The 2 minute Step Test: A reliable and valid measure of functional capacity in older adults post coronary revascularisation

ABSTRACT

Purpose: Exercise training is the central component in cardiac rehabilitation. A baseline assessment of aerobic capacity is paramount for exercise prescription and safety. The Two-Minute Step Test (2MST) has been used to measure aerobic capacity in healthy older adults. However, the reliability and validity of the 2MST in older adults post-coronary revascularisation (CRV) is unknown.

Methods: A prospective observational study was conducted in a single cardiac rehabilitation centre. Two 2MSTs and one six-minute walk test (6MWT) were completed in a single session. The 2MST measurements were recorded by two raters for each individual. The six-minute walk distance (6MWD) and 2MST steps recorded by both raters were analysed to determine the relationship and agreement between measurements.

Results: 31 participants with a median (IQR) age of 66 (62,73) years old were included in the study post CRV. Strong positive correlations were found between steps achieved during the 2MSTs and the 6MWD ($r= 0.87$, 95% CI 0.82 to 0.91, $p< 0.0001$). Excellent inter-rater reliability was demonstrated between raters during the 2MSTs (ICC = 0.999 to 1.000, $p=0.000$). Excellent relative test-retest reliability was demonstrated in both 2MSTs recorded by both raters (ICC = 0.927 to 0.934, $p=0.000$). However, absolute test-retest reliability may have been limited by a learning effect between repeated 2MSTs.

Conclusions: The results of this study indicate that the 2MST may be used as an alternative to the 6MWT as an outcome measure for aerobic capacity in older adults post-CRV. However, a practice trial is recommended at baseline to account for a learning effect.
INTRODUCTION

Cardiac Rehabilitation (CR) is the key to the comprehensive management of patients post-coronary revascularisation (CRV) and is a Class I intervention in individuals after MI\(^1\). As part of CR, exercise training has been reported to reduce mortality and readmissions and to improve aerobic capacity in older adults post CRV\(^3,4\).

Symptom-limited cardiopulmonary exercise testing (CPX) is the gold standard for assessing baseline exercise capacity and cardiovascular risk prior to exercise training in CR\(^4\). However, predictive submaximal tests such as the six-minute walk test (6MWT) are commonly used in the clinical setting to estimate VO\(_{2\text{max}}\) in older adults\(^6\) as they are more feasible and practical than face considerable difficulty completing the CPX\(^7\). In a Phase II/III CR population, the 6MWT has been shown to be a valid assessment tool in CR, with moderate correlations found between six-minute walk distance (6MWD) and maximal metabolic equivalents (METs) (r=0.687, p<0.001) obtained via a CPX\(^8\).

Considering possible space limitations, the two-minute step test (2MST) has been used as an alternative to the 6MWT to assess aerobic endurance, and is one of the components in the Senior Fitness Test (SFT), an assessment tool designed for independent older adults aged 60 years and above with less than two chronic conditions\(^11\). 2MST scores showed moderate correlations to submaximal treadmill test performance using the Modified Balke’s treadmill protocol (r=0.74, no p value reported), quadriceps isometric strength (r=0.61, p<0.001) and time measured during the TUG test (r=-0.66, p=0.000). Relative test-retest reliability of the 2MST was reported to be excellent in the general older adult population (ICC= 0.90-0.95)\(^11\). Similar results were also seen in another study where the 2MST showed a moderate positive correlation to quadriceps strength (r=0.61, p<0.001) and was reported to be valid against the 6MWT (r=0.44, p<0.001) in patients with stable heart failure\(^26\). Peak VO2 measured by CPX
performed according to the Bruce protocol and 2MST showed a strong correlation ($r=0.70, P<0.001$) in obese with comorbidities and morbidly obese patients$^{40}$. However, there remains a gap regarding its reliability and validity when used with older adults, aged 60 years old and above post-CRV. Given the importance of exercise testing in CR, there is a need for the 2MST to be validated in this population for its use as an alternative test to the 6MWT. It was hypothesised that the 2MST would be a reliable and valid outcome measure for measuring submaximal functional capacity in older adults post-CRV.

Studies included in present review (n=4)
METHODS

Consecutive patients referred for outpatient CR at the Tan Tock Seng Hospital (TTSH) Cardiology clinic were invited to participate in this prospective observational study. Patients were referred to the programme by their attending cardiologist for rehabilitation after a coronary event.

The criteria for inclusion were: older adults aged 60 years and above, independently ambulant with or without a walking aid and had undergone CRV which includes Coronary Artery Bypass Grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), valve replacement or a combination of these.

Patients were excluded if they had; deficits in cognitive function, medical diagnosis of lung disease(s), heart failure, unstable angina, arrhythmias, severe aortic stenosis, mitral regurgitation, aortic aneurysm, recent embolism, active myocarditis or pericarditis or other comorbidities limiting functional testing.

Ethics approval was obtained from the National Healthcare Group Domain Specific Review Board (NHG DSRB Ref: 2015/01101) and from the University College London (UCL) research ethics committee (Project ID: 8225/001). Informed consent was obtained from all participants for this study.

Procedures and standardised instructions (Appendix A and B) for each test were given before addressing all questions from the participants regarding the study.

PROCEDURE

The assessment of each participant was performed in a single day, during the first physiotherapy assessment conducted three weeks after hospital discharge. All three tests (two
2MSTs and one 6MWT) were completed in a single session as according to the procedural flowchart (Appendix C and D).

Tests were administered in the same order for all participants. A single measurement of resting blood pressure (BP), Heart Rate (HR) and Rating of Perceived Exertion (RPE) using the Borg’s CR-10 scale (Appendix E) were taken in sitting, five minutes before the start of each test.

After baseline vital signs were measured, participants completed the first 2 Minute Step Test (2MST1). After a 15-minute rest interval, participants then underwent the 6MWT. The second 2 Minute Step Test (2MST2) was conducted 15 minutes after the 6MWT was completed. The perceived level of dyspnoea was assessed using the Borg’s CR-10 scale immediately at the end of the 2MST and at the 3rd and 6th minute of the 6MWT.

**2-minute step test**

The aim of the 2MST is to determine the number of times an individual can march on the spot within two minutes according to the protocol established by Rikli & Jones\(^{10}\) (Appendix F). For those who were ambulant with a walking aid and for those who required support, a stable chair was placed next to the participant if required to reduce fall risk. The test was demonstrated once to the participants and instructions were given as established by Jones and Rikli\(^{14}\) (Appendix B).

Both investigators recorded the number of performed steps independently with handheld tally counters (Tally Counter SPR24100, SP.Richards Company, Smyrna, United States of America). Blinding was ensured as the tally counter of each investigator was not within the visual field of the other investigator throughout the test. The end result of the test was expressed as the number of steps performed during the two minutes.
6-minute walk test

The aim of the 6MWT is to assess submaximal level of functional capacity by measuring the distance an individual can quickly walk on a flat, hard surface in the period of six minutes. The 6MWT was carried out using a 15 m hospital walkway, with floor markings at every metre. In view of space limitation, guidelines have been established, advocating the administration of the 6MWT on a course of at least 10 m\textsuperscript{15}. Standardised instructions were given throughout the test according to guidelines by the American Thoracic Society (ATS)\textsuperscript{9} (Appendix A). HR and oxygen saturation were measured using a pulse oximeter (PalmSAT® 2500 Nonin Medical, United States of America) and RPE using Borg’s CR-10 score at the third and sixth minute. The end result of the test was expressed as the total distance covered by the participants, measured to the nearest metre.

Sample size and power calculation

Power calculations were performed to estimate the appropriate sample size for this study using MedCalc (MedCalc, Belgium). Based on ICCs obtained\textsuperscript{11}, to demonstrate an ICC of at least 0.70 and to achieve at least 90% power in a 2-sided test at 5% significance level, a minimum of 17 subjects were required.

Statistical Analysis

Data analysis and diagrammatic presentation were performed using the statistical software SPSS version 22.0 (IBM, Portsmouth, UK) and MedCalc (Mariakerke, Belgium). Convergent validity was examined using Pearson or Spearman rank correlations to examine the association between the 2MST and the 6MWT based on the normality of the data when assessed.
Relative Test-retest reliability and inter-rater reliability of the 2MST were examined via Intra-class coefficients (ICC) with 95% confidence interval (CI) for a single trial using a two-way mixed model. Agreement between the two measurements was examined by constructing a 95% limits of agreement (LOA) plot. A change in mean analysis was used to compare the two 2MST measurements to assess for any ‘systematic differences’. Further analysis on absolute reliability of the 2MST scores across trials was conducted using repeated measures analysis of variance (ANOVA) depending on sphericity of the data.

To quantify the amount of change in 2MST steps that must be observed to be considered to have exceeded measurement error, the MDC was calculated at both the 90% (MDC_{90}) and 95% (MDC_{95}) confidence intervals using SEM values calculated.

The SEM was calculated using the following equation:

\[
SEM = SD \sqrt{(1 - ICC)}
\]

The MDC was then calculated using the SEM derived from the equations:

\[
MDC_{95} = SEM \times 1.96 \times \sqrt{2} \text{ and}
\]

\[
MDC_{90} = SEM \times 1.65 \times \sqrt{2}
\]

Statistical significance for all tests was set at p<0.05 in a two-sided test.
RESULTS

PARTICIPANTS

Thirty-five patients who were referred for CR at the Cardiology clinic in TTSH Singapore met the inclusion criteria and were eligible to be included in the study. Four patients were excluded due to refusal to participate. All thirty-one participants completed all three tests (2MST, 6MWT, 2MST) within one single testing session. Baseline descriptive characteristics were obtained for each participant (Table 1).

All three tests were well tolerated by all the study participants with no adverse events reported. None of the participants reported any chest pain, palpitations or worsening cardiac or physical symptoms during testing. The results of all the exercise tests’ performance are presented in Table 2.

VALIDITY

The 2MST demonstrated high convergent validity with the 6MWT from a pooled correlation coefficient of $r = 0.87$ (95% CI 0.82 to 0.91, $p < 0.0001$). Participants who achieved longer distances in the 6MWT performed more steps during both 2MST1 and 2MST2. Strong positive correlations between the 6MWT and 2MST were found in measurements taken by both raters ($r = 0.86 \text{ to } 0.88$) (Table 3). The linear relationship between the 6MWT and the 2MST is further illustrated in Figure 1.

RELIABILITY

Excellent inter-rater reliability was demonstrated between both raters for the 2MST1 and 2MST2 number of steps recorded (ICC =
0.999 to 1.000, \( p=0.000 \). ICC values of \( \geq 0.90 \) represent “very high reliability” \(^{22}\) and an ICC of 1.00 corresponds to perfect agreement \(^{19}\).

Rater 1 counted on average 0.16 less steps for 2MST1 and 0.42 steps less for 2MST2 compared to Rater 2, with a SD suggesting they may be up to one step out on their total counts. Bland-Altman plots highlighting the difference in 2MST scores against the mean 2MST scores (Figure 2) between-raters displayed a bias of -0.2 (95% LOA -2.4, 2.1) to -0.4 (95% LOA -2, -1.2) and summarises the agreement between both raters with >95% of differences lying within ± 1.96SD. The bias for 2MST1 is likely to be not significant as the line of equality is within the CI of the mean difference \(^{23}\).

**Test-retest reliability**

Excellent test-retest reliability was also demonstrated between the number of steps achieved in both 2MST1 and 2MST2 (ICC = 0.962 to 0.966, \( p=0.000 \)). There was an increase in steps from a mean (SD) of 7.48(9.80) to 7.74(10.4) achieved during 2MST2 compared to 2MST1 which proportionally represented a mean (SD) rise of 11.3(15.0) to 11.6 (15.7) %.

Changes in mean values between two tests can consist of a ‘systematic difference’ which is a participant performing better (or worse) during the second test due to factors such as fatigue or learning effect \(^{18}\). As the 95% CI of the mean difference in the results do not include zero, this indicates a systematic difference.

Bland-Altman plots representing the difference in 2MST test scores against the mean 2MST scores (Figure 3) measured during 2MST1 and 2MST2 displayed a bias of -7.5 to -7.7 steps. This bias is likely to be significant as the line of equality is not within the CI of the mean difference \(^{23}\). Agreement in 2MST scores from both trials is shown with majority >95% of differences lying within ± 1.96SD. Precision of measurements are associated with how close repeated measurements are to each other \(^{24}\). From the analysis, the 95% LOA ranging from -
26.7 to 11.7 and -28.1 to 12.6 is wide suggesting that the precision of the 2MST measurements is low.

Based on the data analysis using the Mauchly’s test, the assumption of sphericity has been met from no significant values reported due to results from two time-points which amounts to perfect sphericity\textsuperscript{25}.

Using an ANOVA with repeated measures, the results show that there were significant changes in 2MST steps over time measured by both Rater 1 and Rater 2, $F(1.000, 30.000) = 18.088, p=0.000$ and $F(1.000, 30.000) = 17.290, p=0.000$ respectively. The SEM and MDC values are presented in Table 4.

The SEM, which estimates the difference between measurements obtained from repeated testing and an individual’s ‘true’ score, ranged from 0.992 to 1.084 steps. From the MDC\textsubscript{95} calculated, a change in 2MST steps of 2.75-3.00 should be achieved to be 95% confident that the change represents a ‘true change’\textsuperscript{19}. The bias of -7.5 to -7.7 steps highlighted in the previous Bland-Altman plot between the two 2MST trials exceeds the MDC calculated indicating that the mean difference in measurements is not attributed to measurement error. This possible systematic differences between the two 2MSTs suggests a learning effect.
DISCUSSION

This is the first study investigating the reliability and the validity of the 2MST as an assessment tool to measure aerobic capacity in older adults who underwent CRV.

CONVERGENT VALIDITY

There were strong, positive correlations between the 6MWD and the steps achieved during both 2MSTs found in this study. This was contrary to the findings by Wegrzynowska-Teodorczyk et al (2016)\textsuperscript{26} and Pedrosa and Holanda (2009)\textsuperscript{27} in which low correlations were reported between the 6MWD and 2MST steps as will consequently be discussed.

Differences in disease-associated limitations to test performance

A higher level of leg fatigue experienced during the 2MST compared to the 6MWT was suggested to contribute to low correlations in the study by Wegrzynowska-Teodorczyk et al (2016) on Chronic Heart Failure (CHF) patients. Leg fatigue is commonly reported as a symptom of skeletal muscle dysfunction (SKD)\textsuperscript{28}. This often limits exercise in CHF due the inability to increase cardiac output (CO) appropriately during exercise which results in an insufficient increase in perfusion to exercising muscle leading to early anaerobic metabolism and muscle fatigue\textsuperscript{29}. In this study, it is unclear if subjective ratings of leg fatigue differed between the 6MWT and 2MST as it was not within the objectives to measure leg fatigue. However, SKD may not be the dominant factor limiting exercise test performance in this population of individuals who suffered an acute episode of reduced myocardial perfusion.

CRV can restore functional status by significantly improving angina symptoms and ischaemic heart disease symptoms such as shortness of breath\textsuperscript{30}. In patients post-CRV, test performance may instead be limited by a reduction in aerobic capacity due to factors such as deconditioning from peri-operative stress and prolonged immobility post procedure\textsuperscript{31} rather
than SKD arising from chronic adaptations. With the probability of a slower onset of leg fatigue during the 2MST in patients post-CRV compared to CHF due to reduced likelihood of SKD, this would have led to stronger correlations obtained between the 2MST steps and 6MWD in this study. It must also be considered that differences in correlations may have been contributed to by inclusion of a younger population (mean (SD) of 59(12) years vs median (range) 66(62,73)) with less co-morbidities (hypertension: 49% vs 67.7%, diabetes mellitus: 22% vs 45.2%).

**RELIABILITY**

**Inter-rater reliability**

Although the 2MST were administered by different raters in earlier studies, inter-rater reliability was not established prior to test-retest reliability. Therefore, the difference in scores in the 2MSTs may be attributable to variability between raters instead of a true difference in scores. In this study, the same raters were used throughout the two 2MSTs and excellent reliability was found between them. Based on these results, this could contribute to the evidence of the use of different raters when administering the 2MST which is more reflective of clinical practice.

**Test-retest reliability**

The results of this study complement the results previously reported for the relative reliability of the 2MST in community-dwelling older adults 60-94 years of age by Rikli and Jones (2001) (ICC=0.90) and Miotto et al (1999) (ICC=0.95). In comparison to these studies where there was a time interval of at least two days between tests, this study found evidence of excellent relative test-retest reliability of the 2MST within a single session. However, test performance in the second trial was observed to be better than the first trial, with a mean (SD) increase in scores of 11.3(15.0) % to 11.6(15.7) % demonstrated in the results.
Repeated measurements of the 2MST displayed good agreement between scores. However, when the scores of both trials were compared, there was a statistically significant change in scores from the first to the second 2MST. Despite good agreements displayed in the Bland-Altman plot, there was a tendency of higher scores in the 2MST2 compared to the 2MST1. The wide 95% LOA reported also suggests that the precision of measurements was low, showing evidence of a large variance in scores from the first to the second 2MST. This large degree of variance in measurements could be attributed to a systematic difference, limiting the absolute reliability of the 2MST.

Systematic difference in repeated 2MST measurements was first reported by Dugas (1996) who investigated the relative reliability between three 2MST trials. From higher ICCs obtained from the second and third trial, it was concluded that an initial learning effect exist with repeated 2MST administration. When the 2MST was repeated three times with a time interval of two to five days between tests in the study by Miotto et al (1999), there were significant differences between the first 2MST and the third 2MST. No significant differences were found between the first and second trial as well as the second and third trial. Despite significant differences between the first and third trials, excellent relative reliability were found across the three trials (ICC=0.96). However, no study reported on the absolute reliability of the 2MST which could be a better analysis in highlighting systematic difference. Hence, from these studies, it was difficult to justify the need for a practice trial in the 2MST.

The systematic change in mean scores from the first to the second 2MST in this study combined with the findings from earlier studies would suggest that there is evidence of a learning effect which should be taken into account when using the 2MST as an outcome measure. Despite the need for a practice test, the administration of two 2MSTs would likely be favourable in the clinical setting as it is more efficient compared to other validated
outcome measures used in CR such as the 6MWT and the incremental shuttle walk test (ISWT) which warrants practice tests as well due to a learning effect\textsuperscript{38,39}. 
CONCLUSION

The convergent validity of the 2MST against the 6MWD was shown by a strong and significant relationship between steps achieved in the 2MST and the 6MWT in this study. Excellent inter-rater reliability of the 2MST was demonstrated in this study. Excellent relative test-retest reliability of the 2MST was also demonstrated in this study. However, despite good agreements between scores measured between the 2MSTs, absolute test-retest reliability was limited by evidence of a systematic difference between tests, suggesting a learning effect in repeated 2MSTs.

The 2MST can be used as an alternative to the 6MWT as an outcome measure for aerobic capacity in older adults post-CRV. However, a practice trial is recommended at baseline to account for a learning effect.’

IMPLICATION ON PHYSIOTHERAPY PRACTICE

The use of the 2MST in CR based on the results from this study are limited to older adults aged 60 and above who had undergone CRV and cannot be generalised to all patients attending CR. With the 2MST scores obtained, reference to the normative values developed by Rikli and Jones (2001) can be made to determine if the individual’s aerobic capacity is normal if the score falls under “normal” according to the individual’s age. As there are no MCID values available currently, any increase in scores is considered as an improvement when using the 2MST as an outcome measure in CR.

In this study, two participants had undergone a CABG surgery where a left internal mammary artery graft was used for the bypass. Hence, results from this study cannot be generalised to all patients who have undergone a CABG due to limitations they may face while carrying out 2MST compared to 6MWT due to restrictions from the leg vein harvesting site.
REFERENCES


Figure Legend

Figure 1: Scatterplot displaying the relationship between 2MST steps and 6MWD

Figure 2: Bland-Altman Plots displaying differences in steps recorded by raters during 2MST1 and 2MST2

Figure 3: Bland-Altman Plot displaying difference in steps recorded during 2MST and 2MST2 by Rater 1 (Left) and Rater 2 (right)
Table Legend

Table 1: Baseline descriptive characteristics

Table 2: Exercise test performance assessed by 6MWT and 2MST of patients post CRV (n=31)

Table 3: Correlations between six minute walk distance (6MWD) and steps achieved during 2MST

Table 4: ICC, Standard error of measurement for repeated measures (SEM) and minimal detectable change (MDC) scores at the 95% and 90% CI for the 2MST
Appendices

Appendix A: 6MWT

Standardized Instructions:

“The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I’m going to show you. Please watch the way I turn without hesitation.” Demonstrate by walking one lap yourself. Walk and pivot around a cone briskly. “Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don’t run or jog. Start now, or whenever you are ready.”

Stop test if the following are observed: chest pain, intolerable dyspnea, leg cramps, staggering, diaphoresis, and pale or ashen appearance.

Standardized Encouragement:

After every minute, tell the patient the following (in even tones):

“You are doing well. You have _ minutes to go.”

Do not use other words of encouragement (or body language to speed up).
If the patient stops walking during the test and needs a rest, say this: “You can lean against the wall if you would like; then continue walking whenever you feel able.” Do not stop the timer.

If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping prematurely.

When the timer is 15 seconds from completion, say this:

“In a moment I’m going to tell you to stop. When I do, just stop right where you are and I will come to you.” When the timer rings (or buzzes), say this: “Stop!” Walk over to the patient.

Consider taking the chair if they look exhausted.

Mark the spot where they stopped by placing a bean bag or a piece of tape on the floor.

Appendix B: 2 Minute Step test

Standardized Instructions:

1. Stand next to a wall. Measure the height of the iliac crest and patella and mark it on the wall. Then place a piece of tape on the wall half the distance between the two.
2. When I say “go”, raise each knee to the mark on the wall, for as many times as possible in the 2 minute period
3. I will be counting the number of times your right knee reaches the required height and that will be your score.
4. If you cannot maintain the proper knee height cannot be maintained, I will ask you to slow down, or to stop until you can regain the proper form, but the stopwatch will continue to run.
5. At the end of the test, you can walk slowly for a minute to cool down
6. If you feel unsteady you may hold on to the back of a chair for stability
7. After this test, you will rest and we will measure your vital signs
8. This test will be repeated after 5 minutes
Appendix C: Flowchart of Procedure (New patients)

- Patients referred for Cardiac rehabilitation at Clinic 3B
- Meets Inclusion Criteria
  Participants who went through coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty, valve replacement or a combination of the above
  - Physiotherapist (PT) in charge approaches participant and attains approval before Informed consent is obtained by the primary investigator
  - Proceed with normal standard PT initial assessment by PT in charge. Both investigators will then conduct the first 2MST and 6MWT with a 15-minute interval between tests, ensuring that vitals return to baseline
- Meets Exclusion Criteria
  Patients with deficits in cognitive function, medical diagnosis of lung diseases, heart failure, unstable angina, arrhythmias, severe aortic stenosis or mitral regurgitation, aortic
- Refused informed consent
  Proceed with normal standard PT initial assessment
Appendix D: Flowchart of Procedure (Current Patients)

Both investigators will proceed with measuring the second 2MST.

15-minute rest interval (for continuation of usual patient education)

End of session. Vitals will be checked to have returned to baseline before patient leaves the clinic

Patients currently undergoing weekly cardiac rehabilitation exercise sessions

Meets Exclusion Criteria
Patients with deficits in cognitive function, medical diagnosis of lung diseases, heart failure, unstable angina, arrhythmias, severe aortic stenosis or mitral regurgitation, aortic aneurysm, recent embolism, active myocarditis or pericarditis or other comorbidities limiting functional testing are excluded.

Meets Inclusion Criteria
Participants who went through coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty, valve replacement or a combination of the above

PT in charge identifies suitable participants 1 week before and asks participants for willingness to participate in study

Refused
Proceed with exercise sessions as per normal by PT in charge

Agreed
Participants are informed to return the following week on a scheduled visit and report 40-minutes before appointment time. Informed consent is obtained by the primary investigator on the day itself
Both investigators will proceed with measuring the 2MST\textsubscript{1}, 6MWT and 2MST\textsubscript{2} with a 15-minute interval between tests, ensuring that vitals returned to baseline before initiating the next test.

End of session. Vitals will be checked to have returned to baseline before patient proceeds with exercise class.

Appendix E: Borg's CK-10 scale
<table>
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<th>Description</th>
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<td>None</td>
</tr>
<tr>
<td>0.5</td>
<td>Very, very light</td>
</tr>
<tr>
<td>1</td>
<td>Very light</td>
</tr>
<tr>
<td>2</td>
<td>Light</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>A little intense</td>
</tr>
<tr>
<td>5</td>
<td>Intense</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
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<td>9</td>
<td>Very, very intense</td>
</tr>
<tr>
<td>10</td>
<td>Maximum</td>
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</tbody>
</table>

Appendix F: Standardised 2MST protocol
2-Minute Step-in-Place (An Alternative to the 6-Min Walk Test)

Purpose. To assess aerobic endurance.

Equipment. Stopwatch, tape measure or 30-in. piece of cord, masking tape, mechanical counter (if possible) to ensure accurate counting of steps.

Set-Up. The proper (minimum) knee-stepping height for each participant is at a level even with the midway point between the patella (middle of the knee cap) and the iliac crest (top hip bone). This point can be determined using a tape measure or by simply stretching a piece of cord from the patella to the iliac crest, then folding it in half to determine the midway point. To monitor correct knee height when stepping, books can be stacked on an adjacent table or a ruler can be attached to a chair or wall with masking tape to mark the proper knee height.

Protocol. On the signal "go" the participant begins stepping (not running) in place, starting with the right leg, and completes as many steps as possible within the time period. Although both knees must be raised to the correct height to be counted, the tester only counts the number of times the right knee reaches it. The counter also serves as a spotter in case of loss of balance and ensures that the participant maintains proper knee height. As soon as proper knee height can no longer be maintained, the participant is asked to stop—or to stop and rest until proper form can be regained. Stepping may be resumed if the 2-min time period has not elapsed. If necessary, the participant can place one hand on the table or chair to assist in maintaining balance.

To assist with proper pacing and to improve scoring accuracy, a practice test should be given prior to the test day. On test day, the examiner should demonstrate the procedure and allow the participants to practice briefly to recheck their understanding of the protocol.

Safety. At the end of the test the participant should slowly walk around for about a minute to cool down.

Scoring. The score is the total number of times the right knee reaches the minimum height. To assist with pacing, participants should be told when 1 min has passed and when there are 30 s to go.