Clinical and Demographic Predictors of the Need for Pharmacotherapy in Neonatal Abstinence Syndrome

A thesis submitted by

Shawana Bibi, MD

in partial fulfillment of the requirements for the degree of

Master of Science

in

Clinical and Translational Science

Tufts University

Graduate School of Biomedical Sciences

August 2022

Advisor: Jonathan M. Davis, MD

Abstract

Background:

Timely and accurate prediction of the need for pharmacotherapy (PT) for Neonatal Abstinence Syndrome (NAS) remains elusive. Significant variation in clinical expression of NAS in terms of onset, severity, need for PT, and duration of physiologic instability leads to prolonged birth hospitalization, with a significant increase in the healthcare costs.

Objective:

To develop and validate a clinical prediction model of need for PT in NAS using patient specific clinical and demographic data.

Methods:

Pooled patient level data was used from 3 separate cohorts and comprised of infants ≥37 weeks gestation born to mothers with history of opioid use disorder (OUD) during pregnancy. Primary outcome was need for PT utilizing a modified Finnegan Neonatal Abstinence Scoring System (FNASS). A stepwise multivariable logistic regression model was built using variables available at the time of admission and internally validated by bootstrapping. Leave-one-out validation was performed using data from two study cohorts to develop the model and external validation using the third cohort. This procedure was repeated for three unique combinations of cohorts, allowing us to examine stability of validation. Model performance was evaluated using discrimination (area under the receiver operating characteristic curve, AUROC) and calibration.

Results:

A total of 698 infants were included of which 430 required PT. The final model included seven predictors of the need for PT: gestational age, any exposure to breast milk, type of maternal opioid for treatment of OUD, exposure to heroin, cocaine, benzodiazepines, and/or antipsychotic medications. The model had an AUROC of 0.68 (95 % CI: 0.64-0.72; optimism corrected 0.65).

Conclusion:

Our clinical prediction model that incorporated data from several cohorts was parsimonious with seven predictors identified for need for PT. The model appeared to calibrate well however discrimination was modest. Our results indicate that currently available predictive models of NAS are not sufficient to accurately determine opioid exposed infants at high vs low risk of requiring PT. Additional work is needed to improve their predictive performance.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AAP (American Academy of Pediatrics)
ACOG (American College of Gynecology)
AUROC (Area Under the Receiver Operator Curve)
CCH (Cape Cod Hospital)
CI (Confidence Interval)
C section (Cesarian Section)
EMR (Electronic Medical record)
ESC (Eat, Sleep, Console)
FNASS (Finnegan Neonatal Abstinence Scoring System)
GA (Gestational Age)
LOS (Length of Stay)
MAR (Missing at Random)
MAT (Medication Assisted Therapy)
MICE (Multivariate Imputation by Chained Equations)
MOTHER (Maternal Opioid Treatment: Human Experimental Research)
NAS (Neonatal Abstinence Syndrome)
NICU (Neonatal Intensive Care Unit)
NIS (National Inpatient Sample)
NOWS (Neonatal Opioid Withdrawal Syndrome)
OPRM1(Opioid Receptor Mu 1)
OR (Odds Ratio)
OUD (Opioid Use disorder)
PRS (Polygenic Risk Score)
PT (Pharmacotherapy)
RCT (Randomized Controlled Trial)
Rx (Treatment)
SD (Standard Deviation)
SSRI (Selective Serotonin Reuptake Inhibitor)
TJU (Thomas Jefferson University)

Chapter 1: Introduction

Neonatal Abstinence Syndrome (NAS), also commonly referred to as Neonatal Opioid Withdrawal Syndrome (NOWS) is a constellation of signs affecting newborns with chronic exposure to opioids in utero, often with co exposure to other psychotropic substances. The change in terminology to NOWS reflects the fact that clinically important neonatal withdrawal results from intrauterine opioid exposure.

There has been an exponential rise in Opioid Use Disorder (OUD) in pregnancy over the past two decades, concurrent with the worsening of the opioid crisis resulting in a significant increase in the incidence of NAS[1,2]. From 2000 to 2016, incidence of NAS increased from 1.2 to 8.8 per 1000 hospital births[3], with a disproportionate rise in rural populations[4] and Medicaid enrolled infants[5] with notable state to state variation[6]. Using National Inpatient Sample (NIS) data (2004 through 2014), Winkelman et al showed that by 2014, one infant was born every 15 minutes with NAS and Medicaid financed births related to NAS contributed $462 million in hospital costs[5]. These data highlight the downstream effects of NAS on our public health system, adding layers of complexity to the opioid crisis.

1.1 Clinical Presentation of NAS

NAS has a highly variable clinical expression in terms of onset, severity and duration of signs and symptoms which primarily involve central nervous system (e.g., tremors, increased muscle tone and seizures in severe cases) and gastrointestinal system (e.g., loose stools, feeding intolerance) due to the concentration of opioid receptors in these sites[7]. Onset of signs depends on the type of opioid used during pregnancy (short or long acting) and typically varies from as early as 24 hours of life in case of heroin use to

72 hours of life in case of methadone exposure which has a longer half-life and sometimes can even be delayed up to 5-7 days[8]. Other factors that contribute to variation in onset and severity of NAS include maternal co-exposure to other psychoactive agents such as sedative -hypnotics, barbiturates, anxiolytics, cocaine, gabapentin and tobacco[9]. Additionally, factors such as timing of most recent drug use prior to delivery, net placental transfer of drug and pharmacokinetic properties of drug in an infant are also important to consider[9].

Infants with *in utero* opioid exposure are therefore typically hospitalized for several days to monitor for the signs/symptoms of NAS and to determine need for pharmacotherapy[10]. Average national length of stay in the hospital for opioid exposed infants is reported to be 16 days and prolonged hospitalization tremendously adds to the health care costs[5,11].

1.2 Screening and testing for NAS

The American College of Obstetrics and Gynecology (ACOG) recommends substance use screening at the time of first prenatal visit using a validated instrument. A positive screen typically leads to further investigation by a thorough maternal history of drug use and testing by urine toxicology. While universal testing has often been recommended based on studies that have shown up to 20 % of pregnant mothers testing positive on urine toxicology had screened negative for substance use, legal issues associated with positive testing makes adoption of universal testing more complex.

Infants can be tested for potential in utero drug exposure in several different ways including urine, meconium, and umbilical cord toxicology with wide institutional variation in testing methodology. Each of these methods have certain limitations that

need to be considered before making clinical decisions. For example, urine specimens need to be obtained soon after birth, before the drug is excreted and eliminated by the kidneys. In addition, urine toxicology only captures recent exposures to substances during pregnancy. In contrast, meconium toxicology, reflects remote exposures as early as 20 weeks of gestation and is therefore considered the gold standard. However, it may miss any period of abstinence by the mother closer to delivery. Although umbilical cord testing has recently been suggested to be comparable to meconium testing with the added advantage of easier collection however, it still has not been widely adopted[8]. Additionally, clinicians need to understand that the testing is not necessarily required in situations where mother is receiving treatment for OUD and is followed and tested regularly in medication assisted therapy (MAT) programs.

1.3 Diagnosis and Management

For the past four decades, the diagnosis and management of NAS has been based on a clinical scoring system called FNASS (Finnegan Neonatal Abstinence Scoring System) proposed in 1975 by Dr Loretta Finnegan[12]. More widely used tools today are modified/shortened versions of FNASS including the MOTHER (Maternal Opioid Treatment: Human Experimental Research) trial modification[13]. However, all of these tools are based on assessing signs and symptoms affecting the central/autonomic nervous systems and the gastrointestinal systems and are inherently subjective in nature. Infants are typically scored every 2-4 hours and decision to initiate pharmacotherapy is usually based on 2 or 3 scores > 8 or one to two scores > 12, per institutional protocols. While studies have suggested that the cutoff of 8 is useful in differentiating opioid exposed from non-opioid exposed infants[14], the rationale for using this cutoff for initiating treatment

and /or to guide treatment weaning decisions is not supported by robust evidence. It has also been observed that there may be a delay in initiation of treatment based on FNASS where in an infant must demonstrate higher scores every two to three hours for at least two to three times before treatment with opioids can be initiated. This can result in delay in establishing physiologic stability, with infant requiring higher doses of opioid therapy and take longer to wean off the medications and be safely discharged home. In 2014, a new tool called Eat, Sleep, Console (ESC) was proposed by investigators at Yale and has since then been adopted by many institutions due to its ease of use and potential for reducing length of hospital stay (LOS)[15] in the hospital. Instead of characterizing all the signs /symptoms of withdrawal, this scoring system focuses on an infant's ability to feed at least 1 oz per feed, sleep uninterrupted for at least an hour and able to be consoled within 10 minutes. Institutions using an ESC approach, reported a significant reduction in hospital LOS and proportion of infants treated with pharmacotherapy. However, all these institutions implemented this approach alongside other non-pharmacologic and pharmacologic interventions that are known to impact LOS and need for pharmacotherapy e.g., rooming in practices, breastfeeding, and use of as needed medication[16,17]. Not all institutional environments can support a heavy reliance on non-pharmacologic measures such as cuddler programs so there are limitations to wide adoption of this tool.

1.4 Clinical prediction tools

Despite years of research, timely and accurate prediction of NAS severity remains elusive. Significant variation in the clinical expression makes it challenging for the clinicians to predict the risk profile for a given infant at or shortly after birth and

make informed decisions regarding the optimal duration of observation in the hospital and if/when to initiate pharmacotherapy.

Several clinical and demographic factors have been shown to be associated with NAS severity[18-25]. Recently developed risk prediction tools have attempted to predict an infant's risk of need for pharmacotherapy[26-28]. These tools, while promising for the clinicians, have not yet been widely adopted due to several limitations such as reliance on FNASS for assessment of opioid exposed infants. This has led to an increased interest in exploring other factors e.g., genetics that potentially contribute to the variation in NAS severity[29]. Several small studies have identified genetic variants in opioid receptor genes (e.g. OPRM1) and genes related to the stress response, dopamine pathways, and opioid metabolism that are associated with differences in the need for pharmacotherapy as well as LOS in NAS[29-33].While these studies suggest that genetic variation could help explain some of the variability seen in NAS expression, inclusion of genomic information to the existing risk prediction models and its clinical implementation will largely depend on availability of large scale genomic databases and advancement in computational biology approaches. There is urgent need for developing better risk prediction models using clinical and demographic information with larger sample sizes.

1.5 Scientific Premise

The scientific premise guiding this study is that application of predictive modeling methodology using known clinical and demographic variables can help estimate the probabilistic susceptibility of an infant requiring pharmacotherapy for NAS with greater precision. Precise assessment of this risk can improve management of these newborns by helping clinicians make decisions regarding pharmacotherapy in a timely

manner. The short-term goal is to evaluate the utility of a clinical risk prediction score for stratifying the NAS patient population into distinct risk categories such that individualized decision making regarding length of observation and initiation of therapy is possible. Currently, the American Academy of Pediatrics (AAP) recommends observing opioid exposed infants in hospital for 3-7 days depending on the type of opioid they are exposed to in utero, while recognizing that there is limited data to inform such decision making[8]. Infants determined to be high risk could have low dose opioids started immediately after birth and weaned rapidly which may minimize the development of severe NAS and improve outcome. Conversely, low risk infants could be sent home earlier and followed by a visiting nurse, greatly reducing hospital days and associated costs.

Chapter 2: Clinical and demographic predictors of the need for pharmacotherapy in Neonatal Abstinence Syndrome (NAS)[1]

[1] Bibi S, Singh R, Breeze JL, Nelson J, Kraft WK, Davis JM. To be submitted to *Journal of Perinatology*

2.1 Introduction

Neonatal Abstinence Syndrome (NAS), now commonly referred to as Neonatal Opioid Withdrawal syndrome (NOWS) is a withdrawal syndrome that affects newborns with chronic exposure to opioids in utero but often with co exposure to other substances. There has been an exponential rise in Opioid Use Disorder (OUD) in pregnancy over the past two decades, concurrent with the worsening of the opioid crisis. Consequently, there has been a several fold increase in the incidence of NAS[1,2]. From 2000 to 2016, a steep rise in the incidence of NAS was noted from 1.2 to 8.8 per 1000 hospital births[3], with disproportionate rise in rural areas[4] as well as among Medicaid enrolled infants[5] and with a notable state to state variation[6]. Using National Inpatient Sample (NIS) data (2004 through 2014), Winkelman et al showed that by 2014, one infant was born every 15 minutes with NAS and Medicaid financed births related to NAS contributed $462 million in hospital costs in 2014[5]. This data highlights the downstream effects of NAS on our public health system, adding layers of complexity to the opioid crisis.

Accurate stratification of an opioid exposed infant's risk of need for pharmacotherapy for NAS at the time of birth has several potential advantages. First, evidence based, and resource intensive care measures could be targeted for opioid exposed newborns at highest risk of requiring pharmacotherapy. Examples include admission to NICU with enhanced nursing ratio and potentially starting pharmacotherapy prior to high FNASS scores. Second, accurate risk stratification can inform shared decision making with parents. Finally, accurate risk stratification allows enrichment of patient population for clinical trials evaluating novel therapies.

Several predictive tools have been proposed to inform clinical decision making for pharmacotherapy for NAS[27-29]. However, these tools are yet to be adopted routinely in clinical practice primarily due to lack of objective assessment of NAS and reliance on FNASS, paucity of external validation and generalizability and heterogeneity in the number and type of variables used in the models.

In this study we aimed to utilize pooled patient level data from two randomized control trials (RCTs) and three observational cohorts to derive a clinical predictive model that can be utilized to risk stratify opioid exposed infants into two distinct groups based on need for pharmacotherapy.

2.2 Methods

2.2.1 Data source and study cohorts

We pooled data from three cohorts (derived from two RCTs, two prospective observational research studies led by two sites and a retrospective community hospital cohort). Cohort size ranged from 79 to 392 infants with the pooled cohort consisting of 698 infants. Inclusion and exclusion criteria for all cohorts is outlined below.

2.2.1.1 Tufts Medical Center (N = 392)

This cohort included prospective data from an eight-site RCT that compared methadone with morphine for the treatment of NAS (conducted 2014-2018, led by Tufts Medical Center)[34] as well as a concurrent observational study of newborns whose parents gave consent for the clinical trial but did not require treatment or whose parents refused consent for randomization in the clinical trial but consented to data collection with similar. inclusion and exclusion criteria. Infants were eligible for inclusion if their mothers received opioid agonist treatment for OUD during pregnancy with Methadone or

Buprenorphine or received an opioid prescription for chronic pain. Infants with maternal history of psychotropic drug use for a known psychiatric diagnosis or illicit drugs during pregnancy were also included. Infants born at 37 weeks gestation or later were included. Exclusion criteria included prenatal exposure to more than 3 oz of alcohol per week during pregnancy. Infants with evidence of sepsis, major congenital anomalies or genetic disorders were also excluded.

2.2.1.2 Cape Cod/Falmouth Hospital Retrospective Cohort (N = 79)

This cohort had retrospective data from Cape Cod and Falmouth Hospitals, community hospitals in Massachusetts and had similar inclusion and exclusion criteria as the Tufts Medical Center trial[35].

2.2.1.3 Thomas Jefferson University (N = 227)

This cohort was composed of eligible participants in a single center clinical trial of sublingual buprenorphine for NAS as well as a prospective observational study at the same center that enrolled all neonates at-risk for NAS based upon a history of in utero opioid exposure[36]. The trial included infants ≥ 37 weeks gestation, who were exposed to opioids in utero and excluded infants with major congenital malformation, birth weight < 2200 g, serious medical or neurologic illness, seizures, hypoglycemia requiring treatment with intravenous glucose and hyperbilirubinemia (serum bilirubin level > 20 mg/dl). Infants with maternal exposure to benzodiazepines more than 30 days prior to delivery were excluded.

2.2.2 Inclusion and exclusion criteria

For this study all infants ≥ 37 weeks gestation born to pregnant women with history of OUD during the current pregnancy were eligible for inclusion. Preterm infants

(less than 37 weeks gestation) were excluded given their variable length of and response to opioid exposure in utero.

2.2.3 Primary Outcome

The primary outcome was the need for pharmacotherapy for NAS. This was determined based on modified Finnegan Scoring criteria referred to as MOTHER NAS scale used to assess severity of NAS symptoms. A score was assigned every 4 hours and treatment was initiated for a single score of ≥12 or 2 (Tufts) or 3 (Jefferson) consecutive scores of ≥8.

2.2.4 Covariates

Key independent variables in our data set that were considered to predict the binary primary outcome of need for pharmacotherapy are shown in Table 2.1. Data on these variables is typically available at the time of birth. Co-exposures were determined by maternal self-report, maternal toxicology screens and subsequently by infant urine toxicology screens. Exposure to opioids was limited to medication assisted therapy (MAT) with methadone or buprenorphine and illicit opioids such as heroin. Prescription opioid exposure was not considered as a key predictor given its increasingly limited use in practice. In this data set, prescription opioid use was reported in only 32 out of 730 mother- infant dyads.

2.2.5 Sample Size

The current analytic data set with data on demographic and clinical variables as well as the primary outcome has a sample size of 698 (430 treated, 62%) after exclusion of infants with maternal exposure to prescription opioids. With 268 non-events

(38 %), our data set could evaluate up to 13 predictors in the model to avoid model overfitting, following the 20 events per variable guideline[37].

Table 2.1 Key Independent Variables Considered for Inclusion in Prediction Model	
Demographics	Gestational Age Sex
Infant Characteristics	Birth Weight Maternal breast Milk
Maternal Characteristics	Maternal Race Cesarean Section Delivery Type of opioid for treatment of maternal OUD Methadone Buprenorphine
Co-exposures	Heroin Cocaine Benzodiazepines SSRIs Antipsychotics Alcohol Tobacco Amphetamines Gabapentin

2.2.6 Missing data

Data on infant characteristics was almost complete, however there was missing data on some maternal exposures. Among the key independent variables of interest, data on heroin exposure was missing for 107 infants (15.3%), cocaine exposure was missing for 85 infants (12.2 %), type of maternal treatment opioid was missing for 17 infants (2.4%), amphetamine was missing for 115 infants (16.5%) and alcohol was missing for 240 infants (34.4 %). Data on gabapentin was missing for more than 50 % of subjects.

2.2.7 Statistical Analysis

Potential candidate variables for model building were selected based on expert opinion and previously published data. Variables were assessed for collinearity and none of the covariates were found to have a strong collinear relationship. Variables that had data missing entirely across a cohort were also excluded e.g., maternal race and alcohol exposure. Univariable comparisons were made between selected candidate predictors and need for pharmacotherapy.

2.2.7.1 Handling of missing data

Missing data on key independent variables was addressed using multiple imputation[38]. Data on these infants were retrieved from electronic medical records (EMR) with inconsistent documentation on exposures, and therefore were handled under the assumption of missing at random (MAR). Values for these missing variables were imputed 10 times to generate 10 complete datasets utilizing "MICE" (Multivariate Imputation by Chained Equations) package in R studio. For each missing baseline variable, a regression model was generated to model the distribution of the missing variable as a function of all available data. This preserved the underlying variability and distributional relationships present in the underlying data. We included all variables to be used in subsequent analyses as well as the outcome variable in the imputation model. These included need for PT, gestational age (weeks), infant birth weight (grams), infant sex, any breast milk, type of maternal opioid for treatment of OUD (Methadone vs Buprenorphine), exposure to tobacco, heroin, cocaine, benzodiazepines, SSRIs and/or antipsychotic medications. Additionally, we included study cohort in the imputation model as this variable might be predictive of missing values. Variables with greater than

50% missingness were not imputed, as this could lead to unreliable imputation. Only variable meeting this criterion was Gabapentin.

2.2.7.2 Model derivation

Final model was derived using pooled data from all three study cohorts using multivariable logistic regression modeling and specified using backward stepwise variable selection procedure. P value criterion of 0.157 was used to exclude or include variables at each step of model building. Variable selection in multiple imputed data sets can be challenging, since different variables may be selected between imputed data sets when variable selection is applied in each imputed data set. To enable variable selection while using multiple imputation, all 10 imputed datasets were "stacked" into a single large dataset or "superset". To account for the multiple observations for each subject, each entry in superset was weighted by (1-f)/M, where f equals the average fraction of missing data across all variables used in the imputation models and M is the number of imputed data sets i.e. 10 in this case[38,39]. Eleven predictors were included in the model building procedure: Infant birth weight, gestational age, sex, exposure to breast milk, maternal opioid, heroin, cocaine, SSRIs, benzodiazepines, antipsychotics, and tobacco.

2.2.7.3 Model validation

Due to lack of an independent cohort of infants for external validation, we internally validated the model using bootstrap validation[40]. We utilized "boot_MI" function in "psfmi" package (R studio)[41], which bootstraps from the incomplete dataset and applies multiple imputation in each bootstrap sample. Five hundred bootstrap samples were generated from the original dataset. Multiple imputation was used to generate 10 datasets for each bootstrap sample. Internal validation was conducted with

backward variable selection using P value criterion of 0.05, for each bootstrap sample, including all candidate variables. The performance measures in the multiply imputed bootstrap samples were tested in the original multiply imputed datasets (pooled) to determine the optimism. Estimated slope value was used as a shrinkage factor to prevent our model from being overfitted in new data. This was done by multiplying the pooled coefficients with the shrinkage factor and also to determine a new intercept value that is aligned with the shrunken coefficients.

Leave-one-out validation (internal- external validation)[42] was then performed using data from two study cohorts to develop the model and conducting validation on the excluded third cohort. This procedure was run for three unique combinations of cohorts, allowing us to examine stability of validation while also performing external validation.

2.2.7.4 Estimation of model performance

Model performance was evaluated by measuring discrimination and calibration in each of the three cohorts. Model discrimination was determined by examining the area under the receiver operating characteristic curve (AUROC). Calibration was assessed graphically by plotting observed risk of pharmacotherapy against deciles of predicted risk and by assessing calibration slope based on boot strap validation. Shrinkage factor was applied to adjust for optimism.

All statistical analyses were performed using R software, version 4.0.5 (R foundation for statistical computing, Vienna, Austria).

2.3 Results

Observations from the three study cohorts were restricted to the 698 infants with complete data on primary outcome and excluded infants < 37 weeks gestational age

since preterm infants are known to have variable expression from in utero opioid exposure.

2.3.1 Maternal and infant characteristics

Univariable comparisons were made between selected candidate predictors and need for pharmacotherapy. Results of the univariate analyses for the 698 infants are given in Table 2.2. Infants who received pharmacotherapy were more likely to have been exposed to maternal opioid treatment for OUD with Methadone than Buprenorphine (69 vs 31 %) and were less likely to have received breast milk (46 vs 63 %).

Table 2.2 Maternal and infant characteristics and co-exposures by need for PT

	No PT (N= 268)	PT (N = 430)	P value	Missing N (%)
Infant characteristics				
Gestational age (GA) weeks	39.1 (1.2)	39.3 (1.2)	0.09	0 (0.0)
Female sex	133 (49.6)	223 (51.9)	0.62	0 (0.0)
Birth weight (grams)	3092.1 (482.7)	3105 (470.5)	0.73	0 (0.0)
Breast milk exposure	166 (63.1)	193 (46.3)	<0.001	18 (2.6)
Maternal characteristics				
White Race	147 (93.6)	262 (89.7)	0.22	249 (35.6)
Type of MAT: Methadone Buprenorphine	 147 (57.2) 110 (42.8)	 293 (69.1) 131 (30.9)	0.002	17 (2.4)
C section delivery	69 (31.7)	128 (34.6)	0.52	110 (15.8)
Co-exposures				
Heroin	39 (17.3)	105 (28.8)	0.002	107 (15.3)
Cocaine	11 (4.7)	44 (11.5)	0.007	85 (12.2)
Benzodiazepines	9 (3.4)	65 (15.6)	<0.001	17 (2.4)
SSRIs	28 (10.5)	64 (15.1)	0.11	6 (0.9)
Antipsychotics	13 (4.9)	42 (9.8)	0.03	5 (0.7)
Alcohol	8 (5.0)	21 (7.1)	0.50	240 (34.4)
Tobacco	201(75.6)	349 (81.9)	0.05	6 (0.9)
Amphetamines	5 (2.3)	9 (2.5)	1.0	115 (16.5)

Categorical variables expressed as frequencies and %, continuous variables as mean and SD

There were no significant differences in demographic characteristics such as maternal race, gestational age, sex, and mode of delivery (c- section vs vaginal birth) between the two groups. Distribution of predictor variables across three study cohorts is shown in Table 2.3. The proportion of infants with the outcome across the three study cohorts ranged from 54 to 67 %. This wide variation in outcome rates may have been influenced by the subtle differences in outcome definition. For example, PT was initiated at TJU when 3 consecutive FNASS scores were ≥ 8 while at Tufts it was initiated for 2 consecutive scores of ≥8.

Table 2.3 Maternal and infant characteristics and co-exposures by study cohort

	Overall (n = 698)	Tufts (n = 392)	TJU (n = 227)	CCH (n = 79)
Infant characteristics				
NAS PT	430 (61.6)	262 (66.8)	123 (54.2)	45 (57.0)
GAa (weeks)	39.2 (1.24)	39.3 (1.3)	38.9 (1.2)	39.6 (1.3)
Female sex	356 (51)	206 (52.6)	111(48.9)	39 (49.4)
Birth weight (grams)	3100.1 (474.9)	3139.3 (503.4)	2985.2 (421.7)	3235.4 (406.2)
Breast milk exposure	359 (52.8)	226(57.7)	91 (43.5)	42 (53.2)
Maternal characteristics				
White Race	409 (91.1)	345 (90.3)	NA	64 (95.5)
Type of MAT: Methadone Buprenorphine	 440 (64.6) 241 (35.4)	 196 (50.0) 196 (50.0)	 212 (98.6) 3 (1.4)	 32 (43.2) 42 (56.7)
C- section delivery	197 (33.5)	139 (35.5)	29 (24.2)	29 (38.2)
Co-exposures				
Heroin	144 (24.4)	75 (19.4)	53 (40.2)	16 (22.2)
Cocaine	55 (9.0)	41 (10.7)	9 (5.9)	5 (6.6)
Benzodiazepines	74 (10.9)	57 (15.0)	11 (4.9)	6 (8.0)
SSRIs	92 (13.3)	50 (12.9)	33 (14.5)	9 (11.7)
Antipsychotics	55 (7.9)	35 (8.9)	16 (7.1)	4 (5.3)
Alcohol	29 (6.3)	23 (6.0)	NA	6 (8.2)
Tobacco	550 (79.5)	301 (77)	187(83.9)	62 (79.5)
Amphetamines	14 (2.4)	8 (2.0)	4 (3.3)	2 (2.6)

a Gestational Age; Categorical variables expressed as frequencies and %, continuous variables as mean and SD

2.3.2 Need for pharmacotherapy (PT)

The final model was derived using data from all study cohorts and yielded 7 predictors of the need for PT: gestational age, any maternal breast milk, type of maternal opioid for treatment of OUD (methadone vs buprenorphine), exposure to heroin, cocaine, benzodiazepines, and/or antipsychotic medications. Table 2.4 displays the results of the final model.

Except maternal breast milk exposure, all other predictor variables in the final model were associated with higher odds of requiring PT. Exposure to methadone was associated with higher odds of requiring PT compared to buprenorphine, (aOR: 1.57).

Table 2.4 Multivariable logistic regression model				
	Estimate	aORsa	95 % CIb	P-value
Intercept	-8.04			
Infant characteristics				
Gestational age (weeks)	0.21	1.23	1.08 – 1.41	0.003
Breast milk exposure	-0.58	0.56	0.39 - 0.79	<0.001
Maternal characteristics				
Type of MAT: Buprenorphine Methadone	 0.45	 Ref 1.57	 1.10-2.24	 0.01
Co-exposures				
Heroin	0.43	1.53	1.00 - 2.34	0.05
Cocaine	0.56	1.75	0.82 – 3.73	0.15
Benzodiazepines	1.59	4.89	2.34 – 10.21	<0.001
Antipsychotics	0.66	1.94	0.97 - 3.85	0.06
a adjusted Odds Ratios cAntipsychotics: included Zyprexa, Seroquel, Risperdal b confidence intervals				

2.3.3 Model Performance

The final model derived using data from all three cohorts had AUROC was 0.68 (95 % CI: 0.64 - 0.72; optimism corrected 0.65 via bootstrapping). This decrement in discrimination from 0.68 to 0.65 reflects a percent change of approximately 17 %

calculated as [(Validation C-statistic—0.5) −(Derivation C-statistic—0.5)]/ (Derivation C-statistic—0.5) x100[43]. A C- statistic of 0.7 to 0.8 is generally considered acceptable and 0.8- 0.9 considered excellent[44,45].

The model derived from the combination of Tufts and CCH cohorts achieved better discrimination with AUROC of 0.73 (Table 2.5) however it did not perform as well on external validation in TJU cohort (AUROC 0.65). Rest of the derivation cohorts had a C-statistic similar to the final model (Table 2.5).

Table 2.5 Model training and validation results

Training			Validation		
Cohorts	AUROC	95 % CI	Cohorts	AUROC	95 % CI
Tufts, CCH	0.73	0.64 – 0.78	TJU	0.65	0.56 – 0.72
TJU, CCH	0.67	0.60 – 0.73	Tufts	0.67	0.61 – 0.72
TJU, Tufts	0.68	0.64 – 0.73	CCH	0.64	0.51 – 0.76
Tufts, TJU, CCH are the three study cohorts. TJU = Thomas Jefferson University, CCH= Cape Cod Hospital					

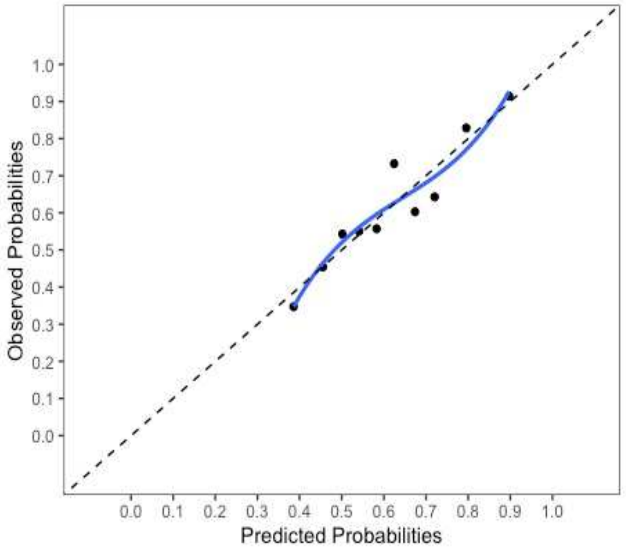

Figure 2.1: Calibration plot for the final model

The final model and the three training models appeared to calibrate well (Fig 2.1) however models showed poor calibration within the external validation cohorts except the one derived from the combination of TJU and Tufts Cohorts (Appendix, Fig

4.1-4.6). Calibration slope for the final model was 0.84 based on boot strap validation reflecting some overfitting.

2.4 Discussion

Our multicenter, pooled cohort observational study identified seven specific maternal and newborn clinical variables associated with NAS severity and need for PT. There is an urgent need to develop objective clinical tools to accurately predict NAS severity and need for PT to facilitate the optimal precision medicine approach for newborns with *in utero* opioid exposure. Our study has attempted to overcome the current limitations for establishing clinical utility of the existing predictive models such as validity, small sample size, data from a single center or claims based with variation in coding for NAS.

Of the two infant variables, gestational age (GA) significantly increased the odds of need for PT while maternal breast milk provision significantly decreased the odds of need for PT. The relationship between GA and NAS severity is not clearly understood. In a retrospective cohort study, Gibson et al failed to demonstrate a relationship between increasing GA and need for PT[46]. Another retrospective cohort analysis showed that preterm infants were less likely to require NAS PT, a finding consistent with our results[47]. There are several possible explanations for this that are biologically plausible. Premature infants are exposed for shorter durations during pregnancy and may have immature opioid receptors altering their symptomatology and hence their scores on FNASS. Nevertheless, this relationship needs to be explored further in future. Maternal breast milk exposure has consistently been associated with less severe expression of NAS and is a potentially modifiable predictor of the need for PT. Maternal breast milk not only

is well tolerated with less feeding intolerance in NAS but also may have trace amounts of maternal MAT medication, both of which can help reduce NAS severity. Additionally, while documented as maternal breast milk, many of these infants are also breastfed, which allows for reinforcement of non-pharmacologic care practices as skin-to-skin care and holding of the infant. Nonetheless it is important to note that provision of breast milk is impacted by need for transfer to higher level of care for severe withdrawal syndrome and need for treatment. Furthermore, in such cases rooming in can be hard to practice. Based on institutional guidelines, provision of maternal breast milk can be highly variable as well, due to specific eligibility criteria if illicit drug exposure is confirmed on drug screening.

There was no significant association between infant sex and need for PT in our model unlike some prior work showing male infants to have higher risk of receiving PT for NAS. In a recently published clinical predictive model, Singh et al reported association of need for PT with male sex[28]. While a clear plausible biological reason is not yet evident in literature, further research is warranted to explore this association[18].

Maternal medication assisted therapy (MAT) with methadone was associated with higher odds of requiring PT compared to buprenorphine which is consistent with existing literature and reinforces the accuracy of our model[19,24,28]. Other notable maternal exposures that were associated with higher odds of PT in the final model were use of heroin, benzodiazepines, and antipsychotic agents. Co -exposure to psychotropic agents is now increasingly recognized as contributory to severe NAS necessitating not only prolonged therapy but also use of second line agents[21,25]. A notable exclusion in the final model was SSRI exposure, since SSRIs are widely prescribed agents in pregnant mothers

with anxiety and depression and their use has been associated with increased severity of NAS in a recently published clinical predictive model[28]. In another recent study Bakhireva et al found that neonates co exposed to maternal opioids and SSRIs were >3 times more likely to need PT than those exposed to opioids alone[23]. Potential mechanisms underlying this severity of NAS profile remain unclear. Theoretically a drug- drug interaction between SSRIs and opioids could cause worse NAS. However, it has also been proposed that SSRIs could cause neurobehavioral alteration independently from opioid withdrawal that can artificially inflate NAS severity scores (designed specifically for opioid exposure)[48]. In short, to have a better understanding of the true association of individual psychotropic agents with NAS treatment, more research is needed with adequately powered, well designed studies. While the importance of addressing maternal mental health problems during pregnancy certainly cannot be denied, it is prudent to exercise caution when prescribing multiple psychotropic agents to pregnant women and there is need for establishing best practice guidelines in this regard.

Overall, our model is parsimonious utilizing seven predictors. The model discrimination was broadly consistent across the three derivation cohorts (AUROC 0.67-0.73) with only one model (Tufts, CCH) reaching threshold of good discrimination. As frequently observed[49,50] the discrimination on external validation was worse (AUROC 0.64-0.67) except one model (TJU, CCH). Nonetheless, the final model did not demonstrate acceptable level of discrimination for identifying opioid exposed infants at high risk of requiring PT (AUROC 0.68). Lack of data on some clinically important variables that resulted in their exclusion could have potentially contributed to a relatively modest discrimination, hence limiting its clinical utility. Variables of interest in this

regard include exposure to gabapentin and maternal race, data on which was either missing entirely across cohorts or had significant amount of missingness. Additionally, lack of accurate means of measurement of certain exposures also compounds prediction challenges e.g., alcohol which is dependent on self-report.

The internal-external validation demonstrated poor calibration within the external validation cohorts except the model derived from Tufts and TJU cohorts which was externally validated in Cape Cod Hospital cohort. Poor calibration likely reflects overfitting within derivation cohorts or could be attributed to unaddressed differences in eligibility criteria and outcome rates across the study cohorts.

This study to our knowledge is the first of its kind that utilized geographically diverse multicenter patient level data to predict the risk of PT in opioid exposed neonates. As a strength internal and leave-one-out validation enhanced the methodological rigor of the study. We do acknowledge several important limitations. First, data on certain exposures of interest was missing such as gabapentin (>50 % missingness) and was therefore not included in the modeling. Gabapentin is increasingly being prescribed to pregnant women and literature suggests worse outcomes in opioid exposed newborns with co-exposure to gabapentin. We were unable to study the association of maternal race with need for PT in our study because data on maternal race was missing entirely across some study cohorts. Nonwhite race however was underrepresented across all three study cohorts which is a consistent with prior studies. Likewise, data on alcohol use was also missing entirely for one cohort and was therefore excluded. Another notable covariate exclusion was amphetamine exposure. In our pooled data set exposure to amphetamine was fairly limited amongst pregnant women and due to nonevent rate being a limiting

factor for inclusion of predictors in the model, we decided to exclude amphetamine exposure. It should however be noted that co-exposure to amphetamine may be more common and therefore more relevant in some geographic locations. We also did not have data on exposures such as non-pharmacologic measures including rooming in practices and cuddling programs that are implemented across some units and have been associated with improved outcomes.

Second, there was fair heterogeneity across the study cohorts due to varying inclusion/exclusion criteria and wide variation in outcome frequency (54 -67%). This heterogeneity limits the inter-study comparability in terms of reported exposure rates and subsequent model performance and was not addressed during modeling. Finally, our model has not been validated in a fully independent external validation cohort with data not available at the time of prediction model development and as such is not suitable for reliable risk prediction in a broader opioid exposed newborn population.

Our study demonstrates that prediction of an infant's risk of requiring pharmacotherapy for NAS at the time of birth remains a challenging task. While several demographic and clinical factors including maternal exposures and infant characteristics exist that have been identified as predictive of PT, their predictive power at present is not sufficient to enable risk prediction at an individual patient level. Nevertheless, these frequently highlighted predictors need to be further investigated. With substantial increase in polysubstance use (licit and illicit) among pregnant women and unknown interactions among psychotropic agents and opioids in this patient population, it has become difficult to understand what precise drug combination substantially enhances risk of NAS in an individual infant. Better understanding of the biologic pathways in which

these drugs interact and are metabolized will help delineate exposures or combination of exposures that significantly increase risk of an infant being treated for NAS. Furthermore, challenges in prediction are also magnified by the lack of a gold standard definition for diagnosing NAS across clinical as well as research settings[51]. Majority of NAS definitions and therefore diagnosis is linked to infant's scores on modified versions of Finnegan NAS Scoring System or use of administrative coding data[52]. These scoring tools are inherently subjective and greatly influenced by inter- rater variability. This variation in NAS definition across centers and studies is likely to impact predictive model performance in external validation by limiting inter-study comparability in the event rates.

In summary, a dire need for identification of novel predictors continues to be felt and as such existing clinical predictive models are not sufficient to accurately and precisely determine which infants exposed to in utero opioids are at a high versus low risk of requiring treatment for NAS. In this regard genomic data and Polygenic Risk Score (PRS) can be a promising avenue to explore besides directing research efforts towards identification of metabolomic and proteomic biomarkers of interest.

2.4.1 Conclusion

In conclusion, results of our clinical predictive model were mostly consistent with existing clinical models in a geographically diverse cohort. Despite the methodological rigor, our clinical predictive model demonstrated modest discrimination of infants at high versus low risk of need for PT. Overall, our modeling results reflect inherent challenges with lack of an objective tool for diagnosing NAS, measuring clinically important variables and lack of a standard definition for NAS. Future research work should focus

on addressing issues with missing data on variables of clinical interest and external validation in a true independent cohort.

2.5 Collaboration

The statistical analysis was performed by Shawana Bibi in consultation with Jason Nelson and Janis Breeze. Manuscript was prepared by Shawana Bibi and reviewed and edited by Drs. Davis and Singh, Janis Breeze and Jason Nelson.

Chapter 3: Discussion

Timely and judicious use of pharmacotherapy in opioid exposed newborns with NAS is of paramount importance for clinicians as OUD continues to remain a major public health issue globally. Accurate stratification of opioid exposed newborns as high risk for requiring PT is needed to enable timely treatment with Morphine instead of waiting for days until the infants demonstrate signs of withdrawal. Accurate risk prediction is also likely to save millions of dollars in health care costs by decreasing hospital LOS for observation of these infants and potentially expedite recovery if timely treatment is initiated in high-risk individuals. However, existing clinical prediction models do not sufficiently describe risk for need of PT in NAS. Challenges to future development of such prediction tools include lack of a standardized and objective definition of NAS, lack of large sample sizes and incomplete understanding of the impact of polysubstance use in pregnant population on NAS. Research efforts need to be geared towards overcoming these challenges by developing large multicenter NAS data registries and exploring novel risk stratification tools.

3.1 Predicting need for pharmacotherapy in opioid exposed newborns

Our multicenter, pooled cohort observational study identified seven specific maternal and newborn clinical variables associated with NAS severity and need for PT. There is an urgent need to develop objective clinical tools to accurately predict NAS severity and need for PT to facilitate the optimal precision medicine approach for newborns with *in utero* opioid exposure. Our study has attempted to overcome the current limitations for establishing clinical utility of the existing predictive models such as

validity, small sample size, data from a single center or claims based with variation in coding for NAS.

Of the two infant variables, gestational age significantly increased the odds of need for PT while maternal breast milk provision significantly decreased the odds of need for PT. Maternal breast milk exposure has consistently been associated with less severe expression of NAS and is a potentially modifiable predictor of the need for PT. Maternal breast milk not only is well tolerated with less feeding intolerance in NAS but also may have trace amounts of maternal MAT medication, both of which can help reduce NAS severity. Additionally, while documented as maternal breast milk, many of these infants are also breastfed, which allows for reinforcement of non-pharmacologic care practices as skin-to-skin care and holding of the infant. Nonetheless it is important to note that provision of breast milk is impacted by need for transfer to higher level of care for severe withdrawal syndrome and need for treatment. Furthermore, in such cases rooming in can be hard to practice. Based on institutional guidelines, provision of maternal breast milk can be highly variable as well, due to specific eligibility criteria if illicit drug exposure is confirmed on drug screening.

There was no significant association between infant sex and need for PT in our model unlike some prior work showing male infants to have higher risk of receiving PT for NAS. In a recently published clinical predictive model, Singh et al reported association of need for PT with male sex[28]. While a clear plausible biological reason is not yet evident in literature, further research is warranted to explore this association[18].

Maternal medication assisted therapy (MAT) with methadone was associated with higher odds of requiring PT compared to buprenorphine which is consistent with

existing literature and reinforces the accuracy of our model[19,24,28]. Other notable maternal exposures that were associated with higher odds of PT in the final model were use of heroin, benzodiazepines, and antipsychotic agents. Co -exposure to psychotropic agents is now increasingly recognized as contributory to severe NAS necessitating not only prolonged therapy but also use of second line agents[21,25]. A notable exclusion in the final model was SSRI exposure, since SSRIs are widely prescribed agents in pregnant mothers with anxiety and depression and their use has been associated with increased severity of NAS in a recently published clinical predictive model28. In another recent study Bakhireva et al found that neonates co exposed to maternal opioids and SSRIs were >3 times more likely to need PT than those exposed to opioids alone23. Potential mechanisms underlying this severity of NAS profile remain unclear. Theoretically a drug- drug interaction between SSRIs and opioids could cause worse NAS. However, it has also been proposed that SSRIs could cause neurobehavioral alteration independently from opioid withdrawal that can artificially inflate NAS severity scores (designed specifically for opioid exposure)[48]. In short, to have a better understanding of the true association of individual psychotropic agents with NAS treatment, more research is needed with adequately powered, well designed studies. While the importance of addressing maternal mental health problems during pregnancy certainly cannot be denied, it is prudent to exercise caution when prescribing multiple psychotropic agents to pregnant women and there is need for establishing best practice guidelines in this regard.

Overall, our model is parsimonious utilizing seven predictors. The model discrimination was broadly consistent across the three derivation cohorts (AUROC 0.67-0.73) with only one model (Tufts, CCH) reaching threshold of good discrimination. As

frequently observed[49,50] the discrimination on external validation was worse (AUROC 0.64-0.67) except one model (TJU, CCH). Nonetheless, the final model did not demonstrate acceptable level of discrimination for identifying opioid exposed infants at high risk of requiring PT (AUROC 0.68). Lack of data on some clinically important variables that resulted in their exclusion could have potentially contributed to a relatively modest discrimination, hence limiting its clinical utility. Variables of interest in this regard include exposure to gabapentin and maternal race, data on which was either missing entirely across cohorts or had significant amount of missingness. Additionally, lack of accurate means of measurement of certain exposures also compounds prediction challenges e.g., alcohol which is dependent on self-report.

The internal-external validation demonstrated poor calibration within the external validation cohorts except the model derived from Tufts and TJU cohorts which was externally validated in Cape Cod Hospital cohort. Poor calibration likely reflects overfitting within derivation cohorts or could be attributed to unaddressed differences in eligibility criteria and outcome rates across the study cohorts.

3.2 Strengths and limitations

This study to our knowledge is the first of its kind that utilized geographically diverse multicenter patient level data to predict the risk of PT in opioid exposed neonates. As a strength internal and leave-one-out validation enhanced the methodological rigor of the study. We do acknowledge several important limitations. First, data on certain exposures of interest was missing such as gabapentin (>50 % missingness) and was therefore not included in the modeling. Gabapentin is increasingly being prescribed to pregnant women and literature suggests worse outcomes in opioid exposed newborns

with co-exposure to gabapentin. We were unable to study the association of maternal race with need for PT in our study because data on maternal race was missing entirely across some study cohorts. Nonwhite race however was underrepresented across all three study cohorts which is a consistent with prior studies. Likewise, data on alcohol use was also missing entirely for one cohort and was therefore excluded. Another notable covariate exclusion was amphetamine exposure. In our pooled data set exposure to amphetamine was fairly limited amongst pregnant women and due to nonevent rate being a limiting factor for inclusion of predictors in the model, we decided to exclude amphetamine exposure. It should however be noted that co-exposure to amphetamine may be more common and therefore more relevant in some geographic locations. We also did not have data on exposures such as non-pharmacologic measures including rooming in practices and cuddling programs that are implemented across some units and have been associated with improved outcomes.

Second, there was fair heterogeneity across the study cohorts due to varying inclusion/exclusion criteria and wide variation in outcome frequency (54 -67%). This heterogeneity limits the inter-study comparability in terms of reported exposure rates and subsequent model performance and was not addressed during modeling. Finally, our model has not been validated in a fully independent external validation cohort with data not available at the time of prediction model development and as such is not suitable for reliable risk prediction in a broader opioid exposed newborn population.

Our study demonstrates that prediction of an infant's risk of requiring pharmacotherapy for NAS at the time of birth remains a challenging task. While several demographic and clinical factors including maternal exposures and infant characteristics

exist that have been identified as predictive of PT, their predictive power at present is not sufficient to enable risk prediction at an individual patient level. Nevertheless, these frequently highlighted predictors need to be further investigated. With substantial increase in polysubstance use (licit and illicit) among pregnant women and unknown interactions among psychotropic agents and opioids in this patient population, it has become difficult to understand what precise drug combination substantially enhances risk of NAS in an individual infant. Better understanding of the biologic pathways in which these drugs interact and are metabolized will help delineate exposures or combination of exposures that significantly increase risk of an infant being treated for NAS. Furthermore, challenges in prediction are also magnified by the lack of a gold standard definition for diagnosing NAS across clinical as well as research settings[51]. Majority of NAS definitions and therefore diagnosis is linked to infant's scores on modified versions of Finnegan NAS Scoring System or use of administrative coding data[52]. These scoring tools are inherently subjective and greatly influenced by inter- rater variability. This variation in NAS definition across centers and studies is likely to impact predictive model performance in external validation by limiting inter-study comparability in the event rates.

In summary, a dire need for identification of novel predictors continues to be felt and as such existing clinical predictive models are not sufficient to accurately and precisely determine which infants exposed to in utero opioids are at a high versus low risk of requiring treatment for NAS. In this regard genomic data and Polygenic Risk Score (PRS) can be a promising avenue to explore besides directing research efforts towards identification of metabolomic and proteomic biomarkers of interest.

3.3 Conclusion

In conclusion, results of our clinical predictive model were mostly consistent with existing clinical models in a geographically diverse cohort. Despite the methodological rigor, our clinical predictive model demonstrated modest discrimination of infants at high versus low risk of need for PT. Overall, our modeling results reflect inherent challenges with lack of an objective tool for diagnosing NAS, measuring clinically important variables and lack of a standard definition for NAS. Future research work should focus on addressing issues with missing data on variables of clinical interest and external validation in a true independent cohort.

Chapter 4: Appendix

Internal - External Validation:

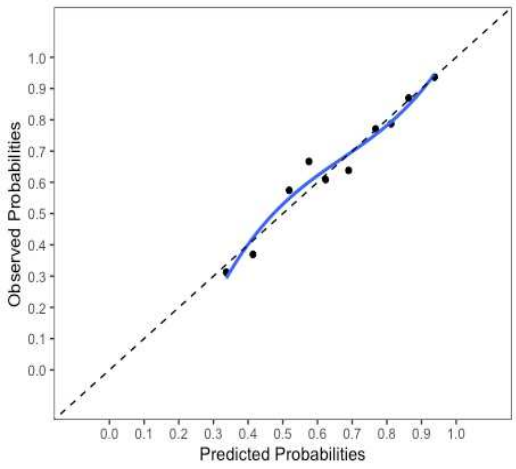

Figure 4.1: Calibration Plot for Model 1 derived from Tufts and Cape Cod Cohorts

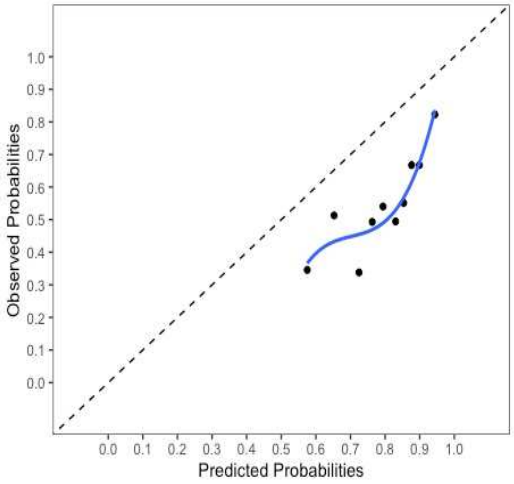

Figure 4.2: External Validation of Model 1 in TJU Cohort

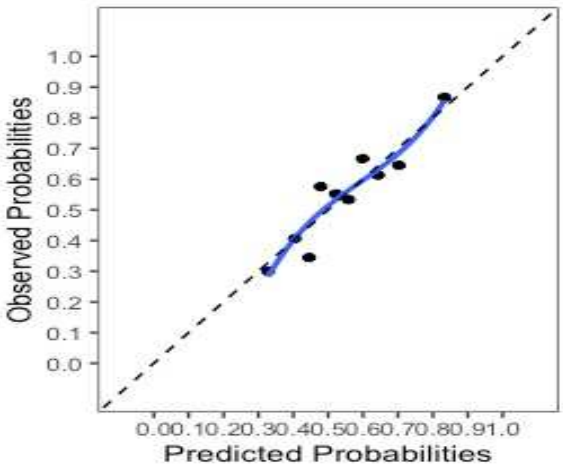

Figure 4.3: Calibration Plot for Model 2 derived from TJU and Cape Cod Cohorts

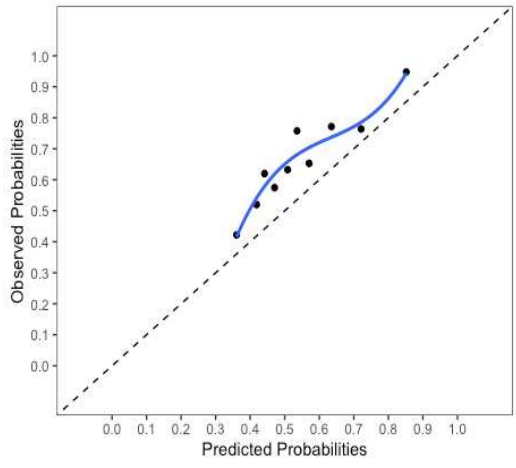

Figure 4.4: External Validation of Model 2 in Tufts Cohort

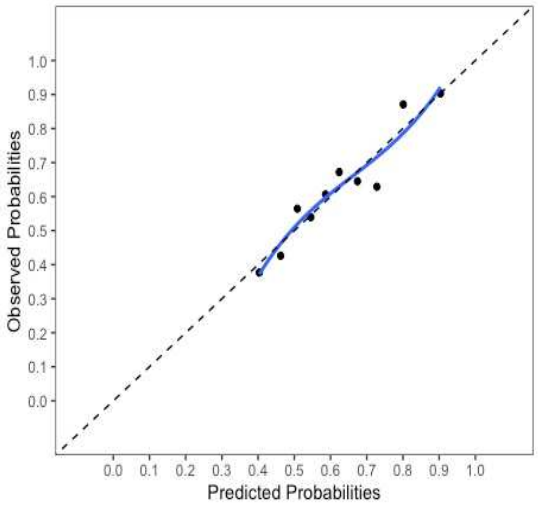

Figure 4.5: Calibration Plot for Model 3 derived from Tufts and TJU Cohorts

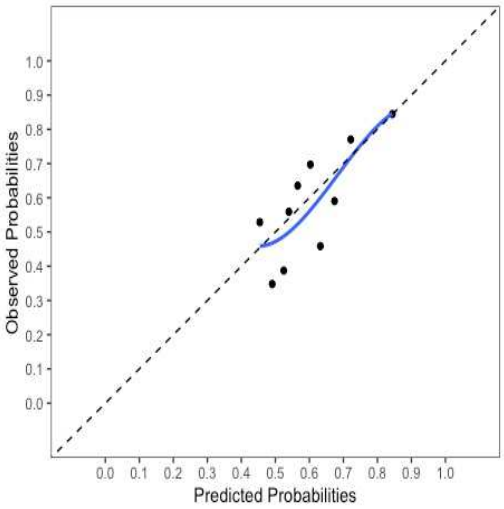

Figure 4.6: External Validation of Model 3 in Cape Cod Cohort

Chapter 5: Bibliography

1. Haight SC, Ko JY, Tong VT, Bohm MK, Callaghan WM. Opioid use disorder documented at delivery hospitalization: United States, 1999-2014. MMWR Morb Mortal Wkly Rep.2018;67(31):845-849.
2. U.S. Agency for Healthcare and Quality (AHRQ) Trends in neonatal abstinence syndrome births in the United States. Updated January 18, 2018. Accessed March 3,2020.https://www.hcupus.ahrq.gov/reports/Trends_NeonatalAbstinenceSyndrome_Births_UnitedStates.pdf
3. Patrick SW, Schumacher RE, Benneyworth BD, Krans EE, McAllister JM, Davis MM. Neonatal abstinence syndrome and associated health care expenditures: United States, 2000–2009. JAMA. 2012;307(18):1934–1940
4. Villapiano NLG, Winkelman TNA, Kozhimannil KB, Davis MM, Patrick SW. Rural and urban differences in neonatal abstinence syndrome and maternal opioid use, 2004 to 2013. JAMA Pediatr. 2017;171(2):194–196
5. Winkelman TNA, Villapiano N, Kozhimannil KB, Davis MM, Patrick SW. Incidence, and costs of neonatal abstinence syndrome among infants with Medicaid: 2004–2014. Pediatrics.2018;141(4): e20173520
6. Ko JY, Patrick SW, Tong VT, Patel R, Lind JN, Barfield WD. Incidence of neonatal abstinence syndrome - 28 states,1999–2013. MMWR Morb Mortal Wkly Rep. 2016;65(31):799–802
7. Jansson LM, et al. Neonatal abstinence syndrome. Pediatr Clin N Am 2019; 266:353-67. PMID: 30819342
8. Patrick SW, Barfield WD, Poindexter BB, AAP COMMITTEE ON FETUS AND NEWBORN, COMMITTEE ON SUBSTANCE USE AND PREVENTION. Neonatal Opioid Withdrawal Syndrome. Pediatrics.2020;146(5): e2020029074
9. Hudak ML, Tan RC; Committee on Drugs; Committee on Fetus and Newborn; American Academy of Pediatrics. Neonatal drug withdrawal. Pediatrics.2012;129(2).
10. Devlin LA, Davis JM. A practical approach to neonatal opiate withdrawal syndrome. Am J Perinatol. 2018;35(4):324-330. PMID: 29100261
11. Strahan AE, Guy GP, Bohm M, et al. Neonatal Abstinence Syndrome Incidence and Health Care Costs in the United States, 2016. JAMA Pediatr. 2020;174(2):200-202
12. Finnegan LP, Connaughton JF Jr., Kron RE, Emich JP. Neonatal abstinence syndrome: assessment and management. Addict Dis. 1975;2(1–2):141–158
13. Jones HE, Kaltenbach K, Heil SH, et al. Neonatal abstinence syndrome after methadone or buprenorphine exposure. The New England Journal of Medicine. 2010;363(24):2320–2331
14. Zimmermann-Baer U, Notzli U, Rentsch K, Bucher HU. Finnegan neonatal abstinence scoring system: normal values for first 3 days and weeks 5-6 in non-addicted infants. Addiction.2010;105(3):524–528. doi:10.1111/j.1360-0443.2009.02802

15. Grossman MR, Berkwitt AK, Osborn RR, et al. An Initiative to Improve the Quality of Care of Infants with Neonatal Abstinence Syndrome. Pediatrics. 2017;139(6). doi:10.1542/peds.2016-3360.
16. Wachman EM, Grossman M, Schiff DM, et al. Quality improvement initiative to improve inpatient outcomes for Neonatal Abstinence Syndrome. J Perinatol. 5 2018. doi:10.1038/s41372-018-0109-8.
17. Blount T. Decreasing length of stay and use of pharmacotherapy for neonates with NAS on an inpatient floor: a quality improvement initiative. In: Pediatric Academic Societies Toronto, Canada; 2018.
18. Charles MK, Cooper WO, Jansson LM, Dudley J, Slaughter JC, Patrick SW. Male sex associated with increased risk of neonatal abstinence syndrome. Hosp Pediatr. 2017;7(6):328-334. PMID: 28465360
19. Brogly SB, Saia KA, Walley AY, Du HM, Sebastiani P. Prenatal buprenorphine versus methadone exposure and neonatal outcomes: systematic review and meta-analysis. Am J Epidemiol. 2014;180(7):673- 686. PMID: 25150272
20. Jones HE, Heil SH, Tuten M, et al. Cigarette smoking in opioid-dependent pregnant women: neonatal and maternal outcomes. Drug Alcohol Depend. 2013;131(3):271-277. PMID: 23279924
21. Huybrechts KF, Bateman BT, Desai RJ, et al. Risk of neonatal drug withdrawal after intrauterine co- exposure to opioids and psychotropic medications: cohort study. BMJ. 2017;358: j3326. PMID: 28768628
22. MacMillan KDL, Rendon CP, Verma K, Riblet N, Washer DB, Volpe Holmes A. Association of rooming-in with outcomes for neonatal abstinence syndrome: a systematic review and meta-analysis. JAMA Pediatr. 2018;172(4):345-351. PMID: 29404599
23. Bakhireva, L. N. et al. Severity of neonatal opioid withdrawal syndrome with prenatal exposure to serotonin reuptake inhibitors. Pediatr Res. (2021). Epub ahead of print
24. Lemon LS, Caritis SN, Venketaramanan R, Platt RW, Bodnar LM. Methadone versus Buprenorphine for Opioid Use Dependence and Risk of Neonatal Abstinence Syndrome. Epidemiology. 2018 Mar;29(2):261-268.
25. Kanemura A, Masamoto H, Kinjo T, Mekaru K, Yoshida T, Goya H, Aoki Y. Evaluation of neonatal withdrawal syndrome in neonates delivered by women taking psychotropic or anticonvulsant drugs: A retrospective chart review of the effects of multiple medications and breastfeeding. Eur J Obstet Gynecol Reprod Biol. 2020 Nov; 254:226-230.
26. Patrick SW, Slaughter C, Harrell F, Hartmann K, Martin P, Dudley J, Stratton S, Cooper WO. Development and Validation of a Clinical Prediction Tool for Neonatal Abstinence Syndrome. Peer reviewed, platform presentation. Pediatric Academic Societies Annual Meeting. Toronto, Ontario. Canada. 2018
27. Isemann BT, Stoeckle EC, Taleghani AA, Mueller EW. Early Prediction Tool to Identify the Need for Pharmacotherapy in Infants at Risk of Neonatal Abstinence Syndrome. Pharmacotherapy.2017;37(7):840–848
28. Singh R, Houghton M, Melvin P, Wachman Elisha, Diop H, et al. Predictors of pharmacologic therapy for neonatal opioid withdrawal syndrome: a retrospective analysis of a statewide database. Journal of Perinatology (2021) 1381–1388

29. Wachman EM, Farrer LA. The genetics and epigenetics of neonatal abstinence syndrome. Semin Fetal Neonatal Med. 2019;24(2):105-110. PMID: 30709700
30. Smith AH, Jensen KP, Li J, et al. Genome-wide association study of therapeutic opioid dosing identifies a novel locus upstream of OPRM1. Mol Psychiatr 2017; 22:346–52.
31. Wachman EM, Hayes MJ, Brown MS, et al. Association of OPRM1and COMT single nucleotide polymorphisms with hospital length of stay and treatment of neonatal abstinence syndrome. J Am Med Assoc 2013; 309:1821–7.
32. Mactier H, McLaughlin P, Gillis C, Osselton MD. Variation in infant CYP2B6 genotype Associated with the need for pharmacologic treatment for neonatal abstinence syndrome in infants of methadone-maintained opioid-dependent mothers. Am J Perinatol 2017; 34:918–21.
33. Oei JL, Xu HX, Abdel-Latif ME, et al. Dopamine D2 receptor gene polymorphism in newborn infants of drug-using women. Arch Dis Child Fetal Neonatal 2012;97: F193–8.
34. Davis JM, Shenberger J, Terrin N, et al. Comparison of safety and efficacy of methadone vs morphine for treatment of neonatal abstinence syndrome: a randomized clinical trial. JAMA Pediatr. 2018;172(8):741-748. PMID: 29913015
35. Friedman H, Gregory P, et al. Pharmacologic treatment of infants with neonatal abstinence syndrome in community hospitals compared to academic medical centers. Journal of Perinatology (2018) 38:1651–1656
36. Kraft WK, Adeniyi□Jones SC, et al. Buprenorphine for the treatment of the Neonatal Abstinence Syndrome. N Engl J Med 2017; 376:2341-8.
37. Ogundimu EO, Altman DG, Collins GS. Adequate sample size for developing prediction models is not simply related to events per variable. J Clin Epidemiol. 2016 Aug; 76:175–182.
38. Wood AM, White IR, Royston P. How should variable selection be performed with multiply imputed data? Stat Med. 2008; 27:3227–46.
39. Austin PC, Lee DS, Ko DT, White IR. Effect of variable selection strategy on performance of prognostic models when using multiple imputation. Circ Cardiovasc Qual Outcomes. 2019 Nov;12(11): e005927.
40. Steyerberg EW, Harrell Jr FE, et al. Internal validation of predictive models: Efficiency of some procedures for logistic regression analysis. J Clin Epidemiol. (2001)774-781.
41. Martijn W Heymans (2021). psfmi: Prediction Model Pooling, Selection and Performance Evaluation Across Multiply Imputed Datasets. R package version 1.0.0. https://mwheymans.github.io/psfmi/
42. Steyerberg EW, Harrell Jr FE. Prediction models need appropriate internal, internal-external, and external validation. J Clin Epidemiol. 2016; 69:245-247.
43. Wessler BS, Ruthazer R, et al. Regional validation and recalibration of clinical predictive models for patients with acute heart failure. J Am Heart Assoc. 2017;6: e006121. doi: 10.1161/JAHA.117.006121
44. Schneeweiss S, Seeger JD, Maclure M, Wang PS, Avorn J, Glynn RJ. Performance of comorbidity scores to control for confounding in epidemiologic studies using claims data. Am J Epidemiol. 2001 Nov 1;154(9):854-64.

45. Hosmer DW, Lemeshow S. In: Applied logistic regression. 2nd ed. New York, NY: John Wiley & Sons, 2000.
46. Gibson KS, Stark S, Kumar D, Bailit JL. The relationship between gestational age and the severity of neonatal abstinence syndrome. Addiction. 2017 Apr;112(4):711-716.
47. Amiri S, Nair J. Gestational Age Alters Assessment of Neonatal Abstinence Syndrome. Pediatr Rep. 2022 Jan 28;14(1):50-57.
48. Lester B, Davis JM. Disarray in the perinatal management of neonatal abstinence syndrome. Pediatric Res (2022) 91:727–728
49. Venema E, Wessler BS, Paulus JK et al. Large - scale validation of prediction model risk of bias assessment Tool (PROBAST) using a short form: high risk of bias models show poorer discrimination. J Clin Epidemiol. 2021 Oct; 138:32
50. Wessler BS, Nelson J, Park JG et al. External Validations of Cardiovascular Clinical Prediction Models: A Large-scale Review of the Literature. Circ Cardiovasc Qual Outcomes. 2021 Aug; 14(8): e007858
51. Doherty KM, Scott TA et al. Evaluating Definitions for Neonatal Abstinence Syndrome. Pediatrics (2021) 147 (1): e2020007393.
52. Jilani SM, Jordan CJ, Jansson LM, Davis JM. Definitions of neonatal abstinence syndrome in clinical studies of mothers and infants: an expert literature review. J Perinatol.2021; 41(6): 1364–1371