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Correlation between diminished vagal tone and somatic dysfunction severity in very and extremely low birth weight preterm infants assessed with frequency spectrum heart rate variability and salivary cortisol

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Abstract

Osteopathic manipulative treatment (OMT) is evolving in the neonatal intensive care unit (NICU) setting. Studies showed its efficacy in length of stay and hospitalization costs reduction. Moreover, it was suggested that OMT has a modulatory effect on the preterm infants' autonomic nervous system (ANS), influencing saturation and heart rate. Even if OMT is based on the palpatory examination of the somatic dysfunctions (SD), there are controversies about its identification and clinical relevance. The objective of this study was to evaluate the inter-rater reliability, clinical characteristics, and functional correlation of the SD Grade score with the heart rate variability (HRV) and the salivary cortisol (sCor) using a multivariate linear model approach. To evaluate those features, we implemented an ad hoc SD examination for preterm infants that was performed by 2 trained osteopaths. It was based on the new variability model of SD that includes an SD Grade assessment procedure. The ANS features were assessed by frequency parameters of HRV studying high frequency (HF), low frequency (LF), and HF/LF, whereas sCor was tested with a radioimmunoassay. The ANS assessment was standardized and performed before SD testing. Sixty-nine premature infants were eligible. SD Grade showed excellent concordance between the blinded raters. Using SD Grade as a grouping variable, the infants presented differences in GA, Apgar, pathological findings, length of stay, and ventilatory assistance. In our multivariate model, HF, LF, and LF/HF resulted linearly correlated with SD Grade. Instead, sCor presented a linear correlation with 5' Apgar and respiratory distress syndrome but not with SD Grade. SD Grade was in line with the natural history of the underdevelopment due to prematurity. Our models indicate that the cardiac vagal tone is linearly related with SD Grade. This finding may improve the multidisciplinary decision making inside NICU and the management of modifiable factors, like SD, for cardiac vagal tone regulation.

Abbreviations: ANS = autonomic nervous system, GA = gestational age, HF = high frequency, HPA = hypothalamic–pituitary–adrenal, HRV = heart rate variability, ICC = interclass correlation, LF = low frequency, LOS = length of stay, NICU = neonatal intensive care unit, NZ = neutral zone, OMT = osteopathic manipulative treatment, PMA = postmenstrual age, RDS = respiratory distress syndrome, sCor = salivary cortisol, SD = somatic dysfunction, TAR = time to autonomous respiration, TT = tissue texture, VM = variability model.

Keywords: heart rate variability, neonatology, neutral zone, osteopathic manipulation, premature infants, variability model

1. Introduction

The maturation of the autonomic nervous system (ANS) is a critical milestone for preterm infants. Preterm birth can expose infants admitted to the neonatal intensive care unit (NICU) to a moderate or severe underdevelopment of the ANS, linked to a deregulation between sympathetic and parasympathetic nervous system[1] with minor adaptation of system. Heart rate variability (HRV) and salivary cortisol concentration (sCor) are 2 markers commonly used for the assessment of sympatho-vagal functionality, the pain response, and the hypothalamic–pituitary–adrenal (HPA) activity in premature infants.[2–4]

HRV in preterm infants was studied to determinate its predictive value on some clinical conditions that may occur during their permanence inside the NICU. Altered sympathovagal balance, specifically a diminished parasympathetic activity, assessed with HRV frequency domain, was correlated with low gestational age (GA)[5] and low postmenstrual age (PMA).[6] Moreover, diminished vagal tone was identified as a predictive biomarker for necrotizing enterocolitis onset[7] and was described in newborns diagnosed with respiratory distress syndrome (RDS) or symptomatic patent ductus arteriosus.[8]

Correspondingly, salivary cortisol – as measure of distress – was studied in premature infants because of its relationship with pain response to invasive or noninvasive stimuli. High humidity high flow nasal cannula was associated with lower salivary cortisol levels versus nCPAP,[9] whereas eye-screening examination and heel lance increased the salivary cortisol level.[10,11]

Complementary medicines studied the HRV variations, focusing on the interaction of massage and kangaroo care in the regulation of sympathovagal activity in infants, observing in both cases a reduction of low frequency to high frequency (LF/HF) and an increased vagal activity, via HF parameter, after treatment.[12–14] Among complementary therapies, the effects of the osteopathic manipulative treatment (OMT) on HRV were investigated in healthy adults and in patients with chronic pain, outlining significant variations on HF parameters and LF/HF ratio, and a modulatory effect of OMT on heath–brain interactions.[15,16] The osteopathic clinical practice relies on palpatory findings and distinctive physical examination, which main goal is the recognition of the somatic dysfunction (SD) (Code ME93.0) that is an altered function of related components of the body framework system: skeletal, arthrodial, and myofascial structures, and their related vascular, lymphatic, and neural elements.[17] In the recent years, osteopathy has improved its methodological and clinical approach, especially in neonatal setting, showing efficacy in reducing LOS and timing to oral feeding attainment.[18,19]

Notwithstanding this, there is a lack of knowledge in the clinical recognition of SD and whether it is correlated with vagal tone. Recently, a new model for the assessment of SD was theorized, the variability model (VM),[20] which considers the evaluation of the movement in the neutral zone (NZ), meant as the movement around the rest position with minimum internal passive and active resistance,[21–23] and the biological function of fascia and fascial system as a connective tissue network which provides an environment that enables all the body systems to operate in an integrated manner and become a proprioceptive organ.[24–27] The VM and the related clinical signs, useful in identifying DS, use the assessment of the motor variability in the NZ as an indicator of the altered somatic function in the osteopathic palpatory diagnosis. The VM of SD incorporates a grading scale of SD (SD Grade) that assumes the existence of tissues response adaptability to allostatic load, and their possible predisposition to a loss of their adaptation potential when allostatic load exceeds. Therefore, the aim of this study was to analyze the preterm infants' clinical characteristics, related to the grade of SD assessed with the proposed VM, specifically the correlation of HRV frequency parameters and sCor with the severity of SD using a multivariate model approach.

2. Methods

2.1. Study design

In order to investigate the autonomic regulation in preterm infants and its correlation with SD Grade, we performed a single-center cross-sectional study based on the data recorded during the daily clinical practice in the period between February 2017 and October 2018 in the tertiary level of the NICU of Del Ponte Hospital in Varese. All the infants' families provided the signed informed consent for the use of these data for research purposes. The Provincial Ethics Committee of Varese approved the study on February 14, 2017 and all the peri- and postnatal data were retrieved from an electronic dataset; they included information about demographic entities, delivery, medical procedures, and perinatal and maternal information. We assessed SD referring to the examination of the VM within NZ.[20]

2.2. Population and patients

All the stable very and extremely low birth weight infants of both sexes aged between 24 [+0] weeks and 33 [+6] weeks of GA admitted at the NICU during the period of observation at their 34th week of PMA were screened for eligibility. Body weight at birth < 800 and > 1500 g, genetic disorders, congenital abnormalities, cardiovascular or pulmonary congenital abnormalities, neurological disorders, abdominal obstruction, malignant arrhythmias, RDS at 34th weeks of PMA, adrenal disorders or malformations, adrenal insufficiency, high dosage corticosteroid therapy, intraventricular hemorrhage, absence of parental consent, and preterm infants transferred from/to other hospitals were considered as exclusion criteria. In addition, infants who experienced complications during the study period were excluded. The patients who were screened for eligibility and who had main data missing completely at random (HRV, sCor, or SD Grade) were excluded from the final analysis. Since to date there is no study estimating prevalence of SD according to VM osteopathic assessment neither in preterms nor in adults, no sample size calculation was performed. The characteristics of the 69 infants observed are represented in Table 1.

2.3. Outcomes and variables

2.3.1. SD grading. The assessment of SD based on VM has been developed with the application of the palpatory examination finding within the NZ taking into consideration movement variability in that range.[20] In vitro and in vivo studies on spine motion evidenced that the NZ is the area of passive joint shift where the movements are produced with a minimal internal resistance and low tissue deformation.[28] Therefore, the NZ assessment can be more precise and reliable, potentially improving tactile perception, allowing a greater sensory discrimination of NZ. Indeed, a light force for quality of motion and an initial resistance-free movement are more effective than using a larger force for quantity of motion.[29,30] Furthermore, due to the gentle force applied, tissue deformation is minimized enhancing reliability and so the assessment of the manual skills. The SD assessment was performed by 3 trained osteopaths through a 0 to 3 grading scale, using a standardized palpatory procedure in order to maximize inter-rater reliability.

All the palpatory findings exams were performed at the 34th PMA, after HRV recording and sCor assessment, with the infants showing stable conditions and laying supine. The examiner's approach lasted about 5 minutes and was composed as follows:

1. The examiner assessed the movement variability within the NZ of the spine with a sacro-occipital stance. The examiner checked the motion reaction of the spine fascia with a light reverse rotary, opposite lateral and traction and compression stimulus (micro motion), induced between the head and the sacral regions.
2. The myofascial tissue texture (TT) assessment was performed using a bimanual compression in the scapular, pelvic girdle, lower chest, upper chest, abdominal, and spinal regions as well as a gentle compression of the spine fascia with the same sacro-occipital stance. An altered elastic response to compression defined an atypical tissue resilience and so an abnormal TT.

Table 1

Overall sample characteristics and stratification of somatic dysfunction grade.

Variable	OverallN = 69	Grade 2N = 29	Grade 3N = 40	*P* value
Male, n (%)	35 (50.7)	14 (40.0)	21 (60.0)	.729
Twins, n (%)	35 (46.4)	15 (42.7)	20 (57.1)	.888
Birth weight, kg	1.12 (0.92–1.33)	1.20 (1.00–1.43)	1.07 (0.87–1.15)	.006
Discharge weight, kg	2.01 (1.83–2.25)	1.98 (1.81–2.23)	2.03 (1.84–2.27)	.712
Weight gain, kg	0.83 (0.63–1.17)	0.73 (0.55–1.07)	1.03 (0.70–1.45)	.048
VLBW, n (%)	42 (60.9)	22 (52.4)	20 (47.6)	–
ELBW, n (%)	22 (31.9)	7 (31.8)	15 (68.1)	.117
Gestational age, wk	29.0 (27.4–30.5)	30.3 (28.9–31.4)	28.3 (26.3–29.4)	<.001
GA < 28 d, n (%)	45 (65.2)	23 (51.1)	22 (48.9)	-
GA > 28 d, n (%)	24 (34.8)	6 (25.0)	18 (75.0)	.036
APGAR 1 min, pt	6.5 (5–8)	7 (5–8)	5 (5–7)	.131
APGAR 5 min, pt	8 (8–9)	8 (8–9)	8 (7–8)	.025
C-section, n (%)	39 (56.5)	20 (51.3)	19 (48.7)	.076
IUGR, n (%)	9 (13.1)	6 (66.6)	3 (33.3)	.108
SGA, n (%)	4 (5.8)	1 (25)	3 (75)	.477
RDS, n (%)	44 (63.7)	14 (31.8)	30 (68.1)	.023
PDA, n (%)	33 (47.8)	13 (39.3)	20 (60.6)	.671
BPD, n (%)	9 (13.4)	4 (44.5)	5 (55.6)	.875
Jaundice, n (%)	26 (37.6)	7 (26.9)	19 (73.1)	.048
Infections, n (%)	17 (24.6)	6 (35.3)	11 (64.7)	.517
HF, ms^2	13.2 (9.14–20.1)	17.15 (13–22.7)	10.3 (8.4–16.6)	.001
LF, ms^2	86.7 (79.5–90.3)	82.8 (77.2–87)	88.9 (83.2–91.2)	.003
LF/HF, ms^2	6.3 (3.9–10)	4.8 (3.5–6.7)	8.7 (5–11.4)	.001
Salivary cortisol, ng/mL	13 (8–21.5)	10 (8–15)	16.5 (10.5–26)	.021
LOS, dd	50 (41–69)	43 (31–61)	54 (46.5–82)	.012
TOF, dd	40 (31–57)	36.5 (27–34)	41 (35–29)	.165
TAR, dd	17.5 (2–48)	1 (2–44)	28 (12–59)	.006

Mann–Whitney *U* test was used to compare continuous variables and Chi-squared test was calculated for categorical variables with *P* value < .05.

BPD = bronchopulmonary dysplasia, C-section = Cesarian section, ELBW = extremely low birth weight, GA = gestational age, HF = high frequencies, IUGR = intrauterine growth restriction, LF = low frequencies, LF/HF = low frequencies/high frequencies, LOS = length of stay, N = number of infants, PDA = patent ductus arteriosus, RDS = respiratory distress syndrome, SGA = small for gestational age, TAR = time to autonomous respiration, TOF = time to oral feeding, VLBW = very low birth weight.

3. Finally, to evaluate the tenderness status, the reaction during slight compression between head and sacrum was evaluated using the facial expression, the presence of reflex-moves, the crying, or the sudden increase of heart rate.

The integration of the 3 assessment phases completes the SD Grade. The examiners were blinded toward the clinical, demographic characteristics of the infant and all the testing performed in the study.

SD Grade was classified as follows:

- Grade 0 to G0 (Absent SD), movement variability in NZ present;
- Grade 1 to G1 (Mild SD), altered movement variability in NZ;
- Grade 2 to G2 (Moderate SD), altered movement variability in NZ + myofascial TT abnormalities or tenderness;
- Grade 3 to G3 (Severe SD), altered movement variability in NZ + myofascial TT abnormalities + tenderness.

Moreover, during each assessment, the osteopaths counted the specific regions of the palpatory findings where a segmental SD was detectable, according to the ICD 11 classification (Code ME 93.0). The examiner assessed the variability of movement within the NZ is the fundamental analysis guiding all other assessments.

2.3.2. Heart rate variability.
A mathematical model was used for the detection of RR peaks and their representation of the frequency domain; after that, 3 main parameters were observed in this study: LF, HF, and LF/HF. For the HRV recording we used a 10-minute standard time monitoring using HRV module of the Kubios software (version 2.2, Kubios). The frequency domain was analyzed via its power spectrum of linear method and its frequency, considering LF ranging 0.02 to 0.2 Hz, HF ranging 0.2 to 2 Hz, and the ratio of both frequencies (LF/

HF).[31] Very low frequencies and time domain series were not considered in this study. The whole recording was obtained at least 30 minutes after feeding (between 8 and 12 AM with a distance of at least 4 hours from any painful or stressful procedure) with the awoken infants and in supine decubitus with a recommended ambient temperature (22–26°C) and lighting level (10–600 lux) for NICU. The registration was not considered valid when the neonatal clinical routine changed, in particular when the infant received cardiac medication or general anesthesia in the previous 7 days, considering the influence on HRV.

2.3.3. Salivary cortisol concentration.
The measurement of the salivary cortisol is a reliable out-of-laboratory marker, which provides useful indications about the HPA axis characteristics and distress state as a subclinical research tool.[32] The HPA axis starts its feedback regulation during the second trimester of gestation. Cortisol is fundamental for the stress response and the lung maturation, both relevant in premature infants.[33] The protocol for salivary assay was standardized in the 69 premature infants and it was collected 2 times, before and after HRV. The sCor was recorded as the main assessment of the 2. The salivary samples were collected at the same time of HRV, at least 30 minutes after the scheduled feeding and every 2 to 3 hours after any disruptive event and invasive medical procedure, in order to have a stable condition. A commercially available kit for radioimmunoassay was used to analyze the cortisol level, performing the detection while the infant was neither crying nor in an active alert state.[34]

2.4. Statistical analysis

Demographic and clinical data were tabulated as appropriate and are shown as mean, median, standard deviation, and

Figure 1. Osteopathic examination for the assessment of somatic dysfunction and its grade. The numbers on the figure illustrate the procedure as described in the text.

interquartile range. In Table 1, variables were stratified for SD severity and compared to Mann–Whitney U test for continuous variables and to Chi-squared test for categorical variables. We analyzed the agreement of 2 experienced raters on the SD Grade examination through the average measure and 95% confidence interval of interclass correlation (ICC), adopting a 2-way random effects model. To study the independent course of HRV frequency parameters and sCor with respect to SD grading, a multivariate linear regression model including GA, Apgar 5′, and RDS was implemented. The possible interacting variables were tested within the demographic, perinatal, and clinical features reported in Table 1. Specifically, we previously tested different variables combinations for the models, assessing their goodness-of-fit by the coefficient of determination (R^2). To improve the interpretation of the simple models, we graphically represented them with scatter plots, line fits, and 95% confidence interval, reporting the P value of the regression coefficient and its relative R^2 on the plot. STATA version 15 (Stata Corporation, College Station, TX) and the GraphPad Prism version 8 (GraphPad Software, Inc., La Jolla, CA) were used to compute the statistical analyses as appropriate.

3. Results

3.1. Study population and ICC

Sixty-nine patients were examined, 35 males (51%) and 34 females (49%), with a mean GA of 28.9 ± 2.4 days and a mean birth weight of 1103.4 ± 274.1 g.

The SD grading assessment revealed 0 patients with SD Grade 0 or Grade 1; this result makes us understand how a premature baby always shows significant fascial adaptations. Within the sample, 40 (58%) patients showed a SD Grade 2 and 29 (42%) a SD Grade 3. At birth, the mean Apgar 5′ score of the entire sample was 7.96 ± 1.18 and 39 (56%) of them were delivered by Cesarian section.

Among clinical variables stratified by SD Grade, the body weight, GA, Apgar 5′, RDS, Jaundice, length of stay (LOS), and time to autonomous respiration (TAR) demonstrated a statistically significant difference, as reported in Table 1. The reliability of the SD Grade was measured based on the results reported by 2 experienced, board-certified, osteopaths who were blinded to the exam results of the peer-rater. Notably, in this specific setting, the global agreement for SD Grade
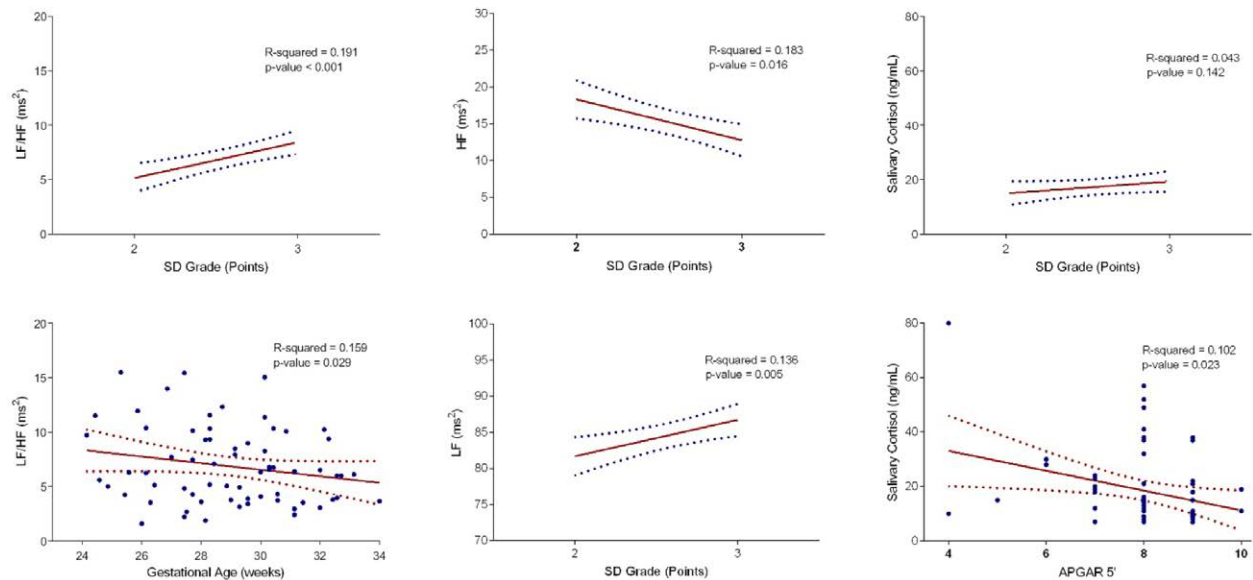
Figure 2. These regression plots show how the independent variables, SD Grade and APGAR 5', affect LF, HF, LF/HF, and sCor. The Y-axis represents the LF/HF values after frequency domain transformation expressed in ms². Statistical significance (P value), coefficient of determination (R-squared), 95% confidence interval and line of best fit are showed for each plot. HF = high frequency, LF = low frequency, sCor = salivary cortisol, SD = somatic dysfunction.

on 69 preterm infants was excellent with an ICC of 0.864 (0.773–0.918).

3.2. Regression models

All 8 regression models were statistically significant with ($P < .05$) in both simple and multiple regression models, described in Figures 2 and 3, and Table 2.

Figure 2 depicts the univariate linear models trajectories for the ANS outcomes and SD Grade, with the description of the statistical significance and R^2. As shown in the central panels, SD Grade and HF had a negative linear correlation with a slope of −5.59 (−8.94 to −2.17), whereas SD Grade and LF had a positive correlation with a slope of 5.04 (1.58–8.50). Moreover, the left panel and the right panel of Figure 2 depicted univariate linear regressions for LF/HF and sCor. In these panels, LF/HF increases with the increase of SD Grade observing a coefficient of 3.56, while it decreases with a GA of about −0.31 for each gestational week increase, as shown in the correspondent right panel. Analyzing the contribution of SD Grade to sCor in the upright panel of Figure 2, it shows that SD Grade does not increase the sCor concentration, while the Apgar 5' was negatively correlated to the sCor with a slope of −3.64 for each increasing point of Apgar 5'. In this case, Apgar 5' – and not GA – seems to affect the mean sCor at assessment. Considering the above cited relationships of HRV and sCor trajectories, a multivariable linear regression was implemented using HF, LF, LF/HF, and sCor as dependent variables and GA, Apgar 5', RDS, and SD Grade as covariates for the different models. Table 2 shows that HF, LF, and LF/HF had linear trajectories with SD Grade, independently from the GA and Apgar 5'. Specifically, SD Grade had a negative interaction with HF with a slope between the lower and the higher grade of SD of −6.43 ms², and had a positive correlation with LF with a slope between the 2 grades of SD of 5.68 ms². Moreover, Table 2 describes sCor with respect to GA, Apgar 5', RDS, and SD Grade in a multiple regression model. In this case, we notice that RDS and Apgar score are linearly related with salivary cortisol level with a slope of 9.70 and −4.44 ng/mL, respectively. SD grade showed a marginal, quasi-statistically significant, positive linear interaction with sCor. Figure 3 shows the absence of a linear interaction between LF/HF and sCor.

4. Discussion

In this study, we report the application of the SD Grade method from the VM for SD examination in very and extremely low weight preterm infants. SD Grade showed excellent concordance between 2 blinded raters, hence we aimed to establish whether the SD Grade could be related to differences of infant's clinical and perinatal characteristics.

Although OMT was considered effective in reducing LOS, hospitalization costs, and it was suggested to improve the gastrointestinal functionality and to reduce the time to full oral feeding attainment, SD assessment remains controversial. Among the most important limitations of OMT, there is the lack of adequate clinical assessment and classification of the myofascial system adaptation capability to postural stress, which could be relevant for both the clinical decision making and infants' clinical benchmarks into the NICU setting.

We were able to overcome some of those issues implementing an ad hoc version of the VM for preterm infants for SD examination and SD Grade scoring (min 0, max 3 points). Among the 69 preterm infants we examined, 42% were diagnosed with a grade 2 of SD and 58% of the infants with a grade 3. No infants presented grade 1 and 0 of SD. This is probably because of the immaturity and reduced adaptation capability of the population we studied. In fact, using SD Grade as grouping variable, mean GA emerged to be lower in the group of infants with severe SD Grade, suggesting the possible interaction of immature development and reduced fascial system adaptation capabilities. Other differences emerged about the 5 minutes Apgar score, which was lower in the group with severe SD, while the LOS and the TAR were longer in the SD group. Moreover, RDS and jaundice relative frequencies were higher in the babies with severe SD, 31.8 versus 68.1 and 26.9 versus 73.1, respectively. Based on those results, we can speculate that SD Grade was in line with the natural history of underdevelopment due to premature birth.

Moreover, recent studies described the modulatory effects of OMT on ANS, especially on the cardiac vagal tone, measured with frequency parameter of HRV and verified with functional MRI in adults.[16,35] Furthermore, Manzotti et al observed that the exteroceptive stimulation of C-tactile afferent fibers influences the autonomic regulation[36] and, in another study, OMT was effective on heart rate downregulation and on spO2
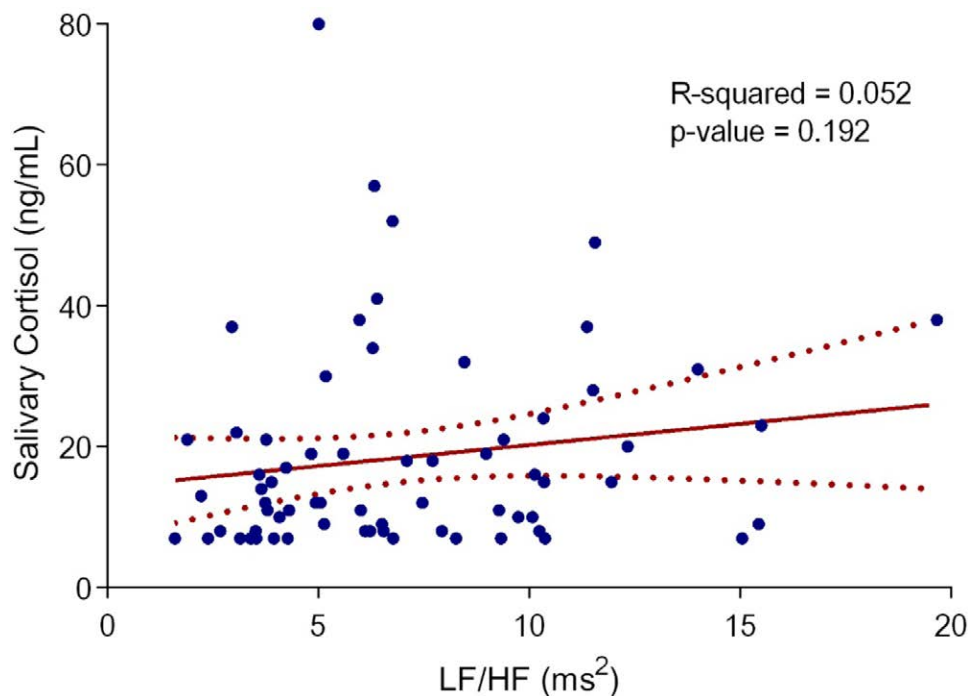
Figure 3. Regression plots show the interaction between salivary cortisol and HRV. The *Y*-axis represents the LF/HF values after frequency domain transformation expressed in ms². Statistical significance (*P* value), coefficient of determination (*R*-squared), 95% confidence interval, and line of best fit are shown for each plot. HF = high frequency, HRV = heart rate variability, LF = low frequency.

Table 2

Results of multivariate linear regression LH/HF and salivary cortisol (sCor).

	HF[¹]			LF[¹]		
	Estimate	**95% CI**	***P* value**	**Estimate**	**95% CI**	***P* value**
Gestational age	−0.78	−2.07; 0.51	.248	0.56	−0.77; 1.88	.406
SD Grade	−6.43	−10.6; −2.2	.003	5.68	1.36; 10.1	.011
RDS	0.82	−3.85; 5.48	.728	−1.44	−6.24; 3.36	.551
APGAR 5'	1.39	−0.99; 3.77	.248	−1.23	−3.69; 1.21	.317

	LF/HF[⁺]			sCor[¥]		
	Estimate	**95% CI**	***P* value**	**Estimate**	**95% CI**	***P* value**
Gestational age	−0.04	−0.80; 0.72	.920	−0.44	−2.91; 2.02	.722
SD Grade	2.84	0.37; 5.31	.025	7.39	−0.61; 15.4	.061
RDS	−0.30	−3.05; 2.44	.827	9.70	0.81; 18.5	.033
APGAR 5'	−0.25	−1.65; 1.15	.719	−4.44	−8.91; −0.01	.050

A multivariate linear regression model was implemented for HF, LF, LF/HF, and sCor separately. The goodness of fit, as expressed by R^2, was 0.19[¹], 0.16[¹], 0.14[⁺], and 0.18[¥].

CI = confidence interval, HF = high frequencies, LF = low frequencies, RDS = respiratory distress syndrome, sCor = salivary cortisol, SD Grade = somatic dysfunction grade.

stabilization in preterm infants.[37] Notwithstanding this, the SD assessment and its correlations with the cardiac sympathovagal activity were not directly investigated in this trial. Therefore, to model the features of ANS, we used a statistical approach on frequency spectrum HRV parameters (HF, LF, LF/HF) and the concentration of sCor before a palpatory evaluation. Our models showed a negative correlation between SD Grade and HF and a positive trajectory between LF, LF/HF, and SD Grade, in a model adjusted for GA, Apgar 5', and RDS (see Table 2). Conversely, SD Grade was not correlated with sCor in both simple and multiple linear models. Interestingly, in our model, RDS and Apgar 5' were inversely correlated with sCor, suggesting an influence even at time distance on the activity of the cortisol pathway, whether GA and SG Grade were not. This finding is in

line with the data obtained by Neu et al that revealed a negative correlation between Apgar 1' and sCor (see Fig. 2).[38]

These results suggest a specific correlation between the SD Grade severity and cardiac vagal tone, which can potentially explain the sensitization status that the raters revealed when examining stable-infants in the incubator. Other authors theorized a possible relevant role of sensitization and interception as important features in SD, reporting an upregulation of vagal tone after OMT.[39,40] To the authors best knowledge, this study reports the first available data on SD Grade examination and cardiac vagal activity in preterm infants.

This finding might have a relevant clinical impact as the vagal tone is nowadays considered a relevant biomarker of premature

infants' health and ANS maturation.[41–43] Moreover, in a rodent model, Doheny et al showed how the low vagal tone especially assessed with HF parameter is linked to a reduced gastric motility and favors pro-inflammatory state in the intestinal tract.[44] Likewise, altered frequency spectrum HRV parameters have been considered as predictive biomarkers for necrotizing enterocolitis[7] and correlated with a critical condition as RDS.[8] It should be emphasized that the SD Grade can be intended as an indicator for an altered status of vagal tone. In a NICU setting, this pragmatic assessment may be valuable to identify the infant's ability to handle with stressors and the child's adaptation to life. Also, SD Grade and HRV could be useful tools in OMT research and clinical practice inside NICU, with possible advances toward multidisciplinary decision making, the prognosis, and OMT-targeted therapies.

In addition, it is remarkable how the osteopathic assessment based on the VM proved to be a feasible procedure to adopt in such a critical condition.[45] No adverse events nor any other type of practical problems were detected during the study period. Beside this, the assessment we used and, more in general, the osteopathic palpation revealed to be a safe, easy-to-apply, and rapid procedure, also applicable in specific settings such as NICU. Finally, it is important to stress the peculiarity of this approach specifically based on the qualitative – rather than quantitative – assessment of motion.[20] The myofascial connective system is an important network for interaction between internal and external environment.[27] As for general movements, the motion assumes a central role; the palpation of the quality of the movement, through the VM, may be an important indicator for the premature infant's health and adaptation.

Limitations to this observational study include some non-modifiable possible confounders, such as the different exposure of the infants to acoustic stimuli, breathing patterns, the slightly different positioning of electrodes and the osteopaths' required specific training for VM. Furthermore, the limits of APGAR score should be disclosed. According to the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, the APGAR score alone is not predictive of asphyxia or neurological disorders.[46] In this study the APGAR score was used to balance the infants' condition at birth in the multivariate model. Future research should focus on the comparison of term infants with premature infants using the VM and SD Grade examination. Moreover, the correlations we described cannot be generalized to all preterm infants because we assessed healthy and stable preterm infants within the 34th week of PMA.

In summary, we concluded that SD Grade assessed with VM showed an excellent inter-rater concordance and was coherent with natural history of underdevelopment related to prematurity. Preterm infants who presented severe SD (grade 3) had lower GA and 5' Apgar, higher frequency of RDS and Jaundice, and longer LOS and TAR. Moreover, a negative linear correlation between vagal tone and SD Grade was found, leading to an understanding of the preterm infant's myofascial system adaptation and to new perspectives for multidisciplinary decision making regarding OMT in a complex setting as NICU. This study suggests that SD Grade could be a new tool that together with the HRV recording could help with the identification of the adaptation capacity in stable preterm infants.

Acknowledgments

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Correction

Affiliations b-e were corrected from "b Department of Neurosciences, University of Torino, Torino, Italy, c Division of Paediatric, Manima Non-Profit Organization Social Assistance and Healthcare, Milan, Italy, d Department of Research, SOMA –Istituto Osteopatia Milano, Milan, Italy, e Neonatal Intensive Care Unit, Woman and Child Department, Del Ponte Hospital, Varese, Italy, f PPCR, Harvard T.H. Chan School of Public Health – ECPE, Boston, MA, USA." to "b. Division of Paediatric, Manima Non-Profit Organization Social Assistance and Healthcare, Milan, Italy. c. Department of Research, SOMA - Istituto Osteopatia Milano, Milan, Italy d. Woman and Child Department, Varese Hospital, Insubria University, via Ravasi 2 21100 Varese, Italy. e. PPCR, Harvard T.H. Chan School of Public Health – ECPE, Boston, USA."
The examiner assessed the variability of movement within the NZ is the fundamental analysis guiding all other assessments

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