

# Different Doses of Oral Folic Acid for Homocysteine-Lowering Therapy in Patients on Hemodialysis

## A Randomized Controlled Trial

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**Introduction.** We compared the effect of higher and lower doses of folic acid compared to our routine daily dose on plasma homocysteine levels, in our hemodialysis patients.

**Materials and Methods.** Eighty patients on hemodialysis receiving oral folic acid, 10 mg/d, were randomized to receive folic acid at either doses of 5 mg/d (group 1) or 15 mg/d (group 2) for 2 months. Plasma levels of total homocysteine were measured before and after the study period.

**Results.** Hyperhomocysteinemia was seen in 75 patients (93.8%) before, and in 37 patients of group 1 (92.5%) and 39 of group 2 (97.5%) after the study period. In group 1, a nonsignificant decrease occurred in plasma homocysteine level ( $29.67 \pm 12.26 \mu\text{mol/L}$  to  $27.78 \pm 9.94 \mu\text{mol/L}$ ,  $P = .30$ ), while in group 2, there was a significant decrease in homocysteine level ( $32.40 \pm 9.76 \mu\text{mol/L}$  to  $29.58 \pm 9.62 \mu\text{mol/L}$ ,  $P = .01$ ). Changes in homocysteine level correlated with its baseline level ( $r = -0.42$ ,  $P < .001$ ). In both groups, significant reductions in homocysteine level were seen mostly in those patients with high baseline homocysteines.

**Conclusions.** Routine folic acid supplementation of 10 mg/d could not normalize plasma homocysteine levels in most of our patients. Increasing folic acid dose made a statistically significant but clinically trivial decrease in homocysteine levels, and could not normalize homocysteine level in most patients. Patients with a higher baseline homocysteine level achieved a greater reduction, which may be explained by primary noncompliance of some patient. Further investigation of folic acid dosage is suggested.

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### INTRODUCTION

Homocysteine is an independent risk factor of atherothrombotic diseases and its role as a predictor of cardiovascular disease is tantamount to the traditional risk factors, especially in patients with end-stage renal disease (ESRD).<sup>1</sup> Homocysteine induces oxidative stress, inhibits antioxidant enzymes, accelerates endothelial dysfunction, and increases thrombogenicity.<sup>2,3</sup> Wald and colleagues showed a causal role for homocysteine

in cardiovascular events in their meta-analysis.<sup>1</sup> However, the mechanism of contribution of homocysteine to atherothrombotic changes is not yet completely unraveled. In patients with ESRD, hyperhomocysteinemia is mainly due to impairment in remethylation of homocysteine caused by uremia, as well as the deficiency of the cofactors such as folate.<sup>4,5</sup> Muntner and associates found that glomerular filtration rate is strongly associated with higher levels of total homocysteine.<sup>6</sup>

Homocysteine-lowering therapy has proved to be effective in prevention of cardiovascular morbidity and mortality.<sup>1,7</sup> The Dialysis Outcomes and Practice Pattern Study showed that supplementation of water-soluble vitamins, including folic acid, vitamin B6 and vitamin B12, reduced the rate of cardiovascular events and the overall mortality in patients receiving long-term dialysis.<sup>8</sup> However, folic acid, is proven to be the main effective factor, and the additional effect of vitamin B12 or B6 has been disputed.<sup>9-11</sup> Folic acid lowers plasma homocysteine level, and thus reduces the burden of cardiovascular disease in patients with ESRD.<sup>5,7</sup>

In spite of the evidenced effect of folic acid in lowering plasma homocysteine level, it does not always normalize homocysteine level in patients on hemodialysis.<sup>3,12,13</sup> Since folate is filtered by hemodialysis,<sup>14</sup> treatment with high doses of folic acid is recommended in these patients. According to the National Kidney Foundation Disease Outcomes Quality Initiative, daily oral doses of 1 mg to 60 mg of folic acid have been studied<sup>15</sup>; however, the higher doses have not had a better result compared to lower doses in terms of plasma homocysteine levels.<sup>15</sup> Usually, homocysteine-lowering therapies in patients with ESRD cannot reduce homocysteine to levels lower than 15  $\mu\text{mol/L}$ .<sup>16</sup> Even intravenous route and treatment with active forms of folic acid have been tried, with controversial results.<sup>13,17,18</sup> Folic acid is routinely administered in many dialysis centers; however, there is no consensus on the effective dose.<sup>8,19</sup> A recent study on a group of Iranian patients on hemodialysis showed that homocysteine could be reduced by only 30% after a long-term treatment with oral folic acid at a dose of 1 mg/d.<sup>19</sup>

Currently, we administer a daily dose of 10 mg/d folic acid at our hemodialysis center. We designed this study to determine whether our protocol of folic acid administration has been successful as a homocysteine-lowering strategy, and if higher or lower doses of folic acid affect plasma homocysteine levels. To answer these questions, we prospectively investigated the effect of folic acid in two doses 5 mg/d and 15 mg/d on plasma homocysteine levels of 80 patients on long-term hemodialysis.

## PATIENTS AND METHODS

### Patients

We recruited patients on long-term hemodialysis

at the dialysis unit of Hasheminejad Kidney Center, Tehran, Iran. The inclusion criteria were being on hemodialysis for at least 3 months and 18 year of age and over. In addition, since folic acid is routinely administered in our center at a dose of 10 mg/d, we included only those patients who had been receiving 10 mg/d of folic acid, for at least 3 months. The exclusion criteria were smoking, severe cardiovascular disease, cancer, hypothyroidism, planned kidney transplantation in near future, and administration of drugs affecting plasma folate or total homocysteine levels (antiepileptic drugs, steroids, sulfonamides, triamterene, sulfasalazine, estrogens, methotrexate, and cyclosporine). Eligible patients who consented to participate in the study were enrolled. The study was approved by the Research Deputy of Iran University of Medical Sciences.

### Methods

The study consisted of a 2-month period of treatment with two different doses of oral folic acid.<sup>20</sup> Ninety patients, previously taking 10 mg/d of folic acid, were randomly assigned into 2 groups to receive folic acid at either of the new doses of 5 mg/d (group 1) or 15 mg/d (group 2). Folic acid was administered to the patients weekly in the form of 5-mg tablets (Jalinus, Tehran, Iran), in order to be administered once or thrice daily, depending on group assignment. The patients also continued to receive their routine daily oral vitamin B preparations (1 tablet of vitamin B6, 40 mg, and 1 tablet of vitamin B complex, containing vitamin B1, 5 mg; vitamin B2, 2 mg; vitamin B6, 2 mg; and nicotinamide, 20 mg) throughout the study. All of the patients underwent bicarbonate-based hemodialysis 3 times a week and 4 to 4.5 hours per session, with conventional polysulphone dialyzers. Dialysis dose was targeted for a single-pool KT/V of 1.2. The patients were asked to keep on their usual diet during the study.

### Laboratory Investigations

Plasma levels of folate and total homocysteine were measured before starting the new doses of folic acid and after the 2-month study period. One milliliter of nonfasting predialysis blood samples were collected in ethylenediamine tetraacetic acid (EDTA)-coated tubes and placed on ice immediately. Plasma was separated within 2 hours

by centrifugation at 2000 g for 10 minutes at 4°C and stored at -20°C, to be analyzed with one kit at the end of the study. Folate levels were measured in plasma samples (nmol/L) using the Elecsys folate II assay with Roche Elecsys 2010 Chemistry Analyzer (Roche, Basel, Switzerland). Plasma concentration of total homocysteine (μmol/L) was measured using the enzyme cycling method (Diazyme, La Jolla, CA, USA) according to the manufacturer's instructions, with Hitachi 902 analyzer (Roche, Hitachi, Tokyo, Japan). The intra-assay and interassay coefficient of variations values were less than 5%, and the linear range was between 2 μmol/L and 50 μmol/L. The reference range for the total homocysteine level in plasma is not elucidated and may vary in different populations. We defined a high homocysteine level to be greater than 12 μmol/L.<sup>21</sup>

### Statistical Analyses

Data analyses were performed with the SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were examined for normality by use of the Kolmogorov-Smirnov test. Comparisons of homocysteine levels between the two groups were done with the *t* test and the Mann-Whitney test, where appropriate. Comparison between homocysteine levels before and after changing the folic acid dose was done using the paired *t* test and the Wilcoxon signed rank test, where appropriate. The chi-square test was used to compare the nominal variables. Spearman rho

correlation coefficient test was used for testing the correlations of the measured homocysteine levels. A *P* value less than .05 was considered significant.

### RESULTS

Of 140 patients on hemodialysis, 90 were eligible and consented to participate in this study. Ten patients (5 in each group) were excluded from analyses due to noncompliance with the prescribed folic acid dose. Therefore, 40 patients in each group were included in data analyses. The study group consisted of 80 patients (37 women and 43 men) with a mean age of 60.2 ± 14.9 years who were on hemodialysis for a mean of 78.9 ± 70.4 months. Table 1 summarizes the patients' characteristics.

Before the study period, 3 patients in group 1 (folic acid, 5 mg/d) and none of the patients in group 2 (folic acid, 15 mg/d) had a plasma folate level lower than the reference range (< 20 nmol/L). After the randomization period, the mean plasma folate level was 328.09 ± 289.99 nmol/L in group 1 and 625.55 ± 503.16 nmol/L in group 2 (*P* = .002). Two patients in group 1 had a folate level lower than the reference range. Plasma folate levels after the study period did not significantly correlate with serum albumin or the KT/V (*r* = 0.07, *P* = .60 and *r* = 0.08, *P* = .51; respectively).

Plasma homocysteine levels were higher than normal before the study period in 75 patients (93.8%) (36 [90.0%] in group 1 and 39 [97.5%] in group 2). After the study period, there were 76 patients (95%) with hyperhomocysteinemia;

**Table 1.** Characteristics of Patients on Hemodialysis Who Received Different Doses of Folic Acid for 2 Months\*

Characteristics	Group 1 (Folic Acid, 5 mg/d)	Group 2 (Folic Acid, 15 mg/d)	<i>P</i>
Age, y	62.7 ± 15.3	57.7 ± 14.3	.13
Sex			
Male	20 (50.0)	23 (57.5)	
Female	20 (50.0)	17 (42.5)	.05
Dialysis duration, mo	83.2 ± 73.6	74.6 ± 67.6	.59
Cause of ESRD			
Diabetes mellitus	13 (32.5)	7 (17.5)	
Hypertension	5 (12.5)	7 (17.5)	
PCKD	3 (7.5)	5 (12.5)	
Urologic	1 (2.5)	6 (15.0)	
Others	2 (5.0)	1 (2.5)	
Unknown	16 (40.0)	14 (35.0)	.32
KT/V	1.17 ± 0.34	1.21 ± 0.30	.61
Serum albumin, g/dL	3.77 ± 0.41	3.87 ± 0.52	.38
Serum protein, g/dL	6.93 ± 0.73	7.18 ± 0.66	.14

\*Values in parentheses are percents. ESRD indicates end-stage renal disease and PCKD, polycystic kidney disease.

37 patients in group 1 (92.5%) and 39 in group 2 (97.5%). Plasma homocysteine levels after the study period did not significantly correlate with serum albumin or the KT/V ( $r = 0.08$ ,  $P = .51$  and  $r = -0.05$ ,  $P = .64$ ; respectively).

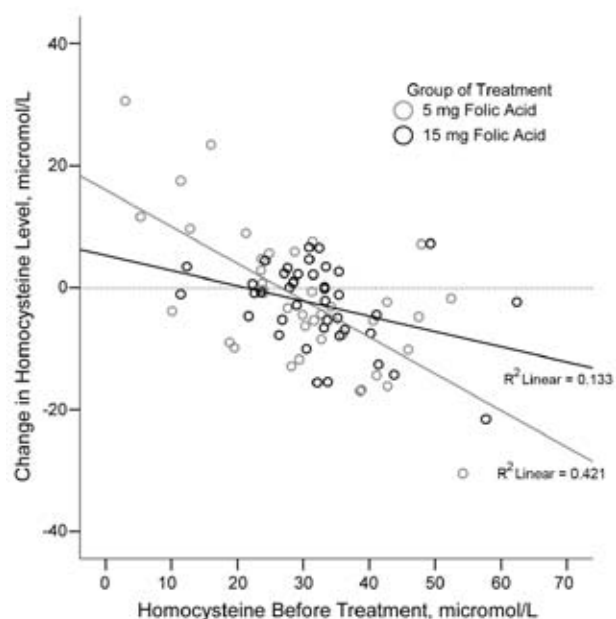
Table 2 shows the mean plasma homocysteine levels before and after the study in each group. Plasma levels of total homocysteine were not significantly different between the patients of group 1 and group 2, neither before nor after the study period (Table 2). In group 1, a nonsignificant decrease was seen in plasma total homocysteine level after reducing the folic acid dose from 10 mg/d to 5 mg/d ( $29.67 \pm 12.26 \mu\text{mol/L}$  versus  $27.78 \pm 9.94 \mu\text{mol/L}$ ,  $P = .30$ ; 95% CI, -1.75 to 5.54), while in group 2 with 15 mg/d of folic acid, there was a significant decrease in homocysteine level ( $32.40 \pm 9.76 \mu\text{mol/L}$  versus  $29.58 \pm 9.62 \mu\text{mol/L}$ ,  $P = .01$ ; 95% CI, 0.68 to 4.96).

After the study period, 26 patients in group 1 and 24 in group 2 had a decrease in homocysteine level (mean decrease,  $8.17 \pm 6.82 \mu\text{mol/L}$  and  $6.79 \pm 5.62 \mu\text{mol/L}$ , respectively), while 14 patients in group 1 and 16 in group 2 had an increase in homocysteine level (mean increase,  $9.75 \pm 6.62 \mu\text{mol/L}$  and  $3.13 \pm 2.27 \mu\text{mol/L}$ , respectively). Accordingly, more than half of the patients in each group did experience a decrease in plasma homocysteine levels.

In search of the characteristics of these subgroups, we evaluated factors related to the outcome, ie, plasma homocysteine level. Plasma folate level did not correlate with the plasma homocysteine level after the study period in neither of the study groups (group 1,  $r = -0.05$ ,  $P = .76$ ; group 2,  $r = 0.09$ ,  $P = .58$ ), while the percentage of changes in homocysteine level reversely correlated with the baseline homocysteine level ( $r = -0.42$ ,  $P < .001$ ; Figure). This correlation was only reflected in group 1 (group 1,  $r = -0.50$ ,  $P = .001$ ; group 2,  $r = -0.28$ ,  $P = .08$ ), showing that patients with a higher baseline homocysteine level achieved a

greater reduction of homocysteine with 5 mg of folic acid.

We analyzed the homocysteine trend in patients with high or low baseline homocysteine levels, considering the median homocysteine level of  $31 \mu\text{mol/L}$  as the cutoff point. Patients with baseline homocysteine levels greater than  $31 \mu\text{mol/L}$  had significant decreases in homocysteine levels after the study period (group 1, from  $40.1 \pm 7.3 \mu\text{mol/L}$  to  $32.6 \pm 9.6 \mu\text{mol/L}$ ,  $P = .004$ ; group 2, from  $38.3 \pm 8.3 \mu\text{mol/L}$  to  $33.4 \pm 9.8 \mu\text{mol/L}$ ,  $P = .01$ ). In contrast, no significant alterations in homocysteine levels were seen in patients with baseline homocysteine levels lower than  $31 \mu\text{mol/L}$ , in neither of the groups (group 1, from  $21.1 \pm 8.1$



Association of changes in plasma total homocysteine level with its baseline value after treatment with different doses of folic acid ( $r = -0.47$ ,  $P < .001$ ). Most patients with a high baseline plasma homocysteine level (right side of the graph) had a greater decrease in homocysteine level after the study period. In contrast, most of those with a low baseline homocysteine level (left side of the graph) faced an increase in homocysteine levels. It is noticeable that the patients with the highest amounts of increase in homocysteine were from group 1 and had a lower baseline homocysteine (left-upper side).

**Table 2.** Plasma Levels of Total Homocysteine and Its Changes After Treatment With Different Doses of Folic Acid\*

Plasma Parameters	Group 1 (Folic Acid, 5 mg/d)	Group 2 (Folic Acid, 15 mg/d)	P
Homocysteine before treatment, $\mu\text{mol/L}$	$29.67 \pm 12.26$	$32.40 \pm 9.76$	.27
Homocysteine after treatment, $\mu\text{mol/L}$	$27.78 \pm 9.94$	$29.58 \pm 9.62^*$	.41
Homocysteine changes, %	$28.4 \pm 170.5$	$-7.2 \pm 18.9$	.19

\*In group 2, the difference between values of before and after treatment was significant ( $P = .01$ ).

$\mu\text{mol/L}$  to  $23.8 \pm 8.4 \mu\text{mol/L}$ ,  $P = .48$ ; group 2, from  $25.2 \pm 5.6 \mu\text{mol/L}$  to  $24.8 \pm 7.1 \mu\text{mol/L}$ ,  $P = .92$ ). Of note, of the 22 patients in group 1 whose baseline homocysteine levels were lower than  $31 \mu\text{mol/L}$ , 12 had increased levels ranged  $0.7 \mu\text{mol/L}$  to  $30.6 \mu\text{mol/L}$  after lowering folic acid dose to  $5 \text{ mg/d}$ .

## DISCUSSION

Hyperhomocysteinemia is known to occur mainly secondary to genetic defects, deficiency of water-soluble vitamins, certain drugs, aging, and kidney failure.<sup>3,13,22</sup> Thus, lowering plasma homocysteine in patients on dialysis is a part of the attempt to prevent cardiovascular events.<sup>8</sup> Although impaired homocysteine metabolism in patients on dialysis is not merely due to the severe vitamin deficiency, high doses of folic acid, the major component of homocysteine-lowering therapy, proved to be effective in lowering plasma homocysteine.<sup>7,13</sup> However, the main controversy lies in the degree of this effect and the appropriate dosage to reach an optimal homocysteine level. In the present study, we found that our routine treatment with  $10 \text{ mg}$  of oral folic acid could not normalize plasma homocysteine levels in 93.8% of the patients. Furthermore, reducing the routine  $10 \text{ mg/d}$  dose of folic acid to  $5 \text{ mg/d}$  for 2 months did not lead to any significant alteration in the mean homocysteine level. On the other hand, increasing the daily folic acid dose to  $15 \text{ mg}$  resulted in a clinically trivial, albeit statistically significant, decrease in the mean homocysteine level.

Different doses of folic acid, ranging from  $7.5 \text{ mg/w}$  to more than  $100 \text{ mg/w}$ , have been studied on patients receiving hemodialysis; however, the effect had been similar in many reports and plasma homocysteine levels could not be generally normalized.<sup>12,13,15,16</sup> In two studies, low folic acid doses ( $7.5 \text{ mg/w}$  and  $15 \text{ mg/w}$ ) were compared with higher doses ( $35 \text{ mg/w}$  and  $70 \text{ mg/w}$ ), and in line with our results, the authors found no superior effects with the higher doses.<sup>3,12</sup> Righetti and colleagues compared folic acid therapy at two doses of  $35 \text{ mg/w}$  and  $105 \text{ mg/w}$  with no folic acid supplementation, for 1 year. Folic acid was effective regardless of its dose; however, they could successfully normalize homocysteine levels only in 12% of the patients.<sup>7</sup> Sanchez Alvarez and associates confirmed these findings in a same

setting.<sup>13</sup> Therefore, we speculate that homocysteine cannot be lowered only by supraphysiologic doses of folic acid supplementation. Other influencing factors have been proposed including uremia itself, malnutrition, and inflammation.<sup>5,23,24</sup> Malnutrition and its consequences may contribute to the *reverse epidemiology* in patients on hemodialysis, which can explain the differences between trials.<sup>25</sup> In addition, methylenetetrahydrofolate reductase gene polymorphism affects homocysteine levels; however, its influence on treatment with folic acid is a matter of controversy.<sup>13,22</sup>

Contrary to the above studies, some investigators have achieved favorable homocysteine levels with folic acid in combination with variable doses of B vitamins.<sup>26,27</sup> Alvares Delfino and colleagues administered  $30 \text{ mg/w}$  of folic acid for 6 months and yielded an overeager homocysteine level of  $8.4 \mu\text{mol/L}$ .<sup>26</sup> Even,  $5 \text{ mg/w}$  of folic acid could normalize homocysteine level in 67% of the patients when accompanied with intravenous administration of B vitamins for 20 weeks.<sup>28</sup> These successful outcomes have been claimed to be a result of higher compliance with a supervised drug administration program.<sup>25</sup> Also, to eliminate malabsorption and noncompliance, some have tried intravenous folic acid and reported favorable outcomes.<sup>18,23</sup>

Another factor which may influence the effect of homocysteine-lowering therapy is the length of the treatment period. The maximal effect of folic acid therapy in patients on dialysis can be achieved after 6 months.<sup>13,18,26</sup> We had a slight decrease in homocysteine level after 2 months of treatment with  $15 \text{ mg/d}$  of folic acid, and it was likely that the decreasing trend would continue with a longer treatment course. However, homocysteine-lowering therapy had been started long before the study period in our patients, and regarding the abovementioned evidence on the trivial effect of the dosage, our short-term study might not be a major concern in interpretation of the results. All of our patients had been receiving folic acid at a dose of  $10 \text{ mg/d}$  for at least 6 months before the study (mean, 78.9 months). Surprisingly, the treatment duration with  $10 \text{ mg/d}$  of folic acid did not have any relationship with the plasma homocysteine levels (data not shown in this article), and 93.8% of our participants still had a high homocysteine concentration. We assume that noncompliance with the treatment could have been a potential

cause of unfavorable results of treatment with 10 mg/d of folic acid.

We found that those patients with a higher baseline homocysteine level experienced significant reduction after the study period. The association of baseline homocysteine levels with the outcome of treatment has been indicated by other investigators, as well.<sup>3,13</sup> One explanation may be that the patients with higher homocysteine levels may have been the noncompliant ones, in whom the plasma homocysteine reduced after supervised regular folic acid intake. In group 2, there was a greater proportion of patients with higher baseline homocysteine levels (Figure), which may be one of the reasons for the statistically significant, although clinically trivial decrease, of homocysteine levels.

The appropriate dosage of folic acid for the treatment of hyperhomocysteinemia is not yet defined in patients on hemodialysis. We found no relationship between plasma levels of folate and homocysteine, which means that homocysteine does not necessarily decrease with higher plasma folate levels. This finding, which was also shown by Arnadottir and associates,<sup>3</sup> makes it more difficult to draw conclusions. However, choosing a lower dose of folic acid for routine therapy in patients on dialysis has been discouraged by some authors.<sup>7,29</sup> First, excessive intake of folic acid does not impose significant side effects or additional costs to the patients. Second, folic acid has antioxidant and anti-atherosclerosis effects independent of homocysteine.<sup>26,27</sup> Folic acid was shown to improve total plasma antioxidant capacity and reduce C-reactive protein.<sup>26,27</sup> Also, folic acid could reduce oxidative stress with a higher studied dose, while both of the low and high doses had a same effect on homocysteine levels.<sup>12</sup> Third, Righetti and colleagues showed that a maximal dose of 5 mg/d lowered cardiovascular events in 3 years, although high homocysteine levels were not corrected.<sup>5</sup> Thus, treatment with folic acid, especially at a high dose, might be beneficial for the patients with ESRD even if not be able to normalize plasma homocysteine level. However, imposing additional pills to the patients' medication may lead to patient noncompliance without much clinical benefit, and we do not recommend higher doses of folic acid according to the results of this study.

On the other hand we found no significant changes in homocysteine levels with the reduced

dose of folic acid. However, as shown in the Figure (left-upper part), those patients with a lower baseline homocysteine level tended to have increased levels in the group of 5 mg/d folic acid. Thus, decreasing our routine folic acid dose may cause an increase in the homocysteine levels of those patients who have had relative improvement of hyperhomocysteinemia with the 10 mg/d dose. It has been shown that every unit of reduction in homocysteine can improve long-term cardiovascular outcome.<sup>1</sup> According to the mentioned findings, although we may not achieve normal homocysteine levels in most of the patients, we do not recommend lower doses, either, and will hold on to our strategy of 10 mg/d of folic acid in patients on long-term hemodialysis.

## CONCLUSIONS

We could not normalize plasma homocysteine levels with routine folic acid supplementation, and could not find any clinically significant differences in the homocysteine-lowering effect of folic acid with higher or lower doses than our routine oral dose (10 mg/d). Noncompliance with oral intake of folic acid, nutritional factors, intake of other vitamins, and the effect of uremia per se may be the confounders that should be investigated in controlled studies.

## CONFLICT OF INTEREST

None declared.

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