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Abstract:

Carotid intima-media thickness (C-IMT) measurements provide a non-invasive assessment of sub-clinical atherosclerosis. The aim of the study was to assess the inter- and intra-observer variability of automated C-IMT measurements undertaken by two novice operators using the Panasonic CardioHealth Station.

Participants were free from cardio-metabolic disease and each underwent serial bilateral C-IMT ultrasound measurements. Immediate inter operator measurement variability was calculated by comparing initial measurements taken by two operators. Immediate retest variability was calculated from two consecutive measurements and longer-term variability was assessed by conducting a further scan one week later.

50 apparently healthy participants (n=20 females), aged 26.2 ± 5.0 years were recruited. Operator 1 recorded a median (inter-quartile range) right and left-sided C-IMT of 0.471mm (0.072mm) and 0.462mm (0.047mm). Female's right and left C-IMT was 0.442mm (0.049mm) and 0.451mm (0.063mm) respectively. The limits of agreement (LoA) for immediate inter-operator variability were -0.063 to 0.056mm (mean bias -0.003mm). Operator 1's immediate retest intra-operator LoA were -0.057 to 0.046mm (mean bias was -0.005mm). One week LoA were -0.057 to 0.050mm (mean bias -0.003mm).Operator 2 recorded median right and left sided C-IMT of 0.467mm (0.089mm) and 0.458mm (0.046mm) for males respectively whilst female measurements were 0.441mm (0.052mm) and 0.444mm (0.054mm) respectively. Operator 2's intra-operator immediate retest LoA were -0.056 to 0.056 (mean bias <-0.001mm). Intra-operator LoA at one week were -0.052 to 0.068mm (mean bias 0.008mm).

Novice operators produce acceptable short-term and one week inter- and intra-operator C-IMT measurement variability in healthy, young to middle aged adults using the Panasonic CardioHealth Station.

Keywords: reliability; reproducibility; C-IMT; ultrasound

Introduction

The process of atherogenesis often occurs in areas of high oscillatory shear stress within human vasculature (Kolodgie*, et al.* 2007; Lee*, et al.* 2001). Early stages of atherosclerosis often present as thickened arterial walls, a phenotype that can be observed using B-mode ultrasound imaging. Pignoli and co-workers (Pignoli*, et al.* 1986) established that the distance between two parallel echogenic lines observed when imaging human arteries, is a valid measurement of combined intima and media thickness of the carotid artery (C-IMT) when compared to histopathological samples. Increased C-IMT has repeatedly been shown to predict cardiovascular disease (CVD), decreased coronary flow reserve, and increased risk of mortality (Lorenz*, et al.* 2007; Lorenz*, et al.* 2012; Rohani*, et al.* 2005; Salonen*, et al.* 1993; Sonoda*, et al.* 2004; Takiuchi*, et al.* 2003). A thickened intima-media layer in the carotid artery is indicative of long-term exposure to CVD risk factors and is a visually meaningful measurement that can provide important information on the progress of arterial disease. It is now one of the most widely used methods of assessing subclinical atherosclerosis (O'Leary*, et al.* 2010) and a valuable surrogate end point for primary and secondary prevention studies. However, the clinical significance of C-IMT measurements are not fully appreciated as its typical measurement variability remains under reported.

Only in recent years have international consensus statements provided guidance on the standardisation of imaging protocols (Stein*, et al.* 2008; Touboul*, et al.* 2012), and equipment refinement has meant that current systems are more "user friendly". Automated edge detection systems may be a way to reduce measurement variability by standardising image selection and measurement (O'Leary & Bots 2010). These record measurements based on pre-defined criteria and may provide opportunities for less experienced sonographers to reliably measure C-IMT (Vanoli*, et al.* 2013). However, there are few reported data that confirm this

technical benefit. The aim of our study was to quantify the variability of C-IMT measurements taken using a specific automated edge detection system (Panasonic CardioHealth Station, Panasonic Biomedical Sales Europe BV, Leicestershire, UK). We hypothesised that novice operators could produce acceptable inter and intra-operator variability from measurements taken at short term and one-week intervals during C-IMT measurements in healthy, young-to-middle aged adults.

Methods

Participants

Ethical approval for the study was reviewed and approved by the Department of Sport, Health & Exercise Science Research Ethics Committee and meets the ethical standards of this journal. Participants were recruited from the local community and written informed consent was taken from each volunteer. All participants were aged 18 to 40 years and were free from any underlying medical conditions including cardiometabolic disease. Female participants were excluded if they were pregnant. All participants were instructed to attend in a euhydrated state. Participants were asked to refrain from moderate physical activity and to avoid beverages containing caffeine on the day of testing.

Study Protocol

Each participant was required to attend on two occasions, seven days apart. Appointments were scheduled at the same time of day to control for circadian variation. Resting heart rate and blood pressure was taken after 10 minutes of rest using a sphygmomanometer (Accoson

Works, Essex, UK) and a Littman stethoscope (3M Healthcare, St Paul, Minnesota, USA). Body mass was measured using SECA balance scales (Vogel & Halke, Hamburg Germany) and stature was measured using a stadiometer (Holtain Ltd, Crymych, Dyfed). Waist and hip circumference measurements were also taken using an inflexible tape measure.

Device Specification

The Panasonic CardioHealth Station is a commercially available ultrasound system which uses a broadband probe (5-13 MHz) with a centre frequency of 9 MHz optimised for carotid imaging. When the probe is correctly positioned over the carotid artery, onboard software automatically identifies the far wall with a region of interest (ROI) tool (figure 1). The system captures a sequence of near R-wave triggered (end-diastole) images automatically by monitoring the vessel distension characteristics and stops when pre-defined C-IMT boundary quality criteria are met. A mean, minimum, and maximum C-IMT in mm are calculated based on real-time raw data over multiple lines in the region of interest and values are displayed to 3 decimal places. The probe is also fitted with an integrated accelerometer and gyroscope that track its insonation angle relative to ground. The angle of the probe (°) is displayed onscreen (figure 1) and recorded with the images to allow the operator to review and reproduce similar angles on repeated scans.

Carotid Ultrasound Measurement Technique

Two right-handed novice operators (<10 practice scans each) performed three C-IMT investigations. At visit 1, operator one (O1) and operator two (O2) scanned the same subject consecutively (initial scan); followed by a second examination by both operators

approximately 10 minutes later (immediate repeat scan). At one week follow up, O1 and O2 performed a third consecutive scan (one week scan).

Participants were positioned supine on a 180° examination bed with their head rotated against a gauge angled to 45°. The probe angle was standardised using software that indicated the probe angle (°) relative to ground. All images were taken from the far wall of the distal common carotid artery (CCA), 1cm proximally from the bifurcation. When the region of interest was correctly identified and software image criteria were met, an image was automatically frozen at end-diastole and C-IMT was calculated over a 10 mm length using automated boundary detection. Each measurement was visually checked for accuracy. Images were taken at four angles following the order; right lateral (125-145°), right anterior (170-190°), left lateral (215-235°), left anterior (170-190°).

Statistical Analysis

SPSS Version 19 (IBM, New York, USA), SigmaPlot Version 12 (Systat Software, California, USA) and Microsoft Excel 2007 (Microsoft, Washington, USA) were used for analysis. Continuous variables are presented as mean with 95% confidence intervals (CI) and standard deviation where specified (SD); non-normally distributed data as medians (interquartile ranges) and categorical data as percentages. Skewness and kurtosis were checked visually with histograms and Kolmogorov-Smirnov (K-S) tests were used to assess normality. Log_{10} transformations were conducted to attempt to correct for deviations from normality and where parametric assumptions could not be met Wilcoxon and Mann-Whitney U tests were used to identify significant differences between variables. Heteroscedasticity was evaluated using the Breusch-Pagen test. An arbitrary level of 5% statistical significance was used throughout (two-tailed).

Bland-Altman plots were used to calculate mean bias and limits of agreement (LoA) (Bland*, et al.* 1999) and because the CardioHealth Station calculates C-IMT to the nearest μm, results were reported as mm rounded to 3 decimal places. The significance of variability shown by LoA and bias depends on whether differences reach clinically meaningful levels. Intra-class correlation coefficients (ICCs) using a two-way mixed effect model for agreement of single measurements were performed. A consensus on ICC strength has not been reached, however, for our purposes, moderate agreement was defined as an ICC of 0.6-0.75, good agreement between 0.75 and 0.9 and excellent >0.9 (Atkinson*, et al.* 1998). Coefficient of variation percentage (CoV%) was calculated as within-subject standard deviation divided by the group mean multiplied by 100 (Atkinson & Nevill 1998). A CoV% was calculated for each angle interrogated by O1 and O2. Immediate inter operator measurement variability was calculated by directly comparing operator 1's measurement with operators 2's at any given time point, for example, O1 initial scan versus O2 initial scan. Longer term inter-operator variability was calculated by comparing the initial scans of one operator to the one-week scan of the other operator. This also allowed a comparison of inter-operator measurement variability under conditions similar to those where study participants return for repeated visits but where scans are conducted by different operators. Short term and one-week intra-operator variability was calculated by comparing an operators initial scan to their immediate repeat scans and initial scan to one-week scan respectively.

Results

Fifty participants (60% male; age 26.2 ± 5.0 years; BMI 24.6 Kg·m^{-2} (interquartile range 3.2Kg·m^{-2}) were recruited. A significant reduction in resting heart rate and resting systolic blood pressure was noted at the follow up visit, all other supplementary measurements

remained unchanged (Table 1). No scans were excluded due to poor image quality. Both operators noted that males had significantly larger C-IMT than females, however all C-IMT measurements were within normal healthy ranges (Simon, *et al.* 2002).

Angle Consistency

The CoV% for insonation angle was small for both operators indicating good angle consistency. For O1, right and left anterior measurements were taken at angles of 174 ± 4° and 185 ± 4° with CoV% of 2.1 and 1.9 respectively. Right and left lateral measurements were taken at 135 ± 5° (CoV% 3.4) and 222 ± 5° (CoV% 2.4). O2 showed similar results: right anterior 172 ± 3° (CoV% 1.8), left anterior 185 ± 4° (CoV% 2), right lateral 137 ± 5° (CoV% 3.7) and left lateral 219 ± 6° (CoV% 2.5) Statistically significant (P<0.001) differences of ~3° between O1 and O2's measurement angle for right anterior, right lateral and left lateral (mean difference 1.4°, 1.9° and 3.4° respectively) were recorded.

Immediate Inter-Operator Variability

Bland-Altman plots were used to assess the inter-operator variability of measurements taken at the same time point. Bland-Altman plots require the differences between two measurements to be normally distributed, an assumption that was not met (p<0.05). Log_{10} transformation did not correct for non-normal distribution but graphical histograms showed near normality with low kurtosis and skewness and a Breush Pagan test showed homoscedasticity (p=0.0002) so untransformed data was used. The LoA are still useful and are likely to be too wide rather than too narrow when data is not normally distributed (Bland & Altman 1999). Results from O1 and O2 (Figure. 2) showed low variability from all

measurements (mean bias -0.003mm; LoA -0.063 to 0.056mm) and good ICC strength (0.840; 95% CI 0.815 to 0.862). Similar results were shown when data was binned into separate scans. Initial scan mean bias was -0.009mm (LoA -0.068 to 0.051mm; ICC 0.834 95% CI 0.775 to 0.877), immediate repeat scan mean bias was -0.003mm (LoA -0.063 to 0.057mm; ICC 0.840 95% CI 0.794 to 0.877) and the one-week follow up scan had a mean bias of 0.003mm (LoA -0.054 to 0.060mm; ICC 0.840 95% CI 0.815 to 0.926). When measurements were broken down further and analysed by ultrasound view and time point (table 3), the largest measurement error was from images taken on the left lateral or anterior aspects at the immediate repeat scan, however all correlations reached statistical significance (P<0.05).

One-week Inter Operator Variability

One-week variability (initial scan versus one-week scan) remained similar to immediate variability for both operators. O1's initial measurement had good agreement with O2's 7 day measurement (mean bias -0.001; LoA 0.067 to 0.066mm) and O2's initial measurement had good agreement with O1's 7 day measurement (mean bias 0.005; LoA 0.056 to 0.066mm) whilst ICCs were 0.815 (95% CI: 0.763 to 0.857) and 0.814 (95% CI 0.760 to 0.856) respectively.

Short term and One-week Intra-Operator Variability

O1's short term mean bias (initial scan versus immediate repeat scan) was -0.005mm and LoA were -0.057 to 0.046mm (ICC 0.883; 95% CI: 0.847 to 0.911). One-week mean bias and LoA (initial scan versus one-week follow up scan) were -0.003mm and -0.057 to 0.050mm (Figure. 3) with ICC of 0.868 (95% CI: 0.829 to 0.899). O2's short-term mean bias and LoA were

<-0.001mm and -0.056 to 0.056mm respectively (ICC 0.858; 95% CI: 0.816 to 0.890) whilst mean bias and LoA for one-week measurements were 0.008mm and -0.052 to 0.068mm (Figure. 4) with ICC 0.828 (95% CI: 0.771 to 0.870). All ICC were significant (p<0.001) however the strength of the ICCs appeared to be dependent on the angle of measurement. For O1, right lateral measurements demonstrated the strongest ICC (0.929; 95% CI 0.890 to 0.957) whilst left anterior measurements were weakest (ICC: 0.821; 95% CI 0.733 to 0.888). Right anterior ICC was 0.863 (95% CI: 0.793 to 0.915) and left lateral was 0.833 (95% CI: 0.749 to 0.895). O2 also recorded the strongest ICC for right lateral measurements (0.914; 95% CI: 0.867 to 0.947) however the weakest ICC was for left lateral measurements (0.711; 95% CI: 0.586 to 0.812). Right anterior ICC was (0.872; 95% CI 0.804 to 0.921) and left anterior ICC was 0.721 (95% CI: 0.598 to 0.819).

Learning effect

All C-IMT measurements were chronologically organised and were categorised by the first 25 participants and final 25 scans to examine if measurement variability improved with experience. Short term LoA improved after 25 scans for both operators whereas one-week LoA only improved for O2 (Table 4). ICC strength decreased with experience for one-week variability for both operators but agreement still remained good (O1, ICC= 0.842; O2, ICC= 0.785).

Discussion

We have shown that novice operators produce acceptable short term and one-week inter and intra-operator C-IMT measurement variability in healthy, young to middle aged adults using

the Panasonic CardioHealth Station. We report lower intra-operator mean bias than previously reported; Kanters *et al.,* (Kanters*, et al.* 1997) stated that mean measurement differences ranging from -0.02 to 0.63mm in their systematic review whereas we report a mean bias of -0.011mm. Intra-operator LoA (O1: -0.049 to 0.039mm; O2: -0.048 to 0.048) show that novice operators are capable of detecting clinically meaningful C-IMT increases of 0.1mm (Lorenz, Markus, Bots, Rosvall & Sitzer 2007; Salonen & Salonen 1993) using this ultrasound device.

As documented by other investigators, intra-operator variability was lower than inter-operator variability (Kanters, Algra, van Leeuwen & Banga 1997; Lundby-Christensen*, et al.* 2010; Stensland-Bugge*, et al.* 1997), however our findings show improved levels of inter-operator agreement in comparison with other investigators (Lundby-Christensen, Almdal, Carstensen, Tarnow & Wiinberg 2010; Stensland-Bugge, Bønaa & Joakimsen 1997). A recent study using the CardioHealth Station to evaluate novice user trainability showed substantially wider inter-operator LoA than our study (-0.103mm to 0.096mm versus -0.063 to 0.056mm) (Vanoli, Wiklund, Lindqvist, Henein & Näslund 2013). Variable examination angle and the presence of increased IMT (and therefore potentially less homogenous C-IMT) may have contributed to their results and the discrepancy between the study findings. However this remains speculative as the authors do not provide data on ultrasound probe angle consistency. Future research may wish to focus on the replication of specific angles and artery segments using integrated angle sensors to minimise measurement error.

The probe angle that novice operators take images from also appears to affect measurement variability and right lateral scans provided less measurement variability than other views. This finding indicates that specific angles and hand positions (for right handed operators) may demand a greater degree of practice before novice operators acquire the same level of measurement accuracy as more experienced operators. The LoA for measurements taken from

the right side (LoA~-0.037 to 0.053mm) by our novice operators are comparable to recent research (Saba*, et al.* 2012) using sonographers with 3 to 10 years experience (~-0.041 to 0.049mm). However when all angles of examinations were combined, the LoA for inter-operator variability were wider (-0.063 to 0.056mm) reflecting the higher variability from images acquired at different hand positions.

A learning effect for C-IMT measurements (examined by comparing variability measures among the first 25 participants compared to the remainder of the sample) was also identified. Although the measurements taken at the start of the study are still within acceptable limits, there is evidence that even limited experience can improve measurement variability. In contrast, measurement variability over one week may not improve irrespective of experience suggesting that the familiarity developed between the short term scans may influence measurements. To our knowledge, the study by Vanoli and colleagues (Vanoli, Wiklund, Lindqvist, Henein & Näslund 2013) is the only recent study to assess the effect of operator experience on measurement variability. Our results are in concordance with Vanoli and colleague's findings (Vanoli, Wiklund, Lindqvist, Henein & Näslund 2013) confirming that experience does improve short-term measurement variability.

A limitation of our study is that measurement variability was assessed over a relatively short time frame. A longer follow period would help to further assert the variability of C-IMT measurements and may be useful for studies that wish to monitor changes over an extended period. However, studies should bear in mind the dynamic nature of atherosclerosis progression and regression when planning longer term studies especially if studies involve the use of elderly or clinical cohorts.

Our results will allow researchers and clinicians to monitor clinical changes in C-IMT measurements and appropriately take measurement error into consideration. The applicability

of this work is restricted to patient primary prevention due to the effect that vascular remodelling may have on measurement variability. Future work should evaluate the variability of C-IMT in clinical cohorts and attempt to control the angle of investigation between scans. Investigations using C-IMT as an end-point should present their own measurement variability data or cite a suitable variability studies to assist with clinical interpretation.

In conclusion, novice operators produce acceptable short term and one-week inter- and intra-operator C-IMT measurement variability in healthy, young to middle aged adults using the Panasonic CardioHealth Station.

References

Atkinson G and Nevill A. Statistical Methods For Assessing Measurement Error (Reliability) in Variables Relevant to Sports Medicine. *Sports Med* (1998); **26**: 217-238.

Bland JM and Altman DG. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research* (1999); **8**: 135-160.

Kanters SDJM, Algra A, van Leeuwen MS and Banga J-D. Reproducibility of In Vivo Carotid Intima-Media Thickness Measurements: A Review. *Stroke* (1997); **28**: 665-671.

Kolodgie FD, Burke AP, Nakazawa G and Virmani R. Is Pathologic Intimal Thickening the Key to Understanding Early Plaque Progression in Human Atherosclerotic Disease? *Arteriosclerosis, Thrombosis, and Vascular Biology* (2007); **27**: 986-989.

Lee RT, Yamamoto C, Feng Y, Potter-Perigo S, Briggs WH, Landschulz KT, Turi TG, Thompson JF, Libby P and Wight TN. Mechanical Strain Induces Specific Changes in the Synthesis and Organization of Proteoglycans by Vascular Smooth Muscle Cells. *Journal of Biological Chemistry* (2001); **276**: 13847-13851.

Lorenz MW, Markus HS, Bots ML, Rosvall M and Sitzer M. Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness: A Systematic Review and Meta-Analysis. *Circulation* (2007); **115**: 459-467.

Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Völzke H, Tuomainen T-P, Sander D, Plichart M, Catapano AL, Robertson CM, Kiechl S, Rundek T, Desvarieux M, Lind L, Schmid C, DasMahapatra P, Gao L, Ziegelbauer K, Bots ML and Thompson SG. Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data. *The Lancet* (2012); **379**: 2053-2062.

Lundby-Christensen L, Almdal TP, Carstensen B, Tarnow L and Wiinberg N. Carotid intima-media thickness in individuals with and without type 2 diabetes: a reproducibility study. *Cardiovascular diabetology* (2010); **9**: 40.

O'Leary DH and Bots ML. Imaging of atherosclerosis: carotid intima–media thickness. *European Heart Journal* (2010); **31**: 1682-1689.

Pignoli P, Tremoli E, Poli A, Oreste P and Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* (1986); **74**: 1399-1406.

Rohani M, Jogestrand T, Ekberg M, van der Linden J, Källner G, Jussila R and Agewall S. Interrelation between the extent of atherosclerosis in the thoracic aorta, carotid intima-media thickness and the extent of coronary artery disease. *Atherosclerosis* (2005); **179**: 311-316.

Saba L, Mallarini G, Sanfilippo R, Zeng G, Montisci R and Suri J. Intima Media Thickness Variability (IMTV) and its association with cerebrovascular events: a novel marker of carotid therosclerosis? *Cardiovascular diagnosis and therapy* (2012); **2**: 10.

Salonen J and Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation* (1993); **87**: II56.

Simon A, Gariepy J, Chironi G, Megnien J-L and Levenson J. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. *Journal of hypertension* (2002); **20**: 159-169.

Sonoda M, Yonekura K, Yokoyama I, Takenaka K, Nagai R and Aoyagi T. Common carotid intima–media thickness is correlated with myocardial flow reserve in patients with coronary artery disease: a useful non-invasive indicator of coronary atherosclerosis. *International Journal of Cardiology* (2004); **93**: 131-136.

Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM and Post WS. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *Journal of the American Society of Echocardiography* (2008); **21**: 93-111.

Stensland-Bugge E, Bønaa KH and Joakimsen O. Reproducibility of Ultrasonographically Determined Intima-Media Thickness Is Dependent on Arterial Wall Thickness: The Tromsø Study. *Stroke* (1997); **28**: 1972-1980.

Takiuchi S, Rakugi H, Fujii H, Kamide K, Horio T, Nakatani S, Kawano Y, Higaki J and Ogihara T. Carotid intima-media thickness is correlated with impairment of coronary flow reserve in hypertensive patients without coronary artery disease. *Hypertension research: official journal of the Japanese Society of Hypertension* (2003); **26**: 945-951.

Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Hernandez Hernandez R, Jaff M, Kownator S, Naqvi T, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E and Woo KS. Mannheim Carotid Intima-Media Thickness and Plaque Consensus (2004–2006–2011). *Cerebrovascular Diseases* (2012); **34**: 290-296.

Vanoli D, Wiklund U, Lindqvist P, Henein M and Näslund U. Successful novice's training in obtaining accurate assessment of carotid IMT using an automated ultrasound system. *European Heart Journal – Cardiovascular Imaging* (2013).

Tables

Table 1 - Baseline and follow up participant characteristics (mean ± SD and median with interquartile range)

Variable	Visit 1	Visit 2	P value
BMI (Kg·m^{-2})	24.6 (3.3)	24.5 (3.4)	0.125^2
W/H Ratio	0.84 ± 0.69	0.83 ± 0.68	0.142^1
Resting SBP (mmHg)	118.3 ± 11.7	114.4 ± 10.0	0.002^1*
Resting DBP (mmHg)	70.9 ± 8.5	69.3 ± 7.7	0.144^1
RHR (BPM)	64.5 (10)	63 (11)	0.032^2*

BMI = Body Mass Index; W/H = Waist to Hip Ratio; SBP = Systolic Blood Pressure;
DBP = Diastolic Blood Pressure; RHR = Resting Heart Rate; ^{1}= paired sample t-test; ^{2} = Wilcoxon test;
* = Significant Difference

Table 2 – C-IMT for males and females taken by Operator 1 & 2 (Median with interquartile rage)

Operator 1	Right (mm)	Left (mm)	P value
Male	0.471 (0.072)	0.462 (0.047)	0.237
Female	0.442 (0.049)	0.451 (0.063)	0.179
P-value	<0.001*	0.033*	
Operator 2			
Male	0.467 (0.089)	0.458 (0.046)	0.021*
Female	0.441 (0.052)	0.444 (0.054)	0.054
P value	<0.001*	0.025*	

Significant difference calculated using Mann-Whitney U and Wilcoxon tests
* = Significant difference

Table 3 – Immediate inter-operator variability by time point and angle

Time Point	Ultrasound View	Mean Bias (mm)	LoA (mm)	ICC	ICC 95% CI	P value
Initial Scan	Right Anterior	-0.009	-0.070 to 0.052	0.867	0.773 to 0.923	<0.001*
	Left Anterior	-0.007	-0.066 to 0.052	0.748	0.595 to 0.848	<0.001*
	Right Lateral	-0.011	-0.064 to 0.042	0.894	0.803 to 0.942	<0.001*
	Left Lateral	-0.008	-0.073 to 0.058	0.744	0.590 to 0.846	<0.001*
Immediate Repeat Scan	Right Anterior	0.001	-0.047 to 0.049	0.908	0.843 to 0.947	<0.001*
	Left Anterior	0.001	-0.061 to 0.063	0.763	0.616 to 0.858	<0.001*
	Right Lateral	-0.004	-0.055 to 0.047	0.920	0.864 to 0.954	<0.001*
	Left Lateral	-0.010	-0.085 to 0.065	0.674	0.488 to 0.801	<0.001*
1 Week Follow Up	Right Anterior	0.008	-0.037 to 0.053	0.912	0.841 to 0.951	<0.001*
	Left Anterior	0.010	-0.054 to 0.074	0.719	0.547 to 0.831	<0.001*
	Right Lateral	-0.004	-0.059 to 0.051	0.902	0.835 to 0.943	<0.001*
	Left Lateral	-0.004	-0.062 to 0.054	0.772	0.632 to 0.864	<0.001*

* = Statistically significant

Table 4 –The Effect of Experience on Measurement Accuracy

Operator	Scan	First/Final Scan	Mean Bias (mm)	LoA (mm)	ICC	ICC 95% CI	P value
Operator 1	Initial Scan Vs Immediate Repeat Scan	First 25 Patients	-0.006	-0.064 to 0.053	0.875	0.819 to 0.914	<0.001*
		Final 25 Patients	-0.005	-0.049 to 0.039	0.897	0.848 to 0.930	<0.001*
	Initial Scan Vs 1 Week Follow up Scan	First 25 Patients	-0.006	-0.060 to 0.047	0.887	0.834 to 0.923	<0.001*
		Final 25 Patients	<-0.001	-0.054 to 0.053	0.842	0.774 to 0.891	<0.001*
Operator 2	Initial Scan Vs Immediate Repeat Scan	First 25 Patients	<0.001	-0.063 t0 0.064	0.851	0.786 to 0.897	<0.001*
		Final 25 Patients	<0.001	-0.048 to 0.048	0.869	0.812 to 0.910	<0.001*
	Initial Scan Vs 1 Week Follow up Scan	First 25 Patients	0.010	-0.050 to 0.071	0.854	0.776 to 0.904	<0.001*
		Final 25 Patients	0.005	-0.054 to 0.064	0.785	0.687 to 0.850	<0.001*

* = Statistically significant

Figures

Figure 1 – A screenshot showing C-IMT investigation where angle of examination is displayed and recorded using an integrated system (Bottom Right). The region of interest (ROI) is located within the white box (centre) and is parallel to a white vertical dotted line (centre -left). The distance between these two features measures 1cm and allows the operator to measure the distance between the flow divider and the C-IMT measurement site during the scan.
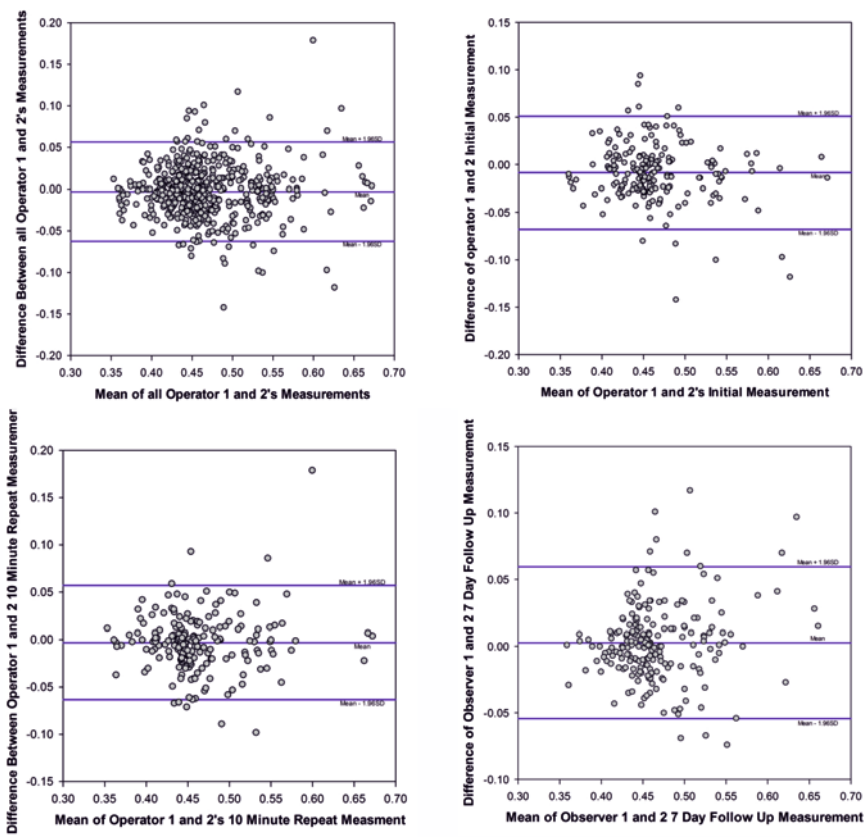
Figure 2 - Bland –Altman plots showing mean bias and LOA for immediate inter operator measurements (O1 = operator 1; O2 = operator 2).
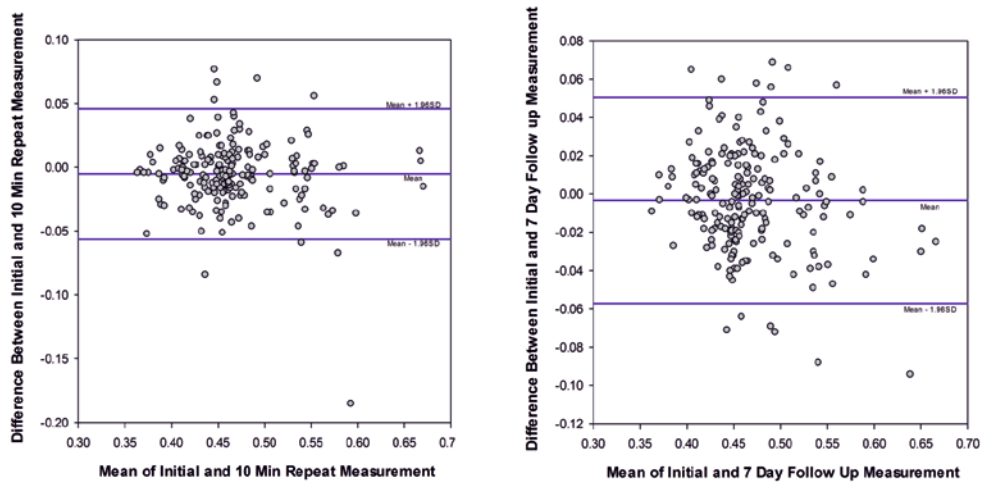
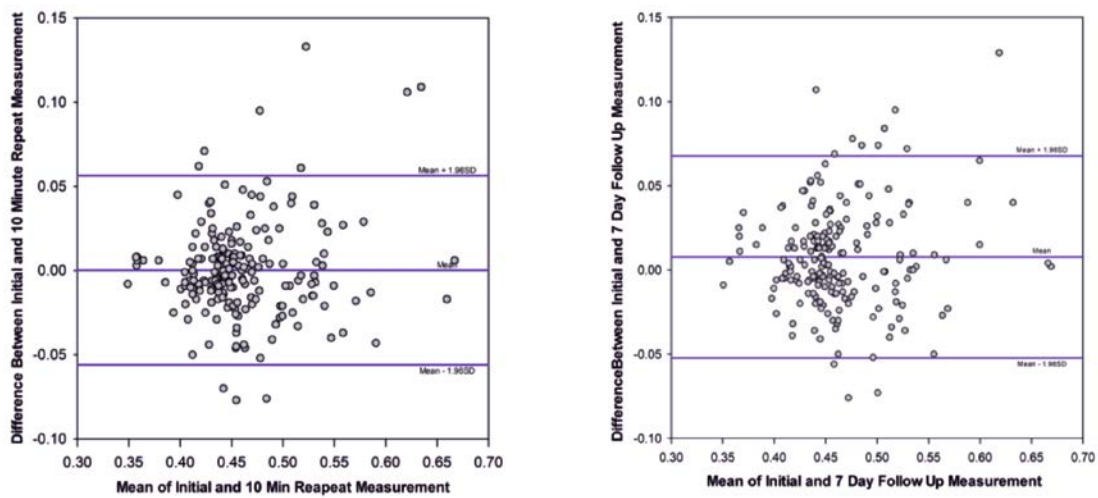**Figure 3** – Operator 1's short and longer term measurement variability.

Figure 4 – Operator 2's short and longer term measurement variability.