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Effects of osteopathic treatment versus static touch on heart rate and oxygen saturation in premature babies: A randomized controlled trial

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ABSTRACT

Background: Osteopathic manipulative treatment (OMT) has been successfully tested in the context of preterm infants. No studies, however, have been conducted to investigate the OMT immediate effects on physiological measurements, such as partial oxygen saturation (SpO2) and heart rate (HR). The purpose of the present study was to assess the effect of osteopathic treatment on SpO2 and HR values and to compare it with 10 min of static touch.

Materials and methods: Ninety-six preterm infants (41 male), aged 33.5 weeks (±4.3) with mean weight at birth of 2067gr (±929) were recruited from the neonatal intensive care unit (NICU) of the Buzzi Hospital in Milan, and randomly allocated to two groups: OMT and Static Touch. Each protocol session consisted of: a) 5-min Pre-touch baseline recording, b) 10-min touch procedure, c) 5-min post-touch recording. Primary and secondary outcomes were, respectively, the baseline changes of HR and SpO2.

Results: The 2 × 2 repeated measure ANOVA for HR showed a statistically significant effect ($F(1,94) = 5.34$; $p < 0.02$), revealing that the OMT group decreases the HR value at T2 ($p = 0.006$). In contrast, SpO2 analysis showed an increase of SpO2 value where the OMT group demonstrated higher values at T2 ($p = 0.04$).

Conclusion: Results from the present study suggest that a single osteopathic intervention may induce beneficial effects on preterm physiological parameters.

Trial registration: ClinicalTrials.gov identifier: NCT03833635 – Date: February 7, 2019.

1. Introduction

Premature birth, before 37 weeks of gestational age, is a common condition affecting 1/10 newborns, with a worldwide estimate of 15 million births per year [1]. Complications of prematurity are estimated to cause 35% of global neonatal deaths and are considered the second leading cause of child death under 5 years after pneumonia [2,3]. During the first year of life, a premature baby shows a weaker health condition in association with developmental and cognitive delays than a full-term newborn [4,5]. In order to protect such fragile life, different methods of monitoring are adopted in neonatal intensive care (NICU), as the evaluation of partial oxygen saturation (SpO2) and the measurement of heart rate (HR) [6,7]. These measures are among the most suitable for determining the biological response to stress in NICU [8].

Postpartum HR in full-term children with spontaneous breathing is higher than 100 bpm; in the preterm infant, HR is relevant and determines a threat if it shows an alteration in respect to the expected levels [9,10]. HR abnormalities could occur in sepsis or in other conditions that cause autonomic nervous system (ANS) functional problems. The heartbeat is influenced by sympathetic (norepinephrine) and parasympathetic (acetylcholine) activity, which leads to frequent small accelerations and decelerations in rate, respectively [11]. Stress and potentially painful stimuli enhance sympathetic activity, thus generating an increase in HR [8,16].

SpO2 is fundamental in the clinical management of preterm babies in the NICU. Low SpO2 values could be correlated with a reduced chance of survival, whereas high SpO2 rates could have harmful effects and results associated to retinopathy of premature and a high incidence pathology that afflicts preterm babies [6]. The regulation of the correct SpO2 parameters could be influenced by various conditions that affect the

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cardio-respiratory system of the premature, such as respiratory distress syndrome, or by the presence of high levels of stress-related to the hospitalization and handling in the NICU [12,13].

Therefore, attention should be paid to stress management and its fundamental role in the care of preterm infants, their growth and neuropsychological development [17]. Several studies have found in the premature baby, a decrease in stress levels associated with a particular mode of touch, called "gentle touch" [14,17–19]. This manual contact mode was observed to have direct effects on the child's ANS: it reduces the levels of cortisol produced by the stress response mediated by the sympathetic activity [20,21], thus potentially improving adaptability and health in the preterm infant [22,23]. Gentle touch has well defined neurophysiological characteristics: it is a soft, slow and moderate pressure tactile stimulus able to stimulate the C-tactile (CT) fibers present in the skin [17]. These fibers are part of the "interoceptive system", which collects afferent signals from the entire body and integrate them all especially in the insular cortex, to elaborate the best possible response to environmental stressors, directing stress axis, ANS and immune system activation [23,24].

Osteopathy is a form of medicine based on manual evaluation and treatment, in which "touch" has a fundamental role, in particular in the clinical management of the premature newborn [17]. Osteopaths use a wide range of techniques to promote adaptation and support the homeo/allostasis that has been altered by impaired function of skeletal, arthrodial, and myofascial components of the body framework and their related vascular, lymphatic, and neural elements [25]. The effectiveness of osteopathic manipulative treatment (OMT) is increasingly evident in the treatment of various problems such as low back pain [26], migraine [27], and in the pediatric field [28]. Several studies conducted on newborns proved the safety and efficacy of OMT in the reduction of length of stay (LOS) and hospital costs [22,28,29], of gastrointestinal symptoms [30], of cranial asymmetries, and in the management of clubfoot and of sucking dysfunction [31]. Furthermore, the osteopathic diagnostic touch seems to be able to modulate the activity of ANS, with an anti-inflammatory and hyper-parasympathetic action [23]. This effect on the ANS is confirmed by studies on adult subjects, whose HRV measurements after OMT showed an increase of parasympathetic activity and a reduction of sympathetic activity [32,33]. Other studies showed the efficacy of OMT on biological parameters *in vitro* [34]and *in vivo* [35], in particular, the reduction of pro-inflammatory cytokines, thus attesting an anti-inflammatory role [36,37] clinically. Moreover, according to recent theories, similar to "gentle touch", "osteopathic touch" would be able to interact with neurological structures via the stimulation of CT fibers, thus modifying for the better the interoceptive processes taking place in the insular region and their influence on the organism [17,23]. This hypothesis leads to great intervention possibilities in terms of neurodevelopment and prevention in children who show, even after growing up, the signs of the constant allostatic overload suffered as preterm newborns.

Based on these assumptions, the hypothesis that osteopathic touch could be a valid support in the improvement of preterm babies' physiological parameters in NICU was conceived: if OMT should change HR and SpO2, it could mean OMT is a viable strategy to help preterm newborns in controlling their stress levels and in positively assisting their development in NICU. The purpose of the present study was thus to test the effects on SpO2 and HR outcomes, and to compare it with 10 min of static, non-CT optimal touch procedure. The present study is the first one investigating the just mentioned hypothesis to our knowledge.

Static touch as comparative procedure was chosen since various OMT techniques, especially on preterm newborn, are similar to a kind of static touch [22]; however, the focus of the therapist performing OMT is different than during a mere static touch, since he has to pay particular attention to the newborn response, as argued by Cerritelli and colleagues in a recent neuroimaging study [38].

2. Materials and methods

2.1. Trial design

The present study was a randomized clinical trial with two groups: (1) OMT and (2) Static Touch. The allocation ratio of participants was 1:1.

2.2. Participants

The preterm infants were recruited from the NICU of the Buzzi hospital in Milan, Italy, from March 2019 to June 2019.

To be included in the study infants should be born at Buzzi hospital, with gestational age (GA) between 28.0 and 36.6 weeks and without clinical (i.e. respiratory or cardiovascular instability, surgical pathologies, born of an HIV-positive or drug-dependent mother, sepsis) and/or congenital/genetic diseases. All infants were enrolled within 1 week of birth.

Written informed consent was obtained from parents or legal guardians before the infant's enrollment in the study. The study was approved by the local Research Ethics Committee (38657/2017), and the trial was registered on ClinicalTrials.gov (identifier: NCT03833635). All infants continued receiving routine neonatal clinical care during the study period.

2.3. Sample size

Due to the lack of research in this field, a formal sample size calculation based on data published in the osteopathic literature was not possible to perform. Thus, arbitrarily considering a medium effect size ($d = 0.5$, according to Cohen's classification), an $\alpha = 0.05$ and $\beta = 0.8$, we obtained a sample size of 64 participants per group ("pwr.t.test" and "cohen.ES" functions of "pwr" R software package). To account for the possible drop-out rate, we increased that number by 10%, reaching 71 participants per group for a total sample of 142 preterm infants.

Therefore, we enrolled 145 preterm infants, of which 45 were excluded for not meeting inclusion criteria or for not signing written consent (Fig. 1).

2.4. Randomization

Enrolled preterm infants were randomly allocated to two groups: (1) OMT or (2) Static Touch. The randomization sequence was computer-generated in blocks of ten, without stratification. The coordinating centre performed and stored the randomization, and the process was upon the responsibility of an information technology consultant.

Thus, 100 preterm infants were randomized into an OMT ($N = 50$) and Static Touch group ($N = 50$). In the latter group, 4 infants did not receive the planned intervention due to clinical complications, reducing the Static Touch group to 46 participants (Fig. 1).

2.5. Allocation concealment

NICU staff was not informed regarding the outcomes and study design as well as patients' allocation. The person performing statistical analysis was also blinded to patients' allocation and did not have any contacts with either the patients or the osteopaths or the NICU staff. Only osteopaths were informed of patients allocation, but they had no role in decisions concerning patient care.

2.6. Interventions

Included preterms underwent a single 20-min protocol, receiving either OMT or Static Touch. The interventions were delivered by researchers with experience in the field of neonatology and expert osteopaths (at least 5 years experience in NICU).

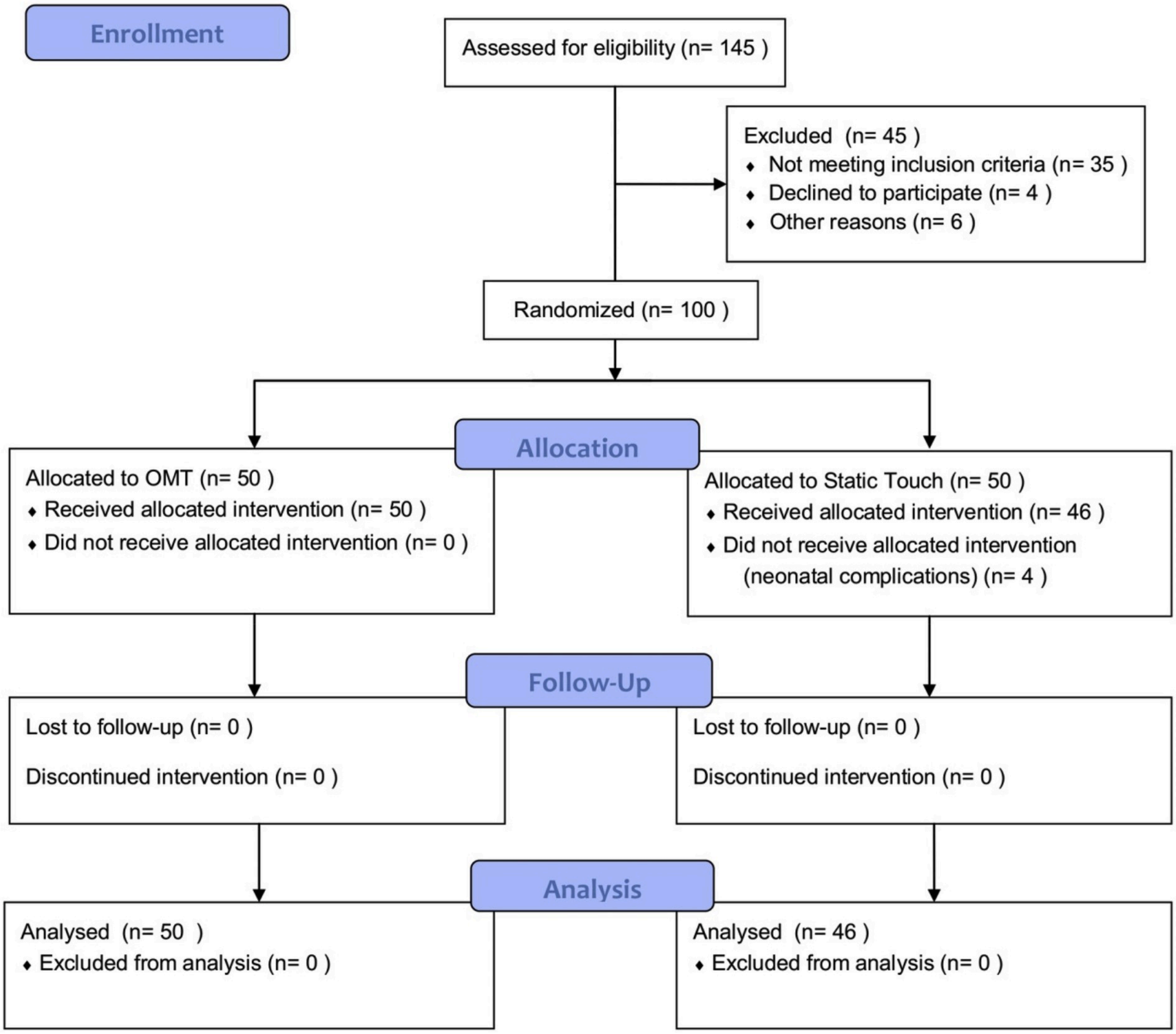

Fig. 1. CONSORT flow diagram.
The figure shows the distribution of patients throughout the study. OMT = osteopathic manipulative treatment.

Each protocol session consisted of: a) 5-min pre-touch baseline recording, b) 10-min touch procedure, c) 5-min post-touch recording. During the baseline period, the hands were placed in the incubator to match the skin temperature of the infant.

The osteopathic procedures followed previous studies [31] and were divided into manual assessment and treatment procedures. For the manual assessment, the osteopath stood up aside the crib, placing the palm of the cranial hand on the baby's occiput covering with the fingers the entire bone surface. The caudal hand is placed on the sacrum, covering as much surface as possible of the sacral bone. The manual assessment aims to identify any posture or mobility restriction areas. It lasted approximately 1 min.

The second part of the procedure relies on the treatment, which is based on the palpatory findings of the initial assessment. It lasted approximately 9 min and aimed at releasing detected changes in the tension and mobility of the tissue. The techniques chosen were those already used in previous studies [27–31] and demonstrated to be safe in the context of preterm infants. Specifically, indirect techniques (e.g. cranial, functional, balanced ligamentous tension) were used.

Static Touch was administered using the dominant hand of the researcher. During the 10-min touch procedure, the infant's dorsum was the area of choice. Specifically, the researcher placed their hand between the first thoracic to the last lumbar vertebra, covering approximately 10 cm. The hand was kept in that position for the duration of the 10-min block, maintaining the same approximate force (~0.3 N).

The contact was always bare hand to bare skin. All preterms were laid on their right side in the crib and kept this position for the entire period of the intervention. The right-sided position was adopted based on clinical reasoning as it was recognized as the most favorable position to prevent potential tubes or probes interference. Preterm infants were put in the right-sided position approximately 2 min before the recording started, with the pulse oximetry probe already attached to the right foot.

The fraction of inspired oxygen (FiO2) was maintained constant throughout the whole session. Besides, in the case of drug administration, the latter was carried out at least 3 h before the experiment/treatment started.

2.7. *Outcomes and physiological monitoring*

The primary outcome was the baseline change of HR, whereas the secondary outcome was the baseline change of SpO2.

SpO2 and HR rate were monitored with a pulse oximeter (Masimo Corporation, Irvine, CA, USA). The pediatric pulse oximetry probe was attached around the dorsal aspect of the infant's right foot. The physiological signals were digitized and recorded at 2 Hz using the New Life

Box physiological recording system (Advanced Life Diagnostics, Weener, Germany) with Polybench software (Advanced Life Diagnostics, Weener, Germany).

2.8. Data analysis

Physiology data was output as .csv file with a data point for every 0.5 s of recording (2 Hz sampling frequency). On an individual participant basis, oxygenation and heart rate data were averaged into 30-s-long time bins and divided into baseline, touch and post-touch periods. For both groups, both baseline and post-touch periods contain 10 data points per time block, whereas the touch period contains 20 data points.

Pulse oximeters can be sensitive to movement artifacts: therefore, to account for non-experimental movements that might produce extreme values, for each period (baseline, touch and post-touch) individual participant's data points that were more than three standard deviations (SDs) above or below the sample mean for that period were identified. Such data-points were determined to be artifacts and replaced by the mean of that participant's non-artifactual epochs of that period.

Considering SpO2 – 9 participants in the baseline period, 7 in the touch period and 2 in the post-touch period were identified as having artifactual epochs; across participants, this represented 3.5% (34 over 960), 1.6% (31 over 1920) and 0.3% (3 over 960) of data points in the baseline, touch and post-touch periods respectively. For HR – 5 participants in the baseline period, 5 in the touch period and 3 in the post-touch period were identified as having artifactual epochs; across participants, this represented 0.5% (5 over 960), 0.6% (11 over 1920) and 0.6% (6 over 960) of data points in the baseline, touch and post-touch periods respectively.

One participant from the Static Touch group was excluded from SpO2 analysis as no non-artifactual data points in the baseline period were revealed. However, that participant was included in the heart rate analysis.

Subsequently, for both oxygenation and heart rate data, each data point from the touch and post-touch periods were converted to change from baseline by subtracting, for every participant, the baseline mean value from each epoch in the touch and post-touch periods.

2.9. Statistical analysis

General characteristics of the OMT and Static Touch groups, GA, weight at birth and baseline heart rate and oxygen saturation were compared with independent samples t-tests. Gender distribution between groups was compared using χ^2 test.

To explore differences between Groups (OMT vs Static) over Time (touch, or T1-T0, vs post-touch, or T2-T0) for HR and SpO2, a 2 × 2 repeated measures ANOVA using Group as between factor and Time as within factor was performed. This procedure was performed by using the "aov_ez" function of the "afex" R package. The data used for this model was obtained calculating the individual change from baseline values of the heart rate and oxygen saturation in both the touch and post-touch periods.

It is worth noting that ANOVA is considered a robust method also when data show violation of normality, which does not necessarily imply the violation of homogeneity of variance [39]. To further explore any between- and within-comparisons, six pairwise post-hoc Welch 2-sample t-tests using the Bonferroni correction ("pairwise.t.test" R function) were applied on the individual participant's data. Welch 2-sample *t*-test is a robust method to be also applied in case of violation of normality and heteroscedasticity [40,41]. The significance level was set at $\alpha = 0.05$ and adjusted for the post-hoc tests.

A post-hoc analysis was conducted in order to investigate the study power further since we performed the intervention only on 96 patients: in fact, they should have been 142 according to the a priori power calculation. Besides, to compute sample size estimates based on different methodological, statistical hypotheses (i.e. power and alpha levels) [42], two Monte Carlo simulation analyses were conducted [43,44]. The details of these analyses can be found in the Supplementary Material.

Data were analyzed using the free software R (Version 3.4.1, The R Foundation for Statistical Computing).

3. Results

3.1. Sample characteristics and baseline measures

We enrolled 145 preterm infants, of which 45 were excluded for not meeting inclusion criteria or for not signing written consent (Fig. 1). Thus, 100 preterm infants were randomized into an OMT (N = 50) and Static Touch group (N = 50). In the latter group, 4 infants did not receive the planned intervention due to clinical complications, reducing the Static Touch group to 46 participants. The 96 newborns (41 male) who completed the study were aged 33.5 weeks (±4.3) with a mean birth weight of 2067gr (±929).

At baseline, these two groups were comparable in terms of gestational age, weight at birth and gender but not for heart rate and oxygen saturation (Table 1).

3.2. Heart rate analysis

Table 2 summarized the data for HR at the different time points. To further explore the difference between groups across time, a 2 × 2 repeated measures ANOVA was applied showing a statistically significant effect for time $F(1,94) = 5.34$;$p = 0.018$, but not for group ($F(1,94) = 0.2910$; $p = 0.59$). To further explore these differences (Fig. 2), six pairwise Welch 2-sample t-tests corrected via Bonferroni were applied to show the OMT group statistically reducing the HR at T2 ($p = 0.006$). The Static group did not show any significant change between post-touch and touch ($p = 0.93$).

3.3. Oxygen saturation analysis

Table 2 reported data on SpO2 at the different time points. The ANOVA found a non-significant effect of group ($F(1,93) = 0.81$; $p = 0.37$) and time ($F(1,93) = 0.1482$, $p = 0.70$). However, as illustrated by Fig. 3, the two groups showed opposite trends. Indeed the OMT sample showed an increase in oxygen saturation both during the touch and the post-touch period, whereas the Static Touch group showed a reduction in oxygen saturation during the same periods. Pairwise t-tests showed a statistically significant difference between the OMT and Static Touch groups at T2-T0 ($p = 0.04$).

4. Discussion

To the best of our knowledge, the present study was the first one

Table 1
General characteristics of the study population at Baseline.

	Osteopathic manipulative treatment (n = 50)	Static Touch (n = 46)	p>|t|
Gestational age (weeks)	32.9 ± 4.4	33.9 ± 4.2	0.27
Weight at birth (grams)	1967 ± 910	2173 ± 948	0.28
Gender	20 (40)	21 (46)	0.72
Heart rate	139.8 ± 16.3	145.6 ± 12.4	<0.001[b]
Oxygen saturation	96.3 ± 3.0	96.7 ± 2.6[a]	0.017[b]

Values shown are mean ± Standard Deviation and all *p* values are from t-tests, except Gender expressed as N (%) and whose *p*-value is from χ^2.

[a] The subjects considered in the Static Touch group for oxygen saturation were 45, since one participant had no non-artifactual data points for oxygen saturation in the baseline period.

[b] $p < 0.05$.

Table 2
Analysis of outcomes: values at T0, T1, and T2 and change at T1 and T2 from the baseline.

Outcome	Group	T0	T1	T2	T1-T0	T2-T0
Heart rate	Static Touch	145.6 ± 12.4	146.8 ± 14.6	145.7 ± 15.3	1.1 ± 8.76	0.1 ± 10.1
	OMT	139.8 ± 16.3	140.5 ± 18.8	138.5 ± 19.0	0.7 ± 10.9	−1.2 ± 13.1[a]
Oxygen saturation	Static Touch	96.7 ± 2.6	96.5 ± 3.3	96.7 ± 2.6	−0.03 ± 1.45	−0.09 ± 1.5
	OMT	96.3 ± 3.0	96.2 ± 3.3	96.6 ± 3.6	0.1 ± 2.16	0.3 ± 2.4[b]

Values are mean ± Standard Deviation. In the manuscript are described the details on how the baseline change scores were calculated.
[a] OMT T2-T0 vs OMT T1-T0: $p < 0.01$.
[b] OMT T2-T0 vs Static Touch T2-T0: $p < 0.05$. OMT = osteopathic manipulative treatment.

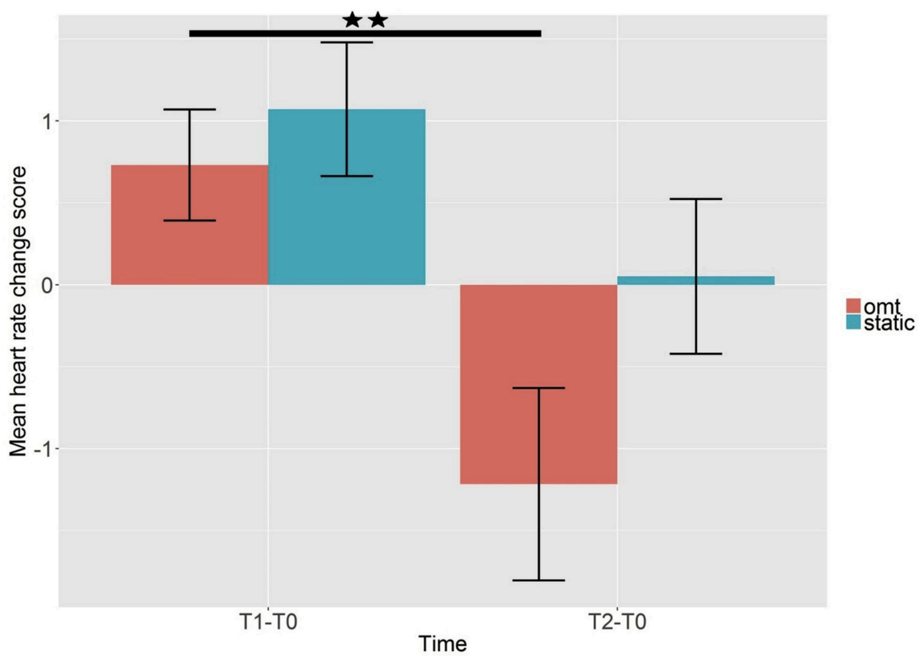

Fig. 2. Mean heart rate change score during the touch (T1-T0) and post-touch (T2-T0) periods. Data are presented as change in heart rate from baseline. OMT: osteopathic manipulative treatment. Error bars show ± Standard Error. The black line indicates the significant effect of Time for the OMT group as detected via 2 × 2 repeated measures ANOVA and Bonferroni corrected pairwise t-tests. $^{**}p < 0.01$.

evaluating the immediate OMT effect on both HR and SpO2 in preterm newborns. Since HR and SpO2 are frequently used physiological indicators in clinical monitoring of newborn infants' health [6–8], our objective was to assess if OMT could have positive effects on preterm newborns' health and stress levels via modification of HR and SpO2.

The results show that one 10-min OMT session produces a reduction of heart rate of preterm infants, and this effect was notably shown in the 5-min post-touch period. In contrast, static touch did not produce a significant change in the heart-rate of preterms. Besides, OMT was also associated with an increase in partial oxygen saturation level, which was not revealed in the static, non-CT optimal touch group.

These findings extend previous work by examining the immediate physiological effects of osteopathic intervention in a sample of preterm babies. Indeed, Cerritelli et al. showed that the use of OMT was significantly associated with a reduction of LOS [28,45]. These data were further confirmed by a recent systematic review, where Lanaro and colleagues synthesized the pooled effect of OMT as compared to control group by reducing the LOS of 2.71 days (95% CI: 3.99, −1.43; $p < 0.001$) [22]. Moreover, a very recent study confirmed these results in a different NICU setting [46]. Therefore, while published research studied the clinical effect of OMT focusing on clinical outcomes (i.e. reduction of LOS, costs), here the effect of OMT was observed during the intervention and immediately after the session by using a well-established and reliable tool for monitoring preterm's conditions.

These results showed to be consistent with previous data on touch stimulation, by using dynamic stroking touch or massage, reporting a parasympathetic activity, a reduction of heart-rate and eventually blood pressure [47–51].

We did not include a control group without any physical stimulus since static touch is a type of touch frequently used in perinatal care, and thus we wanted to assess possible differences with another kind of touch, meaning OMT. The comparison between two types of touch has the advantage of allowing the study of the placebo effect: indeed, preterm newborns could reduce their stress response just for being touched [52]. However, several studies showed different results after various types of touch (e.g. static vs dynamic stroking, light vs moderate pressure) [47–52], as the present study did, although static touch and OMT techniques on newborns could be very similar [22]. These findings point to the direction that, although the placebo effect could be involved, various kinds of touch could elicit different organic response due to the specific nervous pathway it activates [17].

To further discuss the hypothesis that osteopathic intervention using a specific touch can elicit immediate autonomic and metabolic effect, recent studies can be considered. Indeed, McGlone and colleagues hypothesized that the osteopathic intervention might be based on non-specific and specific elements of touch and the meaningful effects of both components are essential for understanding the treatment effects [17]. That is, the osteopathic intervention by using a CT-based

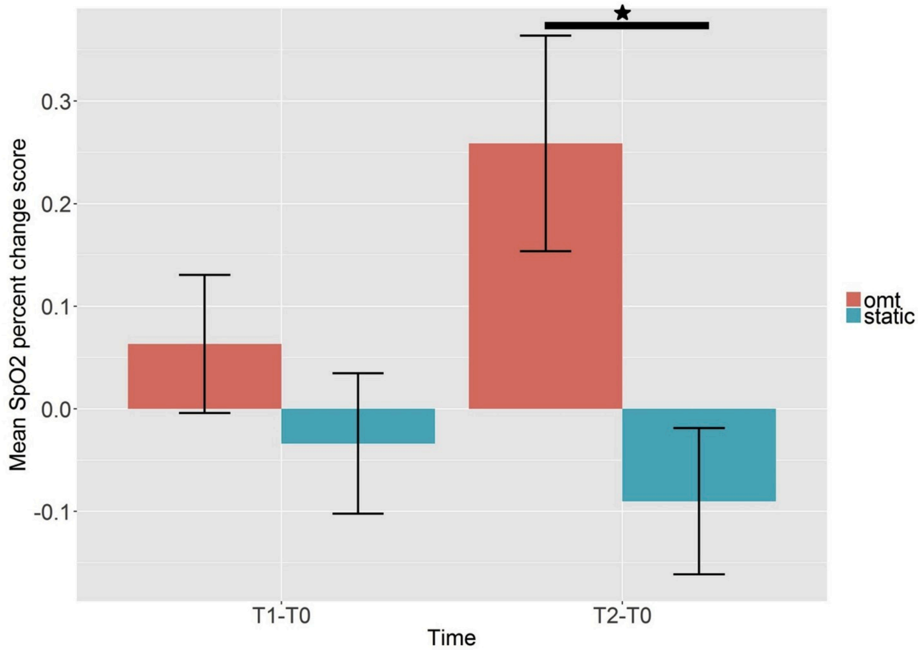

Fig. 3. Mean SpO2 change score during the touch (T1-T0) and post-touch (T2-T0) periods. Data are presented as a change in oxygen saturation from baseline. SpO2 = partial oxygen saturation. OMT = osteopathic manipulative treatment. Error bars show ± Standard Error. The black line indicates a significant difference between groups in the post-touch period as detected via Bonferroni corrected pairwise t-tests. *$p < 0.05$.

mechanism might induce a parasympathetic response as previously hypothesized [23,33]. Indeed, as happens in the majority of manual therapies, touch can be referred to as a mechanical stimulus, that, if respecting given features, can activate an interoceptive pathway modulating the internal homeo/allostasis milieu [23,38].

Furthermore, it has also been hypothesized that OMT might generate a reduction of pro-inflammatory cytokines [26,34,35], suggesting an anti-inflammatory role [36]. Although robust metabolic measurements were not included in the present study, from basic animal research massage-like stroking in mice showed to boost the metabolic system [53]. Arguably, this effect might also be relevant in the context of preterm newborns.

The present findings showed an immediate increase of SpO2 values which may indicate a rapid metabolic reaction in preterm infants receiving OMT, whereas the post-OMT heart rate reduction might reflect a subsequent parasympathetic effect. Therefore, it can be hypothesized that OMT in preterm infants might produce an initial metabolic-vascular effect followed by an autonomic (parasympathetic) response. In other words, OMT could favor a better oxygen exchange activating a cascade of events resulting in a parasympathetic vagus nerve-based response. This phenomenon could start as a neural process which led to a heart rate decrease [54]. It is noteworthy that this inverse (lower HR and higher SpO2) effect might indicate a lower stress level [12,13] and thus interpreted as a possible reduction of preterm newborn psycho-physical stress [8,52]. Indeed, it has been demonstrated that an improvement in the efficiency of the stress response (better equilibrium between sympathetic and parasympathetic divisions) increases the adaptability and health in the preterm infant [22,23]. Thus, further research is necessary to determine the effect of the specific osteopathic intervention on autonomic and metabolic functions.

The lack of significant differences between the two groups during the touch period, specifically for the heart-rate, needs to be discussed with the similarities between the osteopathic intervention and the static touch. Indeed, the osteopathic techniques used in the present study are applied in a way that the therapist induces, with the hands, a meaningless movement of the bodily part touched. This touch might be perceived as a “static” touch. For example, a pilot-RCT investigating the effects of craniosacral therapy on the development of general movements in healthy preterm newborns did not demonstrate any difference in respect to the control group [55]. However, a closer look of Raith and colleagues’ data shows that the non-significant differences might be due to the sample size as the study seems to be significantly underpowered. Thus, further methodological and data-driven approaches are needed to determine the appropriate numbers to be enrolled when similar “static” approaches are used.

The sample size used in the present study was arbitrary computed based on the current literature. However, findings support the fact that the study is underpowered, posing the risk of false-positive results [56]. In order to test it, advanced simulation methods (Supplementary material) were used, showing that bootstrapping the data would produce adequate power both for the SpO2 and HR, reducing the risk of false-positive results. Interestingly, to detect a between-group difference in HR requires a sample that is doubled compared to SpO2. Indeed, considering the second simulation, the one based on the present study means and standard deviations, under the assumptions of normality, homoscedasticity and homogeneity of measures, a power higher than 0.8 for detecting a significant between-group difference for SpO2 is reachable with 200 participants per group (400 total), whereas 500 participants per group (1000 total) are required to detect the same effect for HR. If, instead, the sample size computation is based on ANOVA analysis method, in order to detect a significant effect by group, keeping power of 0.80, the sample sizes are reduced to 100 per group (200 total) for SpO2 and 250 (500 total) for HR. Therefore, choosing between SpO2 and HR as the primary outcome might have significant consequences for the study planning, that in turn might significantly impact the sample size, the study power and thus the study results.

To further understand the sample size differences amongst HR and SpO2, there is a need to consider how the measurements were taken, that is by a pulse oximeter and with a 2 Hz sampling frequency. This device has been proven to be sensitive to both oxygen and HR changes [57–59]. Moreover, it can be suggested that oxygen saturation is a fast-reactive physiological parameter as compared to HR, especially in preterm newborns (indeed, there is a positive correlation between gestational age and HR variability) [15,60]. Thus these elements might strengthen the hypothesis mentioned above that in preterm newborns, the forefront OMT effect is metabolic-vascular, i.e. the oxygen saturation change, followed by an autonomic reaction, i.e. the heart rate change.

It is noteworthy that one of the accepted OMT neurobiological underpinning hypotheses linked the osteopathic intervention to interoception, central sensitization and ANS [23,61,62], where the ANS might play a fundamental role in modulating afferent and efferent signals. Findings from this study, however, opened a slightly different perspective, at least in preterm infants. Indeed, the immediate metabolic (systemic) change would induce a bottom-up effect, that in turn might act on the ANS activity. Further neurobiological studies, systematically comparing the physiological response of the autonomic and metabolic/vascular system are needed to address this question.

The present research evaluated only the acute effect on HR and SpO2 of a 10-min OMT session as compared to static touch. Future studies are needed to determine whether long-term clinically significant effects are detectable, what are the differences with dynamic touch CT-tuned procedures and what dose, i.e. duration and frequency, is necessary to elicit optimal responses. Certainly controlling for baseline values might strengthen the results; however, the high intrinsic variability of the parameters in this fragile sample is unpredictable.

5. Conclusion

The present study showed that OMT produces a reduction of HR of preterm infants, and this effect was shown in the 5-min post-touch period. Static touch did not produce a significant change in the HR of preterms. OMT was also associated with an increase of partial oxygen saturation. These results suggest that a single osteopathic intervention may induce beneficial effects on preterm physiological parameters. This opens new insights for the optimization of modern perinatal care approaches.

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Declaration of competing interest

None.

CRediT authorship contribution statement

Andrea Manzotti: Conceptualization, Methodology, Investigation, Resources, Supervision, Project administration, Writing - review & editing. **Francesco Cerritelli:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Erica Lombardi:** Investigation, Writing - original draft. **Simona La Rocca:** Investigation, Writing - review & editing. **Marco Chiera:** Formal analysis, Writing - original draft, Writing - review & editing. **Matteo Galli:** Data curation, Writing - original draft. **Gianluca Lista:** Validation, Writing - review & editing, Resources, Supervision, Project administration.

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Abbreviations

ANS	autonomic nervous system
CT	C-tactile
FiO2	fraction of inspired oxygen
GA	gestational age
HR	heart rate
LOS	length of stay
NICU	Neonatal Intensive Care Unit
OMT	osteopathic manipulative treatment
SpO2	partial oxygen saturation
SDs	standard deviations

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctcp.2020.101116.

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