Neurological Evaluation of Infants with Evidence of a Hypoxic-Ischemic Event treated with Therapeutic Hypothermia

Thesis by

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August 2017

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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Acknowledgements

There are so many people who merit recognition for their support throughout the realization of my Masters and during the completion of this thesis. Most importantly, I'd like to thank my supervisor Dr. Guilherme Sant'Anna for exposing me to the dynamic and complex domain of neonatology. With his help I was able to delve deeper into the scientific world and explore the many facets of clinical research. This Masters was a very enriching experience that presented me with a challenging and engaging working environment, which also stimulated me both professionally and academically.

I am especially grateful to Samantha Latremouille, for her guidance and input throughout my various Masters projects. Her positivity and advice throughout these two years undoubtedly made the process much more agreeable. I cannot thank her enough for imparting her knowledge about research, statistics, and the overall graduate studies process.

I'd also like to thank my fellow Masters student Monica Bhuller, research assistant Smita Rao, and PhD candidate Dr. Wissam Shalish for providing both a friendly and cooperative working environment.

Finally, I'd like to thank my loved ones and friends for their encouragement in the pursuit of my academic endeavors. Their guidance and never-failing support has given me the confidence to complete my graduate work and seek further study.

Preface and Contribution of Authors

This manuscript style thesis is formatted to include 5 chapters: Chapter 1 - overall introduction on the subject of all studies; Chapters 2 and 3 - two completed investigations with manuscripts in progress; Chapter 4 - prospective ongoing observational study and Chapter 5 - general conclusion.

Trainees and members of staff have participated in some studies: Chapters 1 and 2 - Dr. Alicia Lambrinakos-Raymond (neonatal perinatal medicine fellow) retrospectively collected data from the medical charts of approximately 30 patients. Samantha Latremouille - helped in the statistical analysis for chapter 1, participated in the setup of the apparatus used to collect data for the prospective study, and stepped in for the recruitment and enrollment of patients when I was unavailable. During the period of my Master's I performed: a) all data collection and analysis, b) abstracts preparations and poster presentations at international meetings and MUHC neonatal rounds, c) preparation of research proposal, REB submission, patients screening, enrollment and recordings and d) manuscripts drafts (edits in progress, not yet submitted for publication). Dr. Guilherme Sant'Anna maintained a supervisory role for all projects leading to the completion of my Masters, by giving guidance, directing the analyses and providing his input and edits for the abstracts, posters, presentation and manuscripts.

Abstract

Background: Whole body therapeutic hypothermia (TH) is the standard of care for infants with moderate or severe encephalopathy secondary to a hypoxic-ischemic insult. In infants with gestational age (GA) ≥ 36 weeks and birth weight (BW) ≥ 1800g a ladder approach is used to assess eligibility for the hypothermia treatment. This approach includes an initial identification of physiological criteria, followed by the presence of moderate or severe encephalopathy, which is classified according to the modified Sarnat exam. In our Institution this neurological evaluation should be performed by certified examiners using a standardized form at admission, and throughout the hypothermia treatment, including the day after rewarming. The Sarnat exam includes six categories, however, assessment of some of these categories are challenging. Specifically, evaluation of the autonomic nervous system (ANS) is subjective, can be affected by several variables, and therefore may not add much value to the final neonatal encephalopathy (NE) stage.

Objectives: The objectives of the studies included in this thesis are: 1) assess the adherence to the use of a standardized neurological form, 2) evaluate the contribution of each category of the modified Sarnat exam to the overall assessment of NE and 3) investigate a more comprehensive analysis of ANS and its association with the final NE stage and clinical outcomes.

Methods: All patients initiated on TH during the study periods were eligible. For the first two studies, data was retrospectively collected using a pre-defined data collection form to extract information related to completeness of the forms, as well as details of the neurological exam. Specific methodology was used for each of the first two studies to achieve the pre-defined

objectives. For the ongoing prospective study, new biological signals and technologies are being used to record data that will be analyzed when an adequate sample size is achieved.

Results: Adherence to the use of the standardized neurological form was sub-optimal as it decreased significantly from admission to day 3 of TH, with critical information severely under-reported. The autonomic nervous system (ANS) was the only category that was not significantly associated with the final NE stage at all time points. A total of 8 infants have been enrolled in the prospective study and important issues related to the methodology have been identified.

Conclusion: Although medical documentation is an important way to standardize care and audit practice, adherence to the use of a neurological form in infants with moderate or severe NE treated with TH was sub-optimal. Clearly, ongoing efforts need to be made to ensure a better adherence. Additionally, ANS evaluation using the modified Sarnat exam contributes poorly the final NE stage. Therefore, evaluation of the ANS may be rather imprecise and requires improvement, which is being investigated in the ongoing prospective study. Important limitations of the study design have been identified.

Résumé

Contexte: L'hypothermie thérapeutique du corps entier est la norme de soins pour les nourrissons présentant une encéphalopathie modérée ou grave secondaire à une insulte hypoxique-ischémique. Chez les nourrissons atteints d'âge gestationnel ≥ 36 semaines, et poids à la naissance ≥ 1800 g, une approche par étapes est utilisé pour évaluer l'admissibilité au traitement de l'hypothermie thérapeutique. Cette approche comprend une identification initiale des critères physiologiques, suivie de la présence d'une encéphalopathie modérée ou sévère, classée selon l'examen modifié de Sarnat. Dans notre institution, cette évaluation neurologique devrait être effectuée par des examinateurs certifiés, utilisant une forme standardisée à l'admission, et tout au long du traitement par l'hypothermie, y compris le lendemain du réchauffement. L'examen Sarnat comprend six catégories, mais l'évaluation de certaines de ces catégories est difficile. Plus précisément, l'évaluation du système nerveux autonome est subjective, peut être affectée par plusieurs variables et donc n'ajoute peut être pas beaucoup plus de valeur au stade final de l'encéphalopathie néonatale.

Objectives: Les objectifs des études incluses dans cette thèse sont: 1) évaluer l'adhésion à l'utilisation d'une forme neurologique standardisée, 2) évaluer la contribution de chaque catégorie de l'examen Sarnat modifié à l'évaluation globale de l'encéphalopathie néonatale et 3) enquêter une analyse plus complète du système nerveux autonome et son association avec le stade final de l'encéphalopathie néonatale et les résultats cliniques.

Méthodes: Tous les patients initiés sur l'hypothermie thérapeutique pendant les périodes d'étude étaient éligibles. Pour les deux premières études, les données ont été recueillies rétrospectivement à l'aide d'un formulaire de collecte de données prédéfini pour extraire

l'information relative à l'exhaustivité des formulaires, ainsi que des détails sur l'examen neurologique. Une méthodologie spécifique a été utilisée pour chacune des deux premières études pour atteindre les objectifs prédéfinis. Pour l'étude prospective en cours, de nouveaux signaux et technologies biologiques sont utilisées pour enregistrer des données qui seront analysées lorsqu'une taille d'échantillon adéquate est atteinte.

Résultats: L'adhésion à l'utilisation de la forme neurologique standardisée était sous-optimale car elle a diminué de manière significative de l'admission au jour 3 de l'hypothermie thérapeutique. De plus, les informations critiques étant gravement sous-déclarées. Le système nerveux autonome était la seule catégorie qui n'était pas significativement associée au stade final de l'encéphalopathie néonatale à tous les moments. Au total, 8 nourrissons ont été inscrits à l'étude prospective et des questions importantes liées à la méthodologie ont été identifiées.

Conclusions: Bien que la documentation médicale soit un moyen important de normaliser les soins et la pratique de l'audit, l'adhérence à l'utilisation d'une forme neurologique chez les nourrissons atteints de l'encéphalopathie néonatale modérée ou sévère traité avec l'hypothermie thérapeutique était sous-optimale. De toute évidence, des efforts continus doivent être faits pour assurer une meilleure adhésion. En outre, l'évaluation du système nerveux autonome à l'aide de l'examen Sarnat modifié contribue de manière insuffisante au stade final de l'encéphalopathie néonatale. Par conséquent, l'évaluation du système nerveux autonome peut être plutôt imprécise et nécessite une amélioration, ce qui est étudiée dans l'étude prospective en cours. Des limites importantes liées à la conception de l'étude ont été identifiées.

List of Abbreviations

NE – Neonatal Encephalopathy

DALYs – Disability Adjusted Life Years

HIE – Hypoxic Ischemic Encephalopathy

NO – Nitric Oxide

nNOS – Nitric Oxide Synthase

ROS – Reactive Oxygen Species

ACOG – American College of Obstetricians and Gynecologists

AAP – American Academy of Pediatrics

NICHD – National Institute for Child Health and Human Development

EEG – Electroencephalogram

ANS – Autonomic Nervous System

TH – Therapeutic Hypothermia

aEEG – Amplitude Integrated Electroencephalogram

NRN – Neonatal Research Network

MCH – Montreal Children's Hospital

NICU – Neonatal Intensive Care Unit

LOC – Level of Consciousness

SA – Spontaneous Activity

HR – Heart Rate

HRV – Heart Rate Variability

ECG – Electrocardiogram

BPU – Blood Perfusion Unit

BE – Base Excess

GA – Gestational Age

BW – Birth Weight

MAS – Meconium Aspiration Syndrome

PPHN – Persistent Pulmonary Hypertension of the Newborn

NEC – Necrotizing Enterocolitis

HOL – Hours of Life

HC – Head Circumference

HI – Hypoxia Ischemia

FHR – Fetal Heart Rate Decelerations

RDS – Respiratory Distress Syndrome

IR – Infrared

Ta – Axillary Temperature

Tes – Esophageal Temperature

SD – Standard Deviation

LDF – Laser Doppler Flowmetry

BSC – Light Backscatter

NPI^{TM} – Neurological Pupil Index^{TM}

Chapter 1 – Introduction

1.1 Neonatal Encephalopathy

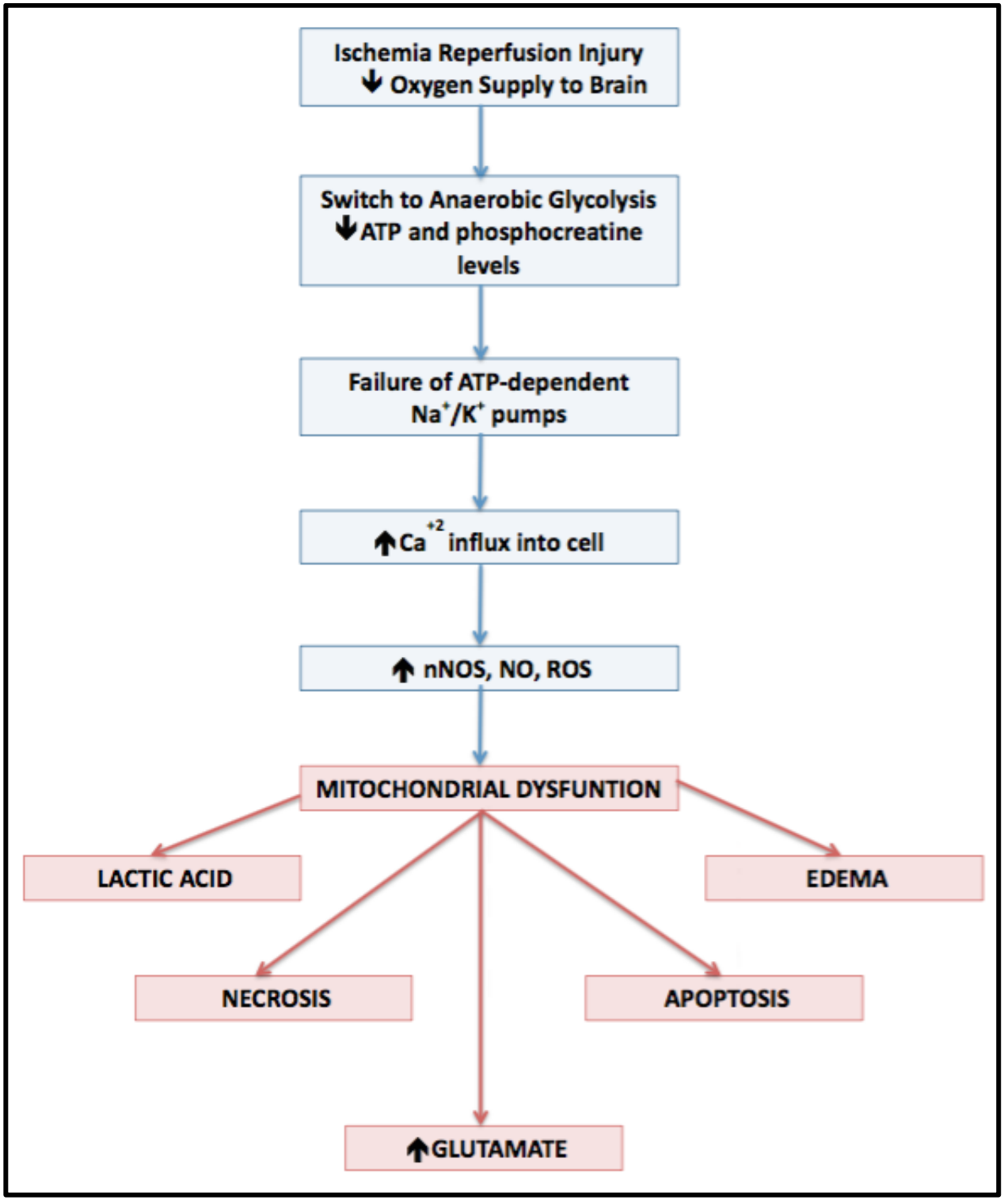
Globally, an estimated 1.8 to 7.7 infants per 1000 live term births suffer from neonatal encephalopathy (NE), which remains an important cause of neurodevelopmental disabilities such as cerebral palsy, and death (1-3). Indeed, NE is the third global cause of death for children under 5 years of age, and represents an important cause of disability-adjusted life years (DALYs) in child and adolescent populations (4). In Canada, between July 2015 and June 2016, approximately 392,902 infants were born alive (5) giving an estimated number of 700 to 3000 infants suffering from NE.

Approximately 30 to 50% of NE cases are secondary to perinatal asphyxia (1, 6, 7) which may occur due to complications of pregnancy, labor or delivery (**Table 1.1**) (8). Such events may result from disturbance of placental gas exchange with partial or complete lack of oxygenation (hypoxia) and/or reduction or cessation of blood flow (ischemia) to multiple organs, most importantly the brain (6, 8). Thus, following a hypoxic-ischemic insult, it is important to assess for the presence of brain involvement, known as hypoxic-ischemic encephalopathy (HIE), which may develop within the first 36 hours of age (9). HIE occurs mainly due to reperfusion injury that can be attributed to several mechanisms including an excitotoxic cascade of biochemical events (neuronal hyperexcitability) with a shift from oxidative to anaerobic metabolism (glycolysis), causing a drop in energy-containing metabolic substrates (**Fig. 1.1**) (1, 10). Ischemia reperfusion injury ultimately initiates the cascade, decreasing the brain's oxygen supply, ATP and phosphocreatine levels (1, 11-18). Next, there is a failure of the ATP-dependent Na+/K+ pumps which leads to Ca^{+2} entry, activation of nitric oxide synthase (nNOS), and

increases in nitric oxide (NO) and reactive oxygen species (ROS), essentially leading to mitochondrial dysfunction (1, 10). Consequently, there is a rise in lactic acid, apoptosis, necrosis, edema, and the excitatory neurotransmitter glutamate (1, 11-18). This prolonged glutamate exposure and hyperexcitability finally results in the development of variable degrees of encephalopathy (1).

The definition of perinatal asphyxia and identification of a hypoxic insult resulting in NE remains a major challenge that requires understanding of all the processes involved. Thus, the next chapters of this thesis will review the criteria used for definition of perinatal asphyxia and identification of a hypoxic insult resulting in NE.

Figure 1.1. *Mechanisms of neurotoxicity following ischemia-reperfusion injury* (1, 11-18).



1.2 Evidence of a Hypoxic-Ischemic Insult

The diagnosis of perinatal asphyxia is quite challenging since there is no single gold standard and most markers have very low specificity and sensitivity (19). According to the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP), "to determine the likelihood that an acute hypoxic–ischemic event that occurred within close temporal proximity to labor and delivery contributed to NE, it is recommended that a comprehensive multidimensional assessment be performed. This assessment should include the neonatal status and all the following potential contributing factors: maternal medical history, obstetric antecedents, intrapartum factors (including fetal heart rate monitoring results and issues relating to the delivery itself), and placental pathology" (19). The criteria used to define an acute intrapartum hypoxic event as sufficient to cause cerebral palsy based on the American College of Obstetricians and Gynecologists is shown in **Table 1.1**.

Therefore, based on the criteria defined by the National Institute of Child Health and Human Development (NICHD), in order to identify a perinatal hypoxic-ischemic event, the following ***physiological criteria*** are necessary: a) presence of significant fetal acidemia as demonstrated by a pH < 7.0 or base deficit > -16 (cord blood or blood gas done at < 1 h of life) or b) moderate fetal acidemia with a pH between 7.0 to 7.15 and base deficit between -10 to -16 (cord blood or blood gas done at < 1 h of life) in the context of an acute perinatal event and low Apgar scores (Apgar score < 5 at 10 min of life) or need for continuous assisted ventilation at 10 min of life (20).

There is a notable rationale behind the aforementioned physiological criteria. A direct correlation was noted between umbilical artery base deficit and the development of moderate or severe NE; high deficits were associated with greater likelihood of NE (**Table 1.2**) (21, 22). A

similar association was determined for the umbilical artery pH where lower pH values were correlated with greater chances of NE in comparison to other clinical dysfunctions (**Table 1.3**) (23). Moreover, the relative risk of neonatal death and neurological impairment increases in term infants with lower Apgar scores when a significant umbilical artery acidemia is also present (24).

Table 1.1. *Criteria used to define an acute intrapartum hypoxic event as sufficient to cause cerebral palsy based on the American College of Obstetricians and Gynecologists*

Essential Criteria (must meet all four) 1. Evidence of a metabolic acidosis in fetal umbilical cord arterial blood obtained at delivery (pH < 7 and base deficit ≥12 mmol/L) 2. Early onset of severe or moderate neonatal encephalopathy in infants born at 34 or more weeks of gestation 3. Cerebral palsy of the spastic quadriplegic or dyskinetic type 4. Exclusion of other identifiable etiologies, such as trauma, coagulation disorders, infectious conditions, or genetic disorders
Criteria that collectively suggest an intrapartum timing (within close proximity to labor and delivery, e.g. 0-48h) but are nonspecific to asphyxia insults: 1. A sentinel (signal) hypoxic event occurring immediately before or during labor 2. A sudden and sustained fetal bradycardia or the absence of fetal heart rate variability in the presence of persistent, late, or variable decelerations, usually after a hypoxic sentinel event when the pattern was previously normal 3. Apgar scores of 0-3 beyond 5 min 4. Onset of multisystem involvement within 72h of birth 5. Early imaging study showing evidence of acute nonfocal cerebral abnormality

Retrieved from American College of Obstetricians and Gynecologists, 2003 (25).

Table 1.2. *Umbilical artery base deficit in relation to the level of neonatal encephalopathy*

Neonatal Encephalopathy	**Umbilical Artery Base Deficit**		
	4-12 mmol/L (n = 116)	**12-16 mmol/L** (n = 58)	**>16 mmol/L** (n = 59)
None	89%	72%	39%
Mild	10%	19%	20%
Moderate	1%	7%	29%
Severe	0%	2%	12%

Adapted from Low J.A., et al, 1997 (22).

Table 1.3. *Umbilical artery pH in relation to the specific clinical dysfunction*

Clinical Dysfunction	**Umbilical Artery pH**			
	6.61-6.70	**6.71-6.79**	**6.80-6.89**	**6.90-6.99**
HIE	80%	60%	33%	12%
Renal	60%	53%	26%	16%
Cardiac	60%	60%	30%	18%
Pulmonary	80%	47%	30%	12%
None	20%	40%	48%	75%

Adapted from Low J.A., et al, 1992 (23).

1.3 Neurological Evaluation

After a hypoxic-ischemic event, a neurological evaluation should be performed to assess for the presence of encephalopathy. The clinical characteristics and classification of NE in infants with evidence of perinatal asphyxia were originally described by Sarnat and Sarnat over 40 years ago and have since been modified and adapted.

1.3.1 Original Sarnat Study

In 1976, Sarnat and Sarnat described a cohort of infants with evidence of perinatal asphyxia with the aim to provide a systematic clinical method for identifying the neurological signs that appear sequentially following the asphyxiated process, and to suggest a relationship between the duration of these signs and the prognosis of the neonate (26). They hypothesized that the postnatal course of the infant, together with electroencephalograph (EEG) changes would offer the best indication of future neurological impairment (26). Three distinct clinical staging levels of hypoxic-ischemic encephalopathy were described: Stage 1 (mild), Stage 2 (moderate) and Stage 3 (severe) (26). Stage 1 was distinguished by hyperalertness, uncontrolled primitive reflexes, sympathetic activation, normal or decreased spontaneous motor activity, and a normal EEG. This stage was usually brief (26). Stage 2 lasted on average 5 days and was characterized by hypotonia, obtundation (altered level of consciousness), strong flexion of the distal joints, and multifocal seizures, with a periodic pattern often introduced by continuous delta activity on the EEG (26). Lastly, infants in Stage 3 had marked depression of brain stem and autonomic functions, distinguished by infrequent periodic discharges on the EEG (26). Stage 3 lasted anywhere from a few hours to 4 weeks and infants were described as being stuporous and flaccid (26). The neurological characteristics used to distinguish the various stages of encephalopathy are listed in **Table 1.4**.

Table 1.4. *Distinguishing features of the three clinical stages of postanoxic encephalopathy in the full-term newborn infant*

Features	**Stage 1**	**Stage 2**	**Stage 3**
Level of consciousness	Hyperalert	Lethargic or Obtunded	Stuporous
Neuromuscular Control			
Muscle Tone	Normal	Mild Hypotonia	Flaccid
Posture	Mild Distal Flexion	Strong Distal Flexion	Intermittent Decerebration
Stretch Reflexes	Overactive	Overactive	Decreased or Absent
Segmental Myoclonus	Present	Present	Absent
Complex Reflexes			
Suck	Weak	Weak or Absent	Absent
Moro	Strong; low threshold	Weak; incomplete; high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tone neck	Slight	Strong	Absent
Autonomic Function	Generalized Sympathetic	Generalized Parasympathetic	Both Systems Depressed
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart Rate	Tachycardia	Bradycardia	Variable
Bronchial and Salivary Secretions	Sparse	Profuse	Variable
Gastrointestinal Motility	Normal or Decreased	Increased Diarrhea	Variable
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
Electroencephalogram Findings	Normal (awake)	Early: low-voltage continuous delta and theta	Early: periodic pattern with isopotential phases
		Later: periodic pattern (awake)	Later: totally isopotential
		Seizures: focal 1-to 1 1/2-Hz spike-and-wave	
Duration	Less than 24 hours	Two to 14 days	Hours to weeks

Retrieved from Sarnat H, Sarnat M, 1976 (26).

1.3.2 Neurological Features of Neonatal Encephalopathy

Level of consciousness was described mainly using *Plum and Posner's criteria* for the diagnosis of stupor and coma (27), taking into account that only certain features are applicable to neonates (26). Various levels of consciousness were observed: a state of *hyperalertness* was characterized by wide and unblinking eyes, full wakefulness (infants who did not sleep for 24 hours), and normal or decreased ***spontaneous motor activity*** (26). *Lethargy* was defined as complete but delayed responses to stimuli, decreased spontaneous movements, whereas *obtundation* was described as infants with delayed but incomplete responses, little or no motor activity (26). Finally, *stuporous* infants responded solely to strong noxious stimuli (26).

Characteristic ***posture*** was also observed. Abnormal posture included strong flexion of the distal joints, fingers and toes; with thumbs adducted and opposed across the palms (cortical thumbs), and flexed wrists (26). These abnormal postures were magnified with any type of stimulation and became more prominent in Stage 2 (26). A decerebrate posture characterized by a spine in opisthotonos (muscle spasm causing backward arching), extended elbows, protonated wrists, and abducted hips, was part of Stage 3 (26).

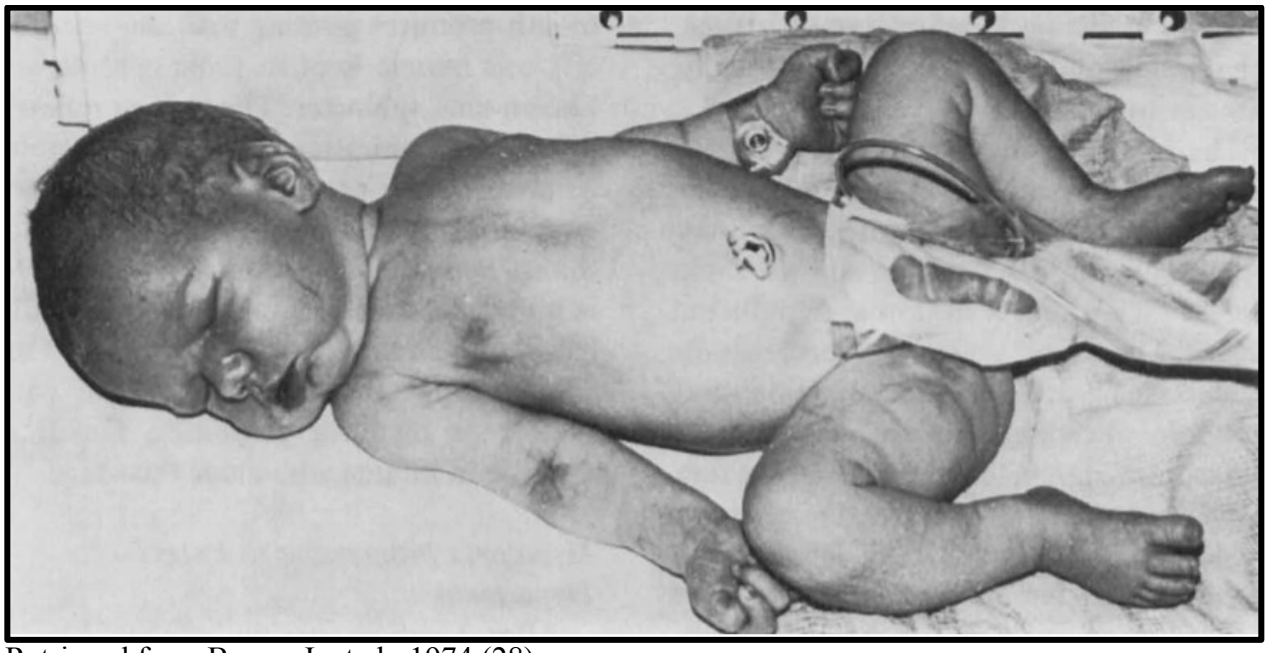
There were distinct patterns of muscle ***tone*** ranging from variable degrees of *hypotonia* (**Fig. 1.2**) to *hypertonia* (**Fig. 1.3**). *Hypotonic* infants were described as floppy, with absent recoil or adduction of their arms or legs, and no shoulder righting (28). In a hypotonic state, the heels of the infant could easily be placed behind the ears, the thighs were not flexed in a prone position but were abducted sideways, and finally the upper limbs were abducted at the side of the head or extended by the side (28). These infants were also generally apathetic and showed marked head lag on pulling to sit (28). Severe hypotonia manifests when brain stem reflexes are disconnected

from higher centers (28). The infant appeared flaccid with little or no spontaneous movement (28). Conversely, *hypertonic* infants exhibited trunk incurvation and became jittery and more alert (28). Hypertonia was more evident in the legs than arms, with both legs and toes becoming rigidly extended (spontaneous Babinski reaction) (28). 'Doggy paddling' movements in the arms may occur as well as cycling movements in the legs (28). A severely hypertonic infant increasingly became more rigid.

Furthermore, certain complex or ***primitive reflexes*** such as ***suck*** and ***Moro*** become weak, incomplete or absent as the stage of encephalopathy worsened. The *Moro reflex* or body-startle response is a seemingly spontaneous bilateral spreading and abduction of the upper extremities outward from the body, followed by their inward movement toward each other in the form of a bow (**Fig. 1.4**) (29). Concurrently, there is an extension of the spine, digits and lower extremities, and the infant may begin to cry (**Fig. 1.4**) (29). The response can be elicited in a number of ways: pulling the infant up and then releasing, light tactile stimuli to the trunk, or sudden loudness (26). The *suck reflex* occurs when the infant begins to suck after the roof of the mouth is touched.

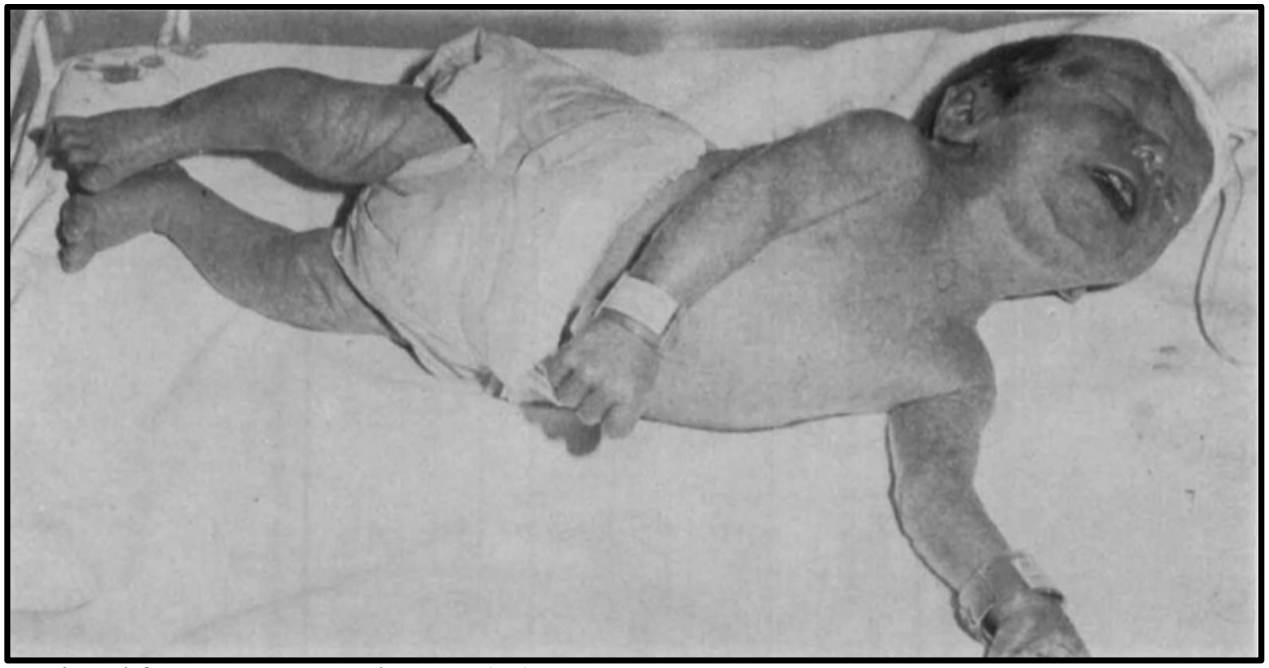
Lastly, there were numerous changes in the ***autonomic nervous system (ANS)*** that act to differentiate the various stages of NE (26). In general, stage 1 (mild NE) was characterized by activation of the sympathetic nervous system; increased alertness, dilation of the pupils (mydriasis), and increased heart rate (tachycardia) (26). Stage 2 (moderate NE), was distinguished by activation of the parasympathetic system; constricted pupils, decreased heart rate, and an increased amount of secretions, gastrointestinal motility, and peristalsis (26). A marked suppression of both the sympathetic and parasympathetic system was noted on stage 3 (severe NE) where pupils were constricted and responded poorly to light and infants were unable to regulate body temperature becoming hypothermic (26).

Figure 1.2. *Infant with neonatal encephalopathy exhibiting hypotonia. Note absent recoil with adduction of the arms and no shoulder righting.*



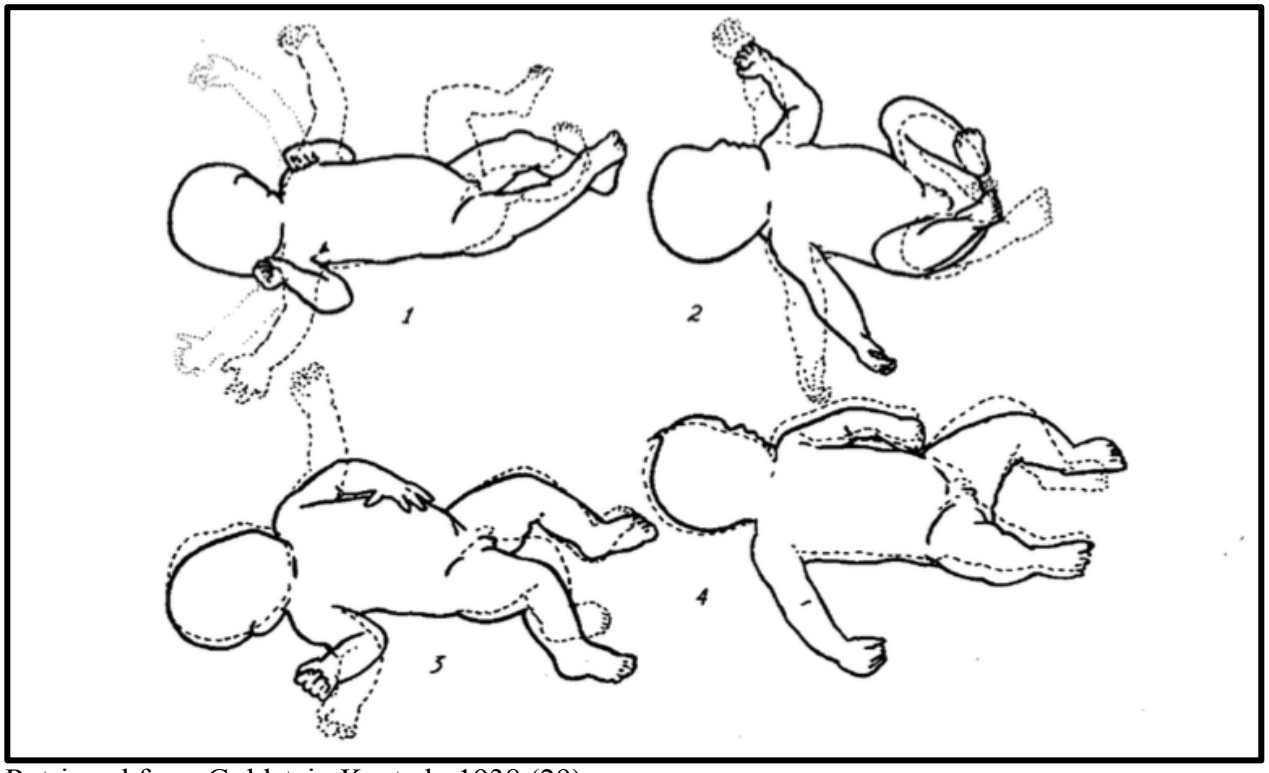
Retrieved from Brown J, et al., 1974 (28).

Figure 1.3. *Infant with neonatal encephalopathy exhibiting hypertonia. Note trunk incurvation with extension of the legs and arms.*



Retrieved from Brown J, et al., 1974 (28).

Figure 1.4. *Line drawings depicting the somatic movements in the several phases of the Moro reflex. Note that the first line drawing is the newborn phase (see text for full description of the Moro reflex in this phase). Notice a decrease in somatic movement activity, as the infant gets older (line drawings 2, 3, and 4 representing the 2nd, 3rd, and 4th month of life).*



Retrieved from Goldstein K, et al., 1938 (29).

1.4 Therapeutic Hypothermia: Eligibility and Neurological Evaluation

Following the positive results of animal experiments (30-39) mild to moderate hypothermia was tested by several randomized control trials in newborn infants with moderate or severe NE secondary to a hypoxic-ischemic insult. Selective head cooling with mild body hypothermia or whole body hypothermia were the two methods tested. In whole body hypothermia, the infant is placed on a blanket connected to a cooling system (Blanketrol II or III Hyper-Hypothermia system, Cincinnati Sub-Zero, Cincinnati, OH) and the esophageal temperature is decreased to 33.5 °C for 72 hours (20). Slow rewarming, at a rate of 0.5 °C per hour up to 36.5 °C, is done afterwards (20). Together, all the studies demonstrated a clear beneficial effect of this intervention. Therapeutic hypothermia (TH), if initiated within the first 6 h of life, increases the chances of survival without increasing the rates of neurodevelopmental disability (20, 40-44). Indeed, TH represents the most significant advancement for preventing reperfusion injury for infants at risk of developing brain injury (45) through its effects on multiple pathways in the biochemical cascade (**Table 1.5**) (46).

In those trials, infants were eligible for TH if there was evidence of a hypoxic-ischemic event (***physiological criteria***) followed by a neurological evaluation showing *moderate* or *severe* encephalopathy (***neurological criteria***) (20). However, there was some variability in the assessment/definition of NE between the trials (**Table 1.6**) (20, 40-44); 3 studies used a neurological exam and a amplitude integrated electroencephalogram (aEEG) classification (20, 40-44), whereas the other three used only the neurological examination.

The NICHD Neonatal Research Network (NRN) trial was the only study where examiners were certified on the performance of a modified Sarnat exam in order to provide a

standardized neurological evaluation as a basis for eligibility criteria (20). The use of a standardized neurological examination was later demonstrated to have significant clinical value at admission and over time (47-50). Indeed, serial standardized neurological examinations not only provided information on the evolution of encephalopathy during hospitalization, but were also shown to be good predictors of clinical outcomes (47-50). A severe NE stage on the neurological exam at 72 hours of life was shown to significantly increase the risks of death and/or disability (49).

Given the importance in performing an accurate neurological exam at admission (eligibility) and over time (prognosis) in infants treated with TH, the Neonatal Division of the Montreal Children's Hospital organized specific training to provide certification to all neonatologists. Following this workshop, a standardized form was developed to be used daily in all patients treated with TH.

Table 1.5. *Mechanism of action of therapeutic hypothermia post brain injury*

Mechanism of Action of Hypothermia	Explanation
Metabolic Changes	↓Cerebral Metabolic Rate ↓in core T→ ↓O_2 and glucose consumption, ↓CO_2 production (51, 52)
Apoptosis and Mitochondrial Dysfunction	Early blockage of apoptotic pathway: ↓mitochondrial dysfunction, caspase enzyme activation, excitatory neurotransmitters Modifies intracellular ion concentrations (51, 53-55)
Ion pumps and Neuroexcitotoxicity	↓Damage from the neuroexcitatory cascade (11-18, 51)
Inflammation	↓Inflammatory and immune responses ↓NO production ↓WCC and neutrophil/macrophage function (51, 56-58)
Free Radicals	↓Free radicals ↑Endogenous anti-oxidative mechanisms attenuate oxidative damage (15, 51, 59)
Acidosis and Cellular Metabolism	↓Toxic metabolite accumulation and acidosis ↑Cell membrane integrity and improves brain glucose metabolism which has positive effects on harmful processes such as ion pump failure, cellular hyperactivity and the mitochondrial dysfunction caused by HIE (51, 60, 61)
Blood Brain Barrier/ Vascular Permeability	↓Brain edema by ↓vascular permeability after IR injury (51, 62, 63) ↓ICP which leads to neurological injury (51, 52)
Vasoactive Mediators	Attenuates imbalances in the production of vasoactive substances such as endothelin and TxA_2 (vasoconstrictors) and prostaglandin I_2 (vasodilator) in order to maintain perfusion homeostasis (13, 51, 64)
Epileptic Activity	↓Epileptic activity which is neuroprotective (51, 65, 66)
Brain Temperature	↓Hyperthermia related adverse effects such as fever (51, 67-69)

Adapted from Moore EM, et al., 2011 (51).

Legend: ↓: decrease, →: leads to, T: temperature, O_2: oxygen, CO_2: carbon dioxide, NO: nitric oxide, WCC: white cell count, HIE: hypoxic-ischemic encephalopathy, IR: ischemia reperfusion, ICP: intracranial pressure, TxA_2: thromboxane A_2.

Table 1.6. *Specificities on the 6 major therapeutic hypothermia trials in HIE infants* (20, 40-44).

Trial	GA	ELIGIBILITY CRITERIA		
		Evidence of Birth Asphyxia	Clinical Evidence of Encephalopathy	aEEG or EEG Finding
1. Shankaran, 2005	**≥ 36 wks**	**1. Cord/1st-hr blood gas: pH ≤ 7, or base deficit ≥ 16** **OR** **2. Cord/1st-hr blood gas: pH 7.01-7.15, base deficit 10-15.9, or Not available** **PLUS** 1. acute perinatal event **PLUS** 2. Apgar at 10 min ≤ 5 or Continued assisted ventilation at 10 min of life	**Moderate or Severe Encephalopathy on Modified Sarnat score**	**None**
2. Gluckman, 2005	**≥ 36 wks**	**≥ 1 criteria of:** 1. Apgar at 10 min < 5 2. Continued assisted ventilation at 10 min of life 3. Cord/1st-hr blood gas: pH < 7 or base deficit ≥ 16 mmol/L	**Moderate to Severe Encephalopathy defined as =** Lethargy, stupor or coma + ≥ 1 of: 1. Hypotonia 2. Abnormal reflexes (oculomotor or pupils) 3. Absent or weak suck 4. Clinical seizures	**Abnormal background** OR **Seizure on aEEG**
3. Azzopardi, 2009	**≥ 36 wks**	**≥ 1 criteria of:** 1. Apgar at 10 min < 5 2. Continued assisted ventilation at 10 min of life 3. Cord/1st-hr blood gas: pH < 7 or base deficit ≥ 16	**Moderate to Severe Encephalopathy defined as =** Lethargy, stupor or coma + ≥ 1 of: 1. Hypotonia 2. Abnormal reflexes (oculomotor or pupils) 3. Absent or weak suck 4. Clinical seizures	**Abnormal background** OR **Seizure on aEEG**
4. Simbruner, 2010	**≥ 36 wks**	**≥ 1 criteria of:** 1. Apgar at 10 min < 5 2. Continued assisted ventilation at 10 min of life 3. Cord/1st-hr blood gas: pH < 7 4. Base deficit > 16 mmol/L at < 1 hr of life	**Moderate to Severe Encephalopathy defined as =** Lethargy, stupor or coma + ≥ 1 of: 1. Hypotonia 2. Abnormal reflexes (oculomotor or pupils) 3. Absent or weak suck 4. Clinical seizures	**Abnormal aEEG or EEG**
5. Zhou, 2010	**≥ 37 wks**	**All criteria of:** 1. Apgar at 1 min ≤ 3 **AND** at 5 min ≤ 5 2. Continued assisted ventilation at 5 min of life 3. Cord blood gas: pH < 7 **OR** base deficit ≥ 16	**Moderate to Severe Encephalopathy defined as =** Lethargy, stupor or coma + ≥ 1 of: 1. Hypotonia 2. Abnormal reflexes (oculomotor or pupils) 3. Clinical seizures	**None**
6. Jacobs, 2011	**≥ 35 wks**	**≥ 2 criteria of:** 1. Apgar at 10 min < 5 2. Continued assisted ventilation at 10 min of life 3. Cord pH < 7 **OR** 1st-hr blood gas: pH < 7 **OR** base deficit ≥ 12	**Lethargy, stupor, coma, abnormal tone, and/or seizures**	**None**

Legend: HIE: Hypoxic-Ischemic Encephalopathy, aEEG: amplitude integrated encephalogram, EEG: electroencephalogram, wks: weeks.

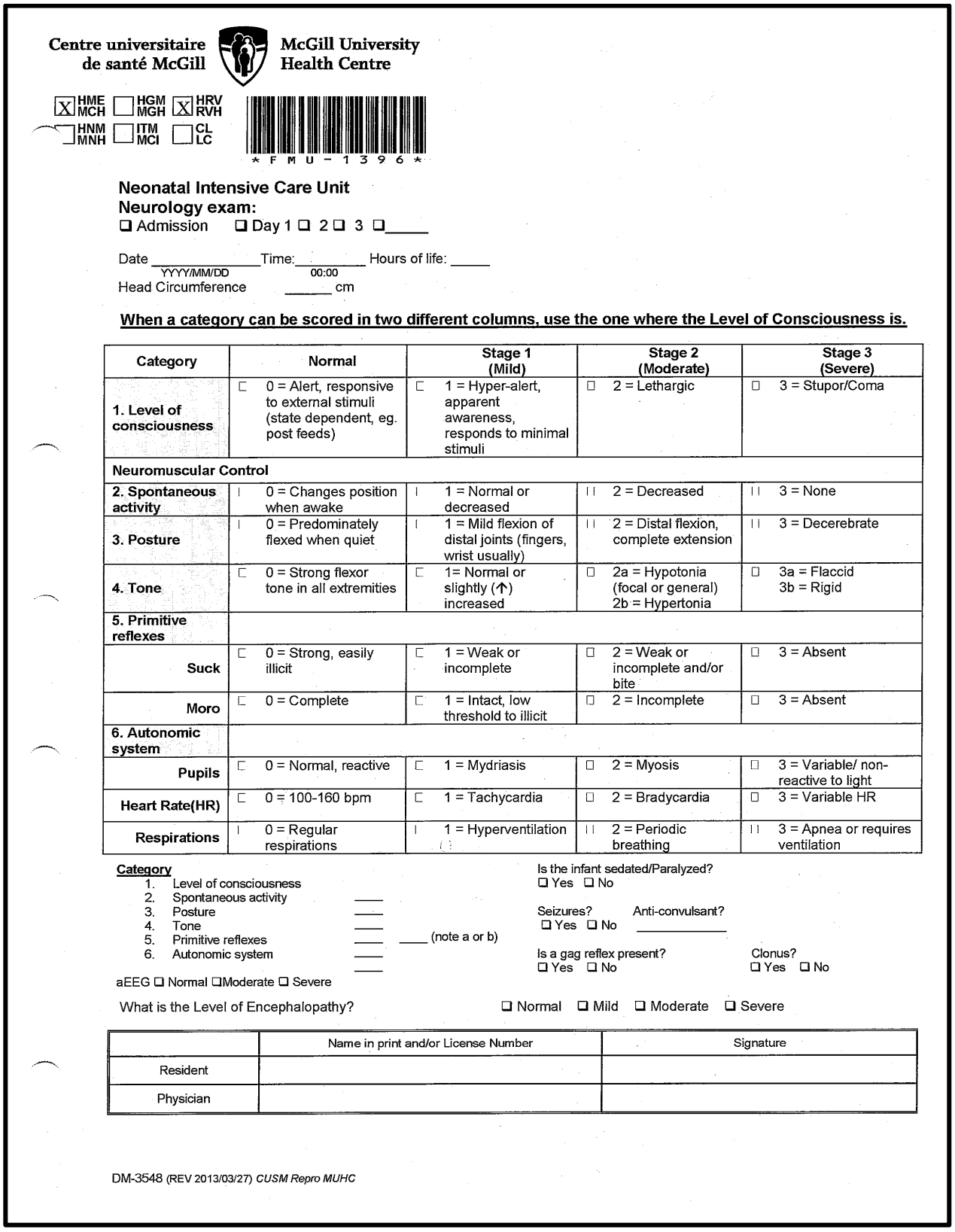
1.5 Standardized Neurological Exam Training & Form Implementation at the MCH

The neurological exam implemented at the Montreal Children's Hospital (MCH) Neonatal Intensive Care Unit (NICU) was based on the modified Sarnat exam developed by the NICHD (20). As part of a quality assurance process, training on the neurological exam was provided by the NICHD principal investigators through a specific workshop given in June 2013 to all neonatologists working in the neonatal unit. In this workshop all steps of the exam and the details on how to score each category were reviewed.

As part of a quality improvement initiative an updated form of the standardized exam (**Fig. 1.5**) was developed and implemented in July 2013. This form must be completed only by trained examiners and includes information on the modified Sarnat exam as well as date, time/day of the exam, use of medications, aEEG, name of the examiner and signature, and extended neurological features such as the presence of gag reflex and/or clonus. For the modified Sarnat exam, six **categories** are assessed separately: 1) level of consciousness (LOC), 2) spontaneous activity (SA), 3) posture, 4) tone, 5) primitive reflexes (suck and Moro), and 6) ANS, which is evaluated by assessments of pupil's size and reactivity, heart rate (HR) and respirations. A score of 0 (normal), 1 (mild), 2 (moderate), or 3 (severe) is given to each of the categories. Subcategories such as suck and Moro, and pupils, HR and respirations are also given scores. If subcategories receive different scores, the highest score is assigned to that respective category. The most frequent score from all 6 categories is then used to assign a final NE stage. If there are an equal number of categories for two different scores, the final NE stage is taken to be the score designated to the LOC. Despite the standardization of the modified Sarnat exam, it still has some issues, which require further improvement. As can be noted in the form, some categories are clear and easy to score whereas others are more subjective and prone to variability,

such as the evaluation of ANS. Furthermore, whether or not each category should carry the same weight on the contribution to the final NE stage is unknown.

Figure 1.5. *Standardized neurological exam form used at The Montreal Children's Hospital Neonatal Intensive Care Unit (NICU)*



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☒ HME MCH ☐ HGM MGH ☒ HRV RVH
☐ HNM MNH ☐ ITM MCI ☐ CL LC

* F M U – 1 3 9 6 *

Neonatal Intensive Care Unit
Neurology exam:
❑ Admission ❑ Day 1 ❑ 2 ❑ 3 ❑_____

Date _____ Time: _____ Hours of life: _____
YYYY/MM/DD 00:00
Head Circumference _____ cm

When a category can be scored in two different columns, use the one where the Level of Consciousness is.

Category	Normal	Stage 1 (Mild)	Stage 2 (Moderate)	Stage 3 (Severe)
1. Level of consciousness	☐ 0 = Alert, responsive to external stimuli (state dependent, eg. post feeds)	☐ 1 = Hyper-alert, apparent awareness, responds to minimal stimuli	☐ 2 = Lethargic	☐ 3 = Stupor/Coma
Neuromuscular Control				
2. Spontaneous activity	☐ 0 = Changes position when awake	☐ 1 = Normal or decreased	☐ 2 = Decreased	☐ 3 = None
3. Posture	☐ 0 = Predominately flexed when quiet	☐ 1 = Mild flexion of distal joints (fingers, wrist usually)	☐ 2 = Distal flexion, complete extension	☐ 3 = Decerebrate
4. Tone	☐ 0 = Strong flexor tone in all extremities	☐ 1= Normal or slightly (↑) increased	☐ 2a = Hypotonia (focal or general) 2b = Hypertonia	☐ 3a = Flaccid 3b = Rigid
5. Primitive reflexes				
Suck	☐ 0 = Strong, easily illicit	☐ 1 = Weak or incomplete	☐ 2 = Weak or incomplete and/or bite	☐ 3 = Absent
Moro	☐ 0 = Complete	☐ 1 = Intact, low threshold to illicit	☐ 2 = Incomplete	☐ 3 = Absent
6. Autonomic system				
Pupils	☐ 0 = Normal, reactive	☐ 1 = Mydriasis	☐ 2 = Myosis	☐ 3 = Variable/ non-reactive to light
Heart Rate(HR)	☐ 0 = 100-160 bpm	☐ 1 = Tachycardia	☐ 2 = Bradycardia	☐ 3 = Variable HR
Respirations	☐ 0 = Regular respirations	☐ 1 = Hyperventilation	☐ 2 = Periodic breathing	☐ 3 = Apnea or requires ventilation

Category
1. Level of consciousness
2. Spontaneous activity ____
3. Posture ____
4. Tone ____
5. Primitive reflexes ____ ____ (note a or b)
6. Autonomic system ____
____

aEEG ❑ Normal ❑Moderate ❑ Severe

Is the infant sedated/Paralyzed?
❑ Yes ❑ No

Seizures? Anti-convulsant?
❑ Yes ❑ No _____

Is a gag reflex present?
❑ Yes ❑ No

Clonus?
❑ Yes ❑ No

What is the Level of Encephalopathy? ❑ Normal ❑ Mild ❑ Moderate ❑ Severe

	Name in print and/or License Number	Signature
Resident		
Physician		

DM-3548 (REV 2013/03/27) *CUSM Repro MUHC*

1.6 Limitations of Current ANS Evaluation

Despite its potential in providing important information, no systematic evaluation of the ANS function in infants with NE secondary to a hypoxic-ischemic insult has been performed. The current methods of evaluation using pupils, heart rate, and respiratory assessments are limited and prone to significant intra and inter-examiner variability as detailed in the next sections.

1.6.1 Pupils

The pupil's evaluation is rather subjective and based on individual perception of the size (normal, constricted or dilated) and reactivity to the light stimuli (brisk, sluggish, or non-reactive). In neonates without encephalopathy, both pupils should be of relatively the same size (70) but size determination is imprecise and prone to error as the values are in millimeters. In adult studies, individual measurements of pupil size and reactivity suffer from inter-examiner variability up to 39% and thus are extremely inexact (71). In neonates this information is not available but can be even more problematic due to the smaller pupil size of this population and lack of cooperation during the exam. The terms used to describe the pupillary response are also quite arbitrary and generally applied in practice without a standard definition (70). Also, the *degree* and *velocity* of pupillary constriction have never been examined despite their potential to provide useful information. A normal pupillary reaction would occur when the pupil constricts in the presence of light and dilates when the light is removed (70, 72). Infants with severe brain damage generally experience variable pupil size that is unreactive in the presence of light (70, 72). Therefore, objective values of size and reactivity are measurements of clinical relevance in assessment of infants that have suffered from a hypoxic-ischemic insult.

1.6.2 Heart rate

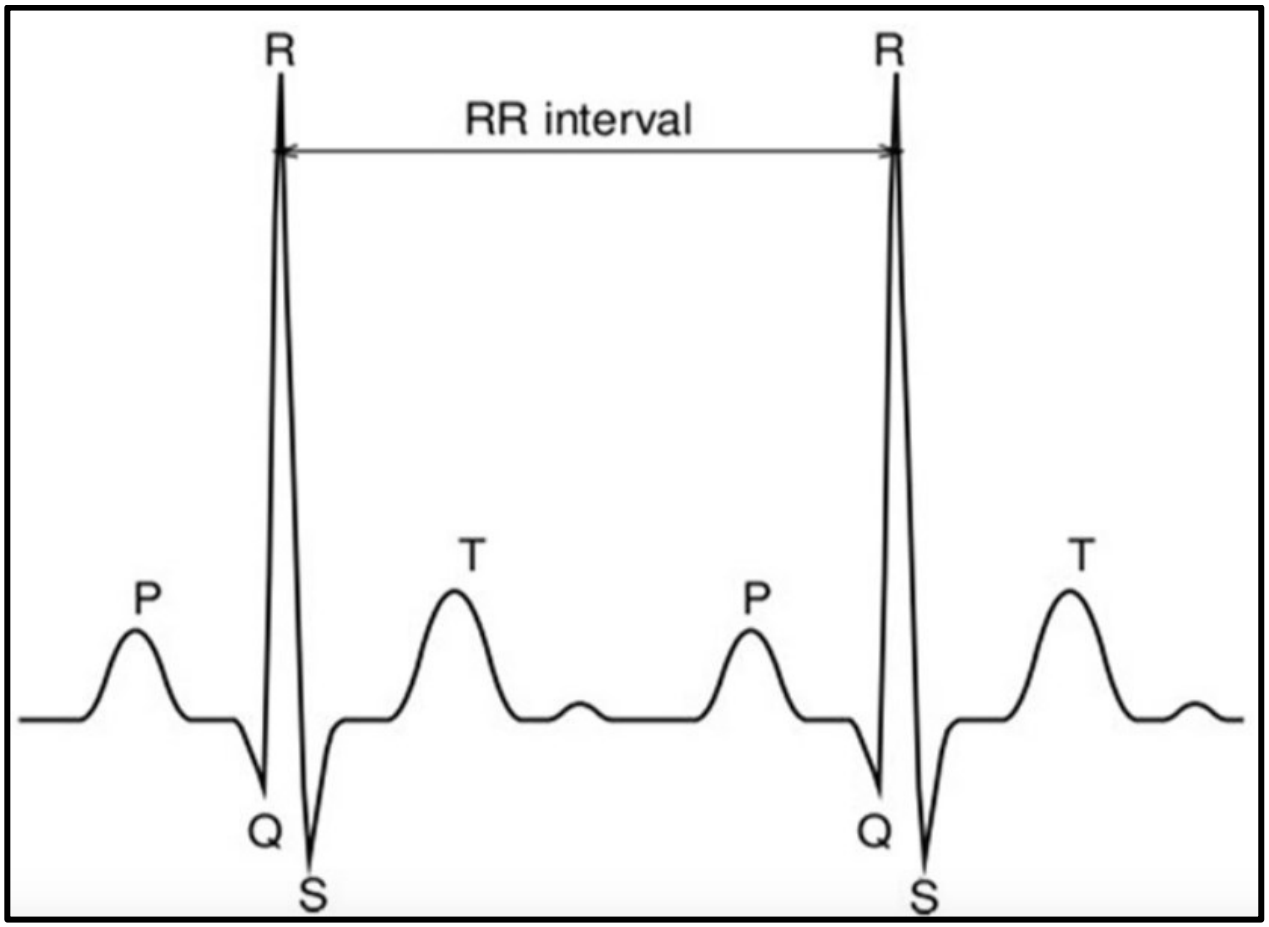
Heart rate assessment is based only on the *average* heart rate obtained from the bedside monitor during the neurological exam and classified as: normal (100-160 bpm), tachycardic (>160 bpm), and bradycardic (<100bpm) or irregular. However, the neural control of the human resting heart rate is significantly more complex, and as such requires a more in depth evaluation. Indeed, a *heart rate variability (HRV)* analysis offers a noninvasive evaluation of both the parasympathetic and sympathetic control of heart rate (73, 74). HRV analysis examines beat-to-beat fluctuations in heart rate during sinus rhythm, which is assumed to arise from fluctuations in the autonomic inputs to the heart (75). One example of a HRV measurement is the normalized-RR interval or NN interval, which estimates the magnitude of the variation in time between successive heartbeats (76). In other words, an RR interval is the interval between adjacent QRS complexes in a continuous electrocardiogram (ECG) recording, resulting from sinus node depolarization **(Fig. 1.6)** (77). The NN interval (normal to normal interval), results from the RR interval correction or normalization; essentially every RR interval that differs by a given percentage from the previous beat will be discarded in the HRV analysis (77).

ECG recordings of HRV accurately reflect the level of autonomic modulations to the heart rhythm, and therefore are considered good prognostic indicators in several pathological conditions (73, 75). Hypoxia-induced alterations in HR have been documented in animal models (78), as well as in adult human studies which reveal that normal cyclic changes in heart rate variability are decreased following traumatic brain injury (79). Indeed, HRV has also been investigated in adult cardiac arrest patients submitted to TH, and values were significantly higher in the hypothermia group when compared to controls (not cooled); loss of variability was

associated with increased neurological damage (75). Thus, in infants with NE, *heart rate variability* analysis may provide a more comprehensive assessment of ANS function.

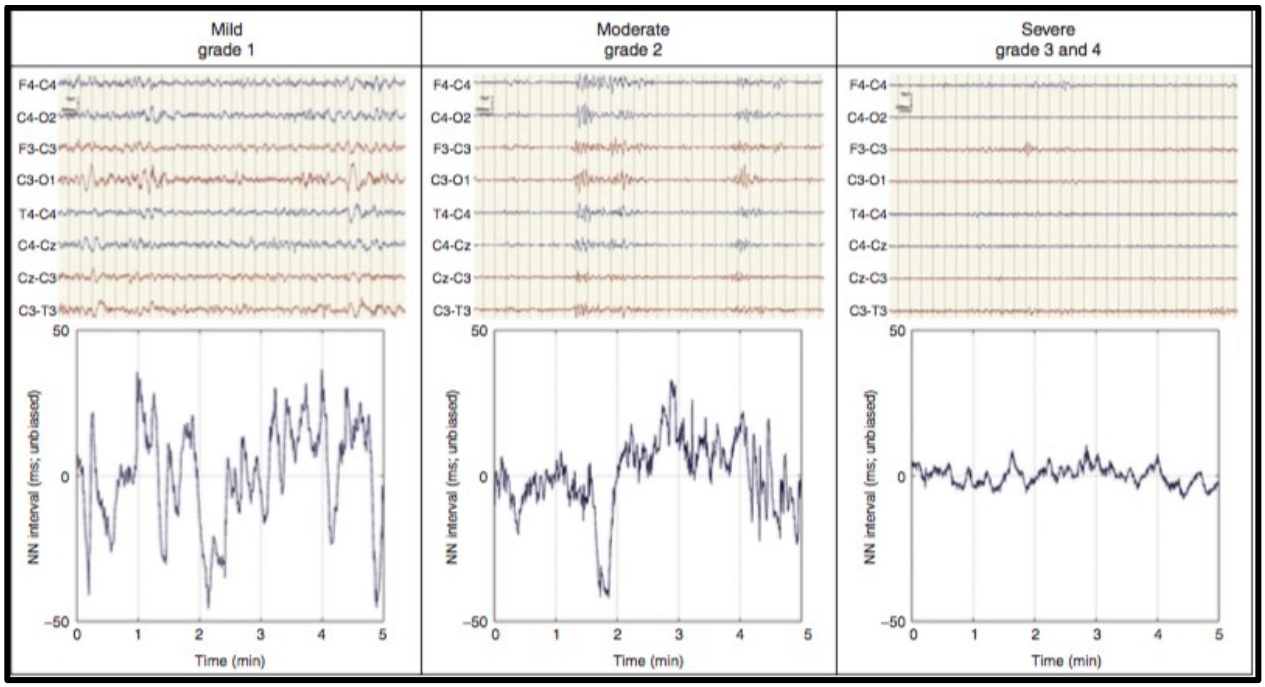
The value of HRV as a predictor of neurodevelopmental outcome has been evaluated (76, 80) and worsening levels of encephalopathy, graded by using EEG recordings, were associated with decreased HRV; in other words, there is a statistically significant negative correlation between HRV and NE severity as shown in **Fig. 1.7** (76).

Figure 1.6. *Diagram showing the RR interval between adjacent QRS complexes in a continuous ECG recording.*



Retrieved from Singh YN, et al., 2012 (81).

Figure 1.7. *NE severity in HIE infants and the corresponding HRV of recordings made prior to the initiation of TH. The inverse correlation between NE severity and HRV can be observed.*



Retrieved from Goulding RM, et al., 2015 (76).

1.6.3 Temperature Control

Temperature control, although an ANS function, was not included in the modified Sarnat exam. Temperature evaluation is quite difficult since several regulatory systems (thermogenesis, vasoconstriction mechanisms, sweating, and breathing rate), as well as external factors (ambient temperature) affect body temperature regulation (82). In addition, in infants submitted to whole body hypothermia, core temperature is artificially forced towards the target temperature; i.e. 33.5°C (20). Despite these limitations, a more complex temperature analysis may provide information on the ANS function.

Brain damage or illness may blunt the thermoregulatory response by decoupling or isolating the thermoregulatory system from its surroundings (82). For instance, in HIE infants not treated with TH, elevated body temperature was observed during the first 3 days of life and associated with greater degrees of brain injury (83). Moreover, significant oscillations of body temperature were also observed both before and during TH, suggesting that this may be an important marker of ANS dysfunction (20, 40).

Low levels of temperature variability or complexity have also been associated with poor prognosis in adult patients with multiple organ failure, and were linked with increased mortality in critically ill patients (84-86). Physiological signals fluctuate irregularly over time as part of normal biological processes. When a system is injured, input is reduced and processing restricted, simplifying the system's output. Consequently, assessing and quantifying temperature complexity in moderate or severe HIE infants, may provide significant information about ANS functioning.

1.6.4 Skin Perfusion

It has been recognized that in asphyxiated infants, changes in cutaneous blood flow is caused by a centralization of the fetal circulation or circulatory redistribution; this means that as a result of hypoxemia and asphyxia, blood flow is directed primarily to the brain, heart and adrenals, at the expense of the peripheral organs such as the lungs, carcass, skin and scalp. (87-92). Arterial chemoreceptor mechanisms increase sympathetic nervous system activity during asphyxia causing a widespread peripheral vasoconstriction through the release of various mediators (87, 93, 94). Therefore, the overall effect of TH on a moderately asphyxiated neonate would be a decrease on peripheral skin perfusion (95). However, when brain insult is more severe, cerebral auto regulation may be impaired leading to circulatory decentralization (52, 87, 95). Hence, infants with a more extensive brain damage may have an inability to regulate circulatory redistribution (52, 87, 95) and exhibit higher peripheral perfusion rates when compared to infants with moderate NE.

Skin perfusion is a very sensitive indicator of circulatory centralization since in comparison to any other peripheral organ, blood flow to the skin decreases to a greater extent under hypothermia (87). This makes skin perfusion an attractive measurement to assess neurological injury in neonates. Laser-Doppler Flowmetry is a continuous, non-invasive, real-time measurement of blood cell perfusion in the microcirculatory beds of the skin tissue (96). By illuminating the surface of the skin tissue, this technique is able to measure the amount of backscattered light, which is then converted into a continuous Laser Doppler signal computing the flux of the illuminated area (96). Blood perfusion in the skin is measured in blood perfusion units (BPU) (96). This method may provide more information regarding ANS function in infants with varying degrees of NE.

1.7 Thesis Objectives

The objectives of this thesis are threefold:

1. To assess the adherence to the use of a standardized neurological form required for the evaluation of infants with NE secondary to a hypoxic-ischemic insult at the Montreal Children's Hospital NICU.
2. To analyze the contribution of each category of the modified Sarnat exam to the overall assessment of NE in a cohort of patients treated with TH at the Montreal Children's Hospital NICU.
3. To investigate if a more comprehensive analysis of ANS function will improve its predictive value, i.e. the association between this category and clinical outcomes, including the final NE stage.

Chapter 2 – The neurological exam of infants treated with therapeutic hypothermia: Institutional adherence to a standardized neurological form

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Abstract submitted to the Pediatric Academic Societies Annual Meeting (Baltimore, 2016)

2.1 Objectives and Hypothesis

The primary objective of this study was to assess the institutional adherence to the use of a standardized neurological form (**Fig. 1.5**) developed and implemented at our Institution, at admission to the NICU, during TH treatment (days 1 to 3) and the day after re-warming (day 4).

Secondary objectives included assessment of the completion of all data included in the form: a) final NE stage, b) scores for each of the 6 Sarnat categories c) name and signature of the certified examiner, d) day, time and hours of life e) head circumference, f) use of sedation and/or other medications, g) seizures and treatment, h) gag reflex, i) clonus, j) aEEG interpretation. We also planned to perform an analysis to assess the reasons for the lack of adherence at the end of the TH treatment and evaluate if adherence changed over the first 2 years of implementation.

We hypothesized a good adherence at admission to the NICU (>90%) that would decrease during TH treatment (from day 1 to day 4), as a function of the severity of the NE stage. We also hypothesized that adherence would decrease from Year 1 to Year 2.

2.2 Materials and Methods

2.2.1 TH Protocol

A whole body TH protocol was implemented in our level III unit in September 2008. The eligibility criteria were those described by the National Institute of Child Health and Human

Development and included neonates with gestational age ≥ 36 weeks and birth weight ≥ 1800g. In order to qualify for TH, a ladder approach was adopted and included: a) physiological criteria - severe acidosis (pH < 7.0 or base deficit > -16), or moderate acidosis (pH = 7.0 to 7.15 and base deficit = -10 to -16) with history of an acute perinatal event and low Apgar scores (Apgar score < 5 at 10 min of life) or need for assisted ventilation (at 10 min of life), and b) moderate or severe NE according to a standardized neurological exam (modified Sarnat exam) (20). In eligible patients, TH was initiated within the first 6 hours of life by placing the neonate on a blanket connected to a Blanketrol II or III Hyper-Hypothermia system (Cincinnati Sub-Zero, Cincinnati, OH). Hypothermia was provided for 72 hours at a target esophageal temperature of 33.5 °C, followed by rewarming at a rate of 0.5 °C per hour up to 36.5 °C.

2.2.2 Neurological Exam

In our unit, the final NE stage is determined on admission by the neonatologist. Subsequent neurological evaluations are performed daily at 24, 48, and 72 hours of TH treatment, and after rewarming. As part of a quality assurance process, certification on the neurological exam was provided to all neonatologists through a specific training given in June 2013. This certification process has been detailed in the introduction section of this thesis.

2.2.3 Study Population

All patients that fulfilled the physiological criteria and were referred for evaluation of NE and possible TH were identified from the unit database for the period of two years immediately following the certification process: Year 1 (August 2013 to August 2014) and Year 2 (September 2014 to September 2015). The medical records of each patient were reviewed by two of the investigators (CS & AL) and clinical data was collected only from patients submitted to TH by

using a pre-defined data collection form. Information regarding the completeness of the form was also extracted.

2.2.4 Clinical data

The following clinical variables were collected: a) maternal characteristics: age, gravida, chronic maternal illness, gestational complications, fever during labor, GBS status, rupture of membranes, chorioamniotis, urinary tract infection, antepartum hemorrhage, placental abnormalities, b) perinatal: abnormal cardiotocography (fetal heart rate decelerations, prolonged fetal bradycardia, fetal tachycardia); cord accidents (prolapsed cord, cord rupture), and/or uterine rupture; shoulder dystocia; mode of delivery (C-section, vaginal, or vaginal with instrument); Apgar scores (1, 5 and 10 min); positive pressure ventilation, endotracheal intubation, need for chest compressions, use of epinephrine or other drugs required during resuscitation, and continuous need for resuscitation in the first 10 min of life; and pH, partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3) and base excess (BE) from umbilical cord or blood gas collected within the first hour of life; c) neonatal: date and time of birth, gestational age (GA), birth weight (BW), head circumference, sex, drugs used during TH, meconium aspiration syndrome (MAS), persistent pulmonary hypertension of the newborn (PPHN), and/or necrotizing enterocolitis (NEC).

Other collected data included information on EEGs (12-lead), brain MRI (performed at 10-30 days of life), gavage feeding (number of days on feeds during hospitalization or need of feeds or gastrostomy at discharge), length of hospital stay, and death during hospitalization.

2.2.5 Standardized Neurological Exam Form

The standardized neurological form contained the following detailed information concerning the neurological evaluation: a) final NE stage, b) each of the 6 Sarnat categories: level of consciousness, spontaneous activity, posture, tone, primitive reflexes (suck and moro), and ANS (pupils, heart rate, and respirations), c) name and signature of a certified examiner, d) date, time and hours of life (HOL) at the time of the exam, e) head circumference (HC), f) sedation and type of sedative used, g) seizures and medication used, h) presence of gag and/or clonus, and i) aEEG classification (**Fig. 1.5**). The final NE stage, the score of each of the 6 individual Sarnat categories, and the aEEG interpretation were given a score of 0, 1, 2, or 3 representing mild, moderate, and severe NE, respectively. A score of zero was normal, i.e. no encephalopathy or abnormalities on each of the categories. The aEEG background was classified as follows: a) continuous normal voltage (CNV, maximum voltage = 10 to 50 μV, and minimum voltage = 5 to 10 μV); b) discontinuous normal voltage (DNV, periods of low voltage below 5 μV, while upper border voltage is >10 μV); c) burst suppression (BS, periods of very low voltage [<5 μV] without variability intermixed with bursts of higher amplitude >25 μV); d) continuous low voltage (CLV, continuous background and maximum voltage <5 μV); or e) flat tracing (FT, inactive background and very low voltage [<5 μV]) (97, 98). The raw EEG was also reviewed for any evidence of seizures. Moreover, all information contained in the neurological form was also extracted for assessment of completeness. For each patient all data was obtained at 5 time points: at admission (before the initiation of TH), days 1 (D1), 2 (D2), and 3 (D3) of TH and after re-warming (D4). Ambiguous neurological forms were defined as those where the time point (admission, days 1 to 4) could not be determined with 100% certainty either due to lack or conflicting information about the day, time and/or hours of life.

2.2.6. Data Analysis

Adherence to the use of the standardized form was determined by the number of forms identified for each patient, at each of the 5 time points of the study period; a comparison was also made between Years 1 and 2. The level of completion for each variable in the forms was expressed as a percentage of the available forms.

To evaluate reasons of adherence to the neurological exam during TH treatment, the population demographics were compared between two groups of infants: a) with forms completed at the end of TH (day 4) and b) no form completed on day 4. Finally, to evaluate if adherence over time was associated with the stage of NE, the percentages of infants with a final NE stage of normal, mild, moderate, or severe were calculated for those two groups and compared over time (admission, and days 1 to 3 of TH).

2.2.7 Statistical Analysis

Descriptive statistical analysis was performed. Data was presented as n (%), mean ± SD, and median (interquartile range). For the primary and secondary outcomes, the Chi-square and Fisher's Exact tests were used to determine statistical significance where applicable.

2.3 Results

During the study period, a total of 144 neonates with evidence of a HI insult were referred for evaluation of NE and possible TH. Of these, 65 did not qualify for TH, and in 3 patients, medical charts could not be ret9rieved. Therefore, a total of 76 patients treated with TH were included in the study (**Fig. 2.1**). The population demographics are detailed on **Tables 2.1 and 2.2**.

Primary outcome. Adherence to the standardized neurological form at admission (before the initiation of TH), from D1 to D3 of TH, and on D4 is presented in **Table 2.3**. Due to the lack of completion of the forms, for the most part, it was unclear whether or not the evaluation on D4 was performed before or after re-warming. In addition, due to the lack of completion, and/or the errors associated with properly filling the day, time and hours of life, 7% of the forms were considered ambiguous.

At admission, neurological forms were completed in 75/76 (99%) patients. Surprisingly, of the completed neurological forms, a final NE stage was only reported in 57 (76%) patients, with 55 (73%) having the signature of the examiner. Moreover, aEEG classification was only reported in 26 (35%) patients on admission. Adherence with the completion of forms decreased significantly over time, from admission to D4 (**Fig. 2.2**). Indeed, on D4, neurological forms were filled in only 26 (37%) patients.

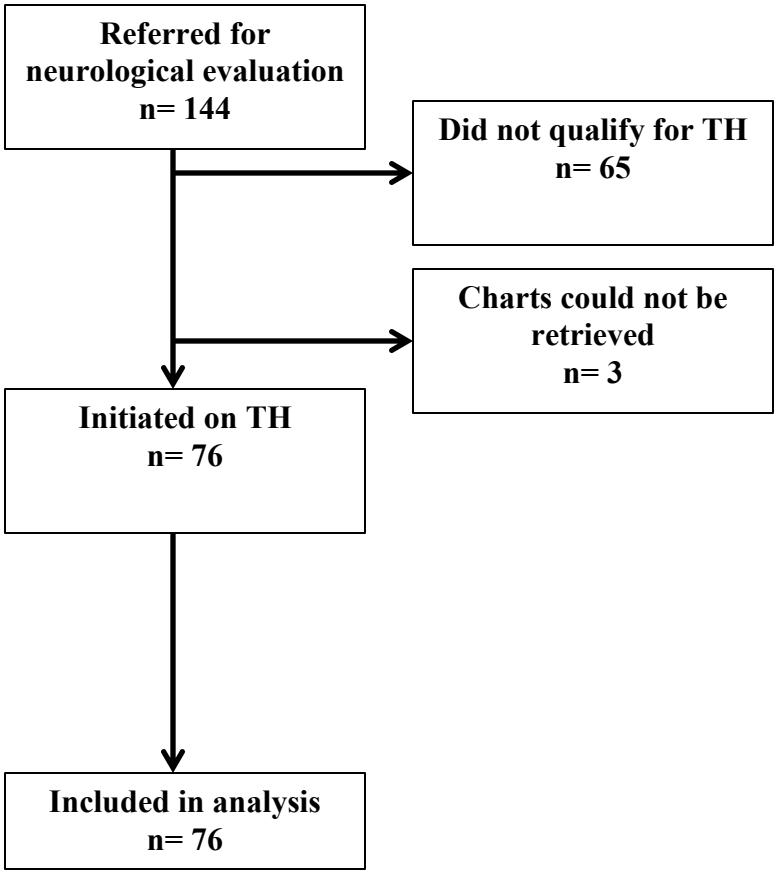
Secondary outcomes. A steady decrease over time was not observed for the final NE stage, signature of the examiner and aEEG classification, as the completion of these sections varied over time (**Fig. 2.3a**). A total of 279 forms were completed for the 5 time points (**Table 2.4**). The final NE stages were reported in 230 (82%); 163 (58%) of which included the signature of the examiner; and 145 (52%) incorporated an aEEG classification (**Fig. 2.3b**).

Of the six categories included in the Sarnat exam, primitive reflexes and ANS evaluation have significantly lower rates of completion when compared to the others (**Fig. 2.4)**. In addition, *all six categories* (each one of the 6 Sarnat categories, excluding the final NE stage) were filled in only 60% of the forms (**Fig. 2.3b**). The level of completion of additional information was also underreported (**Fig. 2.5**); hours of life was recorded in 170 (61%) and head circumference in 65 (23%) forms (**Table 2.4**).

No differences were observed in population demographics (**Tables 2.5 and 2.6**) between infants with a completed neurological form on D4 (n=26) and those without (n=50) except for the presence fetal heart rate decelerations, which was significantly higher in infants with completed forms (38% vs. 12%, $p<0.05$). Adherence to the neurological form on D4 was associated with the final NE stage on the previous day. Neonates with a worse NE stage tended to have a completed neurological form on Day 4 (**Fig. 2.7**). Of infants with a completed neurological form on D4, 5% had a final NE stage of 3 (severe) on D3, and 53% of 2 (moderate). No infants without a neurological form on D4 had a final NE stage of 3 on D3, and only 39% had a final stage of 2. Therefore, as hypothesized, completion of the neurological form on D4 was more likely to occur in infants with worse stages of NE on the previous day. These results however were not statistically significant.

Of the 76 infants, 31 were treated with TH on Year 1 and 45 on Year 2. The completion of the neurological forms decreased from Year 1 to 2 at each time point except at admission. However, results were not statistically significant (**Fig. 2.6**).

Figure. 2.1. *Flow diagram of patient enrollment*



Legend*:* TH: Therapeutic Hypothermia

Table 2.1. *Maternal Demographics*

Maternal Age	30.5 +/- 5.8 [66] (min 17 max 42)
Gestation	
First	35/75 (47)
Second	16/75 (21)
Third	17/75 (23)
Fourth	5/75 (7)
Fifth	1/75 (1)
Seventh	1/75 (1)
Maternal Illness	
Diabetes Mellitus	3/76 (4)
Hypertension	2/76 (3)
Both	1/76 (1)
Neither	70/76 (92)
Gestational Complications	
Pre-eclampsia	1/76 (1)
Gestational Diabetes	1/76 (1)
Both	1/76 (1)
Neither	73/76 (96)
Maternal Fever	9/76 (12)
GBS Status	
Positive	21/76 (28)
Negative	39/76 (51)
Unknown	16/76 (21)
Premature Prolonged ROM	0/76 (0)
Prolonged ROM	8/76 (11)
Chorioamnionitis	
Yes	5/76 (7)
No	70/76 (92)
Unknown	1/76 (1)
Urinary Tract Infection	2/76 (3)
Antepartum Hemorrhage	
Placenta Previa	0/76 (0)
Placenta Abruption	9/76 (12)
Both	1/76 (1)
Neither	66/76 (87)
Placental Abnormalities	
Velamentous Cord Insertion	1/76 (1)
Low Lying Placenta	1/76 (1)
None	74/76 (97)
Cord Accidents	
Prolapsed Cord	5/76 (7)
Cord Rupture	0/76 (0)
Velamentous Cord Insertion	1/76 (1)
Nuchal Cord	8/76 (11)
None	62/76 (82)
Uterine Rupture	4/76 (5)

Results are presented as mean +/- SD [n] (min, max) or n/N (%).
Legend: ROM: Rupture of Membranes

Table 2.2. *Neonatal Characteristics*

	Total	Year 1	Year 2
Outborn Delivery	75/76 (99)	31/31 (100)	44/45 (98)
Male Sex	38/76 (50)	14/31 (45)	24/45 (53)
Gestational Age (weeks)	39.1 +/- 1.60 [76]	39.1 +/- 1.59 [31]	39.1 +/- 1.63 [45]
Birth Weight (g)	3364 +/- 620 [76]	3188 +/- 568 [31]	3485 +/- 631 [45]
Head Circumference (cm)	34.4 +/- 1.63 [65]	34.2 +/- 1.51 [24]	34.6 +/- 1.70 [41]
Length of Stay (days)	19.6 +/-27.6 [76]	20.2 +/-32.0 [31]	19.1 +/-24.5 [45]
Abnormal Cardiotocography			
FHR Decelerations	16/76 (21)	7/31 (23)	9/45 (20)
Prolonged Fetal Bradycardia	12/76 (16)	7/31 (23)	5/45 (11)
Fetal Tachycardia	4/76 (5)	2/31 (7)	2/45 (4)
FHR Decelerations & PFB	6/76 (8)	3/31 (10)	3/45 (7)
FHR Decelerations & Fetal Tachycardia	4/76 (5)	0/31 (0)	4/45 (9)
Other	6/76 (8)	2/31 (7)	4/45 (9)
None	28/76 (37)	10/31 (32)	18/45 (40)
Shoulder Dystocia	6/76 (8)	1/31 (3)	5/45 (11)
Mode of Delivery			
C-Section	40/72 (56)	16/30 (53)	24/42 (57)
Spontaneous Vaginal	23/72 (32)	10/30 (33)	13/42 (31)
Vaginal with Instrumentation	9/72 (13)	4/30 (13)	5/42 (12)
APGAR Scores			
1 min	1 [1-2]	1 [1-2]	1 [0-2]
5 min	3 [2-5]	3 [1.5-5]	4 [2-5]
10 min	5 [3-6]	4 [2-5.3]	5 [4-6]
Interventions in the first 10 min of life			
Positive pressure ventilation	61/76 (80)	22/31 (71)	39/45 (87)
Intubation	42/76 (55)	21/31 (68)	21/45 (47)
Chest Compressions	25/76 (33)	9/31 (29)	16/45 (36)
Epinephrine	11/76 (15)	5/31 (16)	6/45 (13)
Need for resuscitation	47/76 (62)	25/31 (81)	22/45 (49)
Other drugs required during resuscitation	8/76 (11)	2/31 (7)	6/45 (13)
Cord blood gas or blood gas in the 1st h of life			
pH	7.06 +/- 0.18 [76]	7.06 +/- 0.18 [31]	7.05 +/- 0.18 [45]
PCO_2	59.5 +/- 25.8 [66]	54.4 +/- 24.7 [28]	63.3 +/- 26.3 [38]
HCO_3	15.2 +/- 5.10 [66]	15.6 +/- 5.85 [29]	15.0 +/- 4.48 [37]
Base Excess	-12.7 +/- 7.78 [62]	-13.4 +/- 6.70 [23]	-12.3 +/- 8.41 [39]
Meconium Aspiration Syndrome	20/76 (26)	6/31 (19)	14/45 (31)
Persistent Pulmonary Hypertension	17/76 (22)	8/31 (26)	9/45 (20)

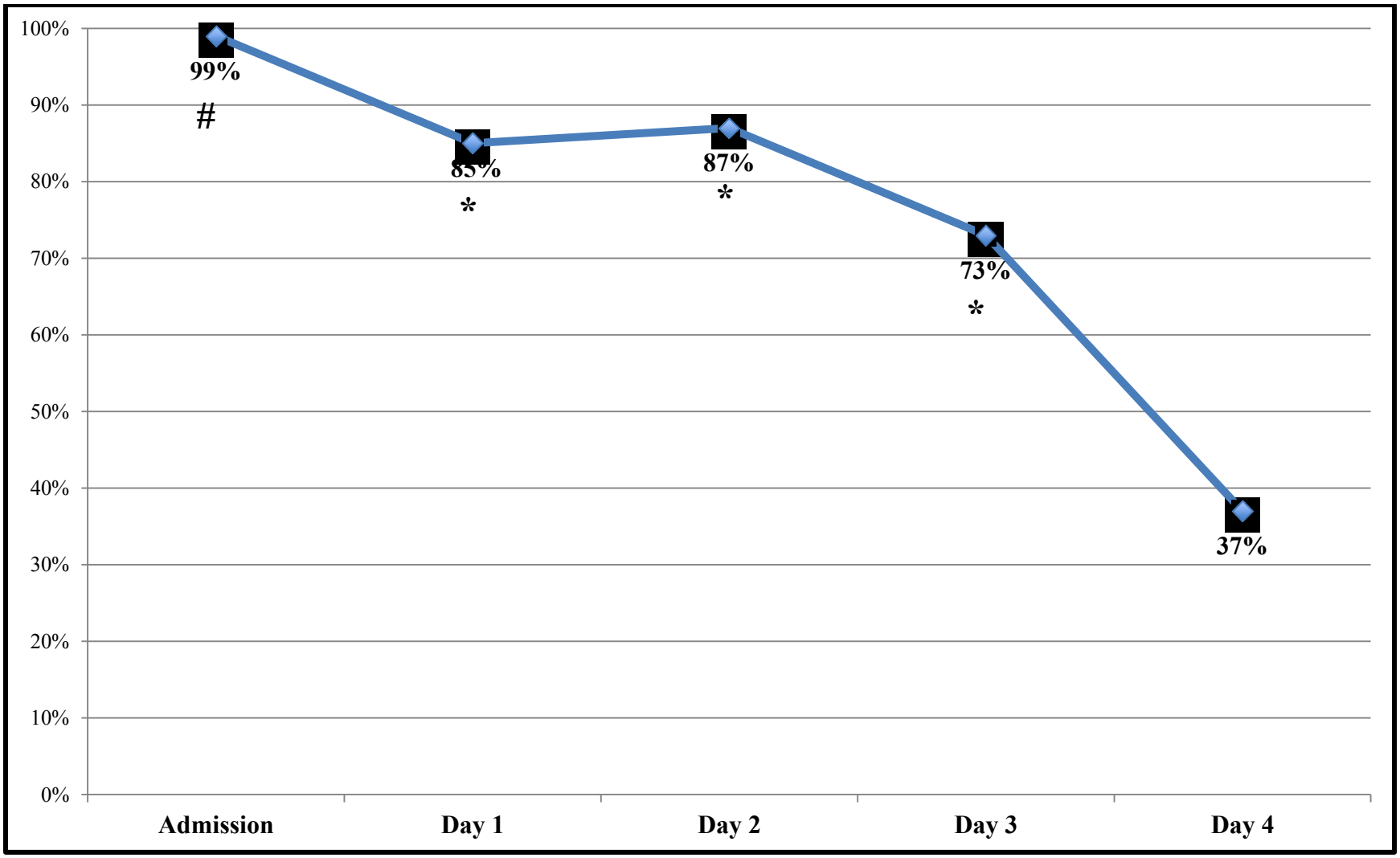
Results are presented as mean +/- SD [n], n/N (%) or median [IQR].
Legend: FHR: Fetal Heart Rate; PFB: Prolonged Fetal Bradycardia

Table 2.3. *Adherence to the Standardized Neurological Form*

	Admission (n=76)	**Day 1 (n= 76)**	**Day 2 (n=76)**	**Day 3 (n=76)**	**Day 4 (n=76)**
Uncompleted Forms due to Death	0 (0)	1 (1)	5 (7)	5 (7)	6 (8)
	Admission (n=76)	**Day 1 (n= 75)**	**Day 2 (n=71)**	**Day 3 (n=71)**	**Day 4 (n=70)**
Ambiguous Forms	5 (7)	6 (8)	5 (7)	5 (7)	6 (9)
Completed Forms	75 (99)	64 (85)	62 (87)	52 (73)	26 (37)
Sarnat Categories	**Admission (n=75)**	**Day 1 (n= 64)**	**Day 2 (n=62)**	**Day 3 (n=52)**	**Day 4 (n=26)**
Level of consciousness	75 (100)	64 (100)	62 (100)	52 (100)	26 (100)
Spontaneous activity	75 (100)	64 (100)	62 (100)	52 (100)	25 (96)
Posture	74 (99)	64 (100)	61 (98)	52 (100)	26 (100)
Tone	75 (100)	64 (100)	61 (98)	51 (98)	25 (96)
Primitive Reflexes	64 (85)	49 (77)	50 (81)	45 (87)	24 (92)
Autonomic nervous system	59 (79)	51 (80)	50 (81)	45 (87)	25 (96)
All 6 Sarnat Categories	44 (59)	37 (58)	38 (61)	32 (62)	17 (65)
Final NE stage	57 (76)	54 (84)	55 (89)	42 (81)	22 (85)
Signature of the Certified Examiner	55 (73)	36 (56)	32 (52)	24 (46)	16 (62)
Other Information					
Day	65 (87)	58 (91)	58 (94)	45 (87)	23 (88)
Date	69 (92)	61 (95)	62 (100)	49 (94)	25 (96)
Time	67 (89)	62 (97)	60 (97)	45 (87)	24 (92)
Hours of life	55 (73)	43 (67)	37 (60)	28 (54)	7 (27)
Head circumference	27 (36)	14 (22)	8 (13)	10 (19)	6 (23)
Sedation	61 (81)	58 (91)	60 (97)	49 (94)	24 (92)
Medication for Sedation	59 (79)	48 (75)	55 (89)	46 (88)	23 (88)
Seizures	66 (88)	60 (94)	60 (97)	51 (98)	26 (100)
Medication for Seizures	64 (85)	59 (92)	58 (94)	49 (94)	25 (96)
Gag reflex	62 (83)	51 (80)	53 (85)	43 (83)	23 (88)
Clonus	48 (64)	49 (77)	50 (81)	39 (75)	19 (73)
aEEG Classification	26 (35)	42 (66)	34 (55)	36 (69)	7 (27)

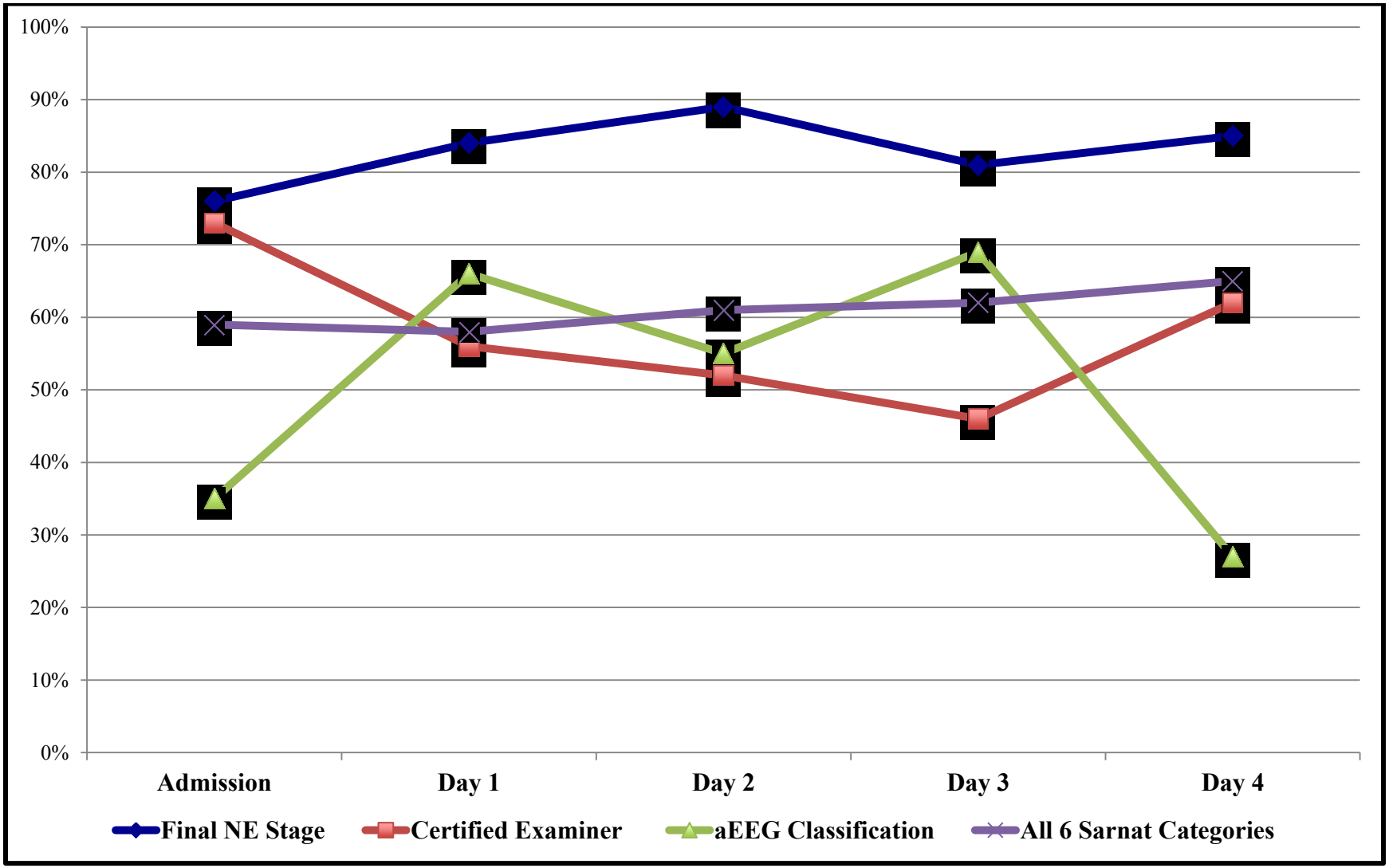
Results are presented as N (%): N = data filled/completed out of a total n for that specific variable.
Legend: aEEG: amplitude-integrated electroencephalogram

Figure. 2.2. *Adherence to the use of the Neurological Form*



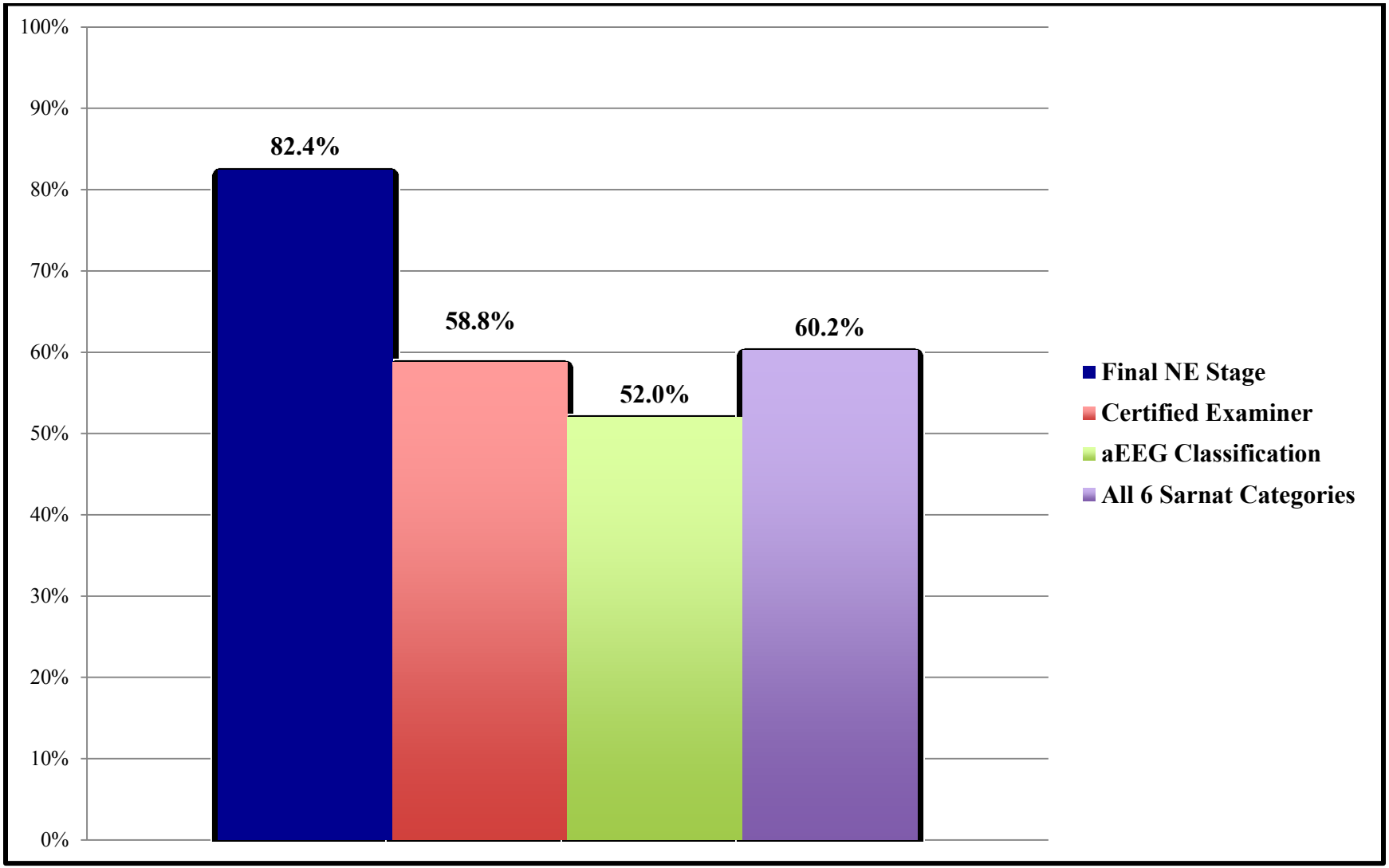
Legend: * $p<0.05$ significantly different from day 4 and # $p<0.05$ significantly different from all other days

Figure. 2.3a. *Completion of Data on the Neurological Form*



Legend: NE: Neonatal Encephalopathy

Figure. 2.3b. *Completion of Data on the Neurological Form*



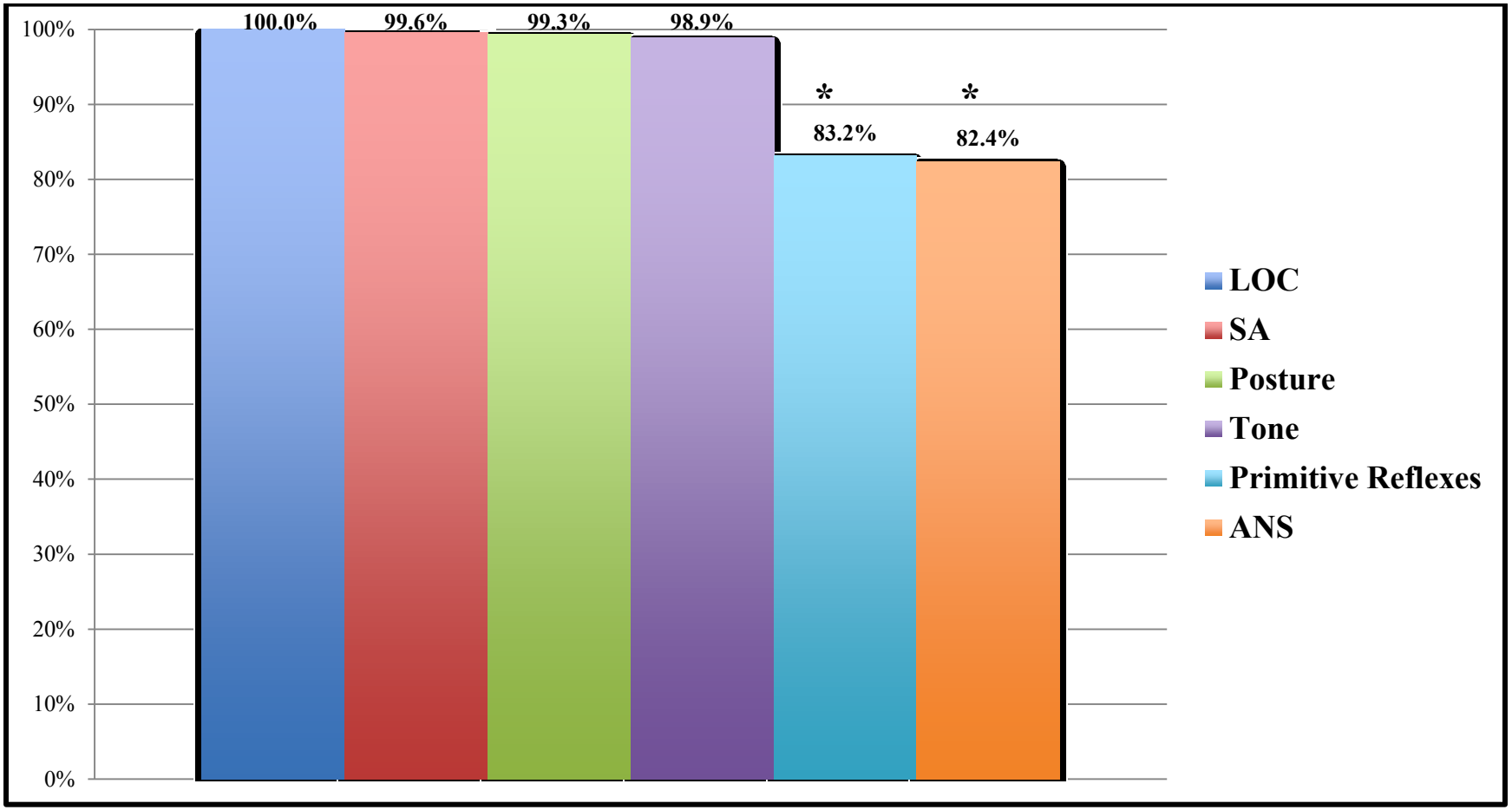
Legend: NE: Neonatal Encephalopathy

Table 2.4. *Overall Adherence to Neurological Form*

Uncompleted Forms due to Death		17/380 (5)
Ambiguous Forms		27/380 (7)
Completed Forms		279/363 (76.9)
Sarnat Categories		
	Level of consciousness	279/279 (100)
	Spontaneous activity	278/279 (99.6)
	Posture	277/279 (99.3)
	Tone	276/279 (98.9)
	Primitive Reflexes	232/279 (83.2)
	Autonomic nervous system	230/279 (82.4)
All 6 Sarnat Categories		168/279 (60.2)
Final NE Stage		230/279 (82.4)
Certified Examiner		163/279 (58.4)
Other Information		
	Day	249/279 (89.2)
	Date	266/279 (95.3)
	Time	258/279 (92.5)
	Hours of life	170/279 (60.9)
	Head circumference	65/279 (23.3)
	Sedation	252/279 (90.3)
	Medication for Sedation	231/279 (82.8)
	Seizures	263/279 (94.3)
	Medication for Seizures	255/279 (91.4)
	Gag reflex	232/279 (83.2)
	Clonus	205/279 (73.5)
	aEEG classification	145/279 (52.0)

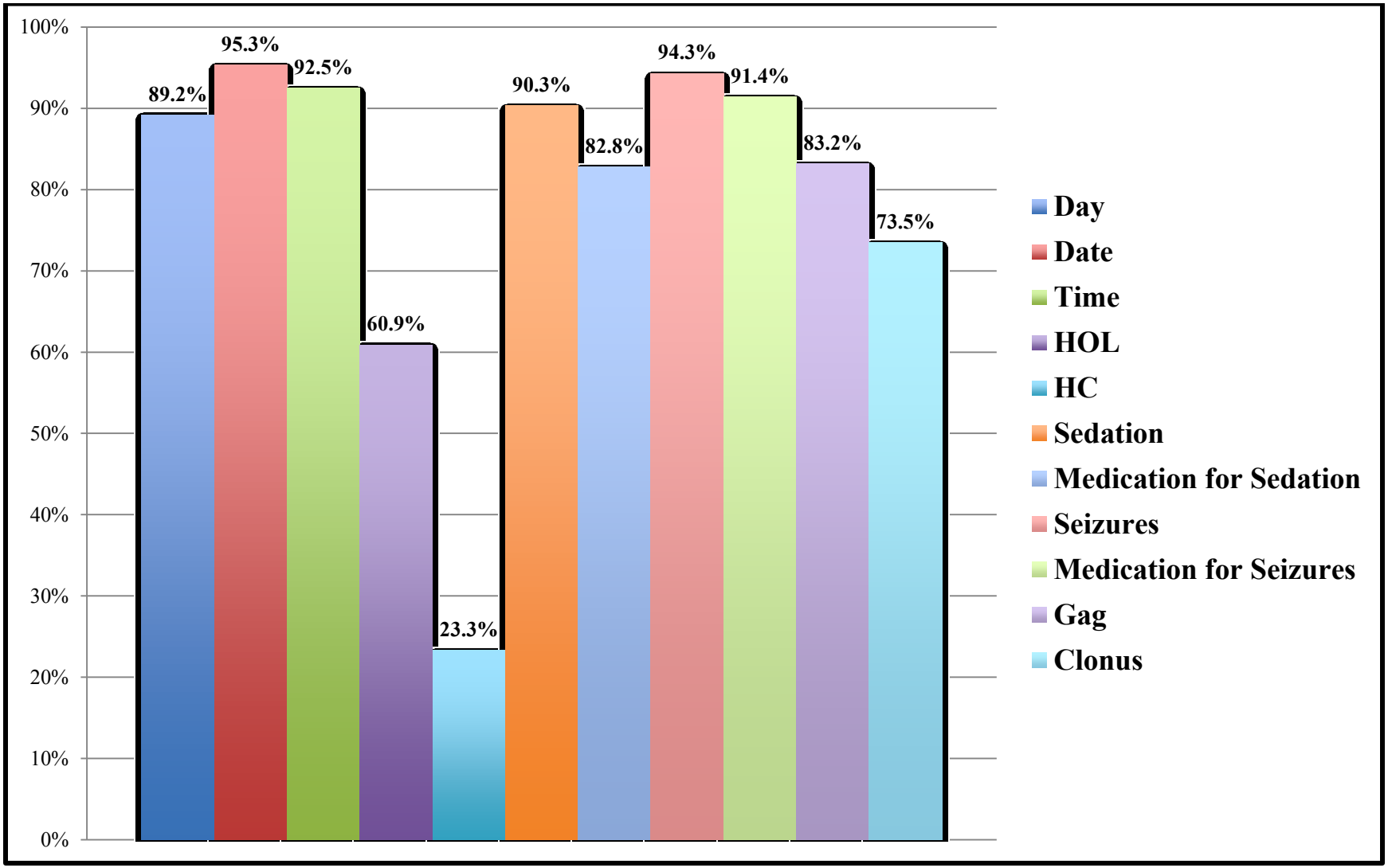
Results are presented as N (%): N = data filled/completed out of a total n for that specific variable.
Legend: aEEG: amplitude-integrated electroencephalogram

Figure. 2.4. *Completion of Individual Sarnat Categories on the Neurological Form*



Legend: LOC: Level of Consciousness; SA: Spontaneous Activity; ANS: Autonomic Nervous System
* $p < 0.05$ significantly different from LOC, SA, Posture and Tone

Figure. 2.5. *Completion of Other Information on Neurological Form*



Legend: HOL: hours of life; HC: head circumference

Figure. 2.6. *Adherence to Neurological Form Year 1 to 2*

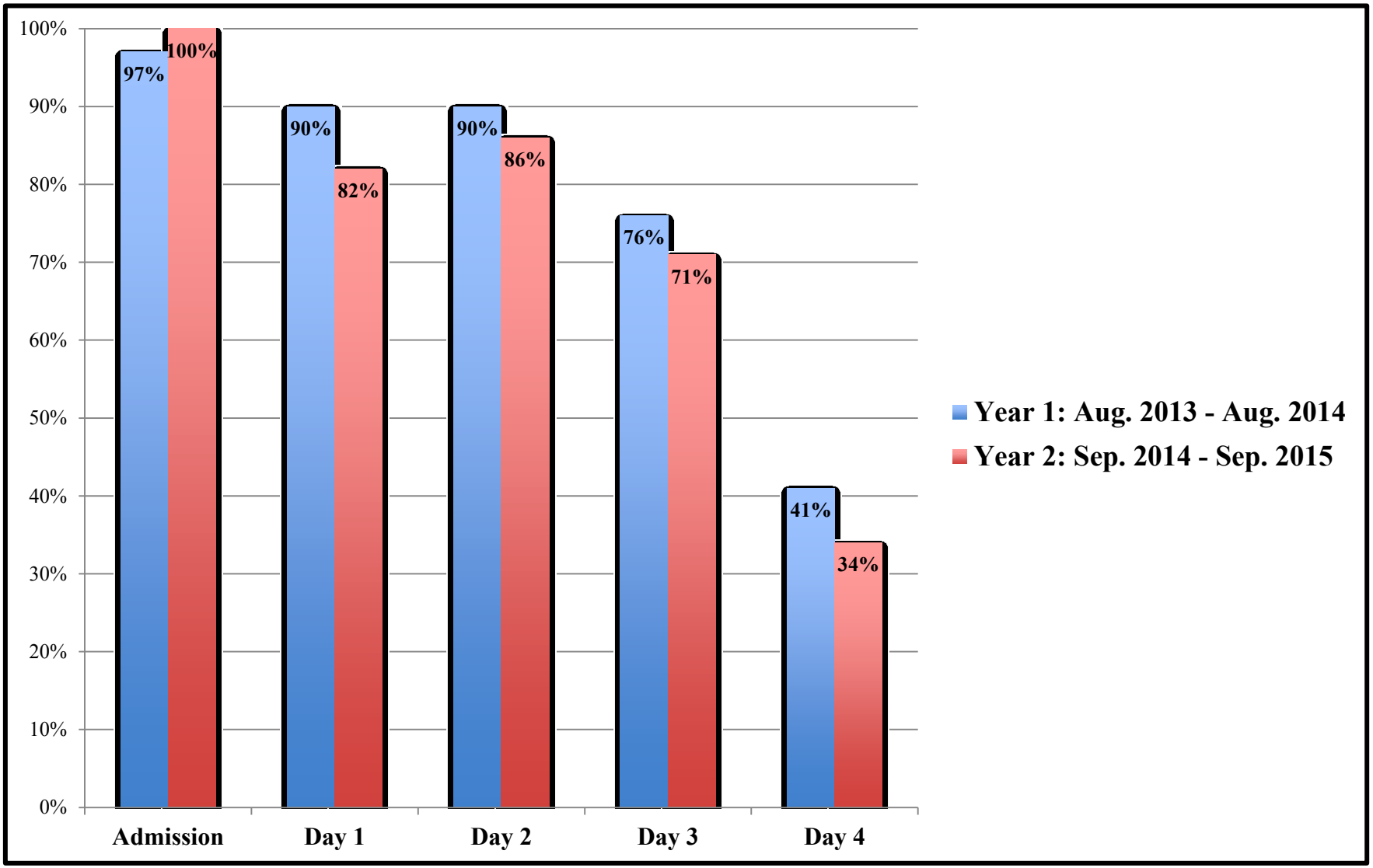


Table 2.5. *Neonatal Characteristics*

	Non-Adherence D4	**Adherence D4**
Outborn Delivery	49/50 (98)	26/26 (100)
Male Sex	27/50 (54)	11/26 (42)
Gestational Age	39.0 +/- 1.56 [50]	39.3 +/- 1.68 [26]
Birth Weight	3339 +/- 622 [50]	3412 +/- 624 [26]
Head Circumference	34.7 +/- 1.66 [40]	34.1 +/- 1.54 [25]
Length of Stay	16.6 +/-14.6 [50]	25.2 +/- 42.5 [26]
Abnormal Cardiotocography		
FHR Decelerations*	6/50 (12)	10/26 (38)
Prolonged Fetal Bradycardia	10/50 (20)	2/26 (8)
Fetal Tachycardia	3/50 (6)	1/26 (4)
FHR Decelerations & Prolonged Fetal Bradycardia	5/50 (10)	1/26 (4)
FHR Decelerations & Fetal Tachycardia	2/50 (4)	2/26 (8)
Other	5/50 (10)	1/26 (4)
None	19/50 (38)	9/26 (35)
Shoulder Dystocia	4/50 (8)	2/26 (8)
Mode of Delivery		
C-Section	28/47 (60)	12/25 (48)
Spontaneous Vaginal	15/47 (32)	8/25 (32)
Vaginal with Instrumentation	4/47 (9)	5/25 (20)
APGAR Scores		
1 minute	1 [0.25-2]	1 [1-3]
5 minutes	3 [2-5]	4 [1-5]
10 minutes	5 [3-6]	5 [3-6.5]
Intervention in the in first 10 min of life		
PPV	41/50 (82)	20/26 (77)
Intubation	28/50 (56)	14/26 (54)
Chest Compressions	17/50 (34)	8/26 (31)
Epinephrine	6/50 (12)	5/26 (19)
Need for resuscitation	32/50 (64)	15/26 (58)
Other drugs required during resuscitation	5/50 (10)	3/26 (12)
Cord blood gas or blood gas in the 1st h of life		
pH	7.04 +/- 0.18 [50]	7.08 +/- 0.17 [26]
PCO2	62.2 +/- 27.5 [44]	54.3 +/- 21.8 [22]
HCO3	15.1 +/- 4.68 [43]	15.4 +/- 5.91 [23]
Base Excess	-11.9 +/- 8.28 [41]	-14.5 +/- 6.55 [21]
Meconium Aspiration Syndrome	12/50 (24)	8/26 (31)
Persistent Pulmonary Hypertension	11/50 (22)	6/26 (23)
Necrotizing Enterocolitis	2/50 (4)	0/26 (0)
Need of gavage during hospitalization	17/50 (34)	8/25 (32)
Need of gavage at discharge	4/48 (8)	5/25 (20)

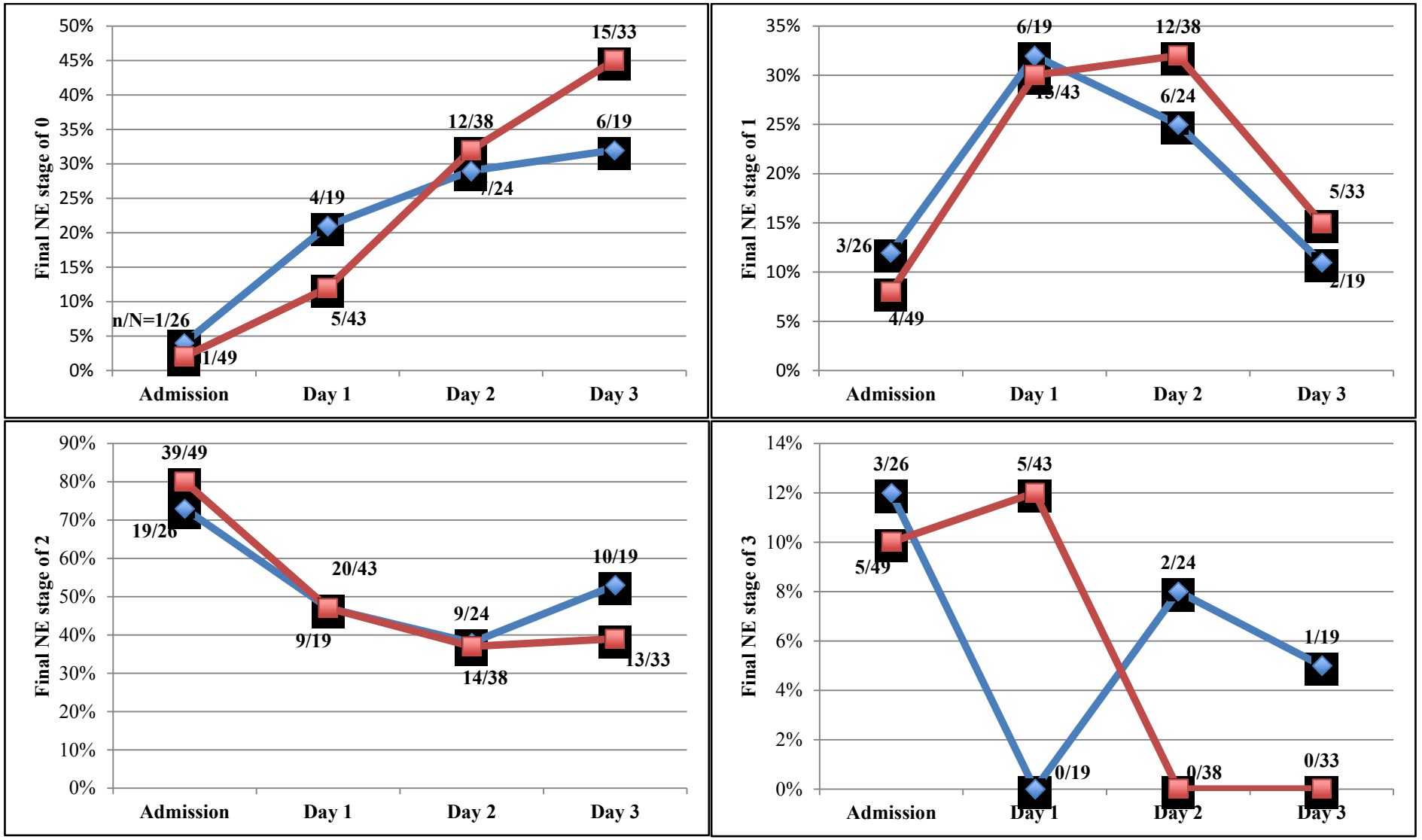
Results are presented as mean +/- SD [n], n/N (%) or median [IQR].
Legend: FHR: Fetal Heart Rate; PPV: Positive Pressure Ventilation.
* $p<0.05$ significantly different between the adherence and non-adherence group

Table 2.6. *Maternal Demographics*

	Non-Adherence D4	**Adherence D4**
Maternal Age	30.5 +/- 5.6 [44] (min 17 max 42)	30.4 +/- 6.28 [22] (min 19 max 41)
Number of Gestations		
One	20/49 (41)	15/26 (58)
Two	13/49 (27)	3/26 (12)
Three	12/49 (24)	5/26 (19)
Four	2/49 (4)	3/26 (12)
Five	1/49 (2)	0/26 (0)
Seven	1/49 (2)	0/26 (0)
Maternal Illness		
Diabetes Mellitus	1/50 (2)	2/26 (8)
Hypertension	2/50 (4)	0/26 (0)
Both	1/50 (2)	0/26 (0)
Neither	46/50 (92)	24/26 (92)
Gestational Complications		
Pre-eclampsia	1/50 (2)	0/26 (0)
Gestational Diabetes	0/50 (0)	1/26 (4)
Both	1/50 (2)	0/26 (0)
Neither	48/50 (96)	25/26 (96)
Maternal Fever	6/50 (12)	3/26 (12)
GBS Status		
Positive	16/50 (32)	5/26 (19)
Negative	25/50 (50)	14/26 (54)
Unknown	9/50 (18)	7/26 (27)
Premature Prolonged ROM	0/50 (0)	0/26 (0)
Prolonged ROM	6/50 (12)	2/26 (8)
Chorioamnionitis		
Yes	4/50 (8)	1/26 (4)
No	46/50 (92)	24/26 (92)
Unknown	0/50 (0)	1/26 (4)
Urinary Tract Infection	1/50 (2)	1/26 (4)
Antepartum Hemorrhage		
Placenta Previa	0/50 (0)	0/26 (0)
Placenta Abruption	6/50 (12)	3/26 (12)
Both	1/50 (2)	0/26 (0)
Neither	43/50 (86)	23/26 (88)
Placental Abnormalities		
Velamentous Cord Insertion	1/50 (2)	0/26 (0)
Low Lying Placenta	1/50 (2)	0/26 (0)
None	48/50 (96)	26/26 (100)
Cord Accidents		
Prolapsed Cord	3/50 (6)	2/26 (8)
Cord Rupture	0/50 (0)	0/26 (0)
Velamentous Cord Insertion	1/50 (2)	0/26 (0)
Nuchal Cord	5/50 (10)	3/26 (12)
None	41/50 (82)	21/26 (81)
Uterine Rupture	2/50 (4)	2/26 (8)

Results are presented as mean +/- SD [n] (min, max) or n/N (%).
Legend: ROM: Rupture of Membranes

Figure. 2.7. *Progression of NE stages based on Day 4 Adherence*



Legend: Blue represents infants <u>with</u> completed forms and red <u>without</u> completed forms on Day 4. NE: Neonatal Encephalopathy; n/N: n= number of patients with a given NE stage and N= number of completed neurological forms on a given day.

2.4 Discussion

In infants with NE secondary to a hypoxic-ischemic (HI) insult and treated with TH the adherence to the use of a standardized neurological assessment form was almost perfect for the final NE stage at admission but sub-optimal for other important variables included in the form. Also, adherence decreased during hypothermia treatment and over the first two years, with critical information severely under-reported.

The development and implementation of a specific form, for the assessment and comprehensive documentation of neurological evaluation in this critical population was an important way to standardize the care provided to these infants in our NICU. Early identification of an infant that would benefit from TH relies strongly on a neurological evaluation performed at < 6 h of life (47) and the progression of the encephalopathy over the first week of life is an important predictor of long-term outcomes, reinforcing the need of a regimented neurological examination at all-time points during hospitalization (99). Furthermore, appropriate documentation is essential in critical patients to ensure quality and continuity of care as well as compliance with protocols. It also provides opportunities to audit practice. Results of the primary outcome of this quality assurance study reinforce the need for continuing educational sessions to ensure proper adherence with documentation of the standardized neurological form of these patients during hospitalization.

Analysis of the secondary outcomes revealed that the least reported category of the modified Sarnat exam was the ANS. This may be related to the fact that current methods for evaluation of pupils, heart rate, and respiration are limited and prone to significant intra and inter-examiner variability, resulting in the inability to comprehensively evaluate this category.

Another possibility is that clinicians may not believe this category adds much value to the overall assessment.

A poor completion of certain sections of the neurological form, such as the final NE stage, aEEG and HC was noted. This finding raises major concerns on the awareness of examiners on the importance of proper documentation of the final NE stage and aEEG interpretation, or shows some difficulty in determining those 2 variables due to uncertainty or lack of appropriate training. HC may not be filled due to time constraints or because practitioners may not consider it relevant information for the neurological examination.

Although a decrease in the level of completion of the neurological form was noted from Year 1 to 2, the results were not statistically significant, but reinforce the need for strategies to ensure compliance to protocols. We hypothesized that adherence with the forms' completion would decrease during hypothermia treatment and after re-warming and this would be related to the severity of the encephalopathy. Indeed, a significant drop in adherence occurred from D3 to D4. A secondary analysis performed showed no differences in population demographics between infants with a completed neurological form on D4 and those without, except for a greater frequency of fetal heart rate (FHR) decelerations in the former group. Also, use of the form on D4 was more frequent in infants with a worse NE stage on the previous day (D3). These differences however were not statistically significant, likely due to the small sample size. Other limitations of the study are discussed in chapter 5 of this thesis.

2.5 Conclusion

This quality assurance study demonstrates that despite the critical role of the neurological evaluation in infants with evidence of moderate or severe encephalopathy secondary to a

hypoxic-ischemic event and treated with TH, institutional adherence to a standardized form was sub-optimal and decreased during hospitalization (from D1 to D4), with relevant information underreported. Thus, ongoing efforts are required to ensure adherence to guidelines developed for TH treatment, especially in what concerns neurological evaluation, which is critical to determine eligibility to the treatment, and provides prognostic information.

Chapter 3 – The contribution of the categories of the modified Sarnat exam to the final neonatal encephalopathy stage

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Abstract submitted to the Pediatric Academic Societies Annual Meeting (San Francisco, 2017)

3.1 Objective

The objective of this study was to analyze the contribution of each of the six categories that are part of the modified Sarnat exam (**Fig. 1.5**) to the final NE stage.

3.2 Materials and Methods

3.2.1 TH Protocol

The whole body TH protocol was described in detail in chapter 2 (methodology section).

3.2.2 Certification on Neurological Exam

In our unit, the attending physician determines the final NE stage on admission, and must perform subsequent neurological evaluations at 24h, 48h, and 72 h during the TH protocol, and after rewarming. As part of a quality assurance process, a specific training on the neurological exam was provided in June 2013 to all neonatologists. Details on this certification process were also described in chapter 2. An updated form of the neurological exam was developed and implemented in July 2013, and must be completed by health care practitioners certified in the neurological examination.

3.2.3 Modified Sarnat Exam

The modified Sarnat exam is composed of six categories: 1) level of consciousness, 2) spontaneous activity, 3) posture, 4) tone, 5) primitive reflexes (suck and Moro), and 6) ANS

(pupils, heart rate, and respirations). Using a standardized form, the examiner scores each of the 6 categories separately (sub-categories are also scored separately with the highest score of a subcategory dictating the final score of that respective category) and a final NE stage is determined based on a majority of categories having a given score. In the case where an equal number of categories have different scores (i.e. no majority can be determined), the final NE stage is based on the score attributed to the LOC. There are four possible scores that can be assigned to each of the categories and subcategories: 0 (normal), 1 (mild), 2 (moderate), and 3 (severe).

3.2.4 Study Population

In order to assess the contribution of each of the 6 categories to the final NE stage of the modified Sarnat exam, patients referred for possible TH were identified from the unit database over a period of three years, from August 2013 to September 2016, and only infants treated with TH were included. (**Fig. 3.1**) Patient demographics and details on the neurological exam were extracted from the medical records.

3.2.5 Clinical Data

The following variables were recorded: a) maternal characteristics: age, number of gestations, chronic maternal illness, gestational complications, fever during labor, GBS status, rupture of membranes, chorioamniotis, urinary tract infection, antepartum hemorrhage, placental abnormalities, b) perinatal: abnormal cardiotocography (fetal heart rate decelerations, prolonged fetal bradycardia, fetal tachycardia); cord accidents (prolapsed cord, cord rupture), and/or uterine rupture; shoulder dystocia; mode of delivery (C-section, vaginal, or vaginal with instrument); Apgar scores (1,5 and 10 min); positive pressure ventilation, endotracheal intubation, need for

chest compressions, use of epinephrine or other drugs required during resuscitation, and continuous need for resuscitation in the first 10 min of life; and pH, partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3) and base excess from umbilical cord or blood gas collected within the first hour of life; c) neonatal: date and time of birth, GA, BW, head circumference, sex, drugs used during TH, meconium aspiration syndrome, persistent pulmonary hypertension, and/or necrotizing enterocolitis.

Other data collected included information on EEGs (12-lead), brain MRI performed (10-30 days of life), gavage feeding (number of days on feeds during hospitalization or need of feeds or gastrostomy at discharge), length of hospital stay, and death during hospitalization.

3.2.6 Neurological Exam

Data Collection

Information concerning the neurological evaluation was extracted from the standardized neurological form and included the scores of each of the 6 Sarnat categories, as well as the final NE stage (**Fig. 1.5**). Each of the categories and the final NE stage were given scores of 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). For each patient, data was obtained at 4 time points: before the initiation of TH, and on days 1 to 3 of cooling.

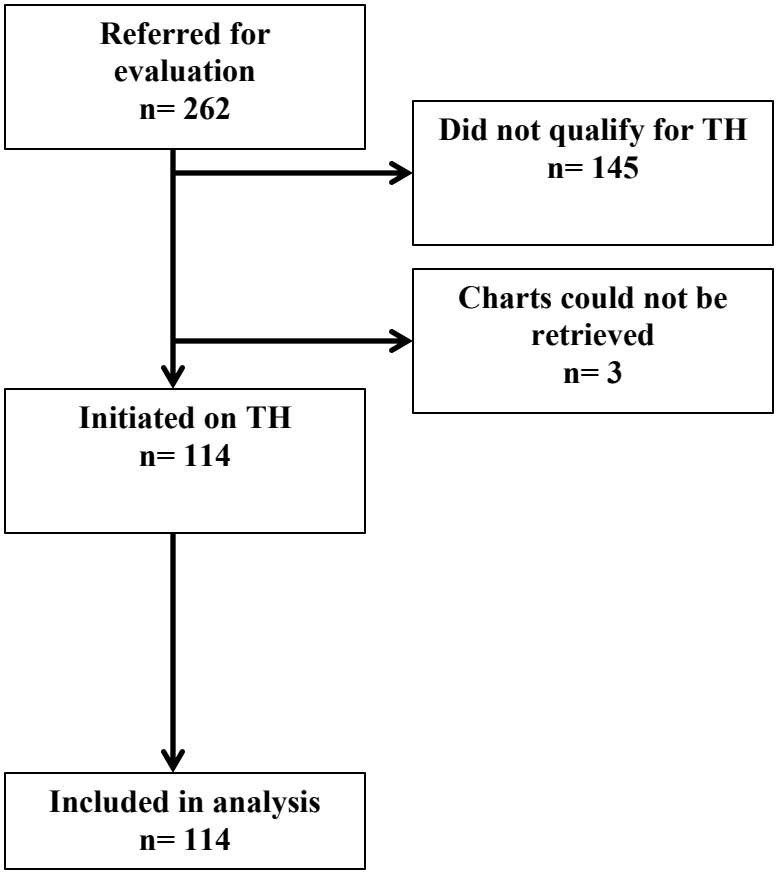
Data Analysis

The final NE stage was plotted against the scores assigned to each of the 6 categories, for each patient, and linear regression lines were fitted for each category. Coefficients of determination (R^2) were calculated to assess goodness of fit and slopes for each of the respective categories were determined. A slope of 1 would represent a perfect correlation between the final NE stage and any given category score.

3.2.7 Statistical Analysis

Descriptive statistical analysis was performed. Data was presented as n (%), mean ± SD, median (interquartile range) and confidence intervals where applicable. For the primary outcome, a multivariate linear regression analysis was performed to determine each category's contribution to the final NE stage. A p-value < 0.05 was considered statistically significant.

Figure. 3.1. *Flowchart of patient enrollment*



Legend: TH: Therapeutic Hypothermia

3.3 Results

A total of 114 neonates were included and classified as mild (n=9), moderate (n=84), and severe NE (n=17) before the initiation of TH (**Fig. 3.1**). Infants with mild or no NE (n=3) on the neurological exam were cooled based on aEEG findings. No detailed information on the Sarnat exam was retrieved for 1 patient on admission. Maternal demographics and population characteristics are presented in **Table 3.1** and **Table 3.2**, respectively. The mean gestational age was 39.2 ± 1.60 weeks, birth weight 3371 ± 574 g, cord pH 7.04 ± 0.18 and base deficit– 13.0 ± 8.05.

Of the 6 categories evaluated, ANS had the lowest R^2 and slope value at any time point. The contribution of each category score to the final NE stage is shown in **Table 3.3**. Moreover, the multivariate linear regression analysis revealed that ANS was the only category not significantly associated with the final NE stage at all points whereas LOC and SA were the only categories where the scores significantly contributed to the final NE stage at all points. (**Fig. 3.2)** This is visually depicted by near perfect regression lines with slopes approximating 1. Posture, tone and primitive reflexes showed variable associations with the final NE stage at different time points.

In a post hoc analysis, the ANS category was removed from the neurological evaluation at admission for each patient, and the final NE stage re-assessed using the same standardized method (**Fig. 3.3**). In only 3 patients, the final NE stage changed, representing only 2.6% of the entire cohort. Interestingly, each of the 3 infants would still qualify for TH, as the final NE stage became 2.

Table 3.1. *Maternal Demographics*

Maternal Age (years)	30.8 +/- 5.5 [97] (min 17 max 45)
Number of gestations	
One	45/110 (41)
Two	25/110 (23)
Three	25/110 (23)
Four	9/110 (8)
Five	2/110 (2)
Six	1/110 (1)
Seven	3/110 (3)
Maternal Illness	
Diabetes Mellitus	5/112 (4)
Hypertension	2/112 (2)
Both	1/112 (1)
Neither	95/112 (85)
Other	9/112 (8)
Gestational Complications	
Pre-eclampsia	1/112 (1)
Gestational Diabetes	8/112 (7)
Both	1/112 (1)
Neither	101/112 (90)
Other	1/112 (1)
Maternal Fever	14/112 (13)
GBS Status	
Positive	36/113 (32)
Negative	57/113 (50)
Unknown	20/113 (18)
Premature Prolonged ROM	1/112 (1)
Prolonged ROM	11/112 (10)
Chorioamnionitis	
Yes	8/112 (7)
No	102/112 (91)
Unknown	2/112 (2)
Urinary Tract Infection	3/112 (3)
Antepartum Hemorrhage	
Placenta Previa	1/112 (1)
Placenta Abruption	13/112 (12)
Both	2/112 (2)
Neither	96/112 (86)
Placental Abnormalities	
Velamentous Cord Insertion	2/112 (2)
Low Lying Placenta	1/112 (1)
None	109/112 (97)
Cord Accidents	
Prolapsed Cord	5/112 (4)
Nuchal Cord	8/112 (7)
Other	3/112 (3)
None	96/112 (86)
Uterine Rupture	6/112 (5)

Results are presented as mean +/- SD [n] (min, max) or n/N (%).
Legend: ROM = Rupture of Membranes

Table 3.2. *Population Characteristics*

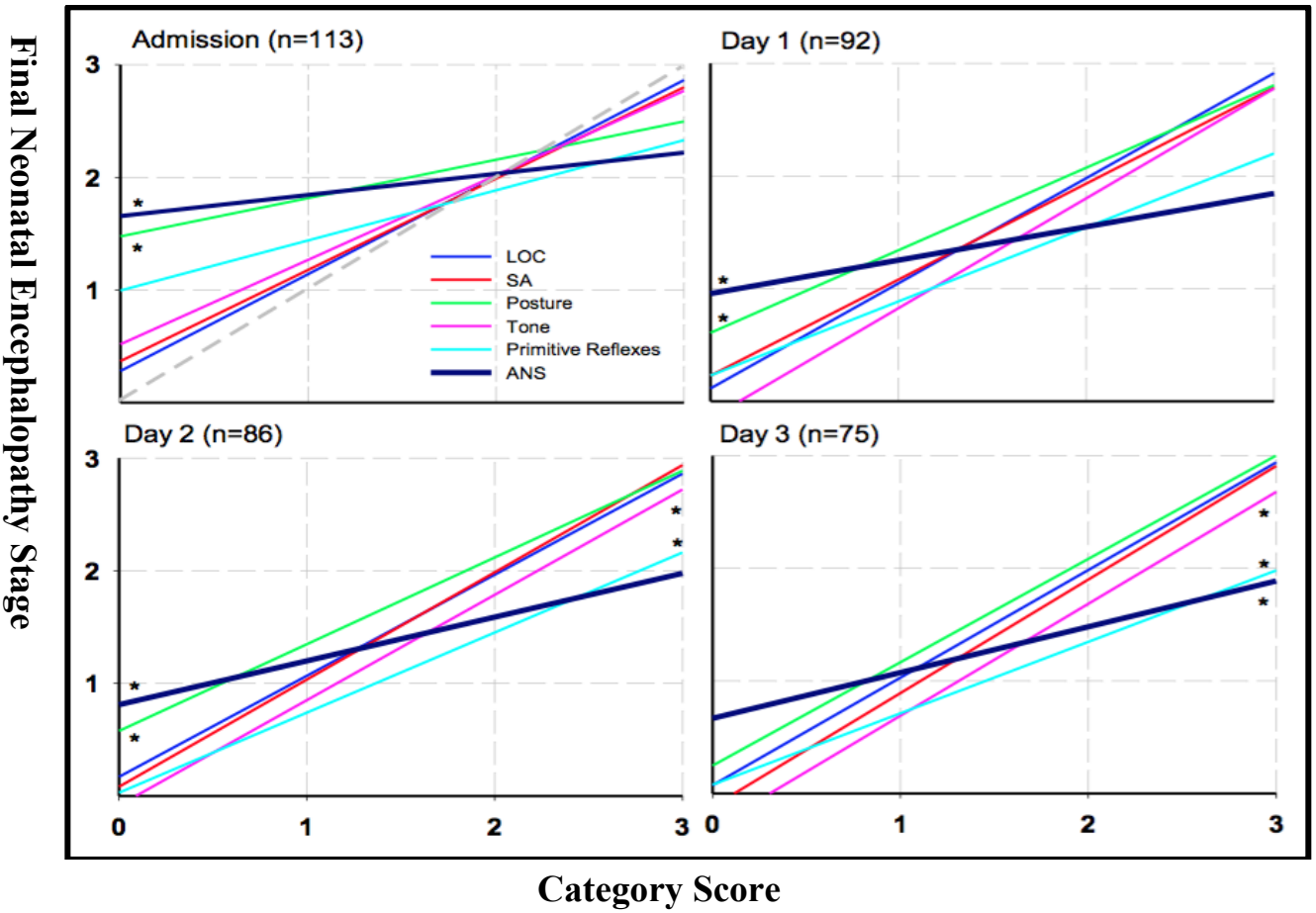
	Total
Outborn	109/114 (96)
Male Sex	58/114 (51)
Gestational Age (weeks)	39.2 +/- 1.60 [113]
Birth Weight (g)	3371 +/- 574 [113]
Head Circumference (cm)	34.3 +/- 1.50 [96]
Length of Stay (days)	19.6 +/-27.5 [114]
Abnormal Cardiotocography	
FHR Decelerations	31/113 (27)
Prolonged Fetal Bradycardia	16/113 (14)
Fetal Tachycardia	4/113 (4)
FHR Decelerations & Prolonged Fetal Bradycardia	7/113 (6)
FHR Decelerations & Fetal Tachycardia	4/113 (4)
Other	10/113 (9)
None	41/113 (36)
Shoulder Dystocia	11/113 (10)
Mode of Delivery	
C-Section	63/108 (58)
Spontaneous Vaginal	30/108 (28)
Vaginal with Instrumentation	15/108 (14)
APGAR Scores	
1 minute	1 [1-2]
5 minutes	4 [2-5]
10 minutes	5 [3-6]
Interventions in the first 10 min of life	
Positive pressure ventilation	88/113 (78)
Intubation	60/114 (53)
Chest Compressions	38/114 (33)
Epinephrine	17/114 (15)
Need for resuscitation	79/113 (70)
Other drugs required during resuscitation	18/114 (16)
Cord blood gas or blood gas in the 1st hour of life	
pH	7.04 +/- 0.18 [113]
PCO2	63.8 +/- 29.0 [96]
HCO3	16.0 +/- 6.26 [91]
Base Excess	-13.0 +/- 8.05 [93]
Meconium Aspiration Syndrome	34/114 (30)
Persistent Pulmonary Hypertension	23/114 (20)

Results are presented as mean +/- SD [n], n/N (%) or median [IQR]
Legend: FHR: Fetal Heart Rate

Table 3.3. *Contribution of Category Scores to Final NE stage*

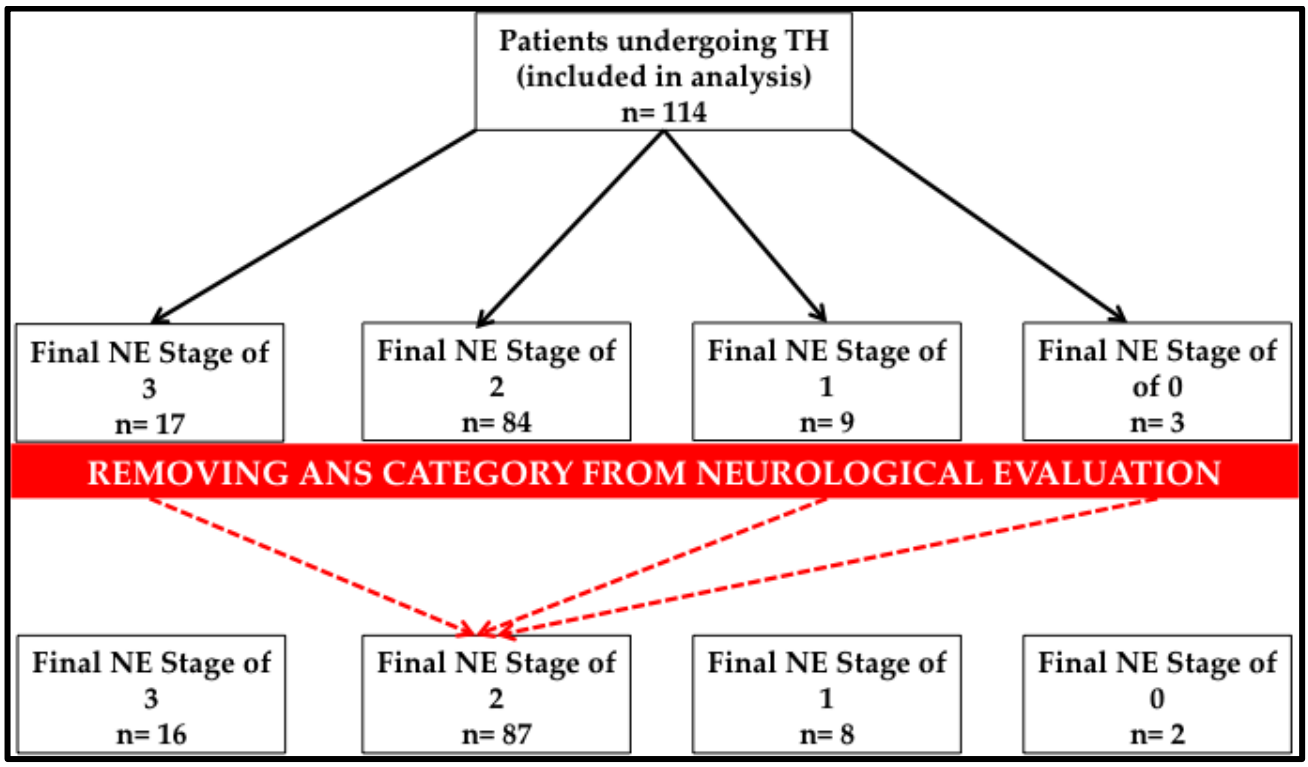
		R^2	Slope	P-Value	95% CI
Admission					
	Level of Consciousness	0.70223	0.8610	<0.0001	(0.28336, 0.51443)
	Spontaneous Activity	0.72441	0.8106	<0.0001	(0.21372, 0.44779)
	Posture	0.16032	0.3402	0.8157	(-0.07697, 0.06075)
	Tone	0.53339	0.7500	<0.0001	(0.15541, 0.35392)
	Primitive Reflexes	0.33322	0.4447	0.0001	(0.06667, 0.20042)
	Autonomic Nervous System	0.14391	0.1875	0.1487	(-0.07087, 0.01089)
Day 1					
	Level of Consciousness	0.88990	0.9332	<0.0001	(0.45693, 0.73108)
	Spontaneous Activity	0.78694	0.8526	0.0111	(0.03989, 0.30070)
	Posture	0.46107	0.7327	0.6831	(-0.11719, 0.07717)
	Tone	0.65758	0.9754	0.0008	(0.08247, 0.30482)
	Primitive Reflexes	0.55994	0.6567	0.0154	(0.01924, 0.17704)
	Autonomic Nervous System	0.18099	0.2967	0.7747	(-0.05092, 0.03807)
Day 2					
	Level of Consciousness	0.82386	0.8970	<0.0001	(0.16537, 0.45637)
	Spontaneous Activity	0.86753	0.9514	<0.0001	(0.39080, 0.71383)
	Posture	0.41437	0.7701	0.2948	(-0.05339, 0.17367)
	Tone	0.48272	0.9350	0.6756	(-0.10398, 0.15958)
	Primitive Reflexes	0.55917	0.7109	0.1137	(-0.01958, 0.17961)
	Autonomic Nervous System	0.25568	0.3890	0.2956	(-0.02893, 0.09389)
Day 3					
	Level of Consciousness	0.89082	0.9555	<0.0001	(0.29538, 0.63442)
	Spontaneous Activity	0.84420	1.0083	0.0009	(0.12734, 0.46859)
	Posture	0.68744	0.9180	0.0006	(0.10259, 0.35740)
	Tone	0.63418	0.9935	0.2293	(-0.05327, 0.21834)
	Primitive Reflexes	0.43943	0.6347	0.9286	(-0.08278, 0.09059)
	Autonomic Nervous System	0.26095	0.4064	0.2782	(-0.02619, 0.08961)

Figure. 3.2. *Linear regression analysis showing the contribution of all category scores to final NE stage (dependent variable). Note that the ANS (thick dark blue line) is the only category not significantly associated with the final NE stage at all time periods.*



Legend:
Grey dashed line on the admission plot represents a hypothetical perfect regression (slope= 1).
* p value not statistically associated with the final NE stage.

Figure. 3.3. *Flow diagram of the final NE stage on admission before and after removal of the ANS category from the neurological evaluation. Note that in only 3 patients the final NE stage changed, but all would still qualify for TH, as the 'new' NE stage became 2.*



3.4 Discussion

In the present study we analyzed the contribution of the six categories of the modified Sarnat exam to the final NE stage. ANS was the only category that did not contribute significantly to the final NE stage at all points, from admission to day 3 of TH. Furthermore, removal of this category changed the final NE stage of only 3 patients (2.6%) but all of them would still qualify for TH, as the 'new' NE stage became 2.

When using the modified Sarnat exam, evaluation of ANS is rather difficult, making this category quite challenging to score. Assessments of the various subcomponents (pupils, heart rate, respirations) are limited and ambiguous. For instance, pupil's evaluation is based on individual perception of the size (normal, constricted or dilated) and reactivity to the light stimuli. Size determination is imprecise and prone to error as the selected values for definition are in millimeters. Adult studies showed that individual measurements of pupil size and reactivity can suffer from inter-examiner variability of up to 39% (71). In neonates this can be even more problematic due to the smaller pupil size and lack of cooperation during the exam. The evaluation of pupillary response to light is performed without a precise tool for such assessment, and therefore also subjective and prone to inter-examiner variability (70).

The second component of the ANS evaluation is heart rate which is assessed based on the *average* heart rate obtained from the bedside monitor or counting with auscultation, during the neurological exam and classified as: normal (100-160 bpm), tachycardia (>160 bpm), bradycardia (<100bpm) or irregular. This simplistic method of scoring heart rate may not be the most accurate representation. Heart rate is affected by body temperature, which might be low during assessment given the increasing practice of initiation of hypothermia soon after birth at

referring centers. Furthermore, neural control of the human heart rate is quite complex, and as such may require a more in depth evaluation (76, 80).

Respirations is also prone to error as neonates that are mechanically ventilated or receiving any type of non-invasive support automatically receive a score of 3 (severe) in the respirations sub-category (and therefore for the ANS category as a whole) regardless as to whether or not the scores given to heart rate and pupils were lower. Respiration abnormalities may also be related to other problems unrelated to asphyxia and brain injury such as air leaks, respiratory distress syndrome or use of sedatives for endotracheal intubation. This may explain part of the discrepancy between the ANS category and the final NE stage.

Based on MRI studies and the pathophysiology of the disease, it is known that asphyxia targets regions of the brain with high metabolic demand, where functional glutamate synapses have already been established in the newborn period (100, 101). Hypoxia ischemia initiates sustained excitatory activity of the cortico-thalamic connections in the brain, which is responsible for the selective neuronal injury in these regions (101). HI preferentially targets primary sensory and forebrain motor systems, which control consciousness, spontaneous and motor activities, and regulation of postural muscle tone (102-104). Additionally, numerous reports have demonstrated that the brainstem, which controls ANS function, is less sensitive to ischemic anoxia in the majority of cases (105-108). Recent evidence suggests that brainstem involvement is a rare event, occurring only in severe cases of fetal asphyxia; these patients with brainstem lesions exhibit severe encephalopathy and die almost immediately after delivery (109, 110). Therefore, this *selective neuronal vulnerability* of the neonatal brain to asphyxia (100, 101, 111-113) may explain the lack of significant contribution of the ANS category to the final NE stage. Lastly, current neurological assessment of ANS is based simply on evaluation of 3

variables (pupils, heart rate, respirations), but there are several other biological processes controlled by the ANS such as skin perfusion, temperature and blood pressure, sweating, and digestion (114). As such, the present evaluation is not comprehensive and may affect the contribution of the ANS to the final NE stage.

There are some limitations to the present study. First, the modified Sarnat exam uses the category LOC as the "tie-breaker" when determining the final NE stage, which may have inherently overestimated the contribution of this category. Second, as in any retrospective study there was some missing data related to incomplete recording of the exam and inability to retrieve medical charts in 3 patients treated with TH. However, we included and analyzed in detail the neurological exam of more than 100 patients from a single center where all examiners were certified in the performance of the modified Sarnat exam. To the best of our knowledge this is the first study that has analyzed in detail each category of the exam and their contribution to the final NE stage from admission to day 3 of hypothermia.

3.5 Conclusion

When using the modified Sarnat exam in infants with evidence of a hypoxic-ischemic insult it is important to know that each of the six categories included in the exam contribute differently to the final NE stage. Moreover, ANS is the only category with no significant contribution at all points, from admission to day 3 of hypothermia. Indeed, removal of this category from the exam did not affect the final NE stage for the majority of the patients and would have not prevented any patient case from receiving the hypothermia treatment. These findings suggest that the current evaluation of ANS may be rather imprecise and requires further investigation and improvement.

3.6 Future directions

Perhaps, instead of removing the ANS category form the neurological examination a more comprehensive assessment should be investigated. For example, analysis of heart rate variability has been demonstrated to have some prognostic value in this population. Thus, research in this domain may improve the overall neurological examination by providing a more accurate classification of the NE stage, and help with the prognostication of these critical patients.

Chapter 4 – A comprehensive evaluation of the Autonomic Nervous System Function in Infants Treated with Therapeutic Hypothermia: A Prospective, Observational Study

4.1 Objectives

The primary objective of this prospective study is to investigate, in infants treated with TH, if a more comprehensive analysis of ANS function is associated with clinical outcomes. The study's specific aim is to record physiological variables controlled by the ANS and to analyze their correlation with clinical outcomes.

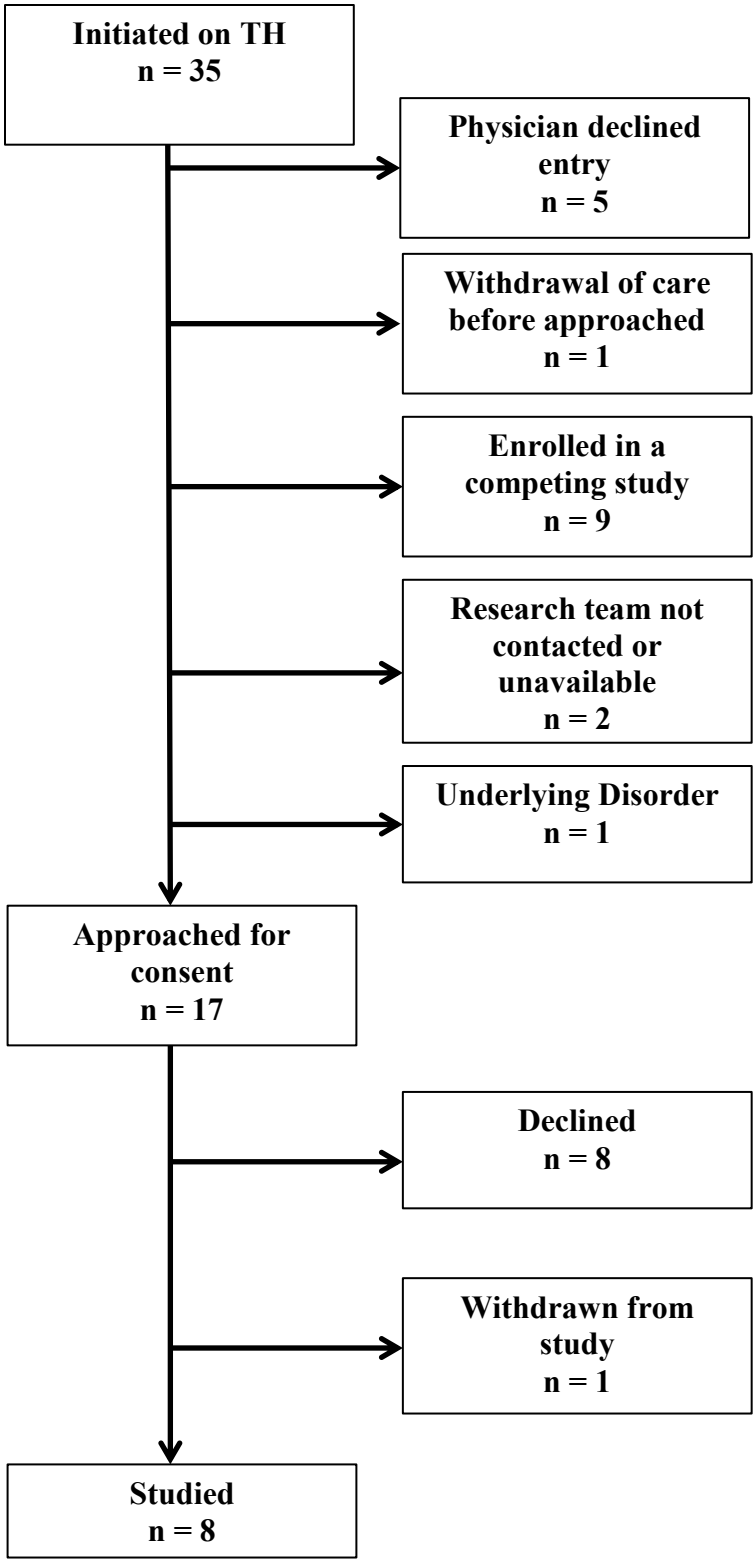
4.2 Materials and Methods

4.2.1 Therapeutic Hypothermia & Study Population

In our unit, whole body hypothermia is used as standard therapy in infants with GA $\geq$ 36 weeks and BW $\geq$ 1800g, with evidence of moderate or severe encephalopathy secondary to a hypoxic-ischemic insult. The hypothermia protocol, including eligibility criteria, equipment, and temperature control, has been described in detail in chapter 2 (methods).

After obtaining study approval from the institutional ethics board in June 2016, infants with moderate or severe NE submitted to TH were screened, and parents were approached for informed consent. Patient demographics and details on the neurological exam were extracted from the medical records of enrolled patients, and specific measurements were obtained during the hypothermia treatment.

Figure 4.1. *Flow diagram of patient enrollment*



Legend: TH: Therapeutic Hypothermia

4.2.2 Clinical Data

The following variables are recorded: a) maternal characteristics: age, number of gestations, number, habits (smoking, alcohol, drugs), chronic maternal illness, gestational complications, fever during labor, GBS status, rupture of membranes, chorioamniotis, urinary tract infection, antepartum hemorrhage, placental abnormalities b) perinatal: abnormal cardiotocography (fetal heart rate decelerations, prolonged fetal bradycardia, fetal tachycardia), cord accidents (prolapsed cord, cord rupture), uterine rupture, shoulder dystocia, mode of delivery (C-section, vaginal, or vaginal with instrument), Apgar scores (1, 5 and 10 min), maximum FiO_2, positive pressure ventilation, endotracheal intubation, need for chest compressions, use of epinephrine or other drugs required during resuscitation, and continuous need for resuscitation in the first 10 min of life, and pH, partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3) and base excess from umbilical cord or blood gas collected within the first hour of life c) neonatal: date and time of birth, inborn/outborn, GA, BW, weight according to gestational age, head circumference, sex, MAS, hypoglycemia, congenital infections, and drugs used during TH d) final outcomes: MAS, PPHN, NEC, respiratory distress syndrome (RDS), surfactant, birth trauma, information on EEGs (12-lead), auditory brainstem evoked potential (hearing screen), visual evoked potentials, somatosensory evoked potentials, congenital infections, death of the infant during hospitalization, abnormal neurological exam at discharge or transfer, abnormal brain MRI at day of life 10, and length of hospital stay.

Neurological Exam

The information extracted from the neurological form included scores for the 6 Sarnat categories: level of consciousness, spontaneous activity, posture, tone, primitive reflexes (suck

and Moro), and ANS (pupils, heart rate, and respirations), as well as the score for the final NE stage (**Fig. 1.5**). The 6 Sarnat categories as well as the final NE stage are given a score of 0, 1, 2, or 3, which represent normal, mild, moderate, and severe NE respectively. Additional information collected from the neurological exam included day, date, time, hours of life, head circumference, degree of aEEG severity, signature of a certified examiner, sedation or seizures and medications used, and presence of gag or clonus. For each patient, the data was obtained at 4 time points: before the initiation of TH, and on days 1, 2 and 3 TH.

4.2.3 Study Design and Recordings

For each patient specific measurements are obtained daily during the hypothermia treatment and included: esophageal temperature (Tes), whole body skin temperature, pupil assessment, skin perfusion, axillary temperature, and electrocardiographic signals. When the patient was considered stable after consultation with the treating team, the following sequence was performed: 1) Infrared picture, 2) Placement of all leads for recordings of skin perfusion, axillary temperature, and electrocardiographic signals, 3) Recordings of these signals for a period of 1 hour and 4) Measurements of pupils using the pupillometer. Details of each specific measurement are provided below.

A. Esophageal Temperature Data

Esophageal temperature (Tes) is recorded in degrees Celsius from the Blanketrol II or III Hyper-Hypothermia system (Cincinnati Sub-Zero, Cincinnati, OH). The temperature is documented in the data collection form every 5 minutes during the one-hour study period, on the three days of TH. Additionally, the esophageal temperature of the infant is also reported at the time the infrared picture is taken (see below).

B. Whole-Body Skin Temperature

Infrared thermography uses a camera to visualize and quantify changes in surface temperature using an accurate, non-contact, high-performance technique. It is non-invasive as no infrared is released to the patient and temperature readings are obtained without disturbing the patient (115-118). In the present study, whole body skin temperature is being assessed by obtaining infrared (IR) images of the whole infant, with the E60bx camera (FLIR Systems, Inc. USA, Boston, MA). As part of the standard of care for all infants treated with TH, the patients are kept in a supine position without any clothes or diaper. Before obtaining the images, the headpiece covering the aEEG leads is removed. The distance between the camera and the patient is adjusted to allow a picture of the whole body. The patient is not handled or touched for at least 5 min prior to the image being obtained, to ensure accurate results. A minimum of three images per patient is obtained for each of the three days of TH, and images are stored for posterior analysis.

C. Pupillometer Data

Pupil size and reactivity are being assessed by using the NPi™-100 pupillometer (NeurOptics, CA, USA), which is a hand-held, portable device that allows for a reliable and objective measurement of pupil's size and light reflexes. It uses a disposable single patient headrest to prevent cross contamination. No calibration is required and an investigator familiar with the technology performs all measurements. The investigator uses gloves to keep the eye open and a picture of each pupil is taken on the three days of TH. Measurements are discontinued if the infant becomes irritable or if a correct reading is not possible. The parameters collected by the pupillometer are described in **Table 4.1** (70). Time of the recordings and

differences in pupil size between the two eyes are also calculated (**Fig. 4.2**). Additionally, a video of the constriction and dilation of both eyes is automatically recorded when an accurate reading is possible (**Fig. 4.3**).

Table 4.1. *Measurements obtained from the pupillometer*

Parameters	Unit of Measure	Definition/Calculation
NPi™ (Neurological Pupil Index™)	Scalar value 0-5	Composite score of pupillary response that uses an algorithm to compare the inputs of the variables below to a normative model.
MAX/MIN	mm	MAX = initial resting pupil size MIN = pupil size at the peak of constriction
%CH	%	Constriction % or Percentage Change ([MAX-MIN]/MAX)
LAT	Seconds	Latency = time difference between the initiation of retinal light stimulation and onset of pupillary constriction
CV/MCV	mm/sec	Average Constriction Velocity (CV) = amount of the constriction divided by the duration of the constriction Maximum Constriction Velocity (MCV) = peak value of velocity during constriction
DV	mm/sec	Average Dilation Velocity (DV) = amount of pupil size recovery (after the constriction) divided by the duration of the recovery

Retrieved from Neuroptics, Neuroptics Interpreting the Information from the Pupillometer, 2011 (70).

Figure 4.2. *Parameters obtained with the pupillometer*

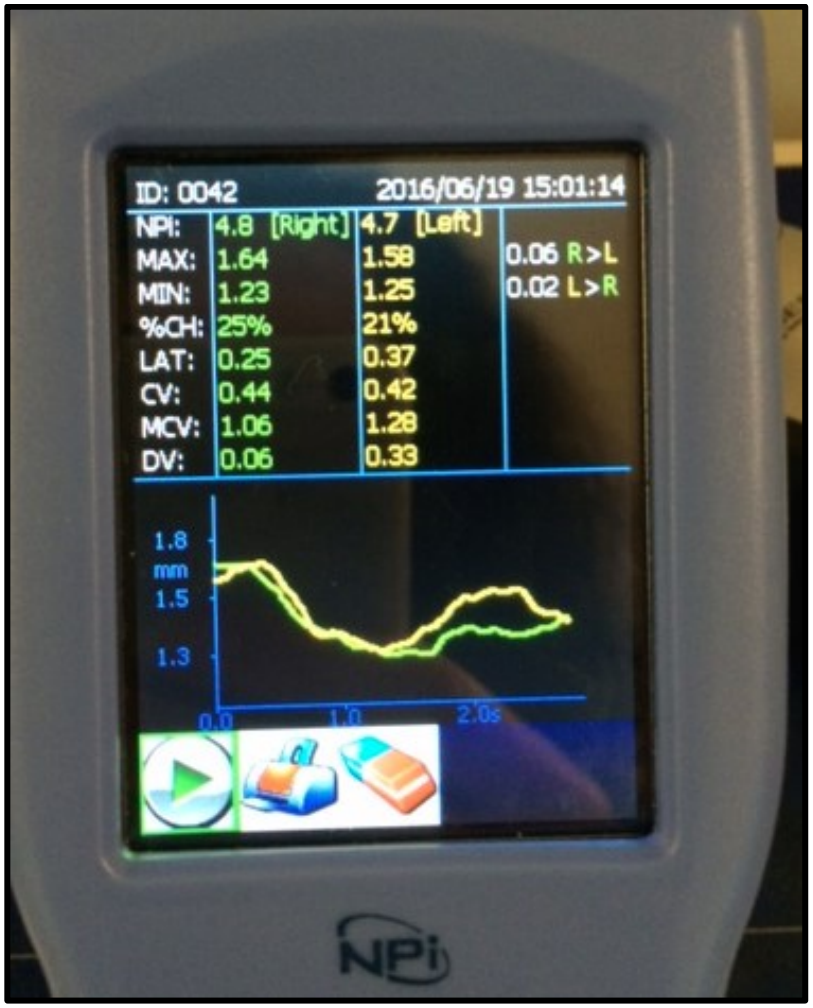
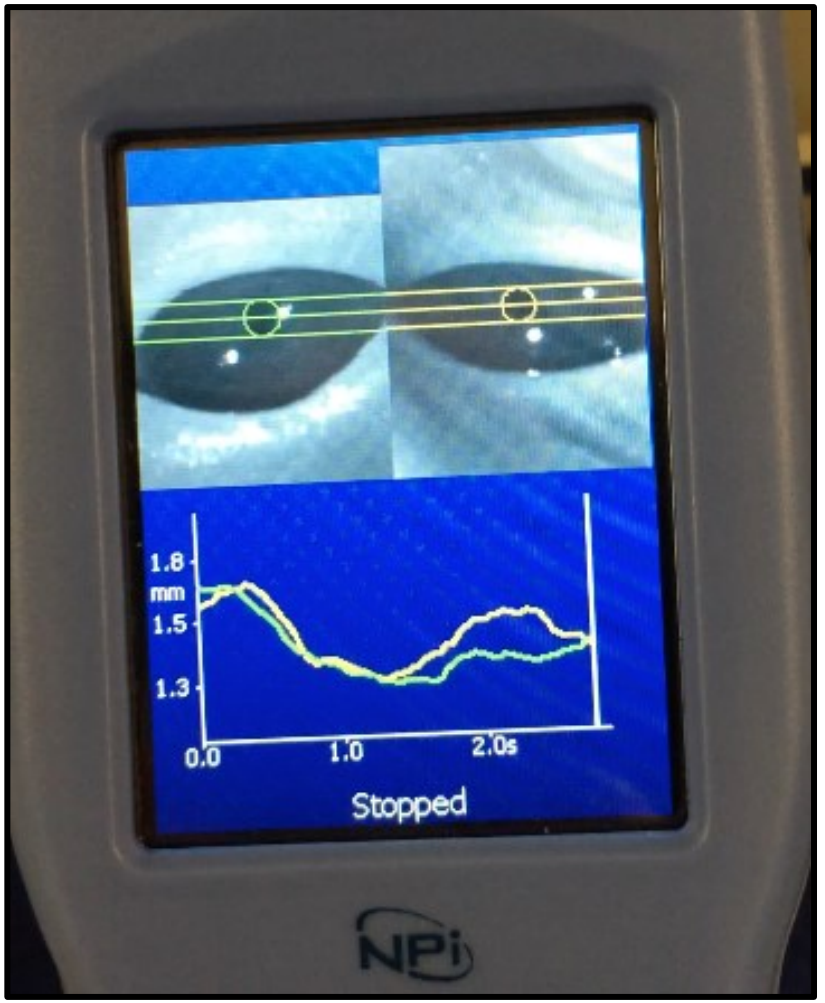


Figure 4.3. *Video comparing the constriction and dilation of the left (yellow) and right (green) eyes.*



D. Skin Perfusion

Skin perfusion is recorded using the skin blood FlowMeter (ADInstruments, Bella Vista, Australia). A small surface probe is secured under the axilla using a self-adhesive ring (**Fig. 4.4**) and measurements are recorded continuously for 1 hour on the three days of TH (**Fig. 4.6**) using the PowerLab data acquisition system (ADInstruments, Bella Vista, Australia).

E. Axillary Temperature

Axillary temperature (Ta) was recorded and measured continuously by using a surface probe placed at the axilla (**Fig. 4.5**) and connected to the PowerLab data acquisition system. Ta was measured continuously for 1 hour during the three days of TH (**Fig. 4.6**).

F. Electrocardiographic signals

Electrocardiographic signals are recorded continuously for 1 hour during the three days of TH by applying 3 ECG leads (**Fig. 4.4**) connected to a BioAmp and the PowerLab data acquisition system (**Fig. 4.6**) and stored for later calculation of heart rate variability by using the LabChart software®.

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Figure 4.4. *Setup showing placement of the 3 ECG leads (red, white, and black) and the skin perfusion probe. The white ECG lead should be placed on the right shoulder, the black on the left shoulder and the red on the left side of the abdomen or hip. The skin perfusion probe should be secured using a self-adhesive ring or sticker, under the axilla of the patient and should remain in good physical contact with the skin during recordings.*

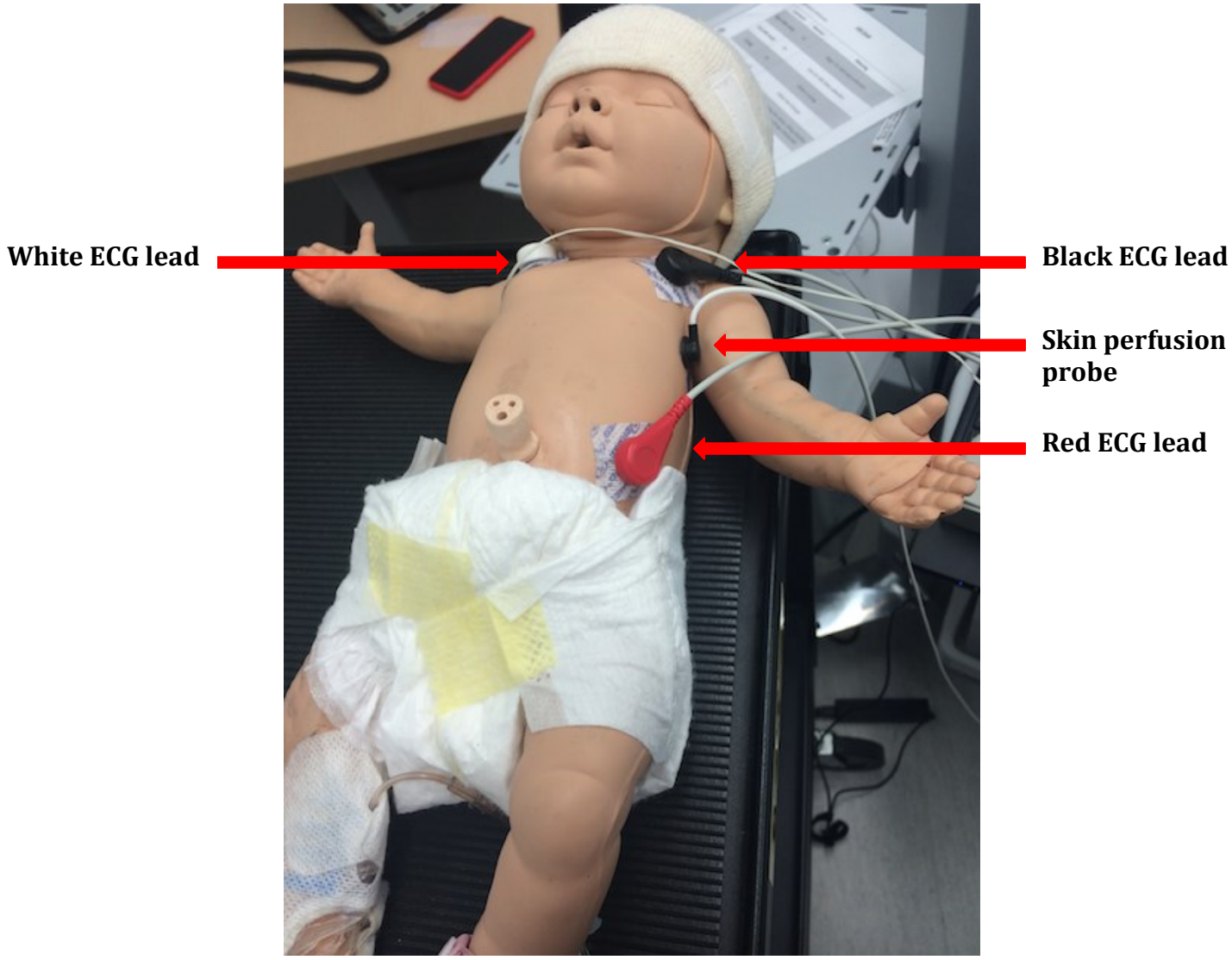

Figure 4.5. *Setup showing placement of the temperature probe under the axilla of the patient. Temperature probe should be secured using a sticker with the white plastic side of the probe in good physical contact with the skin during recordings.*

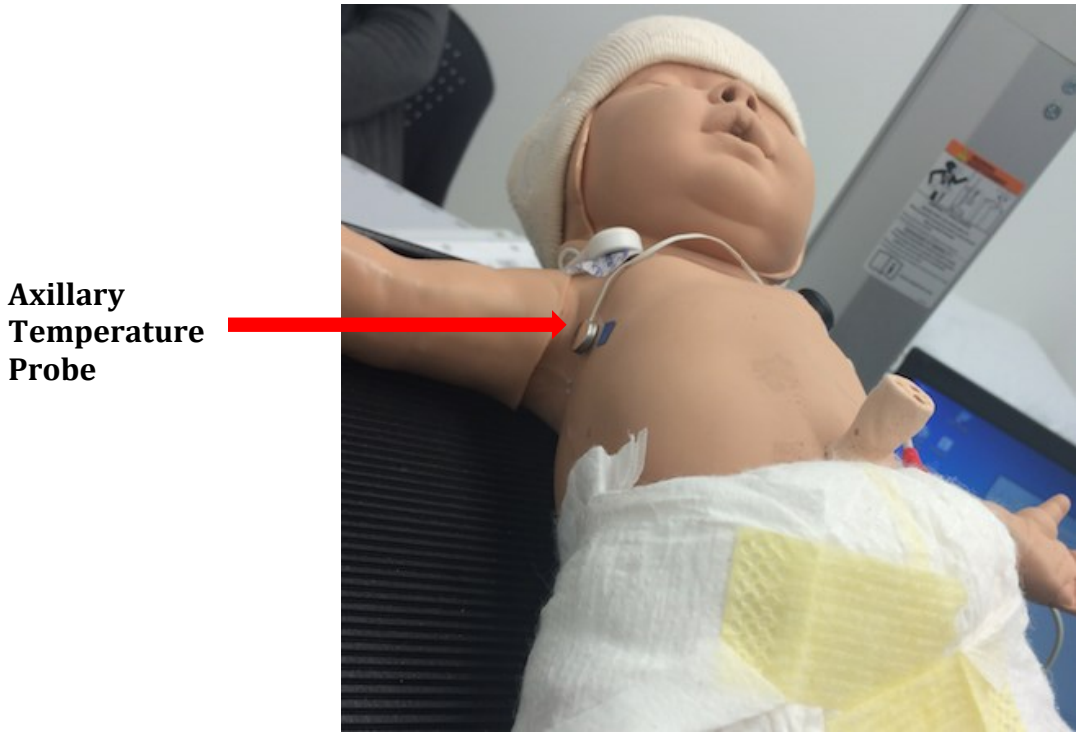

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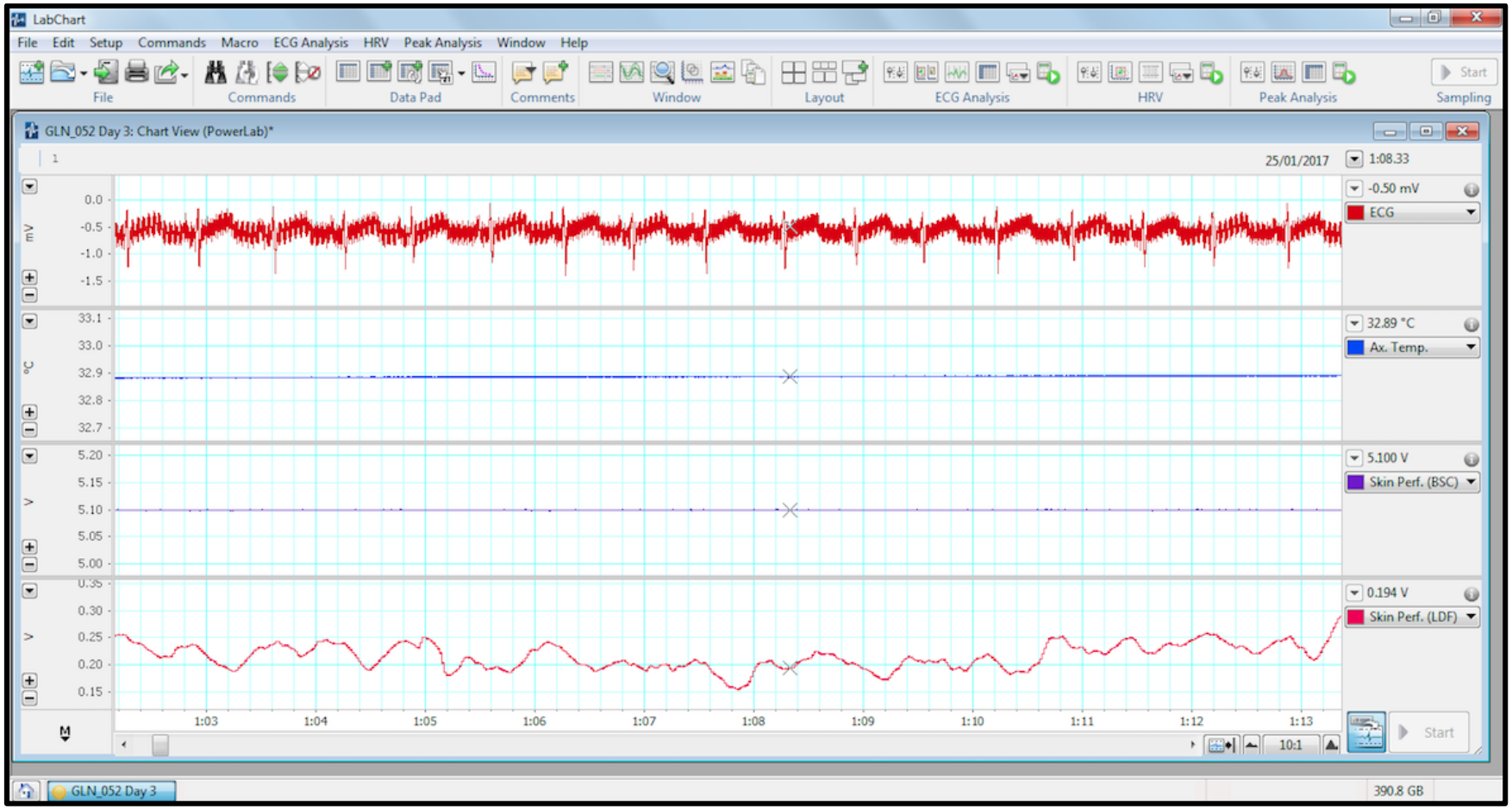
Figure 4.6. *LabChart patient file showing recordings of heart rate (red), axillary temperature (blue) and the two outputs for skin perfusion: the light backscatter (BSC) in purple and Laser Doppler Flowmetry (LDF) in pink.*



4.2.4 Data Analysis

A. Neurological Exam

Data obtained in the neurological exam form will be used to classify infants based on their final outcomes; this will allow us to determine if the new measurements of ANS evaluation are better correlated with an infant's neurological status.

B. Esophageal Temperature Data

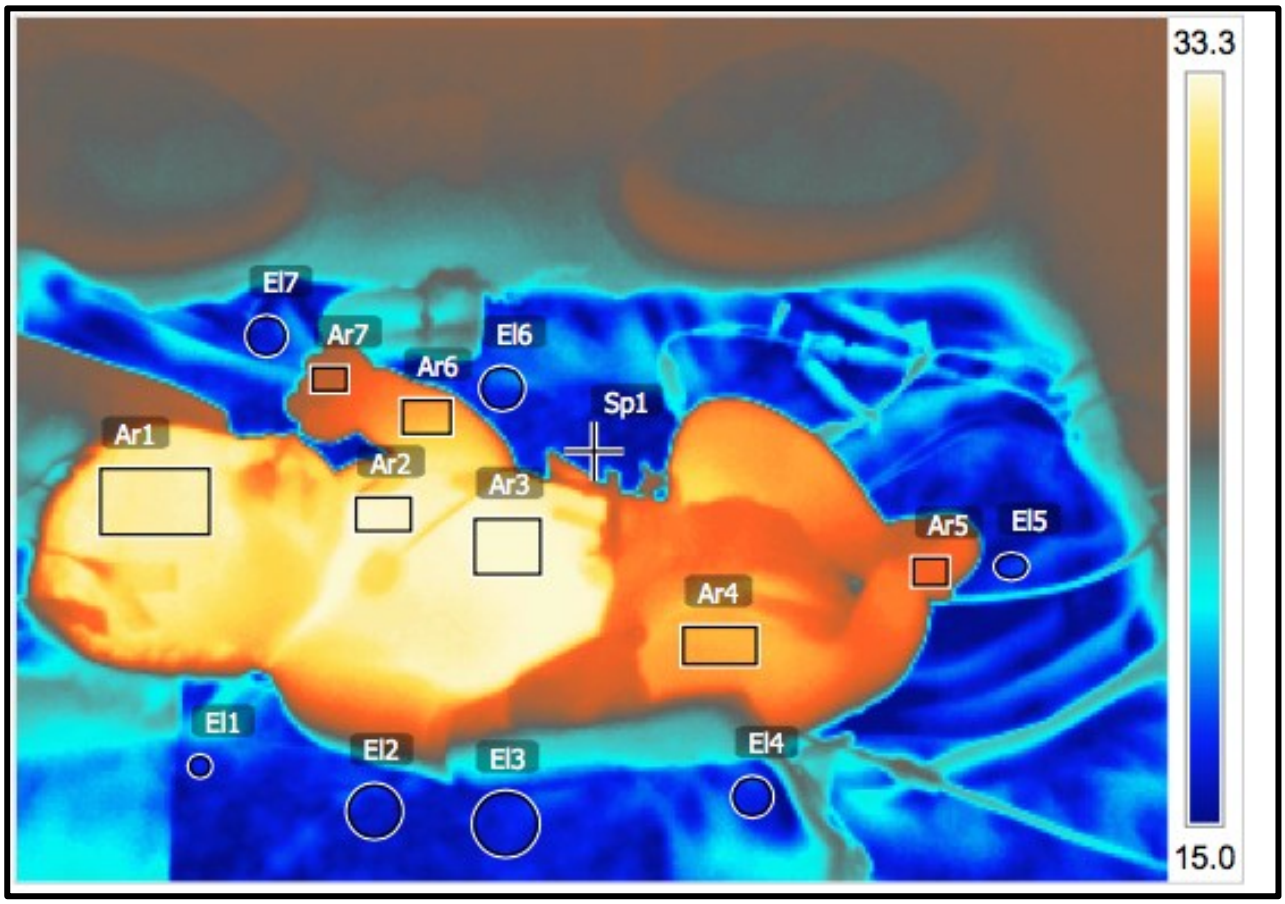
Statistical measures including mean ± SD, median, standard error, range values and the coefficient of variation of esophageal temperature (T_{es}) will be calculated for each patient, for each of the specified study periods.

C. Whole-Body Skin Temperature

Whole body skin temperature will be analyzed my mapping the temperature of the following body areas (Ar): head (Ar1), trunk (Ar2), abdomen (Ar3), legs (Ar4), feet (Ar5), arms (Ar6), and hands (Ar7) (**Fig. 4.7**). As a means of comparison, sections of the cooling blanket (El) will also be captured and analyzed. To allow for an evaluation of *skin temperature distribution*, mean ± SD (standard deviation) and range values (min and max) will be calculated for each area using the specific IR software. Two separate reviewers will analyze each of the chosen three images, per patient, and day of TH as a quality control for the analysis. As there is an inherent subjectivity in manually mapping the different areas of the body an average of the values taken by each of the reviewers will be used. Moreover, the values obtained for each of the parameters will be pooled and averaged between the three chosen images on each day as a separate and additional control mechanism. Additionally, the camera also automatically records the following

parameters at the time each image will be taken: emissivity, reflective temperature, distance, relative humidity, atmospheric temperature, IR window temperature, and IR window transmission. The investigator will ensure that these parameters remain constant for each image and each patient before analysis is performed.

Figure 4.7*. Infrared image of infant submitted to TH, captured with the E60bx camera. Corresponding body areas (indicated with rectangles) and areas of the Blanketrol (indicated with ellipses) are analyzed by the infrared software.*



D. Pupillometer Data

The pupillometer uses a scale to provide an objective and quantifiable score of the pupillary light reflex (70): the NPi^{TM} or the "Neurological Pupil Index^{TM}". This index is an algorithm developed to remove subjectivity from the pupillary evaluation by comparing the collected parameters to a normative model of pupillary reaction to light (70). The NPi^{TM} pupillometer automatically rates measurements on a scale of 0 to 5, with scores equal or above 3 representing normal pupil behavior (with scores closer to 5 representing a more "brisk" response) (70). Scores below 3 are considered abnormal or "sluggish", with this weaker than normal pupillary response shown in red on the device screen (70). NPi^{TM} measurements that appear red on the device screen will be interpreted with caution. Quite frequently, the pupillometer does not accurately measure the pupillary response. Also, NPi^{TM} values shown in red on the device screen may also indicate that the infant blinked or moved during the recording. Mean ± SD will be calculated for each of the pupillometer parameters including the NPi^{TM} index, for each patient during the specified study period.

E. Skin Perfusion

Statistical measures including mean ± SD, median, standard error, and range values of skin perfusion will be calculated for each patient for each of the specified study periods. Signals for Laser Doppler Flowmetry (LDF) and percentage of light backscatter (BSC) will be analyzed separately. The LDF output produces 1 mV for each blood perfusion unit (BPU), with the maximum output of the Blood FlowMeter being 5000 BPU or 5V (119). The BSC output is simply a voltage that represents the percentage of backscattered light or the relative strength of the returned signal (119). For instance, a BSC output signal of 5V would represent a 100%

backscattered signal, which means that the skin perfusion probe is in perfect contact with the skin and that the strength of the signal is the highest (119).

F. Axillary Temperature

Statistical measures including mean ± SD, median, standard error, and range values of axillary temperature will be calculated for each patient for each of the specified study periods.

G. Heart Rate Variability

Heart rate variability will be calculated using the time domain and frequency domain analysis, from 5-minute segments of ECG and using the HRV module of the LabChart software® (ADInstruments, Bella Vista, Australia).

G. Clinical Data

Clinical and demographics data obtained in the data collection form will be used to classify infants based on their final outcomes; this will allow us to determine if the new measurements of ANS evaluation are correlated with an infant's neurological status.

4.2.5 Statistical Analysis

Descriptive statistical analysis will be performed on clinical data. Data will be presented as n (%), mean ± SD, and median (interquartile range). As this is an ongoing study, for the primary outcome, a statistical analysis will be performed at a later time, once a higher sample size can be achieved.

4.3 Study in Progress: Preliminary Results

Throughout the first year of recruitment (June 2016 to 2017), a total of 33 patients were treated with TH and 8 included in the study. Details on patient screening, eligibility and enrollment are provided in **Fig. 4.1**. Of the 8 patients enrolled 4 had all measurements performed on all 3 days of TH and 4 were studied only on days 2 and 3 of cooling. In these 4 patients measurements were not done on day 1 due to unavailability of the parents for consent. Given the lack of studies using the above outlined methodology a convenient sample size of 30 patients was calculated, but we plan to perform an interim analysis after the first 15 patients to re-assess the necessary sample size.

4.4 Study in Progress: Interim Discussion

During the first year of enrollment and recordings, we faced important issues related to this type of study. First, recruitment rate was slower than initially expected due to several factors: a) low consent rate - this is a critical population making the parents hesitant to participate on research involving a few measurements; b) inability to approach parents due to availability, social restrictions, or decision to withdraw care; and c) presence of a competing study recruiting a similar patient population (infants submitted to TH, with an abnormal aEEG on admission). Since this study is testing a drug with a double blind design, which may affect our physiological measurements, we could not share enrollment. Nonetheless, we will continue enrollment for another year and expect to make some important progress since the competing study is close to end.

Another issue identified is the difficulty to perform measurements within the first 24-hours of life, mostly related to obtaining consent from parents. In half of our patients this was not possible. Therefore, measurements on day 2 and 3 are more feasible and will be used in the

primary analysis. Since the competing study selects only neonates with an abnormal aEEG and MRI on day 2 of age, our population is perhaps skewed towards less severe infants. In addition, a control or comparison group was not included. Perhaps, measurements on infants with mild NE could have been done. However, this may be limited, as mild NE infants are not cooled as part of their standard of care, and body temperature may affect our physiological measurements regardless of the infant's neurological status. Nevertheless, we recently decided to include a sub-group of mild NE infants and are applying to REB for protocol amendments.

We also learned that the use of the pupillometer device in this population is challenging. Obtaining measurements for pupil size and velocity of pupillary constriction was rather difficult. The device requires a specific amount of time to accurately register the pupil, the infant cannot move, and their eyes have to remain open; this is difficult in neonates in an irritable state. Lastly, as the color contrast between the pupil and the iris is very light in neonates, the pupillometer device was often unable to identify the pupil. Thus, the device often misread the pupil size returning inexact measurements (red color). Indeed, due to these limitations, reliable measurements during all 3 days of TH could only be obtained for 1 infant. Perhaps, new methods or ways to handle the device can be implemented in the near future to ensure more accurate readings, or this technology cannot by applied to this population.

4.5 Conclusion

This is an ongoing prospective observational study. Up to date, at total of 8 patients were enrolled, and important limitations were identified. This resulted in a proposal for a protocol amendment to include infants with mild NE and a modification to the protocol to perform

measurements only on days 2 and 3 of age. An interim analysis at a future time point will be needed once a higher sample size is achieved.

Chapter 5 – Conclusions

5.1 Overall Conclusions

Early identification of an infant at risk for NE secondary to a hypoxic-ischemic insult, and their initiation on TH relies strongly on the neurological evaluation performed within the first 6 hours of life (47). Also, the evolution of the neurological exam during the first days of age is a good predictor of long-term outcomes (47, 99). Therefore, appropriate documentation of the neurological evaluation may ensure continuity and quality of care. At the Montreal Children's Hospital's NICU, a standardized neurological form was developed and implemented but analysis of its adherence was sub-optimal with a significant decrease of its use from admission to day 4 of age, with critical information severely under-reported. This quality assurance study has demonstrated the need for ongoing efforts to improve adherence to the neurological form.

A modified Sarnat exam is part of the form and used to determine the final NE stage. Of the six categories that comprise the exam, assessment of the ANS was the least reported category at all points assessed, from admission to day 3 of TH. We speculate that the underreporting of this category could be related to the fact that assessment of the 3 components of the ANS category (pupils, heart rate, respirations) is subjective and prone to inter-examiner variability. As such we investigated whether or not this category added value or contributed to the final NE stage. Interestingly, ANS was the only one of the six categories that was not significantly associated with the final NE stage at all points during TH. It is possible that the *selective neuronal vulnerability* of the neonatal brain to asphyxia may also explain the lack of ANS contribution to the final NE stage. A number of investigations have demonstrated that the brainstem, which controls ANS functioning, is less sensitive to episodes of ischemic anoxia (105-108) and rarely involved in the majority of infants with NE. Indeed, brainstem injury is

mainly observed in severely encephalopathic infants, that are more prone to death soon after delivery (109, 110). Thus, infants are either anatomically spared from a hypoxic-ischemic insult and/or die soon after delivery, and as such do not have a neurological examination.

A more comprehensive analysis of the ANS is under investigation since it may be able to provide important information that can be associated with the final NE stage and/or short- and long-term outcomes. This analysis involves recording and analyzing biological signals such as esophageal temperature, whole body skin temperature, axillary temperature, skin perfusion, and heart rate variability. This investigation is also introducing the use of innovative technologies and new concepts such as infrared imaging and an automated pupil device. These new methods will allow for the mapping of skin temperature (infrared skin temperature distribution) and can determine constriction velocity of the pupils, respectively. We hypothesize that this more comprehensive manner of assessing ANS function in asphyxiated neonates treated with TH will be more closely related to the evaluation of the stage of NE and their clinical outcomes. In this ongoing study, 8 patients have been enrolled and studied. An interim analysis is planned at 15 patients and calculation of a proper sample size will be done.

In summary, the work of this thesis provides a scientific review of the literature pertaining to this topic, a quality assurance study on the adherence of a standardized neurological form in asphyxiated infants treated with TH, and determines for the first time the contribution of the various categories included in the modified Sarnat exam to the final NE stage. Furthermore, it includes a prospective observational study protocol aiming to investigate a more comprehensive assessment of the ANS by using sophisticated analyses of biological signals and bedside advanced technologies. Preliminary results and discussion of limitations identified was previously reported.

5.2 Future Work

The prospective observational study was restricted by both time and enrollment opportunities. Continuing recruitment to increase the sample size will provide enough data for a future, comprehensive analysis of ANS function and its association with the final NE stage as well as important clinical outcomes.

In this future endeavor the enrollment criteria will be expanded to include neonates with mild NE on the standardized neurological exam at admission. This group of patients will be used as a control group as previously described in chapter 4 (section 4.4). Also, the study of this sub-group of patients is important given that they may eventually show evidence of brain injury over time.

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