A Randomized Placebo-Controlled Trial of Massage Therapy on the Immune System of Preterm Infants

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KEY WORDS
immune system, natural killer cells, massage, mode of delivery, preterm infants, weight gain

ABBREVIATIONS
AE—adverse event
CI—confidence interval
LU—lytic unit
MOD—mode of delivery
MT—massage therapy
NK—natural killer
PMA—postmenstrual age

This trial has been registered at www.clinicaltrials.gov (identifier NCT00317278).

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WHAT'S KNOWN ON THIS SUBJECT: Stressful events adversely affect the immune system, particularly the natural killer (NK) cells. Infants in the NICUs are exposed to stressful stimuli. The effect of massage therapy on the immune system of preterm infants has not been investigated.

WHAT THIS STUDY ADDS: This randomized placebo-controlled study found daily massage performed in stable preterm infants for a minimum of 5 days was associated with an increase in NK cell cytotoxicity despite lower absolute NK cell numbers compared with controls.

abstract

OBJECTIVES: The aim of this study was to investigate the effects of massage therapy (MT) on the immune system of preterm infants. The primary hypothesis was that MT compared with sham therapy (control) will enhance the immune system of stable premature infants by increasing the proportion of their natural killer (NK) cell numbers.

METHODS: A randomized placebo-controlled trial of MT versus sham therapy (control) was conducted among stable premature infants in the NICU. Study intervention was provided 5 days per week until hospital discharge for a maximum of 4 weeks. Immunologic evaluations (absolute NK cells, T and B cells, T cell subsets, and NK cytotoxicity), weight, number of infections, and length of hospital stay were also evaluated.

RESULTS: The study enrolled 120 infants (58 massage; 62 control). At the end of the study, absolute NK cells were not different between the 2 groups; however, NK cytotoxicity was higher in the massage group, particularly among those who received ≥5 consecutive days of study intervention compared with control (13.79 vs 10 lytic units, respectively; $P = .04$). Infants in the massage group were heavier at end of study and had greater daily weight gain compared with those in the control group; other immunologic parameters, number of infections, and length of stay were not different between the 2 groups.

CONCLUSIONS: In this study, MT administered to stable preterm infants was associated with higher NK cytotoxicity and more daily weight gain. MT may improve the overall outcome of these infants. Larger studies are needed. *Pediatrics* 2012;130:e1549–e1558

Stressful life situations are associated with increased risk of developing infection and cancer in humans,[1] possibly due to the impact on the immune system, particularly natural killer (NK) cell number and function (cytotoxicity).[2,3] NK cells are lymphocytes that exhibit cytolytic activity against tumor cells or cells infected with viruses.[4,5] NK cytolysis of virus-infected cells is an important component of innate immunity and antibody-dependent cellular cytotoxicity.[6] Compared with adult NK cells, neonatal NK cells exhibit decreased cytotoxicity and reach the adult level at 1 to 5 months of age.[7] NK cell numbers and cytotoxic activity are significantly lower in premature infants compared with full-term newborns.[8–10]

There is a correlation between low NK cytotoxicity and susceptibility to viral infections.[11–15] Decreased NK cytotoxicity against herpes simplex virus-infected cells in human cord blood cells has been associated with severe and recurrent herpes simplex virus diseases in infants.[11,12] The reduced NK cell cytotoxicity and deficient antibody-dependent cellular cytotoxicity against HIV-infected cells in premature infants explains in part the increased risk of vertical transmission of HIV infections in preterm infants.[13]

Infants admitted to the NICU are continuously exposed to stressful stimuli such as loud noises, bright lights, and procedure-related activities.[16,17] To reduce the amount of stress in the NICU, the use of tactile interventions such as massage therapy (MT) has been studied. MT has been associated with increased weight gain,[18–21] shorter hospital stay,[18,20,22] cost savings,[18] fewer behavioral signs of stress,[18] and improvement in mental and motor development.[18,20,23]

MT has been shown to increase the immune function including NK cell numbers and cytotoxicity in healthy adults, adults infected with HIV,[24–26] and adults with cancer.[27] Impact of MT on immune parameters (including NK cell numbers) was evaluated in Dominican HIV-infected children aged 2 to 8 years (22 in the massage and 25 in the control group) for 3 months.[28] At the end of the study, increase in NK cells was noted in younger patients (2- to 4-year-olds) who received MT; however, evaluation of NK cytotoxicity was not performed.

Effects of MT on the immune system of premature infants have not been investigated. We therefore conducted a prospective randomized double-blind placebo-controlled trial to examine the effects of MT on the immune system of premature infants, in particular the NK cell numbers and cytotoxicity.

Our primary hypothesis was that MT would enhance the immune system of stable premature infants so that infants receiving MT would report a 35% increase in the proportion of NK cells (in comparison with a 10% increase for infants in the control group). Our secondary hypotheses were that compared with sham therapy, MT would enhance NK cytotoxicity, induce more rapid weight gain, shorten the length of hospital stay, and reduce the number of culture-proven infections. The primary outcome of the study was increase in proportion of NK cells. Secondary outcomes included assessment of NK cytotoxicity, cellular immune function (T and B cells, T-cell subsets), length of hospital stay, and number of culture-proven infections.

METHODS

Inclusion Criteria

Study entry criteria included medically stable premature infants with birth weight between 600 and 1800 g and at 28 to 33 weeks' postmenstrual age (PMA). Stability was defined as lack of need for supplemental oxygen, systemic antimicrobial therapy for infection, or a central line. Subjects were excluded if they had a history of necrotizing enterocolitis, receipt of steroids, history of congenital infection, presence of congenital malformations, chromosomal abnormality, contraindication to blood draw such as bleeding disorder or severe anemia, or receiving human milk feedings. Infants receiving human milk feedings were excluded because human milk is known to contain immune-related compounds that could potentially affect study outcome.[29]

Treatment Groups

The study protocol was reviewed and approved by Wayne State University's Institutional Review Board. Written informed consent was obtained from a parent or legal guardian. Infants were randomized by a research nurse based on PMA on day of randomization in 6 permuted blocks (28–33 weeks) with block sizes of 6 subjects per block generated by the study biostatistician by using a computer-generated sequence provided by Randomization.com (http://www.randomization.com). The investigators, physicians, staff nurses, biostatistician, laboratory research associate, and parents were blinded and were not aware of the treatment status of these infants.

Neonatal research nurses who are certified in infant MT performed all study interventions (massage or sham) behind wide screens. Parents and bedside care delivery staff were asked to leave the infants' bedside during the study intervention. To ensure that the nurses' massage techniques do not drift over time, refresher massage sessions were conducted every 6 months.

Infants randomized to the massage group (method described by Field et al[18]) received MT behind 2 wide screens for 45 minutes a day (three 15-minute sessions) from Monday to Friday for a maximum of 4 weeks or until hospital discharge (whichever came first). Each 15-minute session consisted of three 5-minute phases: tactile, kinesthetic, then

tactile (Fig 1). Infants randomized to the control group received no massage; however, the research nurse remained behind the 2 wide screens and spent the same amount of time without physical contact with the infants. Hands were washed thoroughly before and after contact with each infant. Gowns were also worn.

A "set" of study intervention was defined as 5 consecutive full days of therapy received. The majority of infant massage studies in the United States reported benefit (weight gain, stress reduction) after only 1 set of 5 consecutive days (Monday to Friday) of study intervention (a total of 5 days).[20,21,30–32] Based on these studies, we consider 1 set of 5 consecutive full days of study intervention to be the minimum intervention required to produce evaluable results.

Adverse Events Monitoring

Infants were closely monitored for adverse events (AEs) (eg, apnea, bradycardia, oxygen desaturations, tachypnea, abdominal distention, feeding intolerance, and others) that may arise during study intervention. AEs were reported to the study Data and Safety Monitoring Board.

Assessment of Outcome Measures

After randomization, demographic information was collected by a research nurse including maternal history, delivery, and neonatal characteristics at birth, at time of randomization and throughout the study period. The research nurse also collected blood samples for immunologic assessments. Immunologic assessments included the following.

Absolute T-, B-, and NK Cell Numbers

Lymphocyte immunophenotyping and white blood cell counts were performed in the Immunology Laboratory Section of the Detroit Medical Center University Laboratories. Red blood cells were lysed by using formic acid. Samples were fixed in 0.09% (v/v) formaldehyde before analysis on a Coulter XL flow cytometer equipped with a 488nm Argon laser (Beckman Coulter, Miami, FL). Expression of cell surface markers was determined by staining the cells by using the following combinations of reagents: CD45FITC (Fluorescein isothiocyanate)/CD14PE (Phycoerythrin), CD3FITC/CD19PE, CD3FITC/CD16$^+$56PE, CD3FITC/CD4PE, and CD3FITC/CD8PE (Beckman Coulter, Brea, CA) resulting in the enumerations of total T cells (CD3$^+$), B cells (CD19$^+$), NK cells (CD56$^+$, CD16$^+$, CD3$^-$), and helper (CD4$^+$) and cytotoxic T cells (CD8$^+$).[33]

NK Cell Cytotoxicity

The assessment of NK cell cytotoxicity was performed at the Flow Cytometry Laboratory at Children's Hospital of Michigan, Detroit, Michigan. The study used a modified fluorescent-based cytotoxicity assay developed previously at this laboratory.[34] Target cells were analyzed for their viability at the end of coincubation with the effector cells (NK cells) and cell-mediated cytotoxicity was assessed. Ficoll-separated human effector mononuclear cells were coincubated at various effector:target cell ratios by using the target cell line K562 (ATCC line CCL-243; American Type Culture Collection, Manassas, VA). Target cell killing was assessed by examining Annexin-Fluorescein isothiocyanate (FITC)/Propidium iodide (PI) staining in conjunction with monitoring Flow Count Fluorospheres (Beckman Coulter, Brea, CA) present in each tube to obtain absolute viable target cell counts. Target cell cytotoxicity versus effector:target cell ratios were plotted and allowed determination of lytic unit (LU_{20}) values. LU_{20} values were determined by obtaining the derived effector:target cell ratio that achieved 20% killing of the target cell population. Cytotoxicity values were obtained in triplicate for each effector:target cell ratio.

Statistical Analysis

The study was based on an intent-to-treat design and all statistical analyses were performed by using SPSS Version 17.0 (SPSS Inc, Chicago, IL).

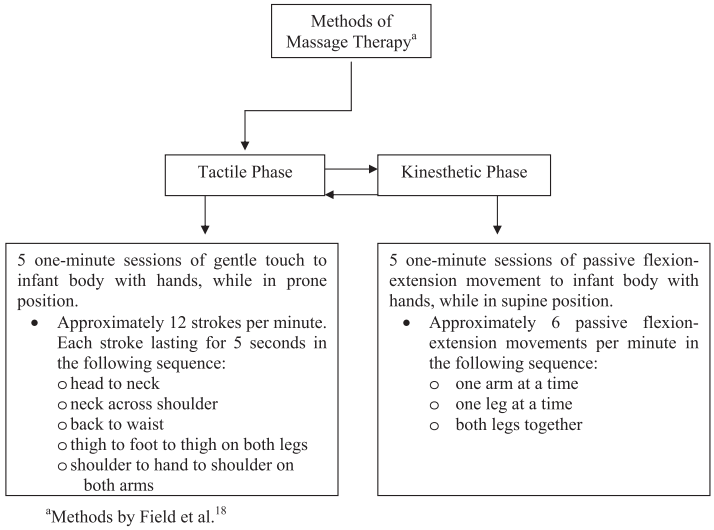

FIGURE 1
MT.

The primary aim of the study was to test the exploratory, null hypothesis that the increase in proportional NK cell numbers was identical in the massage and control groups. Because of the absence of any previous study data in infants, the study was powered based on results of 2 adult studies available at the time of the study.[24,25] We hypothesized that infants receiving MT would report a 35% increase in the proportion of NK cells in comparison with a 10% increase for infants in the control group. With the 25% proportional difference in NK cells hypothesized to occur between study groups, a sample size of 43 infants per group would provide 80% power. To obtain a balanced number randomized to each gestational age group and to account for a possible dropout rate of ~10% to 15%, a total of 120 infants with 60 infants in each study group was planned.

Demographic and baseline clinical data obtained were presented as mean ± SD for continuous variables, and ratios and proportions for categorical variables. The Mann-Whitney *U* tests were performed for unadjusted values and proportional comparisons between the 2 groups were examined by using Fisher exact χ^2 test. A series of 2-factor analysis of covariance models were used to examine mean differences in NK cell numbers and other continuously scaled variables, controlling for baseline differences in NK cell numbers, birth weight, and gestational age at randomization. These results are presented as adjusted findings. Assumptions of normality and homogeneity of variance were checked and verified. Because there was a difference in the mode of delivery (MOD) noted in the baseline characteristics between the 2 study groups, additional analysis on MOD was done. Factor variables were identified as study group (massage or control) and MOD (vaginal or cesarean delivery). Means, 95% confidence intervals (CIs), and *P* values were reported and presented in all study subjects as well as by ≥1 set of 5 consecutive days of study intervention received. Post hoc testing was performed by using the Sidak test to balance possible Type I error and power, given numerous pairwise comparisons conducted. All mean or proportional comparisons between study groups were considered statistically significant at a 2-tailed *P* value ≤.05.

RESULTS

Recruitment

Study subjects were recruited at Hutzel Women's Hospital NICU, between August 2005 and January 2009. A total of 743 infants were screened for study eligibility (Fig 2). Of these infants, 570 (77%) were

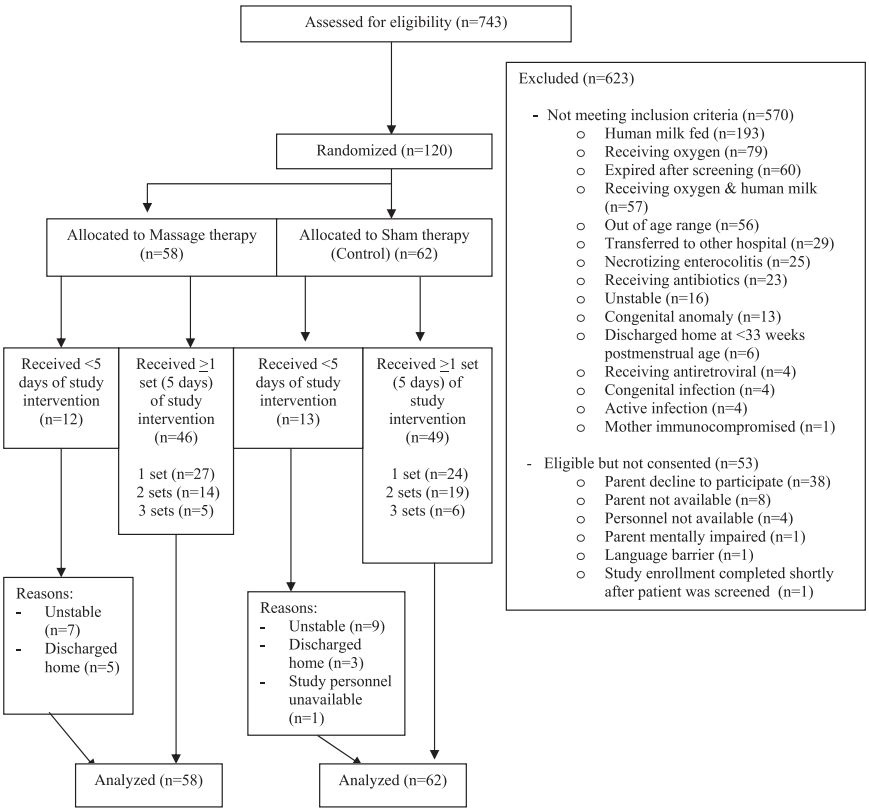

FIGURE 2
Study flow diagram (enrollment, randomization, and follow-up of study patients).

found to be ineligible. One hundred twenty of 173 eligible infants were enrolled; 58 infants were randomized to the massage group and 62 to the control group.

Maternal and neonatal characteristics at birth and randomization are presented in Table 1. Baseline characteristics were similar between the 2 groups with the exception of MOD and birth weight. More infants were delivered vaginally in the massage group (62%) than in the control group (39%) ($P = .03$). Birth weight was higher in the massage than in the control group. There was also a trend to a higher weight at randomization in the massage group compared with the control group.

All baseline laboratory characteristics were similar between the 2 study groups (Table 2).

The days (mean ± SD) of study intervention received (massage 11 ± 4 days; control 12 ± 5 days; $P = .21$) were not statistically different between the 2 groups.

TABLE 1 Maternal and Neonatal Characteristics

	Massage Group ($N = 58$)	Control Group ($N = 62$)	P
Maternal characteristics at birth			
Maternal age, mean ± SD y (range)	26 ± 7 (16–43)	27 ± 7 (17–42)	.27
Gravida, mean ± SD (range)	4 ± 3 (1–14)	4 ± 3 (1–13)	.35
Parity, mean ± SD (range)	3 ± 2 (1–12)	3 ± 2 (1–9)	.30
Maternal infection, No. (%)[a]	4 (7)	9 (15)	.24
Clinical chorioamnionitis, No. (%)[b]	6 (10)	5 (8)	.76
Any antenatal steroids, No. (%)	41 (71)	48 (77)	.41
Antimicrobials before delivery, No. (%)	34 (59)	37 (60)	1.00
Vaginal delivery, No. (%)	36 (62)	24 (39)	.03
Neonatal characteristics at birth			
Infant gestational age, mean ± SD wk (range)	30 ± 2 (26–33)	30 ± 2 (25–33)	.13
Birth weight (grams), mean ± SD (range)	1389 ± 248 (770–1790)	1286 ± 250 (685–1755)	.04
Birth head circumference, mean ± SD cm (range)	28 ± 2 (24–33)	27 ± 2 (23–31)	.71
Male, No. (%)	27 (46.6)	21 (33.9)	.19
Apgar score: 1 min, mean ± SD (range)	6 ± 2 (1–9)	6 ± 2 (1–9)	.79
Apgar score: 5 min, mean ± SD (range)	8 ± 2 (1–9)	8 ± 1 (3–9)	.12
Black race, No. (%)	54 (93)	52 (84)	.16
Delivery room resuscitation			
Use of oxygen, No. (%)	45 (78)	53 (86)	.35
Use of bag and mask, No. (%)	20 (35)	30 (48)	.14
Endotracheal intubation, No. (%)	13 (22)	14 (23)	1.00
Continuous positive airway pressure, No. (%)	14 (24)	16 (26)	1.00
Days on ventilator, mean ± SD (range)	1 ± 3 (0–18)	3 ± 6 (0–37)	.54
Days on oxygen, mean ± SD (range)	5 ± 9 (0–40)	5 ± 9 (0–48)	.76
Bloodstream infection, No. (%)	2 (3)	6 (10)	.27
Grade III or IV intraventricular hemorrhage, No. (%)	2 /17 (12)	3/14 (21)	.64
Neonatal characteristics on day of randomization			
PMA, mean ± SD wk (range)	32 ± 1 (29–33)	32 ± 1 (28–33)	.47
Postnatal age, mean ± SD d (range)	11 ± 12 (1–50)	13 ± 12 (2–57)	.20
Weight at randomization, mean ± SD g (range)	1431 ± 231 (1040–2070)	1349 ± 238 (850–1990)	.06

[a] Documented infection (eg, bacteremia, meningitis, urinary tract infection) during hospitalization resulting in delivery of infant.
[b] Presence of key clinical findings (not culture positive) associated with clinical chorioamnionitis: fever, uterine fundal tenderness, or purulent or foul amniotic fluid.

Primary Outcome

The primary outcome of proportional increase in absolute NK cells was not statistically different between the 2 study groups (Table 3).

Secondary Outcomes

Secondary outcome measures (mean values, adjusted and unadjusted) at the end of study are reported in Table 4. Additional analyses by MOD (vaginal or cesarean delivery) are reported in Table 5.

Absolute NK Cell Numbers and Cytotoxicity

Mean absolute NK cell numbers was not statistically different between the groups (Table 4). However, the adjusted mean NK cell cytotoxicity was higher in the massage group (13.83 LU; 95% CI, 11.42–16.23) than in the control group (10.51 LU; 95% CI, 8.17–12.84; $P = .05$). Among infants who received at least 1 set of 5 consecutive days of study intervention, adjusted mean NK cell cytotoxicity was significantly higher in the massage (13.79 LU; 95% CI, 11.24–16.36) than in the control group (10.06 LU; 95% CI, 7.56–12.56; $P = .04$).

TABLE 2 Baseline Infant Laboratory Characteristics[a]

	Massage Group	Control Group	P
Absolute NK ($CD56^+CD16^+CD3^-$) cell/mm^3, mean ± SD (range)	$n = 58$; 327 ± 244 (28–1092)	$n = 61$; 294 ± 194 (15–932)	.92
NK cell cytotoxicity, LU 20, mean ± SD (range)	$n = 58$; 12 ± 13 (0.71–89)	$n = 61$; 11 ± 10 (0.40–70)	1.0
Absolute T ($CD3^+$), cell/mm^3, mean ± SD (range)	$n = 57$; 4006 ± 1610 (1180–7812)	$n = 60$; 4186 ± 1487 (1969–8660)	.54
Absolute CD4, cell/mm^3, mean ± SD (range)	$n = 57$; 3074 ± 1365 (1062–6539)	$n = 60$; 3166 ± 1175 (1600–7370)	.45
Absolute CD8, cell/mm^3, mean ± SD (range)	$n = 57$; 940 ± 423 (166–2046)	$n = 60$; 1027 ± 513 (247–3459)	.39
Absolute B ($CD19^+$), cell/mm^3, mean ± SD (range)	$n = 57$; 909 ± 810 (52–3278)	$n = 60$; 829 ± 599 (47–2702)	.77
Absolute white blood, $\times 10^3$ cell/mm^3, mean ± SD (range)	$n = 58$; 11 ± 5 (4–21)	$n = 61$; 11 ± 4 (4–25)	.43
Absolute lymphocyte, cell/mm^3, mean ± SD (range)	$n = 58$; 5302 ± 2455 (1379–11 552)	$n = 61$; 5332 ± 2015 (1170–10 584)	.65

[a] Additional analysis by MOD (cesarean delivery and vaginal delivery) not significant between the 2 groups.

TABLE 3 Primary Outcome of Proportional Differences in NK Cells

	Massage Group, *n* (%)	Control Group, *n* (%)	*P*
A ≥35% increase in proportion of absolute NK cell numbers from baseline to end of study for all patients	*N* = 57[a]; 26 (46)	*N* = 60[a]; 26 (43)	.85
Subjects with at least 1 set of 5 consecutive days of study intervention	*N* = 46; 18 (39)	*N* = 49; 19 (39)	1.0

[a] Data on 1 patient were not available.

By MOD, among all infants who were delivered by cesarean delivery, those who received MT also had a higher mean NK cell cytotoxicity compared with the control group (Table 5).

Other Secondary Outcomes

Absolute white blood cells, B and T cells, and T-cell subsets were not statistically different between the 2 groups (Table 4).

Mean adjusted final weight and daily weight gain from randomization to end of study were higher among infants in the massage compared with control group (Table 4).

By MOD, among all infants who were born vaginally, those who received MT were heavier and had significantly more weight gain compared with infants in the control group (Table 5).

The number of culture-proven infections and length of hospital stay from randomization until the end of study were not statistically different between the 2 groups (Table 6).

AEs

MT was well tolerated among the study infants. Overall, AEs were minor (categories 2 and 3) and mostly related to the respiratory and gastrointestinal systems. AEs in these systems were significantly higher in the control group (87%) than in the massage group (57%; $P \leq .001$) (Table 7). All AEs eventually resolved and all were deemed not related to the study interventions.

DISCUSSION

Our present study showed no significant difference in the proportional increase of NK cell numbers between the 2 study groups (massage versus control). However, daily MT performed for ≥5 days in stable premature infants was associated with a significant increase in adjusted mean NK cytotoxicity compared with no MT. Increased NK cell cytotoxicity may be more clinically relevant than increase in NK cell numbers. Absolute NK cell number and NK cytotoxicity may not always show a positive correlation. El-Sameea et al[35] evaluated NK cell numbers and cytotoxicity in early-onset neonatal sepsis and showed that NK cytoxicity was significantly lower among infants with sepsis (3.4% ± 2.1%, range 0.9%–7%) compared with the nonseptic infants (18.3% ± 6.7%, range 10.7%–25.3%; $P < .01$) despite lack of differences in absolute NK cell numbers. They concluded that defective NK cell cytotoxicity plays a more important role in susceptibility to early-onset neonatal sepsis than the absolute number of NK cells. Gradual or lack of increase in NK cytotoxicity was consistently associated with poor outcome. NK cytotoxicity is regulated by activating and inhibitory signals that vary depending on the nature of interacting cells.[36] Therefore, increase or decrease in the absolute numbers of NK cells may not result in increased or decreased cytotoxicity. NK cells that display an increased activation status (by way of massage or other inducible signals) can illicit greater cytotoxicity responses than inactivated NK cells.[37] As such, low absolute NK cells may display relatively high cytotoxicity; similarly, high absolute NK cells may display relatively low cytotoxicity.

Abnormalities in the NK cell number/cytotoxicity and increased susceptibility to infections (varicella zoster virus,[38] herpes simplex virus,[6] HIV,[39] and Epstein-Barr virus[14]) have been reported. Although the role of NK cells has been mainly to provide antiviral and antitumor effects, NK cells may also play a role in antibacterial,[10,35] antifungal,[10] and protozoal defense.[40] Preterm and full-term newborn infants with bacterial and fungal sepsis, pneumonias, and recurrent infections have been shown to have an associated decrease in NK cell cytotoxicity compared with healthy newborns.[10]

Our study also showed that infants who were delivered via cesarean delivery and received MT had the highest NK cytotoxicity compared with those who received no MT and those born vaginally with and without MT. Infants who were born vaginally and received MT also weighed more and gained more weight than those who did not receive MT.

The mechanism of how massage improves the immune system is still not known and may be due to interplay of various cell types, hormones, and cytokines.[41–43] In our study, immune parameters at baseline regardless of MOD were no different between the 2 groups, yet infants born via cesarean delivery who received MT had increased NK cytotoxicity at the end of the study. Cesarean delivery has been associated with lower maternal and fetal stress response compared with normal vaginal delivery[44]; it is possible that MT alleviated stress among infants born via cesarean delivery.

Another important benefit of MT noted in our study was weight gain. Overall, unadjusted daily mean weight gain in MT group was 27 g/d compared with 25 g/d in the control group. A meta-analysis of MT in preterm infants reported better daily weight gain (mean 19–33 g/d, in the massage group vs 1–29 g/day in the control group) and

TABLE 4 Outcome at End of Study[a]

	Unadjusted			Multivariate Adjusted		
	Massage Group, mean ± SD (range)	Control Group, mean ± SD (range)	*P*	Massage Group, mean (95% CI)	Control Group, mean (95% CI)	*P*
Absolute NK, cells/mm^3[b]						
All study subjects	*n* = 57; 355 ± 178 (49–843)	*n* = 60; 379 ± 223 (101–1174)	.70	*n* = 57; 345 (295–397)	*n* = 60; 400 (351–450)	.14
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 358 ± 180 (140–849)	*n* = 49; 358 ± 205 (101–1032)	.95	*n* = 46; 350 (293–407)	*n* = 49; 378 (322–434)	.49
NK cytotoxicity, LU 20[b]						
All study subjects	*n* = 57; 13.68 ± 10.90 (2.17–52.63)	*n* = 60; 10.11 ± 6.97 (0.67–40)	.10	*n* = 57; 13.83 (11.42–16.23)	*n* = 60; 10.51 (8.17–12.84)	.05[c]
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 13.88 ± 10.85 (2.17–52.63)	*n* = 49; 9.63 ± 6.09 (0.672–26.67)	.07	*n* = 46; 13.79 (11.24–16.34)	*n* = 49; 10.06 (7.56–12.56)	.04[d]
Absolute T cells (CD3$^+$), cells/mm^3[b]						
All study subjects	*n* = 57; 4186 ± 1108 (1180–7812)	*n* = 60; 4500 ± 1321 (1969–8660)	.21	*n* = 56; 4210 (3935–4485)	*n* = 59; 4512 (4247–4777)	.12
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 4227 ± 1156 (1180–7812)	*n* = 49; 4371 ± 1239 (1969–8660)	.58	*n* = 45[e]; 4306 (4005–4607)	*n* = 49; 4396 (4106–4685)	.67
Absolute CD4, cells/mm^3[b]						
All study subjects	*n* = 56; 3108 ± 937 (1062–6539)	*n* = 59; 3254 ± 920 (1600–7370)	.13	*n* = 56; 3097 (2891–3303)	*n* = 59; 3285 (3086–3483)	.20
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 45; 3142 ± 977 (1062–6539)	*n* = 49; 3257 ± 945 (1600–7370)	.25	*n* = 45; 3172 (2938–3405)	*n* = 49; 3263 (3038 –3488)	.58
Absolute CD8, cells/mm^3[b]						
All study subjects	*n* = 56; 1081 ± 419 (165–2046)	*n* = 59; 1136 ± 601 (247–3459)	.80	*n* = 56; 1098 (990–1206)	*n* = 59; 1137 (1032–1241)	.62
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 45; 1094 ± 420 (165–2046)	*n* = 49; 1045 ± 438 (247–2346)	.69	*n* = 45; 1109 (1001–1216)	*n* = 49; 1052 (949–1156)	.46
Absolute B cells (CD19$^+$), cells/mm^3[b]						
All study subjects	*n* = 56; 1305 ± 831 (52–3278)	*n* = 59; 1389 ± 735 (47–2701)	.22	*n* = 56; 1262 (1090–1434)	*n* = 59; 1426 (1261–1591)	.18
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 45; 1433 ± 867 (52–3278)	*n* = 49; 1349 ± 711 (47–2538)	.84	*n* = 45; 1364 (1168–1559)	*n* = 49; 1412 (1224–1600)	.72
Final weight, g[b]						
All study subjects	*n* = 58; 2178 ± 548 (1655–5172)	*n* = 62; 1999 ± 284 (1265–2780)	.11	*n* = 58; 2151 (2042–2260)	*n* = 62; 1999 (1893–2104)	.05
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 2161 ± 401 (1665–3384)	*n* = 49; 2006 ± 310 (1265–2780)	.11	*n* = 46; 2142 (2046–2239)	*n* = 49; 2008 (1913–2102)	.05
Weight gain per day from randomization to end of study, g/d[f]						
All study subjects	*n* = 58; 27 ± 7 (8–40)	*n* = 62; 25 ± 6 (11–43)	.02[g]	*n* = 58; 27.6 (26–29)	*n* = 62; 24.5 (23–26)	.01[h]
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 28 ± 6 (13–40)	*n* = 49; 26 ± 6 (11–43)	.01[i]	*n* = 46; 28.22 (26–30)	*n* = 49; 25 (23–27)	.01[j]
Weight gain per day from randomization to end of study, g/kg per d[k]						
All study subjects	*n* = 58; 15.37 ± 3.85 (4.83–22.86)	*n* = 62; 14.99 ± 2.98 (6.30–21.54)	.5	*n* = 58; 15.77 (14.92–16.61)	*n* = 62; 14.55 (13.74–15.36)	.04[l]
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 46; 16 ± 3.76 (6.48–22.86)	*n* = 49; 15.31 ± 3.03 (6.20–21.54)	.25	*n* = 46; 16.16 (15.23–17.10)	*n* = 49; 14.93 (14.01–15.85)	.07

[a] End of study or hospital discharge or transfer.
[b] Adjusted for baseline values, birth weight, and gestational age at birth.
[c] Mean difference = 3.32, SE = 1.71 (95% CI, −0.06 to 6.70).
[d] Mean difference = 3.73, SE = 1.8 (95% CI, 0.14–7.32).
[e] One patient had 1 missing adjusted variable.
[f] Adjusted for weight at randomization and gestational age at birth.
[g] Mean difference = 2.35, SE = 1.13 (95% CI, 0.12–4.6).
[h] Mean difference = 3.09, SE = 1.14 (95% CI, 0.84–5.34).
[i] Mean difference = 2.69, SE = 1.26 (95% CI, 0.2–5.19).
[j] Mean difference = 3.18, SE = 1.26 (95% CI, 0.68–5.67).
[k] Adjusted for birth weight and gestational age at birth.
[l] Mean difference = 1.22, SE = 0.59 (95% CI, 0.04–2.39).

TABLE 5 Outcome by MOD at End of Study[a]

	Vaginal			Cesarean Delivery		
	Massage Group, mean (95% CI)	Control Group, mean (95% CI)	*P*	Massage Group, mean (95% CI)	Control Group, mean (95% CI)	*P*
Absolute NK, cells/mm^3						
All study subjects	*n* = 35; 331 (266–396)	*n* = 24; 446 (374–519)	.02	*n* = 22; 361 (281–441)	*n* = 36; 353 (289–417)	.88
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 340 (268–412)	*n* = 18; 419 (330–507)	.17	*n* = 18; 360 (271–449)	*n* = 31; 338 (269–406)	.70
NK cytotoxicity (LU 20)						
All study subjects	*n* = 35; 12.76 (9.70–15.81)	*n* = 24; 11.99 (8.36–15.66)	.75	*n* = 22; 14.90 (11.15–18.65)	*n* = 36; 9.02 (6.00–12.04)	.02[b]
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 13.14 (9.90–16.38)	*n* = 18; 10.85 (6.87–14.82)	.37	*n* = 18; 14.44 (10.47–18.41)	*n* = 31; 9.27 (6.20–12.35)	.04[c]
Absolute T cells (CD3$^+$), cells/mm^3						
All study subjects	*n* = 35; 4164 (3820–4508)	*n* = 24; 4660 (4249–5070)	.07	*n* = 21; 4256 (3822–4690)	*n* = 35; 4364 (4019–4709)	.70
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 4188 (3815–4561)	*n* = 18; 4494 (4030–4957)	.31	*n* = 17; 4424 (3948–4900)	*n* = 31; 4298 (3943–4653)	.67
Absolute CD4, cells/mm^3						
All study subjects	*n* = 35; 3048 (2791–3306)	*n* = 24; 3335 (3027–3644)	.16	*n* = 21; 3146 (2821–3471)	*n* = 35; 3234 (2975–3493)	.67
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 3069 (2779–3358)	*n* = 18; 3301 (2940–3662)	.32	*n* = 17; 3274 (2904–3645)	*n* = 31; 3225 (2948–3501)	.83
Absolute CD8, cells/mm^3						
All study subjects	*n* = 35; 1071 (936–1206)	*n* = 24; 1191 (1029–1353)	.26	*n* = 21; 1125 (955–1295)	*n* = 35; 1082 (947–1217)	.69
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 1052 (918–1185)	*n* = 18; 1085 (918–1251)	.76	*n* = 17; 1165 (995–1336)	*n* = 31; 1020 (893–1147)	.18
Absolute B cells (CD19$^+$), cells/mm^3						
All study subjects	*n* = 35; 1282 (1069–1495)	*n* = 24; 1436 (1179–1692)	.36	*n* = 21; 1243 (969–1516)	*n* = 35; 1417 (1204–1631)	.32
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 1360 (1119–1600)	*n* = 18; 1407 (1106–1708)	.81	*n* = 17; 1368 (1054–1681)	*n* = 31; 1418 (1190–1646)	.80
Final weight, g						
All study subjects	*n* = 36; 2242 (2104–2379)	*n* = 24; 1975 (1810–2141)	.02[d]	*n* = 22; 2060 (1889–2231)	*n* = 38; 2022 (1888–2150)	.73
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 2215 (2092–2337)	*n* = 18; 2005 (1853–2157)	.03[e]	*n* = 18; 2070 (1918–2222)	*n* = 31; 2010 (1894–2126)	.54
Weight gain per day from randomization to end of study, g/d						
All study subjects	*n* = 36; 27 (25–29)	*n* = 24; 24 (21–26)	.04[f]	*n* = 22; 28 (25–30)	*n* = 38; 26 (24–28)	.28
Subjects with at least 1 set of 5 consecutive days of study intervention	*n* = 28; 28 (26–30)	*n* = 18; 23 (20–26)	.01[g]	*n* = 18; 28 (25–31)	*n* = 31; 27 (25–29)	.62
Weight gain per day from randomization to end of study, g/kg per d						
All study subjects	*n* = 36; 15.18 (14.13–16.24)	*n* = 24; 13.67 (12.39–14.95)	.07	*n* = 22; 16.35 (15.03–17.67)	*n* = 38; 15.34 (14.40–16.46)	.28
	n = 28; 15.83 (14.64–17.01)	*n* = 18; 13.79 (12.32–15.26)	.03[h]	*n* = 18; 16.50 (15.03–17.97)	*n* = 31; 16.06 (14.94–17.19)	.64

[a] End of study or hospital discharge or transfer.
[b] Mean difference = 5.86, SE = 2.39 (95% CI, 1.09–10.66).
[c] Mean difference =5.17, SE = 2.52 (95% CI, 0.17–10.17).
[d] Mean difference = 267, SE = 108 (95% CI, 60–486).
[e] Mean difference = 210, SE = 95 (95% CI, 18–393).
[f] Mean difference = 3, SE = 1.5 (95% CI, 1.1–7.5).
[g] Mean difference = 5, SE = 1.8 (95% CI, 1.5–8.6).
[h] Mean difference = 2.04, SE = 0.95 (95% CI, 0.16–3.92).

TABLE 6 Other Secondary Outcomes at End of Study[a]

Outcome	Massage Group, $N = 58$	Control Group, $N = 62$	P
Length of hospital stay (d) from randomization to end of study, mean ± SD (range)	26 ± 16 (6–97)	26 ± 11 (8–47)	.17
No. of infections[b] from randomization to end of study, No. (%)	8[c] (13.7)	6[d] (10.3)	.56

[a] End of study or hospital discharge or transfer.
[b] All bacteremias due to coagulase-negative staphylococci were associated with clinical deterioration (apnea, bradycardia, oxygen desaturations) and were assessed to be true infections.
[c] Bloodstream infection resulting from *Staphylococcus capitis* (1), *Staphylococcus epidermidis* (2), *Enterococcus faecalis* (1), *E faecium* (1), *Candida parapsilosis* (1); rotavirus gastroenteritis (2).
[d] Bloodstream infection resulting from *S epidermidis* (2), *E faecalis* (3), *Klebsiella pneumoniae* (1).

TABLE 7 AEs

	Massage Group, $N = 58$	Control Group, $N = 62$	P
Categories 1–2[a] AEs, No. (%)	33 (57)	54 (87)	≤.001
Respiratory[b]	18 (31)	36 (58)	.003
Gastrointestinal[c]	15 (26)	18 (29)	.84
Categories 3–4[d] AEs, No. (%)	7 (12)	8 (13)	1.00
Respiratory[b]	4 /12 (33)	7/13 (54)	.53
Gastrointestinal[c]	2/12 (17)	0/13 (0)	.23
Respiratory and gastrointestinal[e]	1/12 (8)	1/13 (8)	1.00

[a] Category 1: Mild/transient event with self-recovery; infant requires no intervention. Category 2: Moderate event requiring minimal intervention.
[b] Apnea, bradycardia, oxygen desaturations, and tachypnea and respiratory distress.
[c] Abdominal distention, feeding intolerance, billous gastric aspirates, fecal occult blood positive, and necrotizing enterocolitis.
[d] Category 3: Severe event but not life-threatening requiring intervention. Category 4: Worse than severe, life-threatening event, requiring intervention.
[e] Apnea, oxygen desaturations, and necrotizing enterocolitis.

mean difference of 5 g (95% CI 3.5–6.7 g),[20] which was higher than our results of 2 to 3 g. Other studies have also shown shorter hospital stay,[20] which was not observed in our study population.

Possible explanation of weight gain in infants who received MT includes increase in parasympathetic (vagal) activity during MT, which stimulates the release of food absorption hormones such as insulin and gastrin.[45]

Strengths of our study include a randomized, double-blind, placebo-controlled study; screened premature infants; and exclusion of human milk–fed infants. Analyses were adjusted and controlled for baseline differences and MOD. Study limitations include study population from a single center. Patients were randomized based on PMA; it is possible that there could be differences in results if randomization was based on postnatal age rather than on PMA. In addition, because this was the first such study conducted in premature infants, at the time the study was designed, there was no justification for randomization by MOD so additional randomization based on MOD was not done. Because of the nature of the study (preliminary, exploratory), clinical significance of increased NK cytotoxicity was not assessed. Our future studies will examine the additional effects of MT on immune system and its mechanisms, potential influence of other factors (MOD, human milk intake), clinical benefit of increased NK cytotoxicity, and neurodevelopmental outcome in preterm infants.

CONCLUSIONS

We found a positive association between MT and NK cytotoxicity as well as MT and weight gain in premature infants. MT appears to be safe and may improve the overall outcome of premature infants in the NICU. This randomized, placebo-controlled study suggests the beneficial effect of MT on premature infants and underscores the need for additional larger studies.

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