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□ ORIGINAL ARTICLE □

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 (^{67}Ga) 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 ^{67}Ga 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-

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Table 1. The Subjects' Characteristics.

Characteristics	
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, % (n=62)	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.

monia, and silicosis, as well as in patients with IPF (5).

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).

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 polyangitis, 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 (PaO_2). 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 (^{67}Ga) scintigraphy

The HRCT findings were evaluated using the semi-quantitative 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

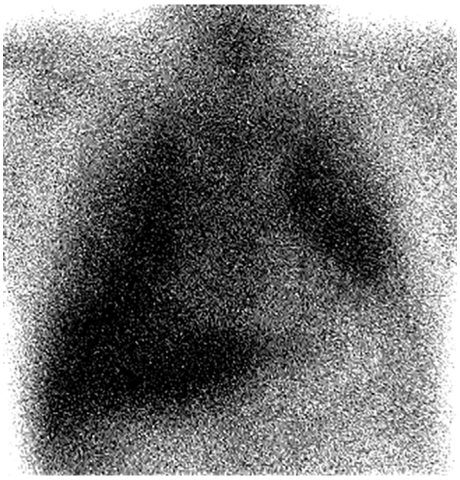

Figure 1. The negative heart sign on ^{67}Ga scintigraphy. The negative heart sign, as shown here, is seen when the amount of gallium taken up by the lungs is greater than the amount of gallium circulating in the blood, and therefore in the heart. This is a robust indication of diffuse pulmonary inflammation.

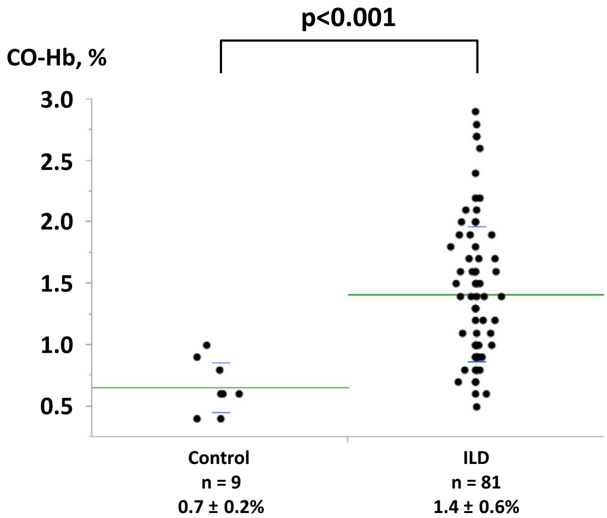

Figure 2. The CO-Hb concentrations in ILD patients and control subjects. The CO-Hb concentration in the ILD patients was higher than that in the control subjects (1.4±0.6% vs. 0.7±0.2%, p<0.001). The center lines show the mean concentrations, while the upper and lower lines show the standard deviations.

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 ^{67}Ga 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 ^{67}Ga scintigraphy, we also categorized according to the presence or absence of the negative heart sign. Both HRCT and ^{67}Ga 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%; CT-ILD, 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 PaO_2, %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-

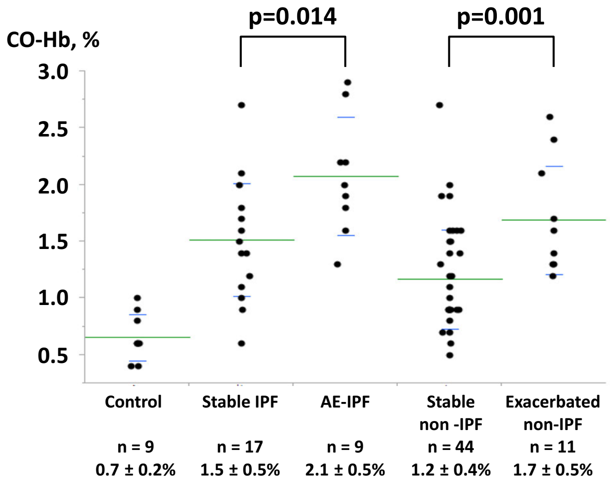

Figure 3. **The CO-Hb concentration during an exacerbation of ILD in comparison to non-exacerbated subjects. The CO-Hb levels were measured in subjects with stable (non-exacerbated) IPF (1.5±0.5%), subjects with an acute exacerbation of IPF (2.1±0.5%), subjects with non-IPF who were stable (1.2±0.4%), and in subjects with an exacerbation of non-IPF (1.7±0.5%). The CO-Hb levels during an acute exacerbation were significantly higher than those observed in stable subjects. The center lines show the mean concentrations, while the upper and lower lines show the standard deviations.**

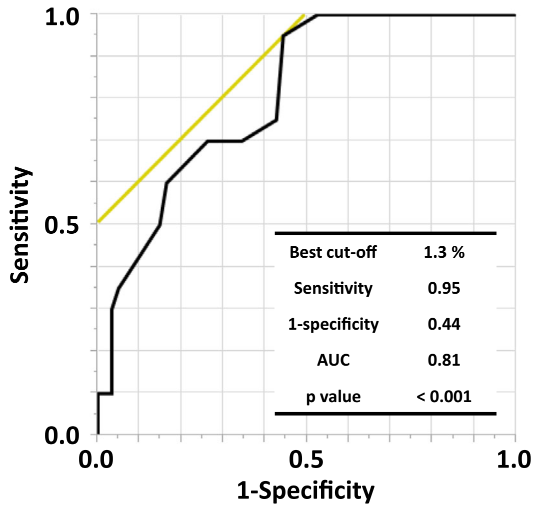

Figure 4. **The receiver operating characteristic (ROC) curve for the CO-Hb concentration. The ROC curve for the CO-Hb concentration was evaluated to discriminate the subjects with an acute exacerbation from the non-exacerbated subjects. The area under the ROC curve was 0.81 and the best cut-off concentration was 1.3%. With this concentration, CO-Hb had a sensitivity of 95% and specificity of 56% for detecting an acute exacerbation.**

Table 2. **The Relationships between the CO-Hb Concentration and the Blood and Pulmonary Function Test Results.**

Parameters	r	n	p value
Partial pressure of oxygen in arterial blood	−0.56	81	<0.001
Lactate dehydrogenase	0.49	81	<0.001
C-reactive protein	0.31	81	0.005
Surfactant protein-A	0.38	77	<0.001
Surfactant protein-D	0.39	77	<0.001
KL-6	0.27	80	0.016
Vital capacity	−0.47	62	<0.001
Forced vital capacity	−0.47	62	<0.001
Diffusion capacity of carbon monoxide	−0.61	57	<0.001

tively. The calculated fibrosis index in subjects with stable IPF, AE-IPF, stable non-IPF, and exacerbated non-IPF were 5.2±5.7%, 5.6±5.8%, 0.5±1.7%, and 1.1±2.3%, respectively. The CO-Hb concentration was significantly correlated with the inflammation index (r=0.57, p<0.001), but not with the fibrosis index (r=0.24, p=0.03) (Fig. 5).

The relationship between the CO-Hb concentration and the uptake on ^{67}Ga scintigraphy

^{67}Ga scintigraphy was performed in 52 of 81 subjects. In the remaining subjects, it was not performed because of severe respiratory failure (n=15) or it was not performed in the month before hospital admission (n=14). Among the subjects who underwent ^{67}Ga scintigraphy, 10 had stable IPF, 37 had stable non-IPF, and 5 had exacerbated non-IPF. Consistent with the greater pulmonary uptake of ^{67}Ga in patients with greater inflammation, the CO-Hb concentration was significantly higher in subjects with a negative heart sign than those without a negative heart sign (1.1±0.3% vs. 1.4±0.5%, p=0.018) (Fig. 6).

Discussion

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 subjects 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 ^{67}Ga 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-

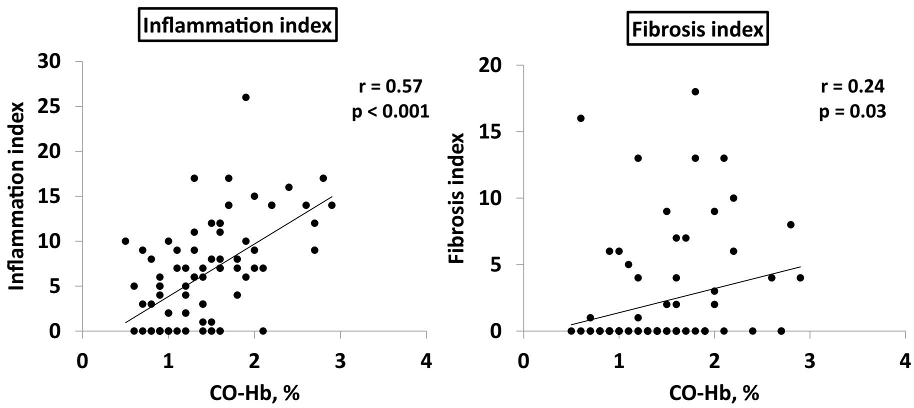

Figure 5. The relationship between the CO-Hb concentration and the HRCT score. There was a significant correlation between the CO-Hb concentration and the inflammation index (r=0.57 p<0.001); however, there was only a weak correlation with the fibrosis index (r=0.24 p=0.03).

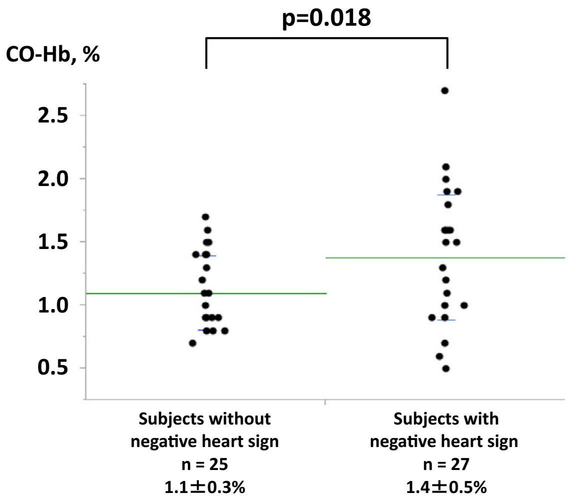

Figure 6. The relationship between the CO-Hb concentration and the uptake on ^{67}Ga scintigraphy. The CO-Hb concentration in subjects with a negative heart sign was higher than that in subjects without a negative heart sign (1.4±0.5% vs. 0.7±0.2%, p=0.018). The center lines show the mean concentrations, while the upper and lower lines show the standard deviations.

sis (20, 21). Due to oxidative stress, HO-1 is overexpressed in the lungs of subjects with an acute lung injury (pathologically diffuse alveolar damage) and the pulmonary overexpression of HO-1 is correlated with a poor prognosis (22, 23). In the present study, we showed that during an AE, the CO-Hb concentration was much higher than observed in non-exacerbated subjects; this most likely reflects heme metabolism and oxidative stress.

ILD is characterized by alveolar inflammation, which leads to progressive fibrosis (24). In the presence of alveolitis, the surfactant apoproteins, SP-A and SP-D, are secreted by type II pneumocytes; these apoproteins can be detected in the serum as a biomarker of alveolitis (24, 25). The concentrations of SP-A and SP-D are reported to be correlated 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 PaO_2, 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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