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Letters to the Editor

To the Editor:

Since its recognition as the ''3rd gaseous mediator,'' the role of hydrogen sulfide (H_2S) has been equivocally discussed in the context of acute lung injury. Depending on the experimental model, both its protective and deleterious effects were reported. However, in viral lung diseases, e.g., paramyxovirus and respiratory syncytial virus infection, both endogenous as well as exogenously delivered H_2S were shown to be protective due to direct antiviral activity in addition to its well-established anti-inflammatory properties (1). Therefore, we read with interest the recent report by Renieris et al. (2) on the relation between serum H_2S concentrations and outcome in patients with SARS-Cov-2-coronavirus pneumonia. The authors reported that survivors presented with significantly higher H_2S levels at days 1 and 7; moreover, mortality was increased when H_2S levels decreased by more than one-third over time. Finally, a threshold value of approximately 150 μM H_2S allowed differentiation between survivors and non-survivors. The authors concluded that serum H_2S concentrations could be a marker of severity in patients with SARS-CoV-2-coronavirus pneumonia. Consequently, maintaining endogenous H_2S availability and/or even exogenous H_2S supplementation *via* slow releasing compounds may represent a therapeutic approach in these patients.

We are struck, however, by the high absolute values of the H_2S concentrations reported in the study, which were measured using the monobromobimane derivatization assay followed by reverse phase high performance liquid chromatography separation (3): according to Figure 1A and B of the study, median serum H_2S concentrations on days 1 and 7 were 188 versus 129 and 177 versus 55 μM in survivors and non-survivors, respectively, the highest individual value measured being approx. 383 μM. These H_2S concentrations are about two orders of magnitude higher than those reported by others using this method for blood and/or tissue H_2S quantification in mice, rats (3), swine (4–6), and humans (healthy volunteers and patients) (7, 8). Administration of Na_2S in rats (bolus injection of 4 mg/kg, continuous i.v. infusion of 20 mg/kg/h (3)) and swine (maximum infusion rate 2 mg/kg/h (4–6)) only increased H_2S levels to a maximum of 2.5 to 6.5 μM. Although the reported plasma levels of H_2S very much depend on the experimental method used, high micromolar H_2S plasma concentrations have to be questioned based on the physico-chemical properties of H_2S: the gas/water coefficient of distribution for H_2S is 0.39, and at physiological pH and at 37°C, ~20% of the total free sulfide is present as dissolved gas (9). Assuming that only 20% of physically dissolved H_2S gas, i.e., 4% to 10% of the total free sulfide, disappears from a blood sample with an H_2S concentration of the above-mentioned approx. 150 μM into the head space due to volatilization (9), this blood sample would smell like rotten eggs, since the human nose' odor threshold is at ~ 1 μM solutions (9). Finally, while baseline H_2S levels in rats measured using the same technique were ~ 0.74 μM, H_2S levels of ~ 51 μM upon Na_2S administration were lethal (10).

Potential pitfalls of the different methods to measure H_2S concentrations in biological samples have been highlighted previously (9). Clearly, the monobromobimane assay *per se* does not solely measure free sulfide concentrations in blood serum or plasma samples due to interferences with the total sulfide pool. Moreover, the measured values largely depend on the analytical conditions, i.e., alkylation time, light exposure, tight temperature control, the actual monobromobimane concentrations used, pH, and/or the presence or absence of chelators (e.g., in the tubing used for blood sampling) (11, 12).

These pronounced discrepancies between reported data on H_2S concentrations have two major consequences: no direct relation between measured blood H_2S concentrations and biologic effects of therapeutic approaches modulating the H_2S are possible, and unless rigorously standardized procedures, which are based on consensus statements (e.g., as for the use of the single-cell gel electrophoresis (''comet assay''), are used even for the same analytical method, the absolute values of the data reported from different studies cannot be compared. Nevertheless, the trends and directions observed within the same study, when all the samples were analyzed by the same method, can, remain valid. Thus, the above methodological concerns (i.e., the uncertain chemical nature of the species measured by the method used here) do not necessarily question the primary conclusions of the study discussed above (2), i.e., that low H_2S concentrations—or probably more broadly, low 'reactive sulfur species concentrations correlate with worse outcomes in SARS-CoV-2-coronavirus pneumonia patients. Nevertheless, follow-up studies to confirm these findings (preferably, using independent, different methods of H_2S or reactive species analysis) are recommended. Moreover—if the inverse correlation between H_2S levels and SARS-CoV-2 outcomes is, indeed confirmed—the potential therapeutic effect of H_2S donation on the outcome of SARS-CoV-2 could also be tested, first in preclinical models, and if positive, potentially in subsequent translational studies as well.

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Peter Radermacher
Enrico Calzia
Oscar McCook
Ulrich Wachter

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

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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_2S) for the prognosis and outcome of severe infection caused by the novel SARS-CoV-2 (also known as Covid-19) (2). Although serum H_2S 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_2S 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_2S 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 10^5 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_2S was measured by the monobromobimane derivatization assay followed by reverse phase HPLC separation (2). Results clearly showed that survivors from Covid-19 had H_2S 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_2S levels, but that H_2S increase may well be an intrinsic characteristic of Covid-19 described for the first time herein. H_2S of healthy was also within reported ranges (7).

To strengthen the finding of increased H_2S in Covid-19 survivors, H_2S 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_2S plays a major role and we wish to thank Rademacher 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.