

3-1-2011

Revised dose schema of sublingual buprenorphine in the treatment of the neonatal opioid abstinence syndrome.

Walter K Kraft
Thomas Jefferson University

Kevin Dysart
Jefferson Medical College

Jay S Greenspan
Jefferson Medical College

Eric Gibson
Jefferson Medical College

Follow this and additional works at: <https://jdc.jefferson.edu/petfp>

Karol Kaltenbach
 Jefferson Medical College Department of Pharmacology Commons

[Let us know how access to this document benefits you](#)

See next page for additional authors

Recommended Citation

Kraft, Walter K; Dysart, Kevin; Greenspan, Jay S; Gibson, Eric; Kaltenbach, Karol; and Ehrlich, Michelle E, "Revised dose schema of sublingual buprenorphine in the treatment of the neonatal opioid abstinence syndrome." (2011). *Department of Pharmacology and Experimental Therapeutics Faculty Papers*. Paper 7.
<https://jdc.jefferson.edu/petfp/7>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Pharmacology and Experimental Therapeutics Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Walter K Kraft, Kevin Dysart, Jay S Greenspan, Eric Gibson, Karol Kaltenbach, and Michelle E Ehrlich

As submitted to:

Addiction

And later published as:

**Revised Dose Schema of Sublingual Buprenorphine in
the Treatment of the Neonatal Opioid Abstinence
Syndrome**

Volume 106, Issue 3, March 2011, Pages 574-580

DOI: 10.1111/j.1360-0443.2010.03170.x

Walter K. Kraft* M.D., Kevin Dysart[♠] M.D., Jay S. Greenspan[♠], M.D., Eric
Gibson[♠] M.D., Karol Kaltenbach[♠] Ph.D., Michelle E. Ehrlich[¥] M.D.

Jefferson Medical College, Philadelphia
Departments of Pharmacology and Experimental Therapeutics*, and Pediatrics[♠]

[♠]A.I. DuPont Hospital for Children, Wilmington, DE

[¥]Mt. Sinai School of Medicine, New York, NY, Departments of Pediatrics and
Neurology

Corresponding Author:

Walter K. Kraft, M.D.
132 South 10th Street, 1170 Main Building
Phone- 215-955-9077; Fax- 215-955-5681
Walter.Kraft@jefferson.edu

The project described was supported by the Commonwealth of Pennsylvania
Tobacco Fund and Grant Number R21DA018207 from the National Institute on
Drug Abuse. The content is solely the responsibility of the authors and does not
necessarily represent the official views of the National Institute on Drug Abuse or
the National Institutes of Health.

Word Count:

Abstract Word Count: 151

References:

Tables: 3

Figures: 2

Keywords (MeSH unique ID#): Neonatal Abstinence Syndrome (D009357), buprenorphine (D002047), Sublingual Drug Administration (D000286), morphine (D009020)

ClinicalTrials.gov Identifier # NCT00521248

ABSTRACT

AIMS: Over half of infants exposed to opioids in utero develop neonatal abstinence syndrome (NAS) of severity to require pharmacologic therapy. Current treatments are associated with prolonged hospitalization. We sought to optimize the dose of sublingual buprenorphine in the treatment of NAS.

DESIGN: Randomized, phase 1, open-label, active-control clinical trial comparing sublingual buprenorphine to oral morphine.

SETTING: Large, urban, tertiary care hospital.

PARTICIPANTS: Twenty-four term infants with signs and symptoms of NAS.

MEASUREMENTS: Outcomes were neonatal safety, length of treatment, and length of hospitalization.

FINDINGS: Sublingual buprenorphine was safe and effective. Infants treated with buprenorphine had a 23-day length of treatment compared to 38 days for those treated with morphine ($p=0.01$), representing a 40% reduction. Length of stay in the buprenorphine group was reduced 24%, from 42 to 32 days ($p=0.05$).

CONCLUSIONS: Sublingual buprenorphine was safe in NAS, with a substantial efficacy advantage over standard of care therapy with oral morphine.

INTRODUCTION

Infants born to mothers receiving methadone maintenance often develop withdrawal symptoms in a complex known as the neonatal abstinence syndrome (NAS). NAS is characterized by signs and symptoms of CNS hyperirritability, gastrointestinal dysfunction, respiratory distress, and autonomic symptoms. (1) Common symptoms in order of frequency include tremors, high-pitched cry, sneezing, increased muscle tone, regurgitation and vomiting, poor sleep, loose stools, sweating, excoriation, mottling, nasal stuffiness, low-grade fever, and tachypnea. NAS reflects a spectrum of disease, and those with milder symptoms respond well to supportive treatments.(2) NAS symptoms severe enough to require pharmacologic treatment occur in 55-94% of infants born to opioid-dependent mothers. (1)

The optimal pharmacologic regimen for NAS has not been established, as reflected in the considerable heterogeneity in the diagnosis (3) and treatment of NAS among different institutions. (4,5) Treatments employed have included opioids, anticonvulsants, benzodiazepines, alpha 2 adrenergic antagonists, chloral hydrate, and the phenothiazine antipsychotic agent chlorpromazine. Cochrane reviews, (6,7) the American Academy of Pediatrics, (1) and expert review (8,9) identify opioid replacement as the ideal treatment for the withdrawal symptoms associated with in utero exposure to opiates. Morphine is the most commonly used agent, and is associated with treatment duration of 8-79 days, (10-15) almost always in an inpatient setting. The use of outpatient treatment in

highly selected patients has been reported to be associated with shorter inpatient stays, but longer total duration of therapy. (16)

Buprenorphine is a long-acting partial mu opioid receptor agonist used in the treatment of adult abstinence therapy. It has the advantage of decreased abuse potential and less respiratory depression than other opioid agonists. We have previously reported the first use of sublingual buprenorphine in NAS at a dose of 13.2 mcg/kg/day in three divided doses. (17) Though designed as a phase 1 safety study, the investigation suggested reduction in lengths of treatment and inpatient hospitalization of approximately 30%. A higher incidence of phenobarbital adjunctive therapy in the buprenorphine group led us to optimize dose parameters. We report here on a second cohort of 24 patients treated with this revised dose schema.

METHODS

Study Design

This was a single site, randomized, open label trial. (Figure 1) Neonates were randomized to treatment with either sublingual buprenorphine or oral morphine in a 1:1 ratio. NAS was graded using the MOTHER NAS score, which is the standard instrument used in the multicenter NIH funded MOTHER study (ClinTrials.gov ID NCT00271219) of buprenorphine in pregnancy. (18) It is also the standard instrument at TJUH. The MOTHER NAS score (hereafter “NAS score”) is based upon the Finnegan score, (19) modifications of which are the most commonly used to monitor NAS. (5,20) Initiation of treatment was based on

any consecutive 3 scores adding up to ≥ 24 . Inclusion criteria included ≥ 37 weeks gestation, exposure to opioids in utero, and demonstration of signs and symptoms of NAS requiring treatment. Exclusion criteria were major congenital malformations and/or intrauterine growth retardation, (21) medical illness requiring intensification of medical therapy, concomitant maternal benzodiazepine or severe alcohol abuse, maternal use of alcohol or of benzodiazepines in the 30 days prior to enrollment (as determined by self-report or intake urine drug screen), concomitant neonatal use of cytochrome P450 3A inhibitors or inducers prior to treatment, seizure activity or other neurologic abnormality, breast feeding or inability of mother to give informed consent due to co-morbid psychiatric diagnosis. The study was approved by the Institutional Review Board of Thomas Jefferson University. Computer-generated randomization was performed by the hospital Investigational Drug Service.

Study Treatments

Patients randomized to sublingual buprenorphine initially received 15.9 mcg/kg/day in 3 divided doses. Buprenorphine solution was prepared by mixing buprenorphine for injection (Buprenex, Reckitt Benckiser) to a final concentration of 0.075 mg/mL in 100% ethanol USP (30% of total volume) and simple syrup USP (85 gm sucrose/100 ml). The solution was administered under the tongue followed by insertion of a pacifier to reduce swallowing. Dose escalation was a 25% increase for NAS scale scores ≥ 24 total on 3 measures or a single score of ≥ 12 . Patients with inadequate control could be administered a rescue dose of

50% of the previous dose, after which the subsequent dose would be advanced 25%. After at least 3 days of dose stabilization, patients could begin weaning for scores < 8. Weaning was at intervals of 10%. Cessation of dosing occurred when buprenorphine was within 10% of the initial dose. All dose calculations were based upon birth weight. If NAS was not controlled with maximally specified dose of 60 mcg/kg/day, patients were administered phenobarbital 20 mg/kg load followed by 2.5 mg/kg twice a day for at least 2 days. Phenobarbital was weaned prior to reduction of the opioid. When NAS scores were reliably <8, the daily dose of phenobarbital was reduced by 50%, followed by complete cessation.

Standard of care treatment at Thomas Jefferson University Hospital consisted of an initial dose of morphine 0.4 mg/kg/day in 6 divided doses with dose escalation of 10%/day for NAS scale scores ≥ 24 total on 3 measures or a single score of ≥ 12 . All dose calculations were based upon daily weight. Adjunctive treatment with phenobarbital was initiated when the dose of morphine reached 1 mg/kg/day. Weaning from the phenobarbital was the same in both the buprenorphine and morphine groups. Infants were weaned with 10% dose reduction every 24 hours in morphine as tolerated until 0.15 mg/kg/day was reached. All patients in the trial were observed for at least two days following the cessation of dosing.

Statistics

This was a follow up pilot study to optimize the dose of sublingual buprenorphine in neonates. Therefore, sample size was not based upon a formal power calculation. Group comparisons for continuous variables were made using the Mann-Whitney U test. Statistical analysis was completed with JMP 5.1.2, (SAS Institute Inc.) Analysis was performed on an intention to treat basis.

RESULTS

Twenty-four infants were enrolled between March 2008 and January 2010. Patient characteristics are presented in Table 1.

Safety

A list of all adverse events observed in the trial is presented in Table 2. There was one serious adverse event in a patient randomized to buprenorphine. This infant randomized to buprenorphine had adverse events of CMV infection, prolonged reflux/poor feeding, elevated transaminases, aminoaciduria, and paronychia of a finger. Poor feeding and enteral hypomotility was initially thought to be part of NAS, but lack of resolution on buprenorphine and phenobarbital, and indeed no worsening when these drugs were removed led to further medical evaluation. CMV was noted in urine, and a lack of cerebral calcifications or microcephaly at birth suggest a late in utero or possibly nosocomial infection. Length of buprenorphine treatment was 45 days and length of stay was 98 days. On the last day of buprenorphine weaning, alanine transaminase (ALT) was

noted to be 30x the upper limit of normal (ULN), while aspartate transaminase (AST) was noted to be 20x ULN. These values waxed and waned over the remainder of the hospitalization, and remained elevated 6 weeks following the cessation of buprenorphine. Bilirubin was never elevated. The DSMB reviewed the case and came to the unanimous judgment that buprenorphine was not responsible for the clinical course of the infant, but did endorse our suggestion to monitor liver function for future enrollees. Liver function testing pre-dose, and at 7 and 21 days post randomization was normal in 6 subsequent patients (three in each treatment arm).

Efficacy

Infants treated with buprenorphine had a 23-day length of treatment compared to 38 days for those treated with morphine ($p=0.01$). (Table 3 and Figure 2) This represents a 40% reduction. Length of stay in the buprenorphine group was reduced 24%, from 42 to 32 days ($p=0.05$). Three infants treated with buprenorphine required phenobarbital compared to one infant treated with morphine.

DISCUSSION

This study builds upon our prior report describing the use of buprenorphine in the treatment of opioid NAS. The goal of the current investigation was to optimize buprenorphine dosing by increasing the 1) initial dose, 2) rate of dose up-titration, and 3) maximum daily dose. An increase in

stock drug concentration was used to maintain manageable drug volume for administration. Sublingual buprenorphine continued to maintain safety at these higher doses. In addition, there was continued advantage over morphine in length of treatment and length of stay, which was now statistically significant. It is arguable that many signs and symptoms observed in the infant with CMV infection did not reflect severity of NAS but instead were manifestations of the multiorgan viral process. Data from this infant was included in the primary analysis. However, removal of this infant causes the length of treatment to drop from 23 to 21 days, and length of stay to drop from 32 to 26.

Three patients in the buprenorphine group required the use of adjunctive phenobarbital compared to one patient in the morphine group. Phenobarbital is often used as a rescue therapy when maximum opioid replacement therapy dose is reached without adequate resolution of symptoms. It has also been used as an initial adjunct in combination therapy with an opioid (12) or as initial monotherapy. (22) Morphine and buprenorphine employ different up-titration rates and number of up-titrations until maximum dose is reached (6 for buprenorphine and 9 for morphine). Thus, the need for adjunctive phenobarbital is not necessarily a surrogate of “treatment failure” in infants with a more severe withdrawal symptom complex. Phenobarbital arguably has a therapeutic index similar to that of opioid treatment in this patient population. It is also not clear where on the dose response curve the present maximum buprenorphine dose lies. Thus it is possible as a partial agonist, buprenorphine may not be able to induce the dense signal generation at the mu opioid receptor obtained with

morphine. Alternatively, it could be hypothesized that a higher maximum dose of buprenorphine could reduce the frequency with which adjunctive phenobarbital would need to be used. However, as there are no definitive adverse events associated with short-term exposure to phenobarbital, it is possible that a short course of phenobarbital may be a useful adjunct to reduce total duration of treatment in children with more severe withdrawal symptoms.

A mechanistic basis for the improvement of buprenorphine over morphine is not immediately clear. The longer duration of action and residence at the mu opioid receptor of buprenorphine relative to morphine may reduce the sudden shifts in receptor agonism and withdrawal symptoms. A more prolonged persistence of drug effect following cessation likely reduces symptoms. Two features of buprenorphine dosing compared to morphine also merit noting. Buprenorphine has a more rapid up-titration of dose compared to morphine, which may allow a quicker attainment of symptom control. Though both regimens employ a 10% weaning schedule, buprenorphine is stopped when a dose within 10% of the starting dose. In contrast, the initial morphine dose is 0.4 mg/kg/day, while cessation of dosing takes place only after drug has been weaned to 0.15 mg/kg/day. Finally, the buprenorphine group dosing was based upon birth weight, while the morphine group was dosed according to daily weight. This difference serves to decrease a relative dose of buprenorphine as the infant grows.

This study was of an open label, randomized, design with primary goals of demonstrating safety and feasibility of sublingual buprenorphine. It is possible

that there was occult bias in the scoring of infants by the nurse evaluators, despite the use of structured training sessions. A double blind, double dummy study would be required to fully evaluate any differential efficacy of buprenorphine over morphine. Another limitation is that the study excluded infants with benzodiazepine exposure and preterm infants. These exclusions serve to limit the generalizability of results. Infants with in utero benzodiazepine exposure have a longer length of stay and treatment compared to those without. (15,23-25) These infants represent a group in whom research should be directed. Premature infants typically have less severe NAS, (26) but buprenorphine does not have an established safety record in this population. Finally, while the length of treatment and length of stay noted in this study is consistent with observational data at Thomas Jefferson University Hospital between 2000-2006, (15) it is longer than has been noted at other institutions. (10,13,27,28) It is not clear if the magnitude of advantage over standard therapy would be maintained at institutions with a shorter length of stay.

In conclusion, buprenorphine at an optimized dose schema has been demonstrated to be safe and efficacious in the treatment of NAS. While indications of a therapeutic advantage over morphine again have been demonstrated, these need to be verified in a double blind clinical trial. Additionally, the unique characteristics of buprenorphine make exploration of outpatient use for treatment of NAS a ripe area for clinical research.

ACKNOWLEDGEMENTS

The authors would like to thank Linda Sailor and Rania Sadaka of the Thomas Jefferson University Hospital Investigational Drug Service, and the nurse practitioners and nurses of the Neonatal Intensive Care and Pediatric Care units.

Table 1. Characteristics of Patients in the trial

		Morphine	Buprenorphine
N		12	12
Sex	Male	4	6
	Female	8	6
Race	White, non Hispanic	11	12
	African-American	1	0
Birth weight (gm)		3010	2680
Gestational Age (weeks)		39.3	39.1
Onset of Treatment. Mean Days after birth		2.4 [SD 0.89]	2.3 [SD 1.4]
1-Minute APGAR Score		8.1	7.6
5-Minute APGAR Score		8.9	9.0
Daily Maternal Methadone Dose (mg)		124 [SD 60]	157 [SD 57]

^{||}Mean

Table 2 : Efficacy Outcomes of Buprenorphine and Neonatal Opium Solution Mean

	Buprenorphine	Morphine	p value
N	12	12	
Length of Treatment (days)	23 [SD 12]	38 [SD 14]	0.01
Length of Stay (days)	32 [SD 24]	42 [SD 13]	0.05
Phenobarbital adjunctive therapy (patients)	3	1	

Table 3: Adverse Events

Subject Allocation Number	Treatment	Adverse Event	Serious AE	Causality
039	morphine	oral thrush	no	unrelated
047	buprenorphine	paronychia of finger	no	unrelated
		reflux/poor feeding	yes	probably not related
		elevated transaminases	no	probably not related
		Cytomegalovirus infection	no	unrelated
		aminoaciduria	no	unrelated
049	morphine	conjunctivitis	no	unrelated
050	morphine	oral thrush	no	unrelated
051	buprenorphine	clavicle birth fracture	no	unrelated
052	morphine	reflux	no	unrelated

Figure 1: Study Schema

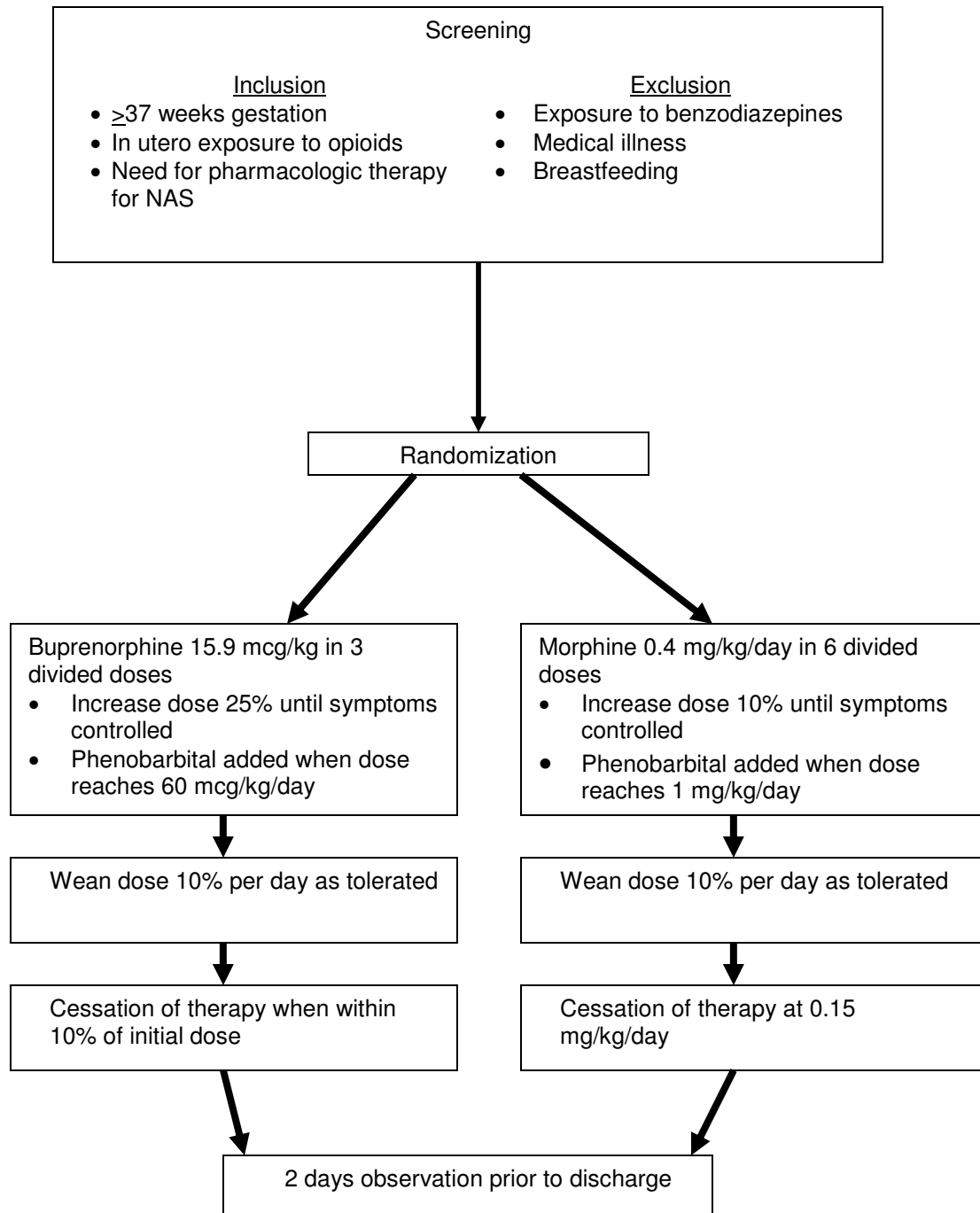
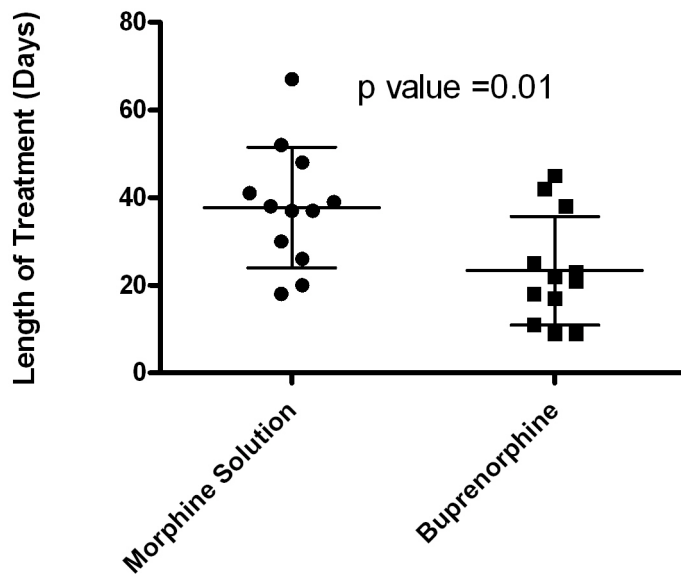
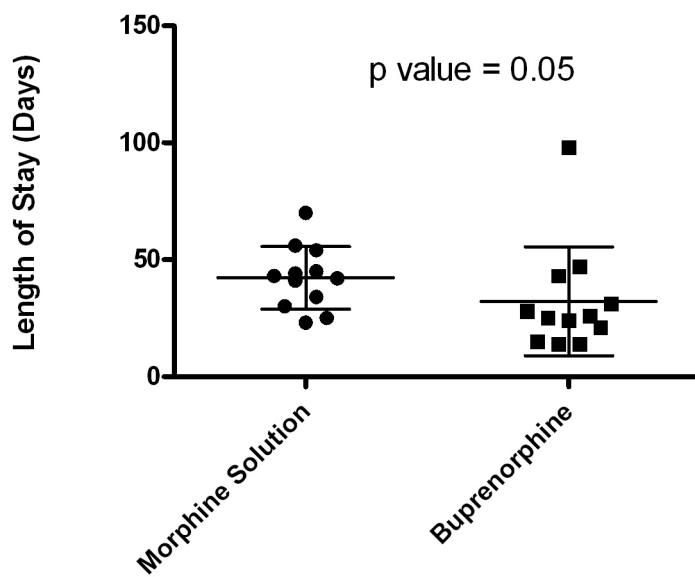


Figure 2: Length of Treatment and Length of Stay

Length Of Treatment, Morphine Solution Compared to Buprenorphine



Length of Stay, Morphine Solution Compared to Buprenorphine



- (1) American Academy of Pediatrics Committee on Drugs. Neonatal drug withdrawal. *Pediatrics* 1998 Jun;101(6):1079-1088.
- (2) Alcorn J, McNamara PJ. Ontogeny of hepatic and renal systemic clearance pathways in infants: part II. *Clin.Pharmacokinet.* 2002;41(13):1077-1094.
- (3) Crocetti MT, Amin DD, Jansson LM. Variability in the evaluation and management of opiate-exposed newborns in Maryland. *Clin.Pediatr.(Phila)* 2007 Sep;46(7):632-635.
- (4) Nandakumar N, Sankar VS. What is the best evidence based management of neonatal abstinence syndrome? *Arch.Dis.Child.Fetal Neonatal Ed.* 2006 Nov;91(6):F463.
- (5) Sarkar S, Donn SM. Management of neonatal abstinence syndrome in neonatal intensive care units: a national survey. *J.Perinatol.* 2006 Jan 1;26(1):15-17.
- (6) Osborn DA, Jeffery HE, Cole M. Opiate treatment for opiate withdrawal in newborn infants. *Cochrane Database Syst.Rev.* 2005 Jul 20;(3)(3):CD002059.
- (7) Osborn DA, Jeffery HE, Cole MJ. Sedatives for opiate withdrawal in newborn infants. *Cochrane Database Syst.Rev.* 2005 Jul 20;(3)(3):CD002053.
- (8) Johnson K, Gerada C, Greenough A. Treatment of neonatal abstinence syndrome. *Arch.Dis.Child.Fetal Neonatal Ed.* 2003 Jan;88(1):F2-5.
- (9) Jansson LM, Velez M, Harrow C. The opioid-exposed newborn: assessment and pharmacologic management. *J.Opioid Manag.* 2009 Jan-Feb;5(1):47-55.
- (10) Lainwala S, Brown ER, Weinschenk NP, Blackwell MT, Hagadorn JI. A retrospective study of length of hospital stay in infants treated for neonatal abstinence syndrome with methadone versus oral morphine preparations. *Adv.Neonatal Care.* 2005 Oct;5(5):265-272.
- (11) Langenfeld S, Birkenfeld L, Herkenrath P, Muller C, Hellmich M, Theisohn M. Therapy of the neonatal abstinence syndrome with tincture of opium or morphine drops. *Drug Alcohol Depend.* 2005 Jan 7;77(1):31-36.
- (12) Coyle MG, Ferguson A, Lagasse L, Oh W, Lester B. Diluted tincture of opium (DTO) and phenobarbital versus DTO alone for neonatal opiate withdrawal in term infants. *J.Pediatr.* 2002 May;140(5):561-564.
- (13) Dryden C, Young D, Hepburn M, Mactier H. Maternal methadone use in pregnancy: factors associated with the development of neonatal abstinence syndrome and implications for healthcare resources. *BJOG* 2009 Apr;116(5):665-671.
- (14) Colombini N, Elias R, Busuttill M, Dubuc M, Einaudi MA, Bues-Charbit M. Hospital morphine preparation for abstinence syndrome in newborns exposed to buprenorphine or methadone. *Pharm.World Sci.* 2008 Jun;30(3):227-234.
- (15) Seligman NS, Salva N, Hayes EJ, Dysart KC, Pequignot EC, Baxter JK. Predicting length of treatment for neonatal abstinence syndrome in methadone-exposed neonates. *Am.J.Obstet.Gynecol.* 2008 Oct;199(4):396.e1-396.e7.
- (16) Oei J, Feller JM, Lui K. Coordinated outpatient care of the narcotic-dependent infant. *J.Paediatr.Child Health* 2001 Jun;37(3):266-270.

- (17) Kraft WK, Gibson E, Dysart K, Damle VS, Larusso JL, Greenspan JS, et al. Sublingual Buprenorphine for Treatment of Neonatal Abstinence Syndrome: A Randomized Trial. *Pediatrics* 2008 Aug 11.
- (18) Jones HE, Johnson RE, Jasinski DR, O'Grady KE, Chisholm CA, Choo RE, et al. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: effects on the neonatal abstinence syndrome. *Drug Alcohol Depend.* 2005 Jul;79(1):1-10.
- (19) Finnegan LP, Kaltenbach K. Neonatal abstinence syndrome. In: Hoekelman RA, Friedman SB, Nelson N, Seidel HM, editors. *Primary Pediatric Care*. Second ed. Mosby: St. Louis; 1992. p. 1367-1378.
- (20) O'Grady MJ, Hopewell J, White MJ. Management of neonatal abstinence syndrome: a national survey and review of practice. *Arch.Dis.Child.Fetal Neonatal Ed.* 2009 Jan 27.
- (21) Mamelie N, Cochet V, Claris O. Definition of fetal growth restriction according to constitutional growth potential. *Biol.Neonate* 2001;80(4):277-285.
- (22) Jackson L, Ting A, McKay S, Galea P, Skeoch C. A randomised controlled trial of morphine versus phenobarbitone for neonatal abstinence syndrome. *Arch.Dis.Child.Fetal Neonatal Ed.* 2004 Jul;89(4):F300-4.
- (23) Abdel-Latif ME, Pinner J, Clews S, Cooke F, Lui K, Oei J. Effects of breast milk on the severity and outcome of neonatal abstinence syndrome among infants of drug-dependent mothers. *Pediatrics* 2006 Jun;117(6):e1163-9.
- (24) Coghlan D, Milner M, Clarke T, Lambert I, McDermott C, McNally M, et al. Neonatal abstinence syndrome. *Ir.Med.J.* 1999 Jan-Feb;92(1):232-3, 236.
- (25) Sutton LR, Hinderliter SA. Diazepam abuse in pregnant women on methadone maintenance. Implications for the neonate. *Clin.Pediatr.(Phila)* 1990 Feb;29(2):108-111.
- (26) Dysart K, Hsieh HC, Kaltenbach K, Greenspan JS. Sequela of preterm versus term infants born to mothers on a methadone maintenance program: differential course of neonatal abstinence syndrome. *J.Perinat.Med.* 2007;35(4):344-346.
- (27) Agthe AG, Kim GR, Mathias KB, Hendrix CW, Chavez-Valdez R, Jansson L, et al. Clonidine as an adjunct therapy to opioids for neonatal abstinence syndrome: a randomized, controlled trial. *Pediatrics* 2009 May;123(5):e849-56.
- (28) Blaser A, Pulzer F, Knupfer M, Robel-Tillig E, Vogtmann C, Nickel P, et al. Drug withdrawal in newborns - clinical data of 49 infants with intrauterine drug exposure: what should be done? *Klin.Padiatr.* 2008 Sep-Oct;220(5):308-315.