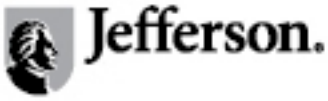

6-1-2019

Quantitative sensory testing in children with sickle cell disease: additional insights and future possibilities.

Robin E. Miller
Nemours/Alfred I duPont Hospital for Children

Dawn S. Brown
Nemours/Alfred I duPont Hospital for Children

Scott W. Keith
Thomas Jefferson University, Scott.Keith@jefferson.edu

Sarah E. Hegarty
Thomas Jefferson University, sarah.hegarty@jefferson.edu

Yamaja Setty
Nemours/Alfred I duPont Hospital for Children

See next page for additional authors

Authors
Robin E. Miller, Dawn S. Brown, Scott W. Keith, Sarah E. Hegarty, Yamaja Setty, Claudia M. Campbell, Suzanne M. McCahan, Suhita Gayen-Betal, Hal Byck, and Marie Stuart

bjh research paper

Quantitative sensory testing in children with sickle cell disease: additional insights and future possibilities

Robin E. Miller,[1] Dawn S. Brown,[1] Scott W. Keith,[2] Sarah E. Hegarty,[2] Yamaja Setty,[1] Claudia M. Campbell,[3] Suzanne M. McCahan,[4] Suhita Gayen-Betal,[1] Hal Byck[5] and Marie Stuart[1]

[1]Nemours Center for Cancer and Blood Disorders, Nemours/Alfred I duPont Hospital for Children, Wilmington, DE, [2]Division of Biostatistics, Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA, [3]Department of Psychiatry and Behavioral Health, Johns Hopkins University School of Medicine, Baltimore, MD, [4]Bioinformatics Core Facility, Nemours Biomedical Research, Alfred I duPont Hospital for Children, and [5]Department of Pediatrics, Nemours/Alfred I duPont Hospital for Children, Wilmington, DE, USA

Received 28 September 2018; accepted for publication 28 January 2019
Correspondence: Robin E. Miller, Nemours Center for Cancer and Blood Disorders, Nemours/Alfred I duPont Hospital for Children, 1600 Rockland Road, Wilmington, DE 19803, USA.
E-mail: rmiller@nemours.org

Summary

Quantitative sensory testing (QST) is used in a variety of pain disorders to characterize pain and predict prognosis and response to specific therapies. In this study, we aimed to confirm results in the literature documenting altered QST thresholds in sickle cell disease (SCD) and assess the test–retest reliability of results over time. Fifty-seven SCD and 60 control subjects aged 8–20 years underwent heat and cold detection and pain threshold testing using a Medoc TSAII. Participants were tested at baseline and 3 months; SCD subjects were additionally tested at 6 months. An important facet of our study was the development and use of a novel QST modelling approach, allowing us to model all data together across modalities. We have not demonstrated significant differences in thermal thresholds between subjects with SCD and controls. Thermal thresholds were consistent over a 3- to 6-month period. Subjects on whom hydroxycarbamide (HC) was initiated shortly before or after baseline testing (new HC users) exhibited progressive decreases in thermal sensitivity from baseline to 6 months, suggesting that thermal testing may be sensitive to effective therapy to prevent vasoocclusive pain. These findings inform the use of QST as an endpoint in the evaluation of preventative pain therapies.

Keywords: sickle cell disease, quantitative sensory testing, vasoocclusive pain, thermal thresholds, pressure pain threshold.

Quantitative sensory testing (QST) evaluates sensory function using a set of standardized psychophysical procedures to measure somatosensory responses to physiological stimuli including temperature and pressure. QST provides an extension of the standard neurological examination by using carefully calibrated stimuli to examine the function of specific peripheral and central nervous system pathways to assess both gain and loss of function. The clinical utility of QST has been well documented in a variety of pain disorders, such as arthritis and neuropathic pain syndromes, and it has been used to characterize pain and predict prognosis and response to therapy, as described in several excellent reviews (Arendt-Nielsen & Yarnitzky, 2009; Backonja *et al*, 2013; Cruz-Almeida & Fillingim, 2014; Bouhassira & Attal, 2016; Edwards *et al*, 2016a; Smith *et al*, 2017). While the majority of this work has been done in adults (Coronado *et al*, 2014; Edwards *et al*, 2016b; Moss *et al*, 2016, 2018; Maher *et al*, 2017), there is a growing body of literature in paediatric disorders (Blankenburg *et al*, 2012, 2018; Kristensen *et al*, 2012; Cornelissen *et al*, 2014; Lieber *et al*, 2018; Teles *et al*, 2018). QST may also be used to elucidate underlying mechanisms in order to evaluate whether targeted treatments may be effective (Grosen *et al*, 2013) and to measure somatosensory profiles and changes in physiological responses over time (Geber *et al*, 2011).

In sickle cell disease (SCD), the nature of vasoocclusive crisis (VOC), factors underlying the transition from acute to chronic pain and the wide variability in the pain experience between individuals are poorly understood. Optimal management for this complex pain syndrome remains highly

 First published online 28 March 2019
British Journal of Haematology, 2019, **185,** 925–934 doi: 10.1111/bjh.15876

problematic despite over four decades of basic and clinical research in the field (Ballas *et al*, 2012). Hydroxycarbamide (HC) and, more recently, L-glutamine are the only medications which have been approved by the US Food and Drug Administration for use in the prevention of VOC (Ware, 2015; Ware *et al*, 2017; Quinn, 2018).

Investigations of new therapies to treat and prevent SCD pain are hampered by the lack of objective outcome measures available to assess efficacy and rely heavily on highly subjective measures, such as patient pain reports and number of days spent in hospital. Analysing SCD pain on a more mechanistic basis through QST may provide a means to develop more patient-specific therapies to treat pain as well as to prevent the transition to chronic or neuropathic pain. In this study we aimed to further evaluate the seemingly ambiguous results in the literature documenting altered QST thresholds in SCD and assess the test–retest reliability of results over time in a paediatric population.

Methods

Subject recruitment

Subjects with SCD and control subjects were recruited from the Pediatric SCD Clinic at Nemours/Alfred I duPont Hospital for Children (NAIDHC). Additional controls were recruited from a Nemours General Pediatric Clinic as well as through advertisements within NAIDHC. Subjects with SCD at their baseline state of health and healthy African American controls aged 8–20 years were included in the study. In addition to the fact that children and young adults were most accessible to the study institution, this population was chosen in an effort to confirm and extend the results of a recent study of QST in SCD (Brandow *et al*, 2013). Patients with SCD genotypes SS, $S\beta^0$ thalassaemia and SC were eligible for the trial. Family groups as well as siblings and other family members of individuals with SCD were included in the control group, and individuals with sickle cell trait (SCT) were not excluded. At enrolment, all subjects were required to be at their baseline state of health and not in the midst of any acute complication of SCD or other acute illness (i.e. steady state). Subjects with SCD were excluded if they had had an acute painful event severe enough to require inpatient treatment with opioids within 2 weeks of enrolment. SCD and control subjects were excluded if they had significant neurocognitive impairment which would prohibit understanding test procedures or had another chronic illness that produced clinical pain. Subjects were compensated $70 after each of the testing sessions.

Testing procedures

All subjects were tested at baseline (day 1) and 3 months later. Subjects with SCD were also tested at 6 months from baseline. Subjects were instructed not to take any pain medication within 24 h prior to a testing session. If they reported taking pain medication within 24 h, the testing session was delayed. Thus, while subjects with chronic pain were not excluded, those on a regular schedule of daily pain medications were not able to participate if they could not tolerate 24 h without medication. For testing, participants were seated in a comfortable chair, positioned so they could not see monitors, in a quiet room with controlled air temperature (68–72°C). Testing was performed without a parent, guardian or observer in the room as presence of a parent during testing has been shown to influence results (Zohsel *et al*, 2006). However, in the presence of the subject, parent/guardian was permitted to undergo a test trial of the planned thermal and pressure testing and was permitted to observe testing procedures via video monitoring from an adjacent room. Subjects were given 10 min to acclimatize to the room temperature prior to beginning study procedures. Each participant completed all testing procedures. All testing was performed by a single research nurse.

Thermal testing

Thermal testing was performed using the TSAII Neurosensory Analyzer (Medoc Ltd., Yishai, Israel) with a 9 cm^2 thermode. Testing was performed in triplicate, on the volar surface of the forearm. For triplicate testing, the thermode was moved up the forearm with each test applied to a different patch of skin to avoid retesting the same area. Thresholds were assessed in the following order: (i) cold detection threshold (cdt), (ii) heat detection threshold (hdt), (iii) cold pain threshold (cpt) and (iv) heat pain threshold (hpt). The baseline temperature of the probe was 32°C. Probe temperatures were not permitted to exceed 48°C nor go below 0°C. For thermal detection thresholds, the thermode temperature was decreased/increased from baseline at a rate of 1°C/s and for thermal pain threshold at a rate of 1.5°C/s. Testing was performed using the Method of Limits, where participants were instructed to press a button when the sensation in question was first perceived. This method has been used in other paediatric QST studies in SCD (Brandow *et al*, 2013; O'Leary *et al*, 2014) as well as in adults (Campbell *et al*, 2016a) and requires the shortest testing duration of all methods, which is particularly desirable for a paediatric study. For temperature detection thresholds (cdt and hdt) participants were asked to indicate the point at which they first felt a sensation of cold or heat, respectively. For pain thresholds (cpt and hpt) participants were asked to indicate the point at which these sensations first produced a painful sensation.

Please note that subjects detecting cold (cdt) or reporting pain (cpt) with cold at higher temperatures than controls or at baseline are exhibiting greater sensitivity. Conversely, for heat detection thresholds (hdt) and heat pain thresholds (hpt), more sensitivity means that subjects detected heat or reported pain with heat at lower temperatures than control subjects or at baseline.

Pressure pain testing

An electronic algometer (SBMedic Electronics, Solna, Sweden) with a 1 cm^2 hard rubber probe was used to assess responses to mechanical pressure (Brennum, 1989). Pressure was increased steadily at a constant rate (50 kPA/s) and subjects indicated when the sensation changed from pressure to the slightest pain, at which point the application was immediately terminated. Pressure pain thresholds were assessed in triplicate at each of two body sites bilaterally: the trapezius muscle and the proximal third of the brachioradialis (forearm). Testing was done on non-overlapping skin sites. Pressure was applied to the muscle belly only. Because of some concern that pressure pain testing (PPT) might cause more anxiety and thus be less well tolerated than thermal testing for this patient population, PPT was included as an optional procedure to be done only at the last study visit for subjects with SCD and controls.

Pain perception and anxiety

Anticipatory anxiety and perceived pain intensity were assessed using a 10-point scale, with one indicating the lowest level and 10 indicating the highest level. Before each thermal or pressure testing session, subjects were instructed on use of the scale. Prior to each testing session, participants were asked to identify, using the scale, how "nervous, afraid or worried" they were about the upcoming task to indicate level of anxiety. Specifically, they were asked "Before we start this testing I have a question. I would like you to tell me how nervous or worried you are about doing this testing on a scale of 1–10? One is not being worried at all and 10 being the most worried you could be." Subjects were queried at the end of the testing session about how intense the experience was for them (Lazaridou *et al*, 2018). Specifically, they were asked "Now that we have finished testing I have another question. On a scale of 1–10, how painful was this test? One being not painful at all and 10 being the most pain you could be in."

Pain diary

Participants with SCD were asked to keep a daily pain diary starting at the time of initial testing and continued until all follow-up testing was completed. An Apple iPad mini™ providing electronic access to the diary was distributed to each subject at the time of enrolment and paper diaries with the same questions as those on the iPad were provided as back up. The electronic Pain Diary was written in the PHP and JavaScript programming languages and was accessible via a username and password protected website. All data, including usernames and passwords, was stored in REDCap via its API (Harris *et al*, 2009). Subjects were asked to document each episode of pain, identify parts of their body affected by pain on the Collaborative Health Outcomes Information Registry (CHOIR) body map (http://choir.stanford.edu), indicate duration of pain symptoms, maximum severity of pain and type of medication administered, and answer some questions regarding the impact of pain on daily functioning. The diary was available between 6:30 pm and 3 am each day. Entries from previous days were not permitted.

Clinical data

Information regarding SCD genotype, current treatment regimen (e.g. chronic blood transfusions, HC), number of health care provider contacts for VOC (clinic visits, hospitalizations and Emergency Room visits) over the past 3 years and history of previous SCD complications was gathered from the electronic medical record (EMR) at NAIDHC. Baseline haemoglobin, white blood cell (WBC) and reticulocyte counts were also collected. Control subjects or their parent/guardian were asked to disclose whether or not they carried SCT, or if they were unsure of their SCT status.

Biomarkers

For SCD subjects only, prior to initial and at 6-month follow-up testing, blood was drawn into citrate anticoagulant tubes for measurement in plasma of lactate dehydrogenase (LDH) activity (a marker of haemolysis), and high-sensitivity C-reactive protein [hs-CRP; a marker of inflammation shown to correlate with hospitalization for vasoocclusive pain in SCD (Krishnan *et al*, 2010)]. LDH and hs-CRP levels were evaluated using standard methods (Krishnan *et al*, 2010).

Statistical methods

Baseline characteristics. Baseline clinical and demographic data were summarized by group (SCD *versus* control) using means and standard deviations or frequencies and percentages, as appropriate.

SCD versus *controls.* To assess differences between SCD and controls on QST values, a longitudinal general linear model was used to model the technical replicate measurements repeated at baseline and 3 months. The raw QST values themselves were shifted for the purposes of modelling to delta temperature values that reflect the absolute temperature deviation from the instrument's reference temperature of 32°C, so that each QST value could be modelled together. Model terms included group, time point, modality, age, sex, pre-test anxiety, the three-way and each two-way interaction between group, time point and modality. A compound symmetric correlation structure was used to account for the strong correlation among the repeated measures within subjects and modality at each time point (Fitzmaurice *et al*, 2011). Robust standard errors were computed using the Huber sandwich estimator (Liang & Zeger,1986). A similar longitudinal general linear model was fitted to only the SCD

patients, and included baseline, 3- and 6-month follow-up data and adjustment for additional covariates, including HC (none, beginning within 5 months before baseline, beginning at least 5 months before baseline), transfusions (none, beginning within 5 months before baseline, beginning at least 5 months before baseline) and genotype. Linear contrasts were used to estimate modality-specific group means and differences between groups or between time points (Casella & Berger, 2002).

Thermal composite score. From these QST models, we estimated a novel thermal composite score (TCS). The TCS can be estimated simply by averaging all the absolute temperature deviations across all modalities (cdt, cpt, hdt and hpt). It is an aggregated measure of a subject's thermal sensitivity across modalities in terms of the absolute temperature deviations of their QST measurements from the instrument's reference temperature of 32°C.

Test–retest reliability. To assess test–retest reliability of QST scores, we fitted longitudinal general linear models adjusted for covariates, as mentioned above, to each QST modality and TCS separately and used the intraclass correlation coefficients (ICCs) from those models as a measure of the within-subject agreement of QST scores for each modality and TCS across technical replicates over time.

Power calculation. In a frequently cited paper, Brandow *et al* (2013) reported significantly increased thermal sensitivity in children with SCD compared with African American controls. We powered our study using data from Brandow *et al* (2013), anticipating between-group effect sizes of 0.46 for heat threshold (difference of approximately 2.5 degrees) and 0.58 (difference of approximately 6.3 degrees) for cold threshold. With 60 subjects per group, we expected to have 80% power to detect an effect size of 0.52 or greater using a two-sided *t*-test with a 5% type I error rate.

Biomarker correlations. Correlations of baseline biomarker levels with baseline QSTs in terms of the averages of technical replicates within participant and modality were computed for the SCD subjects using Spearman's rho.

Analysis of new HC users. As a retrospective nested pre-post study, we analysed a subset of "new" HC users consisting of SCD subjects in whom HC was initiated within 1 month prior to or at any time between their baseline and last QST test session (Table I). To do this, we constructed a longitudinal general linear model and linear contrasts as described above, but without covariate adjustment due to the small subset sample size.

SAS version 9.4 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. The significance level for all tests was set *a priori* at 0.05.

Results

Study population and baseline characteristics

Sixty SCD subjects and 60 control subjects were enrolled in the study. Three subjects with SCD did not complete the study. One patient was lost to follow-up after undergoing baseline testing. One subject was removed from study after baseline testing and another after the second testing session when they were started on long-acting opioids to treat chronic pain and thus were not able to tolerate 24 h without pain medications to permit testing per protocol. Fifty-seven SCD and 60 controls completed all thermal testing. SCD and controls were similar in age and gender. Table II provides information on age, gender and sickle cell diagnosis and summarizes information on pertinent medical history, baseline laboratory data and ongoing treatment (chronic transfusion therapy or HC). There were no subjects on medications to treat neuropathic pain.

Overall, thermal and pressure testing was well tolerated with no reported adverse events, and after baseline testing no patient refused subsequent testing. No subject reported the occurrence of vasoocclusive symptoms related to testing procedures.

Thermal sensitivity

There were no significant differences in thermal thresholds between SCD and control subjects at baseline or month 3 (Table III, Table SI). There was no correlation between

Table I. For the subset of subjects with sickle cell disease who started HC just prior to or shortly after baseline testing, this table describes the timing of HC initiation in relation to QST testing sessions and shows the rise in MCV from baseline test date to 6-month testing.

Subject	Timing of HC initiation in relation to QST testing	Change in MCV from QST1 to QST 3
1	HC initiated 29 days before QST session 1	87 to 88
2	HC initiated 21 days before QST session 1	91 to 100
3	HC initiated on day of QST session 2	95 to 105
4	HC initiated 18 days after QST session 1	77 to 87
5	HC initiated 50 days after QST session 1	100 to 114
6	HC initiated 128 days after QST session 1	77 to 90

HC, hydroxycarbamide; MCV, mean corpuscular volume; QST, quantitative sensory testing.

Table II. Baseline demographic and haematological data of study participants.

	Participants with SCD (n = 60)	Control participants (n = 60)
Age, years (mean ± SD)	13.6 ± 3.8	12.7 ± 3.2
Female, *N* (%)	33 (55.0)	35 (58.3)
Sickle cell trait status, *N* (%)		
Reported positive		17 (28.3)
Reported negative		24 (40.0)
Unsure		19 (31.7)
Sickle cell diagnosis, *N* (%)		
SS	42 (70.0)	
SC	17 (28.3)	
$S\beta^0$ thalassaemia	1 (1.7)	
History of stroke, *N* (%)	8 (13.3)	
History of ≥2 episodes of acute chest, *N* (%)	30 (50.0)	
Number of VOC (inpatient or outpatient), *N* (%)		
0	21 (35.0)	
1–5	25 (41.7)	
6–10	5 (8.3)	
>10	9 (15.0)	
Hydroxycarbamide use, *N* (%)	14 (23.3)	
Chronic blood transfusions, *N* (%)	16 (26.7)	
Baseline hematological data, mean ± SD		
Haemoglobin (g/l)	98.1 ± 14.6	
WBC count (10^9/l)	10.99 ± 4.63	
Reticulocyte count (%)	7.50 ± 4.34	
hs-CRP* (mg/l)	3.78 ± 4.99	
LDH (iu/l)	426.42 ± 166.59	

hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; SCD, sickle cell disease; SD, standard deviation; VOC, venocclusive crisis; WBC, white blood cell.
*n = 58.

Table III. Difference in thermal thresholds and TCS expressed in degrees Celsius, between SCD and controls at baseline and 3-month testing, adjusted for age, sex and pre-test anxiety (TCS was also adjusted for modality).

		Control		SCD		Difference (SCD − Control)			
Modality	Time point	Mean	SE	Mean	SE	Mean	SE	95% CI	*P*
cdt	Day 1	28.28	0.31	28.98	0.22	0.70	0.38	−0.05, 1.44	0.07
cdt	Month 3	27.96	0.40	27.97	0.38	0.01	0.55	−1.08, 1.09	0.99
cpt	Day 1	22.44	0.86	22.73	0.89	0.29	1.24	−2.14, 2.72	0.82
cpt	Month 3	22.68	0.91	22.70	0.76	0.03	1.19	−2.31, 2.36	0.98
hdt	Day 1	35.69	0.32	35.31	0.27	−0.38	0.42	−1.19, 0.44	0.37
hdt	Month 3	35.67	0.27	36.05	0.33	0.38	0.43	−0.46, 1.22	0.38
hpt	Day 1	40.00	0.43	39.94	0.41	−0.06	0.59	−1.23, 1.10	0.92
hpt	Month 3	40.19	0.44	40.32	0.47	0.13	0.64	−1.13, 1.39	0.84
TCS	Day 1	6.24	0.27	5.88	0.26	−0.36	0.37	−1.09, 0.38	0.34
TCS	Month 3	6.31	0.28	6.42	0.26	0.12	0.38	−0.64, 0.87	0.76

cdt, cold detection threshold; CI, confidence interval; cpt, cold pain threshold; hdt, heat detection threshold; hpt, heat pain threshold; SCD, sickle cell disease; SE, standard error; TCS, thermal composite score.

thermal thresholds and pre-test anxiety or post-test pain perception scores (data not shown). Age did not significantly associate with thermal thresholds except among SCD subjects whose cdt increased, on average, by 0.14°C per year (P = 0.01). In subjects with SCD, there was no significant association between thermal thresholds and genotype, gender, chronic transfusion therapy, history of stroke or acute chest syndrome, number of VOCs in the 3 years prior to

Table IV. Thermal test–retest over 6 months for subjects with SCD only, adjusted for age, sex, hydroxycarbamide,* transfusions,* genotype (SS, S Beta 0, or SC), and pre-test anxiety (TCS was also adjusted for modality).

	Day 1		Month 3		Month 6		Difference (Month 3 − Baseline)			Difference (Month 6 − Baseline)			Difference (Month 6 − Month 3)		
Modality	Mean	SE	Mean	SE	Mean	SE	Diff	95% CI	*P*	Diff	95% CI	*P*	Diff	95% CI	*P*
cdt	28.60	0.68	27.78	0.75	27.91	0.71	−0.81	−1.66, 0.04	0.06	−0.68	−1.46, 0.10	0.09	0.13	−1.08, 0.82	0.79
cpt	22.35	1.01	22.52	0.95	21.78	1.19	0.17	−2.10, 2.44	0.89	−0.57	−3.07, 1.92	0.65	−0.74	−1.59, 3.07	0.53
hdt	35.69	0.70	36.23	0.74	36.17	0.72	0.54	−0.30, 1.38	0.21	0.48	−0.40, 1.35	0.28	−0.06	−0.84, 0.96	0.89
hpt	40.32	0.75	40.50	0.79	41.29	0.78	0.19	−1.03, 1.41	0.76	0.97	−0.26, 2.20	0.12	0.78	−2.04, 0.47	0.22
TCS	6.26	0.66	6.61	0.68	6.94	0.73	0.34	−0.38, 1.06	0.35	0.68	−0.10, 1.46	0.09	0.33	-0.40, 1.07	0.37

cdt, cold detection threshold; CI, confidence interval; cpt, cold pain threshold; Diff, difference; hdt, heat detection threshold; hpt, heat pain threshold SCD, sickle cell disease; SE, standard error; TCS, thermal composite score.

*Three level variable: no use (reference level), use started within 5 months, use starting more than 5 months ago.

enrolment, baseline haemoglobin, WBC and reticulocyte counts, LDH or hs-CRP (data not shown). There was a positive correlation between hs-CRP and LDH ($P < 0.001$).

While there was some variability in mean thermal thresholds among SCD subjects over time, there were no significant differences between any two time points (Table IV, Table SII). In addition, there were no significant differences in mean thermal testing results between baseline and 3 months for controls subjects (data not shown) and no significant statistical interactions between modality and study group or time point variables in our models (all $P \geq 0.30$, Tables SI and SII), which suggests the TCS is a reasonable outcome for comprehensively assessing these comparisons. QST measurements were internally consistent in SCD and control subjects for cpt, hdt and hpt (each ICC > 0.55), but not necessarily for cdt (ICC = 0.37) or TCS (ICC = 0.38). We analysed the internal consistency of QST measurement in SCD subjects alone over all three time points and the ICC results were similar.

The group of six subjects with SCD in whom HC was initiated within 1 month prior or between baseline and final testing (described in Table I) demonstrated trends toward reductions in both thermal sensitivity and significant reductions in both thermal pain threshold measurements, as well as the TCS, over time following HC initiation (Table V).

Pressure pain thresholds

Sickle cell disease and control subjects opting in for pressure testing had similar demographics (Table SIII). The SCD subjects showed significantly higher sensitivity to pressure pain than controls (reported pain at lower levels of pressure) in the brachioradialis, but not the trapezius (Table VI, Table SIV).

Pain diary

Compliance with daily entries into pain diary was poor, with only 43.3% of patients entering data ≥50% of the days and 66.6% entering data ≥25% of the days. In these limited data, there was no significant correlation between the number of pain days and thermal thresholds in those who filled out their diaries ≥25% or 50% of the time.

Discussion

Our study using QST did not demonstrate any differences in thermal sensitivity or pain thresholds between subjects with SCD and healthy African American controls. Our results are similar to a recent report by Bakshi *et al* (2017). Interestingly, in our patients who elected to undergo PPT, we have also demonstrated significantly increased sensitivity to pressure pain in the brachioradialis but not the trapezius, as

Table V. Thermal test–retest data over 6 months for "new HC" user SCD subgroup ($n = 6$).

	Day 1	Month 3	Month 6	Difference (Month 3 − Day 1)			Difference (Month 6 − Day 1)		
Modality	Mean	Mean	Mean	Mean	95% CI	*P*	Mean	95% CI	*P*
cdt	29.32	29.00	27.57	−0.32	−2.09, 1.41	0.70	−1.75	−4.62, 1.12	0.22
cpt	23.90	22.38	18.01	−1.52	−4.32, 1.28	0.28	−5.89	−11.15, −0.64	0.03
hdt	35.02	35.37	37.39	0.34	−0.95, 1.64	0.59	2.37	−0.55, 5.29	0.11
hpt	39.34	41.92	43.09	2.58	−0.19, 5.34	0.07	3.75	0.94, 6.56	0.01
TCS	5.29	6.48	8.73	1.19	0.07, 2.32	0.04	3.44	1.63, 5.25	<0.01

cdt, cold detection threshold; CI, confidence interval; cpt, cold pain threshold; HC, hydroxycarbamide; hdt, heat detection threshold; hpt, heat pain threshold SCD, sickle cell disease; TCS, thermal composite score.

Table VI. Model-adjusted* differences in pressure pain thresholds between participants with SCD and control participants by site of test.

	Control		SCD		Difference (SCD − Control)		
Location	Mean	SE	Mean	SE	Mean	95% CI	*P*
Brachioradialis – Left	450.42	19.45	372.50	21.63	−77.92	−136.09, −19.76	0.009
Brachioradialis – Right	408.78	19.45	322.83	21.74	−85.94	−144.26, −27.62	0.004
Trapezius – Left	427.26	19.54	388.33	21.63	−38.92	−97.20, 19.35	0.190
Trapezius – Right	425.72	19.45	402.65	21.63	−23.07	−81.23, 35.09	0.435

CI, confidence interval; SCD, sickle cell disease; SE, standard error.
*Adjusted for age, sex and pre-test anxiety (data reported in kPA/s).

previously demonstrated by Campbell *et al* (2016a). QST was well tolerated by children as young as 8 years of age and was relatively quick, taking only 10–15 min to complete the full set of thermal testing used in this study.

Quantitative sensory testing is a potentially valuable tool that could be useful in assessing the effectiveness of preventative pain therapies in SCD. Outcome measures used in current SCD clinical trials are fairly limited and highly problematic. Pain reports are subjective and, unless daily pain diary entries are used, subject to recall bias. Reduction in pain medication use is similarly difficult to track. As demonstrated by our study, compliance with pain diaries can be poor. Most pharmaceutical intervention trials last 6–12 months. Changes in rate of hospitalization or outpatient visits for VOC are often used, but incidence of severe VOC can vary tremendously from year to year in a given patient, and many patients do not seek medical attention frequently for their crises, making it difficult to document a true change over the typical span of a clinical trial using these measures. Decreased thermal and/or pressure sensitivity and pain thresholds documented in response to a given treatment modality could serve as less subjective markers of response to a preventative pain therapy for use in a short duration clinical trial than these standard outcome measures.

For changes in QST thresholds following an intervention to be useful in clinical trials, there must be relative stability in a given patient's baseline results. To our knowledge, our study is the first to document consistency of QST results in SCD subjects over time. Our ICC results showed that between-subject variability accounted for between 1/3 and 2/3 of the total variance, depending on the QST modality, suggesting that thermal thresholds tend to be consistent over a 3- to 6-month period in subjects with SCD as well as in African American controls. Age was investigated as a possible source of inconsistency among the repeated QST measurements, however, we noted no associations between subject age and QST means or QST replicate standard deviations (data not shown) either in descriptive analyses or in statistical models. Our longitudinal modelling results between the second and third testing session in subjects with SCD were highly consistent, indicating that reliability may improve once subjects have experience with the testing procedures. Based on these data, we would recommend that for clinical trial use, subjects undergo a "practice" QST session to familiarize themselves with the procedures and alleviate any anxiety prior to their baseline QST testing.

Of particular interest is our finding that in six subjects with SCD on whom HC therapy was initiated within 1 month prior to, or between their baseline and final QST test session, all exhibited progressively decreased sensitivity in thermal threshold parameters. While the small sample size and the variable start times of HC relative to QST test sessions limit the conclusions that can be drawn, these results, suggesting that thermal testing may be sensitive to an effective treatment to prevent vasoocclusive pain, should be followed up in larger, more rigorous studies.

Several groups have recently reported on QST in SCD (Brandow *et al*, 2013, 2019; O'Leary *et al*, 2014; Jacob *et al*, 2015; Brandow & Panepinto, 2016; Campbell *et al*, 2016a; Ezenwa *et al*, 2016; Bakshi *et al*, 2017; Veluswamy *et al*, 2018), raising interest in the use of QST as a modality to help elucidate the mechanisms of pain in SCD. Interestingly, Veluswamy *et al* (2018) demonstrated stronger and more rapid vasoconstriction in SCD subjects in response to thermal stimuli, most significantly cold, compared to controls, suggesting that heightened vascular autonomic reactivity may be linked to cold-related VOC in SCD. Overall, however, the results of these studies have been variable, with some groups showing altered thermal thresholds in patients with SCD as compared to control subjects, while other have shown no differences. While Campbell *et al* (2016a) did demonstrate reduced heat pain tolerance in the adult subjects with SCD when compared to African American controls, they were unable to demonstrate any differences in hpts. The volar surface of the forearm was used as the site of testing in our work, as well as in the studies reported by Bakshi *et al* (2017) and Campbell *et al* (2016a).

In contrast, Brandow *et al* (2013) reported significantly increased cdt and cpts and decreased hdt and hpts (indicating increased sensitivity to heat and cold) in children with SCD compared with an African American control group when testing was performed at the thenar eminence. No differences were observed when the foot was the tested area (Brandow *et al*, 2013). In a more recent study, the same

group showed that subjects with SCD had increased sensitivity to cold pain in the hand, but not in the foot, during hospitalization for acute painful events when compared to baseline health (Brandow *et al*, 2019). A study by O'Leary *et al* (2014) showed no significant differences between children with SCD and African American controls in thermal detection or pain thresholds when testing was performed on the forearm. However, when testing was performed on the thenar eminence, they found that children with SCD were less sensitive to cold and heat detection, and had increased sensitivity to cold pain when compared to controls (O'Leary *et al*, 2014). Thus, we suggest that differences between our results and those of Brandow *et al* (2013) may be explained, at least in part, by different body sites used for testing. While our study was similar in size and overall design to the study reported by Brandow *et al* (2013), these authors performed testing on the thenar eminence while in our study, testing was performed on the volar surface of the forearm. Testing on the forearm allows for movement of the probe to a new, non-overlapping skin site between each test. It is possible that repeated testing on the same skin site, as would be necessary when using a small area such as the thenar eminence, could produce sensitization at that site. Additionally, testing on glabrous (thenar) *versus* hairy skin (forearm) could have contributed to the differences observed between our studies, as thermal detection and pain thresholds have been shown to vary based on nociceptive innervation (Granovsky *et al*, 2005).

In a follow-up report, Brandow and Panepinto (2016) proposed cpt and hpt cut-off values indicative of impairment in children with SCD. When we apply these cut-offs to our African American cohort, we find that 50 (83%) of the controls and 49 (82%) of the SCD group would be considered to have impaired cpt and 51 (85%) of the controls and the SCD group would be considered to have impaired hpt. When Bakshi *et al*, (2017) applied the thresholds for impairment suggested by Brandow and Panepinto (2016) for children with SCD, they found that 68% of the SCD and 56% of the control participants had "impaired" cpt values, while 90% of the SCD and 85% of the control participants fell in the abnormal range for hpt. While published studies have established normal standards based on age and sex, African Americans have been shown to have reduced tolerance to experimentally-induced pain (Edwards & Fillingim, 1999; Campbell *et al*, 2005). Normative QST values for a healthy African American paediatric population are not yet well established. Furthermore, ethnicity within the SCD population varies considerably so African American norms may not be directly applicable to all SCD patients. In view of our findings and those of Bakshi *et al* (2017), use of the previously suggested normative QST values warrants caution.

An important facet of our study was the development and use of our novel thermal testing composite score, the TCS. Constructing the delta temperature values necessary for computing the TCS allowed us to model all data together across modalities (cdt, cpt, hdt and hpt). Compared to separately modelling QST modality data, the more parsimonious model of the deltas requires the estimation of far fewer model parameters, making more efficient use of data, and can be used to directly evaluate how interventions interact with modality, as we have done. Modelling the delta temperature values representing the elements of the TCS allows thermal testing response to an intervention to be expressed comprehensively and, if QST responses do not appear to interact with and depend on modality, crude TCS can be estimated in the clinic simply by averaging all of a patient's delta temperature values across modalities. We believe this approach to handling QST replicates is more powerful and more versatile than other methods of handling QST replicates, such as averaging the technical replicates, which tends to underestimate and underutilize the variability in QST assessments.

A significant strength of our study was that all testing was performed by a single, highly trained individual. While we believe that this contributed to the consistency of our results, we recognize that this may not be possible in many clinical or research settings. As such, training and standardization of procedures seem critical. Different methods of QST evaluate different physiological parameters.

Specific testing may measure peripheral sensitivity using thermal or mechanical stimulation; other testing, such as temporal summation, measures central sensitization (hypersensitivity of the CNS), while the combination of two tasks applied heterotopically measure endogenous opioid tone. Central sensitization is a prime suspect for pain facilitation and the transition from acute to chronic pain in patients with SCD. In a recent adult SCD cohort, those who experienced greater central sensitization reported more clinical pain, VOCs, catastrophizing, negative mood and poorer sleep continuity (Campbell *et al*, 2016b). Our study was limited in that we did not utilize some of the more complex QST techniques, such as temporal summation or conditioned pain modulation, where Campbell *et al* (2016a) saw differences between SCD and control subjects. We chose to forgo these modalities out of concern that they might not be feasible in a paediatric age group. Rather, static measures of pressure and thermal sensitivity testing were employed which, in adult populations, have shown good-to-excellent reliability (Marcuzzi *et al*, 2017; Nothnagel *et al*, 2017). However, Bakshi *et al* (2017) was able to complete heat pain tolerance and temporal summation testing successfully in paediatric subjects, supporting the development of these testing modalities for clinical trial use. In addition, our subjects were relatively young and many of them were on HC or chronic transfusion therapy. Thus, as a group, they exhibited a low pain phenotype, which may have resulted in less pronounced threshold differences between SCD and healthy controls. Unfortunately, the current study is unable to assess whether QST is altered secondary to transition from acute to chronic pain, given the short time frame and lack of pain in these young patients. Future studies may benefit from evaluating

temporal summation response and its evolution over time in children as they move through adolescence and into adulthood. Alterations in these responses could potentially be an early warning marker for central sensitization or pain facilitation, while habituation in response could suggest resiliency.

Another limitation of our study was the limited information on baseline pain levels in test subjects. No univariable association was found between the thermal thresholds and number of health care encounters for VOC in the 3 years prior to study entry. Pain managed at home was not quantified due to concern about recall, and there were no measures of impact of pain on quality of life. However, the requirement that subjects be off all pain medications for at least 24 h at the time of testing eliminated patients with chronic pain significant enough to require chronic, scheduled pain medications as well as those with severe acute pain. Poor compliance with the daily pain diary impeded our ability to establish a detailed correlation between the degree of pain experienced and QST results. For an upcoming clinical trial in which our group plans to use a pain diary, we will compensate subjects $1 per day to improve compliance.

Our data shows that, in the absence of the introduction of a new sickle cell-directed, ongoing pharmacological or transfusion therapy, QST results remained relatively consistent over a 6-month period. In contrast, a subset of individuals on whom HC was initiated close to the time of baseline testing, exhibited progressively decreasing thermal sensitivity. Based on our study results, we caution against the use of a predefined "abnormal" range for QST thresholds. Instead, it may be more meaningful to evaluate changes in an individual patient's QST thresholds over time as these changes may indicate a progression of disease (i.e. from an acute to chronic or neuropathic pain phenotype) or response to a new therapy. Our study provides preliminary data to support the future examination of QST as a potentially valuable outcome measure for use in early phase clinical trials to indicate response to a preventative pain therapy.

Acknowledgements

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (NIH/NIGMS) under Award Number P20GM109021 and by an Institutional Development Award (IDeA) from NIH/NIGMS under award number U54-GM104941. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This study was approved by the Nemours Institutional Review Board.

Authorship contributions

R.E.M., C.M.C., S.M.M. and M.S. designed the study. D.S.B, R.E.M., Y.S, S.G.-B. and H.B. performed the research. S.W.K. and S.E.H. analysed the data. All authors (R.E.M., D.S.B, M.S, S.W.K., S.E.H., C.M.C., Y.S, S.G.-B, S.M.M. and H.B.) contributed to the drafting, revision and final approval of the manuscript.

Conflict of interest

The authors have no conflicts of interest to report.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Complete longitudinal general linear model for comparing QST results[‡] between SCD and Control Group subjects.

Table SII. Complete longitudinal general linear model for comparing QST results[‡] for only SCD Group subjects.

Table SIII. Demographics of pressure test opt-in subjects.

Table SIV. Complete general linear model for comparing pressure test results for SCD and control subjects.

References

Arendt-Nielsen, L. & Yarnitzky, D. (2009) Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. *The Journal of Pain*, **10**, 556–572.

Backonja, M., Attal, N., Baron, R., Bouhassira, D., Drangholt, M., Dyck, P.J., Edwards, R.R., Freeman, R., Gracely, R., Haanpaa, M.H., Hansson, P., Hatem, S.M., Krumova, E.K., Jensen, T.S., Maier, C., Mick, G., Rice, A.W., Rolke, R., Treede, R.-D., Serra, J., Toelle, T., Tugnoli, V., Walk, D., Walalce, M.S., Ware, M., Yarnitsky, D. & Ziegler, D. (2013) Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. *Pain*, **154**, 1807–1819.

Bakshi, N., Lukombo, I., Shnol, H., Belfer, I. & Krishnamurti, L. (2017) Psychological characteristics and pain frequency are associated with experimental pain sensitivity in pediatric patients with sickle cell disease. *Journal of Pain*, **18**, 1216–1228.

Ballas, S.K., Gupta, K. & Adams-Graves, P. (2012) Sickle cell pain: a critical reappraisal. *Blood*, **120**, 3647–3656.

Blankenburg, M., Kraemer, N., Hirschfeld, G., Krumova, E.K., Maier, C., Hechler, T., Aksu, F., Magerl, W., Reinehr, T. & Zernikow, B. (2012) Childhood diabetic neuropathy: functional impairment and non-invasive screening assessment. *Diabetic Medicine*, **29**, 1425–1432.

Blankenburg, M., Junker, J., Hirschfeld, G., Michel, E., Aksu, F., Wager, J. & Zernikow, B. (2018) Quantitative sensory testing profiles in children, adolescents and young adults (6-20 years) with cerebral palsy: hints for a neuropathic genesis of pain syndromes. *European Journal of Paediatric Neurology*, **22**, 470–481.

Bouhassira, D. & Attal, N. (2016) Translational neuropathic pain research: a clinical perspective. *Neuroscience*, **338**, 27–35.

Brandow, A. & Panepinto, J.A. (2016) Clinical interpretation of quantitative sensory testing as a measure of pain sensivitity in patients with sickle cell disease. *Journal of Pediatric Hematology Oncology*, **38**, 288–293.

Brandow, A.M., Stucky, C.L., Hillery, C.A., Hoffmann, R.G. & Panepinto, J.A. (2013) Patients with sickle cell disease have increased sensitivity to cold and heat. *American Journal of Hematology*, **88**, 37–43.

Brandow, A.M., Hansen, K., Nugent, M., Pan, A., Panepinto, J.A. & Stucky, C.L. (2019) Children and adolescents with sickle cell disease have worse cold and mechanical hypersensitivity during acute painful events. *Pain*, **160**, 407–416.

Brennum, J. (1989) Measurements of human pressure-pain thresholds on fingers and toes. *Pain*, **38**, 311–317.

Campbell, C.M., Edwards, R.R. & Fillingim, R.B. (2005) Ethnic differences in responses to multiple experimental pain stimuli. *Pain*, **113**, 20–26.

Campbell, C.M., Carroll, C.P., Kiley, K., Han, D., Haywood, C., Lanzkron, S., Swedberg, L., Edwards, R.R., Page, G.G. & Haythornthwaite, J.A. (2016a) Quantitative sensory testing and pain-evoked cytokine reactivity: comparison of patients with sickle cell disease to healthy matched controls. *Pain*, **157**, 949–956.

Campbell, C.M., Mouscou-Jackson, G., Carroll, C.P., Kiley, K., Haywood, C., Lanzkron, S., Hand, M., Edwards, R.R. & Haythornthwaite, J.A. (2016b) An evaluation of central sensitization in patients with sickle cell disease. *Journal of Pain*, **17**, 617–627.

Casella, G. & Berger, R.L. (2002) Statistical Inference, 2nd edn. Duxbury/Thomson Learning, Pacific Grove, CA.

Cornelissen, L., Donado, C., Kim, J., Chiel, L., Zurakowski, D., Logan, D.E., Meier, P., Sethna, N., Blankenburg, M., Zernikow, B., Sundel, R.P. & Berde, C.B. (2014) Pain hypersensitivity in juvenile idiopathic arthritis: a quantitative sensory testing study. *Pediatric Rheumatology Online Journal*, **12**, 39–51.

Coronado, R.A., Bialosky, J.E., Robinson, M.E. & George, S.Z. (2014) Pain sensitivity subgroups in individuals with spine pain: potential relevance to short-term clinical outcome. *Physical Therapy*, **94**, 1111–1122.

Cruz-Almeida, Y. & Fillingim, R.B. (2014) Can quantitative sensory testing move us closer to mechanism-based pain management? *Pain Medicine*, **15**, 61–72.

Edwards, R.R. & Fillingim, R.B. (1999) Ethnic differences in thermal pain responses. *Psychosomatic Medicine*, **61**, 346–354.

Edwards, R.R., Dworkin, R.H., Turk, D.C., Angst, M.S., Dionne, R., Freeman, R., Hansson, P., Saroutounian, S., Arendt-Nielsen, L., Attal, N., Baron, R., Brell, J., Bujanover, S., Burke, L.B., Barr, D., Chappell, A.S., Cowan, P., Etropolski, M., Fillingim, R.B., Gewandter, J.S., Katz, N.P., Kopecky, J.D., Nomikos, G., Porter, L., Rappaport, B.A., Rice, A.S.C., Scavone, J.M., Scholz, J., Simon, L.S., Smith, S.M., Tobias, F., Tockarshewsky, T., Veasley, C., Versavel, M., Wasan, A.D., Wen, W. & Yarnitzky, D. (2016a) Patient phenotyping in clinical trials of chronic pain treatments: IMMPACT recommendations. *Pain*, **157**, 1851–1871.

Edwards, R.R., Dolman, A.J., Martel, M.O., Finan, P.H., Lazaridou, A., Cornelius, M. & Wasan, A.D. (2016b) Variability in conditioned pain modulation predicts response to NSAID treatment in patients with knee osteoarthritis. *BMC Musculoskeletal Disorders*, **17**, 284–292.

Ezenwa, M.O., Molokie, R.E., Wang, Z.J., Yao, Y., Suarez, M.L., Pullum, C., Schlaeger, J.M., Fillingim, R.B. & Wilkie, D.J. (2016) Safety and utility of quantitative sensory testing among adults with sickle cell disease: indicators of neuropathic pain? *Pain Practice*, **16**, 282–293.

Fitzmaurice, G., Laird, N. & Ware, J. (2011) Applied Longitudinal Analysis, 2nd edn. John Wiley and Sons, New York, NY.

Geber, C., Klein, T., Azad, S., Birklein, F., Gierthmuhlen, J., Huge, V., Lauchart, M., Nitzsche, D., Stengel, M., Valet, M., Baron, R., Maier, C., Tolle, T. & Treede, R.D. (2011) Test-retest and interobserver reliability of quantitative sensory testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS): a multi-centre study. *Pain*, **152**, 548–556.

Granovsky, Y., Matre, D., Sokolik, A., Lorenz, J. & Casey, K.L. (2005) Thermoreceptive innervation of human glabrous and hairy skin: a contact heat evoked potential analysis. *Pain*, **115**, 238–247.

Grosen, K., Fischer, I.W., Olesen, A.E. & Drewes, A.M. (2013) Can quantitative sensory testing predict responses to analgesic treatment? *European Journal of Pain*, **17**, 1267–1280.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N. & Conde, J.G. (2009) Research electronic data capture (REDCap) – a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, **42**, 377–381.

Jacob, E., Chan, V.W., Hodge, C., Zeltzer, L., Zurakowski, D. & Sethna, N.F. (2015) Sensory and thermal quantitative testing in children with sickle cell disease. *Journal of Pediatric Hematology Oncology*, **37**, 185–189.

Krishnan, S., Setty, Y., Betal, G., Vijender, V., Rao, K., Dampier, C. & Stuart, M. (2010) Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vasoocclusive crisis. *British Journal of Haematology*, **148**, 797–804.

Kristensen, A.D., Ahlburg, P., Lauridsen, M.C., Jensen, T.S. & Nikolajsen, L. (2012) Chronic pain after inguinal hernia repair in children. *British Journal of Anaesthesia*, **109**, 503–608.

Lazaridou, A., Martel, M.O., Cahalan, C.M., Cornelius, M.C., Franceschelli, O., Campbell, C., Haythornthwaite, J.A., Smith, M., Riley, J. & Edwards, R.R. (2018) The impact of anxiety and catastrophizing on interleukin-6 responses to acute painful stress. *Journal of Pain Research*, **11**, 637–647.

Liang, K.Y. & Zeger, S.L. (1986) Longitudinal data analysis using generalized linear models. *Biometrika*, **73**, 13–22.

Lieber, S., Blankenburg, M., Apel, K., Hirschfeld, G., Driever, P.H. & Reindl, T. (2018) Small-fiber neuropathy and pain sensitization in survivors of pediatric acute lymphoblastic leukemia. *European Journal of Paediatric Neurology*, **22**, 457–469.

Maher, D.P., Ding, W., Singh, S., Opalacz, A., Fishman, C., Houghton, M., Ahemed, S., Chen, L., Mao, J. & Zhang, Y. (2017) Thermal QST phenotypes associated with response to lumbar epidural spinal injections: a pilot study. *Pain Medicine*, **18**, 1455–1463.

Marcuzzi, A., Wrigley, P.J., Dean, C.M., Adams, R., & Hush, J.M. (2017) The long-term reliability of static and dynamic quantitative sensory testing in healthy individuals. *Pain*, **158**, 1217–1223.

Moss, P., Knight, E. & Wright, A. (2016) Subjects with knee osteoarthritis exhibit widespread hyperalgesia to pressure and cold. *PLoS ONE*, **11**, e0147526.

Moss, P., Benson, H.A.E., Will, R. & Wright, A. (2018) Patients with knee osteoarthritis who score highly on the PainDETECT questionnaire present with multimodality hyperalgesia, increased pain and impaired physical function. *Clinical Journal of Pain*, **34**, 15–21.

Nothnagel, H., Puta, C., Lehmann, T., Baumbach, P., Menard, M.B., Gabriel, B., Gabriel, H.H.W., Weiss, T. & Musial, F. (2017) How stable are quantitative sensory testing measurements over time? Report on 10-week reliability and agreement of results in healthy volunteers. *Journal of Pain Research*, **10**, 2067–2078.

O'Leary, J.D., Crawford, M.W., Odame, I., Shorten, G.D. & McGrath, P.A. (2014) Thermal pain and sensory processing in children with sickle cell disease. *Clinical Journal of Pain*, **30**, 244–250.

Quinn, C.T. (2018) L-glutamine for sickle cell anemia: more questions than answers. *Blood*, **132**, 689–693.

Smith, S.M., Dworkin, R.H., Turk, D.C., Baron, R., Polydefkis, M., Tracey, I., Borsook, D., Edwards, R.R., Harris, R.E., Wager, T.D., Arendt-Nielsen, L., Burke, L.B., Carr, D.B., Chappell, A., Farrar, J.T., Freeman, R., Gilron, I., Goli, V., Haeussler, J., Jensen, T., Katz, N.P., Kent, J., Kopecky, E.A., Lee, D.A., Maixner, W., Markman, J.D., McArthur, J.C., McDermott, M.P., Parvathenani, L., Srinivasa, R.N., Rappaport, B.A., Rice, A.S.C., Rowbotham, M.C., Tobias, J.K., Wasan, A.D. & Witter, J. (2017) The potential role of sensory testing, skin biopsy & functional brain imaging as biomarkers in chronic pain clinical trials: IMMPACT considerations. *The Journal of Pain*, **18**, 757–777.

Teles, A.R., Ocay, D.D., Shebreen, A.B., Tice, A., Saran, N., Ouellet, J.A. & Ferland, C.E. (2018) Evidence of impaired pain modulation in adolescents with idiopathic scoliosis and chronic back pain. *The Spine Journal*, 1–10. https://doi.org/10.1016/j.spinee.2018.10.009

Veluswamy, S., Shay, P., Khaleel, M., Puliyel, M., Thuptimdang, W., Chalacheva, P., Sunwoo, J., Denton, C.C., Kato, T.M., Detterich, J.A., Wood, J.C., Sposto, R., Khoo, M., Zeltzer, L. & Coates, T.D. (2018) Sickle cell subjects have a stronger and faster neurally mediated vasoconstriction response to cold pain that correlates with anxiety scores. *Blood*, **132**, 1086.

Ware, R.E. (2015) Optimizing hydroxyurea therapy for sickle cell anemia. *Hematology*, **2015**, 436–443.

Ware, R.E., DeMontalembert, M., Tshilolo, L. & Abboud, M.R. (2017) Sickle cell disease. *Lancet*, **390**, 311–323.

Zohsel, K., Hohmeister, J., Oelkers-Ax, R., Flor, H. & Hermann, C. (2006) Quantitative sensory testing in children with migraine: preliminary evidence for enhanced sensitivity to painful stimuli especially in girls. *Pain*, **123**, 10–18.

