=0.92, $p=2.15\times10^{-20}$). Finally, GGOs at the time of AE were classified as peripheral, multifocal or diffuse parenchymal patterns, as described previously.[16]

Because concurrent emphysema may affect an accurate CT diagnosis of UIP and NSIP,[17] CT images before admission were also reviewed, especially for distinguishing honeycombing from emphysema with GGO. We could access previous CT images for 40 of the 51 patients. In the remaining 11 patients, serial thoracic CT images were also reviewed in addition to the CT image obtained on admission not to misdiagnose emphysema with GGO as honeycombing.

Systemic inflammatory response syndrome

The diagnosis of systemic inflammatory response syndrome (SIRS) was determined at the time of admission, according to the previously defined criteria: temperature >38°C or <36°C, heart rate >90 bpm, respiratory rate >20 breaths/min or $Paco_2$ <32 torr and white blood cell count >12 000/μL, <4000/μL or >10% band forms. Participants who met two or more of the criteria were diagnosed with SIRS.[18]

Data expression and the statistical analysis

Clinical data are expressed as mean±SD for continuous variables. Group comparisons were made using the Mann-Whitney U test for continuous variables. The χ^2 and Fisher's exact tests were used for categorical variables. Logistic regression analysis was performed to determine the relationships between clinical parameters. A Kaplan-Meier model was generated to evaluate survival. Univariate and multivariate analyses using the Cox proportional hazards regression models were used to identify independent patient characteristics, laboratory data and CT predictors of hospital mortality. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing V.2.13.0). More precisely, it is a modified version of R Commander V.1.6-3 designed to include statistical functions frequently used in biostatistics.[19] Statistical significance was defined as a p value of <0.05.

RESULTS

Patient profiles and the symptom duration

The flow diagram in figure 1 shows how the patients were identified. We finally identified 51 patients with AE of idiopathic CFIP (37 men and 14 women) between January 2009 and May 2012. The mean age, male-to-female ratio, smoking history and the mean smoking index were similar in non-survivors and survivors. The mean duration of symptoms (cough or dyspnoea) previous to admission was significantly shorter in non-survivors (p=0.0069, table 1). The mean durations of hospitalisation were 33 days in non-survivors and 72 days in survivors.

Diagnosis of idiopathic CFIP and TSCT findings

In this cohort, 30 patients were diagnosed with IPF according to the 2011 criteria,[14] and the remaining 21

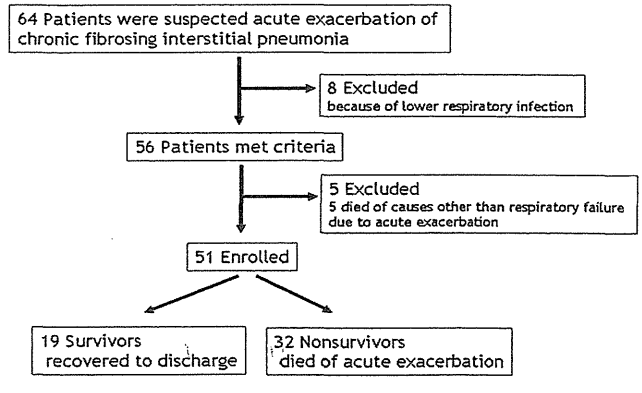

Figure 1 Flow diagram of patients with acute exacerbation of chronic fibrosing interstitial pneumonia.

Table 1 Clinical characteristics of the patients studied

	Non-survivors	Survivors	p Value
Age (years)	72±10	69±5	0.22
Male/female	23/9	14/5	0.91
Smoking history (±)	23/9	15/4	0.48
Smoking index (pack-years)	28±25	45±37	0.071
Duration of symptoms before admission (days)	7±4	14±9	0.0069

Comparison between groups was performed by Mann-Whitney's U test (for continuous variables) or the χ^2 test (for categorical variables).

patients were diagnosed with non-IPF. The latter group included one patient with upper lobe-dominant pulmonary fibrosis and two patients with familiar pulmonary fibrosis. The remaining 18 patients showed findings consistent with fibrotic NSIP on TSCT.[20]

The prevalence of IPF, the extent of GGO and the extent of honeycombing were not significantly different between non-survivors and survivors (table 2). The peripheral distributions of GGO and centrilobular emphysema (CLE) were significantly less prevalent in non-survivors (p=0.0032 and 0.015, respectively).

Laboratory data and SIRS

The mean peripheral blood lymphocyte count was significantly lower in non-survivors (p=0.040, table 3). The mean serum brain natriuretic peptide (BNP) titre was elevated in both groups and significantly higher in non-survivors (p=0.0098). The mean white blood cell counts, serum C reactive protein, serum Krebs von den Lungen-6, serum surfactant protein D, serum procalcitonin (PCT) and the mean D-dimer titres were elevated in both groups, but were not significantly different between groups. The mean Pao_2:Fio_2 (P:F) ratio on admission was significantly lower in non-survivors (p=0.012). On admission, 28 of 32 non-survivors and 4 of 19 survivors fulfilled the criteria for SIRS ($p=9.9\times10^{-7}$, table 3). The mean SIRS score was significantly higher in non-survivors ($p=5.4\times10^{-6}$). The SIRS score and the serum PCT level were positively correlated (p=0.0045, figure 2).

Treatment and the response

Glucocorticoid pulse therapy (methylprednisolone at 1000 mg/day for 3 consecutive days) was performed in 31 of 32 non-survivors and all of the 19 survivors (table 4). Immunosuppressants other than glucocorticoids and mechanical ventilation were used at similar rates in the two groups. The mean P:F ratio on the day after glucocorticoid pulse therapy was significantly lower in non-survivors (p=0.00018).

Prognosis

In non-survivors, the median survival time was 33 days. In survivors, two patients died from a second AE event after discharge from hospital. The overall survival was 67% at 30 days, 43% at 60 days and 40% at 90 days after admission. The Kaplan-Meier estimate for overall survival revealed that the survival rate reached a plateau of approximately 35% at approximately 180 days from admission (figure 3). In this cohort, the TSCT classification (a definite UIP pattern, a possible UIP pattern and inconsistent with the UIP pattern) did not reveal a significant difference in survival by the Kaplan-Meier estimate (figure 4, p=0.51).

Univariate and multivariable analyses of survival

Univariate analysis (table 5) revealed that symptom duration prior to admission, the extent of honeycombing on CT, the presence of CLE, serum PCT level, P:F ratio on admission, the presence of SIRS and the SIRS score were significant predictors of mortality. The P:F ratio on the day after glucocorticoid pulse therapy was also significant in univariate analysis. Multivariable analysis revealed that the serum PCT (HR per 10% increase was 2.7110, 95% CI 1.1770 to 6.4890; p=0.022), the presence of CLE (HR 0.0606, 95% CI 0.0161 to 0.2290; $p=3.6\times10^{-5}$) and the presence of SIRS (HR 6.2810, 95% CI 1.4220 to 27.7500; p=0.015) remained significant predictors after adjusting for age, sex, P:F ratio on admission and extent of honeycombing (table 6). When five patients in whom the cause of death was other than AE were included in the multivariate analysis, PCT (HR

Table 2 Classification of the CFIP and TSCT findings

	Non-survivors	Survivors	p Value
IPF/non-IPF	19/13	11/8	0.94
Extent of honeycombing (%)	17±13	11±10	0.12
Extent of GGO (%)	56±15	49±15	0.10
Distribution of GGO: diffuse/multifocal/peripheral	29/1/2	12/1/6	0.0032
CLE (±)	7/25	11/8	0.015
PSE (±)	15/17	12/7	0.39

Comparison between groups was performed by Mann-Whitney's U test (for continuous variables) or the χ^2 test (for categorical variables).
CFIP, chronic fibrosing interstitial pneumonia; CLE, centrilobular emphysema; GGO, ground-glass opacity; IPF, idiopathic pulmonary fibrosis; PSE, paraseptal emphysema; TSCT, thin-section CT.

Table 3 Laboratory data and SIRS on admission

	Non-survivors	Survivors	p Value
WBC (/μL)	12 041±6450	9712±3315	0.26
Lymphocyte (/μL)	1182±780	1447±570	0.040
CRP (mg/dL)	9.7±6.0	10.4±8.7	0.90
KL-6 (U/mL)	1513±687	1535±1013	0.63
SP-D (ng/mL)	427±321	360±262	0.54
BNP (pg/mL)	168±164	93±171	0.0098
D-dimer (mg/mL)	4.1±4.6	6.2±10.5	0.35
PCT (ng/mL)	0.33±0.60	0.13±0.12	0.17
Pao_2:Fio_2 ratio	157±53	213±81	0.012
SIRS (±)	28/4	4/15	9.9×10^{-7}
SIRS score	2.5±0.9	1.1±1.0	5.4×10^{-6}

Comparison between groups was by Mann-Whitney's U test (for continuous variables) or the χ^2 test (for categorical variables).
BNP, brain natriuretic peptide; CRP, C reactive protein; KL-6, Krebs von den Lungen-6; PCT, procalcitonin; SIRS, systemic inflammatory response syndrome; SP-D, surfactant protein D; WBC, white blood cell count.

2.3980, 95% CI 1.1000 to 5.2240; p=0.028), CLE (HR 0.1752, 95% CI 0.06269 to 0.4894; p=0.00089) and SIRS (HR 5.2600, 95% CI 1.4950 to 18.5100; p=0.0097) continued to be significant and, in addition, age also showed significance (HR 1.0440, 95% CI 1.0020 to 1.0870; p=0.039).

On the basis of the results of multivariate analysis, we divided 51 patients into four subgroups according to the presence or absence of CLE and SIRS (group 1: SIRS− and CLE+, group 2: SIRS− and CLE−, group 3: SIRS+ and CLE+, and group 4: SIRS+ and CLE−). A Kaplan-Meier estimate according to this subgrouping revealed a clear distinction in the prognoses between the four subgroups (figure 5, p=0.0025). All patients in group 1 recovered and survived to discharge; by contrast, all patients in group 4 died of AE.

DISCUSSION

In the present study, the signs and symptoms of 18 of 21 patients diagnosed with non-IPF were suggestive of fibrotic NSIP on TSCT.[20] There is the possibility that

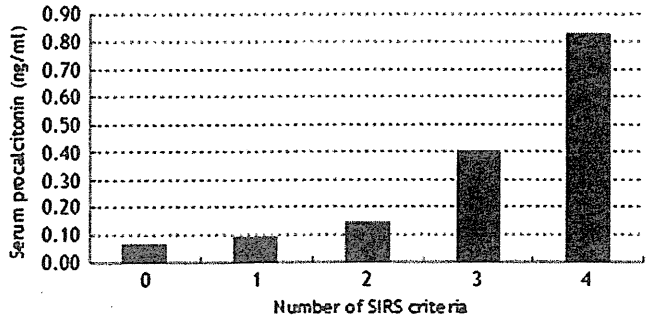

Figure 2 The number of systemic inflammatory response syndrome (SIRS) criteria was positively correlated with the serum procalcitonin concentration (p=0.045). The number of subjects was as follows: 7 (SIRS score 0), 12 (score 1), 11 (score 2), 13 (score 3) and 4 (score 4), respectively. Logistic regression analysis was performed for significance.

patients with IPF were included in the non-IPF group, and vice versa. However, whether a patient had IPF or not was not associated with the outcome in our cohort. Furthermore, the prognostic difference between patients with definite UIP, possible UIP and findings inconsistent with UIP on TSCT was not significant. Thus, we considered that there were convincing reasons to place all participants studied in the CFIP category. There have been a few reports on AE of non-IPF, including a study of patients with idiopathic NSIP[2 3] in which the overall mortality rate was as high as 50%. This suggests that AE, a manifestation of diffuse alveolar damage, may commonly be fatal, irrespective of an individual pattern of CFIP.

Attention should be paid to the differences between survivors and non-survivors in clinical, laboratory and radiographic findings in this cohort. The relatively short symptom duration before admission in non-survivors might be dependent on the severity of the lung injury that has not yet been strictly objectified. Radiographically, factors for discriminating between non-survivors and survivors were the distribution pattern of acute pulmonary infiltrates and the presence of CLE, but not the presence of paraseptal emphysema. CT patterns have been shown to be associated with mortality in patients with AE of IPF;[9] however, there may be a degree of subjectivity in the diagnosis of this condition, and interobserver differences must be considered. Among laboratory data, the serum BNP level and the P : F ratio on the day after glucocorticoid pulse therapy, as well as those on admission, were discriminative. We performed echocardiography in every patient with signs and symptoms clinically suggestive of cardiac dysfunction or those with increased serum BNP, and none showed left ventricular dysfunction. The higher P : F ratio after glucocorticoid pulse therapy suggests that this treatment might be effective for a certain subset of patients. The presence of SIRS and the number of SIRS criteria fulfilled were the factors that provided the most significant discrimination between survivors and non-survivors.

Table 4 Treatment and the response

	Non-survivors	Survivors	p Value
Glucocorticoid pulse therapy (±)	31/1	19/0	1.0
Immunosuppressants other than glucocorticoids (±)	11/21	2/17	0.096
Mechanical ventilation (±)	12/20	3/16	0.12
Pao_2:Fio_2 ratio on the day after glucocorticoid pulse therapy	147±67	267±108	0.00014

Comparison between groups was performed by Mann-Whitney's U test (for continuous variables) or the χ^2 test (for categorical variables).

The univariate analysis revealed that the extent of honeycombing was associated with survival; this was not apparent in the group comparison. On the basis of the results of the univariate analysis, we performed a multivariate analysis of survival, and it revealed some notable results: the most significant factors for predicting in-hospital mortality were the presence of SIRS and the absence of CLE on TSCT, and the serum PCT level was also found to be associated with outcome.

The presence of SIRS, first defined in 1992, indicates a systemic inflammatory response to a variety of severe clinical insults, including non-infectious causes such as trauma, burns, pancreatitis, etc.[18] The definition of SIRS is simple, and thus can be easily applied in routine medical care; however, the clinical significance of SIRS has been controversial. In infected participants, the number of SIRS criteria fulfilled did not influence patient outcome, despite the fact that the presence of organ dysfunction or shock showed prognostic significance.[21] In our study, SIRS was significantly more prevalent in non-survivors, and was one of the most significant predictors of hospital mortality. The influence of SIRS on mortality in patients with AE of CFIP has not yet been examined, probably because the disease process has been recognised as a disorder compartmentalised to the lung. Compared with stable IPF patients, patients with AE of IPF has been reported to show increases in serum biomarkers associated with endothelial cell injury, such as thrombomodulin or plasminogen activator inhibitor-1 (PAI-1).[22] In addition, increases in levels of interleukin 8 or intracellular adhesion molecule 1, known as molecules related to neutrophil recruitment to the lung, were reported to be independent mortality predictors in patients with acute lung injury/acute respiratory distress syndrome.[23] In our cohorts, the mean ratio of bronchoalveolar neutrophils in 17 patients who underwent bronchoalveolar lavage was 20% (data not shown). These data suggest a possibility that acute lung injury mediates systemic inflammation via certain circulating molecules, as in the case of AE of CFIP. The significance of SIRS as a mortality predictor should be confirmed in future studies. Investigations to determine the mechanisms of SIRS development in patients with AE of CFIP may be essential to allow the development of more specific treatments.

PCT, a prohormone of calcitonin, is produced in the medullary C cells of the thyroid gland. It was described as an infection parameter in the early 1990s[24] and is now considered to be a useful serum biomarker for

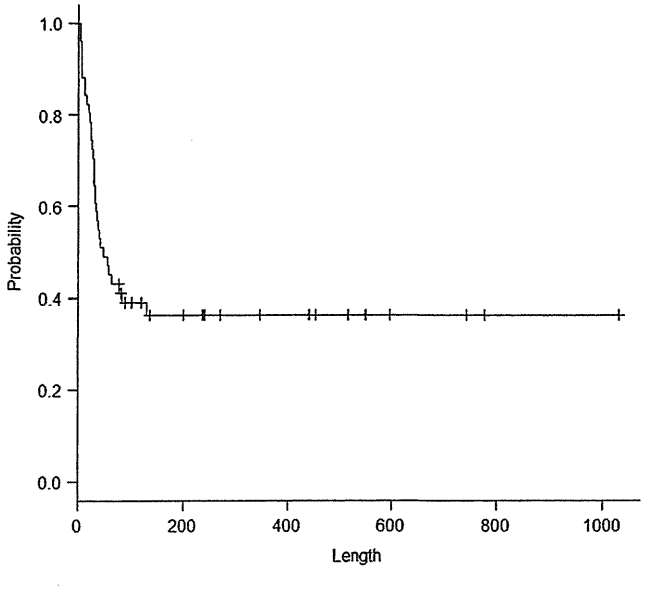

Figure 3 A Kaplan-Meier estimate for overall survival. The x axis indicates days after admission.

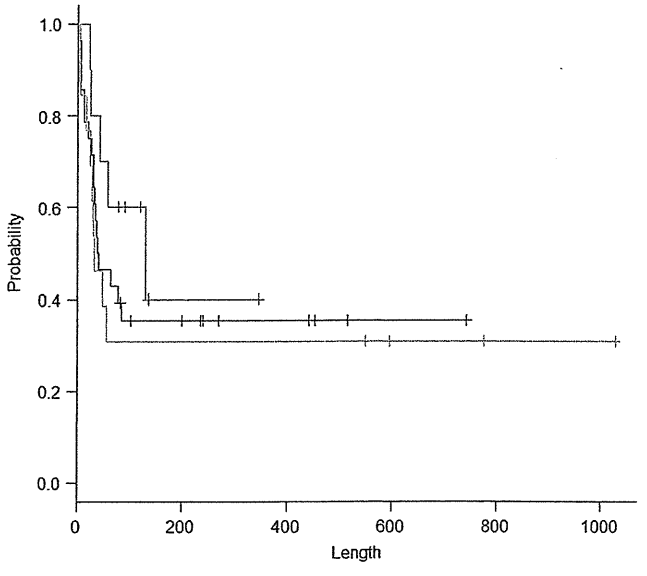

Figure 4 A Kaplan-Meier estimate for survival according to the thin-section CT classification: define usual interstitial pneumonia (UIP) pattern (black, 28 patients), possible UIP pattern (green, 13 patients) and inconsistent with UIP pattern (red, 10 patients). The x axis indicates days after admission.

Table 5 Univariate analysis of survival

	HR	95% CI	p Value
Age (years)	1.018	0.973 to 1.065	0.35
Male sex	1.083	0.50 to 2.345	0.84
Duration of symptoms before admission (days)	0.893	0.824 to 0.969	0.0066
Smoking index (pack-years)	0.9995	0.9989 to 1.0001	0.14
IPF/non-IPF	1.024	0.501 to 2.091	0.95
Extent of ground-glass opacity (%)	1.014	0.990 to 1.039	0.23
Extent of honeycombing (%)	1.031	1.004 to 1.06	0.023
CLE (±)	0.356	0.153 to 0.829	0.017
PSE (±)	0.785	0.392 to 1.574	0.50
Lymphocyte (/μL)	0.9996	0.9990 to 1.0002	0.22
CRP (mg/dL)	0.9929	0.947 to 1.041	0.77
KL-6 (U/mL)	1.000	0.9996 to 1.0004	0.88
SP-D (ng/mL)	1.0005	0.9992 to 1.0019	0.43
BNP (pg/mL)	1.001	0.9992 to 1.0028	0.27
D-dimer (mg/mL)	0.976	0.921 to 1.045	0.49
PCT (ng/mL)	1.889	1.063 to 3.545	0.030
Pao_2:Fio_2 ratio on admission	0.992	0.987 to 0.997	0.0038
Pao_2:Fio_2 ratio on the day after glucocorticoid pulse therapy	0.989	0.984 to 0.994	1.75×10^{-5}
SIRS (±)	11.85	3.551 to 39.54	5.78×10^{-5}
Number of SIRS criteria	1.98	1.448 to 2.707	1.87×10^{-5}

Cox proportional hazards regression models were used for the statistical analysis.
BNP, brain natriuretic peptide; CLE, centrilobular emphysema; CRP, C reactive protein; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; PCT, procalcitonin; PSE, paraseptal emphysema; SIRS, systemic inflammatory response syndrome; SP-D, surfactant protein D.

early diagnosis of bacterial infections.[25] On the other hand, increase of serum PCT levels caused by non-infectious processes have also been reported. In a retrospective analysis of patients who underwent cardiopulmonary bypass surgery but did not have postoperative infection, Hensel *et al*[26] described a significant increase in PCT in patients with both SIRS and acute lung injury, compared with those with SIRS alone. In their cohort, PCT levels were also elevated in patients without SIRS as well as in patients with SIRS alone, and the levels were statistically similar; this indicates that acute lung injury might play a critical role in the facilitated generation of PCT. Rapid increases of PCT levels in patients with non-bacterial pneumonitis caused by inhalational burn have also been reported.[27] In our cases of AE, serum PCT levels were of prognostic significance, and were also positively correlated with the number of SIRS criteria. The measurement of PCT has two key implications in our cohort. First, PCT was useful for excluding the possibility of respiratory infections, because most of the patients had PCT levels of <0.25 ng/mL (data not shown), which is below the level suggestive of severe pneumonia and/or bacteraemia.[28] Second, in the absence of overt respiratory infection, a relatively slight but significant elevation of PCT should not be ignored. PCT was proved to be associated with hospital mortality in both the univariate and multivariate analyses. Although the source of PCT in patients with AE of CFIP has not been confirmed, pulmonary neuroendocrine cells are possible candidates, because an abundance of calcitonin per unit weight of lung tissue has been described.[27 29] The serum PCT level was positively correlated with the number of SIRS criteria, suggesting that PCT could be a novel candidate biomarker for AE of CFIP.

One report has indicated that the absence of smoking history might positively influence the onset of AE in

Table 6 Multivariate analysis of survival

	HR	95% CI	p Value
Age (years)	1.0340	0.9847 to 1.0860	0.18
Male sex	2.4510	0.9260 to 6.4890	0.071
PCT (ng/mL)	2.7110	1.1770 to 6.2450	0.022
Pao_2:Fio_2 ratio on admission	0.9941	0.98232 to 1.0050	0.41
Extent of honeycombing (%)	1.0210	0.9881 to 1.0550	0.22
CLE (±)	0.0606	0.0161 to 0.2290	3.6×10^{-5}
SIRS (±)	6.2810	1.4220 to 27.7500	0.015

Cox proportional hazards regression models were used for the statistical analysis.
CLE, centrilobular emphysema; PCT, procalcitonin; SIRS, systemic inflammatory response syndrome.

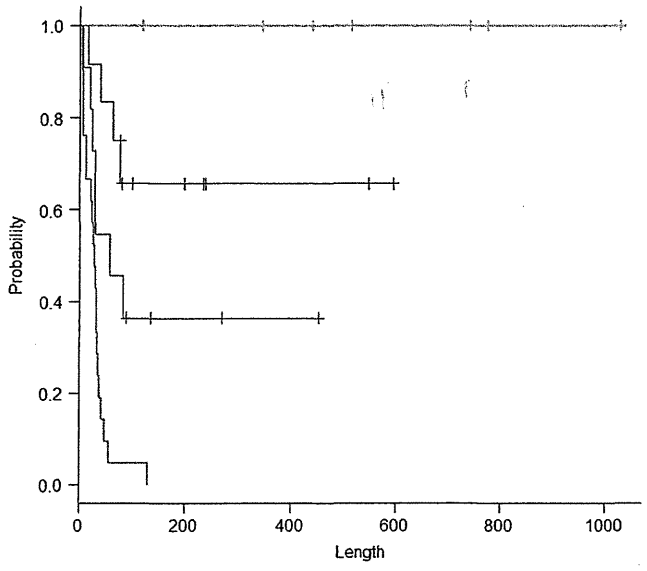

Figure 5 The stratification according to the presence or absence of centrilobular emphysema (CLE) and systemic inflammatory response syndrome (SIRS) revealed distinct survival curves of the four groups. Group 1 (green): SIRS− and CLE+ (n=7), group 2 (black): SIRS− and CLE− (n=12), group 3 (blue): SIRS+ and CLE+ (n=11) and group 4 (red): SIRS+ and CLE− (n=21). The x axis indicates days after admission.

patients with IPF[8]; however, CLE has not been evaluated as a mortality predictor. There are a few possible reasons why the presence of CLE was associated with outcome. First, in some participants with both CFIP and CLE, diffuse alveolar damage, a histopathological hallmark of AE of CFIP, was not present. Second, the pathogenesis of CLE itself antagonises that of ongoing diffuse alveolar damage. Finally, glucocorticoids are more effective for participants who have background CLE. As mentioned above, we ruled out the possibility of obvious cardiac dysfunction by echocardiography in the patients studied. Six of 11 survivors with CLE underwent bronchoalveolar lavage soon after admission (data not shown), and none showed overt alveolar haemorrhage. Although AE in patients with combined pulmonary fibrosis and emphysema has recently been reported,[30] its curability has not been described. In patients with chronic obstructive pulmonary disease (COPD), gene expression analysis and immunohistochemistry showed that urokinase plasminogen activator (PLAU) and urokinase plasminogen activator receptor (PLAUR) were found to be overexpressed in alveolar macrophages and the bronchial epithelium.[31] In infant respiratory distress syndrome, a condition characterised by intra-alveolar fibrin deposition, the ratio of PAI-1 to PLAU in the tracheal aspirates was higher than that in control participants,[32] suggesting a role for PLAU in intra-alveolar fibrinolysis. Lung-specific overexpression of PLAU in mice was shown to reduce the accumulation of collagen in the lung and reduced mortality after bleomycin-induced lung injury.[33] Thus, constitutive activation of the PLAU–PLAUR system in participants with CLE, a histological hallmark of COPD, may antagonise the activation of PAI-1, a possible biomarker of AE of CFIP. Generally, COPD itself has been demonstrated to show glucocorticoid resistance.[34] It remains to be determined whether there are any differences in the histological patterns of AE and the efficacy of glucocorticoids between patients with CFIP alone and those with both CFIP and CLE. The histological patterns could be unravelled by analysis of surgical lung biopsy specimens, and the efficacy of glucocorticoids could be clarified with the accumulation of data from future clinical cases.

The Kaplan-Meier estimate for survival according to the subgrouping by the presence or absence of SIRS and CLE clearly divided the patients into four subgroups. Every patient in group 1 (SIRS−CLE+) was successfully treated with glucocorticoids and discharged. Although an effective pharmaceutical therapy has not been established for patients with AE of IPF,[12] the present study is the first to document a subgroup of CFIP patients who derive a possible benefit from glucocorticoid therapy during AE. Five of the seven cases in group 1 were of IPF. By contrast, all 22 patients in group 4 (SIRS+CLE−) died due to respiratory failure secondary to AE. In this subgroup, any treatment option such as polymyxin B-immobilised fibre column treatment[11] should be considered. An indication for the use of glucocorticoids should also be discussed in the future.

The limitations of the present study are as follows. First, the analysis was retrospective. However, we consecutively enrolled every participant admitted with AE of CFIP from January 2009, and collected the hospitalisation medical records as completely as possible. Second, this study was undertaken in a single medical institution, and therefore the number of patients studied was limited. Third, the diagnosis of CFIP and the classifications were somewhat dependent on the TSCT findings. Because most of the patients were in moderate-to-severe respiratory failure (two-thirds of the patients studied had a P : F ratio of ≤200, data not shown), surgical lung biopsies on admission were difficult to perform. Half of the patients studied had been referred, and among those, none had undergone surgical lung biopsy. Consequently, five patients underwent surgical lung biopsy in this study. We tried, as much as possible, to rule out known entities associated with the development of pulmonary fibrosis. Finally, most of the referred participants had not undergone pulmonary function tests before admission. Therefore, we could not evaluate pulmonary function test results as mortality predictors.

Several mortality predictors have been identified in patients with AE of IPF. However, it is unclear how those factors could contribute to the management of this possibly fatal disorder in routine medical practice. We expect that the prognostic significance of CLE and SIRS, factors by which patients could be subgrouped on admission, could contribute to the rational management of patients with AE of CFIP. The utility of these prognostic factors should be prospectively investigated in future cohorts.

Contributors YU conceived and designed the study. YU, AK, FS, AS, KIK, KH and MK were involved in the acquisition, analysis, interpretation of data and in writing the manuscript before submission. All authors have read and approved the final version of the manuscript.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None.

Ethics approval Saitama Medical University.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Kondoh Y, Taniguchi H, Kawabata Y, *et al.* Acute exacerbation in idiopathic pulmonary fibrosis. Analysis of clinical and pathologic findings in three cases. *Chest* 1993;103:1808–12.
2. Park IN, Kim DS, Shim TS, *et al.* Acute exacerbation of interstitial pneumonia other than idiopathic pulmonary fibrosis. *Chest* 2007;132:214–20.
3. Silva CI, Müller NL, Fujimoto K, *et al.* Acute exacerbation of chronic interstitial pneumonia: high-resolution computed tomography and pathologic findings. *J Thorac Imaging* 2007;22:221–9.
4. Olson AL, Huie TJ, Groshong SD, *et al.* Acute exacerbations of fibrotic hypersensitivity pneumonitis: a case series. *Chest* 2008;134:844–50.
5. Richard L, Kradin RL, Subba R, *et al.* Case records of the Massachusetts General Hospital. Case 12–2010: an 89-year-old man with progressive dyspnoea. *N Engl J Med* 2010;362:1522–31.
6. Ley B, Collard HR, King TE. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:431–40.
7. Kondoh Y, Taniguchi H, Katsuta T, *et al.* Risk factors of acute exacerbation of idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2010;27:103e10.
8. Song JW, Hong SB, Lim CM, *et al.* Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. *Eur Respir J* 2011;37:356–63.
9. Akira M, Kozuka T, Yamamoto S, *et al.* Computed tomography findings in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008;178:372–8.
10. Fujimoto K, Taniguchi H, Johkoh T, *et al.* Acute exacerbation of idiopathic pulmonary fibrosis: high-resolution CT scores predict mortality. *Eur Radiol* 2012;22:83–92.
11. Abe S, Azuma A, Mukae H, *et al.* Polymyxin B-immobilized fiber column (PMX) treatment for idiopathic pulmonary fibrosis with acute exacerbation: a multicenter retrospective analysis. *Intern Med* 2012;51:1487–91.
12. Collard HR, Moore BB, Flaherty KR, *et al.* Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;176:636–43.
13. Cottin V, Nunes H, Brillet PY, *et al.* Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. *Eur Respir J* 2005;26:586–93.
14. Raghu G, Collard HR, Egan JJ, *et al.* An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788–824.
15. Ichikado K, Suga M, Müller NL, *et al.* Acute interstitial pneumonia: comparison of high-resolution computed tomography findings between survivors and nonsurvivors. *Am J Respir Crit Care Med* 2002;165:1551–6.
16. Akira M, Hamada H, Sakatani M, *et al.* CT findings during phase of accelerated deterioration in patients with idiopathic pulmonary fibrosis. *AJR Am J Roentgenol* 1997;168:79–83.
17. Akira M, Inoue Y, Kitaichi M, *et al.* Usual interstitial pneumonia and nonspecific interstitial pneumonia with and without concurrent emphysema: thin-section CT findings. *Radiology* 2009;251:271–9.
18. Bone RC, Balk RA, Cerra FB, *et al.* Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest* 1992;101:1644–55.
19. Kanda Y. Investigation of the freely-available easy-to-use software "EZR" (Easy R) for medical statistics. Bone Marrow Transplant 2013;48:452–8.
20. American Thoracic Society/European Respiratory Society International multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2002;165:277–304.
21. Alberti C, Brun-Buisson C, Goodman SV, *et al.* Influence of systemic inflammatory response syndrome and sepsis on outcome of critically ill infected patients. *Am J Respir Crit Care Med* 2003;168:77–84.
22. Collard HR, Calfee CS, Wolters PJ, *et al.* Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L3–7.
23. McClintock D, Zhuo H, Wickersham N, *et al.* Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury. *Crit Care* 2008;12:R41.
24. Assicot M, Gendrel D, Carsin H, *et al.* High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet* 1993;341:515–18.
25. Gilbert DN. Procalcitonin as a biomarker in respiratory tract infection. *Clin Infect Dis* 2011;52:S346–50.
26. Hensel M, Volk T, Döcke WD, *et al.* Hyperprocalcitonemia in patients with noninfectious SIRS and pulmonary dysfunction associated with cardiopulmonary bypass. *Anesthesiology* 1998;89:93–104.
27. Nylen ES, Jeng J, Jordan MH, *et al.* Pneumonitis-associated hyperprocalcitonemia. *Am J Med Sci* 1996;312:12–18.
28. Berg P, Lindhardt BØ. The role of procalcitonin in adult patients with community-acquired pneumonia—a systematic review. *Dan Med J* 2012;59:A4357.
29. Becker KI, Silva OI, Snider RH. The pathophysiology of pulmonary procalcitonin. *The endocrine lung in health and disease.* Becker KI, Gazdar AF, eds. Philadelphia, *WB Saunders* 1984:277–99.
30. Kishaba T, Shimaoka Y, Fukuyama H, *et al.* A cohort study of mortality predictors and characteristics of patients with combined pulmonary fibrosis and emphysema. *BMJ Open* 2012;2:e000988.
31. Wang IM, Stepaniants S, Boie Y, *et al.* Gene expression profiling in patients with chronic obstructive pulmonary disease and lung cancer. *Am J Respir Crit Care Med* 2008;177:402–11.
32. Cederqvist K, Sirén V, Petäjä J, *et al.* High concentrations of plasminogen activator inhibitor-1 in lungs of preterm infants with respiratory distress syndrome. *Pediatrics* 2006;117:1226–34.
33. Sisson TH, Hanson KE, Subbotina N, *et al.* Inducible lung-specific urokinase expression reduces fibrosis and mortality after lung injury in mice. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1023–32.
34. Barnes PJ. Corticosteroid effects on signaling. *Eur Respir J* 2006;27:413–26.

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2013;161(suppl 2):10–15
DOI: 10.1159/000350335

Published online: May 29, 2013

Effect of Formoterol on Eosinophil Trans-Basement Membrane Migration Induced by Interleukin-8-Stimulated Neutrophils

Akiko Kawashima[a] Tomoko Suzuki[a] Fuyumi Nishihara[a, b]
Takehito Kobayashi[a, b] Yotaro Takaku[c] Kazuyuki Nakagome[a, b]
Tomoyuki Soma[a, b] Koichi Hagiwara[a] Minoru Kanazawa[a] Makoto Nagata[a, b]

[a]Department of Respiratory Medicine and [b]Allergy Center, Saitama Medical University, and [c]Department of Respiratory Medicine, Saitama Cardiovascular and Respiratory Center, Saitama, Japan

Key Words
Asthma · Eosinophil · Trans-basement membrane migration · Neutrophil · Interleukin-8 · Formoterol

Abstract
Background: Neutrophils are often increased in the airways of either chronic severe asthma or acute exacerbations. Neutrophils that have migrated in response to interleukin-8 (IL-8) may lead eosinophils to accumulate in the airways of patients with asthma and possibly aggravate the disease. In this study, we investigated whether formoterol modified the trans-basement membrane migration (TBM) of eosinophils stimulated with neutrophils and IL-8. ***Methods:*** Neutrophils and eosinophils were isolated from peripheral blood obtained from healthy donors. Eosinophil TBM was examined using a modified Boyden's chamber technique. Neutrophils were preincubated with or without formoterol (0.1 μM) at 37°C for 30 min. Eosinophils were added to the upper compartment of a chamber with a Matrigel-coated transwell insert. Medium containing preincubated neutrophils and IL-8 was added to the lower compartment of the chamber. After a 90-minute incubation, the eosinophils that had migrated into the lower chamber were calculated using eosinophil peroxidase assays. ***Results:*** A combination of neutrophils and IL-8 significantly induced the eosinophil TBM; formoterol alone had no effect. However, formoterol modestly but significantly attenuated the TBM of eosinophils stimulated with neutrophils and IL-8. ***Conclusion:*** These results suggest that formoterol may act as a therapeutic agent on enhanced eosinophilic inflammation in acute exacerbation or persistent, severe asthma. The effect of formoterol likely involves the inhibition of neutrophil activation.

Introduction

Inhaled corticosteroids (ICSs) have drastically improved asthma control [1, 2]. However, there is also evidence that increasing ICSs is not effective when asthma is exacerbated [3]. Infiltration of neutrophils into the airways, which is often observed with asthma exacerbation, may be associated with this phenomenon. In general, the infiltration of eosinophils into airways with asthma is well known, and the sputum eosinophil count is often used as a marker of asthma exacerbation [4, 5]. On the other hand, the presence of neutrophilic inflammation is also

KARGER

1018–2438/13/1616–0010$38.00/0

E-Mail karger@karger.com
www.karger.com/iaa

Correspondence to: Dr. Makoto Nagata
Department of Respiratory Medicine and Allergy Center, Saitama Medical University
38 Morohongou, Moroyama-machi, Iruma-gun
Saitama 350-0495 (Japan)
E-Mail favre4mn@saitama-med.ac.jp

observed in persistent asthma [6, 7]. Green et al. [8] reported that asthma patients with predominantly neutrophils in their sputum do not respond as well to treatment with ICSs.

Although sputum neutrophilia is often clinically observed in severe asthma or asthma exacerbation, the mechanisms by which it occurs are still under discussion. According to the ENFUMOSA (European Network Study for Understanding the Mechanisms of Severe Asthma), both neutrophilic and eosinophilic inflammation persist in the airways of patients with severe asthma [9]. We observed a similar phenomenon in severe, persistent asthma in Japanese patients [10]. We recently reported that neutrophils stimulated with interleukin-8 (IL-8) play a role in the regulation of eosinophilic inflammation in asthma [11]. That is to say, in the presence of IL-8, neutrophils induce eosinophil trans-basement membrane migration (TBM) in vitro, which is one of the key processes in which eosinophils accumulate in the airways of asthma patients [11].

The combination of ICSs and long-acting β_2 agonists (LABA) is now widely known to improve asthma control and to reduce exacerbations as demonstrated in a number of studies, including the OPTIMA (Optimal Protocol for Treatment Initiation with Methotrexate and Adalimumab Combination Therapy in Patients with Early Rheumatoid Arthritis) study and the FACET (Formoterol and Corticosteroids Establishing Therapy) study [12, 13]. O'Byrne [14], in a double-blind, randomized, parallel-group study, demonstrated that a combination of budesonide (an ICS) and formoterol (a LABA) could replace short-acting β_2 agonist (SABA) reliever medications in asthma exacerbations. Another study showed that formoterol reduced neutrophilic airway inflammation in asthma patients [15]. Formoterol has been known to have anti-inflammatory effects on neutrophils, inhibiting the release of oxidants [16] and the adhesion to postcapillary venules in rat airways [17]. Another study reported that allergen-induced sputum eosinophilia was significantly reduced by combination treatment with budesonide/formoterol, but not by budesonide alone [18].

Taken together, we hypothesized that formoterol would provide an inhibitory effect on the neutrophil-eosinophil interaction (which may be important in the exacerbation of asthma) via an inhibitory effect on neutrophil activation. In this study, we report that the TBM of eosinophils, which is induced by IL-8-stimulated neutrophils, is inhibited by formoterol.

Materials and Methods

Reagents

Anti-CD16 antibody-coated magnetic beads were purchased from Miltenyi Biotec (Auburn, Calif., USA). HBSS was purchased from Gibco BRL (Grand Island, N.Y., USA). PBS was obtained from Wako (Osaka, Japan). Recombinant human IL-8 was purchased from R&D Systems (Minneapolis, Minn., USA). Fetal calf serum was purchased from MP Biomedicals (Aurora, Ohio, USA). BSA was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Formoterol and budesonide were supplied by AstraZeneca (Lund, Sweden).

Preparation of Neutrophils and Eosinophils

Neutrophils and eosinophils were isolated from peripheral blood collected from nonatopic healthy donors whose eosinophil content was <5% of their peripheral leukocytes. The numbers of males and females were comparable among donors, with similar age distributions ranging from 26 to 34 years. Informed consent was obtained before collection of each blood sample. Briefly, 10 ml of dextran was added to 40 ml of heparinized blood and erythrocytes were removed as sediment. The system-HISTOPAQUE (Sigma-Aldrich) was performed for isolation according to the manufacturer's instructions. Separated lymphocytes were removed, 2 ml of sterile distilled water and 2 ml of 1.8% salt solution were added, followed by 20 ml of the sterilized PBS and the neutrophils (>95% purity and >99% viability) were separated by centrifuge. Neutrophils (0.5×10^5 cells/ml) with 0.2% BSA/HBSS were prepared, and IL-8, formoterol and budesonide were added to the neutrophil suspension and preincubated for 30 min in 5% CO_2 at 37°C. The remaining cells were washed with HBSS at 4°C supplemented with 5% FCS in PBS, then incubated with anti-CD16 antibody-coated magnetic beads for 30 min at 4°C, and finally filtered with a column containing steel wool placed in a magnetic field (Miltenyi Biotec). Eosinophils (>98% purity and >99% viability) which passed through the column were collected and washed, and the number of cells was adjusted to 2.5×10^5 cells/ml with 0.2% BSA/HBSS buffer. Eosinophils (2.5×10^5 cells/ml) were added to the upper compartment of a Matrigel-coated transwell (3 μm; Becton Dickinson Labware, Franklin Lakes, N.J., USA). Neutrophils and IL-8 or formoterol, budesonide and buffer were added to the lower compartment of the chamber and incubated for 90 min in 5% CO_2 at 37°C. The study was approved by the IRB and all subjects gave written informed consent.

Trans-Basement Membrane Migration

The peroxidase activity of migrated eosinophils in the lower compartment of the chamber was determined and the number of migrated eosinophils was calculated from the activity of the standard media which contained known numbers of eosinophils [11]. To determine the peroxidase activity of eosinophils, the medium of the lower compartment was incubated with a substrate (1 mM OPD, 1 mM H_2O_2 and 0.1% Triton X-100 in Tris-HCl, pH 8.0) for 30 min at room temperature. The reaction was stopped by adding 100 μl of 4 N H_2SO_4 and absorbance at 490 nm was determined. The effect of neutrophils on the outer density value in this assay was negligible: 0.046 ± 0.002 for 0% control and 0.064 ± 0.006 for 100% control of neutrophils, respectively (n = 4) [11]. Similarly, the addition of neutrophils to 100% control of eosinophils did not modify the outer density value [1.566 ± 0.023 addition of 0% con-

Int Arch Allergy Immunol 2013;161(suppl 2):10–15
DOI: 10.1159/000350335

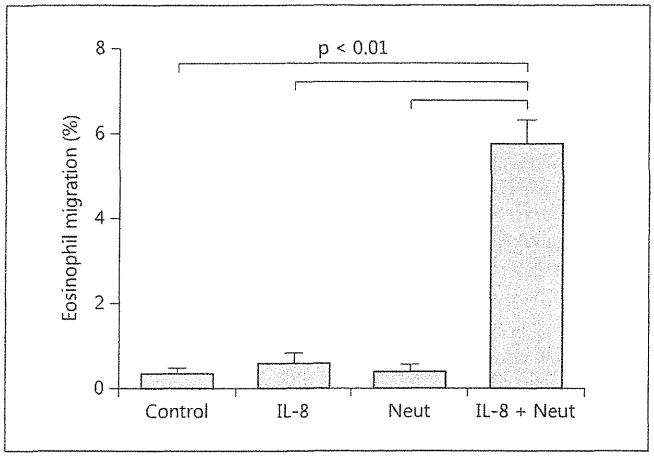

Fig. 1. A combination of IL-8 and neutrophils induced eosinophil TBM. The graph represents the percentage of migrated eosinophils. Buffer (Control), IL-8 in buffer (IL-8), neutrophils (Neut) or the combination of IL-8 and neutrophils (IL-8 + Neut) are in the bottom compartment of the transwell, and eosinophils are added to the upper compartment. Values are mean ± SEM. The combination of IL-8 and neutrophils is compared with control, IL-8 and neutrophils only (n = 8).

trol vs. 1.569 ± 0.012 addition of 100% control of neutrophils (n = 4, p = n.s.)] [11]. The numbers of migrated eosinophils were determined by measuring fluorescence in the medium using the Fluoromark (Bio-Rad Laboratories, Hercules, Calif., USA) microplate fluorometer. The viability of both eosinophils and neutrophils after migration exceeded 98% by trypan blue exclusion.

Statistical Analysis

Values are expressed as means ± SEM. Parametric data were compared by using the Student t test for mean values analysis of variance (ANOVA) with correction for multiple comparisons (Scheffé's constants) when more than two variables were analyzed using GraphPad software.

Results

A Combination of IL-8 and Neutrophils Induced Eosinophil TBM

We have reported that a combination of IL-8 and neutrophils induced eosinophil TBM when both cell types were placed simultaneously in the upper room of a Boyden chamber [11]; this is thought to be a model of asthma exacerbation like that which follows a viral infection. In this study, to test the hypothesis that formoterol may modify eosinophil TBM via the inhibition of neutrophil activation, we first explored whether the induction of eosinophil TBM also occurred when neutrophils were placed in the bottom chamber. Neutrophils (0.5×10^5 cells/ml) were placed in the bottom chamber of the transwell, and eosinophils (2.5×10^5 cells/ml) were added to the upper chamber. The experiments were run in the presence or absence of 10 nM of IL-8 in the bottom chamber. After a 90-minute incubation at 37°C, we counted the migration of eosinophils with eosinophil peroxidase assay as described in Methods. As illustrated in figure 1, only the combination of IL-8 and neutrophils significantly induced eosinophil migration. Neutrophils or IL-8 alone had no effect on eosinophil migration. Therefore, although the experimental condition was different (neutrophils in the lower vs. the upper chamber), we confirmed the results from our previous study [11]; neutrophils stimulated by IL-8 can induce eosinophil TBM with our current system.

Budesonide Did Not Attenuate TBM of Eosinophils by IL-8-Stimulated Neutrophils

It is well known that corticosteroids provide anti-inflammatory effects on airway inflammation such as bronchial asthma [1] and that ICS therapy has a pivotal anti-inflammatory role in the treatment of mild asthma [14], although recent reports have demonstrated that simply increasing ICSs does not improve asthma exacerbations [3, 12, 13]. To investigate the effects of corticosteroids on eosinophil TBM, we added 0.1 μM of budesonide to the bottom compartment of the transwell with the IL-8 (10 nM) and the neutrophils (0.5×10^5 cells/ml). They were preincubated together for 30 min at 37°C. After the incubation, we put eosinophils (2.5×10^5 cells/ml) into the upper compartment and incubated for 90 min at 37°C. IL-8-stimulated neutrophils induced eosinophil TBM (fig. 2). However, budesonide did not attenuate the TBM of eosinophils stimulated by IL-8 and neutrophils (fig. 2). In selected experiments, we also used higher concentrations of budesonide (up to 1 μM) to observe the effect on eosinophil TBM, but no inhibitory effect of budesonide was seen (data not shown).

Formoterol Attenuates the TBM of Eosinophils Stimulated by IL-8 and Neutrophils

The effect of combination treatment with budesonide/formoterol for both the maintenance and relief of severe asthma exacerbation is unbeaten [12, 13]. Following the results shown in figure 2, we investigated the effect of formoterol on eosinophil TBM by adding 0.1 μM of formoterol, rather than budesonide, to the bottom chamber. As illustrated in figure 3, formoterol modestly (18.2% in-

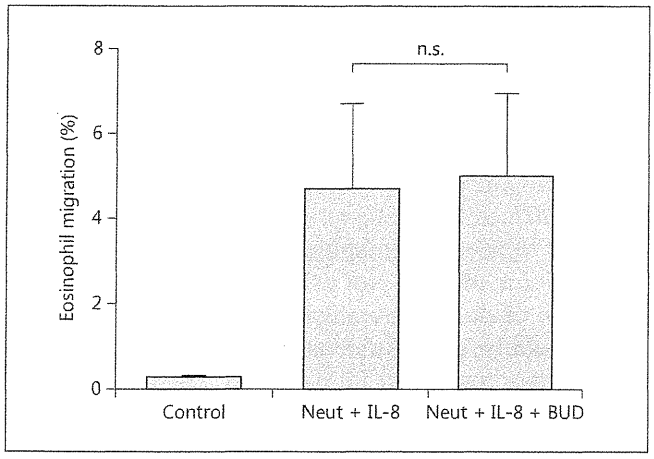

Fig. 2. Budesonide does not attenuate the TBM of eosinophils stimulated by IL-8 and neutrophils. The graph shows the percentage of migrated eosinophils. Buffer (Control), neutrophils and IL-8 (Neut + IL-8) or budesonide with IL-8 and neutrophils (Neut + IL-8 + BUD) are in the bottom compartment of the transwell, and eosinophils are added to the upper compartment. Values are mean ± SEM; n.s. = not significant for the comparison between Neut + IL-8 and Neut + IL-8 + BUD (n = 3).

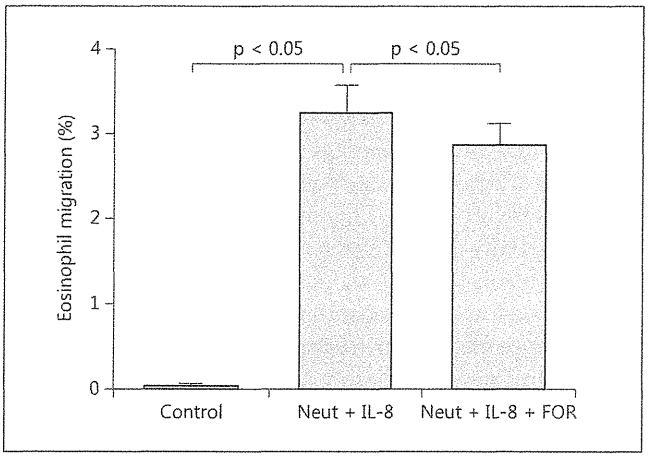

Fig. 3. Formoterol significantly attenuates the TBM of eosinophils stimulated by IL-8 and neutrophils. The graph indicates the percentage of migrated eosinophils. Buffer (Control), neutrophils and IL-8 (Neut + IL-8) or formoterol with IL-8 and neutrophils (Neut + IL-8 + FOR) are in the bottom compartment of the transwell, and eosinophils are added to the upper compartment. Values are mean ± SEM; $p < 0.05$ for Neut + IL-8 versus Neut + IL-8 + FOR or for Control versus Neut + IL-8 (n = 5).

hibition) but significantly attenuated the TBM of eosinophils stimulated by 10 nM of IL-8 and neutrophils. Formoterol inhibited TBM of eosinophils in a dose-dependent manner from 10^{-8} to 10^{-6} M (data not shown). This result suggests that formoterol provides an inhibitory effect via the inactivation of neutrophils.

Discussion

We provide evidence that formoterol inhibits the TBM of eosinophils induced by IL-8-activated neutrophils. Our data suggest that one of the mechanisms by which formoterol inhibits airway inflammation in asthma may be associated with neutrophilic inflammation. Moreover, this may explain why the combination of budesonide/formoterol relieves asthma exacerbation and persistent severe asthma.

The coexistence of eosinophils and neutrophils in the airways of severe asthma and exacerbation following viral infection is well known [9, 10, 19]. Our previous report suggests that neutrophils can regulate the accumulation of eosinophils in asthma airways [10]. As mentioned previously, TBM is the pivotal process by which circulating eosinophils accumulate in the airways of asthma patients. In this study, eosinophil TBM was induced by a combination of IL-8 and neutrophils, but not by either alone (fig. 1). The potential mechanism of this phenomenon is thought to be that neutrophils stimulated with a chemoattractant (such as IL-8) augment the TBM of eosinophils. Further, matrix metalloproteinase (MMP)-9, leukotriene B_4 (LTB_4), platelet-activating factor (PAF) and tumor necrosis factor (TNF)-α are thought to be involved as mediators in the augmentation of eosinophil TBM by IL-8-stimulated neutrophils, because the inhibitors or the antagonists of these factors inhibit this augmentation [11].

ICSs have considerably contributed to our ability to improve asthma control; however, not all patients achieve control of asthma symptoms and airway inflammation with ICS therapy. In particular, increasing ICSs is not effective in treating the exacerbation of asthma [3]. Both sputum eosinophilia and neutrophilia are seen clinically in severe asthma and in asthma exacerbation. Neutrophil accumulation is generally understood to be resistant to glucocorticoid therapy [20]. Our data, in which budesonide does not attenuate the TBM of eosinophils stimulated by IL-8 and neutrophils, (fig. 2) support these clinical findings. In contrast, the question that the effect of budesonide on eosinophil TBM might be underestimated still remains, because budesonide works via binding with glucocorticoid receptor protein. The incubation

time of budesonide might be too short for the inhibition of eosinophil TBM to show enough suppression. The longer incubation time of budesonide was not used in this study; that is another assignment.

The combination therapy of budesonide/formoterol has been efficacious as a reliever medication in cases of asthma exacerbation. The mechanism of this efficacy might partially depend upon an anti-inflammatory effect of formoterol. Kelly et al. [18] demonstrated that allergen-induced sputum eosinophilia was significantly reduced by combination treatment with budesonide/formoterol but not by budesonide monotherapy. Formoterol itself has various kinds of anti-inflammatory effects. Formoterol is known to suppress the production of superoxide-anion and the release of elastase by formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated neutrophils in vitro [21]. Formoterol also attenuates neutrophil numbers and IL-8 levels in the sputum of asthmatic patients [15]. In this study, formoterol significantly reduced the TBM of eosinophils stimulated by IL-8 and neutrophils (fig. 3). Another kind of β_2 agonist is known to have a similar effect, as Procaterol inhibits eosinophil migration and the release of eosinophil chemotactic activity from epithelial cells [22]. Our data suggest that formoterol has an anti-inflammatory effect via the suppression of neutrophil activation.

Following these results, our interest moved to the mechanisms by which formoterol, but not budesonide, attenuates eosinophil TBM by IL-8-stimulated neutrophils. As mentioned previously, there is evidence that MMP-9, LTB_4 or PAF are involved in the augmentation of eosinophil TBM stimulated by activated neutrophils, because the inhibitors for MMP-9, LTB_4 and PAF blocked the eosinophil migration [11]. Therefore, formoterol may affect these chemoattractants and reduce the TBM of eosinophils. A recent study indicates that formoterol attenuates production of superoxide and LTB_4 on activated neutrophils by fMLP via association with increases of cyclic adenosine monophosphate [21]. MMP-9 may be a target as another potential mechanism. Tacon et al. [23] recently demonstrated that formoterol reduces transcription factor, Fos-related Ag-1 (Fra-1, part of the AP-1 family) expression and DNA binding activity and MMP-9 on lung epithelial cells induced by rhinovirus infection, via inhibition of the MAPK pathway. Rhinovirus causes the majority of common colds and is known to cause asthma exacerbation. According to their study, the combination of formoterol/dexamethasone therapy suppressed MMP-9 expression more than either monotherapy did [23]. In this study, we also examined the effect of formoterol/budesonide combination therapy on eosinophil TBM by IL-8-stimulated neutrophils; however, we could not find any additional effects (data not shown).

These discussions remain speculations, however, as the molecular mechanisms by which formoterol attenuates eosinophil TBM by activated neutrophils are still uncertain. Nonetheless, our results support the clinical efficacy of formoterol on sputum eosinophilia in asthma exacerbation, which is not altered by ICS monotherapy. Although we did not investigate MMP-9, LTB_4 and PAF, they should be part of the targets for the mechanism of inhibition of eosinophil TBM by activated neutrophils in further study.

In summary, we have presented the novel finding that formoterol reduced the TBM of eosinophils by IL-8-stimulated neutrophils. In our study, formoterol may have contributed to inhibit airway eosinophilia induced by activated neutrophils (which assumes exacerbation of asthma). Our finding suggests that formoterol may be useful as a therapeutic agent for enhanced eosinophilic inflammation that occurs in persistent, severe asthma. This formoterol effect likely involves inhibiting neutrophil activation.

Acknowledgement

The authors thank Ms. Akemi Yokote for her excellent technical assistance.

Disclosure Statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this paper.

References

1 Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ: Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143–178.

2 Expert Panel Report 3 (EPR-3): Guidelines for the diagnosis and management of asthma: summary report 2007. J Allergy Clin Immunol 2007;120:S94–S138.

3 Harrison TW, Oborne J, Newton S, Tattersfield AE: Doubling the dose of inhaled corticosteroid to prevent asthma exacerbations: randomised controlled trial. Lancet 2004;363:271–275.

4 Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID: Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715–1721.
5 Spahn JD: Asthma biomarkers in sputum. Immunol Allergy Clin North Am 2012;32:387–399.
6 Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, Chu HW: Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001–1008.
7 Gibson PG, Simpson JL, Saltos N: Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001;119:1329–1336.
8 Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID: Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 2002;57:875–879.
9 The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. Eur Respir J 2003;22:470–477.
10 Kikuchi S, Nagata M, Kikuchi I, Hagiwara K, Kanazawa M: Association between neutrophilic and eosinophilic inflammation in patients with severe persistent asthma. Int Arch Allergy Immunol 2005;137(suppl 1):7–11.
11 Kikuchi I, Kikuchi S, Kobayashi T, Hagiwara K, Sakamoto Y, Kanazawa M, Nagata M: Eosinophil trans-basement membrane migration induced by interleukin-8 and neutrophils. Am J Respir Cell Mol Biol 2006;34:760–765.
12 Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A: Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med 1997;337:1405–1411.
13 O'Byrne PM, Barnes PJ, Rodriguez-Roisin R, Runnerstrom E, Sandstrom T, Svensson K, Tattersfield A: Low dose inhaled budesonide and formoterol in mild persistent asthma: the OPTIMA randomized trial. Am J Respir Crit Care Med 2001;164:1392–1397.
14 O'Byrne PM: Daily inhaled corticosteroid treatment should be prescribed for mild persistent asthma. Pro Am J Respir Crit Care Med 2005;172:410–412.
15 Maneechotesuwan K, Essilfie-Quaye S, Meah S, Kelly C, Kharitonov SA, Adcock IM, Barnes PJ: Formoterol attenuates neutrophilic airway inflammation in asthma. Chest 2005;128:1936–1942.
16 Anderson P, Lotvall J, Linden A: Relaxation kinetics of formoterol and salmeterol in the guinea pig trachea in vitro. Lung 1996;174:159–170.
17 Bowden JJ, Sulakvelidze I, McDonald DM: Inhibition of neutrophil and eosinophil adhesion to venules of rat trachea by beta 2-adrenergic agonist formoterol. J Appl Physiol 1994;77:397–405.
18 Kelly MM, O'Connor TM, Leigh R, Otis J, Gwozd C, Gauvreau GM, Gauldie J, O'Byrne PM: Effects of budesonide and formoterol on allergen-induced airway responses, inflammation, and airway remodeling in asthma. J Allergy Clin Immunol 2010;125:349–356 e313.
19 Pizzichini MM, Pizzichini E, Efthimiadis A, Chauhan AJ, Johnston SL, Hussack P, Mahony J, Dolovich J, Hargreave FE: Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. Am J Respir Crit Care Med 1998;158:1178–1184.
20 Ito K, Herbert C, Siegle JS, Vuppusetty C, Hansbro N, Thomas PS, Foster PS, Barnes PJ, Kumar RK: Steroid-resistant neutrophilic inflammation in a mouse model of an acute exacerbation of asthma. Am J Respir Cell Mol Biol 2008;39:543–550.
21 Gravett CM, Theron AJ, Steel HC, Tintinger GR, Cockeran R, Feldman C, Anderson R: Interactive inhibitory effects of formoterol and montelukast on activated human neutrophils. Eur Respir J 2010;36:1417–1424.
22 Koyama S, Sato E, Masubuchi T, Takamizawa A, Kubo K, Nagai S, Isumi T: Procaterol inhibits IL-1beta- and TNF-alpha-mediated epithelial cell eosinophil chemotactic activity. Eur Respir J 1999;14:767–775.
23 Tacon CE, Newton R, Proud D, Leigh R: Rhinovirus-induced MMP-9 expression is dependent on Fra-1, which is modulated by formoterol and dexamethasone. J Immunol 2012;188:4621–4630.

急性呼吸不全における栄養管理
～人工呼吸患者を中心に～

奈良県立医科大学内科学第二講座
吉川（よしかわ） 雅則（まさのり）
木村（きむら） 弘（ひろし）

急性呼吸窮迫症候群（ARDS）や慢性呼吸器疾患の増悪では、しばしば重症呼吸不全に陥り人工呼吸管理を要する。このような病態においては栄養管理の優劣が予後に重大な影響をおよぼす。近年、急性呼吸不全を呈する重症病態の栄養管理に関するエビデンスが集積されつつあり、各国からガイドラインが発表されている。それらの共通の認識として、発症後24～48時間以内の早期経腸栄養の開始、胃管栄養が困難な場合の小腸栄養の選択、overfeedingに対する警鐘とunderfeedingの推奨、蛋白質の十分な補給、ARDSに対する魚油を含む栄養剤の有用性が記載され、高脂質栄養剤や免疫調整栄養剤投与に際する留意事項なども示されている。

はじめに

人工呼吸管理を必要とする重症病態においては、栄養障害は感染症や多臓器不全などの合併につながり、死亡率の上昇や入院期間の延長とも関連することから、適切な栄養管理は重要な課題である。近年、重症患者における栄養、代謝状態に関する理解が深まり、栄養管理に対する考え方も変容しつつある。本稿では重症患者、特に人工呼吸管理を要する急性呼吸不全患者の栄養管理について解説する。

1．栄養療法の基本

栄養療法とは経口・経腸あるいは経静脈的に栄養素を治療目的に投与することである。これらの栄養療法を選択する場合の一般的な手順としては、まず腸管機能がある程度保たれていれば、経腸栄養（enteral nutrition：EN）が選択され、長期の栄養補給が必要であれば投与ルートとして胃ろう、短期ならば経鼻チューブを使用する。消化管機能が低下していない場合は半消化態栄養剤、低下している場合は残渣の極めて少ない消化態栄養剤や成分栄養剤を使用する。（表1）腸管が使用できない場合は静脈栄養（parenteral nutrition：PN）を選択せざるを得ないが、短期的には末梢静脈栄養、長期的には中心静脈栄養を施行する。消化管機能が回復すれば経腸栄養へと移行する（図1）[1]。

2. 侵襲下におけるエネルギー投与

栄養療法を行う場合は、各病態における代謝状態や生体内のエネルギー供給の状況について把握しておく必要がある。慢性下気道感染症やCOPDなどによる慢性呼吸不全では、飢餓から軽度の代謝亢進状態にあると考えられる。一方、ALI/ARDSや慢性期からの急性増悪によって人工呼吸管理が必要となる急性呼吸不全では、高度侵襲に近い代謝状態が想定される。

一般的に栄養療法を行う場合のエネルギー投与量の決定法として、間接熱量測定によって安静時エネルギー消費量（resting energy expenditure：REE）を実測する方法、Harris-Benedictの式から予測基礎代謝量（basal energy expenditure：BEE）を算出し、ストレス係数と活動係数を乗じる方法、簡便にエネルギー初期投与量を体重1kgあたり25～30kcalとする方法がある。

人工呼吸管理を要する急性呼吸不全などの侵襲下において、実測したREEあるいは推算式に基づいて決定した必要エネルギー量を単純に投与エネルギー量とすべきか否かは重要な問題である。すな

表1　経腸栄養剤

成分栄養剤	消化態栄養剤	半消化態栄養剤		
		医薬品	食品	
エレンタール エレンタールP ヘパンED	ツインライン エンテミール ペプチーノ	エンシュア・リキッド エンシュア・H ラコールNF アミノレバンEN	呼吸器	プルモケア・EX ライフフロン・QL
			肝	ヘパスII
			腎	レナウェル リーナレン
			高度侵襲	オキシーパ メイン インバクト アノム
			糖尿	グルセルナEx Inslow
			癌	プロシュア

（すべて商品名、食品の半消化態栄養剤は病態別に代表的なものを呈示）

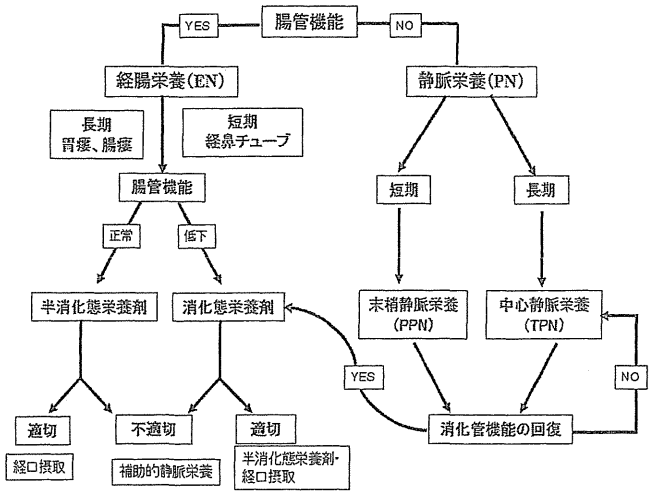

図1　栄養療法のdecision tree（文献1）より引用改変）

わち早期にPNを開始して必要と考えられるエネルギー量を外因性に充足することの妥当性が議論されている。EPaNIC study2）ではICU患者を対象に、後期PN群（最初の7日間はEN単独とし8日目からPNを追加）と早期PN群（1日目からPNを開始し3日目からENを追加して目標摂取カロリーに到達）のアウトカムを比較検討している。後期PN群ではICUからの早期退出や平均ICU滞在期間および人工呼吸管理期間の短縮がみられた。一方、早期PN群では血糖上昇や肝機能異常や新たな感染症の合併が認められ、早期PNによる外因性のエネルギー充足は逆効果であると結論された[2]。その他の大規模研究でもICU患者に対する早期PN併用によるエネルギー充足の有用性が否定されている。

3．内因性エネルギー供給とoverfeeding

侵襲下の病態では各種のストレスホルモンやサイトカインによる異化亢進状態にあり、脂肪分解や筋蛋白の崩壊によって内因性にエネルギーが供

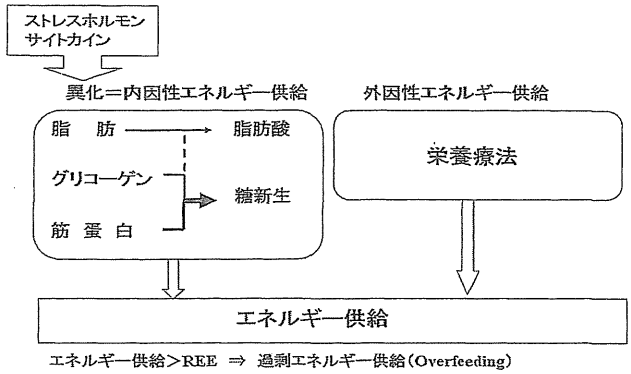

図2　侵襲下のエネルギー供給（文献3）より引用）

給されている。栄養療法による外因性エネルギー供給も加わった場合、総エネルギー供給量がREEよりも大きくなり、結果的に過剰エネルギー供給（overfeeding）となる可能性がある（図2）[3]。

overfeedingに伴う高血糖によるグルコース毒性は厳密な血糖コントロールで抑制可能である。一方、栄養ストレスによって引き起こされるautophagyの障害やREEの増加、CO_2産生の増加、骨格筋蛋白の代謝障害などは血糖コントロールによっても制御できない。特に、細胞障害の修復と感染防御において重要な役割を担っているautophagyは過剰な栄養源の投与と過剰なインスリン分泌で障害され、感染防御能の低下や臓器障害につながる。

栄養療法の実施においてunderfeedingかoverfeedingであるかの判定が重要となる。代謝状態からの評価法としては間接カロリメトリーによるREEの実測が最も信頼性が高い。酸素摂取量に対する炭酸ガス排泄量の比である呼吸商（respiratory quotient：RQ）が1以上であり、エネルギー充足率が実際に100％を超えている場合はoverfeedingと判定される。一方。RQが0.85未満でかつエネルギーの充足率が90％未満であればunderfeedingと考えられる[3]。

4．侵襲下における栄養療法

overfeedingを回避するには、侵襲反応による内因性エネルギー供給と栄養療法による外因性エネルギー供給との合算をREEとして設定することが望ましい（図3）。しかし、基本的に内因性エネルギー供給量の厳密な評価は不可能であり、REEの測定もできない場合には、エネルギー投与量は大まかな指針に従って決定する（表2）。急性期における必要最小限のエネルギー投与量を6～9kcal/kgBWと

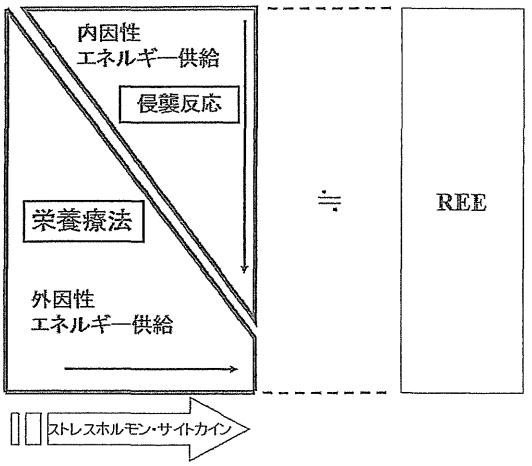

図3　Overfeedingを回避するエネルギー投与法
ー侵襲下ー（文献3）より引用）

表2　重症患者におけるエネルギー投与の指針
（文献3）より引用）

	必要最低限度	エネルギー投与の上限
急性期の極期	6～9kcal/kgBW/日	15kcal/kgBW/日
一般的な急性期	6～9kcal/kgBW/日	20～25kcal/kgBW/日
回復期	25～30kcal/kg/BW/日	
慢性期に移行	6～9kcal/kgBW/日	25（～30）kcal/kgBW/日

して、極期には上限を15kcal/kgBW、一般的な急性期には20～25kcal/kgBWを上限とすることが推奨されている[3]。回復期には25～30kcal/kgBWとして積極的な栄養補給を行う。

蛋白質の投与量を初期には1.0～2.0g/kgとし1.5kg/kgまでは許容範囲とする。脂質投与量は総エネルギー量から蛋白質によるエネルギー量を差し引いた非蛋白エネルギーの約30％と高めの比率に設定する。それによりCO_2産生を抑制し換気系への負荷を軽減できる[4]。また、蛋白質、脂質以外のエネルギーは糖質で投与する（図4）。侵襲下の特殊栄養剤として免疫賦活栄養剤（immune-enhancing diet：IED）や免疫調整栄養剤（immune-modulating diet：IMD）が用いられる。免疫賦活作用が期待される栄養素（immunonutrients）としてはグルタミン、アルギニン、核酸、ω-3脂肪酸、γリノレン酸、食物繊維、抗酸化物質が挙げられる。IEDとしてはアノム®、インパクト®、イムンα®、サンエット-GP®が該当する。また、IMDは過剰な生体反応を抑制する目的で用いられ、アルギニンの配合比が低く、抗酸化物質が強化されている。重症敗血症ではアルギニン投与は代謝産物である一酸化

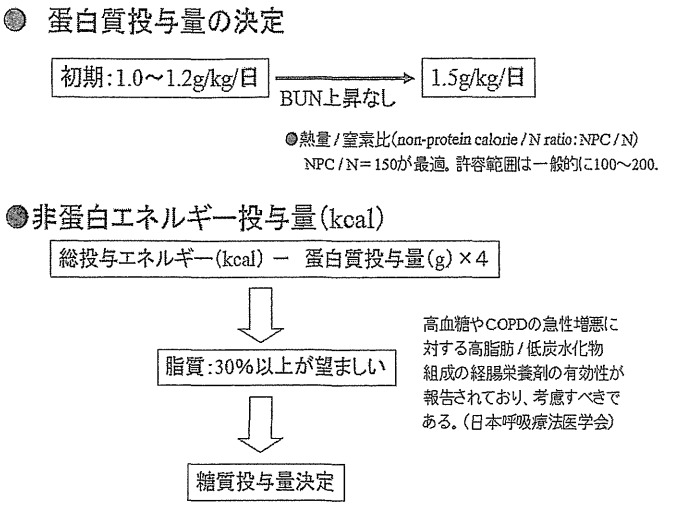

図4　各投与栄養素の決定

窒素（NO）の過剰産生を介して炎症を増悪させる可能性がある。ライフロン-QL®、アノム®、オキシーパ®がIMDに該当する。

5．急性期から回復期への栄養療法の移行

侵襲時にはストレスホルモンやサイトカインの作用によって筋蛋白の崩壊が生じてアミノ酸が供給される。それらは生体防御や組織修復に利用され、同時に急性期蛋白の産生にも用いられる。それに代わってアルブミン合成は低下するため、基本的にアルブミンとCRPとは逆相関すると考えられる。

急性期から回復期に移行していくタイミングをとらえて栄養療法も転換する必要がある。すなわち異化優位の代謝状態では内因性のエネルギー供給が見込まれるため、外因性のエネルギー補給はできるだけ抑制する。一方、同化優位となれば強力な栄養療法とともに積極的なリハビリテーションを実施する（図5）。CRP 3mg/dL程度が同化と異化の境界を決定する目安となりうる。

6．ALI/ARDSに対する栄養療法

1）低カロリー栄養

急性呼吸不全の代表的病態であるALI/ARDSに対する初期の低カロリー経管栄養（約400kcal/day、目標カロリーの25％程度）は高カロリー経管栄養（約1300kcal/day、目標カロリーの80％程度）と比較して、人工呼吸器離脱日数や60日死亡率、感染症の合併などのアウトカムを改善しなかったと

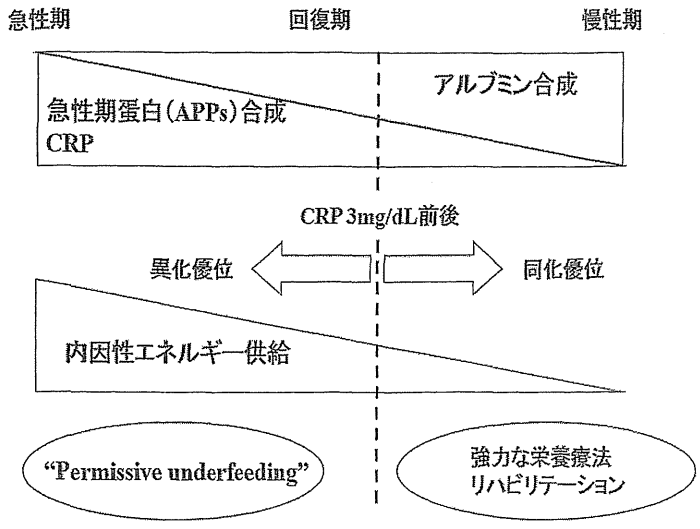

図5　急性期から回復期への栄養療法の移行

報告されている[5]。また、人工呼吸管理中の急性呼吸不全患者を対象とした検討においても同様の結果が報告されている。一方、低カロリー栄養群ではIL-12、IL-1 β、IL-6などの炎症性マーカーやTissue Factor（組織因子）が高カロリー栄養と比較して低値であることから、低カロリー栄養は全身の炎症や凝固能の亢進を軽減する可能性が指摘されている。現時点では初期の低カロリー栄養の有用性を支持する根拠は乏しいとされているが今後さらなる検討を要する。

2）ω-3脂肪酸

敗血症を有するALI/ARDS患者を対象として、ω-3脂肪酸であるエイコサペンタエン酸（EPA）およびγリノレン酸（GLA）や抗酸化物質を強化した栄養剤（オキシーパ®）とコントロールダイエット（エンシュアプラス®HN）の比較検討が行われている。死亡率や人工呼吸管理日数、感染症の合併率に関しては有意差を認めなかったが、ICU滞在期間がオキシーパ群で短縮していた[6]。メタアナリシスでも、死亡率の低下、臓器障害リスクの低下、ICU滞在期間の短縮などのアウトカムを改善することが報告されている[7]。

7．急性期・重症病態における栄養管理ガイドライン

日本呼吸療法医学会[4]および米国集中治療学会（SCCM）/米国静脈経腸栄養学会（ASPEN）のガイドライン以外にも欧州静脈経腸栄養学会（ESPEN）のガイドラインやカナダのCritical Care Nutritionグループによるガイドライン、日本呼吸器学会ALI/ARDS診療のためのガイドライン第2版が参考となる。これらのガイドラインの共通点としては、①経腸栄養が可能ならば、静脈栄養よりも優先する、②治療開始後24～48時間の早期に経腸栄養を開始する、③overfeedingを回避する、④アルギニンは重症度の高い患者では推奨されない、⑤ALI/ARDSに対しては、ω-3脂肪酸、γリノレン酸、抗酸化物質を強化した免疫調整栄養剤の使用を推奨する。⑥グルタミンの経腸栄養による投与は熱傷と外傷で推奨する、などの記載が挙げられる。詳細については各ガイドラインを参照頂きたい。

おわりに

急性期病態における栄養療法を実施する場合、各ガイドラインは参考資料とし、各々の施設の実情に即した治療プロトコールに従って治療を行うことが重要と考えられる。また、現場の医師と看護師に加えてRSTやNSTも参画したチーム医療として管理を行うことが望ましい。実践的には症例ごとに治療プロトコールを綿密に立案するとともに、栄養療法開始後も栄養評価を継続的に行い、病態の変化に応じて適切な変更を加えていくことが重要である。

文　献

1) A.S.P.E.N. Board of Directors and The Clinical Guidelines Task Force : Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN 26：63SA-65SA, 2002.
2) Casaer MP, Mesotten D, Hermans G, et al. Early versus late parenteral nutrition in critically ill adults. N Engl J Med, 365:506-517, 2011.
3) 寺島秀夫，只野惣介，大河内信弘．周術期を含め侵襲下におけるエネルギー投与に関する理論的考え方－既存のエネルギー投与量算定からの脱却．静脈経腸栄養 24：1027-1043, 2009.
4) 日本呼吸療法医学会栄養管理ガイドライン作成委員会：急性呼吸不全による人工呼吸患者の栄養管理ガイドライン．人工呼吸 29：75-120, 2012.
5) The National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trial Network. Initial trophic vs full enteral feeding in Patients With Acute Lung Injury. The EDEN Randomized Trial. JAMA, 307:795-803, 2012.
6) Grau-Carmona T, Morán-García V, García-de-Lorenzo A, et al. Effect of an enteral diet enriched with eicosapentaenoic acid, gamma-linolenic acid and anti-oxidants on the outcome of mechanically ventilated, critically ill, septic patients. Clin Nutr, 30:578-584, 2011.
7) Pontes-Arruda A, Demichele S, Seth A, et al. The use of an inflammation- modulating diet in patients with acute lung or acute respiratory distress syndrome: a meta-analysis of outcome data JPEN J Parent Enter Nutr 32：596-605, 2008.

特集 COPD治療のさらなる展開を目指す—ガイドライン第4版を巡って—

COPDとCPFE(肺気腫合併肺線維症)*

金澤　實[1]

はじめに

Combined Pulmonary Fibrosis and Emphysema(CPFE)はCottinらによって提唱された疾患概念で，HRCT(高分解能CT)において上肺野の気腫と下肺野の線維化を認める臨床的症候群である[1]．本誌特集のメインテーマである慢性閉塞性肺疾患(COPD;chronic obstructive pulmonary disease)との関係では，病因論的にはともに喫煙と加齢を基盤として発生し，気腫化と線維化を共有病変として有するため，実際の臨床像としても共通する部分が多い．CPFEは間質性肺炎の1病型として捉えられることもあるが，COPDの延長上にも存在し，この3者は連続する疾患スペクトラムと考えられる．本稿ではCPFEをCOPDの側から考察し，その病態の本態を示したい．

喫煙に起因する肺病変と肺疾患

生体は慢性的なタバコ煙曝露により，多くの病変や疾患を発現する．死亡の原因としては悪性腫瘍，動脈硬化性の生活習慣病と呼吸器疾患がそれぞれ30%ほどを占める．疾患単位ではCOPDが最多である．これらの疾患の発生は，喫煙に起因して感染の誘発，免疫機能の変化，慢性炎症の発生，悪性腫瘍発生などに関係している[2]．図1には呼吸器系に限って喫煙に関連した基本病変と肺疾患を模式的に示した．COPDもまたCPFEも素因のある個体において，加齢とともに喫煙という外因が加わって発生する病変であり疾患である．最近では加齢の分子機序も次第に解明が進んでおり，2012年の呼吸器学会では加齢と呼吸器疾患のシンポジウムが開催されたが，加齢と気腫・線維化との関係では当教室の臼井が発表し，テロメアの短縮と気腫化・線維化の機序をその後CPFEの総説で記載している[3]．基本病変のなかで，末梢気道病変と気腫化病変がCOPDの病態形成の主役をなすことに異論はないが，中枢気道病変と線維化病変にも一定の役割があると考えられる．一方，CPFEに関しては気腫化病変と線維化病変が主因である．

喫煙に起因する線維化病変について従来の考えは，呼吸細気管支炎(RB;respiratory bronchiolitis)，RBを伴う間質性肺疾患(RB-ILD;RB associated with interstitial lung disease)や剥離型間質性肺炎(DIP;desquamative interstitial pneumonia)が喫煙を主因とする疾患とされてきた．RB-ILDやDIPはかなり稀な疾患であるが，近年，喫煙に起因した特有の線維化病変があり，これまで考えられていたよりも頻回に認められるとの報告が相次いでいる．Kawabataらは下葉の肋骨面

* Chronic Obstructive Pulmonary Disease(COPD)and Combined Pulmonary Fibrosis and Emphysema(CPFE)

[1] 埼玉医科大学呼吸器内科(〒350-0495 埼玉県入間郡毛呂山町毛呂本郷38)　Minoru Kanazawa:Department of Respiratory Medicine, Saitama Medical University

0452-3458/14/¥500/論文/JCOPY

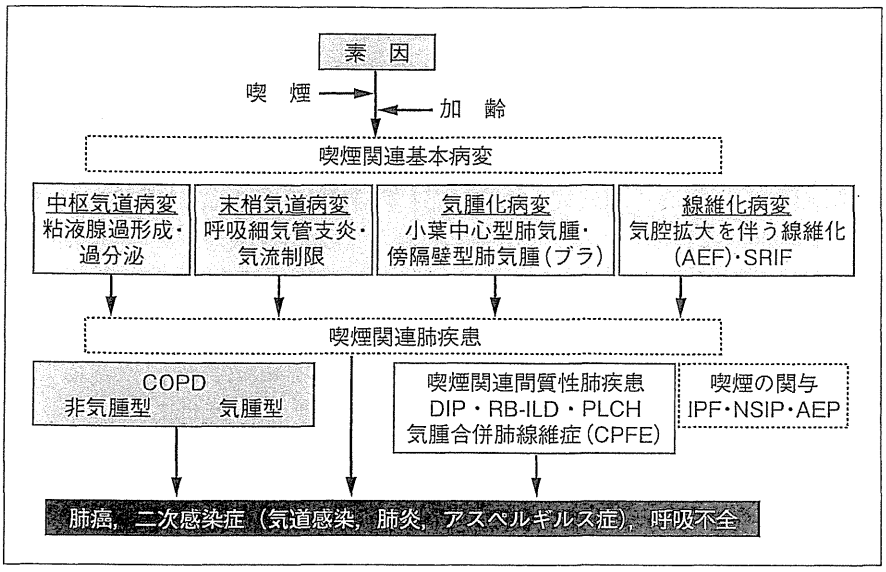

図1　喫煙に関連した病変と疾患
NSIP：非特異型間質性肺炎，AEP：急性好酸球性肺炎．

に優位で，胸膜から少し離れて存在する種々の大きさの壁の薄い嚢胞を伴う網状病変を，気腔拡大を伴う線維化（AEF；airspace enlargement with fibrosis）として報告した．その特徴は，肺構造の改変を伴う硝子化の目立つ間質線維化病変で，気腫性変化を伴い，細気管支中心性の分布を示し，線維芽細胞巣がないこととした[4]．またKatzensteinらは同様の特徴を有する病変を喫煙関連間質性線維化（SRIF；smoking related interstitial fibrosis）として，予後不良の通常型間質性肺炎（UIP；usual interstitial pnuemonia）パターンとは鑑別されるべきものとした．これらの病変の近傍にはいずれも小葉中心性肺気腫や呼吸細気管支炎の所見を伴うことから，これらは喫煙に起因する病変であり，数多くの例でみられる変化とした[5]．気腫性病変と線維化性病変が同一個体に発生することは，それぞれ気腫と線維化が喫煙という共通の損傷機転や炎症反応を有することを示唆する．一方，組織の修復機転が異なると考えられ，COPDと特発性肺線維症（IPF；idiopathic pulmonary fibrosis）の発症機転を理解するうえでも重要な病態・病変といえよう[6]．

これらの基本病変の組み合わせや独特の特性から喫煙が関連する肺疾患群の位置づけを示した（**図2**）．RB-ILD，DIP，そして肺ランゲルハンス細胞組織球症（PLCH；pulmonary Langerhans cell histiocytosis）はいずれも喫煙が主因と考えら

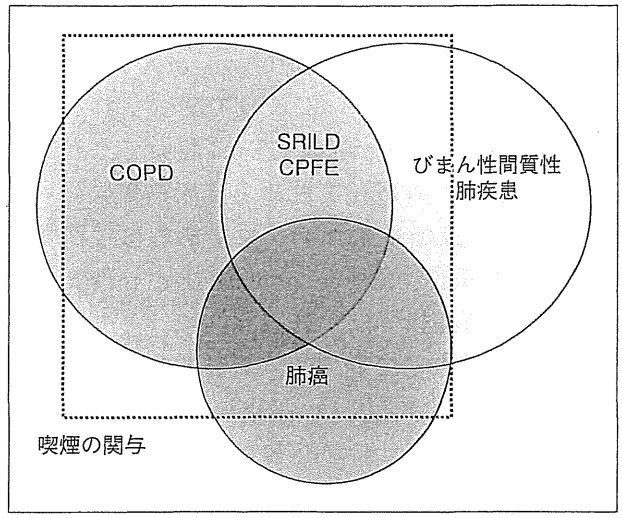

図2　喫煙が関連する肺疾患

れた間質性肺疾患のため喫煙関連肺疾患（SRILD；smoking related interstitial lung disease）と呼ばれていたが，気腫と線維化病変の捉え方は限定的であり，AEFやSRIFほどには気腫と線維化を意識したものではなかった．AEFとSRIFはともに喫煙を原因として強く意識している点が特筆さるべきである．2011年のATSガイドラインでIPFは，喫煙が関連し，最大の危険因子とは認めたものの特発性のラベルを外していない[7]．ただ将来IPFの一部で，気腫を伴う病型が喫煙に起因したものとして独立した疾患とみなされる可能性を残している．

喫煙者のHRCTにおいて，気腫と線維化がど