

Institute for Anesthesiological Pathophysiology and Process Engineering, University Hospital, Ulm, Germany  
Peter.Radermacher@uni-ulm.de

The authors report no conflicts of interest.

Csaba Szabo

Chair of Pharmacology, OMI Department, Section of Science and Medicine, University of Fribourg, Fribourg, Switzerland

## REFERENCES

- Bazhanov N, Ansar M, Ivanciuc T, Garofalo RP, Casola A: Hydrogen sulfide: a novel player in airway development, pathophysiology of respiratory diseases, and antiviral defenses. *Am J Respir Cell Mol Biol* 57(4):403–410, 2017.
- Renieris G, Katrini K, Damoulari C, Akinosoglou K, Psarrakis C, Kyriakopoulou M, Dimopoulos G, Lada M, Koufargyris P, Giamarellos-Bourboulis EJ: Serum hydrogen sulfide and outcome association in pneumonia by the SARS-CoV-2 Coronavirus. *Shock* 54(5):633–637, 2020.
- Wintner EA, Deckwerth TL, Langston W, Bengtsson A, Leviten D, Hill P, Insko MA, Dumpit R, VandenEkart E, Toombs CF, et al.: A monobromobimane-based assay to measure the pharmacokinetic profile of reactive sulphide species in blood. *Br J Pharmacol* 160(4):941–957, 2010.
- Simon F, Scheuerle A, Gröger M, Stahl B, Wächter U, Vogt J, Speit G, Hauser B, Möller P, Calzia E, et al.: Effects of intravenous sulfide during porcine aortic occlusion-induced kidney ischemia/reperfusion injury. *Shock* 35(2):156–163, 2011.
- Osipov RM, Robich MP, Feng J, Chan V, Clements RT, Deyo RJ, Szabo C, Sellke FW: Effect of hydrogen sulfide on myocardial protection in the setting of cardioplegia and cardiopulmonary bypass. *Interact Cardiovasc Thorac Surg* 10(4):506–512, 2010.
- Osipov RM, Robich MP, Feng J, Liu Y, Clements RT, Glazer HP, Sodha NR, Szabo C, Bianchi C, Sellke FW: Effect of hydrogen sulfide in a porcine model of myocardial ischemia-reperfusion: comparison of different administration regimens and characterization of the cellular mechanisms of protection. *J Cardiovasc Pharmacol* 54(4):287–297, 2009.
- Rajpal S, Katikaneni P, Deshotels M, Pardue S, Glawe J, Shen X, Akkus N, Modi K, Bhandari R, Dominic P, et al.: Total sulfane sulfur bioavailability reflects ethnic and gender disparities in cardiovascular disease. *Redox Biol* 15:480–489, 2018.
- Kožich V, Ditrói T, Sokolová J, Křížková M, Krijt J, Ješina P, Nagy P: Metabolism of sulfur compounds in homocystinurias. *Br J Pharmacol* 176(4):594–606, 2019.
- Olson KR: A practical look at the chemistry and biology of hydrogen sulfide. *Antioxid Redox Signal* 17(1):32–44, 2012.
- Klingerman CM, Trushin N, Prokopczyk B, Haouzi P: H<sub>2</sub>S concentrations in the arterial blood during H<sub>2</sub>S administration in relation to its toxicity and effects on breathing. *Am J Physiol Regul Integr Comp Physiol* 305(6):R630–R638, 2013.
- Nagy P, Pálkás Z, Nagy A, Budai B, Tóth I, Vasas A: Chemical aspects of hydrogen sulfide measurements in physiological samples. *Biochim Biophys Acta* 1840:876–891, 2014.
- Ditrói T, Nagy A, Martinelli D, Rosta A, Kožich V, Nagy P: Comprehensive analysis of how experimental parameters affect H<sub>2</sub>S measurements by the monobromobimane method. *Free Radic Biol Med* 136:146–158, 2019.

**Reply to Radermacher et al. on “Serum Hydrogen Sulfide and Outcome Association in Pneumonia by the SARS-CoV-2 Coronavirus”**

*To the Editor:* We read with great interest the comments addressed by Radermacher et al. (1) on our publication

regarding the importance of hydrogen sulfide (H<sub>2</sub>S) for the prognosis and outcome of severe infection caused by the novel SARS-CoV-2 (also known as Covid-19) (2). Although serum H<sub>2</sub>S levels as high as 249 μM and 580 μM have been demonstrated in patients with septic shock (3) and severe asthma (4), we agree that the elevated serum H<sub>2</sub>S is an intriguing finding. We tried to deliver some answers that are based on: the performance of the used assay in healthy volunteers and in patients with other types of severe lung infection; and the reproducibility of the data by using another assay.

We measured levels of H<sub>2</sub>S in 17 healthy volunteers and in 60 patients with ventilator-associated pneumonia (VAP). VAP was diagnosed according to standard definitions (5) and all patients had microbiological confirmation with one Gram-negative pathogen isolated in counts greater than 10<sup>5</sup> colony-forming units/mL from the bronchoalveolar lavage by the culture technique already described (6). Isolated pathogens were *Acinetobacter baumannii* (n = 23), *Pseudomonas aeruginosa* (n = 19), and *Klebsiella pneumoniae* (n = 18). Blood samples were collected within the first 24 h from diagnosis of VAP and H<sub>2</sub>S was measured by the monobromobimane derivatization assay followed by reverse phase HPLC separation (2). Results clearly showed that survivors from Covid-19 had H<sub>2</sub>S levels significantly greater than healthy population and patients with VAP (Fig. 1). This elaborates the hypothesis that it is not the assay that leads to false-positive increased H<sub>2</sub>S levels, but that H<sub>2</sub>S increase may well be an intrinsic characteristic of Covid-19 described for the first time herein. H<sub>2</sub>S of healthy was also within reported ranges (7).

To strengthen the finding of increased H<sub>2</sub>S in Covid-19 survivors, H<sub>2</sub>S was measured in the same samples by a photometric methylene blue assay (8). Despite the lack of specificity of this assay leading to higher measurable levels, the interpretation of the findings was the same (Fig. 2).

We feel that Covid-19 is a new territory of research where modulation of H<sub>2</sub>S plays a major role and we wish to thank Radermacher et al. (1) for paving us the way to strengthen our data.

Georgios Renieris  
Konstantina Katrini  
Evangelos J. Giamarellos-Bourboulis  
Department of Internal Medicine National  
and Kapodistrian University of Athens  
Medical School, Athens, Greece  
egiamarel@med.uoa.gr

The authors report no conflicts of interest.

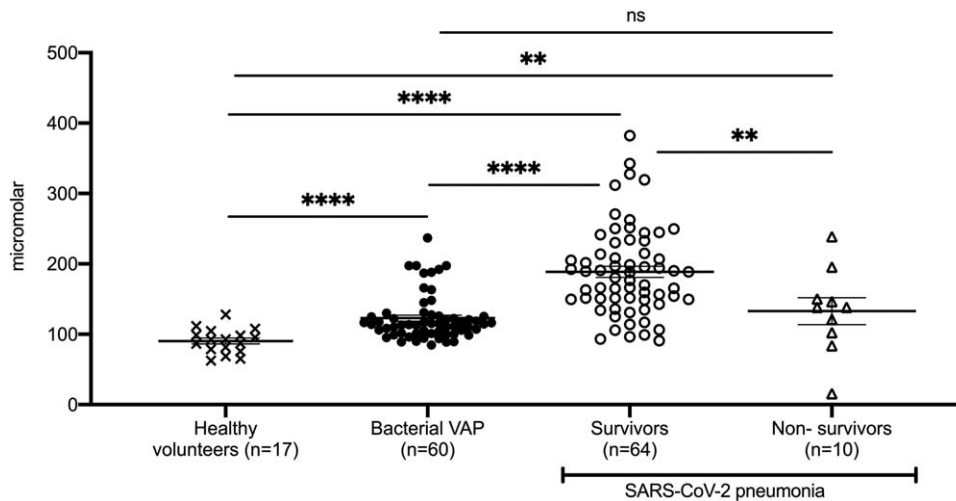


FIG. 1. Hydrogen sulfide (H<sub>2</sub>S) levels in serum of (A) healthy volunteers; (B) patients with ventilator associated pneumonia (VAP) by *P aeruginosa*; *K pneumoniae*; *A baumannii*; and (C) patients with pneumonia by the SARS-CoV-2 coronavirus on day 1 after hospital admission measured by monobromobimane derivatization assay followed by reverse phase HPLC separation. Comparison by the Mann–Whitney *U* test; ns indicates non-significant, \**P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001, \*\*\*\* *P* < 0.0001.

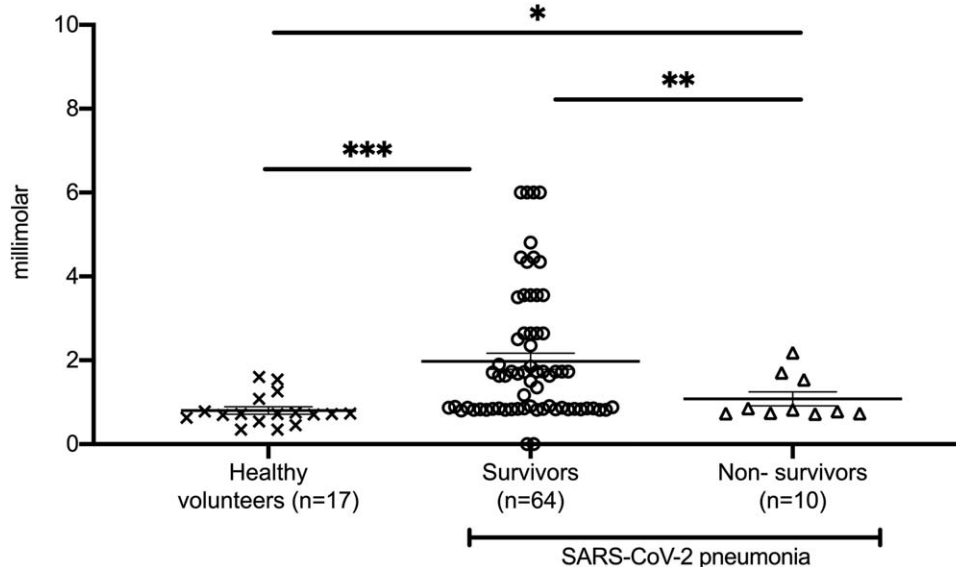


FIG. 2. Hydrogen sulfide (H<sub>2</sub>S) levels in serum of (A) healthy volunteers; (B) survivors; and (C) non-survivors of pneumonia by the SARS-CoV-2 coronavirus on day 1 after hospital admission measured by a photometric methylene blue assay. Comparison by the Mann–Whitney *U* test; ns indicates non-significant, \**P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001, \*\*\*\* *P* < 0.0001.

## REFERENCES

- Calzia E, McCook O, Wachter U, Radermacher P, Csaba S: Letter to the Editor. *Shock*; 2020. Epub Ahead of print.
- Renieris G, Katrini K, Damoulari C, Akinosoglou K, Psarrakis C, Kyriakopoulou M, Dimopoulos G, Lada M, Koufargyris P, Giamarellos-Bourboulis EJ: Serum hydrogen sulfide and outcome association in pneumonia by the SARS-CoV-2 coronavirus. *Shock*; 2020. doi: 10.1097/SHK.0000000000001562.
- Saito J, Zhang Q, Hui C, Macedo P, Gibeon D, Menzies-Gow A, Bhavsar PK, Chung KF: Sputum hydrogen sulfide as a novel biomarker of obstructive neutrophilic asthma. *J Allergy Clin Immunol* 131(1): 232–234.e3, 2013.
- Li L, Bhatia M, Zhu YZ, Zhu YC, Ramnath RD, Wang ZJ, Anuar FBM, Whiteman M, Salto-Tellez M, Moore PK: Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J* 19(9):1196–1198, 2005.
- Calandra T, Cohen J: *The International Sepsis Forum Consensus Conference on definitions of infection in the intensive care unit: critical care medicine* 33(7):1538–1548, 2005.
- Baselski V, Baselski V, Klutts JS, Klutts JS: Point-counterpoint: quantitative cultures of bronchoscopically obtained specimens should be performed for optimal management of ventilator-associated. *J Clin Microbiol* 51(3):740–744, 2013.
- Karunya R, Jayaprakash KS, Gaikwad R, Sajeesh P, Ramshad K, Muraleedharan KM, Dixit M, Thangaraj PR, Sen AK: Rapid measurement of hydrogen sulphide in human blood plasma using a microfluidic method. *Sci Rep* 9(1):3258, 2019.
- Ang AD, Rivers-Auty J, Hegde A, Ishii I, Bhatia M: The effect of CSE gene deletion in caerulein-induced acute pancreatitis in the mouse. *Am J Physiol Gastrointest Liver Physiol* 305(10):G712–G721, 2013.