Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Analgesic effects of breast- and formula feeding during routine childhood immunizations up to 1 year of age

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BACKGROUND: Data on analgesic effects of breast/formula milk sucking while receiving routine childhood immunizations are available only in early infancy, have rarely been compared in the same study, and are not accompanied by information on mothers' satisfaction/acceptance. Here we aimed to compare the analgesic effect of both methods vs. held-only controls up to 1 year of age, and verify mothers' satisfaction.

METHODS: Two to 12 months children subjected to vaccine were allocated into three groups: breastfed, formula-fed, and held-only controls. A video recording was performed to analyze pain parameters: crying latency/duration and specific scales [FLACC (Face, Legs, Activity, Cry, and Consolability), NIPS (Neonatal Infant Pain Scale)]. After the procedure, mothers filled in a satisfaction questionnaire.

RESULTS: One-hundred and sixty-two children were recruited: 54 breastfed, 35 formula fed, and 73 controls. Breastfed showed the longest crying latency, and together with formula fed, had the shortest duration and lowest pain scores. Most mothers appreciated not only the respective feeding-mediated pain mitigation method used, but also the simply-holding procedure. In all cases, they felt reassured, with an unexpected frequent underestimation of their child's pain during the shot.

CONCLUSIONS: The analgesic effect of breastfeeding during vaccination extends also to children >6 months old, and is obtained by formula too. Embracing the child may help to reassure mothers.

Pediatric Research (2021) 89:1179–1184; https://doi.org/10.1038/s41390-020-0939-x

IMPACT:

- We confirmed the analgesic effect of breastfeeding during the vaccination procedures in early infancy.
- We show for the first time that this effect is extended also to children up to 1 year of age, and it may be obtained by formula feeding as well.
- Most mothers appreciated pain mitigation not only through feeding, but also the simply-holding procedure.
- In all cases, mothers felt reassured, with an unexpected frequent underestimation of their child' pain during the shot.
- The promotion of these easily feasible and well-accepted strategies should be further encouraged within health professionals during vaccination procedures.

INTRODUCTION

Vaccinations are among the greatest public health achievements of the twentieth century, but they also represent some of the unavoidably occurring painful procedures performed in early-age settings. As anxiety and distress involve not only children themselves but also their families,[1] pain mitigation during immunization has been proposed to improve the acceptance of vaccine and possibly reduce the vaccination hesitancy as well.[2] Among the treatments proposed to lessen invasive procedure-related pain, studies show that breastfeeding[3] or drinking formula[4] vs. controls reduce in general procedural pain. In studies conducted in infants up to 6 months, breastfeeding has been found to be effective also to reduce acute procedural pain during routine childhood immunizations.[5] The mechanisms involved are multifactorial and seem to include sucking, skin-to-skin contact, warmth, rocking, sound and smell of the mother, and probably endogenous opiates present in the breast milk as well.[6] Interestingly, more recently, it was shown that not only breastfeeding but also formula feeding is effective in mitigating pain due to vaccination shot among young 4–10-week-old infants in comparison with controls, but the two methods of feeding were not compared.[7]

Our study aims to compare the effectiveness of both breastfeeding and formula feeding vs. controls to reduce pain during immunization injection in children aged up to 12 months, and assess mothers' satisfaction.

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Received: 1 December 2019 Revised: 22 February 2020 Accepted: 23 March 2020
Published online: 11 May 2020

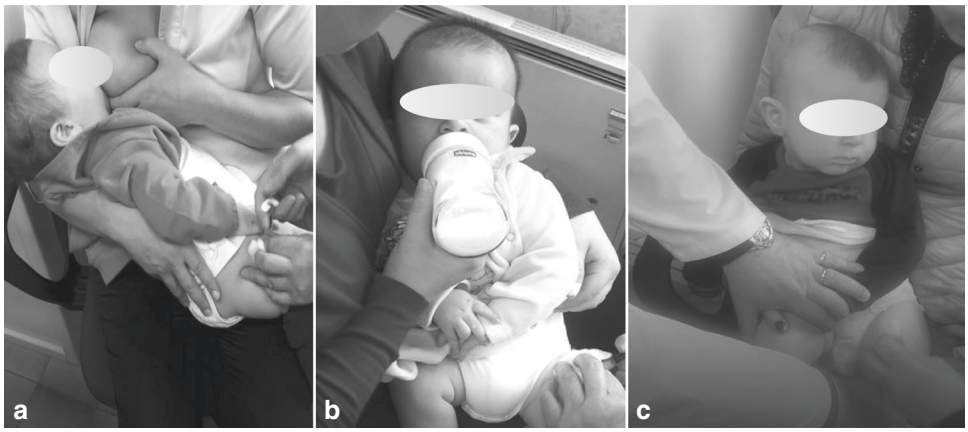

Fig. 1 Mother–child dyad positions and feeding modalities during vaccination. In the control group, children were held in the arms of their mother and received the vaccination without being fed (**c**). In the treated groups, children (**a**, **b**) were also fed during vaccination according to their usual modality of feeding (breast or formula, respectively).

MATERIALS AND METHODS

Aim and study design

This is a non-randomized, non-blinded study that compares three pain parameters (latency of crying, duration of crying, and pain scores) in three groups of children (breastfed, formula-fed, and unfed controls during the procedure) subjected to routine vaccinations of the first year of age [hexavalent (H) and antipneumococcal (P) vaccines] administered according to the current vaccination schedule in three doses at the age of the third, fifth, and tenth–twelfth month.

Study setting and participants

The study was conducted at the Vaccination centers of the Local Health Authority "ASL Salerno" [Salerno and Cava de' Tirreni (Italy)] from March to December 2017.

We recruited children from 2 to 12 months of age subjected to vaccination practice. The procedure was performed in a quiet room. Health professionals provided vaccination conforming to the standardized vaccination protocol: injection in the vastus lateralis muscle without aspiration using 25-G needles. Professionals received training before the start of the study.

Before vaccination, for each child, parents filled a questionnaire to record the following data, name, age, gender, auxological data at birth, type of childbirth, time elapsed between childbirth and the first breastfeed, possible admission in the neonatal intensive care unit, presence of a suspected developmental delay or impairment, chronic illnesses, breast vs. formula feeding, current weight, any vaccinations already made, title of study of parents, citizenship, ethnic origin, and work of parents.

Exclusion criteria included suspected developmental delays or impairments, chronic illnesses, admissions to a neonatal intensive care unit, and be born more than 2 weeks preterm (i.e., 36 weeks of gestation or more). All the eligible infants were enrolled in this cohort study as detailed in the Cohort diagram (Supplementary Fig. 1S).

As the modalities of feeding and position cannot be blinded to participants and personnel, consecutive recruited children were allocated to two treated groups based on their usual/prevalent mode of feeding: treated group 1 [B = breastfeeding during vaccination, as per World Health Organization (WHO) recommendations[2]]; treated group 2 (F = drinking formula milk during vaccination), and control group independent of their usual mode of feeding [C = no feeding during intervention, but held as the other groups as per Center for Disease Control (CDC) recommendations[8]] (Supplementary Fig. 1S).

The recruited children, with exposed thigh, were all held in the arms of their mothers sitting for 2 min before and 3 min after the vaccination shot. In the treated groups, the children were fed according to their usual modality of feeding (breast or formula) (Fig. 1a, b). The formula feeding and the bottles were brought by infants' parents from home. After 2 min of feeding, the vaccine was administered. The feeding continued during the vaccination and 3 min later. In the control group, children were held in the arms of their mother and received the vaccination without being fed (Fig. 1c). The health professional who practiced the vaccination was positioned in front of the mother and warned at the moment both of the entrance and the exit of the needle ("inside", "outside"). The observer was positioned sideways to the group and made a video recording lasting for 3 min to audio–videotape the infant during the procedure according to the majority of previous similar studies.[4–7] Children who practiced both vaccinations in the same session (at an interval of 3 min) received the second injection of vaccine, monitored as above.

All infants received the same commercial brand of vaccine following identical step procedures.

Clinical outcomes, procedures, and measures

The primary outcomes were crying latency and crying duration. Secondary outcomes were pain scores. By the vision of the recorded audios and videos, we analyzed the above parameters according to the following methods:

- *Crying latency*: time in seconds elapsed between the introduction of the needle at the moment when the child starts to cry, by using a digital stopwatch.
- *Crying duration*: time in seconds elapsed from the start of crying to the moment when the child stops crying for a period of silence of at least 5 s, by using a digital stopwatch.
- *Pain scores* assessed by using two standardized pain measurement scales: NIPS (Neonatal Infant Pain Scale)[9] and FLACC (Face, Legs, Activity, Cry, and Consolability).[10] The relative scores were obtained at baseline time 0 (FLACC 0 and NIPS 0 = time of needle insertion), and after 1 min (FLACC 1 and NIPS 1) and 3 min (FLACC 3 and NIPS 3).

The NIPS scale analyzes six parameters with a total score between 0 and 7. The parameters are facial expressions, breathing patterns, action of arms and legs, and state of awareness with 0 or 1 point per issue, and crying patterns with 0, 1, or 2 points per pattern.[9] The FLACC scale analyzes five parameters: Face, Legs, Activity, Cry, and Consolability. To each of them, we assigned a score from 0 to 2, with a total score between 0 and 10.[10]

Each parent who accepted to voluntarily participate in the study was required to sign informed consent, and was allowed to

withdraw from the study at any time. The mothers were also given a satisfaction questionnaire, specific for each group, to ascertain their perception of the efficacy of the analgesic method and propensity to recommend it to others. The figure corresponding in their opinion to the pain felt by their child during the vaccination procedure was compared with the value detected through the NIPS and FLACC scales. The comparison of maternal impression vs. NIPS and FLACC-scored data was obtained by dividing the specific scales into three gross sections, which identified a degree of mild, moderate, and high pain, respectively.

The study protocol received Ethical Committee approval at the University of Salerno, Italy, given its anonymousness, voluntariness of participation, absence of risk or burden as per WHO[2] and CDC[8] recommendations, sponsors, conflicts of interest, and incentives for the responding subjects; however, no approval was considered necessary. The study was conducted according to the Helsinki Declaration of Human Rights.[11]

Statistical analysis

The statistical tests used to evaluate the data are:

- Analysis of the variance for differences in the averages of crying duration, crying latency, and pain scores obtained from the FLACC and NIPS scales of the three groups (B, F, and C).
- Student's *t* test for mean value differences:
  - among clinical, demographic, and social information of the three (B, F, and C) groups
  - between two groups (B and F, B and C, and F and C) as to crying duration, crying latency, and pain scores obtained from the FLACC and NIPS scales
  - between the first and second injection (hexavalent and anti-pneumococcal) practiced in the same vaccination session
  - among pain records obtained in the same (I, II, and III) vaccination session in each of the three groups of infants

In all statistical analyses, $p < 0.05$ was considered as significant.

RESULTS

From March to December 2017, we recruited 162 Caucasian children, allocated to a specific group during the vaccination procedure: breastfeeding (B), formula feeding (F), and no feeding (control, C).

The number of studied procedures was as follows:

- 56 children practiced the I dose, for a total of 108 measurements (vaccines: H 56, P 52).
- 61 children practiced the II dose, for a total of 111 measurements (vaccines: H 58, P 53).
- 39 children practiced the III dose, for a total of 72 measurements (vaccines: H 35, P 37).

The children receiving both vaccinations during the same session were 120.

As summarized in Table 1, except for a slightly higher birth weight in B vs. F, there were no significant differences between clinical, demographic, and social data of the three groups, both at entry and during the phases of the study.

Figure 2a, b shows the cumulative results of latency (Fig. 2a) and duration (Fig. 2b) of crying. The parameters observed during the entire study period indicated that mean latency of crying in group B was longer than that in the other two groups (F and C). Mean crying duration of groups F and B was significantly shorter than group C; these differences were observed at all three doses.

Figure 3 shows the results referring to FLACC and NIPS scales. In both cases, FLACC/NIPS of group B at baseline (at the moment of injection) behaved significantly better than group C. FLACC/NIPS of the two feeding groups (B and F) at 1 min behaved better than group C. The same differences between the groups were confirmed even more at the time 3 min, with scores in the B and F groups of ~90% lower than the controls.

These differences between the three groups were present in children subjected to hexavalent and in those subjected to antipneumococcal vaccine as well.

The analgesic effect of both breastfeeding and formula-feeding practice on vaccine-related pain measured by the duration of crying and FLACC scores was maintained at least up to the time of the third dose (i.e., 10–12 months of age) (Supplementary Fig. S2).

Consecutive vaccination

Differences in pain parameters of children who received hexavalent (first) and antipneumococcal (after) vaccines in the same vaccine session showed a general trend of greater sensitivity to pain in the second vaccination as revealed by a longer crying duration (Supplementary Fig. S3).

Mothers' impression

Mothers' impression and children's NIPS and FLACC pain-scale results agreed in 21/41 cases (51.2%) and 22/41 cases (53.6%), respectively. Among the discordant cases, maternal impression scored lower than their children in 12 cases (equally in NIPS and FLACC) and higher in eight and seven cases, respectively. The analgesic procedures were overall appreciated by mothers and reduced their agitation in the majority of cases (Table 2).

DISCUSSION

Our study confirms in an Italian series the analgesic effect of breastfeeding during routine childhood immunization. As some analgesia over controls was however observed also with formula feeding, we hypothesize that such effect may in part depend on a pacifier effect of the mere milk sucking itself in addition to the proposed intrinsic properties of human milk/closest contact with the mother via breastfeeding.

There is no universally accepted gold standard to measure infant pain. Although most infants show both behavioral and physiological responses to pain, these two groups of measures are either uncorrelated or weakly correlated across many situations and studies.[12] Cry has been suggested as a good behavioral indicator of infant pain, including that in response to procedural pain.[13] In our study, we assessed pain responses by using cry duration and latency to cry, that is, two standard parameters that had been assessed also in other akin studies aimed to determine the effectiveness of a topical anesthetic to decrease pain during vaccination procedures.[14]

We found that *crying latency* was significantly longer in breastfed infants than in control and artificially nursed children groups, respectively. These results agree with two other studies,[15,16] which showed a similar difference between breastfed children and the control group. Interestingly, however, no literature data for comparison of our results regarding formula-fed children are available. Regarding *crying duration*, we could also confirm the results of published works[15–20] in favor of breastfeeding for reduction of crying duration with an average of ~50% (between 20% and 75%). Interestingly, another novel information made available from our study is that this favorable effect may be obtained also beyond the first 6 months of life, up to 12 months, that is, up to the third dose of vaccine. Of note, similar to breastfeeding, also formula milk showed an analgesic effect at all ages, by reducing cry duration by more than 50% compared with

the control group. Pain estimation by FLACC and NIPS scales confirmed that breastfeeding and formula feeding are effective in reducing pain during vaccination.[7,17,19,20]

Table 1. Clinical, anthropometric, and demographic information of the 162 infants enrolled into the study.

	Breastfeeding ($n = 54$)	Controls ($n = 73$)	Formula feeding ($n = 35$)	*P* value	
Measurements	99	127	65		
Males/females	56/43	62/65	33/32	B/C	0.2
				F/C	0.7
				B/F	0.5
Mean age in months within the whole study period (range)	4.6 (2–12)	5.3 (2–12)	5.4 (2–12)	B/C	0.1
				F/C	0.8
				B/F	0.2
Preterm/term	1/53	5/68	2/33	B/C	0.18
				F/C	0.82
				B/F	0.9
Delivery spontaneous/ cesarean	34/54	41/73	19/35	B/C	0.09
				F/C	0.92
				B/F	0.6
Birth weight *z*-score (mean ± 2 SDS)	0.05 ± 0.88	−0.27 ± 1.19	−0.47 ± 1.04	B/C	0.15
				F/C	0.5
				B/F	0.04
Mean maternal age in years (range)	33.7 (21–43)	33 (19–45)	34.6 (25–46)	B/C	0.3
				F/C	0.1
				B/F	0.3
Mean paternal age in years (range)	37.4 (22–48)	36.2 (24–57)	37.6 (27–53)	B/C	0.2
				F/C	0.2
				B/F	0.8
Vaccine sessions (*N*)					
I	26/72	33/72	13/72	I/II	
(B–C–F)	0.9				
II	16/45	20/45	9/45	II/III	
(B–C–F)	0.5				
III	12/45	20/45	13/45	I/III	
(B–C–F)	0.3				

B breastfeeding, *C* control, *F* formula feeding.

Overall, these findings are in agreement with previous literature on breastfeeding and vaccination procedure,[15–20] and also confirm previous trends observed in a meta-analysis by Shah et al.,[21] who found that younger infants who were breastfed before, during, and after the vaccination procedure had less pain and shorter cry duration than those who were not breastfed.

The mechanisms underlying the beneficial effect of breastfeeding against vaccination pain are still undefined. Some authors have suggested that these mechanisms include sucking, skin-to-skin contact,[22] warmth, rocking, sound and smell of the mother, and endogenous opiates present in the breast milk.[6] However, our data and those by Boss-Veneman et al.[7] obtained with formula feeding as well may raise some hesitations in front of these views. Whether the intrinsic sweet taste of milk itself is involved remains a possibility. In fact, Shah et al.,[21] by evaluating several studies conducted with sweet-tasting solutions, found that the administration of sucrose with or without nonnutritive sucking (i.e., use of a pacifier) was associated with less pain compared with no intervention or sterile water with or without nonnutritive sucking. Moreover, the total cry duration was lower in infants who received sucrose than in those who received sterile water. Recent results from Iran obtained during hepatitis B vaccination by measuring the analgesic effects of breast milk vs. formula, bottle feeding mother's milk, and controls appear of much interest, but are not comparable as they were obtained only in 1-day-old neonates and with pain evaluation measures diverse from our and all the other studies.[23]

In our study, we analyzed the differences between pain due to two vaccinations performed in the same session (hexavalent vaccine first and antipneumococcal vaccine later, in the same child). Data suggest a greater sensitivity to pain in children vaccinated with the second vaccination, both as regards the parameters of crying (latency and duration) and scores derived from the scales. Possible explanations for these different behaviors are either a different aptitude to induce pain (more intense with anti-pneumococcal)[7] or a greater reaction to pain simply because given an additional vaccine after the hexavalent vaccine. Interestingly, maternal perception of the pain experienced by the child agreed in 50% of cases, with underestimation unexpectedly more frequent than overestimation. Finally, it appears that most control mothers felt that holding their children was a reassuring measure per se. This aspect warrants further evaluation in future studies as health care professionals may prefer to have the child on the exam table when administering vaccines. According to the CDC recommendations, a comforting hold safely prevents children from moving their arms and legs

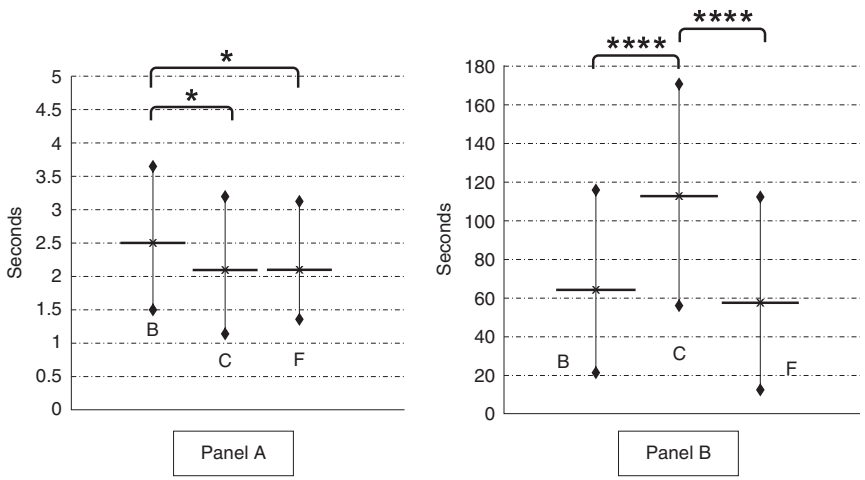

Fig. 2 Latency and duration of crying. Cumulative results (hexavalent and antipneumococcal vaccination) of latency (**a**) and duration (**b**) of crying during all the phases of the study. *$p < 0.05$; ****$p < 0.0001$. B breastfed, C controls, F formula fed.

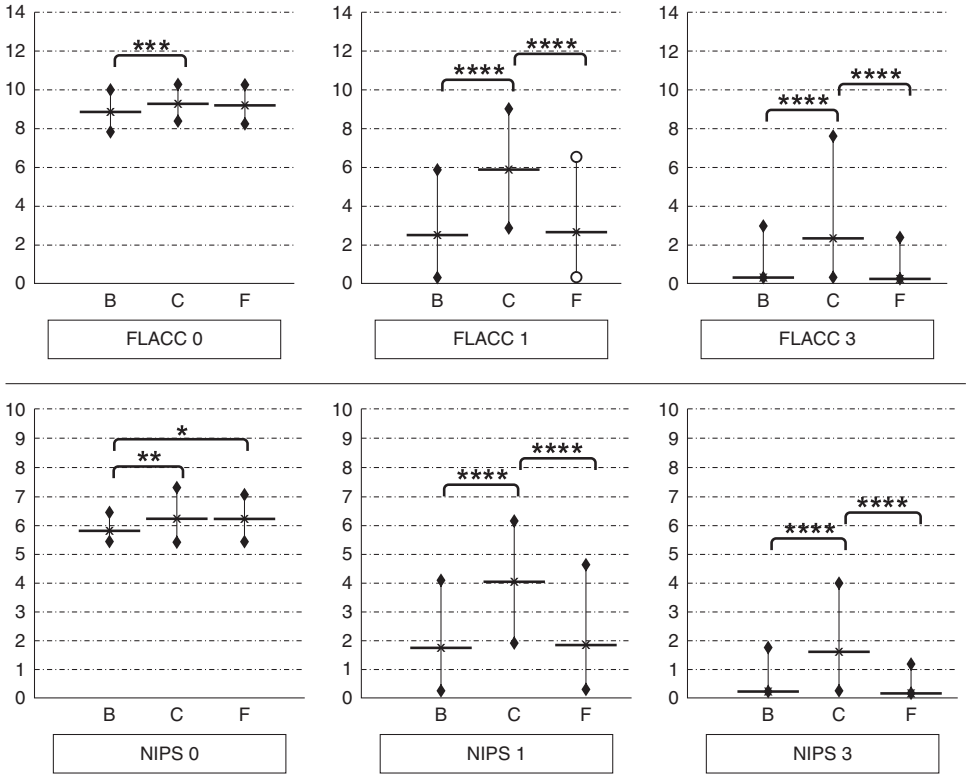

Fig. 3 Cumulative results of scores obtained with FLACC and NIPS scales. Cumulative results (hexavalent and antipneumococcal vaccinations) of the scores obtained with FLACC and NIPS scales at "baseline = time 0 min", at "the 1 min time", and at the "3 min time". *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. B breastfed, C controls, F formula fed, NIPS Neonatal Infant Pain scale, FLACC Faces Legs Activity Cry Consolability Revised scale.

Table 2. Percentage of satisfaction among the mothers of the three groups of children undergoing vaccination.

	Group A	Group F	Group C
Reduction of maternal agitation	70.6	75.1	83.2
Analgesic procedure considered not useful	6.2	8.3	8.3
Mothers willing to repeat the procedure during subsequent vaccinations	88.2	91.7	83.3
Mothers in favor of experimenting also other analgesic systems	88.2	91.7	41.7

Group A, held + breastfeeding; group B, held + bottle feeding; group C, held-only control.

during injections, avoids frightening children by embracing them rather than overpowering them, encourages parents to nurture and comfort their child, and allows the health care professional steady control of the limb and the injection site.[8]

Limitations of the study

Our study has some limitations. First, as the breastfeeding intervention cannot be blinded to participants and personnel, and also our study is at high risk of bias.[6] Regarding pain tools, we used composite pain indicators with both physiological and behavioral components, without measuring other individual physiological indicators, such as heart rate and oxygen saturation. However, the NIPS,[9] which measures facial expression, is considered among the more sensitive indicators in pain expression in infants.[24] As coding discrete facial actions requires extensive training for observers, and may be difficult to score at the bedside, in our study, it was assessed ex post at videotape by experienced persons. The other scale we utilized (FLACC scale)[10] is not specific for the neonatal age, as it is a measurement used to assess pain for children between the ages of 2 months and 7 years, or individuals who are unable to communicate their pain. It was included because of the children >44 days of age, which is the chronological limit for the NIPS scale. In general, the results obtained by FLACC and NIPS scale, however, were in good agreement. Last, but not least, as we do not have comparisons vs. a group with nonnutritive sucking and/or simply lying on the exam table, the effects we attribute to breast and formula milk in relieving pain might be explained at least in part also by the so-called Kangaroo Care (skin-to-skin) effect,[25] rather than the sweet taste of milk.[26]

CONCLUSIONS

Our study results confirm and broaden the knowledge on the analgesic effect of breastfeeding in the course of painful procedures. Namely, we showed that breastfeeding reduces the vaccination pain also over the age of 6 months, that is, up to the 12-month third dose, and that children experience quite comparable analgesic effects also during formula milk feeding at all ages studied. Despite the feeding analgesic effect, the antipneumococcal vaccination injected after the hexavalent vaccine is more painful in all cases.

Last, but not least, we found that maternal perceptions of the pain experienced by their child were matched by half the cases, and underestimation is unexpectedly more frequent than overestimation.

Overall, through a head-to-head comparison, the study for the first time suggests that both maternal and formula feeding may have an intrinsic pacifier effect against the painful stimulus during

vaccination. Because immunizations are among the first unavoidable painful experiences in healthy children and concern the whole population, it is necessary to expand pain reduction strategy studies in this area. Our results also suggest that the simple effect of holding the child has moms' reassuring value endorse the CDC recommendations, and may have a positive impact on the acceptance of vaccination.

ACKNOWLEDGEMENTS

We thank the nurses and pediatricians working at the Vaccination centers and the families of the children participating in this study.

AUTHOR CONTRIBUTIONS

C.V. and A.O.: contributed equally for substantial conception and design, acquisition, analysis and interpretation of data, drafting the article, and final approval of the version to be submitted. M.A.S., C.M., and D.V.: literature revision, substantial contributions to interpretation of data, revising the article critically for important intellectual content, and final approval of the version to be submitted. M.A., A.N., and A.L.C.: logistics, acquisition and interpretation of data, revising the article critically for important intellectual content, and final approval of the version to be submitted. P.V.: senior author with substantial contribution to conception, design and monitoring of the study, drafting of the article, and final approval of the version to be submitted.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-0939-x) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Patient consent: Each parent who accepted to voluntarily participate in the study was required to sign informed consent, and was allowed to withdraw from the study at any time.

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REFERENCES

1. Schechter, N. L. et al. Pain reduction during pediatric immunizations: evidence-based review and recommendations. *Pediatrics* **119**, 1184–1198 (2007).
2. WHO. Reducing pain at the time of vaccination: WHO position paper. *Wkly. Epidemiol. Rec.* **90**, 505–516 (2015).
3. Cramton, R. E. & Gruchala, N. E. Managing procedural pain in pediatric patients. *Curr. Opin. Pediatr.* **24**, 530–538 (2012).
4. Weissman, A., Aranovitch, M., Blazer, S. & Zimmer, E. Z. Heel-lancing in newborns: behavioural and spectral analysis assessment in pain control methods. *Pediatrics* **124**, e921–e926 (2009).
5. Taddio, A. et al. Reducing pain during vaccine injections: clinical practice guideline. *CMAJ* **187**, 975–982 (2015).
6. Harrison, D. et al. Breastfeeding for procedural pain in infants beyond the neonatal period. *Cochrane Database Syst. Rev.* **10**, CD011248 (2016).
7. Bos-Veneman, N. G. P., Otter, M. & Reijneveld, S. A. Using feeding to reduce pain during vaccination of formula-fed infants: a randomised controlled trial. *Arch. Dis. Child.* **103**, 1132–1137 (2018).
8. Center for Disease Control. Benefits of holding your child. https://www.cdc.gov/vaccines/parents/visit/holds-factsheet.html. (last date accessed: 11 may 2020).
9. Lawrence, J. et al. The development of a tool to assess neonatal pain. *Neonatal Netw.* **12**, 59–66 (1993).
10. Merkel, S. I., Voepel-Lewis, T., Shayevitz, J. R. & Malviya, S. The FLACC: a behavioral scale for scoring postoperative pain in young children. *Pediatr. Nurs.* **23**, 293–297 (1997).
11. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* **310**, 2191–2194 (2013).
12. Cong, X., McGrath, J. M., Cusson, R. M. & Zhang, D. Pain assessment and measurement inneonates: an updated review. *Adv. Neonatal Care* **13**, 379–395 (2013).
13. Beacham, P. S. Behavioral and physiological indicators of procedural and postoperative pain in high-risk infants. *J. Obstet. Gynecol. Neonatal Nurs.* **33**, 246–255 (2004).
14. Taddio, A., Nulman, I., Goldbach, M., Ipp, M. & Koren, G. Use of lidocaine–prilocaine cream for vaccination pain in infants. *J. Pediatr.* **124**, 643–648 (1994).
15. Goswami, G. et al. Comparison of analgesic effect of direct breastfeeding, oral 25% dextrose solution and placebo during 1st DPT vaccination in healthy term infants: a randomized, placebo controlled trial. *Indian Pediatr.* **50**, 649–645 (2013).
16. Gupta, N. K. et al. Randomized controlled trial of topical EMLA and breastfeeding for reducing pain during DPT vaccination. *Eur. J. Pediatr.* **172**, 1527–1533 (2013).
17. Dilli, D., Küçük, I. & Dallar, Y. Interventions to reduce pain during vaccination in infancy. *J. Pediatr.* **154**, 385–390 (2009).
18. Efe, E. & Ozer, Z. C. The use of breast-feeding for pain relief during neonatal immunization injections. *Appl Nurs. Res.* **20**, 10–16 (2007).
19. Erkul, M. & Efe, E. Efficacy of breastfeeding on babies' pain during vaccinations. *Breastfeed. Med.* **12**, 110–115 (2017).
20. Abdel Razek, A. & Az El-Dein, N. Effect of breast-feeding on pain relief during infant immunization injections. *Int. J. Nurs. Pract.* **15**, 99–104 (2009).
21. Shah, V., Taddio, A. & Rieder, M. J., HELPinKIDS Team. Effectiveness and tolerability of pharmacologic and combined interventions for reducing injection pain during routine childhood immunizations: systematic review and meta-analyses. *Clin. Ther.* **31**(Suppl. 2), S104–S151 (2009).
22. Johnston, C. et al. Skin-to-skin care for procedural pain in neonates. *Cochrane Database Syst. Rev.* **2**, CD008435 (2017).
23. Hatami Bavarsad, Z. et al. Effects of breast milk on pain severity during muscular injection of hepatitis B vaccine in neonates in a teaching hospital in Iran. *Arch. Pediatr.* **25**, 365–370 (2018).
24. Guinsburg, R. et al. Differences in pain expression between male and female newborn infants. *Pain* **85**, 127–133 (2000).
25. Pandita, A. et al. Is kangaroo mother care effective in alleviating vaccination associated pain in early infantile period? A RCT. *Early Hum. Dev.* **127**, 69–73 (2018).
26. Lima, A. G. et al. Glucose solution is more effective in relieving pain in neonates than non-nutritive sucking: a randomized clinical trial. *Eur. J. Pain* **21**, 159–165 (2017).