

Letter to the Editor

Sputum hydrogen sulfide as a novel biomarker of obstructive neutrophilic asthma

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Hydrogen sulfide (H₂S) is produced by many cell types in the lungs and is the third “gasotransmitter,” along with nitric oxide and carbon monoxide, possessing vasodilator and neurotransmitter functions.¹ H₂S induces vascular smooth muscle relaxation as an endothelium-derived relaxing factor² while suppressing airway smooth muscle proliferation and IL-8 release.³ H₂S might act as a reducing agent to decrease oxidative stress.⁴

We examined the levels of H₂S in serum and sputum from 23 patients with severe asthma, 17 patients with mild-to-moderate asthma, and 15 healthy subjects. Patients with severe asthma were defined according to the need for either continuous or near-continuous oral corticosteroids, high-dose inhaled corticosteroids, or both to achieve a level of mild-to-moderate persistent asthma.⁵ Patients with nonsevere mild-to-moderate asthma had well-controlled disease and no need for reliever medications while using 1000 µg or less of inhaled beclomethasone equivalent per day. Exsmokers with a smoking history of less than 5 pack years were included. Sputum induction was performed with inhaled nebulized 3% NaCl solution, and sputum was processed with 0.1% dithiothreitol, as described previously.⁶

We first evaluated the reliability of H₂S measurements using a sensitive electrode (Model 9616; Orion Research, Beverly, Mass). Antioxidant buffer (SAOB II; Orion Research) was added into standard solutions in a 1:1 ratio. The H₂S concentration of

0.1% dithiothreitol and PBS solution itself was less than the detection limit (<1 µmol/L). Without the buffer added, serum H₂S levels of stored samples decreased over 3 days to half of those assayed at day 0; this was not observed with the prior addition of buffer (see Fig E1, A, in this article’s Online Repository at www.jacionline.org). We spiked 5 different concentrations (from 10 to 100 µmol/L) of H₂S solution to serum samples and found a tight correlation between the measured and predicted H₂S levels (see Fig E1, B[i]). The Bland-Altman plot showed that the differences between measured and predicted H₂S levels were within the range of ±20% (see Fig E1, B[ii]). Serum H₂S levels from 9 healthy volunteers taken twice over a 1-week period showed a mean coefficient of variation of 6.34% (see Fig E1, C).

Table I shows the clinical characteristics of subjects enrolled in the study. H₂S levels in induced sputum from patients with severe and nonsevere asthma were significantly higher than those from healthy subjects (Fig 1, A). Serum H₂S levels were 10 times higher than in sputum, and serum and sputum H₂S levels in patients with severe and nonsevere asthma were higher than those in healthy subjects (*P* < .05; Fig 1, B). There was no significant difference in salivary H₂S levels between the healthy and asthmatic groups (Fig 1, C). There was no significant difference in sputum and serum H₂S levels between the severe and nonsevere groups and between asthmatic patients with and without inhaled corticosteroid treatment. There was a positive correlation between sputum and serum H₂S levels (see Fig E2, A, in this article’s Online Repository at www.jacionline.org) but no significant correlation between salivary and sputum H₂S levels and between salivary and serum H₂S levels.

TABLE I. Subjects’ characteristics

	Healthy nonasthmatic subjects (n = 15)	Patients with nonsevere asthma (n = 17)	Patients with severe asthma (n = 23)
Age (y)	40.8 ± 13.8	36.1 ± 12.2	47.4 ± 10.9*†
Sex (male/female)	9/6	7/10	10/13
Height (cm)	166 ± 6.91	171 ± 9.12	166 ± 11.9
Weight (kg)	61.9 ± 13.8	68.6 ± 13.4	80.1 ± 20.6*
BMI	22.1 ± 3.82	23.5 ± 4.27	29.0 ± 6.61*†
Atopy (%)	0	76.5*	78.3*
FEV ₁ (% predicted)	97.4 ± 11.3	83.2 ± 12.4*	67.2 ± 20.0*†
FEV ₁ /FVC ratio (%)	81.3 ± 6.53	73.0 ± 8.84	65.5 ± 14.1*
Sputum eosinophils (%)	0.73 ± 0.59	4.80 ± 6.25	24.6 ± 31.0*
Sputum neutrophils (%)	35.1 ± 23.1	44.1 ± 22.3	34.5 ± 23.5
Sputum macrophages (%)	61.3 ± 21.5	48.0 ± 23.2	38.9 ± 23.9*
FENO (ppb)	21.1 ± 13.0	37.9 ± 29.5	58.3 ± 38.3*
ACQ score	NA	1.10 ± 0.59	2.78 ± 1.14†
AQLQ score	NA	5.89 ± 0.65	4.08 ± 0.95†
Medication taken, no. (%)			
Inhaled corticosteroid	NA	12 (70.6)	23 (100)
Long-acting β ₂ -agonist	NA	10 (58.8)	23 (100)†
Leukotriene antagonist	NA	2 (11.8)	13 (56.5)†
Xanthine	NA	0	10 (43.5)†
Oral prednisolone	NA	0	16 (69.6)†
ICS (BDP equivalent [µg])	NA	835 ± 778	2043 ± 209†

Data are shown as means ± SDs, where applicable.

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; BDP, beclomethasone dipropionate; BMI, body mass index; FVC, forced vital capacity; ICS, inhaled corticosteroid; NA, not applicable.

**P* < .05 versus healthy subjects.

†*P* < .05 versus patients with nonsevere asthma.

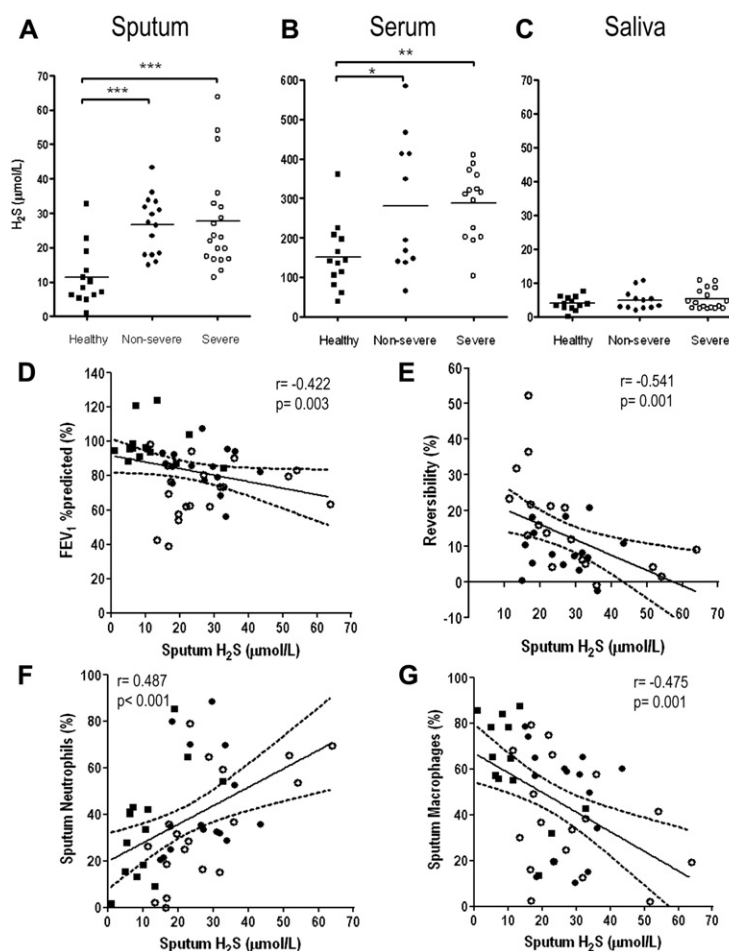


FIG 1. H₂S levels in sputum (A), serum (B), and saliva (C) from patients with severe asthma (open circles), patients with nonsevere asthma (solid circles), and healthy subjects (solid squares) are shown. *** $P \leq .001$, ** $P \leq .01$, * $P \leq .05$. The relationship between (1) sputum H₂S levels and FEV₁ percent predicted (D) and reversibility to salbutamol (E) and (2) sputum H₂S levels and sputum neutrophils (F) and macrophages (G) is also depicted. The line of identity is shown with 95% CIs.

Sputum H₂S levels were negatively correlated with FEV₁ percent predicted ($r = -0.422$, $P = .003$; Fig 1, D), and in patients with asthma, sputum H₂S levels showed negative correlation with reversibility to albuterol ($r = -0.541$, $P = .002$; Fig 1, E). There was a positive correlation between sputum H₂S levels and neutrophil percentages ($r = 0.487$, $P < .001$; Fig 1, F) and an inverse correlation between sputum H₂S levels and macrophage percentages ($r = -0.475$, $P = .001$; Fig 1, G) but not with eosinophil percentages. A negative correlation was found between serum H₂S levels and sputum macrophage percentages ($r = -0.463$, $P = .007$). There was a negative correlation between sputum H₂S and fraction of exhaled nitric oxide (FENO) levels (measured with the portable analyzer NObreath; Bedfont Scientific, Rochester, United Kingdom; $r = -0.522$, $P = .002$) in asthmatic patients but none between serum H₂S and FENO levels (see Fig E2, B). Sputum and serum H₂S levels did not correlate with any other clinical parameters, such as age, height, inhaled corticosteroid dose, or Asthma Control Questionnaire scores, except body mass index ($r = 0.426$, $P = .003$). A multiple linear regression analysis revealed that sputum H₂S levels but not serum H₂S levels were associated with increased sputum neutrophil percentages, increased body mass index,

and decreased FEV₁ (percent predicted, see Table E1 in this article's Online Repository at www.jacionline.org).

In this first study to evaluate H₂S measurements, serum H₂S levels were generally higher than those reported previously,⁵ a discrepancy that might be due to the use of sulfide antioxidant buffer. H₂S levels decreased over time because H₂S readily evaporates and oxidizes, and the addition of sulfide antioxidant buffer prevented the oxidation of the sulfur compounds. The accuracy of this measurement was acceptable within a 20% range, and levels measured were reproducible over the short term.

The sputum H₂S level represents a novel promising biomarker of asthma, particularly as a marker of neutrophilic inflammation and chronic airflow obstruction and also as a reflection of β -adrenergic bronchodilator responsiveness. The sputum H₂S level was better than the serum H₂S level as a biomarker of asthma. Furthermore, because serum H₂S levels are higher than those in sputum and because they can be influenced by systemic nonrespiratory diseases, such as heart, liver, renal, and pancreatic disease,⁷ serum levels might not be specific to asthma. The increased serum H₂S levels in both our severe and nonsevere asthmatic populations might represent the systemic component of their asthma because there was a positive correlation between serum and sputum H₂S levels.

In the circulation it is likely that vascular endothelial cells and vascular smooth muscle cells produce H₂S, whereas in the airways airway smooth muscle cells are likely to be an important source. There is evidence to indicate that H₂S might have anti-inflammatory and antioxidant effects in the lungs and therefore might have a protective effect in asthmatic patients. In support of a potential protective effect of H₂S in asthmatic patients is the observation that exogenous H₂S prevented ovalbumin-induced bronchoconstriction and inflammation in sensitized rats.⁸ Asthma medications and respiratory tract bacterial infections can also influence sputum H₂S levels. H₂S produced by bacteria might sustain bacterial growth and suppress their sensitivity to antibiotics.⁹

The inverse relationship between sputum H₂S and FENO levels is of interest. Because FENO levels are useful in detecting eosinophilic airway inflammation and corticosteroid responsiveness,¹⁰ the use of combined FENO and sputum H₂S measurements might provide a more comprehensive description of asthma phenotypes. This should be explored further.

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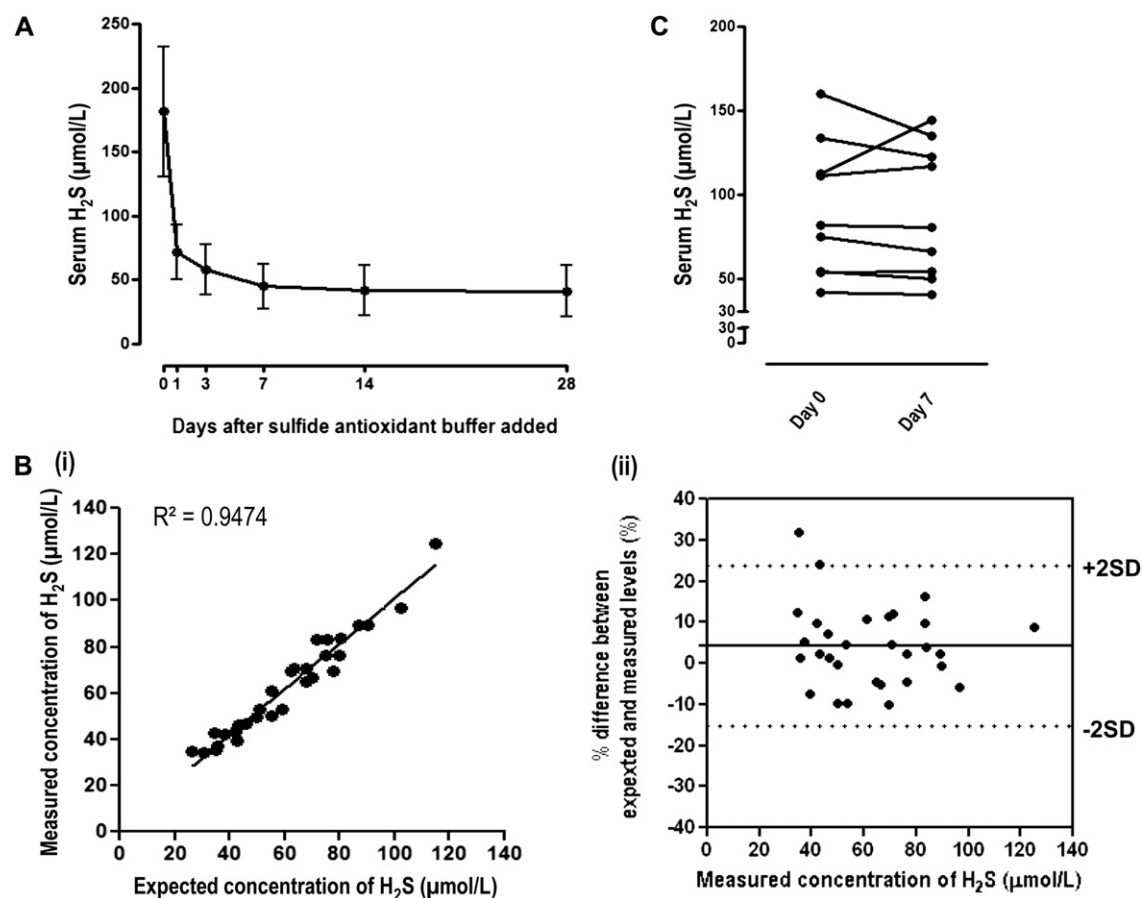


FIG E1. **A**, Changes in H_2S levels in stored samples. **B(i)**, Correlation between measured and predicted serum H_2S levels in samples to which standard H_2S solutions were added. **B(ii)**, Bland-Altman plot between measured and predicted serum H_2S levels. Lines indicate the mean percentage difference and ± 2 SDs, respectively. **C**, Serum H_2S levels measured twice at days 0 and 7.

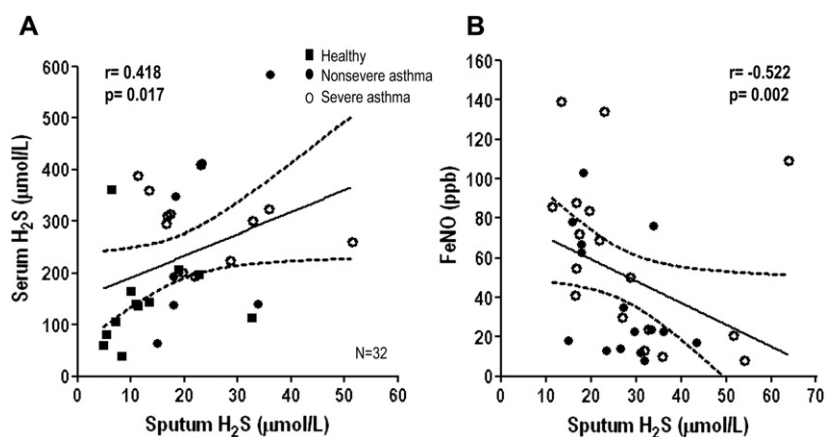


FIG E2. **A**, Relationship between sputum and serum H₂S levels ($r = 0.418$, $P = .017$) for patients with severe asthma, patients with nonsevere asthma, and healthy subjects. **B**, Relationship between sputum H₂S and FeNO levels in patients with severe and patients with nonsevere asthma ($r = -0.522$, $P = .002$). The line of identity is shown with 95% CIs.

TABLE E1. Relation of sputum H₂S levels and other clinical markers using multiple linear regression analysis (n = 55)

Risk factor	β value	95% CI	P value
BMI	0.51	0.58 to 1.82	.01
FEV ₁ (% predicted)	−0.35	−0.49 to −0.003	.04
Sputum neutrophils (%)	0.37	0.04 to 0.39	.02

Overall R^2 = 0.55.

BMI, Body mass index.