

Influence of Blood Pressure Variation on Non-Invasive Myocardial Work in Patients Undergoing TAVI for Severe Aortic Stenosis

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Abstract

Background

Myocardial work (MW) is an emerging, non-invasive method for assessing left ventricular performance by integrating brachial blood pressure (BP) with myocardial deformation imaging. While international hypertension guidelines recommend recording BP in the seated position, transthoracic echocardiography (TTE) is routinely performed in the left lateral decubitus position (LLD). Whether fluctuations in BP recorded in the seated and LLD positions meaningfully alter MW indices remains unclear. This study prospectively evaluated the influence of BP recording position on MW parameters in patients with severe aortic stenosis (AS) prior and following transcatheter aortic valve intervention (TAVI).

Methods

A total of 98 patients with severe AS undergoing TAVI were prospectively enrolled. Blood pressure was recorded in the seated position prior to TTE and repeated in the LLD position at the end of the scan. Global work index (GWI), global constructive work (GCW), global wasted work (GWW) and global work efficiency (GWE) were calculated. Paired analyses compared myocardial work parameters derived from seated versus LLD BP with subgroup analyses based on direction of BP change.

Results

Across 316 paired BP recordings, a small but significantly higher BP was shown in the seated position when compared to the LLD position (147 ± 24 vs. 144 ± 22 mmHg, $p = 0.003$). Modest but statistically significant differences were observed in GWI (+ 42 mmHg%, $p < 0.001$), GCW (+ 54 mmHg%, $p < 0.001$) and GWW (+ 4 mmHg%, $p = 0.003$) when using seated BP with no difference in GWE. Subgroup analyses revealed substantially larger differences in GWI, GCW and GWW indices when larger BP increases in either direction between positions were observed. Linear mixed effects modelling demonstrated that body position does not independently influence MW indices when BP is controlled, signifying that changes in GWI, GCW and GWW are driven by fluctuations in BP.

Discussion

BP recorded in the seated and LLD positions cannot be considered interchangeable for assessment of MW. Even modest positional BP variation produced significant changes in GWI, GCW and GWW, particularly in patients with larger directional BP shifts of > 10 mmHg. These findings highlight the need for standardised BP acquisition protocols which support the consistent recording of BP in the LLD position during TTE image acquisition for accurate assessment of MW.

Introduction

Myocardial work (MW) is a novel and robust assessment of complex left ventricular (LV) mechanics and performance derived by combining myocardial deformation imaging from transthoracic echocardiography (TTE) and non-invasive blood pressure (1, 2). MW offers comprehensive insight into global and regional myocardial contractile function through the analysis of non-invasive pressure-strain loops (PSL) which is a less afterload dependent assessment of LV function (3). Russell et al. (4) first identified that non-invasive PSL correlated very well with invasive micromanometer and sonomicrometry derived stroke work ($r = 0.99$) and glucose metabolism ($r = 0.81$) measured by F-fluorodeoxyglucose positron emission tomography (FDG-PET) in a small number of dogs and patients. Since then, non-invasive MW via TTE has been evaluated in a wide range of cardiovascular conditions from coronary artery disease (5), heart failure (6), mitral valve prolapse (7), and assessment of various forms of cardiomyopathy (8, 9). These studies all conclude that MW provides a more sensitive, prognostic evaluation of LV remodelling or function compared to standard indices currently used in clinical practice. Estimation of LV pressure using non-invasive systemic blood pressure (BP) typically taken from the cubital fossa at the time of the TTE is combined with data from speckle tracking imaging to generate MW parameters such as global work index (GWI), global constructive work (GCW), global wasted work (GWW) and global work efficiency (GWE). A detailed overview of all MW definitions, calculations and normative values can be found at Luke et al. (10).

A comparative review of methodologies across multiple publications reveals a subtle but important inconsistency in how BP is recorded for the purpose of MW. Although non-invasive BP is routinely measured using the brachial artery, body positioning when measuring BP can differ considerably with some recording BP in the seated position prior to TTE, while others obtain measurements with the patient in the LLD position. This lack of standardisation is apparent when reviewing primary publications calculating MW as some measure BP in the supine position at the end of the TTE (11, 12) while one paper acknowledged that BP was measured in the sitting position (13). A larger number of published studies provide limited detail on how BP was recorded beyond the use of a brachial arterial cuff (14–17). Studies offering normative reference values for MW also provide scarce detail about how BP was recorded, but it could be assumed that since MW was derived from retrospective data, BP was recorded in the standardised seated position while the TTE performed in the LLD (18, 19).

Fluctuations in both systolic and diastolic BP can occur due to the recording position when BP is being measured (20). The International Society of Hypertension Practice Guidelines released in 2020 provides an effective set of evidence-based recommendations for performing and measuring BP (21). Among the recommendations for measuring non-invasive BP, the guidelines advocate that a patient should be seated in a quiet room, in a comfortable position with their arm supported at heart level while having their BP taken with a validated device (21). While it is important to standardise how BP is being recorded in clinical practice, the modification of BP due to patient positioning may be particularly relevant for the calculation of MW. During a standard TTE, a patient is positioned in the LLD position for an extended length of time to augment the visualisation of cardiac structures and improve endocardial definition for

the purpose of LV function quantification. It is well recognised that changes in body position can significantly alter BP by up to 10mmHg which could significantly change MW generated scores. A study performed by Netea *et al.* (22) assessing the influence of body position when recording BP concluded that supine systolic ($141 \pm 26\text{mmHg}$ vs $136 \pm 25\text{mmHg}$) and diastolic BP (85 ± 11 vs $80 \pm 10\text{mmHg}$) were significantly higher when compared to seated BP recordings. Similar findings provided by Lu *et al.* (23) confirmed that that systolic BP was $3 \pm 8\text{mmHg}$ higher in the supine position than in the sitting position in 1,487 hypertensive patients ($p < 0.001$). While these measurements appear to be clinically insignificant, if BP is recorded in seated positions while the TTE is performed using the LLD position, these changes could have a profound influence on MW derived by TTE. Findings by Fisicaro *et al.* (24) concluded that BP fluctuates in the supine and LLD positions throughout the scanning period. The results of this investigation identified that higher BP recordings were documented in the supine position at the start of the TTE, reducing in the LLD position when obtaining the apical views, with a significant reduction observed in MWI ($1929 \pm 441 \text{ mmHg\%}$ vs. $1602 \pm 351 \text{ mmHg\%}$, $p < 0.001$) between the two positions. However, this study did not compare MW parameters using BP recorded in the seated position. Therefore, the aim of this prospective study is to investigate if positional BP changes have a significant impact upon MW indices in patients with severe AS undergoing TAVI.

Method

Study population and follow-up:

This prospective, single tertiary centre study recruited 104 consecutive patients referred for a TAVI for severe AS between December 2021 and December 2023. Patients were eligible to participate if they fulfilled the following inclusion criteria shown below:

- ≥ 18 years of age.
- Provided written consent.
- Known severe aortic stenosis by aortic valve area ($< 1\text{cm}^2$), peak velocity ($\geq 4\text{m/s}$) and mean gradient ($\geq 40\text{mmHg}$) and referred for TAVI.
- TTE image quality suitable for strain to be performed.
- Sinus rhythm/bradycardia without significant atrial or ventricular ectopic burden.
- Appropriately rate controlled atrial arrhythmia with stable R-R intervals $< 80\text{bpm}$.

Exclusion criteria include those unable to provide consent, unable to tolerate a standard TTE, have suboptimal endocardial definition, presence of uncontrolled atrial arrhythmias such as atrial fibrillation and flutter, active endocarditis, history of previous valve surgery or more than moderate mitral regurgitation.

Blood pressure and Transthoracic Echocardiography:

All TTEs were performed by experienced, British Society of Echocardiography accredited operators using Vivid E95, Vivid S70 and Vivid iQ ultrasound systems (GE Healthcare, Horten, Norway). Prior to the TTE, all subjects had their height and weight measured to calculate body surface area (BSA).

Non-invasive BP was taken using an automatic brachial cuff (A&D Medical UA-767S-W) initially in the seated position prior to the TTE and at the end of the study in the LLD position. BP was taken once in each position in accordance with departmental protocol. Each patient was invited into a scanning room and requested to sit quietly for five minutes prior to recording a resting BP in accordance with the International Society of Hypertension Practice Guidelines (21). Participants were then instructed to position themselves in the LLD position with a three-lead electrocardiogram attached for the duration of the TTE. A standard British Society of Echocardiography minimum dataset was carried out with emphasis on optimising endocardial definition in the apical four, two and long-axis views for the assessment of myocardial deformation imaging (25). A minimum of two cardiac cycles was used with frame rates over 40 frames per second. A final BP was recorded following the completion of the TTE in the LLD position. All raw images were stored and exported to an encrypted hard drive. The TTE images were analysed off-line using EchoPAC software (GE Healthcare, version 204). All images were obtained by British Society of Echocardiography (BSE) accredited echocardiographers with at least 3 years' of independent echocardiography experience.

All participants received a standard TTE within two hours of their transcatheter aortic valve intervention (TAVI) procedure including aortic Doppler measurements used to grade AS severity. All patients received a standard TTE prior to hospital discharge as part of their routine care. Patients were invited for a standard follow-up TTE at six weeks and 12 months. BP in the seated and LLD was recorded at each appointment where possible.

Assessment of Myocardial Deformation Imaging and Myocardial Work:

Two-dimensional speckle tracking was performed prospectively in the apical four, two and long-axis views of each patient with the use of EchoPAC software (**Figure one**). The automated functional imaging (AFI) speckle-tracking software defined the region of interest (ROI) for each view, automatically marking the borders of the endocardium, myocardium and epicardium. These ROI markers were manually adjusted by the operator if the borders were not clearly tracked or structures were inappropriately included such as the LVOT, mitral valvular annulus, papillary muscles or pericardium. Segments that were deemed untraceable were manually adjusted and the calculation of strain repeated.

Valve timings were automated and checked prior to accepting the strain measurements. Speckle tracking throughout the cardiac cycle was visually inspected in each apical view. Where repeated inappropriate myocardial tracking was observed, another cardiac cycle or optimised image was utilised instead. Following the generation of three successful global longitudinal strain scores, the brachial cuff BP obtained at TTE was included in the MW menu of the EchoPAC software to generate the pressure-strain loops. From this GWI, GCW, GWW and GWE were calculated. Seated and LLD BP was used to generate two separate MW scores for each participant and directly compared. Speckle tracking and MW

was repeated three times to obtain an overall mean score for each MW parameter. The calculation of MW in the presence of severe aortic stenosis and following TAVI was performed by adding the mean aortic gradient (mmHg) to the existing brachial BP measurements.

Statistical analysis:

Positional BP measurements in conjunction with myocardial deformation imaging was used to calculate GWI, GCW, GWW and GWE using seated and LLD BP. Each of the MW parameters was measured three times and expressed as mean ± standard deviation unless specified. A paired t-test was used to compare mean differences between each of the MW parameters and BP. Participants were allocated to one of two groups depending upon their BP response (group one: seated BP ≥ LLD BP; group two: seated BP < LLD BP). Once a Bonferroni adjustment was applied, a p value of ≤0.025 (0.05/2) was deemed statistically significant. Linear mixed-effects regression models were used to evaluate the relationship between myocardial work indices and systolic blood pressure, including body position, scan interval (pre, post 6 weeks and 12 months), and absolute BP change as fixed effects, with repeated measurements modelled as random effects. This approach accounted for within-subject correlation across multiple time points. To minimise the risk of type I error across comparisons of the four myocardial work parameters, a Bonferroni adjusted significance threshold of $p \leq 0.0125$ (0.05 /4 tests) was applied. All analyses were performed using IBM SPSS Statistics (Version 32).

Ethical approval:

All subjects were provided with written informed consent prior to participating in the study. The study protocol was approved by the local and regional (London-Chelsea) research ethics committee (22/LO/0675). All participants were clinically and haemodynamically stable.

Results

A total of 104 participants were included in the study. Six patients were excluded from the study due to four experiencing a stroke post procedure, one death and one TAVI migration which required surgery. The final sample size was 98 participants. Each participant received the opportunity to have a TTE prior to TAVI, immediately post TAVI, six week and twelve months post follow-up. In total, there were 334 TTE performed. BP was successfully recorded in the seated and LLD positions in 316 independent observations. Baseline characteristics of the study population are shown in table 1.

Table 1: Baseline clinical and echocardiographic characteristics of study population.

Baseline data.	Total sample 98
Age, (years)	80.9 ± 6.1
Gender, male (%)	55 (56.1)
Height, (m)	1.65 ± 0.11
Weight, (Kg)	76.1 ± 16.1
BSA, (m ²)	1.83 ± 0.22
Hypertension (%)	60 (61.2)
Diabetes (%)	21 (21.4)
Coronary artery disease (%)	16 (16.3)
LV end diastolic volume indexed (mL/m ²)	47.9 ± 13.7
LV end systolic volume indexed, (mL/m ²)	20.8 ± 8.9
LVEF, (%)	57.5 ± 8.6
LV GLS (%)	-15.3% ± 3.1

Data is represented as mean ± SD or n (%)

Systolic blood pressure changes with position:

The results of the prospective analysis demonstrated that there was a minimal but significant increase in systolic BP recorded in the seated position when compared to the LLD (seated: 147 ± 23mmHg vs. LLD: 144 ± 22 mmHg, p=0.003). However, the minimal difference between the two positions is likely due to the non-uniform response of BP observed in this population. When the participants were grouped based upon their BP response, a larger difference was observed in systolic BP for each of the BP groups (table 2). There were 177 instances where a higher BP recording in the seated position was observed compared to BP recorded in the LLD (154 ± 22mmHg vs 140 ± 21mmHg, p<0.001), while 139 instances demonstrated an increase in BP in the LLD position when compared to seated BP (150 ± 22mmHg vs. 138 ± 22mmHg, p<0.001). There were 100 occasions where seated BP was 10mmHg higher than BP taken in the LLD (161 ± 21mmHg vs. 137 ± 20mmHg, p<0.001) and 71 occasions where a higher systolic BP of ≥10mmHg in the LLD was observed when compared to the seated position (151 ± 17 mmHg vs. 131 ± 17mmHg, p<0.001)

Myocardial work:

The findings of the linear mixed regression model used to investigate the interaction between each of the MW parameters, body position and BP confirmed that body position did not independently influence GWI, GCW, GWW and GWE when BP was controlled. However, the linear mixed effect regression models demonstrated that systolic blood pressure exerted a significant positive linear influence on GWI ($F[1, 538.4] = 199.534, p < .001$), GCW ($F[1, 543.8] = 253.843, p < .001$) and GWW ($F[1, 572.8] = 31.205, p < .001$), whereas no significant association was observed for GWE ($F[1, 561.8] = 0.408, p = .523$). For 1mmHg increase in BP, GWI increases by 16.6mmHg%, GCW by 19.5mmHg% and GWW by 1.3mmHg%. There was a marginally higher but significant change in in GWI, GCW and GWW when BP was measured in the seated position with no difference in GWE was reported (Table 2, Figures 2 and 3).

When patients were categorised based upon BP response, larger differences in BP and therefore GWI, GCW and GWW were observed with no differences remaining for GWE (**table 2**).

Table 2: Illustrating the systolic BP and MW parameters observed during the study across four time points (pre, post, 6 weeks and 12 months).

Whole cohort (n = 316)				
Parameters	Seated	LLD	Mean Difference	Sig
Systolic BP (mmHg)	147 ± 24	144 ± 22	3	0.003
GWI (mmHg%)	2066 ± 613	2024 ± 605	42	<0.001
GCW (mmHg%)	2554 ± 692	2500 ± 682	54	<0.001
GWW (mmHg%)	252 ± 129	248 ± 126	4	0.003
GWE (%)	90 ± 6	89 ± 5	0.18	.318
Seated BP ≥ LLD BP (n = 177)				
Systolic BP (mmHg)	155 ± 22	140 ± 21	15	<0.001
GWI (mmHg%)	2141 ± 585	1979 ± 551	162	<0.001
GCW (mmHg%)	2633 ± 656	2433 ± 624	200	<0.001
GWW (mmHg%)	259 ± 140	239 ± 130	20	<0.001
GWE (%)	88 ± 5	88 ± 5	0	1.0
LLD BP > seated BP (n = 139)				
Systolic BP (mmHg)	138 ± 22	150 ± 22	12	<0.001
GWI (mmHg%)	1977 ± 631	2093 ± 654	116	<0.001
GCW (mmHg%)	2461 ± 713	2601 ± 730	140	<0.001
GWW (mmHg%)	240 ± 113	255 ± 120	15	<0.001
GWE (%)	90 ± 7	90 ± 5	0.40	0.319

When patients were categorised by BP response exceeding ≥10 mmHg difference between seated and LLD positions (**table 3**), the differences in GWI, GCW, and GWW were even larger, with no differences recorded in GWE.

Table 3: Systolic blood pressure and myocardial work indices for patients across four time points (pre, post, 6 weeks and 12 months) where a difference of >10mmHg systolic blood pressure recorded in the seated or left lateral decubitus position (LLD).

Seated BP vs LLD $\geq$ 10mmHg BP (100 occasions)				
Parameters	Seated	LLD	Mean Difference	Sig
Systolic BP (mmHg)	159 $\pm$ 21	137 $\pm$ 20	22	<0.001
GWl (mmHg%)	2191 $\pm$ 605	1934 $\pm$ 558	257	<0.001
GCW (mmHg%)	2698 $\pm$ 685	2382 $\pm$ 639	316	<0.001
GWW (mmHg%)	271 $\pm$ 149	240 $\pm$ 136	31	<0.001
GWE (%)	88 $\pm$ 5	88 $\pm$ 5	0	0
LLD vs seated BP >10mmHg (71 occasions)				
Systolic BP (mmHg)	132 $\pm$ 16	151 $\pm$ 17	29	<0.001
GWl (mmHg%)	1882 $\pm$ 560	2063 $\pm$ 596	181	<0.001
GCW (mmHg%)	2337 $\pm$ 605	2561 $\pm$ 635	224	<0.001
GWW (mmHg%)	238 $\pm$ 99	263 $\pm$ 113	25	<0.001
GWE (%)	89 $\pm$ 5	89 $\pm$ 5	0	0

Agreement analysis:

The findings from the Bland-Altman plots demonstrate a clear positional effect upon systolic BP. Mean seated BP was shown to be consistently higher than in the LLD position with wide limits of agreement highlighting wide variability without proportional bias (Figure 4A). This observation was translated to GWl and GCW where MW parameters were uniformly higher when seated BP was utilised compared to LLD BP (Figures 4B and C). Wide limits of agreement were seen for GWl and GCW with no proportional bias. Interestingly, GWW demonstrated a mean bias closer to zero with modest variability highlighting that GWW is less influenced by BP fluctuations (Figure 4D). As GWE exhibited no fluctuations in BP between seated and LLD positions observed in the linear mixed regression model and paired *t* test results, a Bland-Altman for GWE was not required.

Discussion

Findings from the prospective analysis confirmed a small but statistically significant difference between systolic BP measured in the seated and LLD positions (147 $\pm$ 24mmHg vs. 144 $\pm$ 22mmHg, *p* = 0.003). The magnitude of this difference increased further when the cohort was subdivided according to direction of positional systolic BP response (*p* < 0.001). The differences in MW parameters seen within the entire population were relatively small. These findings were largely attributable to the non-uniform trend of BP behaviour as some BP measurements increased in the LLD position when compared to the

seated position while others demonstrated the opposite effect. When patients were sub-divided into groups depending upon direction of BP change between seated and LLD positions, the results were more significant with GWI, GCW and GWW all demonstrating highly significant changes in each group (table 2). These findings were compounded further in a proportion of patients where ≥ 10 mmHg difference in recorded BP was observed between seated LLD BP (table 3). It is important to note that whilst a significant difference was observed in GWI, GCW and GWW parameters using paired *t* tests, the linear mixed regression analysis clarified that MW is not independently influenced by position when BP is constant. This means that factors influencing BP which include alterations in body position can significantly alter GWI, GCW and GWW. This was confirmed by the level of agreement shown in the Bland-Altman plots (Figure 3) highlighting that the mean differences in systolic BP, GWI and GCW were all elevated when seated systolic BP was adopted compared to LLD BP.

The results from this study identified that mean systolic BP was marginally higher in the seated position than in the LLD position in this AS specific population. Interestingly, while there appears to be a trend in the literature that systolic BP is often higher in the supine position compared to the seated position (22,26,27), although a more recent study containing a larger sample size of 1298 participants concluded that systolic BP recorded in the seated position was higher than the supine position (28). It is well established that systolic and diastolic blood pressure fluctuate with changes in resting position, reflecting normal physiological shifts in vascular tone and venous return (20,29). The International Society of Hypertension Practice guidelines released in 2020 provide an effective set of evidence-based recommendations for performing and measuring BP to minimise these variations (21). While this is essential for consistent serial BP recordings, it is expected that those involved in performing TTE would also be adopting these guidelines to standardise the recording of BP in their local practice.

While it is important to standardise how BP is being recorded in clinical practice, fluctuations in BP due to patient positioning appears to be particularly relevant for the calculation of MW. During a TTE, the LLD position is adopted for an extended length of time to augment the visualisation of cardiac structures and improve endocardial definition (30). The results of the current study adds further weight to the Fiscaro et al. (24) study findings demonstrating that BP recorded in the seated position may not reflect the loading conditions observed in the LLD position for the calculation of GWI, GCW and GWW. These findings justify the need for standardised postural positions when assessing BP for the purpose of calculating MW, as measuring BP in the LLD and sitting position is often incorrectly considered equivalent. This study demonstrates that a proportion of patients with AS exhibit a significant change in BP as recorded in LLD and sitting positions that result in significant differences in GWI, GCW and GWW indices.

Limitations

This prospective study contained several limitations. The data obtained from this study is from a single tertiary centre with a relatively small sample size. However, the study was appropriately powered to detect significant differences within the population. Not all patients were able to attend all TTE follow-up

appointments and some participants immediately post TAVI were unable to offer seated BP during TTE scans. The number of BP recordings is also a limitation as one BP measurement was provided in each position. This choice was made in collaboration with the departmental lead to minimise the impact upon patient appointment times and reflects standards seen in TTE departments around the country when recording BP during TTE appointments. As BP was recorded at two separate intervals (immediately prior and post TTE), there is an argument that BP could have been elevated in the seated position in some due to psychosomatic stress prior to the commencement of the TTE or elevated in the LLD post scans due to the uncomfortable nature of the scan.

Conclusion

BP recorded in the seated and LLD positions cannot be used interchangeably for the accurate assessment of MW. Mean systolic BP was marginally higher when recorded in the seated position when compared to LLD BP with non-uniform changes in BP occurring between the two positions. Significant differences were observed in all MW indices except for GWE which was amplified in a small group of participants who experienced a significant difference of >10mmHg BP change between the two positions. To accurately reflect the loading conditions when performing MW, it is recommended that BP should be measured in the position of TTE image acquisition.

Declarations

Availability of data and materials:

The datasets generated and/or analysed during this study are not publicly available because they form part of an ongoing PhD project, but they can be obtained from the corresponding author upon reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors Contribution:

PL performed the echocardiographic data collection, analysis and interpretation. PL served as the lead author of the manuscript. CE and MA contributed to critical review of the manuscript. All authors read

and approved the final version.

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Figures

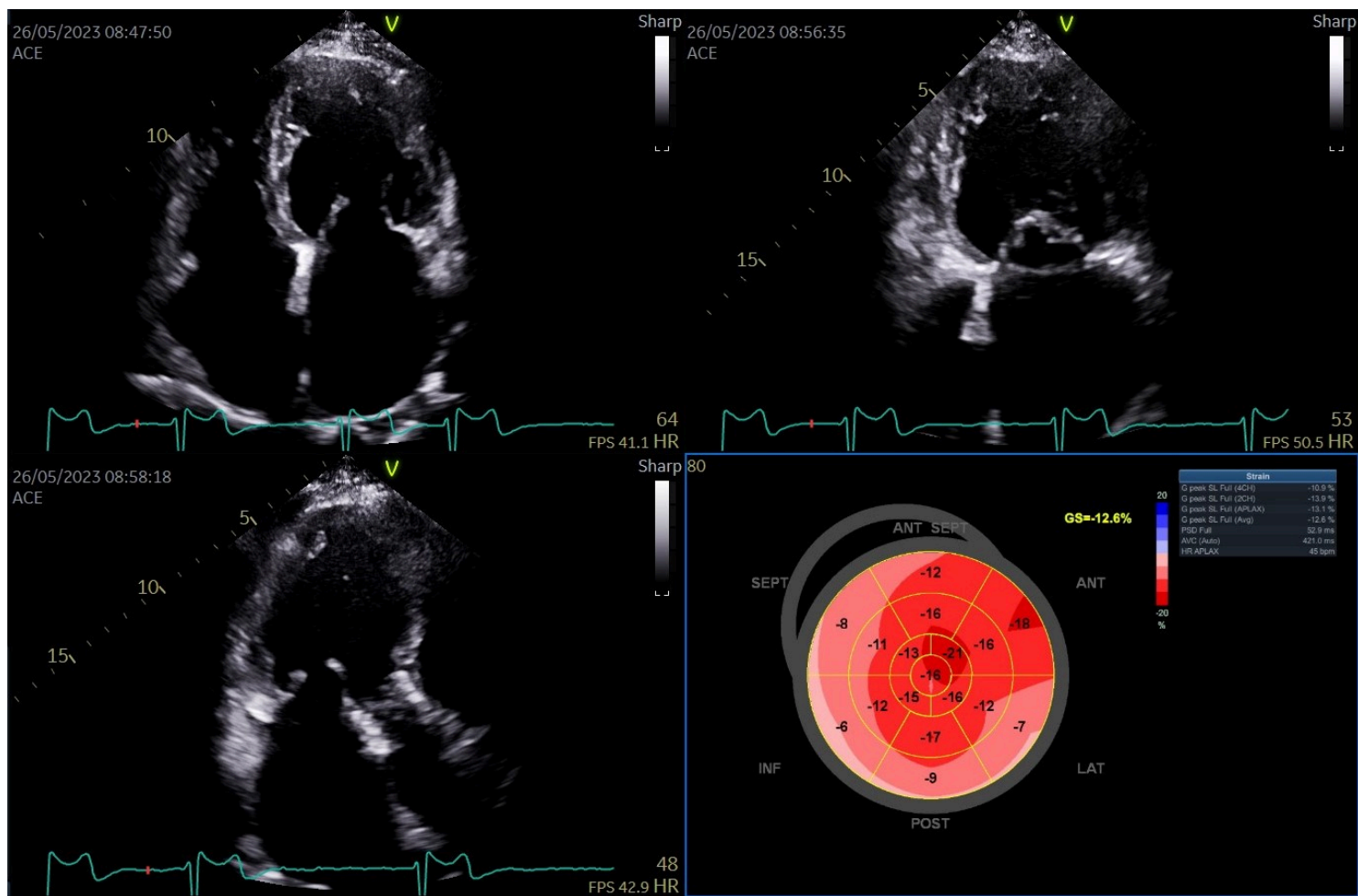


Figure 1

The calculation of myocardial deformation (strain) using the apical four, two and apical long axis. The strain result shown as a bulls-eye plot for a patient included in the study.

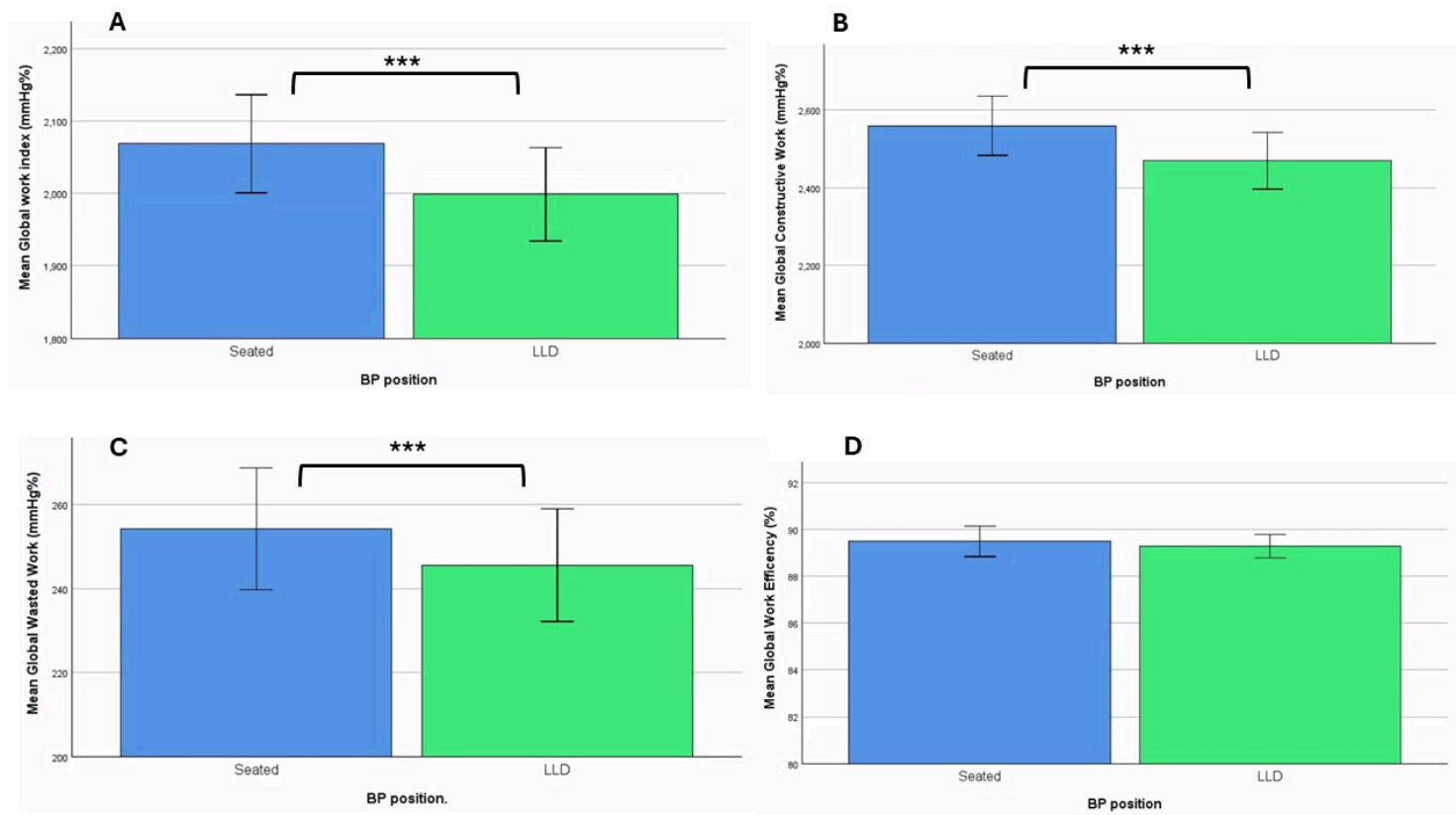


Figure 2

*Shows the mean values $\pm$ 95% CI for each myocardial work indices using BP recorded in the seated and left lateral decubitus (LLD) positions for the whole cohort. Graph A: Mean global work index (mmHg%). Graph B: global constructive work (mmHg%). Graph C: global wasted work (mmHg%). Graph D: global work efficiency (%). *** = $p < 0.001$.*

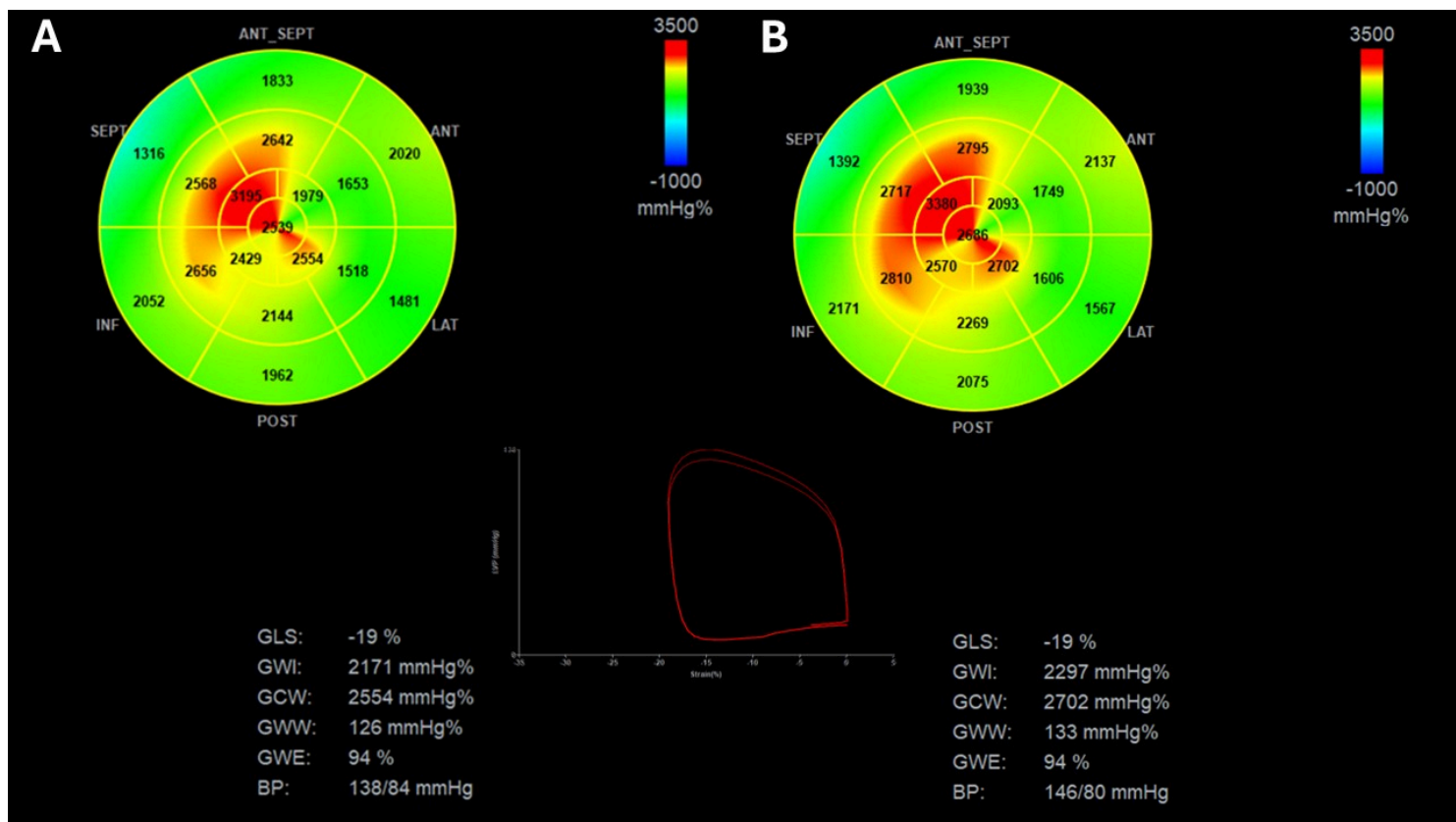


Figure 3

Assessment of myocardial work using blood pressure (BP) measurements taken in the seated position at the start of the transthoracic echocardiogram with a BP of 138/84mmHg (A) and in the left lateral decubitus (LLD) position at the end of the scan with a recorded BP of 146/80mmHg (B). The systolic blood pressure increased in the LLD compared to seated BP. Notice the small change in colour intensity at the apical inferoposterior wall of the bulls-eye plot and minor differences between the two superimposed strain-pressure loop traces for each BP.

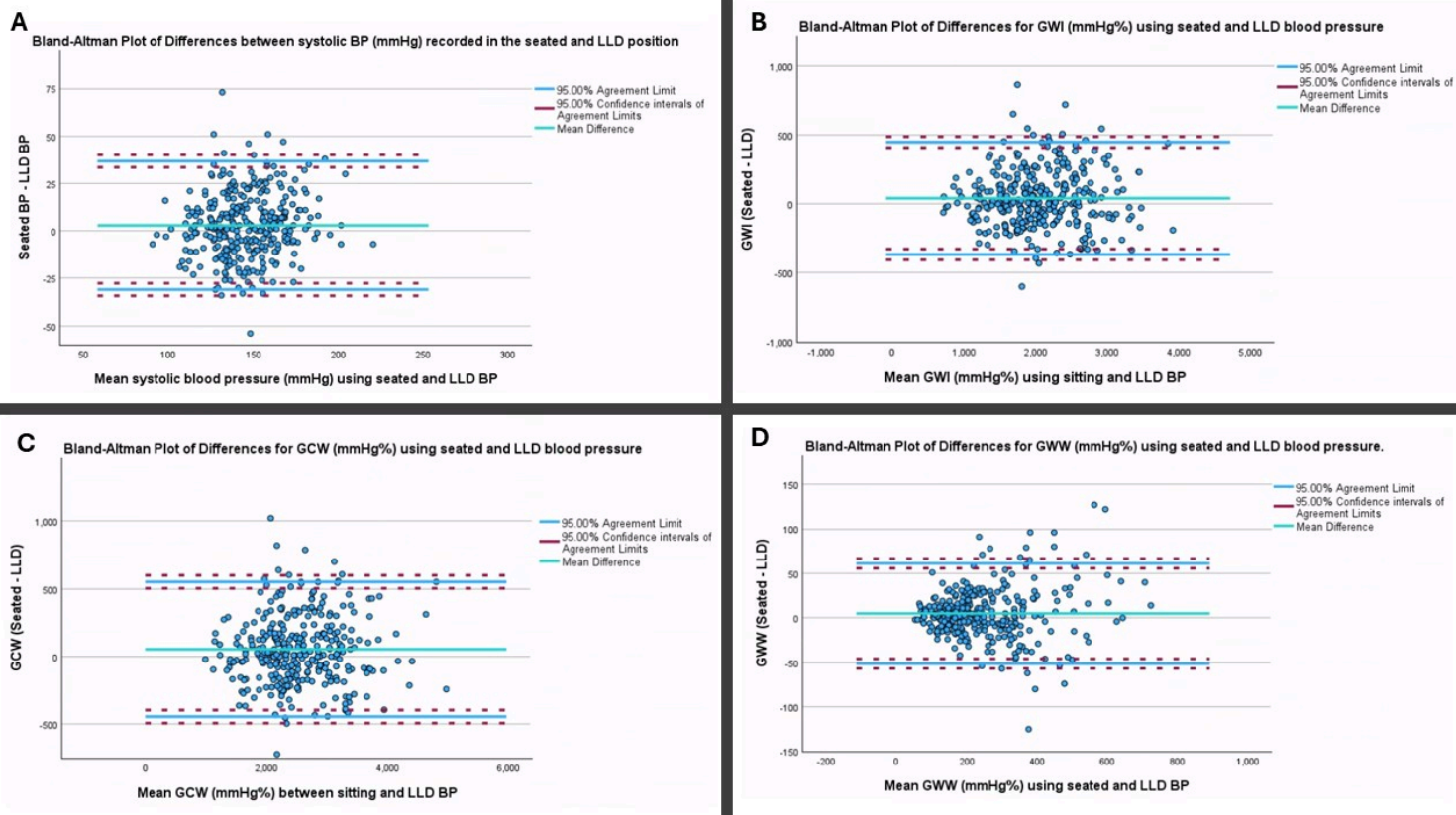


Figure 4

Bland-Altman plots comparing measurements for parameters using seated and left lateral decubitus positions for (A) systolic blood pressure (BP), (B) global work index (GWI), (C) global constructive work (GCW) and (D) global wasted work (GWW). For each panel, the cyan line shows the mean difference in each parameter using BP recorded in the seated and LLD positions, with upper and lower 95% limits of agreement shown by the dashed blue lines and 95% confidence intervals as red dashed lines.