Arterial Carboxyhemoglobin Measurement Is Useful for Evaluating Pulmonary Inflammation in Subjects with Interstitial Lung Disease

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Abstract

Objective The arterial concentration of carboxyhemoglobin (CO-Hb) in subjects with inflammatory pulmonary disease is higher than that in healthy individuals. We retrospectively analyzed the relationship between the CO-Hb concentration and established markers of disease severity in subjects with interstitial lung disease (ILD).

Methods The CO-Hb concentration was measured in subjects with newly diagnosed or untreated ILD and the relationships between the CO-Hb concentration and the serum biomarker levels, lung function, high-resolution CT (HRCT) findings, and the uptake in gallium-67 (67Ga) scintigraphy were evaluated.

Results Eighty-one non-smoking subjects were studied (mean age, 67 years). Among these subjects, (A) 17 had stable idiopathic pulmonary fibrosis (IPF), (B) 9 had an acute exacerbation of IPF, (C) 44 had stable non-IPF, and (D) 11 had an exacerbation of non-IPF. The CO-Hb concentrations of these subjects were (A) 1.5±0.5%, (B) 2.1±0.5%, (C) 1.2±0.4%, and (D) 1.7±0.5%. The CO-Hb concentration was positively correlated with the serum levels of surfactant protein (SP)-A (r=0.38), SP-D (r=0.39), and the inflammation index (calculated from HRCT; r=0.57) and was negatively correlated with the partial pressure of oxygen in the arterial blood (r=-0.56) and the predicted diffusion capacity of carbon monoxide (r=-0.61). The CO-Hb concentrations in subjects with a negative heart sign on 67Ga scintigraphy were higher than those in subjects without a negative heart sign (1.4±0.5% vs. 1.1±0.3%, p=0.018).

Conclusion The CO-Hb levels of subjects with ILD were increased, particularly during an exacerbation, and were correlated with the parameters that reflect pulmonary inflammation.

Key words: carboxyhemoglobin, exacerbation, idiopathic pulmonary fibrosis, negative heart sign, surfactant proteins, pulmonary inflammation

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Introduction

Hemeoxygenase-1 (HO-1) generates biliverdin IXα, ferrous iron, and carbon monoxide (CO) from the oxidation of heme, and exhaled CO reflects the active heme metabolism (1). The levels of exhaled CO are higher in subjects with pneumonia or bronchiectasis, and return to normal after antibiotic therapy (2, 3). The arterial carboxyhemoglobin (CO-Hb) concentration is reported to be well correlated with the level of exhaled CO. Arterial CO-Hb is also increased in subjects with idiopathic pulmonary fibrosis (IPF), in whom it reflects pulmonary inflammation (4). HO-1, which is produced in alveolar macrophages, is greatly increased in subjects with a range of interstitial lung diseases (ILD), including pulmonary sarcoidosis, desquamative interstitial pneu-
Among the 55 subjects with ILD but not IPF (non-IPF), 5 subjects had connective tissue disease-associated ILD (CT-ILD: primary Sjögren’s syndrome, n=2; polymyositis/dermatomyositis, n=1; rheumatoid arthritis, n=1; and autoimmune hepatitis, n=1), 2 had idiopathic nonspecific interstitial pneumonia (iNSIP), 13 had organizing pneumonia (OP: cryptogenic organizing pneumonia, n=9; drug-induced OP, n=3; and radiation-induced OP, n=1), 6 had eosinophilic lung disease (ELD: eosinophilic granulomatosis with polyangiitis, n=2; and eosinophilic pneumonia, n=4), 6 had hypersensitivity pneumonitis (HP), 19 had pulmonary sarcoidosis, and 4 had IgG4-related disease. The diagnosis of CT-ILD was confirmed based on physical findings, serological testing, and HRCT findings that were consistent with ILD. The histological evaluation of lung biopsy specimens was performed to exclude other specific diseases. The diagnoses of iNSIP, OP, ELD, HP, pulmonary sarcoidosis and IgG4-related disease were based on established criteria (6, 8-12). Non-IPF subjects were subdivided into two groups: subjects who were stable at the time of the evaluation (n=44) and those with an exacerbation of non-IPF (n=11), which was defined as acute and progressive disease that required steroid pulse therapy, with symptoms of fever, dry cough, or dyspnea (13). The subjects with an exacerbation of non-IPF included CT-ILD (n=4), OP (n=5), and ELD (n=2). The control subjects included 9 healthy, non-smoking adults (mean age, 59.0±12.6 years) who were admitted to hospital for the evaluation of a pulmonary nodule that was subsequently found to be benign by lung biopsy. The subjects’ characteristics are summarized in Table 1.

### Pulmonary function testing and serum biomarkers

Blood samples were taken from all subjects on admission. The arterial CO-Hb concentrations were measured by spectrophotometry (ABL800 FLEX System, Radiometer, Copenhagen, Denmark). We also measured the lactate dehydrogenase (LDH; normal <225 U/L), C-reactive protein (CRP; normal <0.3 mg/dL), surfactant protein (SP)-A (SP-A; normal <43.8 ng/mL), SP-D (normal <110 ng/mL) and KL-6 (normal <500 U/mL) levels, and the partial pressure of oxygen in the arterial blood (PaO2). Lung function testing was performed in the month prior to admission. The predicted forced vital capacity (%FVC) and predicted vital capacity (%VC) were determined in 62 subjects, while the predicted carbon monoxide diffusing capacity (%DLco) was determined in 57 subjects.

### HRCT and gallium-67 (67Ga) scintigraphy

The HRCT findings were evaluated using the semiquantitative scoring method described by Ooi et al. (14). HRCT abnormalities were categorized as follows: ground glass opacity alone, mixed ground glass and reticular disease, reticular fibrosis alone, and honeycomb lung. These abnormalities were then scored based on the extent of the disease extent (as a percentage) in each of the 6 lobes. A global score was calculated by adding the scores for each

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**Table 1. The Subjects’ Characteristics.**

| Characteristics                      | Number (n) |
|--------------------------------------|------------|
| Total number                         | 81         |
| Age, years (range)                   | 67 (32-86) |
| Male sex, n (%)                      | 49 (60)    |
| Smoking status (Former / never)      | 49 / 32    |
| Pathologically-proven cases, n (%)   | 62 (77)    |
| **Parameters**                       |            |
| Arterial carboxyhemoglobin, %        | 1.4 ± 0.6  |
| Partial pressure of oxygen in arterial blood, mmHg | 76.3 ± 15.3 |
| Lactate dehydrogenase, U/L           | 255 ± 92   |
| C-reactive protein, mg/dL            | 2.7 ± 5.4  |
| Surfactant protein-A, ng/mL (n=77)   | 69.0 ± 3.4 |
| Surfactant protein-D, ng/mL (n=77)   | 206 ± 191  |
| KL-6, U/mL (80 subjects measured)    | 1063 ± 971 |
| Predicted vital capacity, % (n=62)   | 90.8 ± 26.7|
| Predicted forced vital capacity, %   | 89.5 ± 27.1|
| Predicted carbon monoxide diffusing capacity, predicted (n=57) | 71.9 ± 23.4 |
| **Diagnosis, n (%)**                 |            |
| Idiopathic pulmonary fibrosis        | 26 (32)    |
| Acute exacerbation of idiopathic pulmonary fibrosis | 9         |
| Stable idiopathic pulmonary fibrosis | 17         |
| Non-idiopathic pulmonary fibrosis    | 55 (68)    |
| Exacerbation of non-idiopathic pulmonary fibrosis | 11        |
| Stable non-idiopathic pulmonary fibrosis | 44        |
| **All data are shown as the mean ± SD, unless otherwise indicated.** |

We tested the hypothesis that CO-Hb concentrations would be increased during an exacerbation of ILD (evaluating IPF as a separate subgroup) and that the CO-Hb concentrations would be correlated with serum and radiographic biomarkers of inflammation.

**Materials and Methods**

### The study location and subjects

A retrospective data analysis was conducted at the National Defense Medical College Hospital in Japan. Data from 81 non-smoking subjects with ILD (including 62 subjects with confirmatory lung biopsy results) who had been admitted to hospital from April 2009 to December 2013 were evaluated. The data that were extracted included the patients’ medical history, the physical examination findings, the results of an arterial blood gas analysis, and the high-resolution CT (HRCT) findings. Twenty six subjects were diagnosed with IPF based on the IPF consensus classification (6). The IPF patients were subdivided into two groups: those with stable IPF (n=17) and those with an acute exacerbation of IPF (AE-IPF; n=9), which was defined as an unexplained worsening of dyspnea, hypoxemia, or the worsening or severe impairment of gas exchange, new alveolar infiltration on a radiograph, and the absence of an alternative explanation such as infection, pulmonary embolism, pneumothorax, or heart failure (7).

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**The Subject**

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abnormality in all of the lobes. The inflammation index value was calculated as the sum of the ground glass opacity score and the mixed ground glass and reticular disease score. The fibrosis index was calculated as the sum of the reticular fibrosis score and the honeycomb score. In 1989, Cooke et al. described the “negative heart sign” in patients with pulmonary inflammation due to different causes (including sarcoidosis, tuberculosis, acute respiratory distress syndrome, and ILD) and the increased uptake of 67Ga such that the uptake in the lungs was greater than that in the heart, as shown in Fig. 1 (15). The subjects in the present study who underwent 67Ga scintigraphy, we also categorized according to the presence or absence of the negative heart sign. Both HRCT and 67Ga scintigraphy were performed during the month before admission and were independently assessed by two pulmonologists and two radiologists.

Statistical analysis

All of the statistical analyses were performed using the JMP 10 software program (SAS Institute Inc., North Carolina, USA). The data are expressed as the mean ± SD. Group comparisons were made using Wilcoxon’s rank-sum test. We performed a receiver operating characteristics (ROC) curve analysis to determine the most suitable cut-off concentration of CO-Hb. Nonparametric Spearman’s rank correlation coefficients were calculated to assess the correlations between the CO-Hb concentration and other clinical parameters. p values of <0.05 were considered to indicate statistical significance.

Study approval

This study was approved by the institutional review board of the National Defense Medical College Hospital (approval number 75); informed consent was obtained from all of the subjects.

Results

The CO-Hb concentrations in subjects with ILD

The mean CO-Hb concentration in subjects with ILD was 1.4±0.6% and was significantly higher than that in control subjects (0.7±0.2%, p<0.001) (Fig. 2). The CO-Hb concentrations in each type of ILD as follows: IPF, 1.7±0.6%; CTILD, 2.0±0.6%; iNSIP, 1.4±0.9%; OP, 1.5±0.5%; ELD, 1.0±0.4%; HP subjects, 1.4±0.4% pulmonary sarcoidosis, 1.0±0.3%; and IgG4-related disease, 1.1±0.2%. Fig. 3 shows that the CO-Hb values observed during an AE were significantly higher than those observed in stable individuals. The ROC curve for CO-Hb was evaluated to identify subjects who were having an AE (Fig. 4). The area under the ROC curve was 0.81 and the best cut-off concentration was 1.3%. With this concentration, CO-Hb had a sensitivity of 95% and specificity of 56% in distinguishing an AE in subjects who were not acutely ill.

The relationship between the CO-Hb concentration and the blood and pulmonary function test results

The CO-Hb concentration was positively and significantly correlated with the LDH, CRP, SP-A, and SP-D levels, and was inversely correlated with the PaO2, %VC, and %DLco values (Table 2).

The relationship between the CO-Hb concentration and the HRCT scores

The calculated inflammation index in subjects with stable IPF, AE-IPF, stable non-IPF, and exacerbated non-IPF were 5.1±4.9%, 14.1±5.4%, 4.1±3.9%, and 10.6±5.2%, respec-
Sensitivities with greater inflammation, the CO-Hb concentration was significantly correlated with and the uptake on 67Ga scintigraphy.

The relationship between the CO-Hb concentration and serum biomarker levels, lung function, HRCT, and the uptake in CT-ILD, iNSIP, OP, ELD, HP, pulmonary sarcoidosis, and IgG4-related disease. We also compared the differences in the CO-Hb concentrations of patients during an AE and those who were not acutely ill, and evaluated the relationship between the CO-Hb concentration and serum biomarker levels, lung function, HRCT, and the uptake in 67Ga scintigraphy.

Arterial CO-Hb measurement is reported to be useful for monitoring the active heme metabolism and pulmonary inflammation in subjects with inflammatory pulmonary disease (4). In the present study, we measured the CO-Hb concentrations of patients with ILD including those with IPF, CT-ILD, iNSIP, OP, ELD, HP, pulmonary sarcoidosis, and IgG4-related disease. We also compared the differences in the CO-Hb concentrations of patients during an AE and those who were not acutely ill, and evaluated the relationship between the CO-Hb concentration and serum biomarker levels, lung function, HRCT, and the uptake in 67Ga scintigraphy.

Oxidative stress has been implicated in the pathogenesis and progression of ILD (16, 17) and is reported to be correlated with poor clinical outcomes in subjects with ILD (18). Oxidative stress increases the release of HO-1 from alveolar macrophages, bronchial epithelial cells and inflammatory cells, leading to increased CO-Hb concentrations (4, 5, 19). Consistent with these reports, we demonstrated that the CO-Hb concentrations of subjects with ILD were higher than those of control subjects. Thus, the measurement of CO-Hb may be useful for monitoring oxidative stress in subjects with ILD.

The histological pattern of an AE of fibrotic ILD is diffuse alveolar damage or OP superimposed upon fibro-
with the extent of alveolitis (denoted by ground glass opacities on HRCT), but not with the progression of fibrosis (26). Consistent with this observation, we found that the CO-Hb concentration was correlated with the serum levels of SP-A and SP-D and with the PaO2, LDH, and CRP values, which are markers of cellular damage and inflammation (18, 27). The inflammation index, which was calculated by an HRCT scoring system, has also been reported to be a robust indicator of alveolitis (14). In the present study, the CO-Hb concentration was correlated with the inflammation index but not the fibrosis index, which further strengthens the hypothesis that CO-Hb is a marker of oxidative stress and alveolitis.

$^{67}$Ga scintigraphy is also used to evaluate the degree of pulmonary inflammation (28, 29). With an increase in the gallium uptake in the lung, pulmonary images will eventually appear brighter than cardiac (blood flow) images; this is referred to as the negative heart sign. We showed that subjects with ILD who had this negative heart sign had a much higher concentration of CO-Hb than those who did not. Thus, we hypothesize that the measurement of the CO-Hb level may be another non-invasive option (that avoids radiation exposure) for evaluating pulmonary inflammation due to oxidative stress in ILD that can be used in place of $^{67}$Ga scintigraphy.

There are several clinically-accepted methods of assessing alveolitis in patients with ILD. This retrospective study suggests that CO-Hb, which can be easily, rapidly and reproducibly measured, may be as useful in imaging studies and as a serum biomarker. In the future, it will be important to evaluate whether a high CO-Hb concentration reflects alveolitis pathologically and to learn whether the CO-Hb concentration can also be used to evaluate the response to therapy in patients with these diseases.

The authors state that they have no Conflict of Interest (COI).

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References

1. Slebos DJ, Ryter SW, Choi AM. Heme oxygenase-1 and carbon monoxide in pulmonary medicine. Respir Res 4: 7, 2003.

2. Biernacki WA, Kharitonov SA, Barnes PJ. Exhaled carbon monoxide in patients with lower respiratory tract infection. Respir Med 95: 1003-1005, 2001.

3. Horvath I, Loukides S, Wodehouse T, Kharitonov S, Cole P, Barnes P. Increased levels of exhaled carbon monoxide in bronchiectasis: a new marker of oxidative stress. Thorax 53: 867-870, 1998.

4. Yasuda H, Yamaya M, Yanai M, Ohru I, Sasaki H. Increased blood carboxyhaemoglobin concentrations in inflammatory pulmonary diseases. Thorax 57: 779-783, 2002.

5. Lakari E, Pylkäs P, Pietarinen-Runtti P, Pääkkö P, Pääkkö P, Soini Y, Kinnula VL. Expression and regulation of hemeoxygenase-1 in healthy human lung and interstitial lung disorders. Hum Pathol 32: 1257-1263, 2001.

6. Travis WD, Costabel U, Hansell DM, et al.: ATS/ERS Committee on Idiopathic Interstitial Pneumonias. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 188: 733-748, 2013.

7. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 176: 636-643, 2007.

8. Travis WD, Hunninghake G, King TE Jr, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med 177: 1338-1347, 2008.

9. Jeong YJ, Kim KI, Seo IJ, et al. Eosinophilic lung diseases: a clinical, radiologic, and pathologic overview. Radiographics 27: 617-637, 2007.

10. Lacasse Y, Selman M, Costabel U, et al. HP Study Group. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med 168: 952-958, 2003.

11. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al.; ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and Other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis 16: 149-173, 1999.

12. Umehara H, Okazaki K, Masaki Y, et al. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. Mod Rheumatol 22: 21-30, 2012.

13. Oka S, Furukawa H, Shinoda K, et al. Serum biomarker analysis of collagen disease patients with acute-onset diffuse interstitial lung disease. BMC Med 14: 9, 2013.

14. Ooi GC, Mok MY, Tsang KW, et al. Interstitial lung disease in systemic sclerosis. Acta Radiol 44: 258-264, 2003.

15. Cooke SG, Davies ER, Goddard PR. Pulmonary uptake in 67-gallium citrate scintigraphy—the ‘negative heart’ sign. Postgrad Med J 65: 885-891, 1989.

16. Bargagli E, Olivieri C, Bennett D, Prasse A, Muller-Quernheim J, Rottoli P. Oxidative stress in the pathogenesis of diffuse lung diseases: a review. Respir Med 103: 1245-1256, 2009.

17. Walters DM, Cho H, Klebeberger SR. Oxidative stress and antioxidants in the pathogenesis of pulmonary fibrosis: a potential role for Nrf2. Antioxid Redox Signal 10: 321-331, 2008.

18. Kanoh S, Kobayashi H, Motoyoshi K. Exhaled ethane: an in vivo biomarker of lipid peroxidation in interstitial lung diseases. Chest 128: 2387-2392, 2005.

19. Ni L, Venkatesan MI, Miguel A, et al. Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones via the antioxidant-responsive element. J Immunol 165: 3393-3401, 2000.

20. Hyzy R, Huang S, Myers J, Flaherty K, Martinez F. Acute exacerbation of idiopathic pulmonary fibrosis. Chest 132: 1652-1658, 2007.

21. Churg A, Wright JL, Tazelaar HD. Acute exacerbations of fibrotic interstitial lung disease. Histopathology 58: 529-530, 2011.

22. Mumbry S, Upton RL, Chen Y, et al. Lung heme oxygenase-1 is elevated in acute respiratory distress syndrome. Crit Care Med 32: 1130-1135, 2004.

23. Hara Y, Shinkai M, Kanoh S, et al. Clinico-pathological analysis referring hemeoxygenase-1 in acute fibrous and organizing pneumonia patients. Respir Med Case Rep 14: 53-56, 2015.

24. Takahashi H, Shiratori M, Kanai A, Chiba H, Kuroki Y, Abe S. Monitoring markers of disease activity for interstitial lung diseases with serum surfactant proteins A and D. Respirology 11 Suppl: S51-S54, 2006.

25. Ohnishi H, Yokoyama A, Kondo K, et al. Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocye chemoattractant protein-1 as serum markers for interstitial lung diseases. Am J Respir Crit Care Med 165: 378-381, 2002.

26. Takahashi H, Fujishima T, Koba H, et al. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. Am J Respir Crit Care Med 162: 1109-1114, 2000.

27. DeRemee RA. Serum lactate dehydrogenase activity and diffuse interstitial pneumonits. JAMA 204: 1193-1195, 1968.

28. Line BR, Hunninghake GW, Keogh BA, Jones AE, Johnston GS, Crystal RG. Gallium-67 scanning to stage the alveolitis of sarcoidosis: correlation with clinical studies, pulmonary function studies, and bronchoalveolar lavage. Am Rev Respir Dis 123: 440-446, 1981.

29. Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA. Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract (first of two parts). N Engl J Med 310: 154-66, 1984.

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