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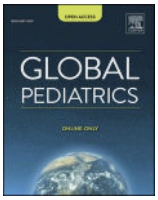

Skin conductance measurement as a selective and continuous pain assessment method during eye examinations for retinopathy of prematurity

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ABSTRACT

Background: Assessing and managing pain in newborns is important for normal development and reduction of morbidity.
Aim: To assess whether skin conductance measurements (SCM) can be used as an objective method for measuring procedural pain during screening for retinopathy of prematurity (ROP) in preterm infants and to identify painful components of the examination.
Methods: 65 separate SCM were performed in 33 infants born at <32 weeks gestational age (wGA) eligible for ROP screening (median 26+4 wGA, range 23+3 to 31+3; median weeks postmenstrual age 37+2, range 31+0 to 49+6). SCM was measured before, during and after eye examination (fundoscopy and/or wide-field digital retinal imaging [WFDRI]), and compared to changes in heart rate (HR), pulse oximetry saturation (SpO2) and behavioral state measured with the Neonatal Pain, Agitation and Sedation Scale (N-PASS).
Results: A major increase of SCM could be seen during both fundoscopy and WFDRI ($p<0.01$, respectively). No correlation was found between SCM and wGA. N-PASS changed significantly during ROP examination ($p<0.01$). While N-PASS could only distinguish painful response from baseline during fundoscopy and WFDRI, SCM detected responses during each stage of the investigations i.e. the application of mydriatics, fundoscopy, anesthetic drops, speculum and WFDRI. HR increased only during digital retinal imaging ($p = 0.049$), while SpO2 decreased only during fundoscopy ($p = 0.042$).
Conclusion: SCM may be used as a continuous and objective method to evaluate pain and its intensity during screening for ROP, enabling the separation of the different painful components of the investigation. Selecting and grading the different painful stages improves the possibility to assess and continue to improve pain management more specifically in these patients.

Abbreviations and acronyms

ROP	retinopathy of prematurity
GA	gestational age
PMA	postmenstrual age
SCM	skin conductance measurements
WFDRI	wide-field digital retinal imaging
nCPAP	nasal continuous positive airway pressure
AUC	area under the curve
N-PASS	Neonatal Pain Agitation and Sedation Scale
PIPP	Premature Infant Pain Profile
CRIES	Crying; Requiring increased oxygen administration; Increased vital signs; Expression; Sleeplessness scale
PIPP-R	Premature Infant Pain Profile – Revised

Introduction

To assess pain in newborn infants submitted to intensive care is important in order to provide adequate pain relief. Both short-term and long-term adverse effects of pain have been described.[1-3] Repetitive procedural pain in preterm infants can lead to altered pain sensitivity, altered brain microstructure, impaired brain development and lower Intelligence Quotient.[1,4,5] In a systematic review repetitive procedural pain showed a delayed postnatal growth and poor cognitive and motor

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development at 1 year of age.[6]

Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the retina and is a major cause of blindness globally in preterm infants.[7-10] Human retinal vascularization begins at approximately 16 weeks' gestational age (GA) and proceeds outward from the optic nerve head to the periphery of the retina. The process is completed at around 40 weeks' GA. In the prematurely born infant there may be an arrest of the normal vascularization, subsequently leading to the development of ROP, detachment of the retina and even blindness.[8] Supplemental oxygen and low GA are important risk factors for the development of ROP.[10,11] The incidence of ROP has increased with improved survival of extremely preterm infants.[7,12] ROP screening for early detection and treatment of threshold ROP significantly decreases the incidence of severe vision loss and blindness.[9] Swedish guidelines for ROP screening include all infants with a GA at birth of 29 weeks or less.[13] ROP screening starts at a postnatal age of 6 weeks, but the first examination is postponed to postmenstrual age (PMA) 31 weeks in infants born < 26 weeks' (25+6) GA.[13] Eye examinations for ROP screening are sometimes painful and stressful, and is dependent on the ophthalmologist's investigative level of skill.[14-16] The methods currently applied for pain relief during ROP screening, such as topical anesthetic eye drops, oral sucrose and non-nutritive sucking, seem to be inadequate.[16-18]

Pain management in preterm infants is important to prevent both short- and long-term adverse effects of pain.[1-5] Skin conductance measurements (SCM) have been introduced as a possible method for assessing pain in infants.[19] Skin conductance is the result of the sympathetic nervous system acting on sweat glands in the skin.[20] The excretion from the sweat gland can be represented as one skin conductance peak.[21] The number and amplitude of the peaks reflect the activity of the sympathetic nervous system.[19] Skin conductance responses during painful procedures have been observed as early as 23 weeks' GA, but study results still vary.[22-25] Some studies report no significant difference in skin conductance activity when comparing painful and non-painful procedures,[23] or low specificity in skin conductance activity,[24] while others report that a stress response to pain can be detected[22] and that skin conductance has a high negative predictive value.[25] In our previous study on SCM, we found that stress response to heel lancing in extremely preterm infants at different postmenstrual ages can be detected by SCM.[22]

The aim of this study was to evaluate skin conductance as a continuous and objective indicator for pain during ROP screening, and to find out which specific intervention, i.e., mydriatic eye drops, fundoscopy, anesthetic eye drops, insertion of the eyelid speculum, or wide-field digital retinal imaging (WFDRI) seems to be most painful. We set out to find an objective, reliable and safe pain measurement tool to use with the ultimate goal of ensuring adequate pain relief in neonates.

Methods

This longitudinal cohort study was conducted at the Neonatal Care Unit at Uppsala University Children's Hospital in Uppsala, Sweden, between August 2010 and August 2014. The Swedish Ethical Review Board approved the study (D:nr 2006/028). Informed parental consent was obtained before the infant was included in the study and reconfirmed before each subsequent measurement.

Patients

Sixty-five skin conductance measurements were performed on 33 different preterm infants that were recruited from the neonatal care unit. The study was conducted from 2010 to 2014. Infants who were in a clinically stable condition and did not receive any anesthetic or sedative medications that might have interfered with their pain response within 24 h before eye examinations were eligible for participation. Patient demographics and morbidity are presented in Table 1. Sixteen infants had ventilatory assistance either by low flow nasal cannula, nasal continuous positive airway pressure (nCPAP) or mechanical ventilation (Table 1).

Table 1
Patient demographics and morbidity.

Demographics	
Gestational age in weeks, median (range)	26+4 (23+3 – 31+3)
Male, n	19
Female, n	14
Twins, n	3
Postnatal age in days, median (range)	73 (33– 174)
Birthweight in grams, median (range)	811 (387 – 1310)
Current weight in grams, median (range)	2188 (955 – 5032)
Apgar, median (range)	
- 1 min	5 (0 – 9)
- 5 min	7 (3– 10)
- 10 min	8 (4– 10)
Ventilatory assistance	
- mechanical ventilation with sedatives, n	0
- mechanical ventilation without sedatives, n	7
- nasal continuous positive airway pressure, n	4
- low flow nasal cannula, n	5
- spontaneous breathing without assistance, n	17
Morbidity	**Number of infants (%)**
Retinopathy of prematurity	
- no retinopathy	13 (39)
- stage 1–3	20 (60)
- stage 4–5	0 (0)
Small for gestational age	1 (3)
Intraventricular hemorrhage	5 (15)
- Grade I-II	4 (12)
- Grade III-IV	1 (3)
Necrotizing enterocolitis, treated surgically	5 (15)
Bronchopulmonary dysplasia[1]	5 (15)

n=number of infants
[1] bronchopulmonary dysplasia defined as supplemental oxygen need >21% at 36+0 weeks' GA

Study design

All infants born < 32 weeks' GA were eligible for ROP screening in the beginning of the study period. The screening criteria were, however, changed to < 31 weeks from January 2012.[26] The first eye examination was initially performed five weeks after birth. In 2012, the first eye examination was postponed until PMA 31 weeks in infants born before a GA of 27 weeks. Thereafter infants were examined with an interval period of twice a week, once a week or even every second week, depending on stage and stability of ROP, until retinal vascularization was complete (i.e., at 40 weeks' gestational age) or until the ROP had regressed. In infants who were treated for ROP, examinations were continued until full regression of the disease was observed.

Eye examinations were performed by experienced pediatric ophthalmologists. The current gold standard screening method for ROP was binocular indirect ophthalmoscopy (fundoscopy) during the study period WFDRI has been increasingly used for documentation of the ROP disease and for telemedical purposes.[27] In the present study, the RetCam® 120 wide-angle fundus camera with a neonatal cone (Clarity Medical Systems, Pleasanton, CA, USA) was used during 2010–2013, and the RetCam 3® (Clarity Medical Systems, Pleasanton, CA, USA) was used during 2014, both systems with a 120-degree view. Forty-two measurements were performed on infants exposed to both fundoscopy and WFDRI and 23 measurements were performed on infants that only underwent fundoscopy.

Before the eye examination, pupils were dilated with one drop of a combination of Cyclopentolate 0.5% and Phenylephrine 0.5% 45 min and 30 min before examination. Two minutes before ophthalmoscopy and/or WFDRI infants received 0.5 ml glucose 30% when medically eligible, according to established unit policy. In case of WFDRI, Tetracaine 1.0% eye drops were instilled before insertion of a newborn eye

speculum. As per hospital unit policy, all infants were recently fed, received oral glucose prior to and were subject to facilitated tucking during the eye examination.

Heart rate in beats per minute (bpm) and oxygen saturation were registered 60 s before, during and 60 s after the different stages of eye examination using pulse oximetry saturation (SpO2; Philips IntelliVue MP50 Neonatal, Böblingen, Germany).

Skin conductance was measured using the Med-Storm Pain Monitor® (Medstorm Innovations, Oslo, Norway). It was measured continuously during the entire ROP examination including instillment of the anesthetic eye drops, insertion of the eye lid speculum, fundoscopy and/or WFDRI. It was also measured before, during and after admission of the Cyclopentolate 0.5% and Phenylephrine 0.5% eye drops in nine infants. By applying three electrodes on the infants' foot, skin conductance fluctuations (the rate of firing in the sympathetic nerves, measured in peaks/second) and area under the curve (AUC) of the peaks (forcefulness of the sympathetic nerve firing) were analyzed. The number of skin conductance fluctuations has been shown to be the more appropriate mode for measuring pain and stress in newborns.[19] Measurements of AUC were only used as a factor to judge the stability of the measurements of number of skin conductance fluctuations and those data are therefore not published in our results.

The multidimensional neonatal pain, agitation, and sedation scale (N-PASS) is a valid and reliable observational pain scale[28,29] and was used since it is the routine pain assessment tool in our neonatal unit. The N-PASS includes five indicators: (A) crying (at intervals or continuous; consolable or inconsolable); (B) behavioral state (restlessness, squirming, and frequent awakening, or arching, kicking and/or constantly awake); (C) facial expression (intermittent or continual); (D) tone of extremities (intermittently or continuously clenched hand/feet; not tense or tense body); and (E) vital signs (heart rate, respiratory rate, and blood pressure increased by 10 or 20% from baseline; and oxygen saturation decreased to 85–75% or <75%).[29] N-PASS is graded 0, 1 or 2, and higher scores indicate higher levels of pain response.[29] The N-PASS scale evaluates procedural pain and has age correction for prematurity, with high scores indicating pain and stress.[30,31] N-PASS observations were performed intermittently before, during and after ROP examination in all monitored infants.

Data analysis

SCM were analyzed by calculating the mean number of peaks/second and AUC during every fifteen-second interval. The first baseline was defined as the mean number of peaks/second of at least four fifteen-second intervals before start of fundoscopy and/or WFDRI. The second baseline was defined as the interval where the number of fluctuations and AUC decreased significantly or returned to zero. Over a four-year period 65 SCMs were performed in 33 infants. In five cases, (two from the combined group and three from the fundoscopy group) the measurements could not be analyzed due to artefacts or failure to acquire relevant measurements. To see a possible maturational aspect in skin conductance, measurements were repeated in 14 of those infants at the time of repeated ROP examination. Time between the first and last examinations and measurements ranged between 7 - 69 days (median 21 days). In most of these infants, the examinations and measurements were repeated more than once.

Statistics

Data was analyzed with Statistical Package for the Social Sciences (SPSS 20.0, Chicago, IL, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). A power of 80% (α=0.20) in this multi parametric study necessitated a sample size of at least 30 registrations for the non-parametric values and less for the continuously registered parametric values to reach a significant level of β=0.05. Student's *t*-test for two-sided paired observations was applied whenever a difference was detected by analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$. Pearson's correlation coefficient was calculated for possible maturational correlations between SCM and GA or PMA, and between SCM and N-PASS where relevant.

Results

Baseline SCM was 0.03 ± 0.04 peaks/second in all infants (Fig. 1), while baseline N-PASS was 0.62 ± 1.03 (Fig. 2).

Instillment of mydriatic eye drops ($n = 9$) given 45 to 30 min before examination did not result in a significant increase in skin conductance (0.06 ± 0.07 peaks/second; $p = 0.13$; Fig. 1).

The infants that were exposed to both fundoscopy and WFDRI ($n = 40$) had a significant increase in peaks/second compared to baseline during fundoscopy (0.14 ± 0.12 peaks/second), application of anesthetic eye drops (0.12 ± 0.13 peaks/second), insertion of the eye lid speculum (0.12 ± 0.10 peaks/second) and WFDRI (0.15 ± 0.15 peaks/second; Fig. 1; $p < 0.01$ respectively). The group of infants that underwent only fundoscopy ($n = 20$) had a significant skin conductance increase when compared to baseline (0.16 ± 018; $p < 0.01$). Differences in skin conductance between fundoscopy and WFDRI were not significant, but it should be noted that a higher increase of peaks/second was seen during WFDRI (Fig. 1; $p = 0.15$).

N-PASS scores were significantly higher than baseline in the entire cohort during both fundoscopy (4.1 ± 1.86) and WFDRI (5.55 ± 2.08; Fig. 2; $p < 0.01$; respectively). N-PASS scores were significantly higher during WFDRI compared to fundoscopy (Fig. 2; $p < 0.01$). N-PASS could not be separated from the other interventions due to the very short intervals between the different stages of the ROP investigation. Heart rate increased significantly only during WFDRI (15 ± 20 beats/minute; Fig. 3A; $p = 0.049$) and oxygen saturation decreased significantly only during fundoscopy (5.3 ± 9.34%; Fig. 3B; $p = 0.042$).

Fig. 4 presents a simultaneous continuous SCM and intermittent N-PASS observational score in one patient, exemplifying the selective pain assessment with SCM during the different stages of ROP examination.

Correlations

N-PASS scores corresponded with SCM on most occasions, but in few instances N-PASS scores were elevated without any increase in skin conductance.

In this cohort of infants investigated mainly after >31 weeks' GA, no significant maturational intra- or inter-individual correlations between GA and SCM was seen at baseline, during instillation of mydriatic eye drops, fundoscopy, application of anesthetic eye drops, insertion of the eye lid speculum or WFDRI ($r = 0.08$, 0.25, 0.14, 0.20, 0.09 and 0.04

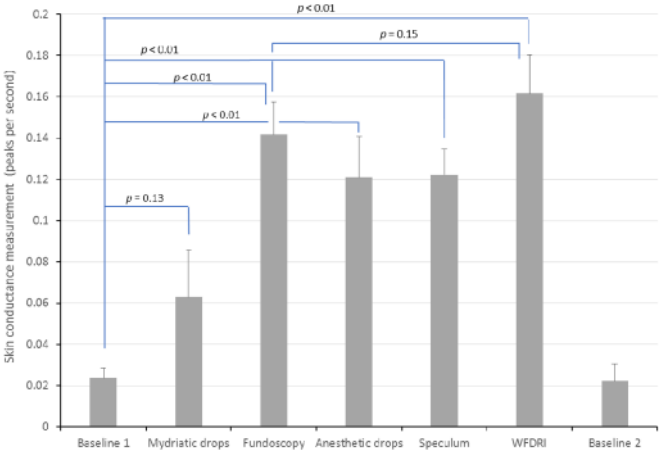

Fig. 1. Skin conductance measurements during different stages of ROP examination. Mean and standard error of mean.

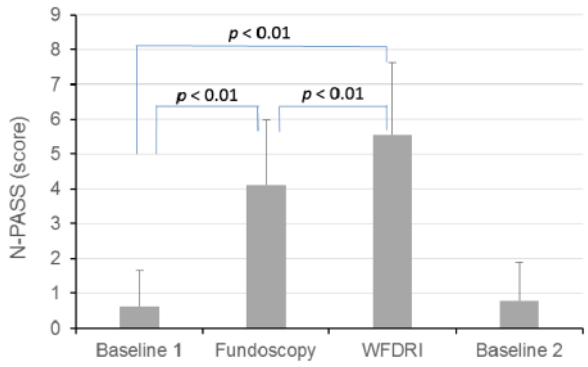

Fig. 2. N-PASS scores during ROP examination. Mean and standard deviations.

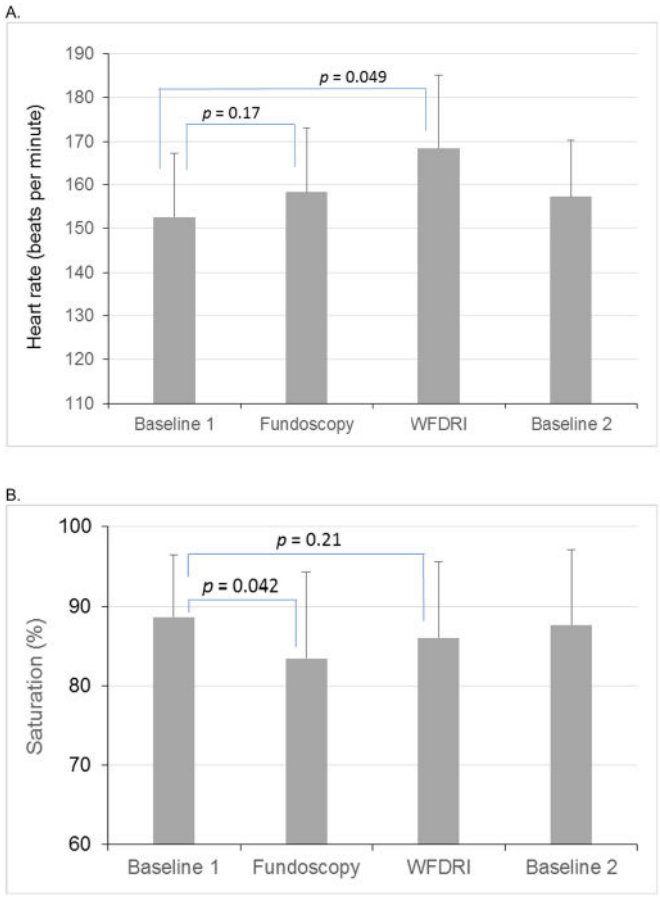

Fig. 3. (A). Heart rate changes during ROP examination. Mean and standard deviations. (B). Oxygen saturation changes during ROP examination. Mean and standard deviations.

respectively; NS). Analysis of a possible maturational aspects of reaction to painful stimuli as measured by N-PASS did not show any correlation ($r = 0.085$; NS). Skin conductance did not increase or decrease significantly with higher PMA in all infants.

Discussion

Skin conductance had a significantly increased response in peaks/second during ROP examination, both during fundoscopy and WFDRI, as compared to baseline recordings. Although skin conductance responses differed in the same infant between the different interventions, there was also an overall significant reaction to anesthetic eye drops and insertion of the eye lid speculum. Gestational age did not seem to affect skin conductance response. Skin conductance results could be validated with N-PASS results, as a significant increase in N-PASS score was also seen during both fundoscopy and WFDRI. The other components of the ROP examination could not be separated by N-PASS, due to the time between most interventions being fairly short.

Our visual observational findings are in line with previous studies that report significant changes in observational pain scales during ROP screening.[14,32,33] Cohen et al. have also described a clinically significant pain response after applying mydriatic eye drops (Tropicamide 1% and Phenylephrine 2.5%), using the PIPP scale before and after instillment of the drops.[32] In a study by Moral-Pumarega et al., a short-term stress response was seen both during fundoscopy and WFDRI when measured by PIPP and CRIES scale.[33] Both PIPP and CRIES scores increased significantly, with a higher increase after fundoscopy than after WFDRI, in contrast to our study, where a higher increase in SCM and N-PASS scores was seen during WFDRI compared to the other interventions. A possible explanation for this difference is that in our study scleral indentation was used only for WFDRI, in contrast to the study by Moral-Pumarega et al., in which fundoscopy was performed with scleral indentation as well.[33] Interestingly, the same study showed similar occurrence of desaturation during fundoscopy and WFDRI.[33] In a recent study, Mataftsi et al. compared the stress induced during fundoscopy with and without the use of an eye lid speculum and scleral indentation, and found lower Premature Infant Pain Profile-Revised (PIPP-R) scores in infants examined without eyelid speculum and scleral indentation.[14] The authors argue that the use of a speculum and indentation should be limited to patients with suspicion of severe disease.[14] In our study a significant increase of skin conductance from baseline was seen during both the instilment of anesthetic eye drops and insertion of the eyelid speculum. Since the time interval was short between these two interventions, it was difficult to fully separate them with SCM.

Physiological indices such as heart rate variability and oxygen saturation are not entirely pain specific as they are also influenced by other factors such as the capacity to produce a response, effects of medication and developmental differences.[34] Evident changes from baseline levels seem to indicate a physiological reaction to acute pain.[35] Bradycardia as well as decrease in oxygen saturation and apnea have also been described as a response to pain.[14,36] During ROP examination, bradycardia might be induced by vagus nerve stimulation (oculo-cardiac reflex or Aschner phenomenon) if applying pressure on the eye with the WFDRI probe.[37] In our study, a general increase in heart rate was seen during WFDRI, and bradycardia was observed in three infants during fundoscopy. Oxygen saturation decreased during fundoscopy, but not during WFDRI, while both SCM and N-PASS scores indicated more intense pain during WFDRI.

There was an increase in N-PASS during all SCM elevations. N-PASS returned to baseline levels within a minute after eye examination, suggesting the absence of prolonged pain. Skin conductance also reached baseline levels again very shortly after the examinations (within 60 s), indicating a short-term stress response to both fundoscopy and WFDRI. Skin conductance results were evaluated after the ROP examinations, and therefore not biased by any factors during examination. Compared to a point observation method such as the N-PASS, continuous SCM may provide more information about pain during longer interventions and can also be of value in observing the duration of pain after interventions, as seen in our study. The different stages of ROP examination could not be separated by N-PASS. With the exception of the instillment of anesthetic eye drops followed by the insertion of eyelid speculum (very short time interval between these two interventions) the different stages of the eye examination can be separated by SCM (Fig. 3). The example of simultaneous SCM and N-PASS scores during one ROP examination in the same patient shown in Fig. 4 illustrates the advantage of SCM in separating the different painful stages of the examination.

Besides being able to assess pain continuously and to differentiate between the painful stages of the examination, SCM might enable more

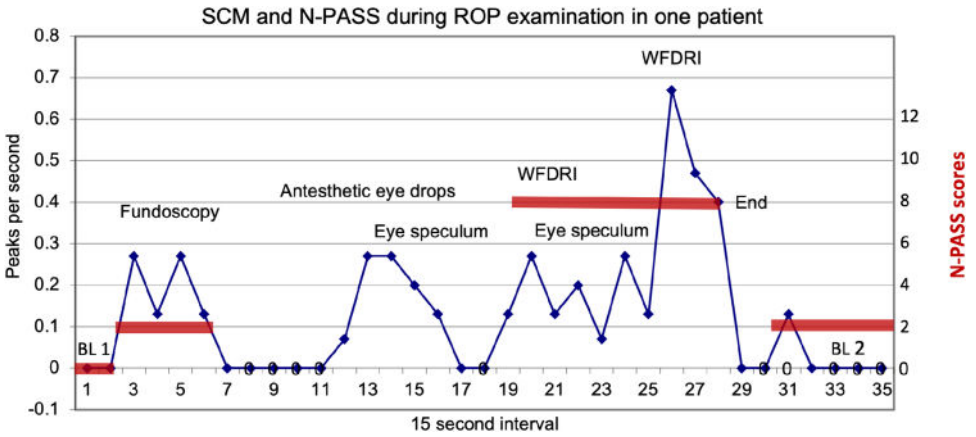

Fig. 4. Example of SCM and N-PASS scores during one ROP examination in one patient.

targeted therapeutical interventions. SCM might also assess the intensity of pain, as the number of peaks/second correlate to the firing rate in the sympathetic nervous system.[38] A disadvantage of SCM is the accidental occurrence of artifacts due to movement and/or pressure on the electrodes. The presence of an observer during SCM is needed to distinguish measurement artifacts from actual increased skin conductance levels, which is in line with other monitoring equipments.

Although results from SCM vary in the current literature[15,22,24] it still seems that the method is capable of evaluating pain during short term interventions. In our previous study on SCM during heel lancing, we found that SCM at baseline took a longer time to stabilize and was higher in infants with a GA of < 28 weeks than in infants with a GA of $\geq 28 + 0$ weeks. In addition, the skin conductance levels took a longer time to return to baseline levels after painful stimuli in infants < 28 weeks' PMA. When the measurements were repeated in each patient at a higher postnatal age, we did not observe a continued sustained response.[22] In the same study, we observed that mean skin conductance values during ROP examinations performed at a later PMA were higher than during heel lancing performed at 1 - 47 days' postnatal age (0.15 versus 0.10 peaks/second), indicating a possible maturational development in pain response with higher PMA.[22] In our present study a maturational pain response could not be correlated between GA and SCM, partly explained by the markedly higher PNA at the time of the investigation as compared to our previous study (mean 73 days vs 15 days), and an overall high PMA (>31 weeks).[22] Avila-Alvarez et al. recently studied SCM during ROP examination and noted a significant increase in both SCM and PIPP-R scores during the procedure, but without a correlation between PIPP-R scores and skin conduction.[15] PIPP-R scores and SCM were both highest during scleral indentation in the study by Avila-Alvarez et al,[15] which is in concordance with our study where N-PASS scores and SCM were highest mainly during WFDRI during which scleral indentation was used. Avila-Alvarez et al. also state that the main advantages of SCM is its objectivity and the possibility of continuous monitoring.[15] What our study adds is that it may also separate the different potentially painful components of the ROP examination.

Adequate pain relief is important during the neonatal intensive care period.[1,22] Current literature suggests that non-pharmacological methods such as oral glucose, non-nutritive sucking and swaddling are beneficial in relieving pain and discomfort to some degree during ROP examinations.[16,36,39,40] Adequate pain relief is also important during potentially painful interventions that take place in regular ward care in older preterm infants.[22]

Our study has its limitations. Some of the infants in our cohort had ventilatory assistance, obscuring part of the face and making N-PASS scoring more difficult. There was also a risk of observer bias while performing the N-PASS, since the observers were not blinded. As skin conductance reflects sympathetic nervous activity,[20] it should be recognized that other factors can induce stress in the newborn and that this could potentially also result in changes in skin conductance. Phenylephrine in the mydriatic eye drops might induce skin conductance changes not related to pain, however, adrenergic acting agents do not affect skin conductance since skin conductance is the result of sweat reaching the skin in response to acetylcholine acting on muscarine receptors.[20] Although the study was performed between 2010 and 2014, the same screening procedure is performed today with similar equipment and pain treatment as previously,[13] thereby highlighting the continued need for pursuing improved pain assessment and reducing stress and pain during these investigations.

To conclude, screening for ROP is important for detecting and treating serious ROP, in order to prevent visual impairment in the preterm infant. This necessitates repeated eye examinations during the neonatal intensive care unit stay and after discharge. In accordance with previous reports, the present study using SCM and N-PASS confirmed that eye examinations for ROP are painful, despite the use of pain-relieving methods. The use of an eye lid speculum seems to be particularly painful and should therefore be used with caution. SCM is a valuable complement to other pain assessment tools as it offers a continuous and objective measurement and might also detect the grade of pain intensity. In contrast to N-PASS, SCM can be used to separate which interventions during ROP examination are most painful, and thereby provide a more selected and specific pain management.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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