Original Experimental

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Concurrent validity of dynamic bedside quantitative sensory testing paradigms in breast cancer survivors with persistent pain

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Abstract

Background – Studies on the concurrent validity of clinically applicable testing protocols for conditioned pain modulation (CPM) and temporal summation of pain (TSP) in breast cancer survivors (BCS) with persistent pain are lacking.

Objectives – This study investigated the concurrent validity of two bedside protocols for CPM and TSP in comparison to a respective reference protocol. The participants' preferences for bedside CPM and TSP protocols were assessed.

Methods – Thirty BCS experiencing persistent pain were included in this study. Each participant underwent a reference test along with two bedside alternatives for assessing both TSP and CPM. For CPM, a cold pressor test (CPT) and blood pressure cuff (BPC) were used as conditioning stimulus. The test stimulus was elicited in parallel by pressure pain threshold after 45 and 90 s of conditioning at the lower limb. The CPM reference test consisted of parallel heat stimuli at the forearms using a two-thermode system. TSP was elicited using a von Frey monofilament (256 mN) and an algometer (98 kPa) at the affected site and opposite lower limb. The TSP reference test consisted of heat stimuli at the affected site and opposite lower limb. Participants' testing preference was examined using a purpose-designed questionnaire. Spearman's rank test examined the correlation between protocols.

Results – The two bedside CPM protocols were strongly correlated (r = 0.787–0.939, $p < 0.005$). A strong correlation was found between the BPC protocol and reference test using the relative effect magnitude (r = 0.541–0.555, $p < 0.005$). The bedside TSP protocols were moderately correlated with each other only at the lower limb using absolute change scores (r = 0.455, p = 0.012). No significant correlation was found between the bedside and reference TSP protocols.

Conclusion – The significantly moderate to very strong correlations between the bedside protocols validate their interchangeability. Researchers and clinicians should be able to choose which bedside protocol they utilize; however, participants favored the use of a BPC and algometer for the evaluation of CPM and TSP, respectively.

Keywords: cancer pain, breast neoplasms, postsynaptic potential summation, pain measurement, conditioning, physiological

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1 Introduction

Breast cancer remains the most prevalent cancer type (11.7%) with 2.3 million new cases reported worldwide in 2020 [1]. Breast cancer survivors (BCS) can experience a myriad of side effects of cancer treatment [2]. Over one-third of women (35%) experience persistent pain, of whom one in four (24%) experience moderate-to-severe pain [3]. These symptoms can have a significant adverse impact on emotional and physical functioning and quality of life [2].

Persistent pain is often related to a dysfunction of the somatosensory system [4]. Aberrations in central somatosensory functioning can be evaluated using dynamic quantitative sensory testing (QST), such as conditioned pain modulation (CPM) and temporal summation of pain (TSP) [5]. CPM relates to the reduction of pain intensity for a test stimulus after or during the application of a conditioning stimulus to a different part of the body. In doing so, CPM evaluates the endogenous inhibitory descending pathways [6]. TSP is a psychophysical measurement focusing on the increment of experienced pain by a repetitive application of a stimulus with equal intensity. As such, TSP serves as a surrogate measure for the neuronal wind-up phenomenon, which is defined as "a high frequency of action potentials in the presynaptic neuron elicits postsynaptic potentials that overlap and summate with each other," thereby evaluating the endogenous facilitatory nociceptive pathways [7,8]. Previous studies in persistent pain after breast cancer treatment reported decreased CPM effects and presence of exaggerated TSP [9,10].

Several experimental methods for CPM and TSP have been investigated in patients with persistent musculoskeletal pain [11,12], neuropathic pain [13,14], and osteoarthritis [15] and in healthy individuals [16]. These studies have used either sophisticated laboratory equipment or simplified bedside alternatives, defined as bedside tests. Previous studies investigating CPM and TSP in BCS have primarily used laboratory-based protocols with computer-controlled thermode systems or computer-controlled cuff algometry [10,17]. Although these protocols are considered the gold standard because of their standardization and control of stimuli, they are mostly unfeasible for use in clinical practice owing to cost, inaccessibility, and required training [18]. Since assessing the somatosensory system and its function is suggested to improve pain management, research into bedside QST methods is warranted [19–21].

Currently, bedside tests for the assessment of CPM and TSP in clinical practice exist, but they have not been investigated in a breast cancer population nor have their concurrent validity, which refers to their ability to produce consistent results when compared to a gold standard protocol, been determined [11,14,15,21]. In addition, patients were never involved in the development of bedside QST protocols, even though they are at the receiving end and can provide valuable information concerning the application of such protocols in clinical practice.

The aim of this study was to investigate the concurrent validity of two bedside protocols for CPM and TSP in BCS experiencing persistent pain by comparing them with each other and with a laboratory-based reference test. Furthermore, participants' preferences for bedside CPM and TSP protocols were assessed.

2 Methods

2.1 Participants

Participants were recruited between November 2020 and August 2022 from a larger cross-sectional study involving cancer survivors at the University of Leuven and University of Antwerp. This parent study investigated different pain mechanisms using different assessment methods in cancer survivors with pain (clinicaltrial.gov NCT03981809) and received ethical approval from the University Hospitals Leuven (s62584) and the University Hospital of Antwerp (B322201940289). All participants provided written informed consent. The study adheres to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement [22].

Eligible participants were women aged ≥18 years, treated for primary breast cancer at least three months prior, and in complete remission (i.e., no active signs or symptoms of cancer). BCS experiencing persistent pain needed to report mean pain intensity during activity >3/10 on the numeric rating scale (NRS) during the past week [23,24]. Initial NRS assessment was conducted via telephone. Persistent pain related to breast cancer treatment was determined based on the location and timing of onset. Pain in the chest, lateral trunk, axilla, arm, or shoulder occurring concurrently or post-treatment was considered related. Exclusion criteria included (1) metastasis, (2) palliative status, (3) cancer recurrence, (4) bilateral cancer, (5) pregnancy, or (6) inability to communicate in Dutch.

2.2 QST

For each participant, a reference test and two bedside alternatives for TSP and CPM were performed. The

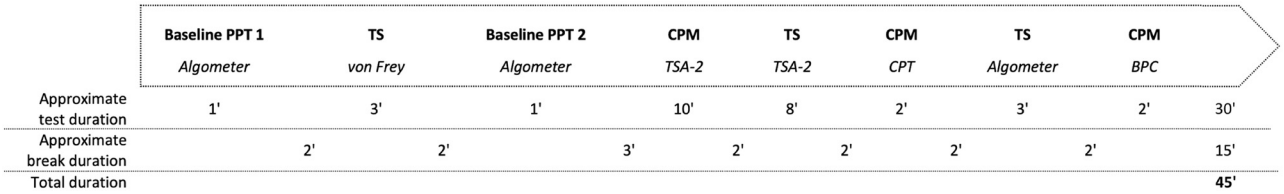

Figure 1: Measurement protocol. PPT = pressure pain threshold, TSP = temporal summation of pain, CPM = conditioned pain modulation, CPT = cold pressor test, BPC = blood pressure cuff, TSA-2 = advanced thermosensory stimulator. The duration is reported in minutes (').

measurements were performed in a quiet room with the participant in a seated position. An overview of these protocols is shown in Figure 1. The order of testing was fixed and between each test, an average wash-out of at least 2 min was foreseen to mitigate any potential sensitization effect due to repetitive stimulation [25].

2.3 CPM protocols

2.3.1 CPM reference test

The reference CPM protocol was performed using the Advanced Thermosensory Stimulator TSA-2 (Medoc, Ramat Yishai, Israel). First, the test stimulus intensity was individualized using a Peltier thermode on the non-affected forearm, opposite to the treated region. Pain4 temperature, evoking NRS-rated discomfort of 4, was determined through a series of heat stimuli. A parallel CPM paradigm followed, applying Pain4 test stimulus to the affected forearm for 45 s, with NRS-rated intensity at intervals. After a 120 s break, the unaffected forearm received a 65 s conditioning stimulus 0.5°C above the Pain4 temperature. Then, after 20 s, Pain4 test stimulus was applied to the affected forearm. NRS-rated pain intensity was assessed at 10, 20, 30, and 40 s [26,27]. More details on the CPM reference protocol are provided in Appendix S1. A schematic overview is presented in Figure 2.

2.3.2 CPM bedside test 1: cold pressor test (CPT)

Pressure pain threshold (PPT) at the tibialis anterior muscle, opposite to the affected region, was used as test stimulus. Baseline PPT was determined using an algometer (Wagner FDX, Greenwich CT, USA). PPT was defined as the pressure which was first perceived as painful and was calculated as the mean of two trials [10]. As conditioning stimulus, the CPT was used with the unaffected hand submerged in 12°C water. PPT was measured at 45 and 90 s during CPT [28–30].

2.3.3 CPM bedside test 2: blood pressure cuff (BPC) occlusion

This protocol employed the same PPT test stimulus. A BPC (BoSo Profitest, Jungingen, Germany) applied pressure on the unaffected upper limb. The cuff was inflated via hand squeeze (20 mmHg per squeeze) and stopped at NRS 5/10 or 220 mmHg. PPT was measured at 45 and 90 s during cuff inflation [8,31].

2.4 TSP protocols

2.4.1 TSP reference test

TSP was assessed at the most painful site and the tibialis anterior muscle using a Peltier thermode. Heat stimuli

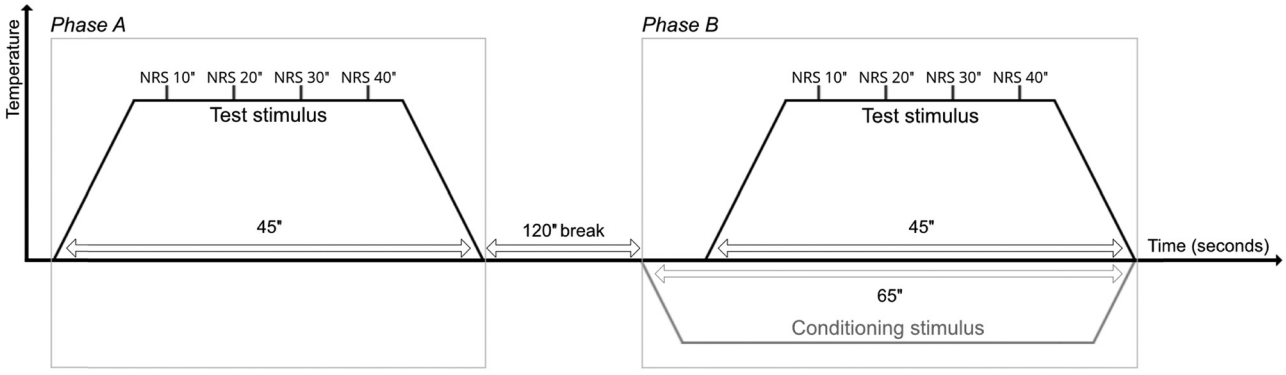

Figure 2: Schematic overview of the reference CPM protocol sequence using the TSA-2. NRS = numeric rating scale.

were individualized to Pain4 temperature from the CPM reference protocol. Subjects received one train of 30 stimuli from 38°C to the individualized peak Pain4 temperature with a ramp rate of 13°C/s, 0.8 s at peak stimulus, and a return rate of 13°C/s. The inter-stimulus interval was set at 1 s with the stimulus frequency approximating 1 Hz. Pain intensity was verbally rated on NRS after the first and last stimuli [32,33].

2.4.2 TSP bedside test 1: von Frey monofilament

TSP was measured at the same locations by applying a series of stimuli using a von Frey monofilament with a stimulation force of 256 mN (Optihair2-Set, Marstock Nervtest, Germany). After the first stimulus, a series of stimuli was delivered for 30 s at a rate of 1 stimulation/s. Pain intensity was verbally rated on NRS after the first and last stimuli [27].

2.4.3 TSP bedside test 2: algometer

For the second bedside TSP test, a digital pressure algometer (Wagner FDX, Greenwich, CT, USA) was used at both locations. Peak pressure was set at 98 kPa with a stimulation frequency of 1 Hz. Amounts of repetitions and instructions to the participant were the same as the bedside TSP test with the von Frey monofilament [8,34].

More detail on the dynamic QST protocols is provided in Appendix S1.

After completion of the assessment, participants were given a purpose-designed questionnaire regarding their experiences and bedside test preferences. The questionnaire contained two questions (yes or no): (1) testing was comfortable and (2) instructions were clear. In addition, participants were asked to indicate their preference for one of the bedside protocols for CPM and TSP at the most painful site and the opposite tibialis anterior muscle (Appendix S2).

2.5 Statistical analysis

Data analysis was performed using R statistical software version 3.6.2 [35]. Normal distribution of the data was checked. Descriptive statistics for continuous variables included median [interquartile range (IQR)] for non-normally distributed and mean [standard deviation (SD)] for normally distributed data. Categorical variables were expressed as frequencies (%).

First, concurrent validity was examined using the absolute and relative effect magnitudes. Spearman's rank (rho) coefficients were calculated for non-normally distributed data and interpreted as follows: <0.3 weak, 0.3–0.5 moderate, 0.5–0.7 good, and >0.7 very good [36,37]. Correlation coefficient of >0.7 is considered to show sufficient evidence of validity [38]. Addition of 0.1 to zero NRS scores enabled relative effect calculations [39].

Second, concurrent validity was explored by comparing the proportion of responders using Fisher's exact test using absolute and relative changes. A meaningful CPM effect was determined by calculating the ±2 SEM (standard error of measurement) [40].

Regarding TSP, responders were defined using the minimal clinically important difference of more than 2 points on the NRS for absolute change and 33% for the relative change [41–43].

Correction for multiple testing was performed using a Bonferroni correction by dividing the alpha (0.05) by the number of tests performed. Participant experiences and preferences were summarized descriptively.

For the reference CPM protocol, the SEM was calculated using the NRS scores at the different time points during phase A (Figure 2): SEM = (pooled SD of NRS scores during phase A) × $\sqrt{(1 - \text{ICC})}$. The interclass correlation coefficient (ICC) was calculated from the mean NRS scores during phase A [40]. Using the ±2 SEM method, participants were classified into three groups of responders: (1) antinociceptive = decrease in NRS of at least 2 SEM during phase B; (2) pro-nociceptive = increase in NRS during phase B of at least 2 SEM; and (3) non-response = no change in NRS or change smaller than 2 SEM. The same methodology was applied for the bedside CPM protocols, using baseline PPT values. The baseline PPT values were logarithmically transformed to normalize the data distribution, after which the ICC was calculated. Using the ±2 SEM method for the bedside protocols, participants were grouped similarly. For all CPM protocols, the ±2 SEM method was used for both absolute and relative effect magnitudes. The relative effect magnitude was calculated by dividing the 2 SEM by the median baseline PPT or NRS scores during Phase A.

3 Results

3.1 Subjects

A total of 30 consecutive participants were included, with a median (IQR) age of 52 (10.5) years. Participant characteristics are summarized in Table 1. A comprehensive overview of the participant characteristics is provided in Table S1.

Table 1: Demographic characteristics of the participants (frequency (%) unless specified otherwise) (n = 30)

Age (years), median (IQR) [range]	52.0 (10.5) [44–70]
BMI (kg/m^2), median (IQR) [range]	25.1 (7.0) [17–34.4]
Pain intensity, median (IQR) [range]	
– VAS at rest	31.0 (29.0) [3–80]
– VAS during activity	43.5 (34.3) [8–80]
– Maximum VAS	71.0 (15.8) [50–100]
– Minimum VAS	23.0 (20.8) [0–65]
– Mean VAS	45.5 (26.8) [0–88]
Location of the most painful site	
– Chest and lateral trunk	11 (36.7%)
– Arm, shoulder, and axilla	12 (40.0%)
– Chest, lateral trunk, arm, shoulder, and axilla	7 (23.3%)
Pain medication: type, n (%)	
– Tricyclic antidepressants, gabapentinoids or SNRI	2 (6.7%)
– NSAID, acetaminophen, or mild opioid	16 (53.3%)
– No medication	12 (40%)

IQR = interquartile range, VAS = visual analogue scale, SNRI = serotonin and norepinephrine reuptake inhibitors, NSAID = non-steroidal anti-inflammatory drugs.

3.2 CPM

One participant was unable to perform the reference CPM protocol because of pain during the application of the conditioning stimulus (46.5°C). Another participant was not able to keep her hand submerged for 90 s during the CPT due to intolerable pain, and one participant did not experience unpleasant pressure during the BPC test and reached the BPC's limits. The CPM data are listed in Table S2.

The correlations between CPM protocols are presented in Table 2. The bedside CPM protocols were significantly and very strongly correlated at each time point, using both absolute and relative CPM effect magnitudes. A significant and strong correlation was found between the BPC protocol and TSA-2 at both timepoints using the relative effect magnitude. No other significant correlations were found after correction for multiple testing (Table 2).

Second, 2 SEM values were calculated to explore meaningful CPM effects. The 2 SEM for the reference CPM protocol using the TSA-2 was 1.74 (43.5%) on the NRS. The 2 SEM for the bedside CPM protocols using baseline PPT was 127.4 (47.6%) kPa (Table S2). The proportions of BCS with anti-nociceptive, pro-nociceptive, and no response are shown in Table S2. Fisher's exact tests showed no significant differences between the proportions of CPM responses with regard to all CPM protocols using either absolute or relative effect magnitudes, indicating good concurrent validity (Table 3).

3.3 TSP

Missing data were highest for the bedside TSP protocol with the algometer at the most painful site (n = 11). Eleven participants were unable to withstand a pressure

Table 2: Correlation (Spearman's rho) between CPM protocols using *absolute* and *relative* CPM effects and comparison (Fisher's exact test) of the proportion of responders

		***Absolute* CPM effect**				***Relative* CPM effect**			
		BPC90	**CPT45**	**CPT90**	**TSA-2**	**BPC90**	**CPT45**	**CPT90**	**TSA-2**
BPC45	Spearman's rho	**0.910†**	**0.877†**	**0.822†**	0.423	**0.939†**	**0.839†**	**0.795†**	**0.541†**
	p-value	<**0.005**	<**0.005**	<**0.005**	0.025	<**0.005**	<**0.005**	<**0.005**	<**0.005**
	Fisher's exact test p-value	*0.942*	*0.938*	*0.878*	*0.056*	*0.853*	*0.858*	*0.927*	*0.074*
BPC90	Spearman's rho	—	**0.840†**	**0.888†**	0.504	—	**0.860†**	**0.812†**	**0.555†**
	p-value	—	<**0.005**	<**0.005**	0.006*	—	<**0.005**	<**0.005**	<**0.005**
	Fisher's exact test p-value	—	*0.827*	*0.823*	*0.038**	—	*0.834*	*0.914*	*0.091*
CPT45	Spearman's rho		—	**0.787†**	0.452		—	**0.856†**	0.455
	p-value		—	<**0.005**	0.014*		—	<**0.005**	0.013*
	Fisher's exact test p-value		—	*1.000*	*0.112*		—	*0.915*	*0.020**
CPT90	Spearman's rho			—	0.370			—	0.347
	p-value			—	0.052			—	0.071
	Fisher's exact test p-value			—	*0.144*			—	*0.070*

CPM = conditioned pain modulation, CPT45 = cold pressor test with 45 s of conditioning, CPT90 = cold pressor test with 90 s of conditioning, BPC45 = blood pressure cuff occlusion with 45 s of conditioning, BPC90 = blood pressure cuff occlusion with 90 s of conditioning, TSA-2 = advanced thermosensory stimulator.

*p < 0.05; †p-value < Bonferroni corrected threshold: 0.05/10 = 0.005.

Fisher's exact test data are shown in italic.

Spearman's rho values and corresponding p-values, and Fisher's exact test p-values that are lower than the Bonferroni correct p-value are shown in bold.

of 98 kPa at this location due to excessive pain. In addition, two and three participants declined TSP with the von Frey monofilament and TSA-2, respectively, as they expected a very painful reaction at the most painful site (Table S3). The presence of exaggerated TSP was overall highest when the stimulus was administered with the von Frey monofilament (36.7–39.3% for absolute NRS change, 63.3–67.9% for relative NRS change) (Table S3). TSP remained modest when it was applied with the TSA-2 (0–3.7% for absolute NRS change, 3.3–3.7% for relative NRS change) (Table S3). No correlation was found between the reference TSP protocol and bedside TSP protocols at the most painful site or the opposite tibialis anterior muscle (Table 3). A significant and moderate correlation was found between the two bedside TSP protocols (von Frey monofilament versus algometer) at the tibialis anterior muscle using the absolute (p = 0.012, rho = 0.455) changes in the NRS (Table 3).

Second, at both locations, Fisher's exact tests showed a significant difference between the reference protocol and bedside protocols in the proportion of participants showing an exaggerated TSP for both absolute and relative effects after correction for multiple testing, indicating low concurrent validity (Table 3).

3.4 Participants experience and bedside test preference

Overall, the majority of participants (1) perceived the testing as comfortable and (2) thought the instructions were clear. The participants' preferred bedside protocol for CPM was the test with BPC as the conditioning stimulus (n = 23, 76.7%). For TSP at the tibialis anterior muscle, 73.3% (n = 22) of the participants preferred the algometer over the von Frey monofilament (Figure 3). For TSP at the most painful site, 36.7% (n = 11) of the participants indicated that TSP using the algometer was too painful and was, therefore, not included in the bedside test preference count (Figure 3). Of the remaining participants, 33.3% (n = 10) preferred the algometer to the von Frey monofilament (Figure 3). Five (16.7%) and three (10.0%) participants remained undecided for their preference (Figure 3).

4 Discussion

This study aimed to explore the concurrent validity of bedside CPM and TSP protocols in BCS with persistent pain. We examined CPM and TSP using absolute and relative effects and the corresponding proportions of responders. In general, the highest correlations were found between the two bedside CPM protocols, both for the absolute and relative effects, and at both the 45 and 90 second time points. Using the relative effect magnitudes, only the BPC protocol was found to be significantly and strongly correlated with the reference protocol at both timepoints. No other significant correlations were found between the BPC protocol and reference protocol using absolute effect magnitudes, or between the CPT protocol and reference protocol in general. However, looking at the proportion of responders to

Table 3: Correlation (Spearman's rho) between the absolute and relative TSP effects of the different test protocols and comparison (Fisher's exact test) of the proportion of responders

			Absolute **change in NRS**		***Relative*** **change in NRS**	
			Algometer	**TSA-2**	**Algometer**	**TSA-2**
Most painful site	**von Frey**	Spearman's rho	0.191	−0.210	0.008	−0.290
		p-value	0.433	0.313	0.975	0.159
		Fisher's exact test p-value	*0.759*	***0.002***†	*1.000*	<***0.001***†
	Algometer	Spearman's rho	—	−0.101	—	−0.423
		p-value	—	0.701	—	0.091
		Fisher's exact test p-value	—	***0.015***†	—	<***0.001***†
Tibialis anterior muscle	**von Frey**	Spearman's rho	0.455	0.077	0.379	0.367
		p-value	***0.012***†	0.685	0.039*	0.171
		Fisher's exact test p-value	*0.252*	<***0.001***†	*0.793*	*0.003**
	Algometer	Spearman's rho	—	−0.036	—	0.016
		p-value	—	0.850	—	0.932
		Fisher's exact test p-value	—	*0.024**	—	<***0.001***†

TSA-2 = advanced thermosensory stimulator, NRS = numeric rating scale.

*p < 0.05; †p-value < Bonferroni corrected threshold: 0.05/3 = 0.017.

Fisher's exact test data are shown in italic.

Spearman's rho values and corresponding p-values, and Fisher's exact test p-values that are lower than the Bonferroni correct p-value are shown in bold.

the different CPM protocols using the 2 SEM method, no significant differences were found between the bedside protocols and the reference protocol, pointing towards some agreement in concurrent validity. A significant and moderate correlation was found between the two bedside TSP tests at the tibialis anterior muscle using the absolute, but not the relative change in NRS. No significant correlations were found between bedside TSP protocols and the reference protocol. The presence of exaggerated TSP was significantly higher for the bedside TSP protocols than for the reference protocols at both locations, confirming limited concurrent validity. Furthermore, the participants favored the bedside CPM test using the BPC and algometer as a bedside TSP test. TSP with an algometer at the most painful site was too painful for 11 participants (36.7%); therefore, a remote body location was preferred.

The lack of correlation between the bedside and reference protocols may be due to the reference protocols used in this study. At this moment, no protocol has been validated as "the reference protocol," probably owing to the variability and complexity of TSP and CPM protocols in addition to the lack of standardization in research paradigms [44,45]. For the CPM, the last recommendation by Yarnitsky and colleagues dates back from 2014 and acknowledges that currently there are insufficient data to identify a specific CPM protocol as most preferred [46]. We utilized the TSA-2 by Medoc for its practicality in standardization and controlling thermal stimuli, and its previous use in studies [27,32,47]. The reference CPM protocol was based on a prior protocol in young healthy subjects [26]; however, it recently showed limited reliability in BCS in a study by Dams et al. [27]. We utilized a parallel CPM protocol rather than a sequential CPM protocol to limit the time required to perform all protocols. Although sequential protocols have been suggested as they limit distraction, parallel protocols do not seem to differ in CPM effect [48]. Furthermore, in our study, not only did the type of stimulus differ between protocols (heat vs cold vs pressure), but the location of the conditioning and test stimuli also differed. It has been shown that the CPM effect can be influenced by its location on the body as different body sites have different distributions of sensory receptors [47]. Alternatively, the CPT may be interesting since it is a well-established and recommended protocol used for the assessment of the endogenous pain-inhibitory systems in different pain population and BCS experiencing pain [10,46,49].

Regarding TSP, protocol recommendations are also lacking. The reference method selected for our study was thermal TSP with the TSA-2 for the same reason it was selected for CPM (i.e., standardization and control of stimuli), but again its validity on its own has not been examined due to protocol variability and lack of gold standard assessment methods [32]. Our reference TSP protocol was based on the protocol of Awali et al. who performed thermal TSP with a Peltier thermode on young, pain-free, healthy participants [32]. This protocol was adapted to suit our pain population with an individualized test intensity set at the NRS for pain of 4/10 (instead of 6 in Awali et al.) [32]. Even though this intensity was in line with previous research, it is possible that this pain intensity was too low and/or that the heat stimulus was set too low for thermal pain summation, as less than 5% demonstrated an exaggerated TSP at both testing locations [27,32]. Also, the Pain4 temperature was determined at the non-affected forearm, whereas the TSP protocol was applied to the most painful

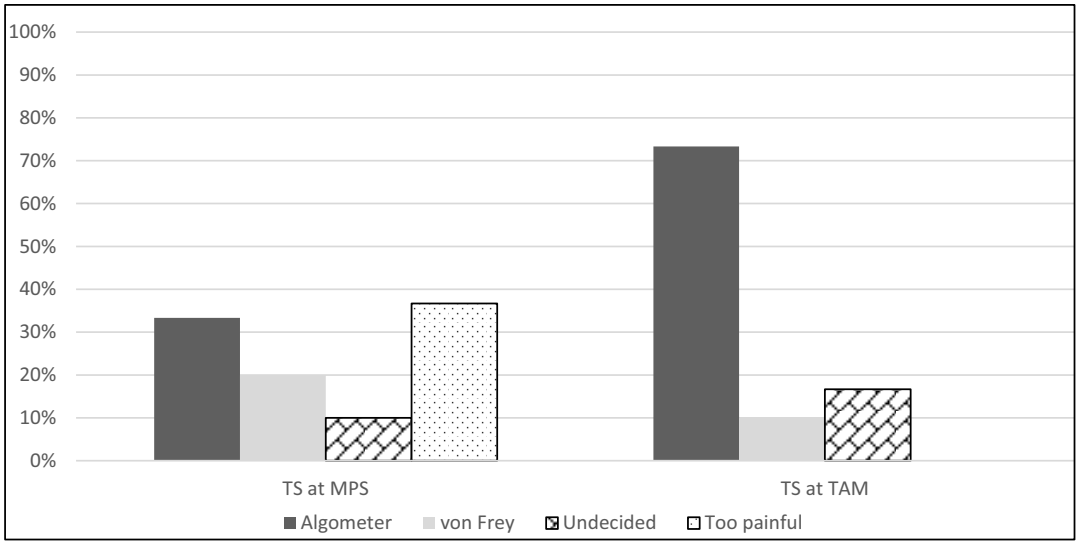

Figure 3: Participants' test preferences for bedside temporal summation of pain (TSP). TAM = tibialis anterior muscle, MPS = most painful site.

site and opposite tibialis anterior muscle. It is possible that differences in sensory receptor distribution between the non-affected forearm and opposite tibialis anterior muscle contributed to the low amount of pain summation [47]. Regarding the bedside TSP protocols, we utilized a von Frey monofilament or an algometer to exert pain summation and, even though prior research has utilized both tools to perform TSP, some considerations are needed [8,27]. The spherical tip of the 256 mN von Frey monofilament is not meant to stimulate nociceptors, resulting in several absent pain scores after the first stimulus. Equally, a pressure of 98 kPa at the tibialis anterior was often perceived as non-noxious. Individualization could increase responder rates and improve the application of TSP with the algometer at a painful site, as illustrated by the missing data (n = 11) due to pain.

This study is the first to investigate CPM and TSP using both absolute and relative effect magnitudes and the corresponding responder analyses in BCS with persistent pain. Until now, most studies have either used the absolute effect magnitude when relying on the NRS because of possible zero ratings or relative effect magnitudes when relying on PPTs, as zero values are uncommon. Solely using the absolute effect to determine an effect has limitations owing to the floor or ceiling effects. To avoid such limitations, we calculated the relative effect magnitudes for the CPM and TSP protocols. Responder analyses for CPM showed similar proportions using both effect magnitudes, whereas for exaggerated TSP, responder rates differed substantially between the methods used. The responder rate could be influenced by the intensity of our protocols, but it is also possible that the cut-off value for absolute and relative change does not match (i.e., an absolute change of 2 on the NRS is not always equal to a relative change of 33%) [50]. Future studies should establish methodological recommendations for assessment of TSP.

4.1 Strengths and limitations

This study has several strengths. Three different protocols, consisting of one reference protocol and two bedside protocols per paradigm were selected for comparison. In addition, different conditioning and test stimuli were used and compared for CPM. Furthermore, this study offers a conservative statistical analysis and comprehensive assessment of CPM and TSP by using absolute and relative changes. A comparison of the proportion of responders in each paradigm provides additional information regarding its concurrent validity. The participants were asked about their experiences and bedside test preferences.

This study also has several shortcomings, the first of which is its limited sample size. Recruitment was ongoing when the COVID-19 restrictions were introduced. Therefore, a convenience sample of 30 participants was used. We did not perform an a priori sample size calculation. Second, during recruitment, we screened BCS based on pain intensity via telephone. BCS were eligible for inclusion if they indicated a mean pain intensity of >3/10 on the NRS. Eligible participants were asked to fill in several pain ratings using a visual analogue scale (VAS): minimum, maximum, during activity, and during rest. Consequently, depending on the type of pain rating, several participants had close to no pain, whereas others had severe pain. This finding is indicative for the dynamic nature pain holds, resulting in a non-normal distribution of pain scores. It is possible that the inclusion of BCS with nearly no pain skewed our results; however, CPM is generally known to be highly variable, even in healthy groups [28]. Third, we did not systematically control wash-out times during our comprehensive assessment. However, the time required to set up each test, together with the standardized instructions, presumably resulted in a sufficient wash-out between tests [25]. Furthermore, CPM effects are predominantly transient [46,48]. Fourth, our study lacked a randomized testing order and familiarization to reduce expectations or anxiety. It is possible this combination influenced our results and test validity due to expectation effects for the first test, and due to learning effects for the last test. Fifth, when participants took pain medication, they were not excluded, nor were they asked to temporarily stop medication. Although only a limited number of participants took tricyclic antidepressants, gabapentinoids, or serotonin and norepinephrine reuptake inhibitors, these medications are known to impact QST outcomes by increasing various thresholds, thereby potentially influencing the results.

4.2 Clinical implementations

Interest in somatosensory evaluation in clinical settings has become popular recently in line with mechanism-based pain approaches and clinical guidelines for the classification of pain [5,19,51,52]. These guidelines propose that QST can aid in the assessment of somatosensory (dys)function [52,53]. However, the clinical applicability and validity of such guidelines remain uninvestigated. This study revealed strong correlations between bedside CPM and TSP protocols. Participants favored the CPM protocol with the BPC and the TSP protocol with the algometer at the tibialis anterior muscle. Clinicians can consider using a BPC as a conditioning stimulus for 45 s to assess CPM, and an algometer at a remote

site to assess TSP. It remains unclear whether evaluating these paradigms affects pain management. In addition, clinical feasibility and utility of these protocols require further investigation.

5 Conclusion

In BCS with persistent pain, bedside CPM protocols using a CPT or BPC are significantly and strongly correlated with each other. Bedside protocols for TSP were only significantly and moderately correlated with each other at a remote location using absolute scores. Participants favored the bedside CPM test using the BPC and the algometer as a bedside TSP test. These results indicate that researchers and healthcare providers can assess CPM using a BPC, PPT, and TSP, using an algometer. Further research on the concurrent validity of dynamic bedside QST protocols is warranted to improve their implementation.

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Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as amended in 2013), and has been approved by the authors' research ethical committee: the University Hospitals Leuven (s62584) and the University Hospital of Antwerp (B322201940289).

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Author contributions: V.H. designed the study and developed the research questions. V.H. collected and analyzed the data with inputs from A.D.G. M.M., N.D., and A.F.V.H. wrote the first draft of the manuscript. M.M., N.D., B.M., L.D., A.D.G., A.F., and A.D.G. contributed to the writing and revision of the manuscript. All the authors critically reviewed and approved the final version of the manuscript.

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Data availability: The raw data can be obtained on request from the corresponding author.

Supplementary material: Table S1: Demographic characteristics of the participants. Table S2: Conditioned pain modulation (CPM) data. Table S3: Temporal summation data.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality Worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49. doi: 10.3322/caac.21660.

[2] Gallaway MS, Townsend JS, Shelby D, Puckett MC. Pain among cancer survivors. Prev Chronic Dis. 2020;17. doi: 10/ghfdr9.

[3] Wang L, Cohen JC, Devasenapathy N, Hong BY, Kheyson S, Lu D, et al. Prevalence and intensity of persistent post-surgical pain following breast cancer surgery: A systematic review and meta-analysis of observational studies. Br J Anaesth. 2020;125:346–57. doi: 10.1016/j.bja.2020.04.088.

[4] Haenen V, Dams L, Meeus M, De Groef A. Altered somatosensory functioning and mechanism-based classification in breast cancer patients with persistent pain. Anat Rec. 2022. doi: 10.1002/ar.25121.

[5] Kosek E, Clauw D, Nijs J, Baron R, Gilron I, Harris RE, et al. Chronic nociplastic pain affecting the musculoskeletal system: clinical criteria and grading system. Pain. 2021;162:2629–34. doi: 10.1097/j.pain.0000000000002324.

[6] Vaegter HB, Petersen KK, Mørch CD, Imai Y, Arendt-Nielsen L. Assessment of CPM reliability: quantification of the within-subject reliability of 10 different protocols. Scand J Pain. 2018;18:729–37. doi: 10.1515/sjpain-2018-0087.

[7] Staud R. The important role of CNS facilitation and inhibition for chronic pain. Int J Clin Rheumatol. 2013;8:639–46. doi: 10.2217/ijr.13.57.

[8] Cathcart S, Winefield AH, Rolan P, Lushington K. Reliability of temporal summation and diffuse noxious inhibitory control. Pain Res Manag. 2009;14:433–8. doi: 10.1155/2009/523098.

[9] Vilholm OJ, Cold S, Rasmussen L, Sindrup SH. Sensory function and pain in a population of patients treated for breast cancer. Acta Anaesthesiol Scand. 2009;53:800–6. doi: 10.1111/j.1399-6576.2009.01938.x.

[10] Edwards RR, Mensing G, Cahalan C, Greenbaum S, Narang S, Belfer I, et al. Alteration in pain modulation in women with persistent pain after lumpectomy: Influence of catastrophizing. J Pain Symptom Manage. 2013;46:30–42. doi: 10.1016/j.jpainsymman.2012.06.016.

[11] Timmerman H, Wilder-Smith OH, Steegers M, Vissers K, Wolff A. The added value of bedside examination and screening QST to improve neuropathic pain identification in patients with chronic pain. J Pain Res. 2018;11:1307–18. doi: 10.2147/JPR.S154698.
[12] Zhu GC, Böttger K, Slater H, Cook C, Farrell SF, Hailey L, et al. Concurrent validity of a low-cost and time-efficient clinical sensory test battery to evaluate somatosensory dysfunction. Eur J Pain. 2019;23:1826–38. doi: 10.1002/ejp.1456.
[13] Reimer M, Forstenpointner J, Hartmann A, Otto JC, Vollert J, Gierthmühlen J, et al. Sensory bedside testing: a simple stratification approach for sensory phenotyping. PAIN Rep. 2020;5:e820. doi: 10.1097/PR9.0000000000000820.
[14] Koulouris AE, Edwards RR, Dorado K, Schreiber KL, Lazaridou A, Rajan S, et al. Reliability and validity of the boston bedside quantitative sensory testing battery for neuropathic pain. Pain Med. 2020;21:2336–47. doi: 10.1093/pm/pnaa192.
[15] Izumi M, Hayashi Y, Saito R, Oda S, Petersen KK, Arendt-Nielsen L, et al. Detection of altered pain facilitatory and inhibitory mechanisms in patients with knee osteoarthritis by using a simple bedside tool kit (QuantiPain). PAIN Rep. 2022;7:e998. doi: 10.1097/PR9.0000000000000998.
[16] Mertens MG, Hermans L, Crombez G, Goudman L, Calders P, Van Oosterwijck J, et al. Comparison of five conditioned pain modulation paradigms and influencing personal factors in healthy adults. Eur J Pain Lond Engl. 2020. doi: 10.1002/ejp.1665.
[17] Schreiber KL, Kehlet H, Belfer I, Edwards RR. Predicting, preventing and managing persistent pain after breast cancer surgery: the importance of psychosocial factors. Pain Manag. 2014;4:445–59. doi: 10.2217/pmt.14.33.
[18] Cruz-Almeida Y, Fillingim RB. Can quantitative sensory testing move us closer to mechanism-based pain management? Pain Med Malden Mass. 2014;15:61–72. doi: 10/f5pd54.
[19] Chimenti RL, Frey-Law LA, Sluka KA. A mechanism-based approach to physical therapist management of pain. Phys Ther. 2018;98:302–14. doi: 10/gdp5wc.
[20] Vardeh D, Mannion RJ, Woolf CJ. Toward a mechanism-based approach to pain diagnosis. J Pain. 2016;17:T50–69. doi: 10/gcsk6b.
[21] Reimer M, Sachau J, Forstenpointner J, Baron R. Bedside testing for precision pain medicine. Curr Opin Support Palliat Care. 2021;15:116–24. doi: 10.1097/SPC.0000000000000556.
[22] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61:344–9. doi: 10.1016/j.jclinepi.2007.11.008.
[23] Kaur N, Kumar R, Jain A, Saxena AK. Sensory Changes and Postmastectomy Pain Following Preservation of Intercostobrachial Nerve in Breast Cancer Surgery: a Prospective Randomized Study. Indian J Surg Oncol. 2021;12:108–13. doi: 10.1007/s13193-020-01193-5.
[24] Belfer I, Schreiber KL, Shaffer JR, Shnol H, Blaney K, Morando A, et al. Persistent postmastectomy pain in breast cancer survivors: analysis of clinical, demographic, and psychosocial factors. J Pain. 2013;14:1185–95. doi: 10.1016/j.jpain.2013.05.002.
[25] Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: a systematic review. Pain. 2016;157:2410–9. doi: 10.1097/j.pain.0000000000000689.
[26] Granovsky Y, Miller-Barmak A, Goldstein O, Sprecher E, Yarnitsky D. CPM test–retest reliability: “standard” vs “single test-stimulus” protocols. Pain Med. 2016. doi: 10.1111/pme.12868.
[27] Dams L, Haenen V, Van der Gucht E, Devoogdt N, Smeets A, Bernar K, et al. Absolute and relative reliability of a comprehensive quantitative sensory testing protocol in women treated for breast cancer. Pain Med. 2021;1162–75. doi: 10.1093/pm/pnab343.
[28] Rolke R, Baron R, Maier C, Tölle TR, Treede DR, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. Pain. 2006;123:231–43. doi: 10.1016/j.pain.2006.01.041.
[29] Pud D, Granovsky Y, Yarnitsky D. The methodology of experimentally induced diffuse noxious inhibitory control (DNIC)-like effect in humans. Pain. 2009;144:16–9. doi: 10.1016/j.pain.2009.02.015.
[30] Lewis GN, Luke H, Rice DA, Rome K, McNair PJ. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. Pain Res Manag. 2012;17:98–102. doi: 10.1155/2012/610561.
[31] Smith A, Pedler A. Conditioned pain modulation is affected by occlusion cuff conditioning stimulus intensity, but not duration. Eur J Pain. 2018;22:94–102. doi: 10.1002/ejp.1093.
[32] Awali A, Alsouhibani AM, Hoeger Bement M. Lean mass mediates the relation between temporal summation of pain and sex in young healthy adults. Biol Sex Differ. 2018;9:42. doi: 10.1186/s13293-018-0200-z.
[33] Kong J-T, Johnson KA, Balise RR, Mackey S. Test-retest reliability of thermal temporal summation using an individualized protocol. J Pain. 2013;14:79–88. doi: 10.1016/j.jpain.2012.10.010.
[34] Middlebrook N, Heneghan NR, Evans DW, Rushton A, Falla D. Reliability of temporal summation, thermal and pressure pain thresholds in a healthy cohort and musculoskeletal trauma population. PLOS ONE. 2020;15:e0233521. doi: 10.1371/journal.pone.0233521.
[35] Core Team R. R: A language and environment for statistical computing. Vienna, Austria: 2022.
[36] Portney LG. Foundations of clinical research: applications to evidence-based practice. Philadelphia, PA: F.A. Davis Company; 2020.
[37] Spearman C. The proof and measurement of association between two things. Am J Psychol. 1904;15:72–101. doi: 10.2307/1412159.
[38] Post MW. What to do with “moderate” reliability and validity coefficients? Arch Phys Med Rehabil. 2016;97:1051–2. doi: 10.1016/j.apmr.2016.04.001.
[39] Kuehl LK, Michaux GP, Richter S, Schächinger H, Anton F. Increased basal mechanical pain sensitivity but decreased perceptual wind-up in a human model of relative hypocortisolism. Pain. 2010;149:539–46. doi: 10.1016/j.pain.2010.03.026.
[40] Kennedy DL, Kemp HI, Wu C, Ridout DA, Rice ASC. Determining Real Change in Conditioned Pain Modulation: A Repeated Measures Study in Healthy Volunteers. J Pain. 2020;21:708–21. doi: 10.1016/j.jpain.2019.09.010.
[41] Salaffi F, Stancati A, Silvestri CA, Ciapetti A, Grassi W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. Eur J Pain. 2004;8:283–91. doi: 10.1016/j.ejpain.2003.09.004.
[42] Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole MR. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain. 2001;94:149–58. doi: 10.1016/S0304-3959(01)00349-9.
[43] Dworkin RH, Turk DC, Wyrwich KW, Beaton D, Cleeland CS, Farrar JT, et al. Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: immpact recommendations. J Pain. 2008;9:105–21. doi: 10.1016/j.jpain.2007.09.005.

[44] Fernandes C, Pidal-Miranda M, Samartin-Veiga N, Carrillo-de-la-Peña MT. Conditioned pain modulation as a biomarker of chronic pain: a systematic review of its concurrent validity. Pain. 2019;160:2679–90. doi: 10.1097/j.pain.0000000000001664.

[45] Bannister K, Hughes S. One size does not fit all: towards optimising the therapeutic potential of endogenous pain modulatory systems. Pain. 2022. Publish Ahead of Print. doi: 10.1097/j.pain.0000000000002697.

[46] Yarnitsky D, Bouhassira D, Drewes AM, Fillingim RB, Granot M, Hansson P, et al. Recommendations on practice of conditioned pain modulation (CPM) testing: CPM consensus meeting recommendations 2014. Eur J Pain. 2015;19:805–6. doi: 10.1002/ejp.605.

[47] Eckert NR, Vierck CJ, Simon CB, Calderon S, Cruz-Almeida Y, Staud R, et al. Methodological considerations for the temporal summation of second pain. J Pain. 2017;18:1488–95. doi: 10.1016/j.jpain.2017.07.009.

[48] Reezigt RR, Kielstra SC, Coppieters MW, Scholten-Peeters GGM. No relevant differences in conditioned pain modulation effects between parallel and sequential test design. A cross-sectional observational study. PeerJ. 2021;9:e12330. doi: 10.7717/peerj.12330.

[49] Schliessbach J, Lütolf C, Streitberger K, Scaramozzino P, Arendt-Nielsen L, Curatolo M. Reference values of conditioned pain modulation. Scand J Pain. 2019;19:279–86. doi: 10.1515/sjpain-2018-0356.

[50] Vigotsky AD, Tiwari SR, Griffith JW, Apkarian AV. What is the numerical nature of pain relief? Front Pain Res. 2021;2:756680. doi: 10.3389/fpain.2021.756680.

[51] Shraim MA, Massé-Alarie H, Hodges PW. Methods to discriminate between mechanism-based categories of pain experienced in the musculoskeletal system: a systematic review. Pain. 2021;162:1007–37. doi: 10.1097/j.pain.0000000000002113.

[52] Finnerup NB, Haroutounian S, Kamerman P, Baron R, Bennett DLH, Bouhassira D, et al. Neuropathic pain: an updated grading system for research and clinical practice. Pain. 2016;157:1599–606. doi: 10.1097/j.pain.0000000000000492.

[53] Nijs J, Lahousse A, Kapreli E, Bilika P, Saraçoğlu İ, Malfliet A, et al. Nociplastic pain criteria or recognition of central sensitization? pain phenotyping in the past, present and future. J Clin Med. 2021;10:3203. doi: 10.3390/jcm10153203.

Appendix S1

A1 CPM protocols

1. CPM reference test

The reference CPM protocol was performed using the Advanced Thermosensory Stimulator TSA-2 (Medoc, Ramat Yishai, Israel). First, the intensity of the stimulus was individualized for each participant. A Peltier 30 mm × 30 mm contact thermode was applied on the volar side of the unaffected forearm [26,27]. The temperature required to evoke an unpleasant sensation with a rating of 4 on the NRS was determined by administering a series of heat stimuli to the non-affected forearm (Pain4). During the first stimulation, the temperature increased to 43°C, starting from a baseline temperature of 32°C. The temperature increased at a rate of 2°C/s and decreased at a rate of 1°C/s. After each stimulus, participants were asked to verbally rate the intensity of pain using an NRS. If a score above or below 4/10 on the NRS was given, the temperature of the next stimulation was decreased or increased by 1°C. A maximum of five stimulations were administered in search of the Pain4 temperature. The minimum and maximum temperatures of the test stimulus were 39 and 46°C, respectively. After determining the Pain4 test stimulus, a parallel CPM paradigm was introduced. The Pain4 test stimulus was administered to the volar side of the affected forearm for 45 s. Participants were asked to verbally rate the intensity of the test stimulus at 10, 20, 30, and 40 s using the NRS. A 120 second break followed, after which the conditioning stimulus was administered to the volar side of the unaffected forearm for 65 s. The conditioning stimulus was set 0.5°C warmer than the Pain4 test stimulus. 20 s after applying the conditioning stimulus, the Pain4 test stimulus was applied in parallel to the volar side of the affected forearm. Verbal ratings of pain intensity for the affected forearm were obtained at 10, 20, 30, and 40 s of stimulation (0–10 NRS). The reliability of the QST protocol is considered to be weak [27] (Figure A1).

1. CPM bedside test 1: CPT

PPT was used as a test stimulus at the upper part of the tibialis anterior muscle opposite to the affected side. First, a baseline PPT without the presence of a conditioning stimulus was determined using a digital pressure algometer (Wagner FDX, Greenwich CT, USA) with a flat round rubber tip and probe area of 1 cm^2. The PPT was defined as the amount of pressure at which the sensation of pressure was first perceived as unpleasant, and was determined by two series of ascending pressure at a rate of approximately 0.98 kPa/s [28]. The final threshold was the arithmetic mean of two trials (kgf) [10]. Participants were blinded to the algometer's screen, making them uninformed of the imposed pressure [29,30].

The conditioning stimulus used in this first bedside CPM protocol consisted of a CPT in which the participants' unaffected hand was submerged in a cold-water bath of approximately 12°C. Tap water was brought to this target temperature by cooling for approximately 45 min using simple household cold packs. The participants' hand was then placed in a cold water bath until the wrist crease. After 30 s participants were asked to verbally rate the intensity of pain in the hand on the NRS. PPT was performed at 45 and 90 s, respectively, providing two PPT outcomes during the presence of a conditioning stimulus. Ascending pressure at a rate of 0.98 kPa/s was used until the participant verbally indicated that the pressure was unpleasant [28].

2. CPM bedside test 2: BPC occlusion

This protocol consisted of the same PPT test stimulus as the bedside CPM test with CPT. For the second bedside CPM test, a single, 8.5-cm-wide chamber BPC (Boso Profitest, Jungingen, Germany) exerted pressure on the unaffected arm, 2 cm superior to the cubital fossa. The occlusion cuff was inflated

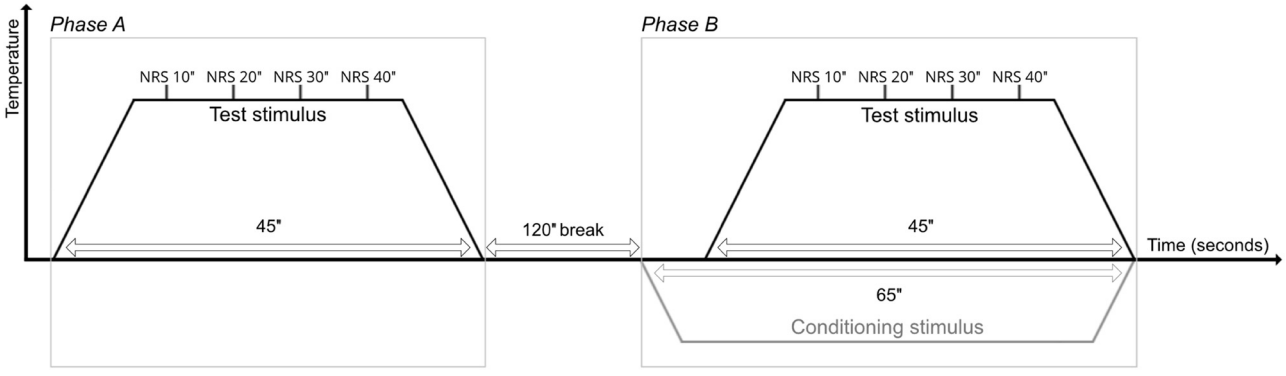

Figure A1: Schematic overview of the reference CPM protocol sequence using the TSA-2. NRS= numeric rating scale.

manually by the examiner via hand squeeze (approximately 20 mmHg per squeeze). After each squeeze, the participant was asked to rate the intensity of the pain on the NRS. The occlusion cuff was inflated until the participant experienced 5/10 on the NRS for pain or until 220 mmHg was exerted by the BPC. Arm ischaemia was not intended to happen [31]. PPT measurements were performed at the same timepoints (45 and 90 s) and rate of pressure as the CPM protocol using CPT [8].

A2 TSP protocols

1. TSP reference test

   TSP was measured at the most painful site (chest, lateral trunk, axilla, arm, or shoulder) and the upper part of the opposite tibialis anterior muscle by applying a series of heat stimuli utilizing the TSA-2 (Medoc, Ramat Yishai, Israel) with a 30 mm × 30 mm Peltier thermode. The intensity of the heat stimuli was individualized for each subject using the Pain4 temperature assessed in the CPM reference protocol. The participants received one train of 30 heat stimuli, starting from a baseline temperature of 38°C and with a peak temperature set at an individualized Pain4 temperature. Thermal TSP was executed with an increase in temperature at a rate of 13°C/s, 0.8 s at peak stimulus, and a return rate of 13°C/s to baseline temperature. The inter-stimulus interval was set to 1 second with the stimulus frequency approximating 1 Hz [32]. Participants were asked to verbally rate the intensity of pain immediately after the first and last heat stimulus on the NRS [33].
2. TSP bedside test 1: von Frey monofilament

   The first bedside TSP test was measured at the same locations by applying a series of stimuli using a von Frey monofilament with a stimulation force of 256 mN (Optihair2-Set, Marstock Nervtest, Germany). After the first stimulus, a series of stimuli was delivered for 30 s at a rate of 1 stimulation/s. Participants were asked to score the pain after the first stimulus on the NRS and immediately after the series of stimuli [27].
3. TSP bedside test 2: algometer

   For the second bedside TSP test, a digital pressure algometer (Wagner FDX, Greenwich, CT, USA) was used at both locations. Peak pressure was set at 98 kPa with a stimulation frequency of 1 Hz. Amounts of repetitions, and instructions to the patient were the same as the bedside TSP test with the von Frey monofilament [8,34].

Appendix S2

Participants' experience

1. The testing was comfortable: yes/no
2. Instructions were clear: yes/no

Participants' test preference

1. Temporal summation at the most painful site
   Algometer vs von Frey monofilament
2. Temporal summation at the tibialis anterior
   Algometer vs von Frey monofilament
3. Conditioned pain modulation
   Cold pressor test vs blood pressure cuff