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ORIGINAL RESEARCH

Inflammatory biomarkers in asthma-COPD overlap syndrome

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Background: The clinical phenotypes and underlying mechanisms of asthma-COPD overlap syndrome (ACOS) remain elusive. This study aimed to investigate a comparison of COPD patients with and without ACOS, focusing on inflammatory biomarkers, in an outpatient COPD cohort.

Methods: We conducted a cross-sectional study analyzing prospectively collected data from the Ishinomaki COPD Network registry. All participants were diagnosed with COPD, confirmed by using spirometry, and were aged 40–90 years and former smokers. Patients with features of asthma including both variable respiratory symptoms and variable expiratory airflow limitation were identified and defined as having ACOS. Then, the inflammatory biomarkers such as fractional exhaled nitric oxide level, blood eosinophil count and percentage, total immunoglobulin E (IgE) level, and presence of antigen-specific IgE were evaluated.

Results: A total of 257 patients with COPD were identified, including 37 (14.4%) with ACOS. Patients with ACOS tended to be younger, have a shorter smoking history, and use more respiratory medications, especially inhaled corticosteroids and theophylline. Mean fractional exhaled nitric oxide level was significantly higher in those with ACOS than in those without ACOS (38.5 parts per billion [ppb] vs 20.3 ppb, $P<0.001$). Blood eosinophil count and percentage were significantly increased in those with ACOS (295/mm^3 vs 212/mm^3, $P=0.032$; 4.7% vs 3.2%, $P=0.003$, respectively). Total IgE level was also significantly higher, and presence of antigen-specific IgE was observed more frequently in patients with ACOS. Receiver operating characteristic curve analysis indicated that the sensitivity and specificity of these biomarkers were relatively low, but combinations of these biomarkers showed high specificity for ACOS diagnosis.

Conclusion: These results provide evidence that these inflammatory biomarkers can be used to support the diagnosis of ACOS.

Keywords: asthma, asthma-COPD overlap syndrome, COPD, biomarkers

Introduction

Asthma and COPD have been regarded as two distinct disease entities that often overlap.[1–3] Asthma and COPD overlap has a prevalence of ~15%–20% in patients with obstructive airway disease (asthma or COPD)[4–6] and is associated with significant health status impairment,[4] increased exacerbations,[5] and increased hospitalizations.[6] In 2014, the Global Initiative for Asthma (GINA) and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) issued a joint document describing that asthma-COPD overlap syndrome (ACOS) is characterized by persistent airflow limitation with several features usually associated with both asthma and COPD.[7] The clinical phenotypes and underlying mechanisms of ACOS have attracted interest during recent years. However, ACOS remains somewhat controversial, and there is no consensus on the best definition of ACOS.[8,9]

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The clinical phenotypes, including biomarkers, of ACOS remain elusive. Inflammatory biomarkers, such as fractional exhaled nitric oxide (FENO), blood eosinophils, and allergen-specific immunoglobulin E (IgE), are sometimes used to distinguish between asthma and COPD.[7] Typically, asthma is characterised by inflammation predominantly involving eosinophils, whereas COPD is characterized by inflammation by neutrophils.[1,2] FENO and blood eosinophil count have been considered as biomarkers of local and systemic eosinophilic inflammation, which increase in patients with asthma.[10,11] Total serum IgE and antigen-specific IgE levels are also elevated in those with allergic asthma.[12] However, the significance of these inflammatory biomarkers in diagnosis of ACOS remains unclear.

We conducted a cross-sectional study to 1) analyze prospectively collected data from a Japanese COPD registry and 2) compare COPD patients with and without ACOS, focusing on inflammatory biomarkers, to investigate clinical phenotypes of ACOS.

Patients and Methods

Study design

We conducted a cross-sectional study to analyze the prospectively collected data of consecutive scheduled visits or newly registered patients from the Ishinomaki COPD Network (ICON) registry[13] between May 2015 and January 2016. Briefly, ICON is a regional medical liaison system aimed at comprehensive care of patients with COPD and has a multicenter interdisciplinary collaboration with health care providers in Ishinomaki, Japan. Patients registered in ICON are regularly treated by general practitioners in Ishinomaki and surrounding cities, and receive scheduled examinations and education at the Japanese Red Cross Ishinomaki Hospital (a 452-bed tertiary community hospital in Ishinomaki) every 6–12 months.

This study is part of an ongoing COPD cohort study registered with the University Hospital Information Network Clinical Trials Registry (identifier: UMIN000017376). All patients provided written informed consent, and the study was approved by the Ethics Committee of Japanese Red Cross Ishinomaki Hospital (approval number: 12-14-1).

Patients

Patients with stable COPD, who were aged 40–90 years and former smokers with a smoking history of at least 10 pack-years, were included in this study. All patients were diagnosed with COPD according to GOLD criteria.[14] Persistent airflow limitation, defined as postbronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <0.7, was confirmed by using repeated spirometry. Exclusion criteria were as follows: current smoker or never smoked, chronic bronchitis or emphysema without airflow limitation, hematologic disease, history of lung resection, use of oral corticosteroids, receiving anti-IgE therapy, and exacerbation of COPD in the 4 weeks preceding data collection.

ACOS criteria

ACOS was defined according to the GINA/GOLD joint document.[7] Participants in this study fulfilled three or more features of COPD, namely age ≥40 years, postbronchodilator FEV_1/FVC ratio <0.7, and exposure to cigarette smoke. Among patients with COPD, we identified those with features of asthma including both history of variable respiratory symptoms and variable expiratory airflow limitation.[15] Respiratory symptoms included wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity. Expiratory airflow limitation was defined as a postbronchodilator increase in FEV_1 >12% and 200 mL from baseline, or increase in FEV_1 >12% and 200 mL (or in peak expiratory flow >20%) from baseline after 4 weeks of anti-inflammatory treatment, in the absence of respiratory infections. We evaluated these diagnostic features based on the medical record review prior to biomarker measurement.

Clinical and physiologic measurements

Sociodemographic characteristics, smoking status, presence of rhinitis, and maintenance treatments were recorded for each patient. Body mass index (BMI) was calculated in kg/m^2. Dyspnea was evaluated by using the modified Medical Research Council dyspnea scale.[16] COPD-related health status was assessed by using the COPD Assessment Test.[17] Frequency of severe exacerbations requiring hospitalization in the previous year was evaluated based on direct patient interviews, diaries kept by patients or caregivers, and medical record review.

Pulmonary function tests were conducted by a well-trained technician following the guidelines under a stable condition.[18] Severity of airflow limitation was classified in accordance with GOLD staging.[14]

Inflammatory biomarker measurements

FENO level was measured by using the NIOX MINO device (Aerocrine, Morrisville, NC, USA) according to the standard operating procedures recommended by the manufacturer. FENO level was classified as follows: normal, <25 parts per billion (ppb); intermediate, 25–50 ppb; and high, >50 ppb.[19]

Blood samples were obtained to determine blood eosinophil count and percentage and total serum IgE level. Presence of antigen-specific IgE was determined by using

the ImmunoCAP Phadiatop test (Thermo Fisher Scientific, Waltham, MA, USA), an in vitro assay for antigen-specific IgE antibodies to common inhalant allergens. Antigen-specific IgE was considered to be present when the results were positive for at least one of the following: house dust mite, cat, dog, *Alternaria tenuis*, *Aspergillus fumigatus*, or ragweed. Blood samples were obtained on the same day as FENO measurement. The cutoff value of high blood eosinophil count was set at ≥500 cells/mm,[3,20] while the cutoff value of total serum IgE level was set at 173 IU/mL according to the reference range of the clinical laboratory at Japanese Red Cross Ishinomaki Hospital.

Statistical analysis

Data are shown as median (interquartile range) or mean ± standard deviation unless otherwise specified. Differences between groups were assessed by using the Student's *t*-test or Mann–Whitney *U*-test for continuous variables, while associations between categorical variables were evaluated by using Fisher's exact test. The value of total serum IgE level was log transformed because the variables were not normally distributed; the results are expressed as geometric mean values. Distribution of GOLD staging between patients with and without ACOS was analyzed by using the Kruskal–Wallis test. Receiver operating characteristic (ROC) curves were plotted in order to estimate the diagnostic cutoff values. An optimal cutoff value was obtained from the highest sum of sensitivity and specificity.

All statistical analyses were performed by using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).[21] *P*-values <0.05 were considered as significant.

Results

Characteristics of the study patients

A total of 334 consecutive patients with COPD were recruited, and 257 eligible patients (236 males, 21 females; median age, 75 years) were identified. Characteristics of the study participants are shown in Table 1. Among the 257 patients with COPD, 48 (18.7%) had a history of variable respiratory symptoms, 57 (22.2%) had variable expiratory airflow limitation, and 37 (14.4%) had both features; these 37 patients were diagnosed as having ACOS.

Characteristics between patients with and without ACOS

Among the 37 patients with ACOS, no patient had respiratory symptoms in childhood, but two (5.4%) had symptoms before the age of 40 years. Marked reversibility with postbronchodilator increase in FEV_1 >12% and 400 mL from baseline was observed in four patients (10.8%).

Table 1 Characteristics of the study patients (n=257)

Age, years	75 (10)
Female	21 (8.2)
BMI, kg/m^2	23.3 (4.6)
Smoking history, pack-years	52.0 (35.1)
Rhinitis	8 (3.1)
FEV_1, L	1.50 (0.93)
%FEV_1, %	63.1 (32.9)
FVC, L	3.03 (1.32)
GOLD stage	
1	54 (21.0)
2	122 (47.5)
3	49 (19.1)
4	32 (12.4)
mMRC dyspnea scale grade	1 (2)
CAT score	5 (6.5)
Regular medication	
LAMA	195 (75.9)
LABA	48 (18.7)
ICS	8 (3.1)
LAMA/LABA	21 (8.2)
ICS/LABA	105 (40.9)
Theophylline	31 (12.1)
Home oxygen therapy	38 (14.8)
Severe exacerbation in previous year	18 (7.0)

Notes: Data are shown as median (interquartile range) or number (%). Used with the permission of the Medical Research Council.[16]

Abbreviations: BMI, body mass index; CAT, COPD assessment test; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; mMRC, modified Medical Research Council.

Characteristics between patients with and without ACOS are shown in Table 2. Patients with ACOS tended to be younger (*P*=0.003) and have a shorter smoking history (*P*=0.019). Sex, BMI, spirometric results, dyspnea, COPD-related health status, and incidence of severe exacerbations requiring hospitalization in the previous year were not significantly different between the groups. Patients with ACOS tended to use more respiratory medications, especially inhaled corticosteroids (ICS) and theophylline, compared with patients without ACOS (86.5% vs 36.8%, 27.0% vs 9.5%, respectively; both *P*<0.001).

Airway and systemic inflammatory biomarkers

Mean FENO levels were significantly higher in patients with ACOS than in those without ACOS (38.5±28.6 ppb vs 20.3±11.0 ppb, *P*<0.001) (Figure 1A). High FENO level were significantly present in those with ACOS, while low FENO level were significantly present in those without ACOS (Table 3). No significant association with FENO

Table 2 Characteristics of patients with and without ACOS

Characteristic	ACOS (+) (n=37)	ACOS (–) (n=220)	*P*-value
Age, years	71.3±7.5	75.0±7.0	0.003
Female	4 (10.8)	17 (7.7)	0.527
BMI, kg/m^2	24.0±3.8	23.4±3.8	0.331
Smoking history, pack-years	47.4±27.4	58.9±27.3	0.019
Rhinitis	3 (8.1)	5 (2.3)	0.092
FEV_1, L	1.68±0.69	1.51±0.65	0.152
$\%FEV_1$, %	64.4±21.9	61.4±22.1	0.441
FVC, L	3.32±0.99	3.09±1.73	0.428
GOLD stage			0.421
1	9 (24.3)	45 (20.5)	
2	18 (48.6)	104 (47.3)	
3	7 (18.9)	42 (19.1)	
4	3 (8.1)	29 (13.2)	
mMRC dyspnea scale score	0.9±0.9	1.1±1.0	0.193
CAT score	5.6±4.0	7.0±6.3	0.443
Regular medication[a]			
LAMA	32 (86.5)	184 (83.6)	0.661
LABA	30 (81.1)	143 (65.0)	0.054
ICS	32 (86.5)	81 (36.8)	<0.001
Theophylline	10 (27.0)	21 (9.5)	<0.001
Home oxygen therapy	3 (8.1)	35 (15.9)	0.216
Severe exacerbation in prior year	1 (2.7)	17 (7.7)	0.268

Notes: Data are shown as mean ± SD or number (%). Used with the permission of the Medical Research Council.[16] [a]Medication could be used alone or in combination. Differences between groups were assessed by using the Student's *t* test or Mann-Whitney *U* test for continuous variables. Distribution of GOLD staging between patients with and without ACOS was analysed by using the Kruskal-Wallis test. $P<0.05$ was considered statistically significant.

Abbreviations: ACOS, asthma-COPD overlap syndrome; BMI, body mass index; CAT, COPD assessment test; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; mMRC, modified Medical Research Council; SD, standard deviation.

levels and ICS treatment was observed in patients with ACOS (P=0.22) and without ACOS (P=0.55).

Mean blood eosinophil count and percentage were significantly higher in patients with ACOS than in those without ACOS (295±160/mm^3 vs 212±223/mm^3, P=0.032; 4.7±3.0% vs 3.2±2.7%, P=0.003, respectively) (Figure 1B and C). High percentage of blood eosinophils (>2%) was observed in those with ACOS (P=0.014) (Table 3). No significant association with ICS use and blood eosinophil count or percentage was observed in patients with (P=0.42 and P=0.78, respectively) and without ACOS (P=0.11 and P=0.61, respectively).

Total serum IgE level was significantly higher in patients with ACOS than in those without ACOS (2989 IU/mL vs 451 IU/mL, P=0.004) (Figure 1D). Presence of antigen-specific IgE also was observed more frequently in those with ACOS (56.8% vs 28.6%, P=0.001).

ROC curve analysis demonstrated that 23 ppb was the best diagnostic cutoff value of FENO level for ACOS (area under the curve [AUC] 0.74; 95% confidence interval [CI] 0.63–0.84). Sensitivity and specificity of 23 ppb for the diagnosis of ACOS were 73.0% and 68.2%, respectively. In addition, ROC curve analysis showed that 156.2/mm^3 was the best diagnostic cutoff value of eosinophil count (AUC 0.70; 95% CI 0.61–0.78) and that 434 IU/mL was the best diagnostic cutoff value of total serum IgE level (AUC 0.64; 95% CI 0.54–0.75). Sensitivity and specificity of 156.2/mm^3 and 434 IU/mL for the diagnosis of ACOS were 83.8% and 49.5%, and 45.9% and 85.9%, respectively.

Various combinations of these biomarkers showed a high specificity for ACOS diagnosis. Combination of FENO >23 ppb and IgE >434 IU/mL showed 94.1% specificity, 37.8% sensitivity, 51.9% positive predictive value (PPV), and 90.0% negative predictive value (NPV). Combination of FENO >23 ppb and eosinophil count >156.2/mm^3 showed 85.5% specificity, 59.5% sensitivity, 40.7% PPV, and 92.6% NPV. Combination of IgE >434 IU/mL and eosinophil count >156.2/mm^3 showed 92.3% specificity, 40.5% sensitivity, 46.9% PPV, and 90.2% NPV. Triple combination (FENO >23 ppb, IgE >434 IU/mL, and eosinophil count >156.2/mm^3) showed 96.8% specificity, 37.8% sensitivity, 66.7% PPV, and 90.3% NPV.

No adverse events were observed during biomarker measurement.

Discussion

We identified patients with ACOS presenting features of asthma, including both variable respiratory symptoms and variable expiratory airflow limitation, in a COPD outpatient cohort, and demonstrated that airway inflammatory biomarkers, including FENO, blood eosinophil count, and IgE, are increased in those with ACOS. The results of the present study demonstrated that inflammatory biomarkers can be used to support the diagnosis of ACOS.

Previous studies have attempted to identify the ACOS phenotypes by using various criteria.[8] Thus, there is an increased awareness of the importance of recognizing ACOS by using biomarkers.[22–25] Recent studies reported that increased FENO levels were identified in a subset of patients with COPD.[23,24] Our findings are congruent with the observation by Donohue et al, who reported increased FENO levels in patients previously clinically diagnosed with both COPD and asthma.[23] In addition, the findings of our study are compatible with those of Kitaguchi et al, who observed increased blood and sputum eosinophil counts in COPD patients with asthmatic symptoms.[25]

The results of this study indicated that inflammatory biomarkers provide additional diagnostic information for ACOS. Although the sensitivity and specificity of these biomarkers

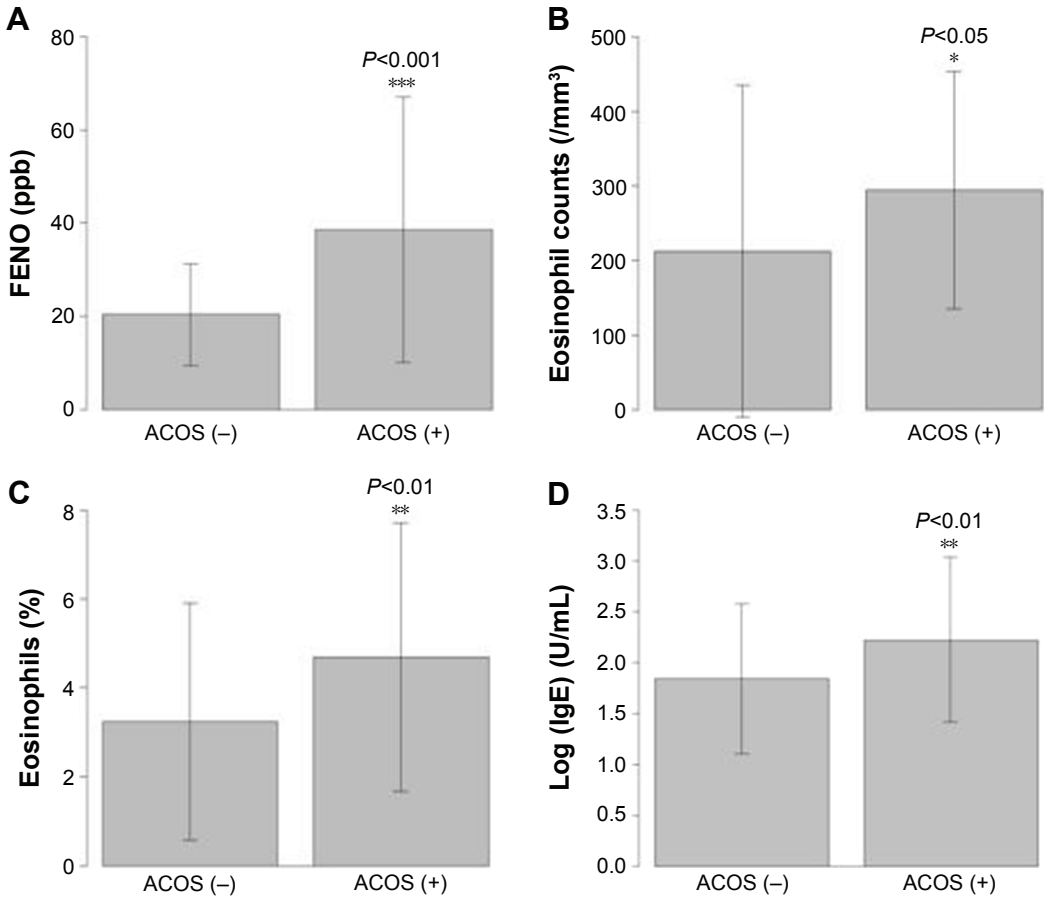

Figure 1 Inflammatory biomarkers in patients with and without ACOS.
Notes: (**A**) FENO level, (**B**) blood eosinophil count, (**C**) percentage of blood eosinophils, (**D**) total serum IgE level. The error bars represent the standard deviation. Differences between groups were assessed by using the Student's *t* test. The value of total serum IgE level was log transformed because the variables were not normally distributed; the results are expressed as geometric mean values. $P<0.05$ was considered statistically significant: *$P<0.05$, **$P<0.01$, and ***$P<0.001$.
Abbreviations: ACOS, asthma-COPD overlap syndrome; FENO, fractional exhaled nitric oxide; ppb, parts per billion; IgE, immunoglobulin E.

were relatively low, combinations of these biomarkers showed high specificity for ACOS diagnosis. Total serum IgE level was not affected by ICS therapy; however, treatment with ICS was reported to decrease FENO level[26,27] and blood eosinophil count.[28] Half of the patients in this study used ICS. Thus, we may have underestimated FENO level and blood eosinophils in these patients. Furthermore, airway eosinophilia is not exclusive to asthma and is present in patients with COPD.[29] Therefore, we propose that overconfidence in such biomarkers may lead to diagnostic errors.

Table 3 Inflammatory biomarkers in patients with and without ACOS

Biomarker	ACOS (+) (n=37)	ACOS (–) (n=220)	*P*-value
FENO level			
<25 ppb	12 (32.4)	161 (73.2)	<0.001
>50 ppb	10 (27.0)	6 (2.7)	<0.001
Blood eosinophils			
≥500/mm³	6 (16.2)	16 (7.3)	0.104
>2%	31 (83.8)	138 (62.7)	0.014
Total serum IgE level >173 IU/mL	19 (51.4)	65 (29.5)	0.013
Presence of antigen-specific IgE	21 (56.8)	63 (28.6)	0.001

Notes: Data are shown as number (%). Associations between categorical variables were evaluated by using Fisher's exact test. $P<0.05$ was considered statistically significant.
Abbreviations: ACOS, asthma-COPD overlap syndrome; FENO, fractional exhaled nitric oxide; IgE, immunoglobulin E; ppb, parts per billion.

Our finding that 14% of patients with COPD fulfilled the ACOS phenotype is in line with the results of well-characterized COPD cohorts, such as the COPDGene study[5] and CHAIN cohort.[30] In this study, patients with ACOS tended to be younger, have a shorter smoking history, and use more respiratory medications, which is compatible with previous studies.[4–6,30,31] However, discrepancies between this and previous studies may have been caused by the older age of the patients in this study, since the participants were enrolled from a regional COPD registry in Japan, one of the most rapidly aging societies in the world.[13]

To date, a standardized treatment for ACOS has not been established because patients with asthma and COPD overlap have been excluded from randomized controlled trials. Treatment with ICS is provisionally recommended

by the GINA/GOLD joint document.[7] High FENO level[32] and blood eosinophilia[33,34] have been identified as surrogate markers of the response to steroids in patients with COPD. The findings of our study support the view that ICS treatment may be beneficial in patients with ACOS and encourage the development of a standardized treatment for ACOS.

The primary strength of our study is that we applied precise diagnostic criteria to identify patients with ACOS. Previous reports included patients with a medical history of physician-diagnosed asthma as the main inclusion criterion. In this study, we confirmed the diagnosis in all patients, with or without features of asthma, according to both asthmatic symptoms and documented reversibility, because we found that some patients had received an inappropriate diagnosis of asthma by a nonrespiratory specialist. In addition, we excluded patients who had characteristics possibly affecting biomarker measurement, including current smokers and oral corticosteroid users.

A potential weakness of our study is that the results were not confirmed by a validation cohort. Thus, the results cannot be generalized directly to a different setting, such as an asthma cohort. Further studies are required to evaluate the diagnostic value of inflammatory biomarkers in untreated patients.

Conclusion

The results of this study provide evidence that inflammatory biomarkers, including FENO, blood eosinophil count, and IgE, can be used to support the diagnosis of ACOS. The results of our study may guide toward better recognition of ACOS and encourage development of specific interventions for ACOS.

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Disclosure

The authors report no conflicts of interest in this work.

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