Clinical Study

Maternal and Cord Serum Cytokine Changes with Continuous and Intermittent Labor Epidural Analgesia: A Randomized Study

Venkat R. Mantha,1 Manuel C. Vallejo,1 Vimala Ramesh,2 Bobby L. Jones,3 and Sivam Ramanathan1,4

1Department of Anesthesiology, Magee-Womens Hospital of UPMC, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
2Department of Anesthesiology, Passavant Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA 15237, USA
3Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, USA
4Department of Anesthesiology, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Correspondence should be addressed to Venkat R. Mantha, manthav@anes.upmc.edu

Received 31 October 2011; Accepted 21 December 2011

Academic Editor: Emmanouil Karteris

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Background. Maternal fever during labor epidural analgesia (LEA) may cause increased maternal and cord serum inflammatory cytokines. We report the effects of intermittent and continuous LEA on these cytokines.

Methods. Ninety-two women were randomly assigned to continuous (CLEA) or intermittent (ILEA) groups, 46 in each. Maternal temperature was checked and blood drawn at epidural insertion (baseline) and four-hourly until 4 h postpartum (4 PP). Cord blood was drawn after placental delivery. Interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), granulocyte macrophage-colony stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) were measured and analyzed according to group randomization, and then combined and reanalyzed as febrile (temperature ≥38°C) or afebrile groups.

Results. Significant intragroup changes from baseline were noted in some groups. Data are pg/mL, median (Q1/Q3). IL-6 rose at all time points in all groups. CLEA: baseline: 18.5 (12.5/31.1), 4 h: 80.0 (46.3/110.8), 8 h: 171.9 (145.3/234.3), and 4 PP: 81 (55.7/137.4). ILEA: baseline: 15.7 (10.2/27.1), 4 h: 68.2 (33.3/95.0), 8 h: 125.0 (86.3/195.0), and 4 PP: 70.2 (54.8/103.6). Febrile group: baseline: 21.6 (13.8/40.9), 4 h: 83.9 (47.5/120.8), 8 h: 186.7 (149.6/349.9), and 4 PP: 105.8 (65.7/158.8). Afebrile group: baseline: 10.9 (2.1/17.4), 4 h: 38.2 (15.0/68.2), 8 h: 93.8 (57.1/135.7), and 4 PP: 52.9 (25.1/78). IL-8 rose at all time points in CLEA: baseline: 2.68 (0.0/4.3), 4 h: 3.7 (0.0/6.5), 8 h: 6.0 (3.3/9.6), and 4 PP: 5.6 (0.8/8.0). Febrile group: baseline: 2.68 (0.0/4.3), 4 h: 3.7 (0.0/6.5), 8 h: 5.2 (2.5/8.0), and 4 PP: 2.0 (0.0/4.0). GM-CSF increased at 8 h and decreased at 4 PP in ILEA baseline: 2.73 (0.0/7.2), 4 h: 2.73 (0.0/7.9), 8 h: 3.9 (2.7/11.5), and 4 PP: 2.0 (0.0/7.2). It increased at 4 h and 8 h and decreased at 4 PP in febrile group: baseline: 2.6 (0.0/4.2), 4 h: 3.2 (2.1/7.0), 8 h: 4.0 (3.2/12.3), and 4 PP: 2.4 (1.7/12.6). There were no intergroup cytokine changes in maternal or cord serum in CLEA versus ILEA or febrile versus afebrile groups.

Conclusions. Some cytokines, especially IL-6, rise physiologically during labor epidural analgesia.

1. Introduction

Maternal intrapartum fever may occur with labor epidural analgesia (LEA) [1]. The mechanism(s) of the fever are not fully understood, but may result from disturbances in thermoregulatory mechanisms [1, 2]. However, there are reports that fever may represent a maternal inflammatory response. Goetzl et al. [3] and Smulian et al. [4] reported a significantly increased maternal serum interleukin-6 (IL-6) levels in febrile versus afebrile subjects.

We previously reported that intermittent, versus continuous, LEA had a lower incidence of maternal and neonatal fever in the first few hours of epidural analgesia initiation [5]. In the same study, we collected, but did not fully analyze, maternal and cord serum cytokine levels; we now report those results. To our knowledge, there are no
studies comparing maternal serum cytokine changes with continuous versus intermittent epidural analgesia.

2. Methods

After Institutional Review Board (IRB) approval, 92 women gave informed consent and were enrolled in this prospective, randomized unblinded study. As required by our IRB, a parent or legal guardian provided consent for enrolled minors. A computer-generated program (Quattro Pro; Corel Corp., Ottawa, Canada) randomized an equal number of women (46) to receive continuous or intermittent lumbar epidural analgesia (CLEA and ILEA groups, resp.). Inclusion criteria were a healthy, nulliparous parturient in spontaneous labor with a term, singleton fetus in vertex presentation. Exclusion criteria were a baseline temperature of ≥37.5°C, suspected chorioamnionitis, history of drug abuse, and pregnancy-related complications such as preeclampsia, intrauterine growth retardation, and gestational diabetes mellitus.

One of the investigators initiated the epidural analgesia at maternal request, using strict sterile precautions (i.e., sterile gloves, shoe covers, operating room scrub, cap, and facemask). In all patients, LEA was initiated with a 3 mL test dose of 1.5% lidocaine with 1:200,000 epinephrine, followed by fentanyl 100 μg and 8 mL of an analgesic solution: either 0.125% bupivacaine with fentanyl 0.0002% or 0.1% ropivacaine with fentanyl 0.0002%. In the CLEA group, a continuous infusion of one of the analgesic solutions was administered at 10–15 mL/hr with a goal of maintaining a T-10 or higher sensory blockade. In the ILEA group, 10–15 mL of the solution was given by manual injections as requested by the patient. The labor and delivery rooms were maintained at a temperature comfortable for the patient, generally between 20 and 22°C.

2.1. Data Collection. Baseline measurements noted at initiation of epidural analgesia included maternal age, height, weight, gestational age, cervical dilation, and tympanic temperature. Peripheral venous blood (5 to 10 mL) was also drawn at this time for cytokine analysis. The temperature measurement and venipuncture were repeated at four-hourly intervals, with the final sampling occurring four hours postpartum (4 PP). Following fetal and placental delivery, umbilical vein cord blood was collected for cytokine analysis. A pediatric nurse measured the neonatal rectal temperature within 30 minutes of delivery.

The blood samples were collected in serum separation tubes and refrigerated immediately. Within 24 hours, the serum was separated by centrifugation at 2500 g for 20 minutes (Sorvall Biofuge Primo, Thermo Fisher Scientific, Asheville, NC, USA). The serum was then placed in duplicate aliquots and immediately frozen and stored at −70°C until analysis. The samples were batched in groups of 50–90 samples for laboratory analysis. They were analyzed within 3 months of collection, by a Multiplex Bead Immunoassay technique (BioSource International Inc., Camarillo, CA, USA). The serum cytokine levels assayed were interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), granulo-

| Variable                        | CLEA n = 46 | ILEA n = 46 | P  |
|---------------------------------|-------------|-------------|----|
| Age (years)                     | 27.0 ± 7.2  | 26.5 ± 6.5  | 0.74|
| Height (cm)                     | 160.4 ± 6.1 | 160.8 ± 7.1 | 0.77|
| Weight (kg)                     | 80.1 ± 17.8 | 81.3 ± 16.7 | 0.73|
| Gestational age (weeks)         | 39.8 ± 1.1  | 39.4 ± 1.3  | 0.12|
| Baseline cervical dilatation    | 3.4 ± 1.5   | 3.5 ± 1.5   | 0.83|
| Baseline temperature (°C)       | 36.7 ± 0.6  | 36.6 ± 0.5  | 0.49|

Data are mean ± standard deviation.

| Variable                        | Febrile °C | Afebrile °C | n   | P  |
|---------------------------------|------------|-------------|-----|---|
| Baseline                        | 36.9 ± 0.44| 36.5 ± 0.55 | 63  | 0.003|
| 4-hour                          | 37.7 ± 0.52| 37.0 ± 0.58 | 57  | <0.001|
| 8-hour                          | 38.3 ± 0.40| 37.3 ± 0.48 | 24  | <0.001|
| 4-hour PP                       | 37.6 ± 0.52| 37.0 ± 0.56 | 63  | <0.001|
| Neonatal                        | 37.0 ± 0.44| 36.7 ± 0.50 | 63  | 0.012|

Data are mean ± standard deviation. 4-hour PP = 4-hour postpartum.

Labor epidural characteristics like the number of vaginal examinations, use of internal monitors or oxytocin augmentation, clinical suspicion of chorioamnionitis, group B streptococcal colonization of the genital tract, times of membrane rupture and full cervical dilation, time and type of delivery, epidural insertion to full dilatation and delivery, and neonatal sepsis evaluation rate were collected. At our institution, clinical chorioamnionitis is diagnosed on the basis of maternal fever (temperature ≥38°C) plus one or more of the following: maternal or fetal tachycardia, uterine tenderness, foul smelling amniotic fluid, or maternal leukocytosis [5]. Clinical suspicion of chorioamnionitis is treated aggressively with intravenous antibiotics and rectal acetaminophen every 4–6 hours as needed. Placentas were sent for histological examination at the discretion of the obstetricians. Guidelines for neonatal sepsis evaluation (NSE) at our institution include prolonged rupture of membranes (>18 hours) and chorioamnionitis; neonatal conditions include hypo- or hyperthermia at 6 hours after birth, apneic spells, respiratory distress or pneumonia, central cyanosis, listlessness, convulsions, poor feeding or vomiting, and cardiovascular instability. NSE at our institution is done at the discretion of the neonatologists.

2.2. Data Analysis. A primary analysis between the two groups was performed. The two groups were then combined,
3. Results

No technical problems or complications were encountered during the insertion or maintenance of epidural analgesia. There were no differences between the CLEA and ILEA groups in demographic and baseline data (age, height, weight, gestational age, cervical dilatation, and temperature) (Table 1).

3.1. Maternal Temperature. Details of the maternal temperature changes with CLEA and ILEA have been previously reported [5]. Briefly, the incidence of fever in ILEA at 4 h was lower than in CLEA (2/42, 4.6% versus 10/44, 22.7%, $P = 0.036$). At 8 h and thereafter, no differences were noted. The overall incidence of fever was 14/46 (30.4%) in CLEA and 12/43 (27.9%) in ILEA ($P = ns$). Regrouping based on the development of fever resulted in 26 women falling into the febrile group and 66 into the afebrile group. Highly significant differences were seen between these two groups at all time points (Table 2).

3.2. Neonatal Temperature. As shown in Table 2, neonates born to febrile mothers had significantly higher temperatures. Two neonates, both in the afebrile group, underwent NSE, which was negative.

3.3. Maternal Serum Cytokines. Intragroup and intergroup analysis of changes from baseline is shown by infusion type in Figure 1 and by fever status in Figure 2. Intragroup analysis showed the following changes: IL-6 showed a highly significant rise ($P \leq 0.0001$) at all time points in all groups—CLEA, ILEA, febrile and afebrile. IL-8 also showed a highly significant rise ($P \leq 0.0075$) at all time points in CLEA and afebrile groups; in the febrile group, it showed a fall at 4 PP, while in ILEA there was no change. GM-CSF increased at 8 h and decreased at 4 PP in ILEA; in the afebrile group, it increased at 4 h and 8 h and decreased at 4 PP, while in CLEA and febrile groups there was no change. There was no change in IL-1β or TNF-α at any time point in any group. Intergroup analysis of CLEA versus ILEA and febrile versus afebrile groups showed no significant difference in any cytokine at any time point.

3.4. Cord Serum Cytokines. There were no significant differences between CLEA and ILEA. Although neonates born to febrile versus afebrile mothers had higher temperatures (Table 2), there were no differences between these groups in any cytokine (Figure 3).

3.5. Labor and Epidural Characteristics. The labor and epidural characteristics of CLEA and ILEA were shown in our previous publication [5], and those in febrile and afebrile subjects are shown in Table 3. Briefly, febrile subjects had significantly lower baseline cervical dilatation, more vaginal examinations and use of internal monitors, longer time intervals between epidural insertion to full dilatation,
Figure 1: Box plots of intra- and intergroup maternal serum cytokine changes by infusion method during labor epidural analgesia. IL-6: interleukin-6, IL-1β: interleukin-1β, IL-8: interleukin-8, GM-CSF: granulocyte macrophage-colony stimulating factor, TNF-α: tumor necrosis factor-α. IL: ILEA group. CL: CLEA group. BL: baseline. 4 h and 8 h: 4 and 8 hours from baseline, respectively. 4 PP: 4 hours postpartum. *: Significant intragroup changes from baseline. There were no intergroup differences.

4. Discussion
To our knowledge, the effect of intermittent versus continuous LEA on maternal and cord serum cytokine expression has not been previously reported.

Our data indicate that some maternal serum cytokines increase with LEA. IL-6 exhibited the most consistent intragroup change, increasing during labor and remaining elevated for at least 4 h postpartum, regardless of CLEA or ILEA and febrile or afebrile group status. There were no CLEA/ILEA or febrile/afebrile intergroup differences in maternal or cord sera. Thus, it is interesting to note that at the 4 h time point, a significantly higher incidence of fever in epidural insertion to delivery, rupture of membranes to full dilatation, and rupture of membranes to delivery.
CLEA versus ILEA group yielded no differences in IL-6 levels. Thus, maternal and cord IL-6 levels appear independent of the method of epidural analgesia, the development of fever, and neonatal temperature.

Our findings differ from some studies that have evaluated maternal serum IL-6 levels and intrapartum fever. Goetzl et al. [3], whose patients received continuous epidural infusions, found that IL-6 levels increased at 4 and 8 h following epidural analgesia initiation. Smulian et al. [4], in a case-control, mixed parity study, found that maternal IL-6 was a strong marker for intrapartum fever; however, only one blood sample per subject was collected within an hour of delivery, and not all subjects received epidural analgesia. De Jongh et al. [7] studied a group of mixed parity patients to evaluate the influence of analgesia (with or without epidural) or anesthesia (general versus epidural)
Figure 3: Box plots of cord serum cytokines in the four groups studied. IL-6: interleukin-6, IL-1β: interleukin-1β, IL-8: interleukin-8, GM-CSF: granulocyte macrophage-colony stimulating factor, TNF-α: tumor necrosis factor-α. IL: ILEA group. CL: CLEA group. NF: afebrile group. F: febrile group. There were no significant CLEA versus ILEA or F versus NF differences in any cytokine.

The rise of maternal serum cytokines, particularly IL-6, may be a normal response to labor [8–13]. Arntzen et al. [8] found that the strength and frequency of uterine contractions were significantly related to serum IL-6 levels. Austgulen et al. [9] observed that IL-6 increased with gestational age and labor activity. Buonocore et al. [10] found that during normal vaginal delivery, IL-6 levels were higher during labor and immediately after delivery than at the beginning of labor. According to Hebisch et al., IL-6 (also IL-8) levels rose with labor onset and remained high through postpartum day 3. Opsjøn et al. observed that maternal serum IL-6 may play a role in the onset of normal labor. Unfortunately, these studies do not mention maternal fever status or use of epidural analgesia.

Serum IL-6 levels also physiologically increase in response to high-intensity exercise in humans [14–19], which may include labor associated frequent, high-intensity
uterine contractions and psychological stresses [7]. IL-6 is a pleiotropic cytokine that has multiple effects on the host including the ability to induce fever. As a potent inducer of acute phase responses, it may have a protective effect on the host [20]. Childbirth can induce an acute phase response [21].

We therefore do not think that a rise in IL-6 represents a pathological response. Our study certainly found that to be true.

We found statistically significant intragroup changes in other cytokines. Clinical significance of these findings is unclear. Our findings have similarities and differences from other studies in the obstetric population. In the Goetzl et al. [3] study, IL-8 levels insignificantly rose at 4 and 8 hours compared to baseline. IL-1 (or 1) or no change found during labor [3, 9, 13], while it was found to be elevated in two other studies [10, 12]. The majority of the studies did not find increases in TNF-α or GM-CSF during labor.

In contrast to our study, Smulian et al. [4] and Goetzl et al. [3] found significantly higher cord blood IL-6 levels in febrile parturients. Goetzl et al. [3] attributed “fetal inflammation” as being caused by maternal inflammation, with greater maternal than cord IL-6 levels. It is unclear whether maternal or cord blood cytokines are interrelated. Although De Jongh et al. [22] found that IL-6 levels in the cord appeared dependent on maternal levels, a number of reports suggest that cytokines, including IL-6, do not cross the term placenta [23, 24].

Limitations in our study include the use of different local anesthetics (ropivacaine and bupivacaine), which may have confounded our results. However, as discussed in our paper that studied the same patients [5], we found little or no difference between these two solutions in VAS pain scores, local anesthetic usage, and duration of labor or mode of delivery.

Another limitation is that we did not have a control group that did not receive epidural analgesia. Such a study would be difficult at our institution, where approximately 90% of laboring women receive epidural analgesia.

In conclusion, we found that maternal serum inflammatory cytokines rise during LEA, irrespective of the method of epidural dosing (intermittent or continuous) or the development of fever. These findings suggest that a rise in maternal serum cytokines during LEA may be physiological, and that there may be no pathological basis for epidural fever based on proinflammatory cytokine analysis.

Presentations

Comparison of Maternal Serum Interleukin-6 Levels in Febrile and Afebrile Parturients during Labor Epidural Analgesia. Oral presentation at the 40th Annual Meeting, Society for Obstetric Anesthesia and Perinatology, Chicago, IL, USA May 2008. Venkat R. R. Mantha, FFARCSI, Vimala Ramesh, FRCA, Manuel C Vallejo, DMD, MD, Sivam Ramanathan, MD.

Acknowledgment

This research was conducted with a seed grant from the Department of Anesthesiology, University of Pittsburgh Medical Center, 1305 A Scaife Hall, Pittsburgh, PA, USA.

References

[1] L. Fusi, P. J. Steer, M. J. A. Maresh, and R. W. Beard, “Maternal pyrexia associated with the use of epidural analgesia in labour,” Lancet, vol. 1, no. 8649, pp. 1250–1252, 1989.
[2] R. C. Goodlin and J. W. Chapin, “Determinants of maternal temperature during labor,” American Journal of Obstetrics and Gynecology, vol. 143, no. 1, pp. 97–103, 1982.
[3] L. Goetzl, T. Evans, J. Rivers, M. S. Suresh, and E. Lieberman, “Elevated maternal and fetal serum interleukin-6 levels are associated with epidural fever,” American Journal of Obstetrics and Gynecology, vol. 187, no. 4, pp. 834–838, 2002.
[4] J. C. Smulian, V. Bhandari, A. M. Vintzileos et al., “Intrapartum fever at term: serum and histologic markers of inflammation,” American Journal of Obstetrics and Gynecology, vol. 188, no. 1, pp. 269–274, 2003.
[5] V. R. Mantha, M. C. Vallejo, V. Ramesh, A. L. Phelps, and S. Ramanathan, “The incidence of maternal fever during labor is less with intermittent than with continuous epidural analgesia: a randomized controlled trial,” International Journal of Obstetric Anesthesia, vol. 17, no. 2, pp. 123–129, 2008.
[6] Y. Benjamini and Y. Hochberg, “Controlling the false discovery rate: a practical and powerful approach to multiple testing,” Journal of the Royal Statistical Society, vol. 57, pp. 289–300, 1995.
[7] R. F. De Jongh, E. P. Bosmans, M. J. Puylaert et al., “The influence of anaesthetic techniques and type of delivery on peripartum serum interleukin-6 concentrations,” Acta Anaesthesiologica Scandinavica, vol. 41, no. 7, pp. 853–860, 1997.
[8] K. J. Arntzen, E. Lien, and R. Austgulen, “Maternal serum levels of interleukin-6 and clinical characteristics of normal delivery at term,” Acta Obstetricia et Gynecologica Scandinavica, vol. 76, no. 1, pp. 55–60, 1997.
[9] R. Austgulen, E. Lien, N.-B. Liabakk, G. Jacobsen, and K. J. Arntzen, “Increased levels of cytokines and cytokine activity modifiers in normal pregnancy,” European Journal of Obstetrics Gynecology and Reproductive Biology, vol. 57, no. 3, pp. 149–155, 1994.
[10] G. Buonocore, M. De Filippo, D. Gioia et al., “Maternal and neonatal plasma cytokine levels in relation to mode of delivery,” Biology of the Neonate, vol. 68, no. 2, pp. 104–110, 1995.
[11] P. C. Greig, A. P. Murtha, C. J. Jimmerson, W. N. P. Herbert, B. Roitman-Johnson, and J. Allen, “Maternal serum interleukin-6 during pregnancy and during term and preterm labor,” Obstetrics and Gynecology, vol. 90, no. 3, pp. 465–469, 1997.
[12] G. Hebisch, P. M. Neumaier-Wagner, R. Huch, and U. Von Mandach, “Maternal serum interleukin-1β, -6 and -8 levels and potential determinants in pregnancy and peripartum,” Journal of Perinatal Medicine, vol. 32, no. 6, pp. 475–480, 2004.
[13] S. L. Opsjøn, N. C. Wathen, S. Tjungulstad et al., “Tumor necrosis factor, interleukin-1, and interleukin-6 in normal human pregnancy,” American Journal of Obstetrics and Gynecology, vol. 169, no. 2, part 1, pp. 397–404, 1993.
[14] K. Ostrowski, T. Rohde, S. Asp, P. Schierling, and B. K. Pedersen, “Pro- and anti-inflammatory cytokine balance in
strenuous exercise in humans,” *Journal of Physiology*, vol. 515, part 1, pp. 287–291, 1999.

[15] D. A. Papanicolaou, J. S. Petrides, C. Tsikos et al., “Exercise stimulates interleukin-6 secretion: inhibition by glucocorticoids and correlation with catecholamines,” *American Journal of Physiology*, vol. 271, no. 3, part 1, pp. E601–E605, 1996.

[16] A. M. Petersen and B. K. Pedersen, “The anti-inflammatory effect of exercise,” *Journal of Applied Physiology*, vol. 98, no. 4, pp. 1154–1162, 2005.

[17] L. L. Smith, A. Anwar, M. Fragen, C. Rananto, R. Johnson, and D. Holbert, “Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise,” *European Journal of Applied Physiology*, vol. 82, no. 1–2, pp. 61–67, 2000.

[18] H. Ullum, P. M. Haahr, M. Diamant, J. Palmo, J. Halkjaer-Kristensen, and B. K. Pedersen, “Bicycle exercise enhances plasma IL-6 but does not change IL-1α, IL-1β, IL-6, or TNF-α pre-mRNA in BMNC,” *Journal of Applied Physiology*, vol. 77, no. 1, pp. 93–97, 1994.

[19] G. G. Wardyn, S. I. Rennard, S. K. Brusnahan et al., “Effects of exercise on hematological parameters, circulating side population cells, and cytokines,” *Experimental Hematology*, vol. 36, no. 2, pp. 216–223, 2008.

[20] C. A. Dinarello, “Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed,” *Journal of Endotoxin Research*, vol. 10, no. 4, pp. 201–222, 2004.

[21] P. A. Mackowiak, “Concepts of fever,” *Archives of Internal Medicine*, vol. 158, no. 17, pp. 1870–1881, 1998.

[22] R. F. De Jongh, M. Puylaert, E. Bosmans, W. Ombelet, M. Maes, and R. Heylen, “The fetomaternal dependency of cord blood interleukin-6,” *American Journal of Perinatology*, vol. 16, no. 3, pp. 121–128, 1999.

[23] R. Aaltonen, T. Heikkinen, K. Hakala, K. Laine, and A. Alanen, “Transfer of proinflammatory cytokines across term placenta,” *Obstetrics and Gynecology*, vol. 106, no. 4, pp. 802–807, 2005.

[24] S. Suzuki and T. Araki, “Fetomaternal interface of cytokine patterns in intrauterine infection,” *Journal of Maternal Fetal and Neonatal Medicine*, vol. 13, no. 3, pp. 211–212, 2003.