

Letter to the Editor

Sputum adiponectin as a marker for western red cedar asthma

To the Editor:

Western red cedar asthma (WRCA) is a form of occupational asthma caused by exposure to dust from western red cedar (*Thuja plicata*). Given the link between adiponectin in asthma and obesity¹ and in response to allergen in animal models,^{2,3} we hypothesized that adiponectin may reflect responses to inhaled cedar dust.

Nineteen subjects with asthmatic symptoms and exposure to cedar dust underwent methacholine and plicatic acid (PA) challenges as previously described.⁴ FEV₁ was measured hourly; sera was obtained before and 2 hours after challenge, and induced sputa were obtained 6 hours post-challenge. Post-challenge FEV₁ decline $\geq 20\%$ was considered positive. A positive response to either challenge was diagnostic for asthma; those with a positive response to PA were diagnosed with WRCA. Early responses occurred within 2 hours after exposure; late responses occurred ≥ 3 hours post-exposure. After processing, sputum supernatants and sera were assayed for adiponectin by ELISA. See this article's [Methods](#) section in the Online Repository at www.jacionline.org.

[Table E1](#) (in the Online Repository available at www.jacionline.org) shows clinical characteristics of the cohort. The majority were overweight or obese (78.9% had a body mass index [BMI] $\geq 25 \text{ kg/m}^2$; the average was $27.3 \pm 0.9 \text{ kg/m}^2$). Most (63.2%) were on inhaled corticosteroids, but only 2 were unable to withhold these medications prior to testing. β -agonists were universally withheld. Most (73.7%) had a positive response to methacholine, PA, or both; 5 did not respond to either agent. Most (63.2%) responded to PA and were diagnosed with WRCA.

Mean sputum adiponectin after methacholine challenge was $36.4 \pm 9.0 \text{ ng/mL}$, whereas after plicatic acid challenge (PAC) it was $119.8 \pm 40.6 \text{ ng/mL}$, representing a significant increase in sputum adiponectin after PAC ($P < .01$, [Fig 1, A](#)). With the outlying post-PAC value excluded, mean sputum adiponectin was $83.5 \pm 19.3 \text{ ng/mL}$ ($P < .02$). Consistent with prior reporting,⁵ neither inhalational challenge affected serum adiponectin (data not shown).

Subgroups were analyzed to determine characteristics, such as age, BMI, baseline FEV₁ (% predicted), and response to challenge, potentially modifying the increase in sputum adiponectin ([Table I](#)). Only in normal-BMI subjects was sputum adiponectin significantly greater post-PAC versus post-methacholine, though the trend was similar in those who were overweight/obese and there was no correlation between the change in sputum adiponectin and BMI ($\rho = 0.065$, $P = .79$). Sputum adiponectin did not differ between asthmatic and non-asthmatic subjects either post-methacholine or post-PAC. However, subjects with asthma had a significant increase in sputum adiponectin, while those without asthma did not.

PAC-positive subjects had significantly more sputum adiponectin after PAC ($166.7 \pm 59.9 \text{ ng/mL}$) than after methacholine challenge ($41.3 \pm 11.9 \text{ ng/mL}$; $P < .02$; [Fig 1, B](#)), even after censoring the outlying value. There was no significant increase in sputum adiponectin in the PAC-negative subjects. There was no significant difference in post-methacholine sputum adiponectin between PAC-positive and PAC-negative subjects. There was,

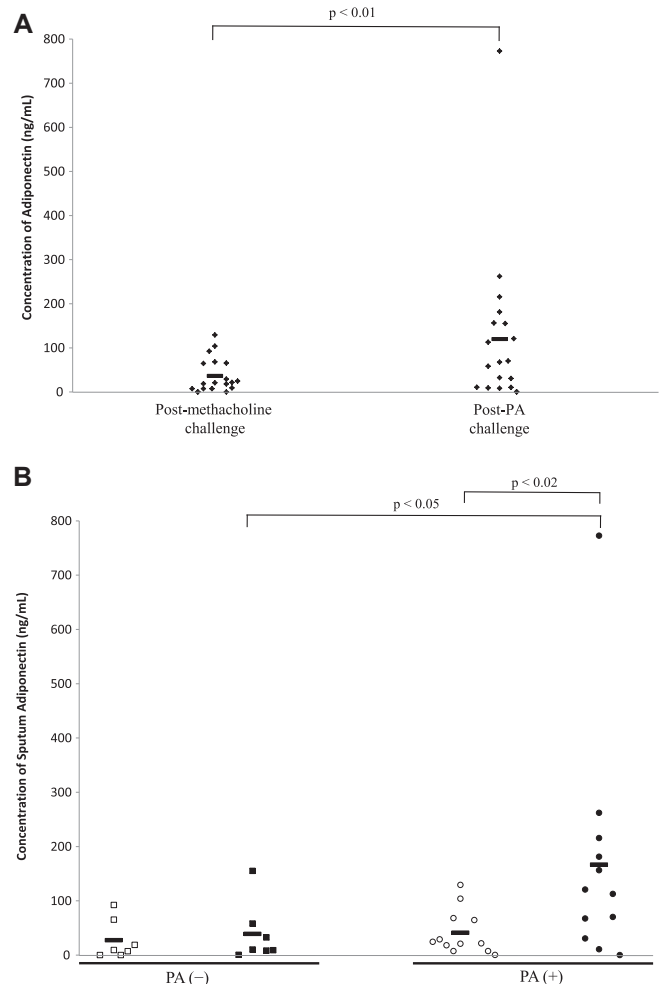


FIG 1. Concentration of sputum adiponectin after inhalational challenge. *Black bars* represent means. Results for all 19 patients (**A**) and stratification by responsiveness to PA (**B**) are shown. *Open shapes* represent adiponectin 6 hours after methacholine challenge; *solid shapes* represent adiponectin concentration 6 hours after PAC.

however, a significant difference in post-PAC adiponectin between the PAC-positive and PAC-negative subjects ($166.7 \pm 59.9 \text{ ng/mL}$ vs $39.2 \pm 20.7 \text{ ng/mL}$, respectively; $P < .05$, [Fig 1, B](#)). Furthermore, subjects with a late response to PA (but not those with an early response) had a significant increase in sputum adiponectin post-PAC ([Table I](#); $P < .001$, with or without the outlying value).

Thirteen subjects had slides of sufficient quality for cell counting. When grouped by PAC positivity (see [Fig E1](#) available in this article's Online Repository at www.jacionline.org), there was an increase in mean sputum eosinophils in both those PAC-positive ($1.5 \pm 0.3\%$ to $14.6 \pm 7.6\%$; $P < .05$) and PAC-negative ($1.9 \pm 0.6\%$ to $5.7 \pm 2.0\%$; $P < .001$). Similar results were found when grouped by response to methacholine challenge (data not shown). Sputum adiponectin outperformed sputum eosinophil counts on receiver-operator curve (ROC) analysis (see [Fig E2](#) available in this article's Online Repository at www.jacionline.org).

TABLE I. Subgroup analysis of post-methacholine challenge and post-PAC sputum adiponectin for 19 subjects evaluated for WRCA. Sputum adiponectin concentrations are expressed as mean $\pm$ SEM

	n	Concentration of sputum adiponectin (ng/mL)		P value (Wilcoxon-signed rank test)
		Post-methacholine challenge	Post-PAC	
Age				
<50 y	9	33.6 $\pm$ 13.8	156.8 $\pm$ 80.6	>.10
$\geq$ 50 y	10	40.4 $\pm$ 12.2	88.37 $\pm$ 86.4	>.05
BMI (kg/m ²)				
Normal (<25)	5	16.6 $\pm$ 13.0	81.9 $\pm$ 46.7	<.001
Overweight or obese ($\geq$ 25)	14	43.3 $\pm$ 10.9	133.3 $\pm$ 53.0	>.05
Baseline FEV ₁ (% predicted)				
1 st Quartile (62.5% to 75.5%)	5	27.4 $\pm$ 11.3	213.2 $\pm$ 148.2	>.20
2 nd Quartile (75.5% to 81.7%)	5	34.1 $\pm$ 24.1	53.0 $\pm$ 20.8	>.20
3 rd Quartile (81.7% to 87.9%)	4	54.0 $\pm$ 23.2	124.9 $\pm$ 43.7	>.20
4 th Quartile (87.9% to 110.8%)	5	38.1 $\pm$ 17.2	88.8 $\pm$ 33.1	>.10
Response to inhalational challenge				
Non-asthmatic	5	23.7 $\pm$ 17.5	46.3 $\pm$ 29.1	>.20
Asthmatic	14	40.7 $\pm$ 10.6	146.0 $\pm$ 53.0	<.05
Methacholine non-responsive	6	21.0 $\pm$ 14.5	64.6 $\pm$ 30.0	>.20
Methacholine responsive	13	43.3 $\pm$ 11.1	145.2 $\pm$ 57.2	<.05
No response to PA	7	27.6 $\pm$ 17.5	98.0 $\pm$ 27.0	>.20
Early response to PA	6	54.6 $\pm$ 21.9	235.5 $\pm$ 114.8	<.001
Late response to PA	6	28.0 $\pm$ 8.6	39.2 $\pm$ 29.1	>.20

Sputum adiponectin was not merely a surrogate for sputum eosinophils. For the PA-positive group, there was a non-significant correlation ($\rho = 0.43$; $P = .29$) between the change in sputum adiponectin and the change in sputum eosinophil percentage upon methacholine challenge. PA-negative subjects demonstrated an opposite trend ($\rho = -0.70$; $P = .19$). There was no significant relationship between the change in sputum adiponectin and change in sputum eosinophils upon PAC, either for those PA-positive ($\rho = -0.17$; $P = .69$) or negative ($\rho = -0.10$; $P = .87$).

Aside from one prior report,⁶ this is the first successful measurement of adiponectin in sputum, although adiponectin has previously been detected on bronchoalveolar lavage of asthmatic subjects.⁷ This is the first report demonstrating variation in adiponectin in humans upon inhalational challenge.

The relationship of adiponectin to airway responsiveness is complex and poorly understood. In mice sensitized to inhaled ovalbumin, artificially increasing the serum concentration of adiponectin abrogated airway hyperresponsiveness to ovalbumin and attenuated the accumulation of inflammatory cells in bronchoalveolar lavage fluid.⁵ Adiponectin-deficient mice have elevated sputum eosinophilia when compared with wild-type mice.³ Human studies are inconsistent regarding serum adiponectin between asthmatic and control subjects,^{8,9} but high sputum adiponectin appears to be associated with low risk for asthma.⁴

Sputum, but not serum adiponectin, changed after inhalational challenge, and the change was driven by the group with a late response to challenge. Thus, the increase in sputum adiponectin after challenge is more likely attributable to increased local production, perhaps due to epithelial damage, rather than increased translocation from serum into the airway. Because sputum adiponectin post-PAC challenge was significantly greater than that post-methacholine only in those with normal BMI, it may be that obesity moderates PA-induced airway adiponectin, perhaps due to elevated baseline adiponectin and/or an attenuated ability to increase adiponectin. However, because the trend was

similar in those who were overweight/obese, and we did not have baseline sputum samples, and our sample size was modest, further work is required to determine the modifying effect of BMI in this context.

Our study only included patients with known or suspected WRCA. Therefore, the effect of inhalational challenge on sputum adiponectin in other types of allergic asthma is unknown, and our data is limited in assessing adiponectin as a robust biomarker.

In conclusion, we have shown for the first time that the concentration of adiponectin in human sputum is responsive to specific inhalational challenge, particularly in those with normal BMI. Adiponectin in sputum may help us understand the pathophysiology of WRCA, as well as potentially other occupational asthma, and the modifying effect of body mass therein.

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METHODS

Study participants

This study was reviewed and approved by the University of British Columbia Clinical Research Ethics Board. Patients with suspected WRCA were recruited from the Lung Centre at Vancouver General Hospital. All patients reported symptoms that were consistent with occupational asthma. Signed informed consent was obtained from each participant.

After a clinical evaluation, patients filled out a questionnaire about their respiratory symptoms, smoking status, and occupational and clinical history. If the questionnaire was incomplete, the patient's clinic chart was reviewed for the relevant information.

Inhalational challenges

Patients were instructed not to use any inhaled short-acting bronchodilators for 12 hours, long-acting bronchodilators for 24 hours, and inhaled corticosteroids for at least 2 weeks prior to testing if it was deemed by the prescribing physician to be safe to do so. They were also instructed to have no caffeine on the day(s) of testing. On day 1 of testing, patients first had baseline spirometry testing, both before and after inhalation of nebulized 0.9% saline solution, followed by methacholine challenge as per the American Thoracic Society guidelines.^{E1} After methacholine challenge, FEV₁ was monitored serially at 20, 30, 45, and 60 minutes, and then hourly, for a total of 6 hours.

On the day after methacholine challenge, all patients underwent inhalational PA challenge (PAC) as previously described.^{E2,E3} Briefly, baseline spirometry was again obtained before and after exposure to 0.9% nebulized saline. Patients were then exposed to nebulized saline containing increasing amounts of PA (0.625, 1.25, 2.5, 5, and 10 mg/mL). Their FEV₁ was monitored at 30 seconds and 90 seconds after each increase in concentration. FEV₁ was then monitored serially at 20, 30, 45, and 60 minutes and then hourly for a total of 6 hours.

A drop in FEV₁ of 20% or greater from baseline in response to challenge with either agent was immediately treated with salbutamol. Monitoring then continued as above.

On both days of inhalational challenges, blood samples were obtained prior to inhalational challenge (0-hour), and at the 2-hour post-challenge time points. Induced sputum samples were obtained at the 6-hour time point.

Induced sputum

Sputum was induced using an aerosol of inhaled hypertonic saline by a modification of the method of Pin et al.^{E4} and sputum samples were prepared using the method described by Pizzichini et al.,^{E5} with minor modifications as previously reported.^{E6}

Sputum cell counts

Two different observers counted at least 400 non-squamous cells, and their differential cell counts were averaged to make a final differential cell count. In the case that the inter-rater variability of the 2 observers was greater than 10%, a third observer counted a cell differential, and the differential counts of the 2 observers with less than 10% inter-rater variability were averaged. Only sputum samples with less than 20% squamous cells and greater than 50% cell viability were used in the final analysis.

Adiponectin assays

Adiponectin concentrations in sputum supernatant and serum were assayed by ELISA using a commercially available assay kit according to the manufacturer's protocols (Invitrogen Corp, Camarillo, Calif, Catalog #KHP0041, and EMD Millipore Corp, St Charles, Mo, Catalog #EZHADP-61K, for sputum and serum, respectively).

Statistical analysis

All data are expressed as the mean $\pm$ SEM unless otherwise noted. Data were analyzed using the Wilcoxon-signed rank test for assays comparing the

subjects before and after inhalational challenge. The Mann-Whitney *U* test was used for comparisons between the full cohort and the subgroup for which sputum cell counts were available, and for comparison of different subgroups of patients after either methacholine or PAC. Spearman rank-order correlation coefficient (ρ) was used to determine the correlation of variables. All tests were 2-tailed, and a *P* value of $\leq .05$ was considered significant. ROC and area under the curve (AUC) analyses were created with SPSS.

RESULTS

Clinical characteristics

Table E1 shows the clinical features of 19 patients evaluated for suspected WRCA. All subjects were male, and all had a history of occupational exposure to western red cedar. The ages of the patients ranged from 21 to 61 years; the average age at the time of presentation was 46.7 ± 2.9 years. Approximately half of the patients (52.6%) were never smokers, and only 1 patient was a current smoker. The length of exposure to cedar dust ranged from 1 month to 40 years, with the average length of exposure being 14.1 ± 3.1 years. Thirteen of the 19 patients (68%) had ongoing occupational exposure to cedar dust, while the others had previously retired from work with cedar dust. The most common symptom was shortness of breath, with 94.7% of patients reporting either exertional or resting shortness of breath. The average duration of symptoms was 5.8 ± 1.2 years, with a range of 1 to 20 years. Spirometry for the cohort is also presented in Table E1. The average baseline FEV₁ was 3.37 ± 0.12 L, and the average forced vital capacity (FVC) was 4.63 ± 0.18 L. The average FEV₁/FVC ratio was 0.73 ± 0.01 . The 13 patients in the subgroup used for analysis of sputum eosinophilia were not significantly different in terms of age, BMI, length of exposure, FEV₁, or history of smoking from those for whom high-quality slides were not available (data not shown). See also main text for further details.

Sputum eosinophilia increases after both positive and negative inhalational challenges

Overall, the 13 subjects with slides available for sputum cell counts had a significant increase in average percent sputum eosinophils, from $1.6 \pm 0.28\%$ after methacholine challenge to $11.2 \pm 5.1\%$ after PAC ($P < .005$). There were no changes in the average percentage of neutrophils ($74.3 \pm 5.5\%$ vs $71.1 \pm 6.1\%$; $P > .20$) or macrophages ($20.7 \pm 5.0\%$ vs $15.3 \pm 3.8\%$; $P > .20$) after methacholine challenge versus after PAC, respectively. Sputum eosinophil counts after methacholine and PAC, grouped by test positivity to PA, are shown in Fig E1. For further details, see the main text.

Sputum adiponectin is a sensitive and specific predictor for WRCA

Fig E2 shows the ROC for post-PAC sputum eosinophils (Fig E2, A) and adiponectin (Fig E2, B). Sputum adiponectin had a favorable AUC of 0.798 ± 0.107 compared with the AUC of 0.625 ± 0.161 for sputum eosinophils. Sputum adiponectin ≥ 62.8 ng/L after inhalational challenge with PA gave the best combination of sensitivity (75.0%) and specificity (85.7%) for a diagnosis of WRCA, outperforming sputum eosinophils (best combination: sensitivity 75% and specificity 60% for sputum eosinophils $\geq 4.0\%$).

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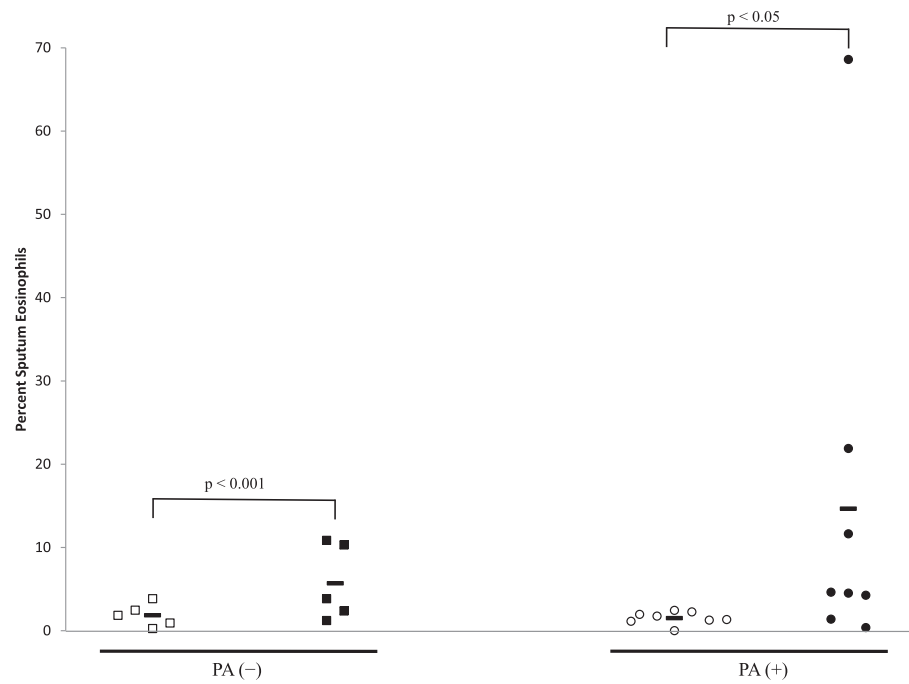


FIG E1. Sputum eosinophils, as a percentage of total cells, for those subjects with adequate sputum after methacholine and after PAC. *Circles* represent subjects with positive PAC, and *squares* represent those with negative PAC. *Open shapes* represent percentages 6 hours after methacholine challenge, and *solid shapes* represent percentages 6 hours after PAC. *Black bars* represent means.

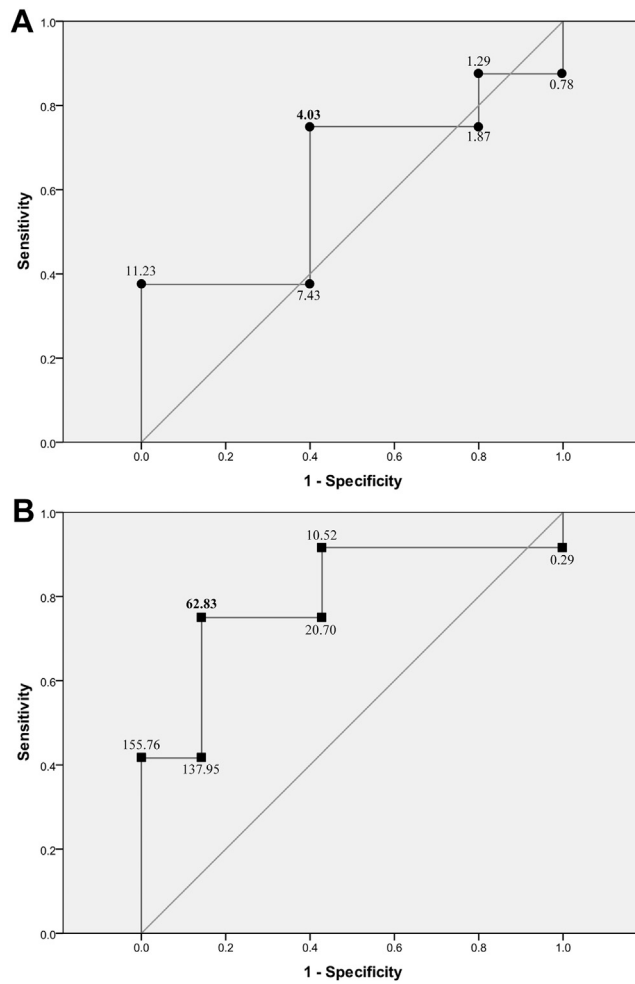


FIG E2. ROC after PAC. ROC for sputum eosinophils, with percent eosinophils represented by *solid dots* (**A**). ROC for sputum adiponectin, with concentration of adiponectin (ng/mL) represented by *solid squares* (**B**). The most relevant cutoffs are shown in *bold*.

TABLE E1. Clinical characteristics of 19 patients evaluated for WRCA

Clinical characteristics	Mean $\pm$ SEM
Age (y)	46.7 $\pm$ 2.9
BMI (kg/m ²)	27.3 $\pm$ 0.89
Length of exposure (y)	14.1 $\pm$ 3.1
Length of symptoms (y)	5.8 $\pm$ 1.2
FEV ₁ (L)	3.37 $\pm$ 0.12
FVC (L)	4.63 $\pm$ 0.18
FEV ₁ /FVC	0.73 $\pm$ 0.01
FEV ₁ (% predicted)	82.1 $\pm$ 2.85
FVC (% predicted)	97.0 $\pm$ 3.20
FEV ₁ /FVC (% predicted)	85.9 $\pm$ 3.34
Symptoms	N (%)
Cough	8 (47.5)
Shortness of breath	18 (94.7)
Sputum	11 (57.9)
Chest tightness	14 (73.7)
Wheeze	14 (73.7)
Medical comorbidities	N (%)
Coronary artery disease	1 (5.3)
Hypertension	2 (10.5)
Dyslipidemia	3 (15.8)
Smoking history	N (%)
Current	1 (5.3)
Prior	8 (42.1)
Never	10 (52.6)
Positive response to inhalational challenge	N (%)
Methacholine only	2 (10.5)
Plicatic acid only	1 (5.3)
Both methacholine and plicatic acid	11 (57.9)
Neither methacholine nor plicatic acid	5 (26.3)