Original Article



Three-dimensional ultrasound for carotid vessel wall volume measurement

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INTRODUCTION

Abstract

Objectives: The intima-media thickness (IMT) of the carotid artery can now be detected on a three-dimensional (3D) plane. The 3D vessel wall volume (VWV) more accurately represents vascular conditions. Through 3D ultrasound, we established a standardized method for carotid VWV measurement. Materials and Methods: A total of thirty patients without stroke or cardiovascular disease who received carotid duplex sonography were retrospectively reviewed. Gray-scale 3D images from the distal common carotid artery (CCA) to internal carotid artery on both sides were acquired using a single-sweep 3D transducer and analyzed offline by using the vascular plaque quantification function of the Philips QLAB software. Then, the 3D IMT (QLAB intima-media thickness [QIMT]), total plaque volume (TPV), and VWV were measured by a neurologist and a technician, and the interobserver variability was assessed. **Results:** The mean two-dimensional (2D) carotid IMT was 0.65 ± 0.12 mm. The mean QIMT, TPV, and VWV measured by observer 1 were 0.68 ± 0.18 mm, 26 ± 12 mm³, and 94 \pm 10 mm³, respectively. The Bland–Altman plot of the mean differences between the QIMT, TPV, and VWV values measured by observers 1 and 2 showed that those of observer 2 were within two standard deviations of those of observer 1. Intraclass correlation coefficients (ICCs) indicated strong correlations in QIMT (ICC = 0.76), TPV (ICC = 0.85), and VWV (ICC = 0.90; P < 0.001) between observers 1 and 2. Both 2D IMT and 3D QIMT exhibited a positive linear correlation with age. Conclusion: This study established a standardized VWV measurement through 3D ultrasound. Reasonable interobserver differences were obtained within a 95% limit of agreement and high reliability (ICC = 0.90). The VWV 1 cm from the CCA bifurcation was quantified with a mean value of 94.2 mm³. Further studies on the 3D ultrasound quantification of carotid arteries are warranted.

Keywords: Interobserver reliability, Intima–media thickness, Three-dimensional ultrasound, Total plaque volume, Vessel wall volume

lith the continual advancements in ultrasound equipment and relevant software, the intima-media thickness (IMT) of the vessel wall and arteriosclerotic plaque of the carotid artery, which were previously detected on a two-dimensional (2D) plane, can now be detected on a three-dimensional (3D) plane. The intima-media volume (IMV) and the total plaque volume (TPV) can be obtained by increasing the artery scanning length and summing the IMTs or plaque volume on the 2D plane, respectively. Thus, smaller values of measured IMT (average, 0.6-0.8 mm in healthy people) can be converted to higher IMV values. Then, the detection of sequential changes in arteriosclerosis, such as short-term or long-term radiation injury, or the shrinkage of atherosclerotic plaques caused by diet control or lipid-lowering medications, is simple [1,2]. Moreover,

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observing the progression of the IMV is more convenient for a shorter duration and with fewer subjects. Focal thickening of the intima-media usually coexists with protuberant plaque and is indistinguishable from each other. Therefore, measuring the thickness of the vessel wall, which comprises the intima-media and protuberant plaque, is more reasonable than measuring the IMT or plaque alone. The 3D vessel wall volume (VWV) thus represents vascular conditions more accurately than the IMV does. Various 3D ultrasound image acquisition techniques and TPV or VWV measurement methods have been reported previously [3-6]. However, no standard protocol is recommended for measuring 3D carotid VWV. A single-sweep

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3D ultrasound scan with semi-automated plaque analysis software has been implemented in routine carotid ultrasound examination since 2017 in the Taipei Tzu Chi Hospital, Taiwan. We used 3D ultrasound to view the carotid artery and established a standardized method for measuring the carotid VWV. Moreover, we analyzed the differences between the TPV measurements at the default setting of the software and the modified manual VWV calculations of the carotid artery.

MATERIALS AND METHODS

A total of thirty patients without stroke or cardiovascular disease referred from the outpatient clinic for carotid duplex sonography were retrospectively reviewed. Ethical approval for this study was provided by the Institutional Review Board of Taipei Tzu Chi Hospital, New Taipei City, on August 7, 2020 (as no. 09-X-063). Informed written consent was waived because the study was a retrospective data analysis. The Philips Affiniti 70 ultrasound system (Philips Healthcare, Bothell, WA, USA) with a linear transducer of 3-12 MHz (L12-3) and a broadband linear volume array transducer of 5-13 MHz (VL13-5) were employed for 2D and 3D carotid scanning, respectively. Carotid duplex sonography was performed by well-trained technicians who had clinical experience of 13 years or more in conducting ultrasound examination. The scan protocol included standard imaging (B-mode, color-coded B-mode, and Doppler study) of the common carotid artery (CCA), internal carotid artery (ICA), external carotid artery, and vertebral artery on both sides. The IMT refers to the distance between the outer vessel wall and the lumen-intima boundary (the inner vessel wall), and it was measured at a mean distance of 1-1.5 cm from the far wall of the distal CCA bilaterally by using the built-in auto-trace function of the 2D scanning equipment [Figure 1]. After completion of the standard protocol, gray-scale 3D images of the carotid artery from the distal CCA to the ICA on both sides were acquired using a single-sweep VL15-3 transducer. The acquisition of 3D carotid images on both sides took only 1-2 min. The scanned digital ultrasound images were stored in hard discs for subsequent offline analysis.

The 3D images were analyzed offline on a computer by using the vascular plaque quantification (VPQ) function of the Philips QLAB software, through which the TPV could be calculated in a semi-automated manner. To establish a standardized method of measuring the carotid VWV, we took

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Figure 1: Online automated measurement of the carotid intima-media thickness

measurements from a 1-cm section of the distal CCA just 1 cm proximal to the carotid bifurcation [Figure 2a]. A total of 42-44 cross-sectional frames of the artery were extracted from this 1-cm section. We selected three frames, namely the starting frame (1 cm proximal to the CCA bifurcation), the ending frame (1 cm proximal to the starting frame), and the key frame (between the starting and ending frames), for contour tracing of the vessel. One or more key frames, which usually represented the most protuberant vessel wall inside the lumen, could be selected to ensure better counter tracing results. The outer vessel wall (media-adventitia boundary) of the artery was manually traced using an elliptical sketching tool and the residual lumen boundary was automatically traced using the software [Figure 2b]. The sensitivity toward boundary recognition could be adjusted to obtain optimal tracing. A preset IMT (the distance between outer and inner walls) of 0.4 mm was input to the system. The intima-media area was defined as the area between the outer and inner vessel walls. Plaque area was defined as the area between the lumen boundary and inner vessel wall, and the wall area was defined as the sum of the intima-media area and the plaque area [Figure 3]. The TPV, which was automatically generated by the software, was the summation of plaque areas of all the 42-44 frames, and the VWV was obtained through summation of the wall areas in all the measured frames. Thus, VWV comprises both IMV and TPV. Measured data from all frames were exported as an Excel file. The VPQ software was originally designed to calculate the volume of large carotid plaques and was capable of only automatically generating the TPV, but not the IMV and VWV. Most plaques were located at the carotid bifurcation and proximal ICA. However, the 3D pixel resolution of the ICA images is usually low and the shape of the bifurcation outer wall is irregular, which limits plaque measurement at the ICA and CCA bifurcation. However, our goal was to measure the VWV instead of the TPV to better represent various vascular conditions, such as the IMV in healthy subjects and the IMV plus TPV in patients with plaques. From the exported Excel file, we manually calculated the VWV of the measured 1-cm section at the junction of the distal CCA and CCA bifurcation by computing the wall areas of all frames as well as the mean IMT (QLAB intima-media thickness [QIMT]) for the measured section.



Figure 2: (a) Selection of the starting frame (1 cm proximal to the bifurcation; red arrow) and the ending frame (1 cm proximal to the starting frame; blue arrow) from the extracted cross sections. (b) Measurement of the cross section of the selected frame. The outer wall was traced manually using the elliptical tracing tool (red coil line; arrow). The inner wall (edge of the 0.4-mm intima-media thickness; yellow coil line; arrow) and the lumen boundary (green coil line; arrows) were traced automatically by the software. BIF, bifurcation.

The interobserver variability was assessed based on two independent observers who were blinded to the observations of the other. The TPV was determined in a semi-automated manner by using the QLAB software, whereas the IMT (QIMT) and VWV were manually calculated from the exported Excel file by a stroke neurologist (observer 1), who is specialized in the field of neurosonology, and a well-trained ultrasound technician (observer 2). The entire process of measurements and calculations required approximately 10 min, which included 5 min for the QLAB TPV measurement and 5 min for QIMT and VWV calculations from the exported Excel file.

Because the distribution of the measured variables was skewed, means and medians in addition to the 25th and 75th percentiles were used to explain the range. The Mann–Whitney *U*-test was used to evaluate differences in continuous variables. P < 0.05 was considered statistically significant. The



Figure 3: Definitions of intima-media area (arrows), plaque area (arrowheads), and vessel wall area (arrows and arrowheads).

Bland–Altman test and mean percentage change were used to determine interobserver variability. The intraclass correlation coefficient (ICC) was analyzed to determine the measurement reproducibility between the two observers. The ICC values ranged from 0 to 1, with values closer to 1 indicating greater homogeneity. The correlation between the measured variables and age was analyzed using linear regression. All statistical analyses were performed using SPSS (version 24, SPSS, Inc., Chicago, IL, USA) and the MedCalc software package (version 18, Mariakerke, Belgium).

Results

A total of thirty patients were enrolled in this study, which included 17 men and 13 women. The average age was 57.9 ± 10.6 years, and it was higher in women than in men (62.1 ± 8.7 years vs. 54.8 ± 11.5 years, P = 0.031). The mean 2D carotid IMT of the sixty carotid arteries in thirty patients, which was automatically traced by the ultrasound equipment, was 0.65 ± 0.12 mm (median, 0.65 mm). The mean QIMT, TPV, and VWV measured by observer 1 were 0.68 ± 0.18 mm (median, 0.70 mm), 26 ± 12 mm³ (median, 25 mm³), and 94 ± 10 mm³ (median, 94 mm³), respectively [Table 1]. No differences in the measured variables were observed between men and women or between the right and left sides of the carotid arteries.

The assessment of interobserver variability of the measured variables in all the sixty arteries revealed no differences in the variable measurements between observers 1 and 2 [Table 2]. The Bland–Altman plot indicated that the mean differences in the QIMT measured by observer 2 were within a standard deviation of \pm 2 of the QIMT measured by observer 1. The bias (mean difference) between the two observers was -0.02 mm, which is only 1.4% relative to the mean QIMT (0.69 mm) for the thirty patients [Figure 4a]. The ICC indicated strong correlations of the measured QIMT between observers 1 and 2 (ICC = 0.76; P < 0.001). The Bland–Altman analyses also revealed that most interobserver values of the TPV and VWV fell within a 95% limit of agreement, with a mean difference of 1.9% (0.5 mm³/26.1 mm³) in TPV and 1.2% (1.1 mm³/94.2 mm³) in VWV [Figure 4b and c].

Table 1: Comparisons of results measured by observer 1 in sixty arteries in thirty patients, by sex and lateralization												
Characteristics	Total patients (n=30)		Gender				Lateralization of carotid arteries					
			Men (<i>n</i> =17)		Women (n=13)		P *	Right (<i>n</i> =30)		Left (n=30)		P *
	Median	Mean	Median	Mean	Median	Mean		Median	Mean	Median	Mean	
Age (years)	56.7	57.9±10.6	54.1	54.8±11.5	60.5	62.1±8.7	0.031	-	-	-	-	
	(50.9-64.9)		(47.6-57.8)		(55.7-70.7)							
IMT (mm)	0.65	0.65 ± 0.12	0.64	$0.65 {\pm} 0.13$	0.65	0.65 ± 0.09	0.679	0.64	$0.64{\pm}0.11$	0.65	0.66±0.13	0.496
	(0.55-0.70)		(0.55-0.72)		(0.61-0.69)			(0.55-0.69)		(0.56-0.72)		
QIMT (mm)	0.70	0.68 ± 0.18	0.64	0.68 ± 0.19	0.72	$0.70{\pm}0.19$	0.228	0.71	$0.69{\pm}0.18$	0.66	0.68 ± 0.18	0.579
	(0.57-0.80)		(0.57-0.77)		(0.5-0.82)			(0.59-0.81)		(0.52-0.79)		
TPV (mm ³)	25	26±12	23	24±12	27	28±13	0.081	24	26±12	24	26±12	0.988
	(15-39)		(13-35)		(18-43)			(14-38)		(13-36)		
VWV (mm ³)	94	94±10	92	95±12	96	94±9	0.715	97	94±9	92	93±11	0.281
· · /	(87-101)		(86-103)		(89-101)			(89-102)		(84-101)		

Data are presented as median (25th-75th percentile) or mean±standard deviation. *Mann-Whitney U-test. IMT: Intima-media thickness, QIMT: QLAB intimamedia thickness, TPV: Total plaque volume, VWV: Vessel wall volume The QLAB analyses revealed a high interobserver reliability, with an ICC of 0.85 for the TPV and of 0.90 for the VWV (P < 0.001).

Table 3 presents the correlation of the 2D carotid IMT with the measured QIMT, TPV, and VWV. The 2D carotid IMT had a positive linear correlation with QIMT (P = 0.001), TPV (P = 0.018), and VWV (P < 0.001). Moreover, QIMT, TPV, and VWV were positively linearly correlated (P < 0.001).

Table 2: Interobserver com	parisons	of the	measured	results	of
sixty arteries in thirty patie	nts				

Variables	Observe	r 1	Observe	P *	
	Median	Mean	Median	Mean	
IMT (mm)	0.65 (0.55-0.70)	0.65±0.12	-	-	-
QIMT (mm)	0.70 (0.57-0.80)	$0.68{\pm}0.18$	0.69 (0.53-0.82)	$0.70{\pm}0.20$	0.838
TPV (mm ³)	25 (19-35)	26±12	24 (13-38)	26±14	0.757
VWV (mm ³)	94 (87-101)	94±10	95 (88-102)	95±11	0.578

Data are presented as median (25th-75th percentile) or mean±standard deviation. *Mann-Whitney U-test. IMT: Intima-media thickness, QIMT: QLAB intima-media thickness, TPV: Total plaque volume, VWV: Vessel wall volume

The 2D carotid IMT had a positive linear correlation with age (P < 0.001) [Figure 5a]. The QIMT also exhibited a positive linear correlation with age (P = 0.022) [Figure 5b]. However, no significant linear correlation was observed between the measured TPV and age. Furthermore, the VWV showed a trend of positive linear correlation with age, but did not reach statistical significance (P = 0.074) [Figure 5c and d].

DISCUSSION

Carotid IMT is the combined thickness of the intimal (20%) and medial (80%) layers of the carotid artery [7]. Numerous large-scale studies have demonstrated the use of carotid IMT to predict cardiovascular risk [8]. A convenient and reliable procedure for measuring the mean carotid IMT is the online carotid ultrasound method, with the most reliable measurement obtained at the far wall of the distal CCA. However, the IMT at the CCA bifurcation and proximal ICA enables more effective risk prediction than that at the distal CCA because of the plaque and a protuberant vessel wall that is usually seen at the CCA bifurcation and proximal ICA [9]. Nevertheless, the completeness of IMT measurement at the carotid bifurcation and ICA is limited by low pixel resolution of the gray-scale images obtained due to vessel tortuosity and

Table 3: Correlation of the intima-media thickness with intima-media thickness variables measured by observer 1									
Variables	IMT				QIMT		TPV		
	coefficient	r ²	<i>P</i> *	coefficient	r ²	P*	coefficient	r ²	P *
QIMT	0.600	0.179	0.001	-	-	-	-	-	-
TPV	31.055	0.094	0.018	56.346	0.605	< 0.001	-	-	-
VWV	42.467	0.244	< 0.001	29.042	0.229	< 0.001	0.436	0.265	< 0.001

*Linear regression test. IMT: Intima-media thickness, QIMT: QLAB intima-media thickness, TPV: Total plaque volume, VWV: Vessel wall volume



Figure 4: Correlation plot (upper half) and Bland–Altman plots (lower half) for interobserver comparison of QIMT (a), TPV (b), and VWV (c) between observers 1 and 2. The blue continuous line labeled as mean indicates the bias, the red dashed lines labeled as 1.96 SD indicate the level of agreement, and the green bars indicate the 95% confidence interval. QIMT: QLAB intima–media thickness, TPV: Total plaque volume, VWV: Vessel wall volume, ICC: Intraclass correlation coefficient

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Figure 5: Linear regression plots for age with IMT (a), QIMT (b), TPV (c), and VWV (d). IMT and QIMT had a positive linear correlation with age. IMT: Intima-media thickness, QIMT: QLAB intima-media thickness, TPV: Total plaque volume, VWV: Vessel wall volume

inconstant insonation angle [10]. Furthermore, the change in IMT on an average is approximately 0.01–0.04 mm annually, which is too small to enable assessment of the treatment effect [11]. IMT is influenced by genetic determinants and is more affected by blood pressure than by atherosclerosis, whereas plaque is more associated with traditional vascular risk factors such as age, sex, hypertension, diabetes mellitus, and hyperlipidemia [12]. Carotid plaque is composed of a thickened intima, smooth muscle cells, macrophages, a lipid core, and various stages of the fibrous cap and is defined as a focal thickening that encroaches into the lumen by 0.5 mm or by 50% of the surrounding IMT or where IMT is >1.5 mm based on ultrasound studies [13]. Studies on carotid plaque number, plaque area, and plaque burden have shown that assessment of plaque is more effective than that of IMT in predicting future cardiac and stroke events [14,15]. Spence and Hackam [16] showed that the average annual change in total plaque area is 10 mm², which facilitates monitoring of the progression or regression of plaque within months and thereby shortens the follow-up period. Population-based studies have shown that increase in the thickness of the carotid IMT was directly associated with an increased risk of myocardial infarction and stroke in older adults without a history of cardiovascular disease [17]. Although a recent meta-analysis failed to demonstrate any added value of IMT compared to the Framingham Risk Score in predicting future cardiovascular disease, many studies emphasize the greater value of measures that include plaque area and thickness, rather than IMT alone, in predicting cardiovascular disease and stroke [18].

The 3D ultrasound technique provides a more precise quantification of plaque. As described in this study, a

1-cm section of the artery can be cut into 42 or more very thin cross-sectional frames at distances of <0.3 mm apart. Semi-automated contour tracing of the intima-lumen boundary in each cross section allows the measurement of a specific lesion in all planes. Furthermore, summation of the measured areas in all frames enables better quantification of the IMV, TPV, and VWV. Moreover, 3D scanning may avoid missing lesions, which is a problem encountered in 2D scanning, and the values measured using 3D scanning are considerably larger than those measured using 2D scanning, which further shortens the follow-up period and reduces the sample size required in observational studies. By using 3D carotid ultrasound, van Engelen et al. [19] evaluated carotid atherosclerosis in patients during a 1-year period and found that changes in both plaque texture and TPV were strongly predictive of vascular events. They recommended 3D ultrasound plaque measurements for vascular event risk prediction in high-risk patients.

The 3D measurement of carotid plaque was first performed using a 2D transducer held by hand or mounted on a motorized rail placed on the neck sweeping over the cross section along the artery [20,21]. With the development of the single-sweep 3D transducer with appropriate software, favorable reproducibility was obtained in the measurement of plaque and artery volume, which makes this technique suitable for serial assessment of carotid plaques [5]. In the present study, acquisition of 3D images of the vessel wall from the distal CCA to the proximal ICA with a single-sweep 3D transducer on both sides took only 2 min. Thereafter, we manually traced the outer wall of the artery using the QLAB VPQ software in three frames, and the intima-media boundary was automatically traced using the same software. By using a scale ruler, the starting frame (1 cm proximal to the CCA bifurcation), ending frame (1 cm proximal to the starting frame), and key frame (between the starting and ending frames) can be easily determined for measurement. This procedure is simple and can be easily performed by experienced physicians and technicians. Moreover, after receiving a short-term training program, even paramedical staff, such as research assistants, who do not have the professional experience of using ultrasound, can perform the measurement easily.

Our target measure in this study was the VWV. However, the default function of the VPQ software generates the TPV, and the IMV was not included in the TPV. We obtained the QIMT and VWV values through calculations from the exported Excel file. Previous studies on IMT prediction or plaque burden evaluation have mostly focused on the measurement of the distance between the outer wall and the inner wall with the highest thickness or plaque. Identifying the boundary between the IMT and the plaque in a protuberant focal thickening vessel wall is difficult. Ideally, the VWV should be measured with a preset IMT of 0 mm. Nevertheless, the resolution of the ultrasound gray-scale images is usually higher at the far wall for IMT measurement. Similarly, in 3D scanning, the uneven resolution of the far and near wall causes bias during automatic tracing of the lumen-intima boundary. However, reducing the sensitivity during automatic boundary tracing to avoid excessive gray-scale speckle noise inside the vessel lumen might lead to undertracing of the near-wall boundary. ultimately resulting in an extremely thin IMT or even absence of IMT in the images [Figure 3]. This reduction of vessel wall area caused by the underestimation of the IMT might not be very prominent in the measurement of a large plaque but may cause an obvious bias when a normal vessel wall or a vessel wall with a small plaque is measured. We observed that the lowest value of 2D IMT was 0.41 mm in our patient group. To avoid the overestimation of the IMT, we proposed a preset IMT of 0.4 mm as an ideal vessel wall distance.

The present study demonstrated good interobserver agreement and reliability. The Bland-Altman plots indicated that the interobserver differences in QIMT, TPV, and VWV in the QLAB analysis were all within the 95% limit of agreement with the bias <2% relative to the mean values. The VWV measured using QLAB had the highest interobserver reliability, with an ICC of 0.90. The QIMT also exhibited a strong correlation with carotid IMT. It is reasonable that the average value of QIMT (0.68 mm) was higher than that of 2D carotid IMT (0.65 mm). When using 2D ultrasound, carotid IMT is measured in the longitudinal section at the far wall of the distal CCA, whereas QIMT is measured in the cross section at the junction of the distal CCA and CCA bifurcation, the anatomical physiology of which may cause an increased IMT. Despite the small sample size in this study, the IMT and QIMT exhibited positive linear correlations with age. Previous studies have shown that men and older people have higher IMT than their female and younger counterparts [22]. In this study, the mean IMT did not differ between men and women because women were older than men, which may have counterweighted the effect of sex. The VWV exhibited a trend of linear correlation with age. This can be explained by the

small sample size and that more complex factors other than age and sex may affect VWV, such as body mass index. By using our proposed standardized method, which is reliable and reproducible, we obtained an average VWV of 94.2 mm³ for the 1-cm section of carotid artery at the junction between the distal CCA and CCA bifurcation. The VWV provides a much greater dynamic range during follow-up than that of the IMT, which has very small dynamic range of <1 mm. This standardized method can be further applied to establish the routine laboratory examination standards for Chinese adults and evaluate pathological conditions related to vascular diseases. The use of multiple 3D ultrasound datasets has been recommended for evaluating all visible CCA and ICA on both sides of the neck to obtain a more effective prediction of vascular risks [15].

This study has some limitations. First, it had a relatively small sample size. However, because this was a pilot study focusing on the reliability of a measuring tool, a large sample size may not be necessary. Second, the gray-scale pixel resolution of the images obtained using the single-sweep 3D transducer was not as high as that obtained using a 2D linear transducer, particularly at the near wall. Moreover, the speckle noise inside the lumen might interfere with the automated contour tracing of the intima-lumen boundary. A newly developed 3D matrix array probe that simultaneously provides real-time transverse and sagittal views can be used to not only reduce the large amount of time required for 3D scanning but also to overcome the problem of pixel resolution [23]. Third, the current QLAB VPQ software demonstrates a limitation in tracing the artery across the CCA bifurcation where the outer vessel wall is irregular in shape. Finally, compared with the online one-step rapid IMT measurement, the time-consuming offline analysis of the VWV limits its clinical applications.

CONCLUSION

This study established a standardized 3D ultrasound measurement of VWV. The 3D VWV measurement yielded reasonable interobserver differences within the 95% limit of agreement and a high reliability with an ICC of 0.90. The VWV of the 1-cm section near the CCA bifurcation was measured with a mean value of 94.2 mm³. Further studies on 3D ultrasound quantification of carotid arteries are warranted.

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Conflicts of interest

There are no conflicts of interest.

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