THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2020, Vol. 75, No. 6, 1089–1094
doi:10.1093/gerona/glz101
Advance Access publication April 25, 2019

OXFORD

Original Article

Glutathione Serum Levels and Rate of Multimorbidity Development in Older Adults

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Received: December 1, 2018; Editorial Decision Date: April 10, 2019

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

We aimed to investigate the association between baseline levels of total serum glutathione (tGSH) and rate of chronic disease accumulation over time. The study population (n = 2,596) was derived from a population-based longitudinal study on ≥60-year-olds living in Stockholm. Participants were clinically assessed at baseline, 3- and 6-year follow-ups. Multimorbidity was measured as the number of chronic conditions from a previously built list of 60 diseases. Linear mixed models were applied to analyze the association between baseline tGSH levels and the rate of multimorbidity development over 6 years. We found that at baseline, participants with ≥4 diseases had lower tGSH levels than participants with no chronic conditions (3.3 vs 3.6 µmol/L; $p < .001$). At follow-up, baseline levels of tGSH were inversely associated with the rate of multimorbidity development (β * time: −0.044, $p < .001$) after adjusting for age, sex, education, levels of serum creatinine, C-reactive protein, albumin, body mass index, smoking, and time of dropout or death. In conclusion, serum levels of tGSH are inversely associated with multimorbidity development; the association exists above and beyond the link between tGSH and specific chronic conditions. Our findings support the hypothesis that tGSH is a biomarker of multisystem dysregulation that eventually leads to multimorbidity.

Keywords: Biomarkers, Multimorbidity, Epidemiology, Biogerontology

Recent studies have detected an association between low levels of glutathione and specific chronic conditions such as diabetes mellitus (1,2), AIDS (3), cystic (4–6) and pulmonary fibrosis (6,7), chronic liver injury (6,8), and some neurodegenerative conditions such as Parkinson disease (6,9) and Alzheimer's disease (6,10–12).

Glutathione is part of the enzymatic antioxidant system and is involved in several essential physiological processes, such as detoxification of endogenous compounds and xenobiotics; transport and storage of cysteine that modulates immune function (13,14); and DNA synthesis, repair, and expression (15,16). Given these multiple biological mechanisms, it is plausible to hypothesize that glutathione plays a role in the aging process. Indeed, an association between low levels of glutathione and biological aging has already been reported (2,6,17,18). However, we still do not know what role, if any, glutathione plays in the development of multimorbidity (ie, the coexistence of multiple chronic conditions in a single individual).

Multimorbidity is the most common clinical condition linked to aging; it affects more than 80% of people aged 65 years or older (19). Moreover, multimorbidity often leads to several negative health outcomes, such as functional and cognitive decline, avoidable hospitalizations, decreased quality of life, and mortality (20,21). A faster accumulation of chronic diseases with time has been previously suggested to be a clinical manifestation of progressive loss of resilience and homeostatic multisystem dysregulation, hallmarks of

the biological aging process (22). The rate of development and expansion of multimorbidity could thus be interpreted as proxies for the speed of biological aging. Few previous studies have explored interindividual heterogeneity in the rate of multimorbidity progression over time (23–27), and found some evidence of the detrimental effects of adverse socioeconomic and lifestyle conditions. So far, only one study looked at biological correlates of multimorbidity development prospectively, showing that higher circulating levels of inflammatory markers are cross-sectionally associated with multimorbidity and predict steeper rates of disease accumulation over time (28).

The aim of our study was to investigate the association between baseline levels of total serum glutathione (tGSH) and the rate of chronic disease accumulation over time in a population-based cohort of older adults.

Methods

Study Population and Data Collection

The study population was derived from the Swedish National study on Aging and Care in Kungsholmen (SNAC-K), a population-based prospective study conducted in the Kungsholmen area of central Stockholm (29). SNAC-K is an ongoing study of a random sample of people aged 60 years or older who live either at home or in an institution. The sample was randomly drawn from 11 age cohorts (60, 66, 72, 78, 81, 84, 87, 90, 93, 96, and ≥99 years). The two youngest and the four oldest age groups were oversampled to make up for the likely high attrition rate in these age groups. Of the 4,590 people invited to join the study, 3,363 participated in the baseline examination (73.3% participation rate). Follow-up assessments were performed every 6 years for younger participants (60–78 years) and every 3 years for older participants (≥78 years). The current study included baseline data, collected between 2001 and 2004, and data from the 3- and 6-year follow-ups.

Data were collected at baseline and each follow-up by trained personnel in accordance with a structured protocol available at http://www.snac.org. Nurses collected demographic data and assessed participants' physical function; psychologists administered cognitive test batteries; and physicians carried out physical, geriatric, and neuropsychiatric examinations. Data on past medical history and vital status were also available through the National Patient Register and the Swedish Death Register, which have been linked to the SNAC-K database.

SNAC-K was approved by the Regional Ethical Review Board in Stockholm, Sweden. Written informed consent was collected from all participants or, if the participant was not capable of providing such consent, from a proxy.

Multimorbidity Assessment

The total number of chronic conditions at baseline and follow-ups was operationalized in accordance with a comprehensive list proposed by Calderón-Larrañaga and colleagues (19). This list of chronic conditions is the result of a consensus reached by an international team of geriatricians, primary care physicians, and epidemiologists. In brief, all codes from the International Classification of Diseases, 10th revision (ICD-10) were first classified as chronic or not chronic, and those classified as chronic were grouped into 60 broader diseases categories. SNAC-K participants' medical histories, laboratory test results, information on current drug use, and data from primary care and hospital medical records were used to identify these chronic conditions.

Glutathione Assessment

Venous blood samples were taken at baseline in nonfasting conditions and stored at −80°C for all SNAC-K participants. For a random sample of 2,785 participants, these were transferred to the University of Oxford, United Kingdom, for further analyses (30). Levels of tGSH (μmol/L), including both the reduced and oxidized form of glutathione, were measured through tandem mass spectroscopy as described previously (31). Although the concentrations of tGSH are higher within cells than in the blood, serum tGSH levels are a good proxy of overall body glutathione (32). The coefficients of variation ranged between 5% and 10%.

Covariates

Education was measured as the highest level of formal education and was categorized as elementary school, high school, or university or above. Serum creatinine, C-reactive protein (CRP), and albumin levels were measured using routine methods at Karolinska Institutet, Stockholm, Sweden. CRP levels were categorized as low (≤5 mg/L), medium (5.1–9.9 mg/L), or high (≥10 mg/L). Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters, and participants were categorized as underweight (<18.5 kg/m^2), of normal weight (18.5–24.9 kg/m^2, reference category), overweight (25–29.9 kg/m^2), or obese (≥30 kg/m^2). Smoking was categorized as never, former, or current smoker. Dropout or death status in the 3- or 6-year follow-up was also included in the analyses to account for potential selection bias.

Statistical Analysis

Differences in sociodemographic and clinical characteristics between people with versus without information on the exposure and covariates were analyzed using the chi-square test for proportions and the Mann–Whitney test for continuous variables. Comparisons of baseline characteristics according to the number of chronic diseases were performed using the chi-square test for proportions and the Jonckheere–Terpstra trend test for continuous variables. Differences in tGSH levels according to age group, sex, and education level were assessed by the Jonckheere–Terpstra, Mann–Whitney, and Kruskal–Wallis tests, respectively.

Linear mixed models were employed to analyze the longitudinal association between baseline tGSH levels and the rate of multimorbidity development over the 6-year follow-up (ie, the number of chronic conditions in each participant in each wave). The interaction term between time and tGSH level was included as a fixed effect; the resulting β-coefficients are interpreted as the average annual change in the number of chronic conditions across the 6-year follow-up. Random effects were defined for the individual and time of follow-up; unstructured covariance was assumed. The exposure (ie, tGSH) was operationalized both as a standardized continuous variable (ie, *Z*-scores), and categorized in quartiles. Models were adjusted for age, sex, educational level, serum creatinine, CRP, and albumin levels, BMI category, smoking (all measured at baseline), and time of dropout/death in a cumulative manner, in order to detect major confounders. Serum creatinine was included as a measure of kidney function, since alterations in protein and amino acid metabolism are present in patients with chronic kidney disease. CRP was included as a marker of inflammation, and albumin as a proxy of nutritional status, which can affect glutathione metabolism (4). BMI was included in the models because it is associated with levels of total serum cysteine, one of the precursors of glutathione (33). Smoking was included given that it is related both to oxidative stress

and to disease occurrence. Additionally, three-way interactions between age and sex and baseline tGSH levels * time were tested.

Sensitivity analyses

First, to evaluate whether tGSH levels were associated with the presence of specific chronic diseases instead of longitudinal changes in multimorbidity, we repeated the analyses excluding cardiovascular, neuropsychiatric, and musculoskeletal diseases from the original total count of diseases in three different models. These three groups of chronic conditions represent the most prevalent patterns of multimorbidity, regardless of the study population and methodology (34). Second, survival analyses were conducted using Cox proportional hazards regression, in order to assess the association between tGSH levels (ie, *Z*-scores) and 14-year all-cause mortality.

In all analyses, *p*-values < .05 were regarded as statistically significant. Analyses were performed using Stata version 14.

Results

The mean age of the study population at baseline was 72.8 years (*SD* = 10.2), 61.3% were women and approximately half had at least high school education (Table 1). The average number of chronic diseases at baseline was 3.8 (*SD* = 2.34), and 6.46 (*SD* = 3.62) at the 6-year follow-up. Participants for whom baseline data on the exposure or the covariates were missing (*n* = 767) were older than the study population (81.6 vs 72.8 years; $p < .001$), more likely to be women (77.1% vs 61.3%; $p < .001$), took a greater number of medications (5.2 vs 3.7; $p < .001$), and had a greater number of chronic conditions (4.9 vs 3.8; $p < .001$). No sex or education differences were found in baseline tGSH levels. However, mean levels of tGSH decreased significantly with increasing age (Figure 1).

Lower levels of baseline tGSH were associated with a higher rate of multimorbidity development and the direction and magnitude of the association remained very stable regardless of potential confounders (Figure 2 and Supplementary Table 1). Specific β-coefficients for the interaction term between follow-up time and 1-*SD* difference in the mean tGSH level were: –0.043 when adjusting for sex, age, and education (95% CI –0.061; –0.025), –0.043 when adjusting additionally for creatinine level (95% CI -0.061; –0.025), –0.043 when adjusting additionally for CRP level (95% CI -0.061; –0.025), –0.043 when adjusting additionally for albumin level and BMI (95% CI -0.061; –0.025), –0.044 when adjusting additionally for smoking habit (95% CI –0.062; –0.026), and –0.044 when adjusting additionally for time of dropout/death (95% CI –0.062; –0.025). Similar findings were obtained when tGSH levels were categorized in quartiles (Figure 3). No significant interactions were found with age and sex.

All models provided similar results when excluding cardiovascular, neuropsychiatric, and musculoskeletal diseases from the original total count of chronic conditions (Figure 2). Finally, lower levels of baseline tGSH were associated with a higher risk of mortality, even in the fully adjusted model (HR 0.91, 95% CI 0.866; 0.980). When tGSH levels were categorized in quartiles with the highest level (4th quartile) as the reference, only the lowest level (1st quartile) was significantly associated with increased mortality (Table 2).

Discussion

In this large longitudinal population-based study of older adults, lower baseline levels of tGSH were associated with a higher rate of multimorbidity development over 6 years. Our results were independent of age, sex, educational level, serum creatinine, CRP level,

Table 1. Baseline Characteristics of the Study Population by Number of Chronic Diseases

	Total Population *n* = 2,596	0 Disease *n* = 91	1 Disease *n* = 292	2–3 Diseases *n* = 966	≥4 Diseases *n* = 1,247	*p*-Value*
Age, mean (*SD*)	72.8 (10.2)	64.3 (6.0)	65.2 (7.0)	69.9 (8.8)	77.3 (9.8)	<.001
Age groups % (*n*)						<.001
60–66	43.9 (1,139)	81.3 (74)	78.4 (229)	55.6 (537)	24.0 (299)	
72–78	30.3 (789)	16.5 (15)	15.1 (44)	29.8 (288)	35.5 (442)	
81–87	17.9 (464)	2.2 (2)	6.5 (19)	11.7 (113)	26.4 (330)	
≥90	7.9 (204)	0.0 (0)	0.0 (0)	2.9 (28)	14.1 (176)	
Women % (*n*)	61.3 (1,591)	49.5 (45)	54.8 (160)	59.9 (579)	64.7 (807)	.001
Education % (*n*)						<.001
Elementary	14.7 (382)	4.4 (4)	6.9 (20)	12.7 (123)	18.9 (235)	
High school	49.5 (1,286)	35.2 (32)	42.3 (124)	47.4 (458)	53.8 (672)	
University	35.8 (928)	60.4 (55)	50.7 (148)	39.9 (385)	27.3 (340)	
BMI % (*n*)						<.001
Underweight	2.5 (64)	0.0 (0)	0.7 (2)	2.1 (20)	3.4 (42)	
Normal weight	44.7 (1,160)	59.3 (54)	57.1 (167)	44.5 (430)	40.8 (509)	
Overweight	40.0 (1,039)	40.7 (37)	38.4 (112)	43.5 (420)	37.7 (470)	
Obese	12.8 (333)	0.00 (0)	3.8 (11)	9.9 (96)	18.1 (226)	
Smoking % (*n*)						<.001
Never	45.5 (1,174)	32.9 (30)	40.5 (117)	45.2 (435)	47.7 (592)	
Former	39.8 (1,029)	42.9 (39)	42.9 (124)	37.2 (358)	40.9 (508)	
Current	14.7 (380)	24.2 (22)	16.6 (48)	17.6 (169)	11.4 (141)	
Number of drugs, mean (*SD*)	3.7 (3.3)	0.92 (1.1)	1.3 (1.7)	2.4 (2.1)	5.5 (3.4)	<.001
tGSH levels in µmol/L, mean (*SD*)	3.6 (1.1)	3.8 (1.1)	3.9 (1.2)	3.6 (1.2)	3.5 (1.1)	<.001

Notes: BMI = body mass index; *SD* = standard deviation; tGSH = total serum glutathione.

*Chi-square test for proportions and Jonckheere–Terpstra trend test for continuous variables.

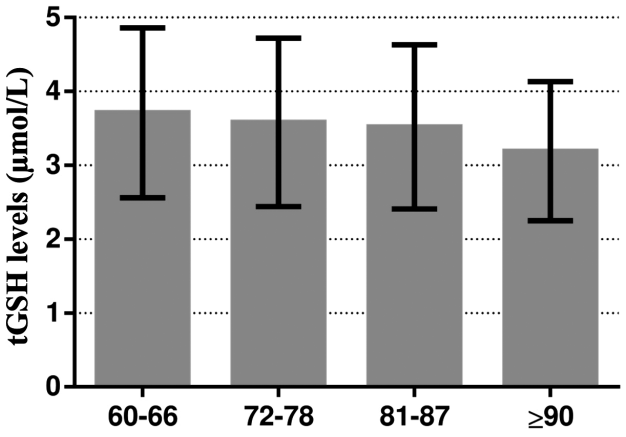

Figure 1. Baseline mean and standard deviation of tGSH levels according to age group. tGSH = total serum glutathione.

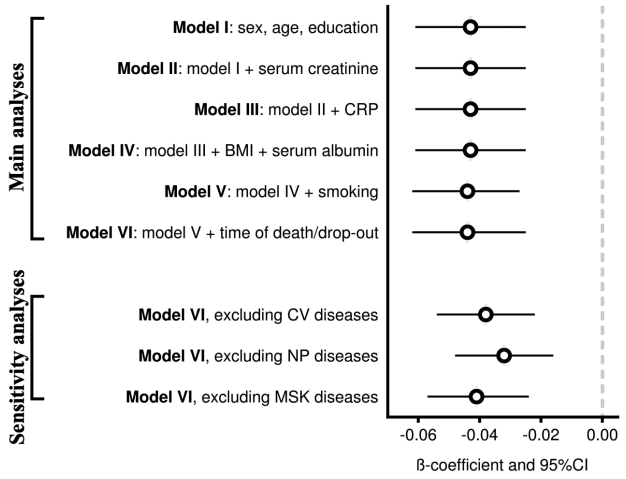

Figure 2. Association between baseline tGSH levels and changes in the number of chronic conditions developed over the 6-year follow-up. Results of the main and sensitivity analyses. BMI = body mass index; CRP = C-reactive protein; CV = cardiovascular; MSK = musculoskeletal; NP = neuropsychiatric; tGSH = total serum glutathione. β-coefficients represent the interaction term between follow-up time and 1-*SD* difference in the mean tGSH level.

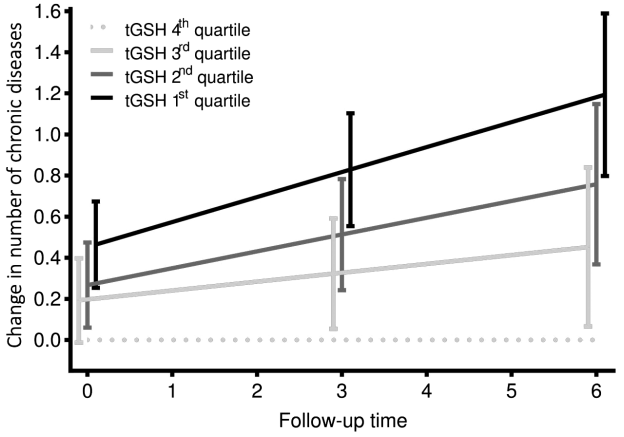

Figure 3. Estimated changes in the number of chronic conditions developed over the 6-year follow-up by baseline tGSH quartile (4th quartile as reference). tGSH = total serum glutathione. 1st tGSH quartile range: 1.31–2.75 µmol/L, 2nd tGSH quartile range: 2.75–3.44 µmol/L, 3rd tGSH quartile range: 3.44–4.23 µmol/L, and 4th tGSH quartile range: 4.23–12.5 µmol/L. Model adjusted for age, sex, education, serum creatinine, C-reactive protein, and albumin, body mass index, smoking, and time of dropout/death.

and albumin, BMI, smoking, and time of dropout or death during follow-up. The association existed above and beyond the link between tGSH and specific chronic conditions; thus, it seemed to be driven by the global burden of multimorbidity. These findings suggest that tGSH may be a relevant biomarker of multisystem failure that eventually leads to multimorbidity.

A number of hypotheses could explain our finding. On one hand, there could be a causal relationship between low tGSH levels and multimorbidity. Glutathione is one of the most important antioxidants in humans and plays a role in several key cell functions, including signaling and transcription processes. An imbalance in glutathione homeostasis may be due to mitochondrial and DNA damage, a state of hypoxia, and/or high levels of oxidative stress that lead to decreased glutathione synthesis or increased glutathione consumption (35). Such an imbalance could contribute to cellular dysfunction and higher susceptibility to stress, leading to multisystem dysregulation and faster development of chronic diseases. On the other hand, low levels of glutathione could also reflect an unknown underlying dysfunction that leads to a breakdown of glutathione homeostasis. In both cases, tGSH levels could be used as a subclinical biomarker to predict the future development of multimorbidity and to better understand who will benefit most from interventions to reduce their future burden of disease.

Previous studies have found lower glutathione levels in older adults than in young populations (2,35,36), which suggest that there is a relationship between age and glutathione levels. Higher concentrations of tGSH levels have also been reported in physically and mentally healthy long-lived older women (32), which highlights the importance of glutathione's role in healthy aging. The association between tGSH and the presence of multimorbidity has been investigated in previous studies, but only in cross-sectional studies with limited information on multimorbidity. Early in the 1990s, Julius and colleagues (37) measured tGSH levels in a small sample of 33 community-dwelling adults older than 60 years. Although the sample was small and only 21 chronic conditions were considered, their results indicated a relationship between lower tGSH levels and a higher number of chronic diseases and worse self-rated health. More recently, in a study of hospital inpatients, Lang and colleagues (18) compared tGSH levels in 74 patients with at least one chronic condition and a control group of 32 patients without any chronic condition. They also observed a cross-sectional association between lower levels of tGSH and the presence of chronic conditions.

The main strength of the current analyses was the use of data from a longitudinal population-based study of a large sample of people living in the community or institutions for whom extensive clinical and laboratory assessments were available. Additionally, chronic conditions were identified with a clinically driven algorithm that integrated different sources of data. The findings are precise and stable, as they remained unchanged despite sensitivity analyses. Moreover, the association found between tGSH levels and mortality reinforces the significance and reliability of our findings.

One of the main limitations is the lack of tGSH measurements during follow-up assessments, which may mean that we underestimated the associations because of regression dilution. Additionally, the technique used to assess serum glutathione levels allowed us to

Table 2. Association Between Baseline tGSH Levels (as Continuous and in Quartiles with 4th Quartile as Reference) and 14-Year All-Cause Mortality

	Fully Adjusted Model		Excluding CV Diseases		Excluding NP Diseases		Excluding MSK Diseases	
	HR (CI 95%)	*p*-Value	HR (CI 95%)	*p*-Value	HR (CI 95%)	*p*-Value	HR (CI 95%)	*p*-Value
tGSH (*Z*-score)	0.91 (0.85–0.98)	.009	0.91 (0.85–0.98)	.008	0.92 (0.85–0.98)	.014	0.91 (0.85–0.98)	.012
4th tGSH quartile	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
3rd tGSH quartile	1.06 (0.87–1.28)	.575	1.06 (0.87–1.29)	.564	1.05 (0.87–1.28)	.613	1.03 (0.85–1.25)	.759
2nd tGSH quartile	1.16 (0.96–1.40)	.122	1.16 (0.96–1.40)	.124	1.14 (0.95–1.38)	.158	1.15 (0.95–1.39)	.145
1st tGSH quartile	1.33 (1.10–1.60)	.003	1.33 (1.11–1.61)	.003	1.31 (1.09–1.58)	.004	1.30 (1.07–1.56)	.007

Notes: CV = cardiovascular; MSK = musculoskeletal; NP = neuropsychiatric; tGSH = total serum glutathione. Results of the main and sensitivity analyses. Models adjusted for age, sex, education, serum creatinine, C-reactive protein, and albumin serum levels, body mass index, smoking, number of chronic diseases at baseline, and time of dropout.

measure tGSH, which is the sum of free and protein-bound glutathione and glutathione disulphide levels. Thus, we could not differentiate between the reduced and oxidized forms of glutathione, which prevented us from ascertaining whether or not there was a direct relationship between the oxidative redox status and tGSH levels. It may be argued that in people with lower tGSH levels, diseases do not accumulate but get diagnosed more rapidly due to more intensive diagnostic workup in people who are already ill. However, the baseline number of chronic conditions each participant suffers from is accounted for in the linear mixed models. Our study population was younger and healthier than those excluded from the study sample, which could bias the association between tGSH levels and multimorbidity. Last, further external validation is needed to discard that our findings are artificially driven by population composition or single-biomarker analysis (38).

Conclusion

Our results show an association between low baseline tGSH levels and a higher rate of multimorbidity development over 6 years in a large population-based sample of older adults. This association might be due to a breakdown of the homeostasis that is safeguarded by the multiple intra- and extracellular functions of glutathione. However, further investigation is needed to better understand the causal relationship between tGSH levels, biological changes, aging, and the development of multimorbidity.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

This work was supported by the funders of the Swedish National study on Aging and Care (SNAC): the Ministry of Health and Social Affairs, Sweden; the participating County Councils and Municipalities; and the Swedish Research Council. Specific grants were obtained from Swedish Research Council (VR), Strategic Research Program in Epidemiology (SfoEpi) of Karolinska Institutet, Loo and Hans Osterman Foundation, Karolinska Institutet's Foundation Grants for Medical Research, Lindhés advokatbyrå AB, and Stiftelsen Gamla Tjänarinnor. None of the funding sources had any role in the design of the study; data collection, analysis, or interpretation; or preparation, review, or approval of the manuscript.

Acknowledgments

We would like to thank all the SNAC-K participants and the SNAC-K Study Group for their invaluable collaboration and contributions in data collection and management. Authors' Contribution: Study concept and design: L.M.P., L.F., A.C.-L. Analysis and interpretation of data: L.M.P., B.H., F.M., L.F., A.C.-L. Critical revision of the manuscript for important intellectual content: L.M.P., B.H., F.M., P.M., A.D.S., H.R., M.I., L.F., D.R., A.C.-L.

Conflict of Interest

None reported.

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