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Cardio-Ankle Vascular Index: Test–Retest Reliability and Agreement in People With Stroke

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Keywords: cardio-ankle vascular index | fatigue | sleep quality | stroke | vascular stiffness

ABSTRACT

The cardio-ankle vascular index (CAVI) is superior to traditional methods of measuring arterial stiffness. However, its application in clinical practice has lagged behind the science. This study aimed to (1) examine its test–retest reliability and agreement between repeated measurements, (2) identify the correlation with fatigue and sleep quality, and (3) compare the CAVI values of the bilateral sides of people with stroke, and those of stroke survivors with and without fatigue. Participants ($n=67$) were assessed using the CAVI, Fatigue Assessment Scale, and Pittsburgh Sleep Quality Index. The test–retest reliability ranged from 0.77 to 0.86. The Bland–Altman plots showed good agreement between test and retest. The standard error of measurement ranged from 0.59 to 0.66. The minimal detectable change ranged from 1.15 to 1.29. The CAVI values of the bilateral sides correlated with fatigue and those at the paretic side correlated with sleep quality. The CAVI values of the paretic side were higher than those of the non-paretic side in people with stroke. Stroke participants with fatigue had higher CAVI values than those without fatigue. CAVI has good test–retest reliability and agreement between repeated measurements for clinical use.

1 | Introduction

Arterial stiffness describes impairment in the distensibility of large arteries. When elastin fibers, in the arterial walls are replaced by collagen fibers, the artery becomes stiffer (Namba et al. 2019). Increased arterial stiffness increases the risk of cardiovascular disease and mortality (Zhong et al. 2018). People with stroke have higher levels of arterial stiffness than those without stroke (Tuttolomondo et al. 2017).

Research has shown that arterial stiffness is associated with fatigue (Gonzales et al. 2015) and sleep quality (Del Brutto et al. 2019) in older people. In people with stroke, arterial stiffness has been associated with obstructive sleep apnea ($B=0.69$, $p=0.039$) probably because sympathetic activation increases arterial stiffness (Chen, Chen, and Yu 2015). It has also been suggested that impaired vascular function may contribute to fatigue in people with chronic fatigue syndrome (Bond, Nielsen, and Hodges 2021), although the relationship between arterial stiffness and fatigue is unclear in people with stroke. With 52.7% of people with stroke experiencing fatigue and 64.3% reporting poor sleep quality (Ho, Lai, and Ng 2021a), understanding the correlations between arterial stiffness and fatigue as well as sleep quality is crucial for optimizing healthcare practices,

Summary

- The cardio-ankle vascular index (CAVI) is a reliable and valid instrument for measuring arterial stiffness in people with stroke.
- There are small differences in CAVI values between the paretic and non-paretic sides of people with stroke.
- Stroke survivors with fatigue have stiffer arteries than those without fatigue.

improving client outcomes, and designing rehabilitation programs for people with stroke.

2 | Background

To accurately measure arterial stiffness, it is necessary to use a reliable technique. Arterial stiffness can be measured using, for example, an imaging technique (such as ultrasound imaging), a pulse wave analysis technique (such as an augmentation index), or pulse wave velocity (such as carotid-femoral pulse wave velocity) (Namba et al. 2019). Of these techniques, carotid-femoral pulse wave velocity is the gold standard, but blood pressure can affect the measurements (Namba et al. 2019). Augmentation index measurements have been found to relate to height and to differ by gender in normotensive healthy people (Hughes et al. 2013).

The cardio-ankle vascular index (CAVI) is a relatively novel indicator of vascular function in clinical settings. It measures the stiffness of the arterial tree from aorta to ankle (Budoff et al. 2022). Although the use of anesthesia may affect its measurements in people aged < 65 (Kim et al. 2011), its measurements are less dependent on blood pressure (Budoff et al. 2022), making it a superior tool for clinical use. The test takes fewer than 5 min to administer, the device that is used can be transported using a trolley, and it is simple and easy to use. CAVI values are derived from the stiffness parameter β developed by Hayashi and Kawasaki and the pulse wave velocity of the Bramwell-Hill equation, using the formula $2\rho \times 1/(Ps - Pd) \times \ln(Ps/Pd) \times PWV^2$, where ρ refers to blood density, Ps and Pd are the systolic and diastolic blood pressure in mmHg, respectively, and PWV is the pulse wave velocity measured between the origin of the aorta and the tibial artery in the ankle (Shirai et al. 2006). The reproducibility of the CAVI was good, with a variability of only 3.8% in healthy adults (Shirai et al. 2006).

Monitoring arterial stiffness may yield information on cardiovascular health (Budoff et al. 2022). Although CAVI is increasingly being used in research settings (Akaida et al. 2024; Cuspidi et al. 2024), its reliability in stroke populations remains unclear. Therefore, this study aimed to (1) investigate the test–retest reliability and agreement of repeated measurements of CAVI values, (2) explore its correlation with fatigue and sleep quality, and (3) compare the CAVI values of the bilateral sides of people with stroke, and those of stroke survivors with and without fatigue.

3 | Methods

3.1 | Setting and Sampling

A cross-sectional design was adopted in this study, and the results are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline. People with stroke were recruited from non-governmental organizations by convenience sampling in Hong Kong in 2021. Community-dwelling people aged 45–80 who had received a diagnosis of stroke at least 6 months ago were recruited. Those with any other comorbid neurological diseases, transient ischemic attacks, or any unstable medical conditions that had not yet been stabilized by treatment, such as uncontrolled diabetes mellitus, were excluded. People who are currently undergoing or who have undergone any rehabilitation therapy or clinical trials within the past 3 months prior to data collection were also excluded.

A previous study demonstrated that the test–retest reliability of the intra-class correlation coefficient for CAVI was 0.80 with a 95% confidence interval (CI) of 0.73–0.87 in a sample of Caucasian adults (Endes et al. 2015). Taking into consideration a minimum acceptable intra-class correlation coefficient of 0.70, an expected intra-class correlation coefficient of 0.85, the number of replicates as 2, a two-tailed level of significance of 0.05, and a power of 0.80, 53 participants would be required (Walter, Eliasziw, and Donner 1998). Assuming that 10% would drop out from the study, at least 59 participants with stroke should be recruited.

3.2 | Outcome Measures

Arterial stiffness was measured using the device VaSera VS-2000 (Fukuda Denshi, Japan). The measurements were conducted with the participants lying in a supine position after having rested for at least 10 mins, as suggested in the operation manual. Cuffs were wrapped around bilateral arms, ankles, and big toes. A microphone was placed on the sternum at the level of the second intercostal space. The measurements of CAVI are shown in Figure 1. A CAVI value of < 8.0, ≥ 8.0 to < 9.0, and ≥ 9.0 represents normal, borderline, and abnormal readings, respectively (Tanaka et al. 2018).

The participants' level of fatigue was assessed using the 10-item Chinese version of the Fatigue Assessment Scale (Ho, Lai, and Ng 2021b) because it assesses both physical and mental fatigue as described by people with stroke (Barbour and Mead 2012). The total score for the scale ranged from 10 to 50, with higher scores indicating higher levels of fatigue. A score of ≥ 20 indicates the presence of fatigue (Ho, Lai, and Ng 2020). The internal consistency (Cronbach's $\alpha = 0.71–0.82$) and test–retest reliability (intraclass correlation coefficient = 0.77–0.95) of the scale were good, without ceiling and floor effects when tested in people with stroke (Ho, Lai, and Ng 2021b).

Subjective measurements of sleep quality were assessed using the Cantonese version of the 19-item Pittsburgh Sleep Quality Index (Chong and Cheung 2012) because it includes a wide range of relevant indicators of sleep quality. The total score

FIGURE 1 | Measurements of the cardio-ankle vascular index.

for the scale ranged from 0 to 21, with higher scores indicating worse sleep quality. Its internal consistency (Cronbach's $\alpha = 0.75$) was good, and no significant difference was found in a retest over a 1-week interval in a Chinese population (Chong and Cheung 2012).

3.3 | Procedures

The Declaration of Helsinki was followed. Ethical approval was granted by the authors' University. Written informed consent from the participants was obtained after they were given a full explanation of the objectives and procedures of the study. Written informed consent was also collected from their guardians when the participants were identified as suffering from cognitive impairment as determined by a score of <6 in the Abbreviated Mental Test (Chu et al. 1995). The CAVI was assessed in two sessions, 7 days apart. The measurements were performed twice at 90-min intervals on day 1 (T1, T2) and once on day 2 (T3). All measurements were conducted by either one of the two well-trained assessors. Other assessments using questionnaires were performed only once at T1.

3.4 | Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software program (version 26.0) was used. The characteristics of the participants were summarized using descriptive statistics.

Test–retest reliability over time was determined using the intraclass correlation coefficients (model 3,1). This was because a two-way mixed-effects model should be used to measure test–retest reliability (Koo and Li 2016) and a single measurement was taken at each time point. Agreement was determined using Bland–Altman plots with 95% limits of agreement. The standard error of measurement and minimal detectable change at the 95% CI were calculated using $S \times \sqrt{1 - r}$ (Stratford 2004), and $S \times 1.96 \times \sqrt{2(1 - r)}$ (Haley and Fragala-Pinkham 2006), respectively, with S denoting the standard deviation of the mean value of CAVI at T1 and T2 or T1 and T3, and r denoting the test–retest reliability coefficient.

The correlation between CAVI values and other measures was examined using scatterplots and Pearson correlation coefficients. The Pearson correlation was used because a combination of the Kolmogorov–Smirnov test of normality, *Z*-tests using skewness and kurtosis, and a visual inspection of histograms and normal Q-Q plots showed that the data were normally distributed.

Comparisons were conducted. The CAVI values of the paretic and non-paretic sides were compared using a paired sample *T*-test because of the need to conduct within-group comparisons. The CAVI values between stroke participants with and without fatigue were compared using the Mann–Whitney Test. A *p*-value of <0.05 was considered significant.

4 | Results

4.1 | Demographic Characteristics

A total of 67 participants with stroke joined this study, and 60 of them completed the retest. The remaining seven participants did not join the retest due to family issues. Their demographic characteristics are shown in Table 1. There were no missing data.

4.2 | Test–Retest Reliability and Agreement Between Repeated Measurements, Standard Error of Measurement, and Minimal Detectable Change

The intra-class correlation coefficients ranged from 0.77 to 0.86 (Table 2). Bland–Altman plots indicated good agreement between T1 and T2 and between T1 and T3, with only three and two plots falling outside the limits of agreement, respectively (Figure 2). Proportional bias was detected for the paretic side at T1–T3 ($B = -0.33$, $p = 0.011$). The bias was resolved when two outliers with a mean value of CAVI ≤ 6 and ≥ 11 were removed from the regression analysis ($B = -0.24$, $p = 0.071$). The standard error of measurement ranged from 0.59 to 0.66 and the minimal detectable change ranged from 1.15 to 1.29 (Table 2).

TABLE 1 | Demographic characteristics of the participants.

	People with stroke ($n = 67$)
	Mean ± standard deviation
Age	63.55 ± 6.35
Time since stroke (years)	6.50 ± 4.59
Body mass index (kg/m^2)	24.22 ± 3.21
Gait speed (meters/s)	0.39 ± 0.48
Pulse rate (beats per min)	67.99 ± 10.47
Fatigue Assessment Scale	22.21 ± 6.42
Pittsburgh Sleep Quality Index	7.64 ± 3.55
	***N* (%)**
Gender	
Female	32 (47.8)
Male	35 (52.2)
Type of stroke	
Ischemic	45 (67.2)
Hemorrhagic	22 (32.8)
Paretic side	
Left	29 (43.3)
Right	38 (56.7)
Dominant side became paretic	
Yes	37 (55.2)
No	30 (44.8)
Presence of hypertension	
Yes	51 (76.1)
No	16 (23.9)
Presence of diabetes	
Yes	17 (25.4)
No	50 (74.6)
Presence of hyperlipidemia	
Yes	47 (70.1)
No	20 (29.9)

	Paretic side	Non-paretic side	p[a]
Handgrip strength (kg)	14.25 ± 8.58	26.21 ± 9.11	< 0.001
Systolic blood pressure (mmHg)	134.06 ± 14.22	131.96 ± 11.69	0.076
Diastolic blood pressure (mmHg)	82.37 ± 8.34	81.40 ± 7.21	0.191

[a]Paired sample *T*-test.

4.3 | Correlations

The CAVI values of the 67 participants at both the paretic ($r = 0.34$, $p = 0.005$) and non-paretic sides ($r = 0.31$, $p = 0.010$) correlated with the fatigue score. Only the CAVI value at the paretic side ($r = 0.25$, $p = 0.041$) correlated with the sleep quality score. Scatterplots depicting the correlations are shown in Figure 3.

4.4 | Comparison

Paired *T*-tests showed that the CAVI value of the paretic side was significantly higher than that of the non-paretic side at all three time points of measurement (Table 3). A Mann–Whitney Test showed that the CAVI values of participants with fatigue ($n = 48$, median = 8.65, interquartile range = 1.45) were significantly higher than those of participants without fatigue ($n = 19$, median = 7.70, interquartile range = 1.50, $p = 0.036$).

TABLE 2 | Test–retest reliability, standard error of measurement, and minimal detectable change in the cardio-ankle vascular index.

	Paretic side	Non-paretic side
Intra-class correlation coefficient (95% confidence interval)		
T1–T2 ($n = 67$)	0.84 (0.76–0.90)	0.77 (0.66–0.85)
T1–T3 ($n = 60$)	0.86 (0.77–0.91)	0.79 (0.67–0.87)
Standard error of measurement		
T1–T2 ($n = 67$)	0.59	0.66
T1–T3 ($n = 60$)	0.61	0.63
Minimal detectable change		
T1–T2 ($n = 67$)	1.15	1.29
T1–T3 ($n = 60$)	1.19	1.25

5 | Discussion

This study demonstrated that the CAVI was reliable for use in people with stroke. The CAVI value of both sides correlated with fatigue, and the value of the paretic side correlated with sleep quality in this sample. Arteries in the paretic side were stiffer than those in the non-paretic side. Participants with fatigue had stiffer arteries than participants without fatigue.

5.1 | Reliability and Agreement

This study is the first to establish the reliability and agreement of the CAVI values in people with stroke. Our examination of the test–retest reliability of the CAVI yielded results similar to those of previous studies (Endes et al. 2015; Yang et al. 2018) (Table 4). The finding from the Bland–Altman plot analysis was also consistent with that found in previous studies (Endes et al. 2015; Kubozono et al. 2007). The mean difference between the test (T1) and retest (T2 or T3) and the 95% limits of agreement between the test and retest in this study were slightly better than those in a previous study of adults (Kubozono et al. 2007) (Table 4), reflecting the CAVI's good agreement for clinical use on people with stroke. That the two well-trained assessors strictly followed the procedures in accordance with the operation manual probably explains the good reliability and agreement of the CAVI.

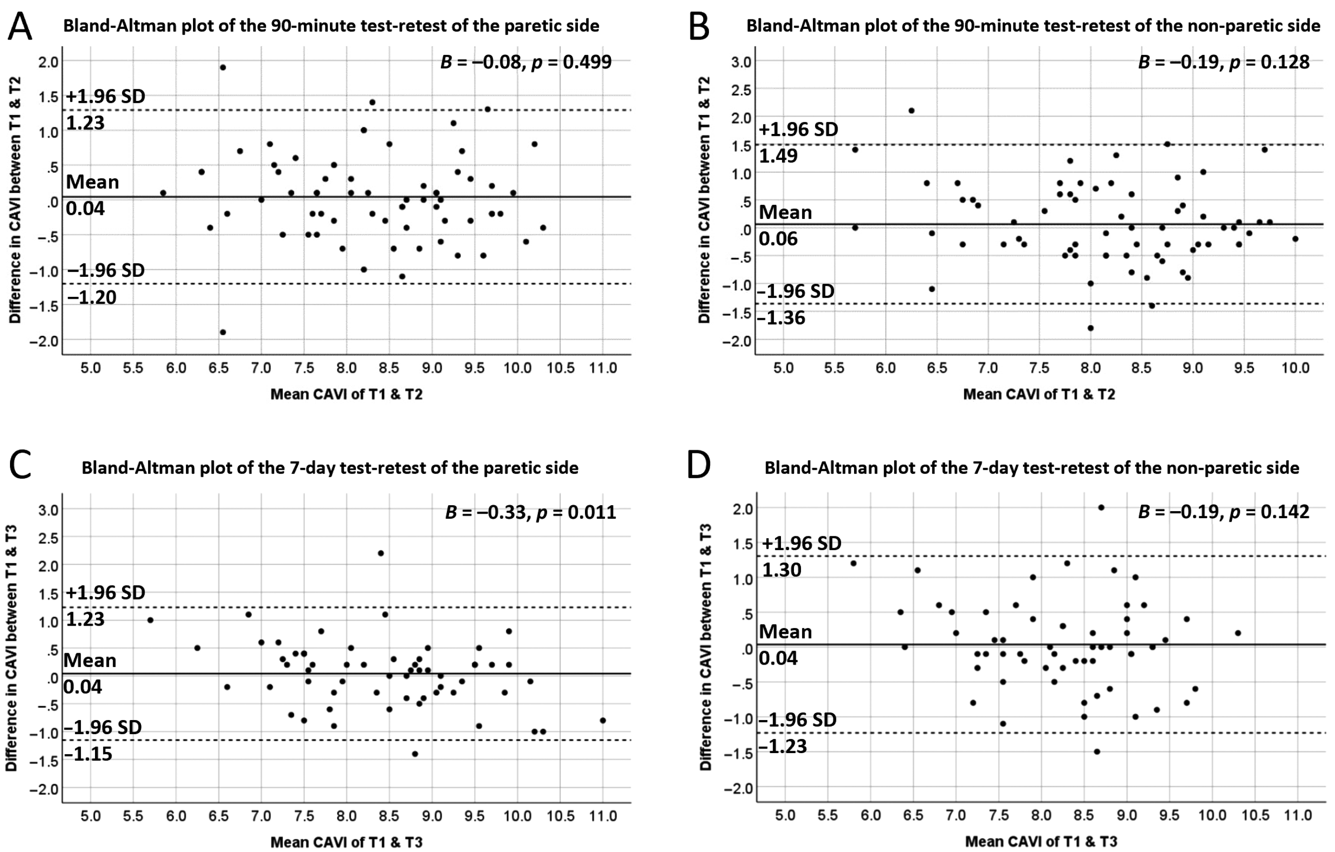

FIGURE 2 | Bland–Altman plots of the cardio-ankle vascular index. (A) T1–T2 test–retest of the paretic side. (B) T1–T2 test–retest of the non-paretic side. (C) T1–T3 test–retest of the paretic side. (D) T1–T3 test–retest of the non-paretic side. The solid line represents the mean difference, and the dotted lines represent two standard deviations above and below the mean difference, i.e., the 95% upper and lower limits of agreement.

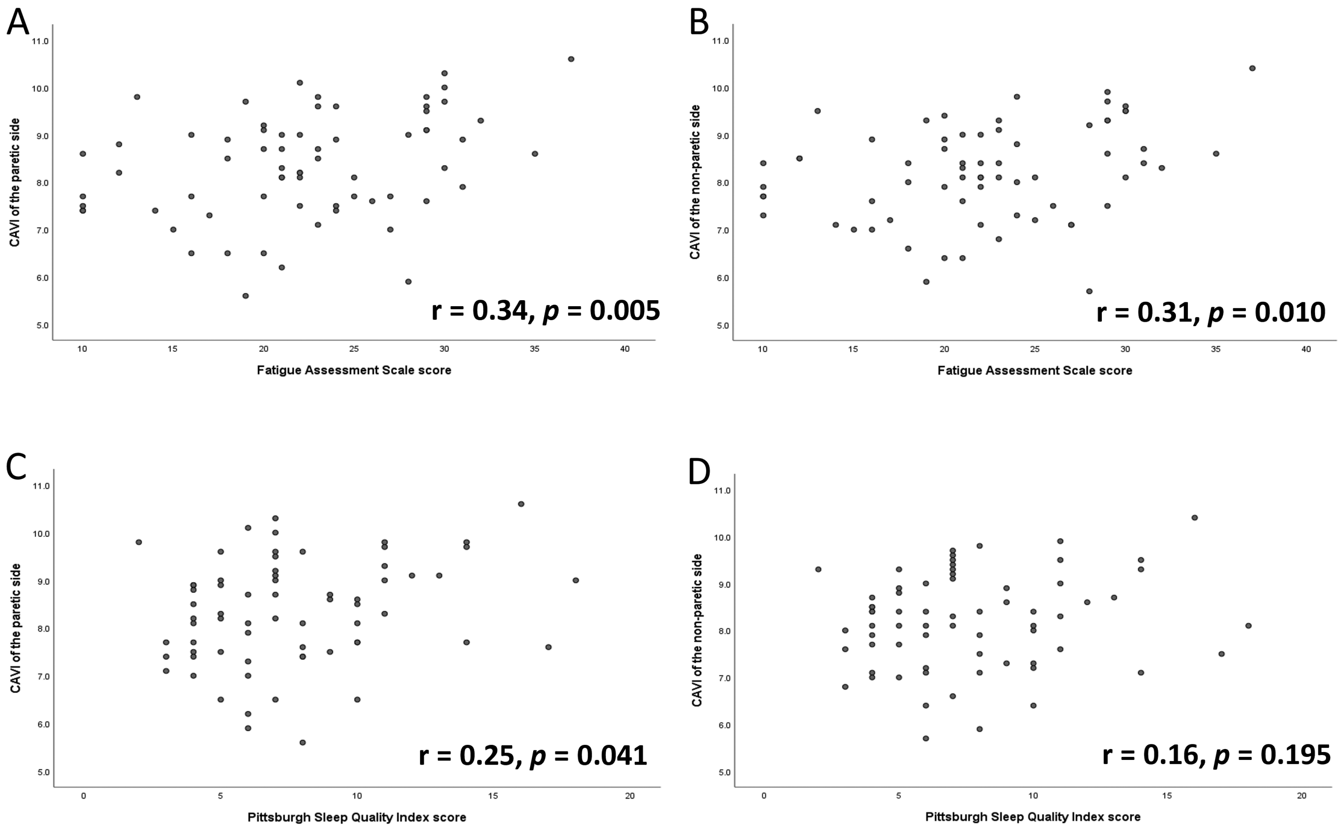

FIGURE 3 | Scatterplots for correlations between (A) the CAVI of the paretic side and the Fatigue Assessment Scale score; (B) the CAVI of the non-paretic side and the Fatigue Assessment Scale score; (C) the CAVI of the paretic side and the Pittsburgh Sleep Quality Index score; and (D) the CAVI of the non-paretic side and the Pittsburgh Sleep Quality Index score.

TABLE 3 | Differences in the cardio-ankle vascular index values (mean ± standard deviation) between the paretic and non-paretic sides with the level of significance.

	People with stroke		
Time points of measurement	**Paretic side**	**Non-paretic side**	***p***
T1 ($n = 67$)	8.34 ± 1.11	8.20 ± 1.02	0.002*
T2 ($n = 67$)	8.29 ± 1.16	8.13 ± 1.14	0.003*
T3 ($n = 60$)	8.40 ± 1.22	8.22 ± 1.06	0.003*

*$p < 0.01$ by paired sample *T*-test.

5.2 | Standard Error of Measurement and Minimal Detectable Change

Measuring change is important to determine the clinical utility of CAVI. The standard error of measurement percentage was acceptable (T1–T2: paretic 7.0%, non-paretic 8.0%; T1–T3: paretic 7.2%, non-paretic 7.7%). Yet, no established standard error of measurement percentage for the value of CAVI is available for comparison. In this study, the minimal detectable change, which is important for clinical interpretations, showed that a difference of 1.15–1.29 would be sufficient to indicate that the change is real and not a measurement error. This finding was comparable to that for people with class III obesity over a 5-min interval, but smaller than that over an interval of 1–10 months (Yang et al. 2018) (Table 4). In this study, the minimal detectable change in CAVI (T1–T2: paretic 13.8%, non-paretic 15.8%; T1–T3: paretic 14.1%, non-paretic 15.1%) was less than 30%, indicating acceptable random measurement errors (Huang et al. 2011).

5.3 | CAVI Values of People With Stroke

The CAVI value of people with stroke in this study was lower than that found in a Japanese study (Saji et al. 2015), in which the median CAVI value of Japanese people with ischemic stroke was 9.9–10.7. CAVI values increase as people age (Asmar 2017) because calcification of the elastic lamellae during aging may stiffen the arterial wall (Schellinger, Mattern, and Raaz 2019). Our participants with stroke were 63.55 years old. They were younger than those in Saji et al.'s study (Saji et al. 2015), where the mean age of their participants with stroke was 69–71 years old. This participant characteristic probably led to the lower CAVI values in our study. Apart from age, other factors would also affect arterial stiffness. A systematic review and meta-analysis showed that habitual physical activity could have beneficial effects on arterial stiffness (Park et al. 2017). Lifestyle factors, such as engaging in aerobic exercise, smoking, diet, and taking medications for chronic diseases such as hypertension also influence arterial stiffness (Wu, Liu, et al. 2015). Yet, such information was not assessed in this study.

TABLE 4 | Test–retest reliability, agreement between repeated measurements, and minimal detectable change in the cardio-ankle vascular index values in this study and previous studies.

	This study	Endes et al. (2015)	Yang et al. (2018)	Kubozono et al. (2007)
Population	People with stroke (mean age 63.55)	Adults (age 18–60)	People with class III obesity (mean age 44)	Adults (age 18–82)
Test–retest interval	90 mins & 7 days	Within 3 months	5 mins & several months	2 weeks
Intra-class correlation coefficient	0.77 to 0.86	0.80	0.72–0.85	
Mean difference between test and retest	0.04 to 0.06			0.2
Limits of agreement	−1.36 to 1.49			−1.2 to 1.7
Minimal detectable change	1.15 to 1.29		1.21–1.25 (5 mins); 1.64–1.71 (1–10 months)	

5.4 | Differences in CAVI Between the Paretic and Non-Paretic Sides of People With Stroke

The differences in CAVI between the bilateral sides of people with stroke may be explained by muscle strength and muscle mass. Consistent with a previous study (Schimmel et al. 2013), handgrip strength in the paretic side of people with stroke was weaker than that in the non-paretic side in this study. It has been shown that arterial stiffness as measured by pulse wave velocity significantly correlated with handgrip strength in older people (Zhang et al. 2021). This is probably because both arterial stiffness and muscular fitness are related to same pathological process, for example, endothelial dysfunction and oxidative stress (Zhang et al. 2021). Thus, weak muscle strength in the paretic side may lead to a higher CAVI value than in the non-paretic side. Arterial stiffness has also been found to relate to muscle mass (Im et al. 2017; Zhang et al. 2021). In stroke populations, the size of the muscles in the paretic limb was about 13%, 5%, and 8% less in the thigh, lower leg, and lean leg mass, respectively, as compared to the non-paretic limb (Hunnicutt and Gregory 2017), leading to differences in CAVI values between the bilateral sides. However, muscle mass was not measured in this study.

5.5 | Arterial Stiffness and Fatigue

Although the correlation between arterial stiffness and fatigue was small and significant, it should not be neglected because it has been pointed out in the literature that fatigue has multiple causes in people with stroke (Nadarajah and Goh 2015). Peripheral arterial stiffness may restrict the flow of blood, resulting in a lower capacity for energy consumption during activities, thus leading to fatigability (Gonzales et al. 2015). It is also possible that psychosocial and behavioral factors such as self-efficacy and reduced physical activity play a more important role in maintaining fatigue at a later stage after stroke (Wu, Mead, et al. 2015). These might explain why people with stroke who experienced fatigue had borderline arterial stiffness (CAVI value of ≥ 8.0 to < 9.0), while those without fatigue had normal arterial stiffness (CAVI value of < 8.0).

5.6 | Arterial Stiffness and Sleep Quality

This study showed a significant positive correlation between the CAVI value of the paretic side and the Pittsburgh Sleep Quality Index score. This finding was consistent with a meta-analysis that found an association between poor sleep quality and increased arterial stiffness in the general population, with an effect size of 0.13 (Saz-Lara et al. 2022). Another study showed that poor sleep quality was the only parameter associated with increased arterial stiffness after adjusting for multiple covariates, lifestyle factors, and potential risk factors for arterial stiffness in people with diabetes mellitus (Osonoi et al. 2015). It is probable that poor sleep quality induces high levels of catecholamines, which may promote cell proliferation and fibrosis in smooth muscles, thus contributing to changes in the structure of a person's arterial walls (Osonoi et al. 2015). Therefore, arterial stiffness is increased. The correlation between arterial stiffness and sleep quality in our study with people with stroke was weak because the relationship between arterial stiffness and sleep quality can be influenced by age, body mass index, dyslipidemia, or diastolic blood pressure (Kadoya et al. 2018). The correlation between the CAVI value of the non-paretic side and sleep quality was insignificant. The reasons for the difference between the bilateral sides warrant further investigation.

5.7 | Limitations

This study had several limitations. First, we included only members of social centers run by non-governmental organizations, who might be more active in engaging in various activities, including physical activities. The findings cannot be generalized to other populations with stroke such as home care patients. Second, despite an adequate sample size, the number of participants was still small, which might have affected the investigation into the correlates of arterial stiffness and the factors mediating the relationship between arterial stiffness and fatigue as well as sleep quality, because such possible correlates are plentiful. Third, confounding factors of arterial stiffness such as level of activity, muscle mass, and use of relevant medications such as anti-hypertensives were not assessed in this study. Fourth, criterion validity, which examines the extent to which CAVI values relate to those found in carotid-femoral pulse wave velocity, a gold standard, was not investigated. Last, clinically important differences perceived as meaningful by clients were yet to be determined.

Future research should involve a larger sample size. The control variables that are included need to be more comprehensive, and confounding factors should be better controlled when analyzing the data. The modifying factors of arterial stiffness, such as lifestyle factors and the use of medications that influence CAVI, could also be examined in the future. The factors or reasons leading to differences in CAVI values between the bilateral sides of people with stroke and the mechanisms through which arterial stiffness contributes to fatigue are worthy of further investigation. Establishing criterion validity and the minimal clinically important difference of CAVI in people with stroke in future studies would further enhance the clinical utility of CAVI. Studies with a longitudinal design would offer a better picture of the changes in CAVI over time to help healthcare professionals to monitor the progress made by clients.

6 | Conclusion

The CAVI has good test–retest reliability and agreement between repeated measurements. The results of this study indicated that arterial stiffness in the paretic side correlated with fatigue and sleep quality. The CAVI values of the paretic side were higher than those in the non-paretic side in people with stroke. The findings add new knowledge on the clinical implications of using CAVI to assess an individual's health after a stroke.

7 | Relevance to Clinical Practice

CAVI is a promising measurement tool for use in monitoring improvements in the health of populations with stroke. With its good test–retest reliability and agreement between repeated measurements, CAVI is useful for assessing arterial stiffness in people with stroke in clinical settings. Healthcare professionals can use CAVI to monitor vascular health and evaluate the effectiveness of interventions for reducing arterial stiffness, particularly during the stroke rehabilitation process.

Author Contributions

Lily Y. W. Ho: conceptualization, data curation, formal analysis, visualization, writing – original draft, project administration, investigation, methodology, software, validation, resources. **Claudia K. Y. Lai:** writing – review and editing, supervision, formal analysis, validation. **Shamay S. M. Ng:** conceptualization, writing – review and editing, supervision, funding acquisition, resources, project administration, formal analysis, validation, methodology, investigation.

Ethics Statement

This study has been approved by Institutional Review Board of The Hong Kong Polytechnic University.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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