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Original Article

Physical function measurement in older long-term cancer survivors

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Abstract

Objective: To establish reliability, validity, and minimal detectable change in measures of function in older long-term cancer survivors. **Methods**: Older cancer survivors were recruited to perform functional measures; 5 Times Sit-to-Stand (5xSTS), 30-second Timed Chair Rise (30sTCR), Short Physical Performance Battery (SPPB), Physical Performance Test-7 (PPT-7). Two testing sessions were completed two weeks apart. Test-retest reliability was examined using the intraclass correlation coefficient ($ICC_{2,1}$), convergent and discriminant validity using Spearman's rho and Minimal Detectable Change (MDC^{95}) was calculated. **Results**: Forty-seven older long-term cancer survivors participated. Test-retest reliability was good for 5xSTS ($ICC_{2,1}$=0.86), 30sTCR ($ICC_{2,1}$=0.89), and SPPB ($ICC_{2,1}$=0.85) and poor for PPT-7 ($ICC_{2,1}$=0.48). Both convergent and discriminant validity was established. **Conclusions**: SPPB, 5xSTS, and 30sTCR are reliable and valid tools to measure function in older long-term cancer survivors. MDC^{95} values were larger than those reported in geriatrics and should be interpreted with caution. Residual effects of cancer treatment, comorbidity, and physical inactivity may contribute to decreased physical function in older long-term cancer survivors, therefore valid and reliable measures like SPPB and the timed chair rise tests should be used objectively measure function throughout the survivorship spectrum.

Keywords: Long-term Cancer Survivor, Neoplasms, Physical Functional Performance, Reliability, Validity

Introduction

Older cancer survivors often face physical conditions that impact function. Cancer and its treatment negatively affect multiple body systems, contributing to impaired functional mobility in cancer survivors[1-5]. With aging, the presence of comorbidity further exacerbates functional declines, putting older cancer survivors at an increased risk for impaired physical function in the years following cancer treatment[3,5,6]. As the long-term survival rates are increasing, cancer has been called a disease of older people[7]. In the United States, nearly two-thirds (64%) of cancer survivors are estimated to be 65 years of age or older[7]. With the increasing number of older cancer survivors, declines in physical function in this population are a large concern[4,8].

In long-term cancer survivors (>10 years since diagnosis), a number of disease associated sequalae are reported which impact physical function and activities of daily living (ADL) performance[9], of which all are influenced by age[10]. Decreased pulmonary function and endurance as well as changes in cognition are reported in long-term survivors of breast cancer and more often in those who received chemotherapy. Deficits in functioning and global health are described in long-term colorectal cancer survivors with more impairment with advanced age[11]. Other symptoms reported across long-term survivors[12] include chronic pain, fatigue[13], and, for those who received chemotherapy interventions, peripheral neuropathy[14], all of which influence overall physical function, balance, and mobility. Additional evidence suggests that physical inactivity is associated with low health-related quality of life in long-term cancer survivors with increased comorbidity and further highlights the need for survivorship care that goes beyond the time of active treatment of the disease and rehabilitation[15]. Although the comprehensive

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geriatric assessment (CGA) is often used initially to detect physical, emotional, cognitive, functional, and psychological issues that may impact diagnosis and treatment[16,17], the presence of long-term effects of cancer on function are evident, yet a consistent mode of measurement is lacking.

While inconsistencies on the definition of physical function in rehabilitation exist in the literature, a recent review of physical function in cancer provides the most common definition[18]. Painter et al. describes physical function as "the ability to perform the basic actions that are essential for maintaining independence and carrying out more complex activities"[18,19]. Declines in physical function in older cancer survivors, therefore, lead to impaired ADL performance, including walking, getting out of a chair or bed (transfers), and personal hygiene[9]. This leads to decreased independence and health-related quality of life and increased mortality[9]. Due to its widespread impact on survivorship, physical function has become a prognostic biomarker in cancer[20]. To address this, exercise and rehabilitative interventions have been reported to promote physical function and improve cancer survivorship[18]. Therefore, older cancer survivors' functional ability should be assessed at set points throughout the survivorship spectrum.

Physical function can be assessed in older adults through either performance of specific functional movements (e.g. sit-to-stand or floor to standing transfers) or by using more comprehensive tools that include a number of functional tasks. For example, the ability to complete multiple sit-to-stand transfers can be assessed through the 5 times sit-to-stand (5xSTS) or the 30-second timed chair rise (30sTCR) tests. These measures both examine lower extremity strength, provide an objective measurement that reflects the individual's transfer capabilities, and may also predict falls in older adults[21-23]. However, another physical function measure, the Short Physical Performance Battery (SPPB), includes not only a sit-to-stand task, but also assessments of balance and gait, thus providing more physical function information[24-27]. An even more comprehensive measure, the Physical Performance Test-7 (PPT-7), has additional items assessing independence in ADL performance[28]. Two systematic reviews were published, one in 2016 and later 2019, describing the clinical utility of measures of function in cancer survivors[29,30]. From those reviews, the authors designated the 5xSTS and SPPB as 'highly recommended or recommended for clinical use' in prostate or breast cancer survivors. However, these measures lack established validity and reliability in older long-term cancer survivors and the evidence describing the clinical utility of other measures of function is lacking. In addition, studies describing the minimal detectable change needed to reflect a true change or improvement in function are limited.

Although the reliability and validity of measures of physical function are established in older adults[23,24,28,31] these properties are not well described in cancer survivors and specifically not in older long-term survivors where impairments or limitations differ from those in the early stage of cancer survivorship let alone those without a history of cancer[5,9]. Therefore, the purpose of this study is to establish test-retest reliability, construct validity, and minimal detectable change of physical functional measures in a mixed group of older long-term cancer survivors.

Methods

Participants

Older cancer survivors ages 65 years and above were recruited from the community as a part of a larger study[32]. Recruitment occurred through advertisements, flyers, and word-of-mouth. Inclusion criteria included being age 65 years or more, English speaking, having a medically confirmed diagnosis of breast, lung, prostate or colorectal cancer, completion of the primary cancer treatment at least 3 months prior to testing, and being able to get up from a chair, stand, and walk 50 feet with or without the use of an assistive device. Participants were excluded if they reported a cancer recurrence or metastasis, a history of chronic neurologic condition (Parkinson's disease, stroke), more than 1 cancer diagnosis, acute illness, or having an unstable medical condition.

Sample Size

Sample size was calculated based on a power of 0.8 and an alpha level of 0.05 (2-tailed). For the reliability analysis, the sample size was estimated using 2 trials of testing by the same rater for test-retest reliability, 2 observations for each participant to create the interclass correlation coefficient ($ICC_{2,1}$). Nineteen participants were required for test-retest reliability analyses. For convergent validity, G*Power 3.1 was used to determine sample size (N=26) assuming a large effect size (p=0.50)[33].

Procedure

Participants individually attended a testing session that was held in a university research lab or in a private room at a senior center. The testing session began with interviewing the participant about demographic information and medical history. The number of comorbidities were used to create a functional comorbidity index (FCI). FCI is a sum of 18 self-reported comorbid conditions with a score of 0-18 with higher scores indicating greater comorbidity[34]. Fall history was also collected and participants were asked to answer yes/no if they had any falls in the past 6 months prior to testing. If they answered "yes," they were asked if they had an injurious fall. Falls were defined as a loss of balance which resulted in a person coming to rest inadvertently on the ground or floor or other lower level.

The Veterans Rand-12 health survey was then completed and physical functional measures were performed. Measures were performed in the same order for each participant. Breaks were given in between

physical tests and measures on an as-needed basis to avoid fatigue. Each measure was performed once to limit the impact of fatigue on performance. One research team member performed the tests and measures and a second researcher recorded the data. All research team members were trained using the same training procedure. The participant returned to the same testing location two weeks later for a second testing session and completed the same tests and measures. At the beginning of the second session, participants were asked if there was a change in health status or falls within the two weeks between testing sessions and their responses were recorded.

Outcome Measures

30-Second Timed Chair Rise (3osTCR)

The 30sTCR is considered as a test of functional fitness and muscular endurance[35]. To perform this measure, the participant began seated with their arms crossed across their chest. When they were told "go" they stood up and sat back down without using their arms as many times as they could in 30 seconds. The tester began timing when they said "go" and counted how many full sit-to-stand repetitions the participant completed in 30 seconds. This test has reported test-retest reliability and criterion validity in community-dwelling older adults[23] and excellent test-retest reliability but poor concurrent validity in head and neck cancer survivors[30].

5 Times Sit-To-Stand (5xSTS)

The 5xSTS, a measure of functional strength and anticipatory balance[35], was performed simultaneously with the 30sTCR to avoid fatigue if completed separately. While the participant performed the 30sTCR, the tester recorded the time that it took to complete the first five sit-to-stand repetitions. Timing for the 5xSTS began when the tester said "go" and stopped when the participant's body touched the chair for the fifth repetition. If the participant was unable to stand without using their hands, their time was not recorded and that was considered a failed test. The 5xSTS has established test-retest reliability[22,31] and construct validity[31,36] in community-dwelling older adults.

Short Physical Performance Battery (SPPB)

The SPPB is a three-part test involving balance, gait speed, and repeated chair stand assessments. Each category is scored 0-4 depending on the time that the task is completed, resulting in a total score of 0-12 points. Higher scores indicate better function. The balance portion involved standing in three positions, feet together, semi-tandem, and tandem, for as long as the participant was able up to 10 seconds. The gait speed portion involved walking at their normal pace for 3 meters, with the time recorded from when they began walking to when they crossed the 3 meter line. Lastly, the repeated chair stand test similar to the 5xSTS with arm position, however timing ended when the participant stood up for the 5th time. The individual scores for component of the measure were summed to create the total SPPB score[37]. This measure has established test-retest reliability and convergent validity in community-dwelling older adults[24].

Physical Performance Test-7 (PPT-7)

PPT-7 is a 7-item test of physical function with standardized instructions for administering the measure. The test involves standing balance, chair rise performance, lifting a book and putting it on a shelf, donning and doffing a jacket, picking up a penny from the floor, turning 360 degrees, and walking 50 feet. Each item is scored 0-4 based on the time that it takes the participant to complete that item with the exception of the 360-degree turn, which is scored based on the quality of movement. The total score for this test can range from 0-28. PPT-7 has established test-retest reliability and construct validity in frail older adults[28]. A similar version of this test, the Physical Performance Test Battery (PPTB), has good to excellent test-retest reliability and construct validity in a mixed sample of cancer survivors[38].

Timed Up and Go (TUG)

TUG was performed to establish convergent validity of the functional measures. The participant began this test seated in a standard-height chair with a back. A piece of tape was placed on the floor 10 feet from the chair. The participant was instructed that when they were told "go" they were to stand up, walk to the line, walk around the line, and return to sit in the chair. Timing began when the tester said "go" and ended when the participant's body made contact with the chair. The TUG test has established validity and test-retest reliability in older cancer survivors[32].

Veterans Rand-12 (VR-12)

The VR-12 is a patient reported outcome measure that assesses health-related quality of life by asking participants questions about their physical and emotional health and the limitations they face due to their health. VR-12 was performed to establish discriminant validity of the functional measures as patient reported outcome measures have weak associations with actual performance on measures of function[39-41]. The scores produced are divided into physical and mental (MCS) component summaries which are calculated based on a norm-referenced algorithm[42]. VR-12 MCS scores were used to establish discriminant validity. Low MCS scores are associated with the presence of depression, functional impairment, and comorbidities in older cancer survivors[43].

Statistical Analysis

Normality of the data was examined using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics including mean and standard deviation or frequencies for those normally distributed and the median scores were reported as measures of central tendency for non-normally distributed demographic, health and cancer related data of

Age, years	73.70 (6.38)
Range	65 - 89
Gender, % Female	31 (66.0%)
Education	
High School Graduate	3 (6.4%)
Some college	8 (17.0%)
Associate Degree	3 (6.4%)
Bachelor's Degree	11 (23.4%)
Education beyond bachelor's degree	22 (46.8%)
Race, % White	43 (91.5%)
Number of Medications	
1-4	16 (40.0%)
5-10	17 (37.8%)
>11	10 (22.2%)
Number of Surgeries	
0	5 (10.9%)
1-3	25 (54.3%)
4-6	10 (32.7%)
>7	6 (13.0%)
FCI	2.60 (1.77)
Range	0-7
BMI, kg/m²	28.07 (4.66)
VR-12 MCS	42.26 (4.36)
Falls History, Yes	15 (31.9%)
Cancer Type	
Breast	28 (59.6%)
Prostate	15 (31.9%)
Lung	3 (6.4%)
Colorectal	1 (2.1%)
Years Since Cancer Diagnosis	11.89 (8.81)
Cancer Stage at Diagnosis	
0	2 (4.3%)
1	22 (46.8%)
2	8 (17.0%)
3	3 (6.4%)
Unknown	12 (25.5%)
Treatment type (n=44)	
Radiation only	6 (12.8%)
Surgery only	14 (29.8%)
Hormonal treatment only	1 (2.3%)
Chemotherapy & Radiation	4 (8.5%)
Chemotherapy & Surgery	5 (10.6%)
Radiation & Surgery	6 (12.0%)
Chemotherapy, Radiation & Surgery	7 (14.9%)
Chemotherapy, Surgery, Hormone	1 (2.1%)

Values shown are mean (SD) or number (%).
Abbreviations: FCI=Functional Comorbidity Index, BMI=Body Mass Index, VR-12=Veterans Rand-12, MCS=Mental Composite Score

Table 1. Participant demographics of older cancer survivors (n = 47).

the population. Reliability was analyzed using the $ICC_{2,1}$ with absolute agreement, average of multiple measurements, with a 95% confidence interval[44]. The literature describes ICC values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively[45]. Construct validity, both convergent and discriminant validity, was examined using Spearman's correlation coefficient between the functional measures and the TUG test for convergent validity. Discriminant validity was examined using Spearman's correlation coefficient and the VR-12 MCS scores. Two-tailed statistical significance was set at $p<0.05$. The minimal detectable change (MDC) was determined by first calculating the standard error of measurement (SEM) by multiplying the SD_{pooled} and $\sqrt{(1-ICC)}$. The MDC at the 95% confidence interval (MDC^{95}) was then calculated by using the formula: $1.96 \times SEM \times \sqrt{2}$. For those measures whose scores are generally obtained using whole points, the MDC^{95} was reported as a whole number. All analyses were performed with IBM SPSS® Version 26 (Armonk, NY).

Results

Characteristics of Participants

Sixty participants were recruited for this study but only 47 had complete information for all measures. Reasons for not having complete information were that 5 were lost to follow up (2 did not return for the second session, 1 became ill and was unable to attend the second session, and 2 were unable to complete the second session due to research stopping in April, 2020 secondary the COVID-19 pandemic). The other 8 potential participants didn't have 5xSTS measurements completed at both time points. Of these, 4 were not able to complete the 5xSTS without upper extremity support at either time point while the other 4 completed the first measure, but were missing data from the second measure. The overall mean age of those excluded was 74.08 (6.70) years. Over 57% were female and breast cancer survivors with a FCI of 2.43 (1.56) comorbidities. An average of 15.4 (10.6) years had passed since cancer diagnosis. Two of the excluded participants had scores that were >3 SD from the mean on all the functional measures. The mean SPPB score was 8.86 (3.33) points, PPT-7 was 20.79 (5.64), and the TUG was 15.46 (13.46) seconds.

The mean age of included participants was 73.70±6.38 years. The majority were female (66.0%), identified as white (91.5%) and had at least a bachelor's degree level of education (70.2%). The average time since cancer diagnosis was over 10 years (11.89±8.81 years). Further demographic information and medical history can be found in Table 1. No participants reported a change in health status or falls after the first testing session. The median times or scores on functional measures were as follows: 5xSTS=12.76 seconds, 30sTCR=12.0 repetitions, SPPB=11.0 points, PPT-7=25 points.

Outcome Measure	Median	Mean, SD	$ICC_{2,1}$	95% CI	MDC^{95}
5xSTS, seconds	12.76	13.02, 3.08	0.86	0.75, 0.92	3.19
30sTCR, repetitions	12.0	11.50, 3.40	0.89	0.80, 0.94	3
SPPB, score	11.0	10.53, 1.77	0.85	0.74, 0.92	3
PPT-7, score	25.0	24.66, 2.40	0.48	0.06, 0.71	4

Abbreviations: SD=Standard Deviation; ICC=Intraclass Correlation Coefficient; CI=Confidence Interval; 5xSTS=5 Time Sit-to-Stand; 30sTCR=30 second Timed Chair Rise; SPPB=Short Physical Performance Battery; PPT-7=Physical Performance Test 7.

Table 2. Scores, test-retest reliability, and MDC^{95} of functional measures in older long-term cancer survivors (n=47).

Outcome Measure	Convergent Validity		Discriminant Validity	
	Timed Up and Go		VR-12 Mental Component Score	
	r_s	*p-value*	r_s	*p-value*
5xSTS	0.53	0.00	-0.03	0.82
30sTCR	-0.69	0.00	-0.01	0.97
SPPB	-0.69	0.00	-0.04	0.77
PPT-7	-0.57	0.00	-0.12	0.41

Abbreviations: VR-12=Veterans Rand 12; 5xSTS=5 Times Sit-to-Stand; 30sTCR=30 second Timed Chair Rise; SPPB=Short Physical Performance Battery; PPT-7=Physical Performance Test 7; r_s=Spearman's rho.

Table 3. Convergent and discriminant validity of functional measures in older long-term cancer survivors (n=47).

Reliability

Reliability analyses can be found in Table 2. Good test-retest reliability was found for 5xSTS ($ICC_{2,1}$=0.86, 95% CI=0.75-0.92), 30sTCR ($ICC_{2,1}$=0.89, 95% CI=0.80-0.94), and SPPB ($ICC_{2,1}$=0.85, 95% CI=0.74-0.92). Poor test-retest reliability was found for PPT-7 ($ICC_{2,1}$=0.48, 95% CI=0.06-0.71).

Validity

Validity of physical functional measures can be found in Table 3. Significant correlations were found between all physical functional measures and TUG, establishing convergent validity. No significant correlations were found between physical functional measures and VR-12 MCS scores, establishing discriminant validity.

MDC^{95}

The MDC^{95} of each measurement can be found in Table 2. For the sit to stand measures of function, the MDC^{95} for the 5xSTS was 3.19 seconds and was 3 repetitions for the 30sTCR. The SPPB had a MDC^{95} of 3 points and the MDC^{95} for the PPT-7 was 4 points.

Discussion

Functional declines with aging may be more severe in older long-term cancer survivors, leading to impaired mobility, ADL performance, and decreased independence. Clinical measures to assess physical function have established reliability and validity in community-dwelling older adults, however, their measurement properties are lacking in long-term cancer survivors. This study establishes the reliability and convergent and discriminant validity of measures of physical function in 47 long-term older survivors of mixed cancer diagnoses.

The physical health status of older long-term cancer survivors is reported in the literature to be somewhat worse than that of comparable persons who have never had cancer[46]. The physical performance measures results of the sample population confirm this as they were generally more impaired than what is reported in community-dwelling older adults[24,47-50] of similar age groups. For example on both the 5xSTS and 30sTCR tests, participants scored worse than what was reported in community-dwelling older adults (5xSTS: 13.02 v. 10.01 seconds[47]; 30sTCR: 11.5 v. 15.5 repetitions[49]). However, the method in which we performed this measure may have influenced the outcomes.

Although we instructed the participants to perform as many sit to stand repetitions as quickly as possible within the 30-second time frame, we recorded the time to complete the fifth repetition as the 5xSTS measure. We recognize that this limits comparison across studies and generalizability of results, however this method was used to limit fatigue.

Despite the differences in measurement on the 5xSTS good test-retest reliability was found the ($ICC_{2,1}$=0.86) which is similar to test-retest reliability reported in 30 community-dwelling older adults[22]. Similarly, the associations between 5xSTS and TUG were similar to what was reported in older adults[31]. Collectively, these results indicate that measuring the fifth repetition as a part of the timed chair rise test may be feasible, but further study is indicated.

The reliability of the 30sTCR ($ICC_{2,1}$=0.89) was also similar to what was reported in a group of community-dwelling older adults indicating that this measure is appropriate for use across both populations[23]. However, we cannot distinguish whether the study population referenced may have had some participants with a cancer history. Regardless, the similarity in ICC values indicates that this measure has comparable reliability in those with a long-term history of cancer as compared to those without a cancer history and should be considered for use.

The MDC^{95} values of the chair rise tests were large indicating that for the 30sTCR the number of repetitions should increase by 3, while the score on the 5xSTS should decrease by greater than 3.19 seconds to be considered as true change. Achieving either of these MDC^{95} values should be considered a large jump in performance on the measures and may not be possible when scores are similar to those reported in this study. The ICC values suggest that performance on these measures may have been slightly different between measurements, which would influence the size of the MDC values calculated. Also, the large standard deviation of each measure suggest that there were likely higher and lower performers on these measures which was reflected in the slightly bimodal distribution of the data therefore, we caution the interpretation of these MDC values for use clinically as they may be overestimate the change in performance needed to reflect true change.

In this study, poor test-retest reliability was found for the PPT-7 ($ICC_{2,1}$=0.48). In a study of a mixed sample of 214 cancer survivors and non-cancer survivors, individual components of the PPTB, a test similar to the PPT-7, had good to excellent test-retest reliability[38]. Average scores on the PPT-7 did not indicate a high degree of impairment which may have contributed to the poor reliability. While convergent and discriminant validity of PPT-7 was established in this study, the reliability of PPT-7 was limited in this group of long-term cancer survivors and further study is indicated.

SPPB is the tool recommended for use across older adult populations as it has the best reliability and validity of measures of function[51]. Reliability for the SPPB ($ICC_{2,1}$=0.85) was similar to results reported in community-dwelling older adults[24]. The EDGE Task Force assigned this measure a rating of "highly recommended"[29]. Therefore, clinicians should consider using the SPPB over the PPT-7 to measure the physical function in older cancer survivors due to its high reliability. However, it is unclear whether the MDC from this study should be used as it is over two times greater than the MDC reported (1.34 points) in a larger sample (n=492) of diverse older adults and is not specific to older cancer survivors[52]. Similar to the chair rise tests, our MDC was influenced by both the standard deviation of the measure (1.7 points) as well as the ICC values, which makes improvements of nearly 3 points on a 12 point scale challenging. As such, we suggest further research be conducted with a larger sample size of cancer survivors with a similar level of function to determine a more accurate MDC^{95} for this population.

A growing body of evidence describes associations between pain and function as well as comorbidity and function in long-term cancer survivors, particularly in those who received chemotherapy, and suggests that a higher degree of pain and increased comorbidity translate into low health-related quality of life and physical function. Although 36.4% of the sample had a history of chemotherapy, we did not examine the results relative to those who received this treatment nor did we gather information on or test participants for the presence of chemotherapy-induced peripheral neuropathy (CIPN)[14] which may translate into increased pain, inactivity, and decreased sensation. In addition, this population had a relatively low level of comorbidity as reflected with the FCI scores. Collectively these data suggest two things. One, this population of older cancer survivors may not reflect the level of function found in survivors with more chronic comorbidity and specifically in those with a history of CIPN. Second, future studies need to be completed to examine the psychometric properties of physical function measures in long-term cancer survivors increased focus on those with cancer-related pain, increased comorbidity, and by cancer treatment history.

Limitations of this study include that of the 60 participants recruited, only 47 completed the outcome measures and most often the most common reason for exclusion was the lack of ability of the sample to perform the 5xSTS measure. This was a convenience sample of community-dwelling older cancer survivors and may have contributed to the lack of diversity in gender, race, and education level of the sample. The sample also lacked diversity in cancer type, as most participants were long-term breast or prostate cancer survivors. Older cancer survivors who are in the early phases of survivorship may perform differently on the measures included in this study, and as such, our findings should only be used for those whose time since diagnosis are similar to the study population.

Future research should examine these tests and measures at various points in the cancer continuum of care, such as pre-treatment, during treatment, and post-treatment. In

this study, the average length of time since cancer diagnosis was 11.89 years. Because a cancer survivor may have different impairments and activity limitations throughout survivorship, the reliability and validity of these measures should be established at different points in survivorship. Future research should also involve a sample of older cancer survivors that is more diverse in race, cancer type, and education.

Conclusion

When considering assessing function in older long-term cancer survivors, the 5xSTS, 30sTCR and SPPB are reliable and valid tools that should be used. The PPT-7 has construct validity but does not have retest reliability in cancer survivors.

Ethical Approval

This study was approved by the Institutional Review Board, approval number HUM00137566. All procedures were performed in accordance with the ethical standards of the Institutional Review Board.

Informed Consent

Informed consent was obtained from all participants in this study.

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