Exosomes, artificial intelligence, and beyond: New paradigms in HIE management

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Disclosure Statements

- I have no relevant financial relationships to disclose or conflicts of interest to resolve.
- I will not discuss any unapproved or off-label, experimental or investigational use of a product, drug or device.

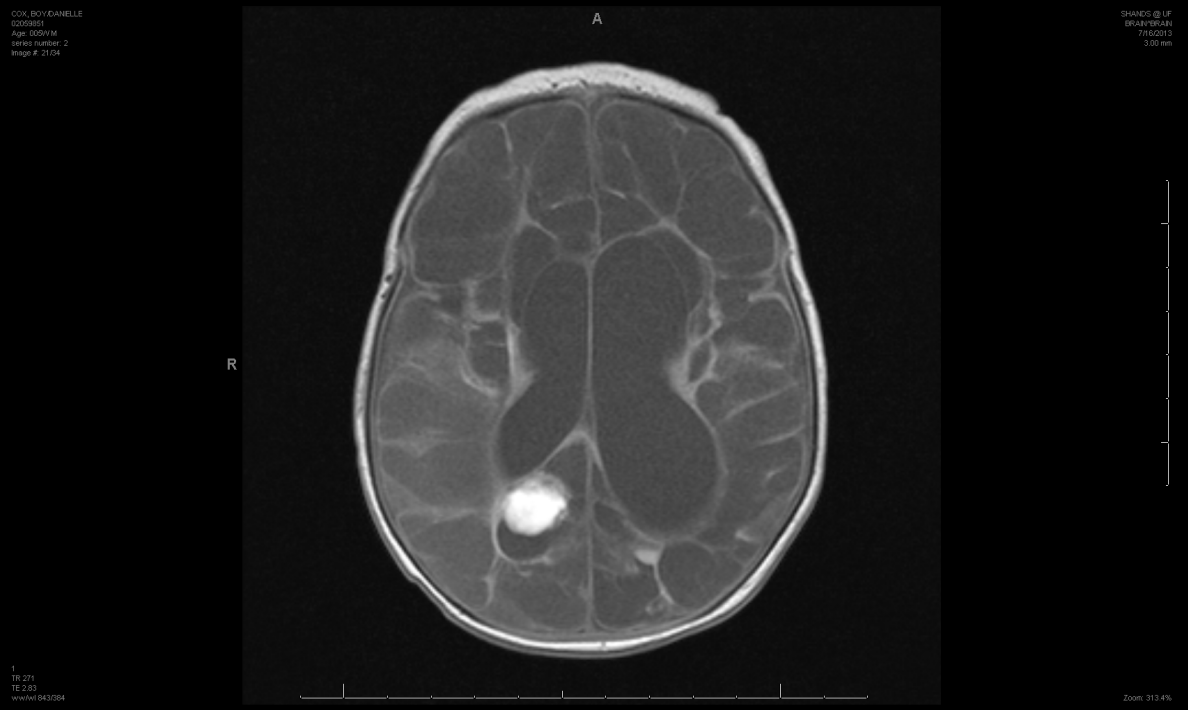
Case 1

- Mother presents 38-weeks of gestation with a placental abruption
- Emergent C-section performed
- At Birth:
  - Requires BMV, intubation, CPR and epinephrine
  - First heart rate detected at 12 minutes of age

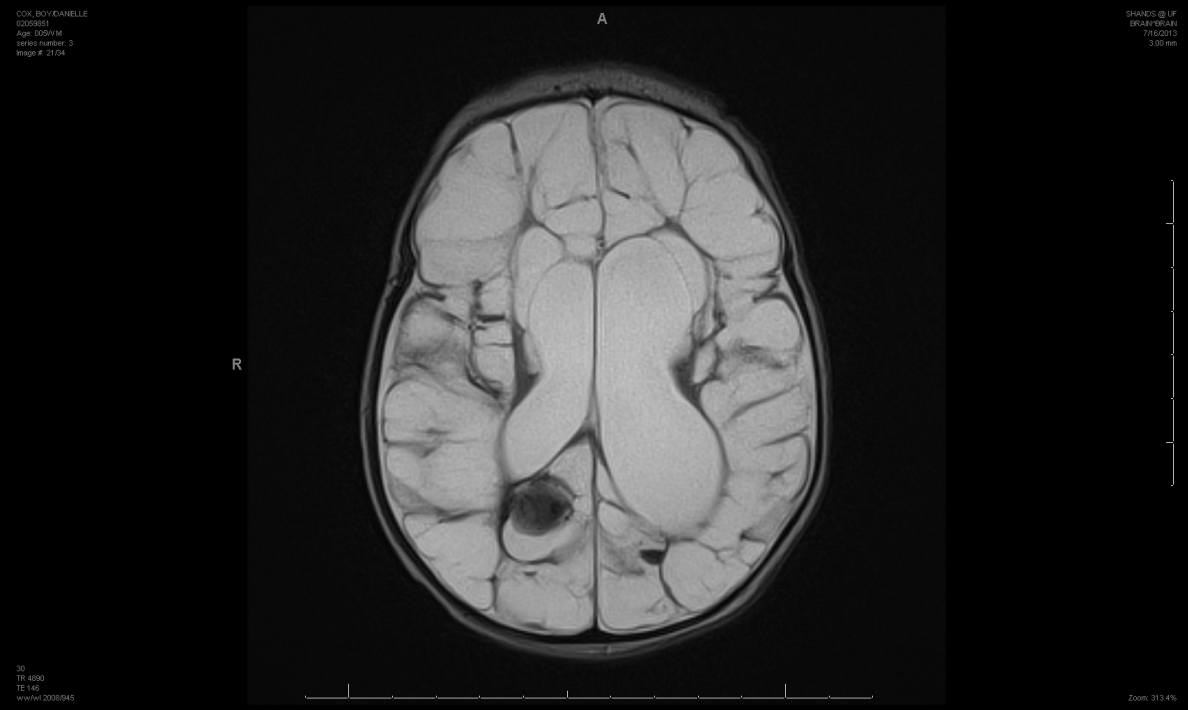
Case 1

- Arterial cord gas= 6.78/102 with a base deficit of -18.
- Neurologic Exam-
  - Lethargic
  - Increased tone
  - Decerebrate posturing
  - No reflexes, no suck
  - Pupils were dilated with no reaction to light.

T1


COX,BOY,DANIELLE
02059851
Age: 005Y/M
series number: 2
Image #: 21/34
A
SHANDS @ UF
BRAIN^BRAIN
7/16/2013
3.00 mm
R
1
TR 271
TE 2.83
ww/wl 843/384
Zoom: 313.4%

T2


COX,BOY,DANIELLE
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Age: 005Y/M
series number: 3
Image #: 21/34
A
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BRAIN^BRAIN
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3.00 mm
R
30
TR 4000
TE 146
ww/wl 2008/945
Zoom: 313.4%

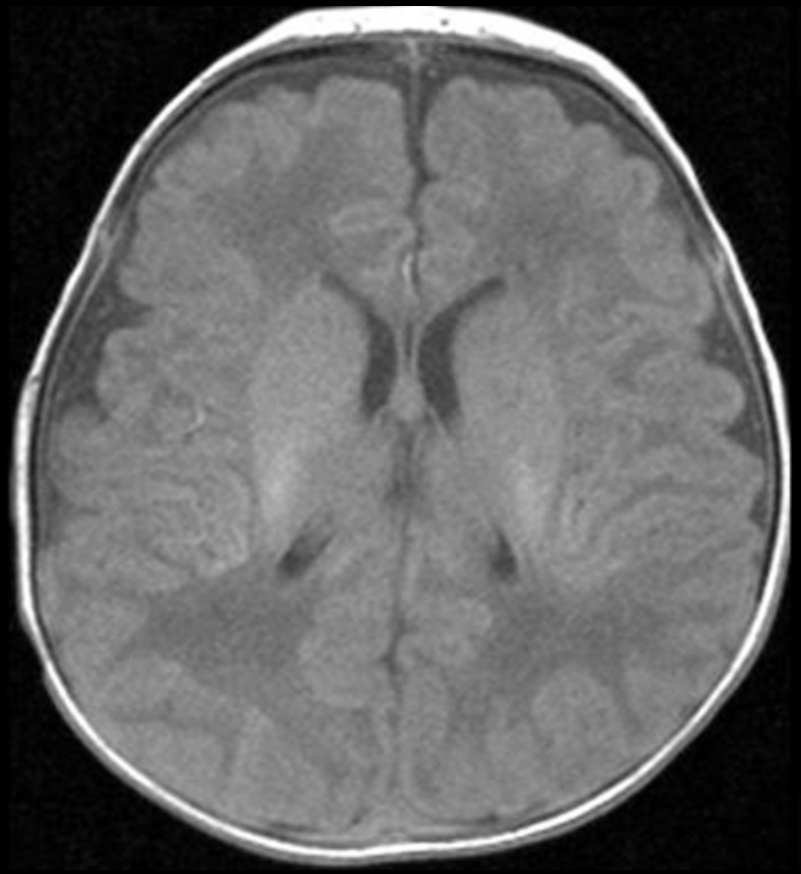
Case 2

- Mother involved in MVA at 38-weeks of gestation
- MVA causes a traumatic placental abruption
- Emergent C-section performed.
- Requires BMV, intubation, CPR and epinephrine
- First heart rate detected at 15 minutes of age
- Initial hematocrit of 18
- Requires emergent mass transfusion protocol

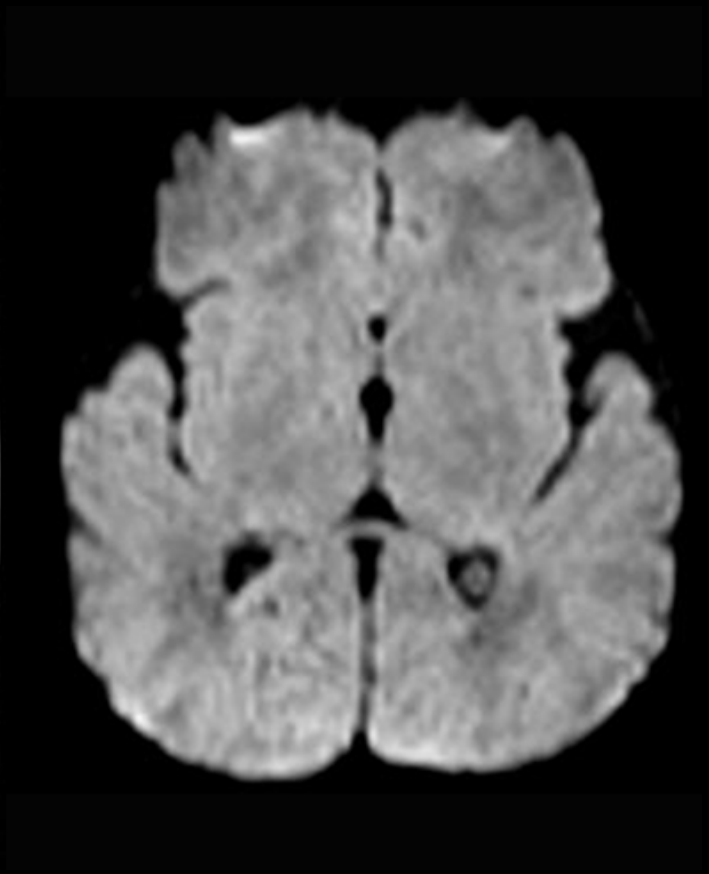
Case 2

- Arterial cord gas= 6.70/98 with a base deficit of -20.
- Neurologic Exam-
  - Lethargic
  - Increased tone
  - Decerebrate posturing
  - No reflexes, no suck
  - Pupils were dilated with no reaction to light
- EEG is found to have an isoelectric background

T1



DWI



Biomarkers

- Response to therapeutic hypothermia
  - Adjunct therapies
    - Clinically
    - Research
- Provide objective data on the severity of hypoxic-ischemic injury
- Mild HIE

Characteristics of Ideal Neuronal Biomarkers

- Cross blood brain barrier
- Collected peripherally and non-invasively (ie blood>CSF)
- Highly correlated with clinical outcomes
- Rapid turnover in circulation to monitor response to treatment or pharmacologic endpoints
- High throughput testing for rapid results

Lecture Outline

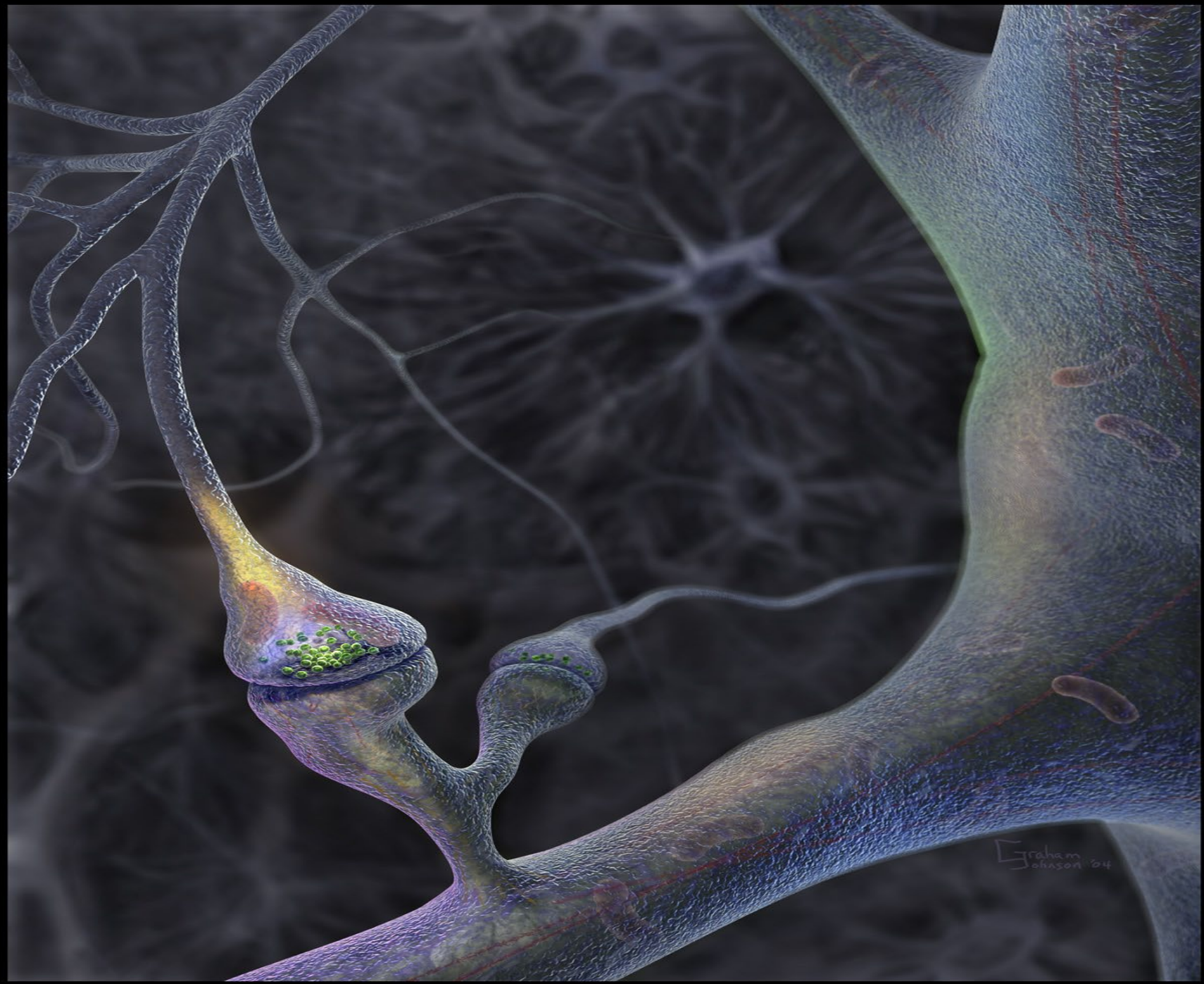
I. HIE
    A. General
    B. Pathophysiology
II. Exosomes
III. Machine Learning/Artificial Intelligence
    A. Predicting Outcomes
    B. Fetal Heart Rate Tracings
IV. Summary
    A. The Future

IA. General

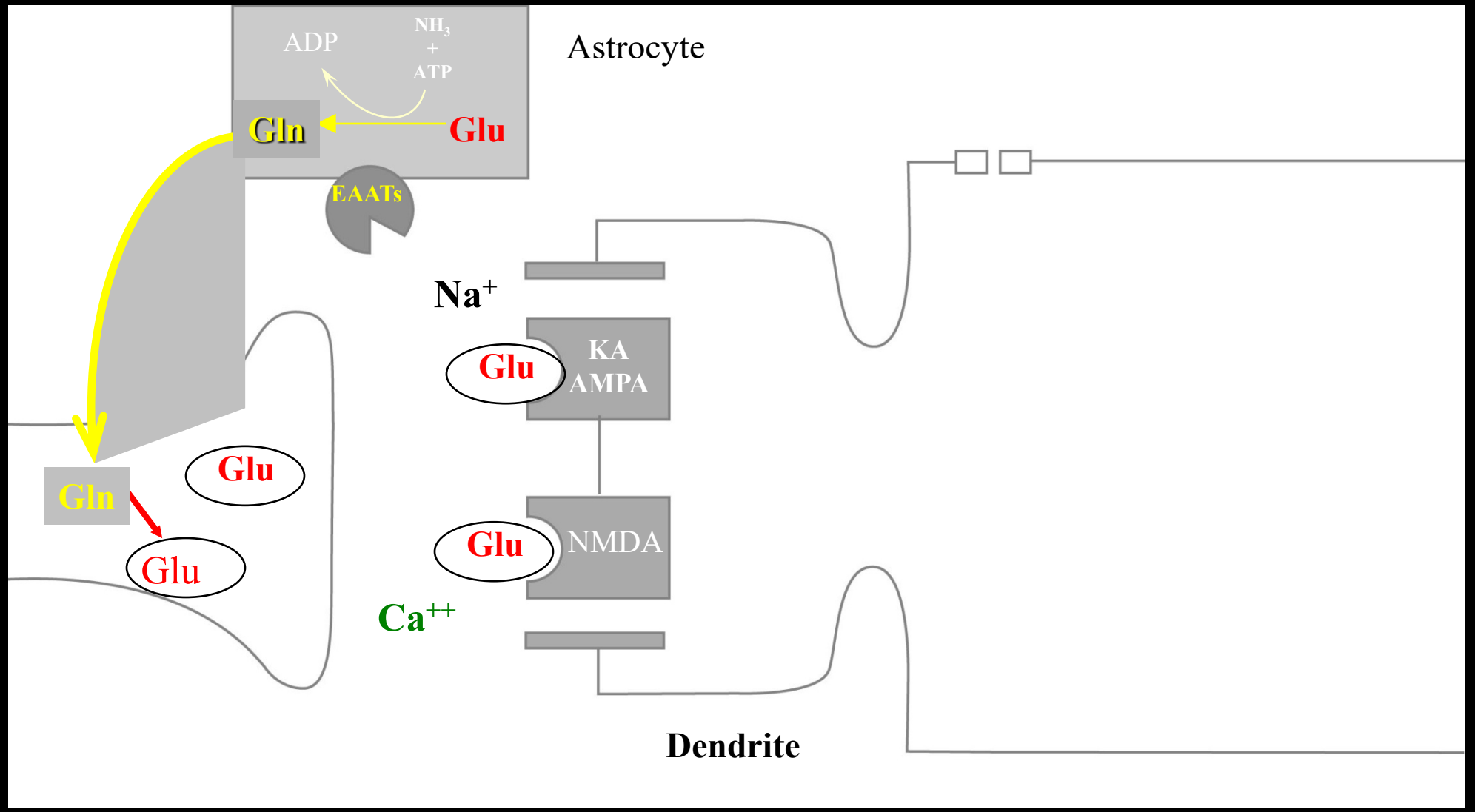
IA. General

- Neonatal encephalopathy due to HI occurs in 2 to 4 out of 1,000 full-term neonates.
- 24-33% of neonates with HIE die during the newborn period.
- Those who survive, 40% develop permanent neurologic handicap.

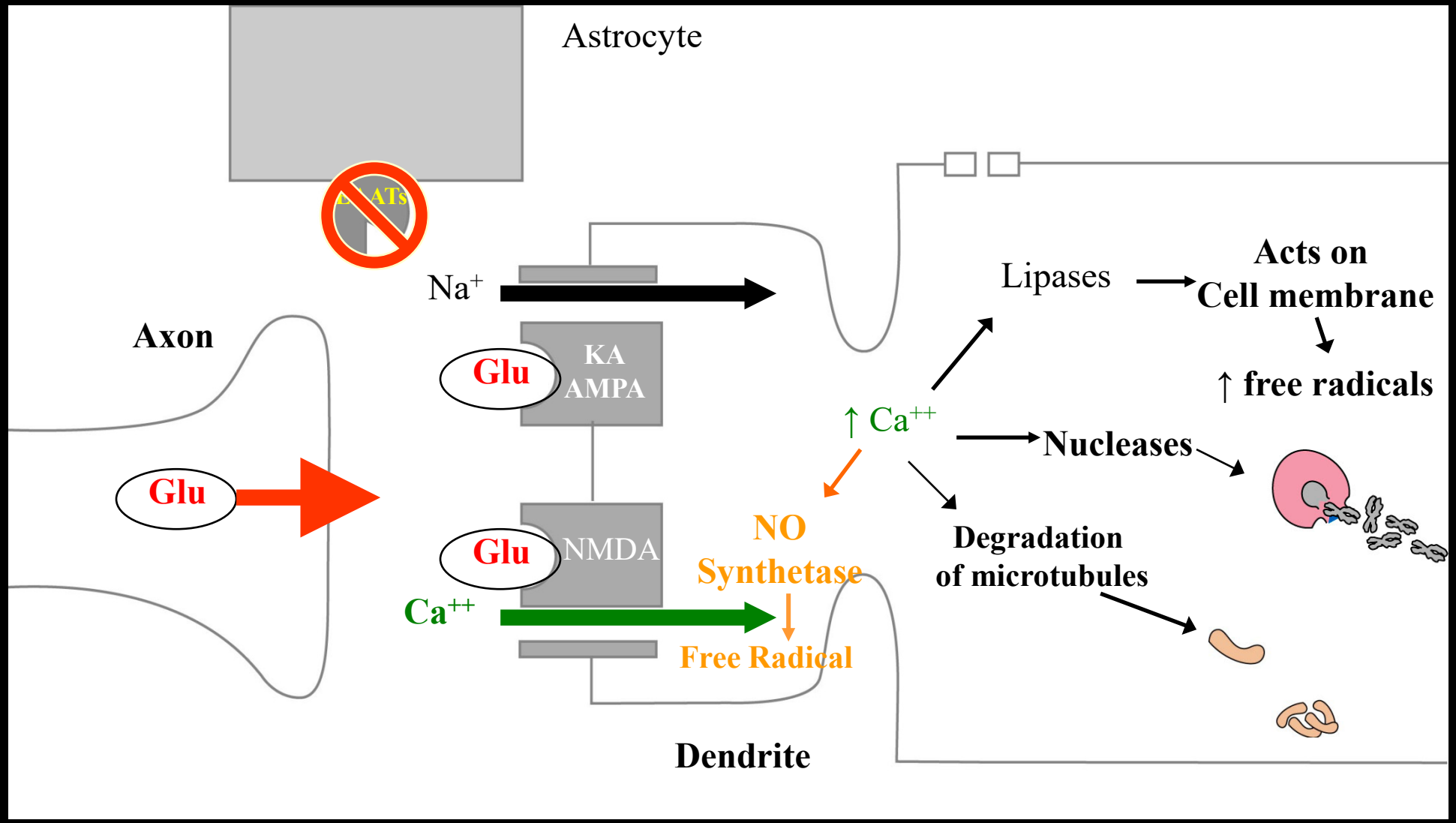
IB. Pathophysiology

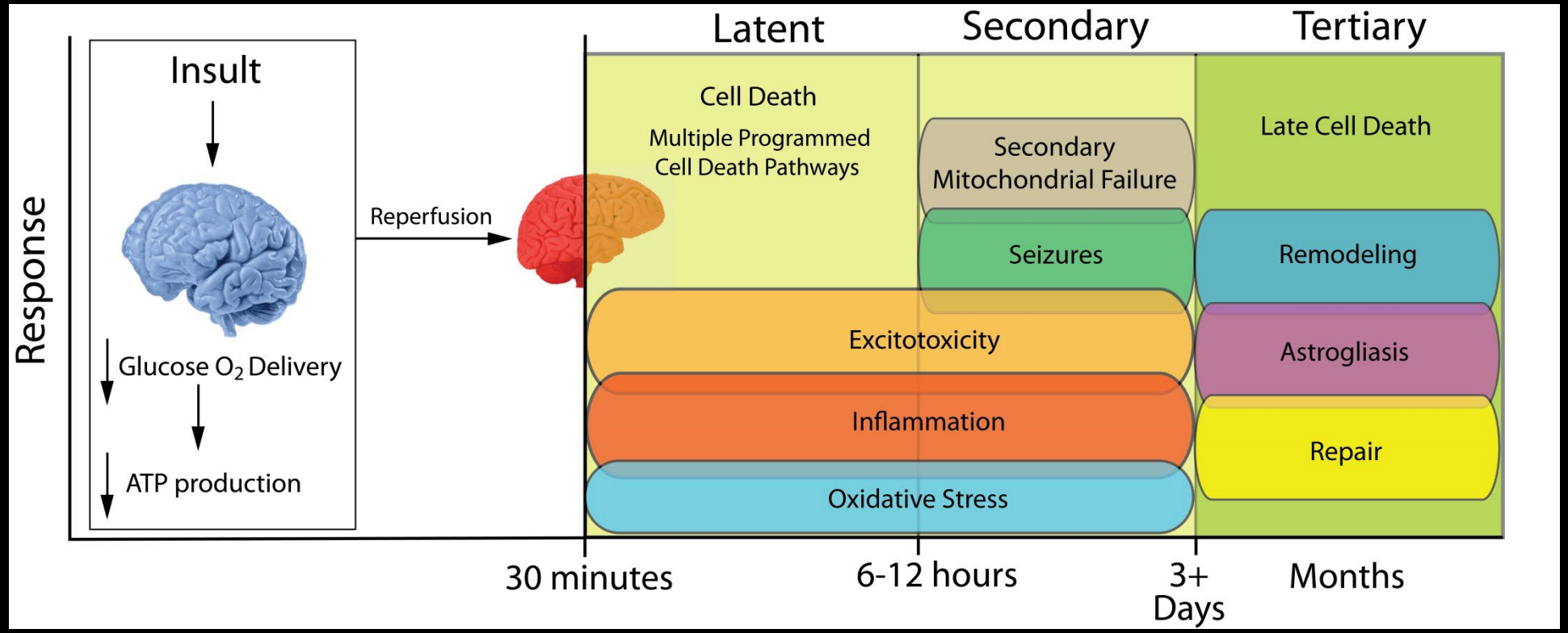

Graham
Johnson 04

II. Pathophysiology

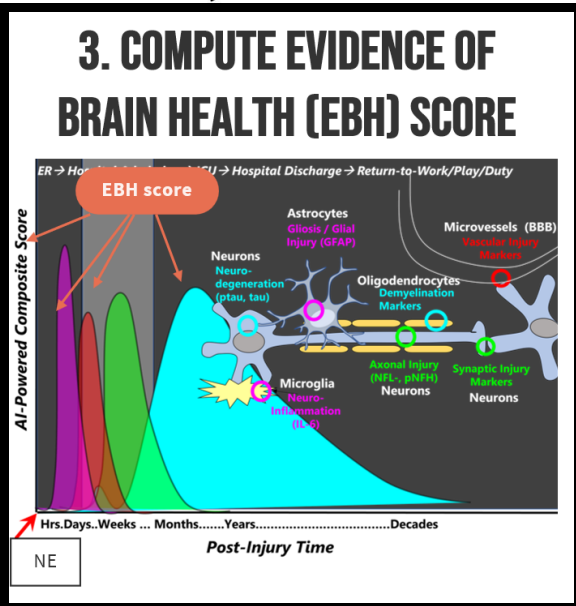


II. Pathophysiology



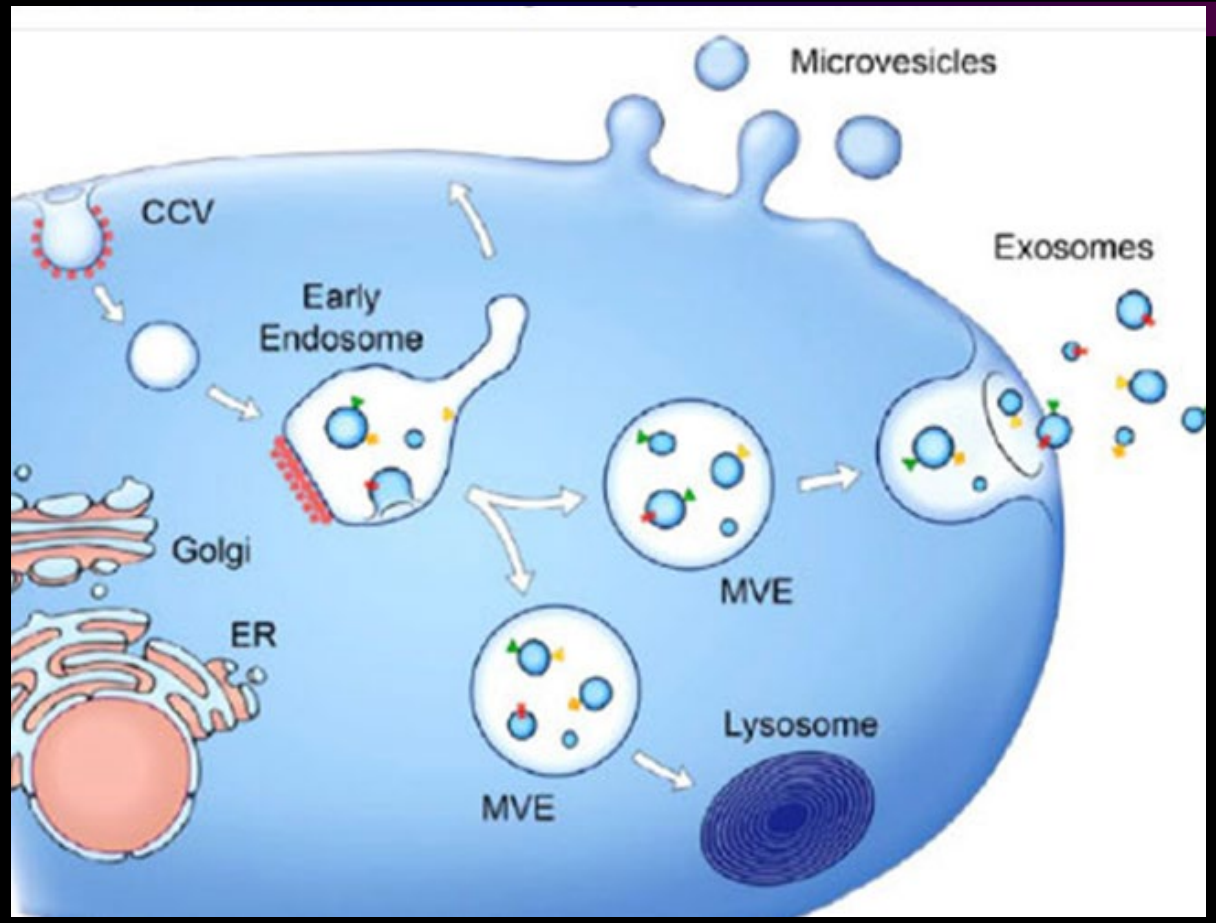


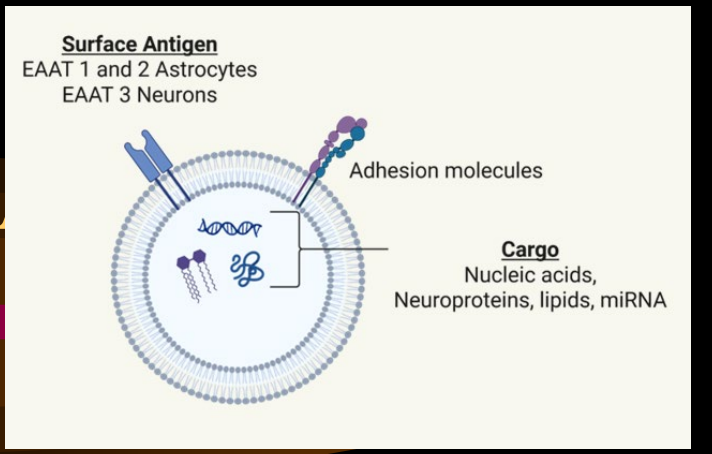
24-40% Sentinel events



II. Exosomes

Exosomes – Definitions and





- Exosomes– characterized by size, composition and density
  - Exosomes 30-120 nm
- Endosomal Sorting Complex Required for Transport (ESCRT) pathway results in formation of endosomes and multivesicular endosomes (MVE)
- Important roles
  - Cell to cell communication
  - Immunoregulation
  - Development of synaptic plasticity
- Diffuse across the blood brain barrier

Rapso & Stoorvogel J Cell Biol 2013

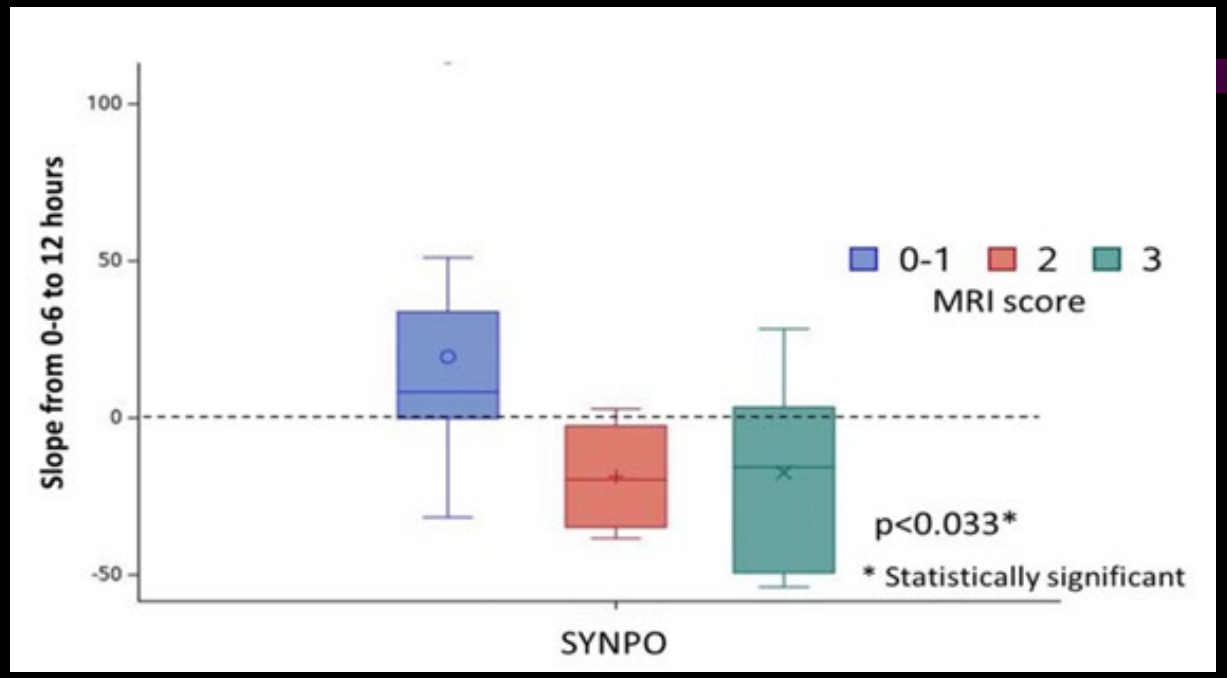
Hypoxic-Ischemic Encephalopathy: Research Questions

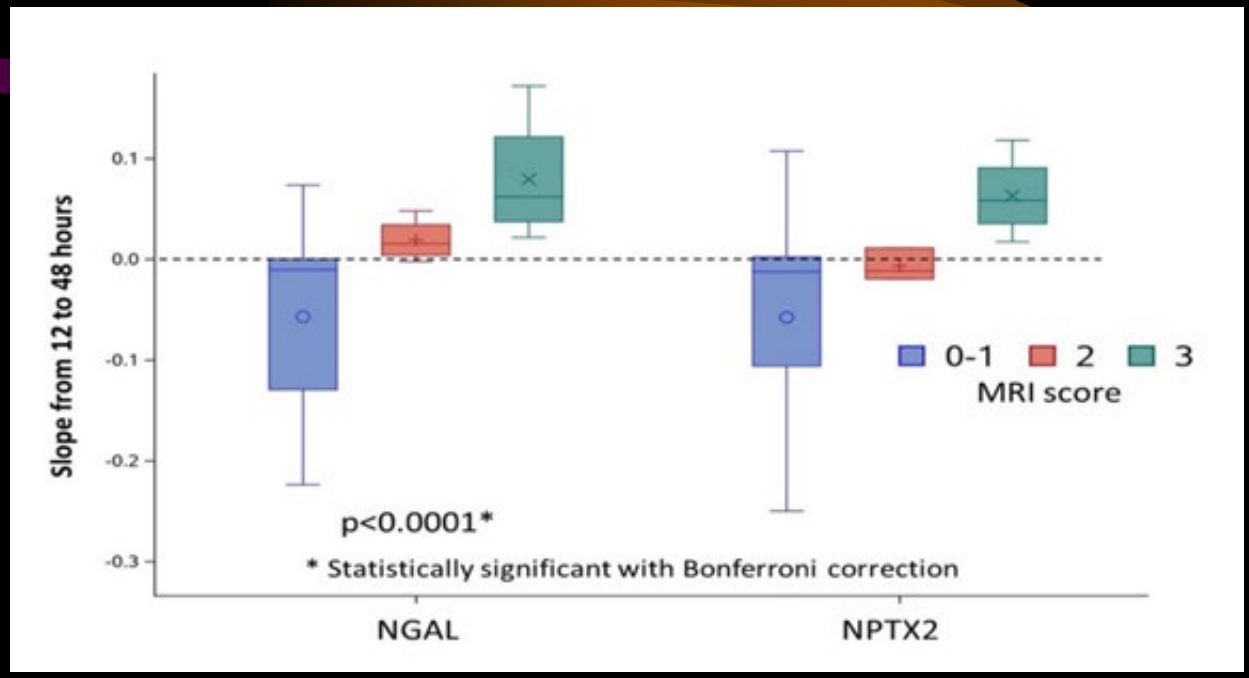
- Can we develop a “troponin like test” to accurately identify the timing and severity of fetal hypoxia-ischemia
  - Predicting neurodevelopmental outcomes
  - Hypothermia
  - Stratification for future neuroprotective trials

Goetzl et al, Ann Clin Transl Neurol 2017

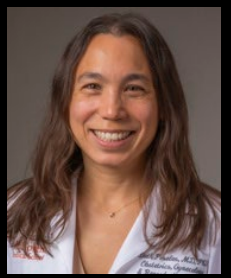
Neutrophil gelatinase-associated lipocalin (lipocalin-2, NGAL) is a glycoprotein that is involved in neuroinflammation.

Exosome based Biomarkers: HIE





Neuropetraxin-2 (NPTX2) is involved with complexes at excitatory AMPA type glutamate receptors and contributing to developmental synaptic plasticity.



Pineles et al; Arch Dis Child Fetal Neonatal, 2021

Free circulating versus Exosomes Biomarkers

Predictive values for MRI-defined injury severity **(PPV positive predict value, NPV negative predictive value)**				
	Serum miRNA	95% CI	EV-derived miRNA	95% CI
Sensitivity	71%	(56-86%)	100%	(99-100%)
Specificity	86%	(73-96%)	95%	(88-100%
PPV	77%	(62-90%)	93%	(84-100%)
NPV	82%	(68-94%)	100%	(99-100%)

Dakroub F, Kobeissy F, Mondello S, et al. MicroRNAs as biomarkers of brain injury in neonatal encephalopathy: an observational cohort study. *Sci Rep*. Mar 19 2024;14(1):6645.

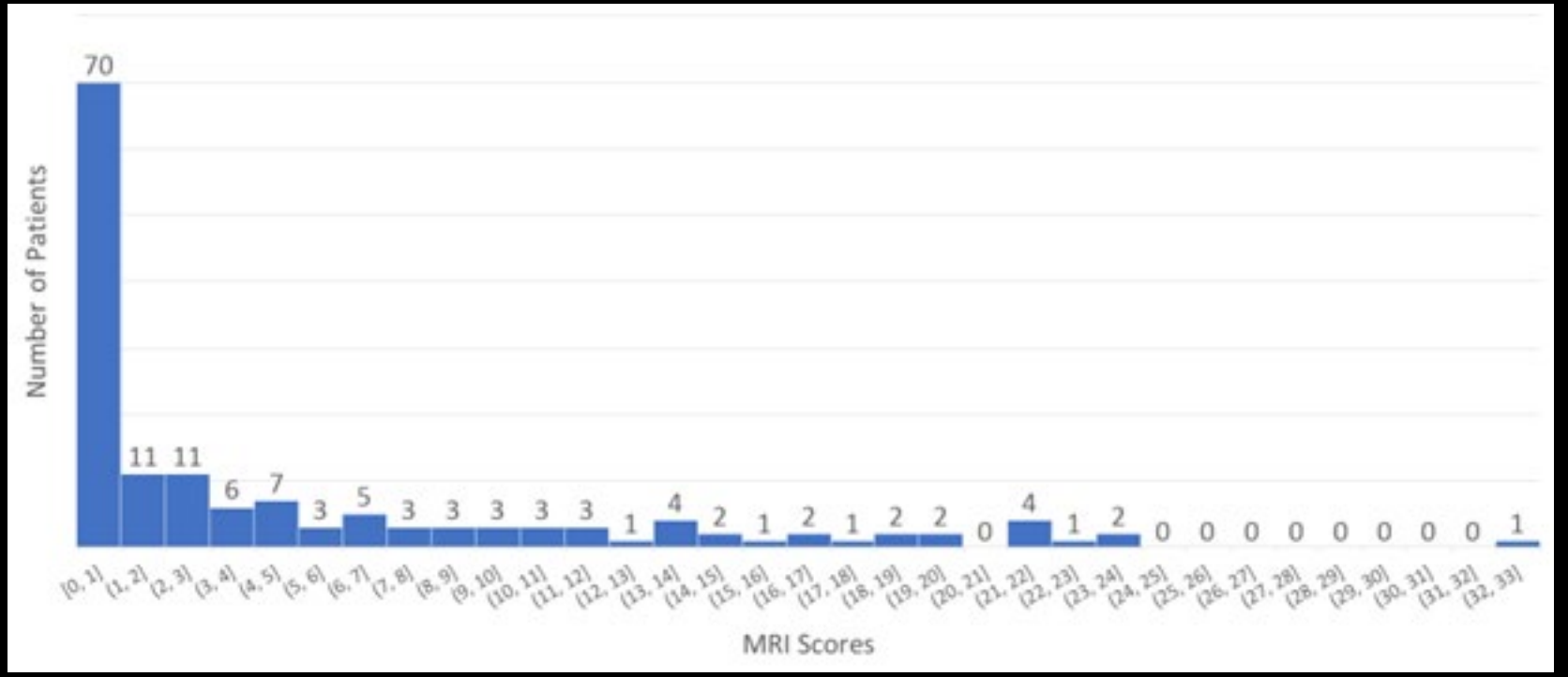
III. Machine Learning/Artificial Intelligence

IIIA. Predicting Outcomes
Digital Biomarkers

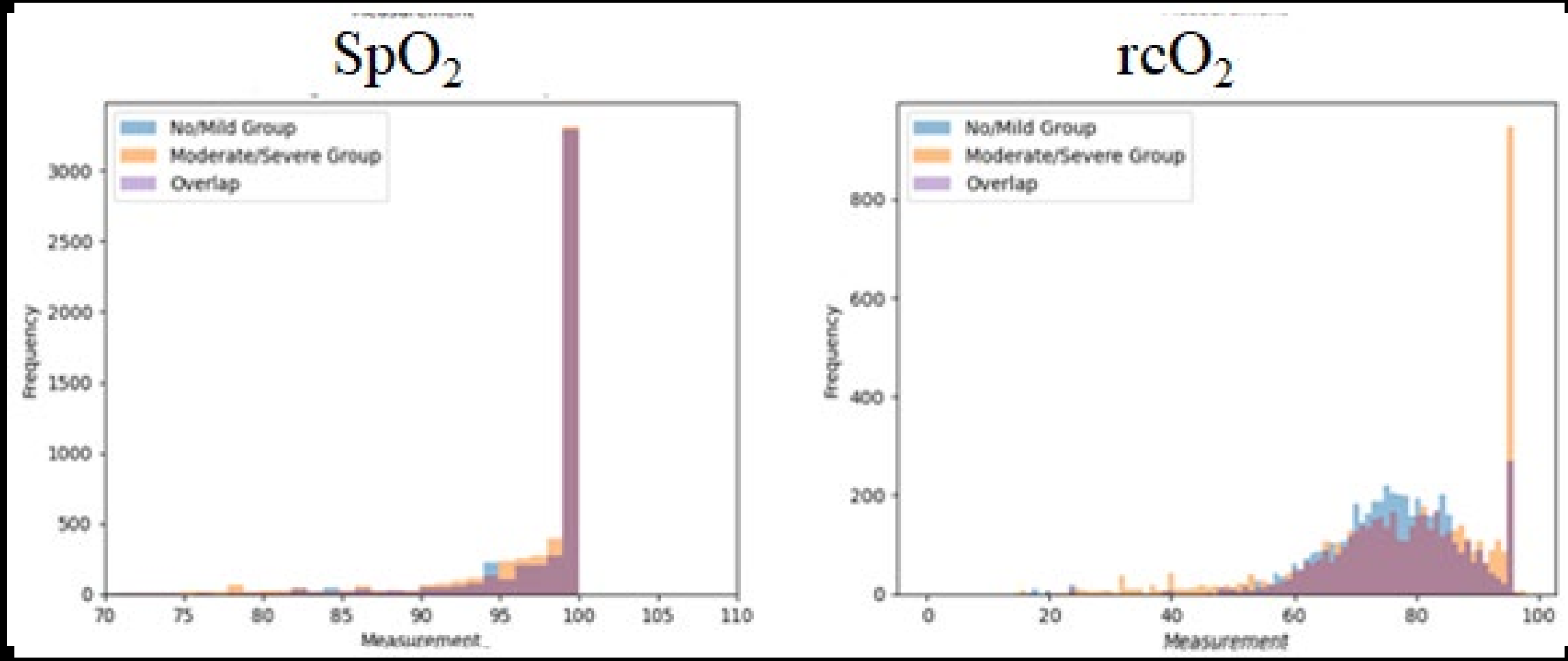
Digital Biomarkers

Digital Biomarkers

- A total of 138 subjects were included in this cohort analysis



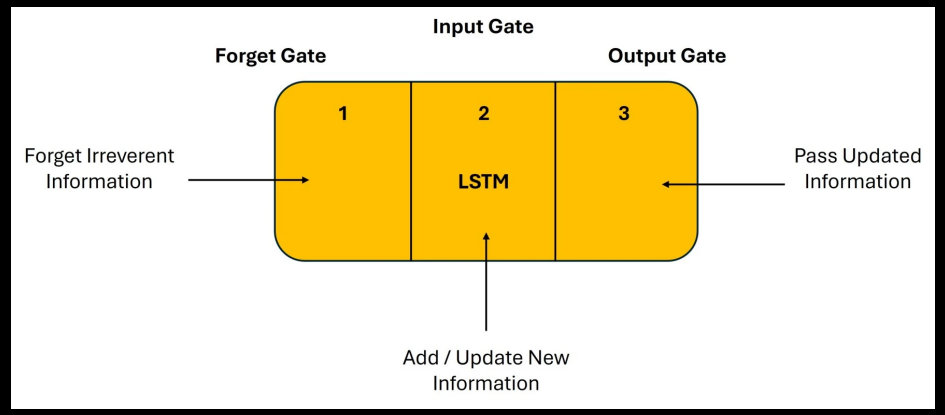
Infant Characteristics at Enrollment	NE (n=65)	NE (n=73)
	No/mild injury on MRI	Moderate/severe injury on MRI
Sex (%)		
Female	46	40
Male	54	60
Gestational Age in weeks (mean ± SD)	38±2	38±2
Birth weight in grams (mean ± SD)	3219±752	3212±740
Apgar score at 1 minute (mean ± SD)	2±2	2±2
Apgar score at 5 minutes (mean ± SD)	4±2	4±2
Apgar score at 10 minutes (mean ± SD)	5±3	5±3
Sentinel Event n (%)	28 (43%)	24 (33%)
C- Section delivery n (%)	42 (65%)	41 (56%)
*Inototropic support n (%)	22 (34%)	44 (60%)
*History of seizures n (%)	9 (14%)	36 (49%)
SARNAT score II n (%)	31 (48%)	25 (34%)
*SARNAT score III n (%)	5 (8%)	27 (37%)
Umbilical cord arterial pH (mean ± SD)	6.96±0.18	6.96±0.18
Umbilical cord arterial Deficit (mean ± SD)	-15±6	-16±6


SpO_2
No/Mild Group
Moderate/Severe Group
Overlap
Frequency
Measurement
rcO_2
No/Mild Group
Moderate/Severe Group
Overlap
Frequency
Measurement

70%/15%/15%

Long ShortTerm Memory (LSTM)

24 Hour
48 Hour



Recurrent Neuronal Network

No/mild injury on MRI

Moderate/severe injury on MRI

Duration	Accuracy on Test Set
24 hours	84.6% ± 3.2%
48 hours	91.2% ± 3.2%

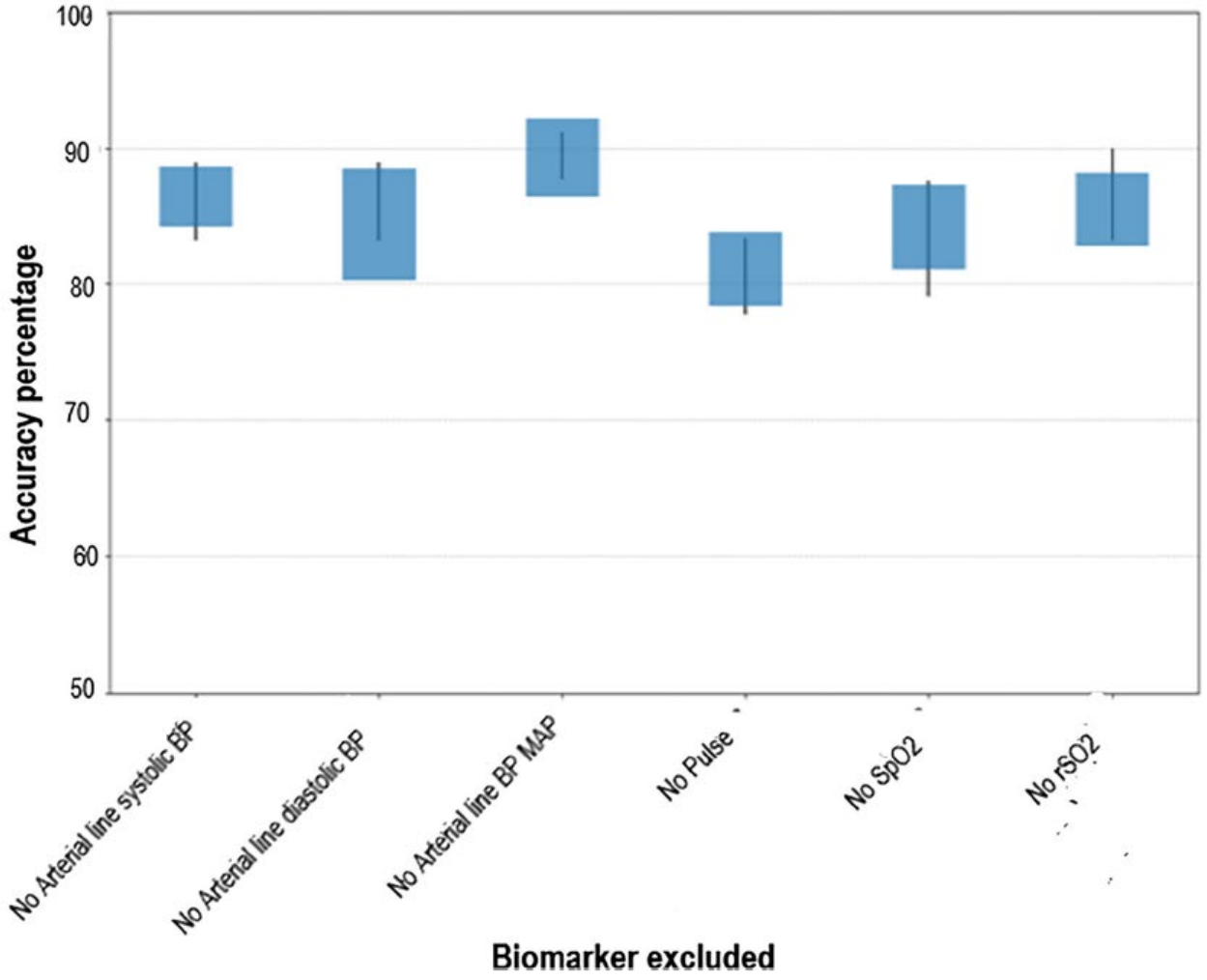
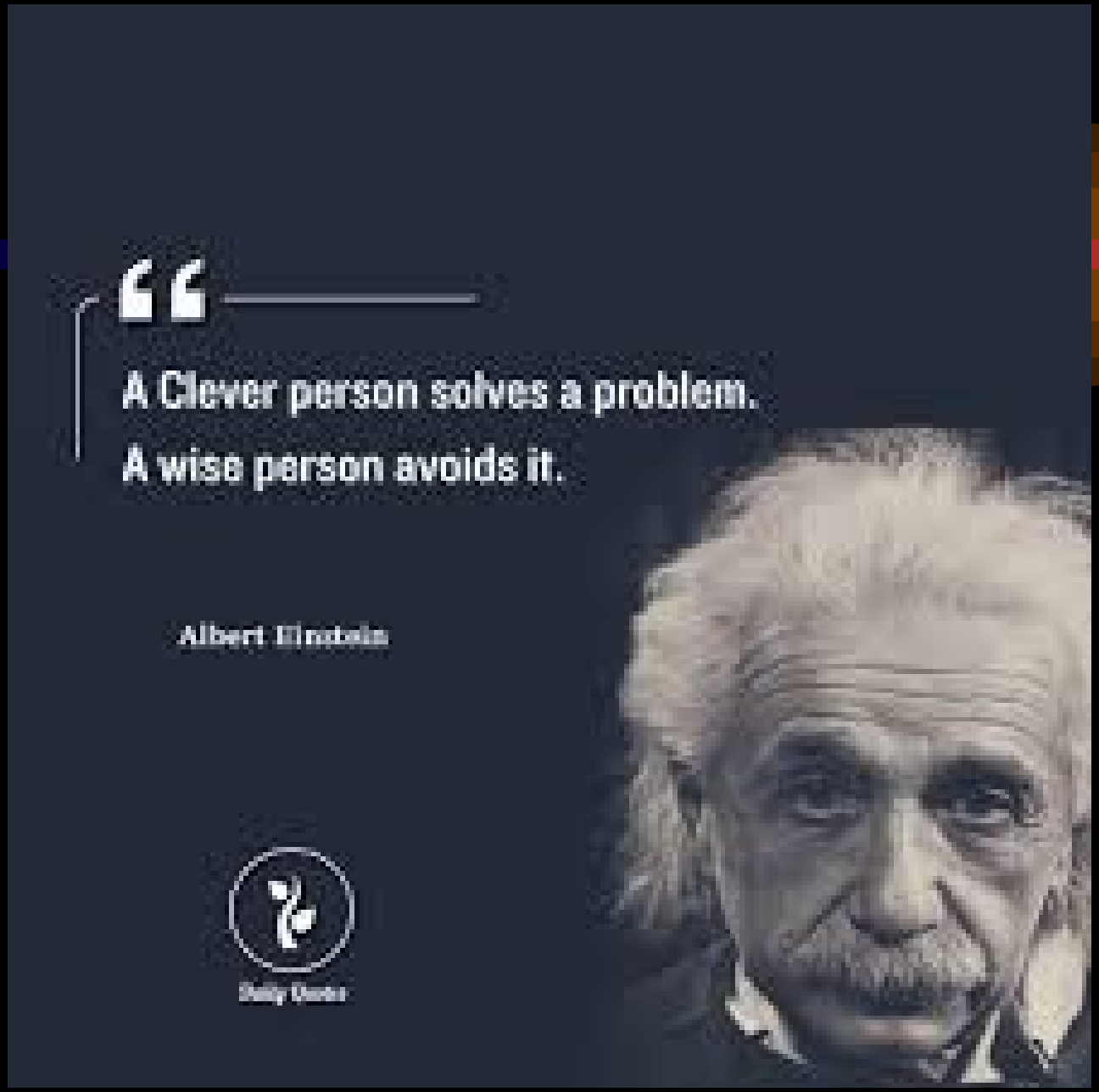


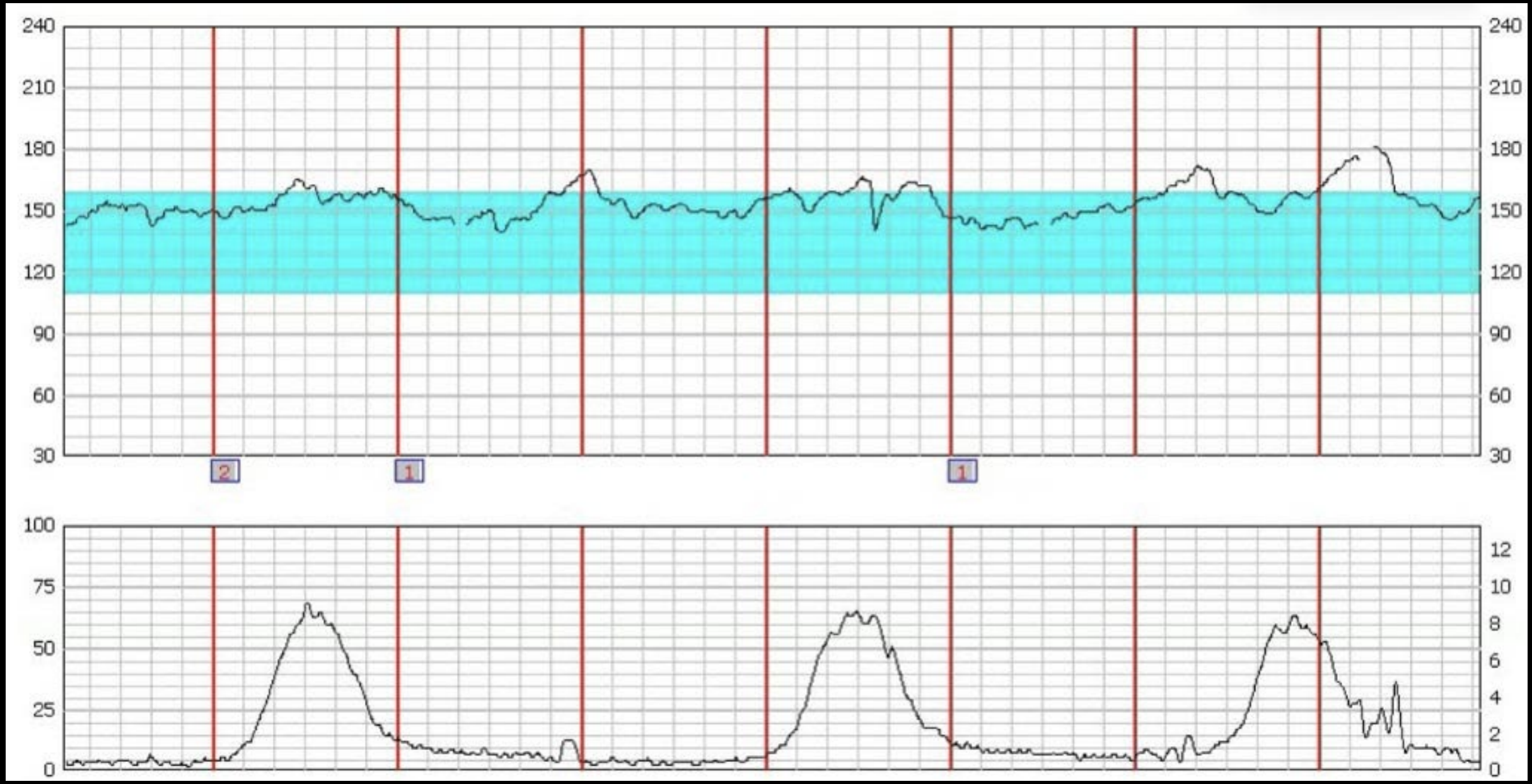
FIGURE 3
Candlestick graph illustrating the variability and range across multiple experiments. The vertical wicks represent the minimum and maximum observed values, while the bodies show the mean ± standard deviation, offering insights into the consistency of results.

IIIAB. Predicting Outcomes Fetal Heart Rate Tracings

Silva LM, Joiner D, Qezelbash-Chamak J, et al. Longitudinal Fetal Heart Rate Analysis Identifies Neonates with Metabolic Acidemia Requiring Therapeutic Hypothermia. Am J Obstet Gynecol. Sep 16 2025;doi:10.1016/j.ajog.2025.09.019

Slides courtesy of Dr. Dillon Joiner


A Clever person solves a problem.
A wise person avoids it.
Albert Einstein


240
210
180
150
120
90
60
30
2
1
1
100
75
50
25
0
12
10
8
6
4
2
0

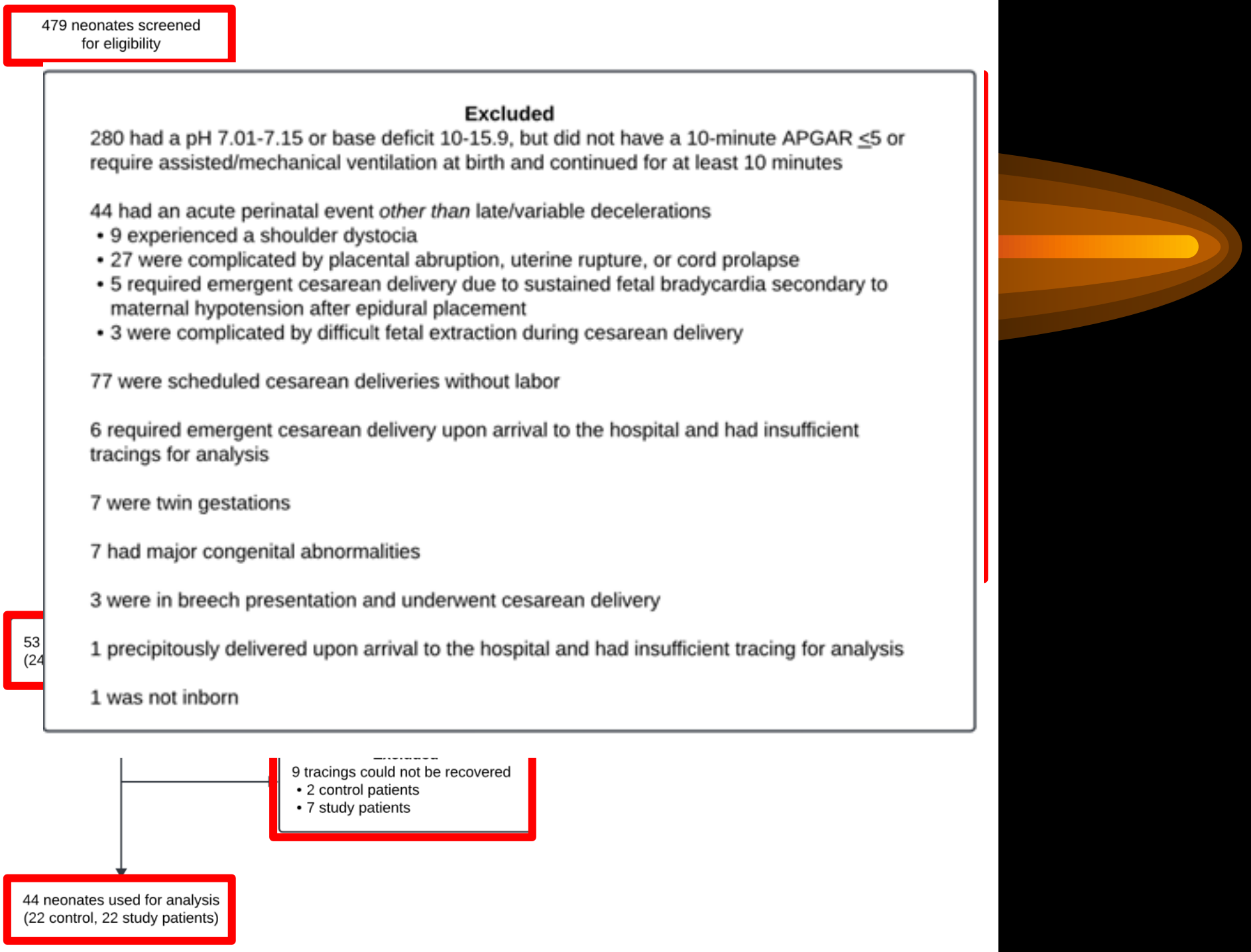
Background

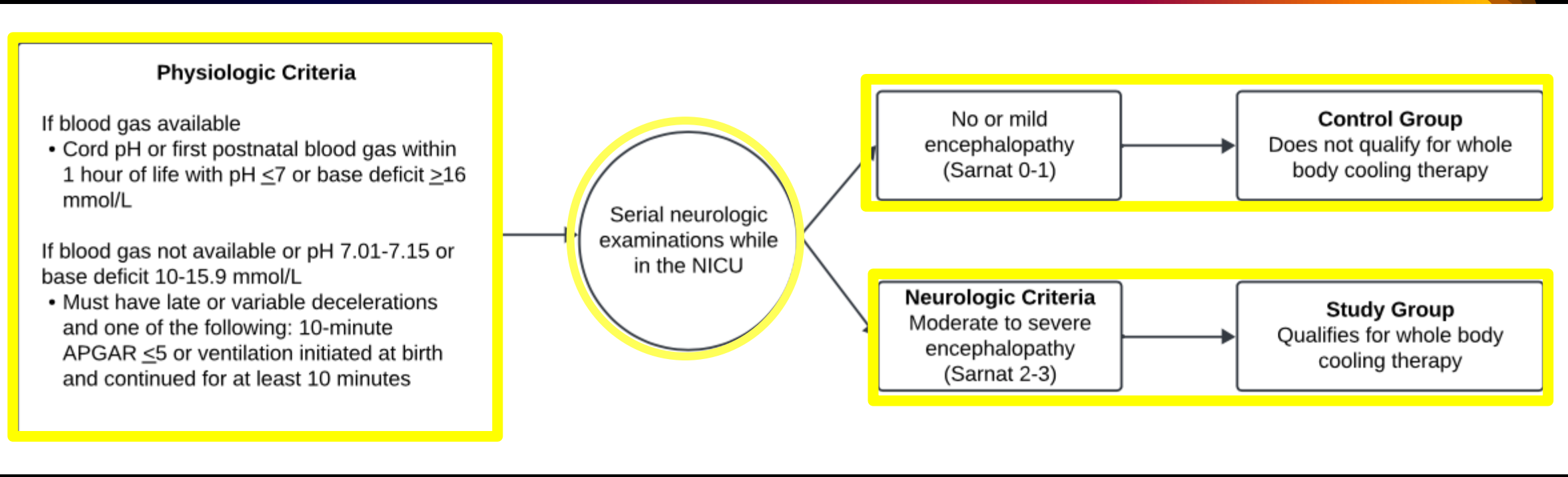
- FHR monitoring– developed to reduce maternal and neonatal morbidity/mortality, by early identification and timely intervention to minimize/prevent neurologic injury
- This increased rates of operative interventions, but no change in rate of neonatal CNS injury
- NICHD later developed a 3-tier classification system for fetal heart tracing
- There remain high rates of intra- and interobserver variability

Summary of 3-tier FHR pattern classification.

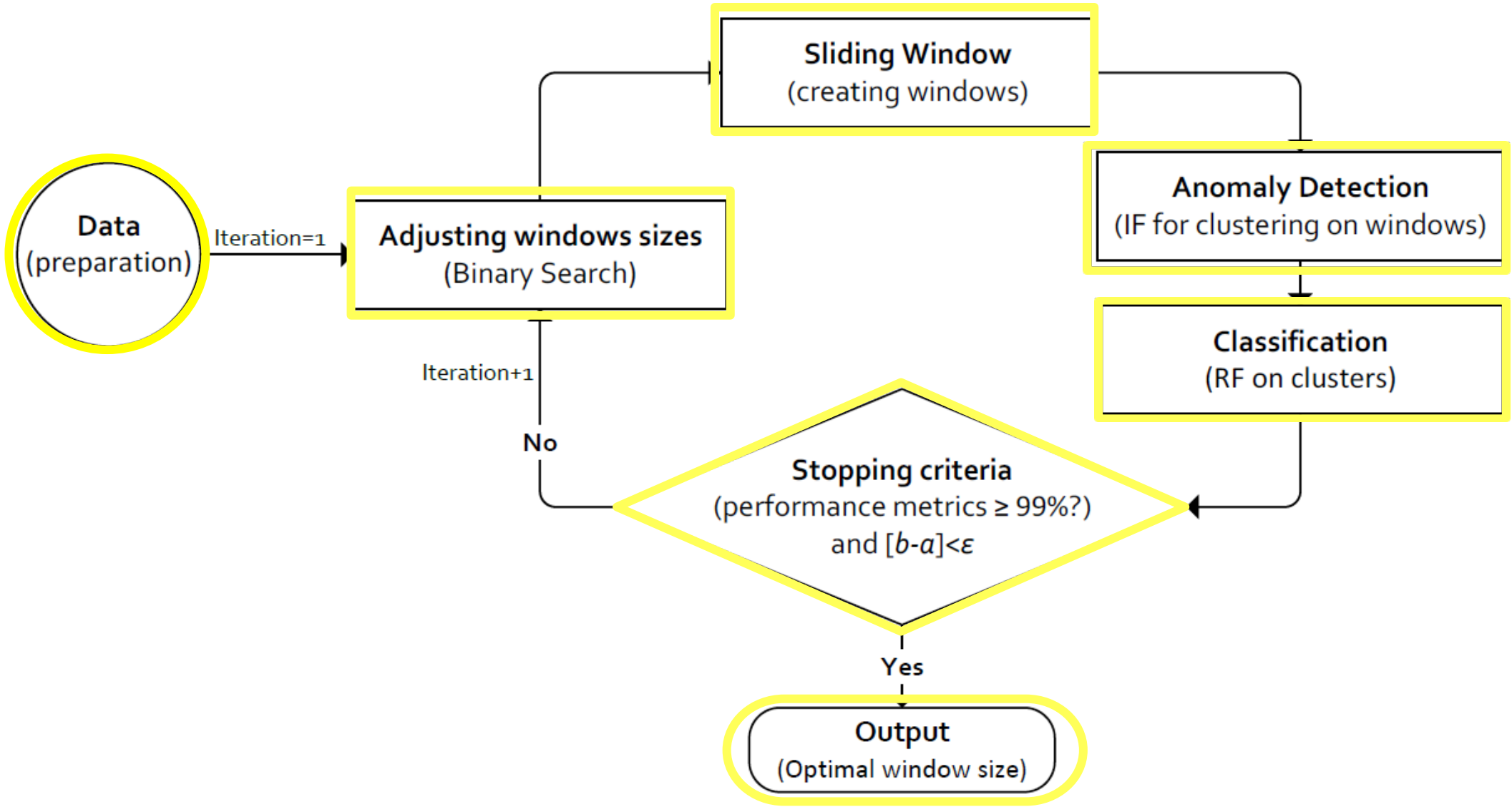
Category	FHR tracing
Category I	Baseline rate: 110–160 beats per minute Baseline variability: moderate Late or variable decelerations: absent Early decelerations: present or absent Accelerations: present or absent
Category II	Includes all tracings not categorized as Category I or III
Category III	Absent baseline FHR variability and any of the following • Recurrent late decelerations • Recurrent variable decelerations • Bradycardia Sinusoidal pattern

(Kikuchi 2020)


479 neonates screened for eligibility
Excluded
280 had a pH 7.01-7.15 or base deficit 10-15.9, but did not have a 10-minute APGAR ≤5 or require assisted/mechanical ventilation at birth and continued for at least 10 minutes
44 had an acute perinatal event other than late/variable decelerations
• 9 experienced a shoulder dystocia
• 27 were complicated by placental abruption, uterine rupture, or cord prolapse
• 5 required emergent cesarean delivery due to sustained fetal bradycardia secondary to maternal hypotension after epidural placement
• 3 were complicated by difficult fetal extraction during cesarean delivery
77 were scheduled cesarean deliveries without labor
6 required emergent cesarean delivery upon arrival to the hospital and had insufficient tracings for analysis
7 were twin gestations
7 had major congenital abnormalities
3 were in breech presentation and underwent cesarean delivery
1 precipitously delivered upon arrival to the hospital and had insufficient tracing for analysis
1 was not inborn
53
(24
9 tracings could not be recovered
• 2 control patients
• 7 study patients
44 neonates used for analysis
(22 control, 22 study patients)

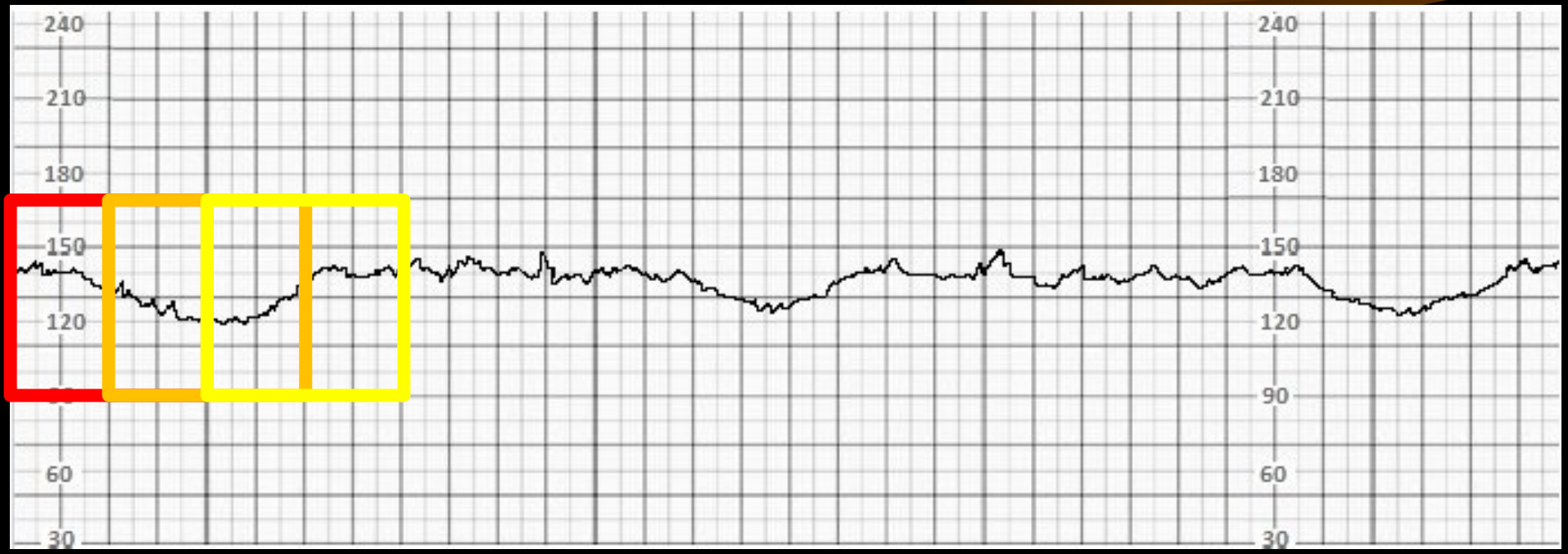


No difference in maternal characteristics between the two groups


Data
(preparation)
Iteration=1
Adjusting windows sizes
(Binary Search)
Sliding Window
(creating windows)
Anomaly Detection
(IF for clustering on windows)
Classification
(RF on clusters)
Iteration+1
No
Stopping criteria
(performance metrics ≥ 99%?)
and [b-a]<ε
Yes
Output
(Optimal window size)

Machine Learning Methods

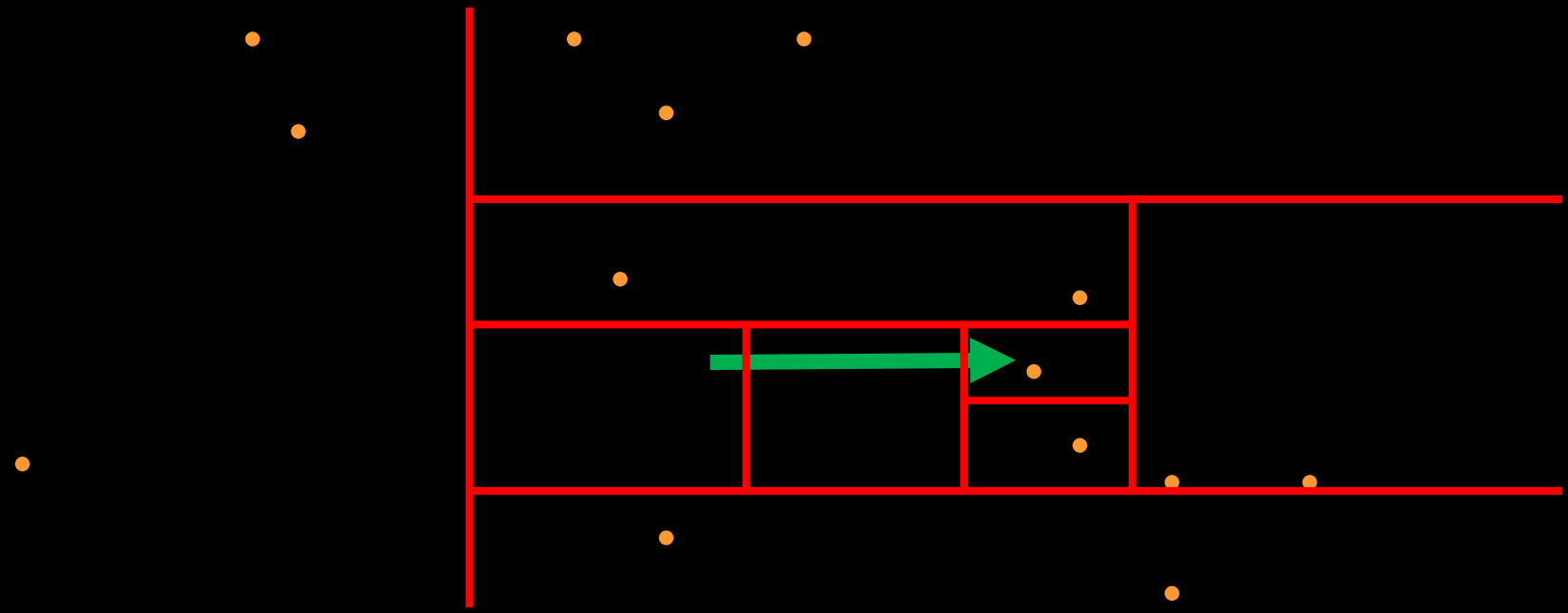
- Entire FHRT for each neonate analyzed via a sliding time window, with 50% overlap



Machine Learning Methods

- Many partitions to isolate a point = “normal”

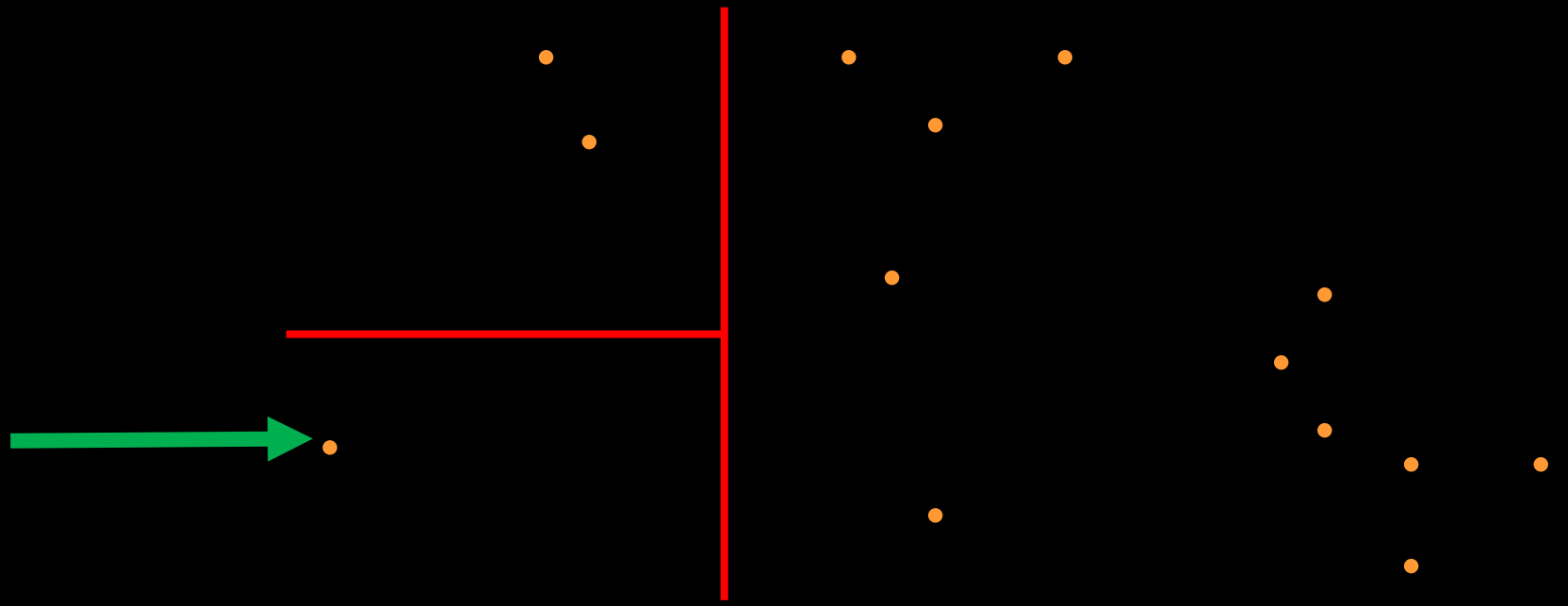
Isolation Forest Demo



Machine Learning Methods

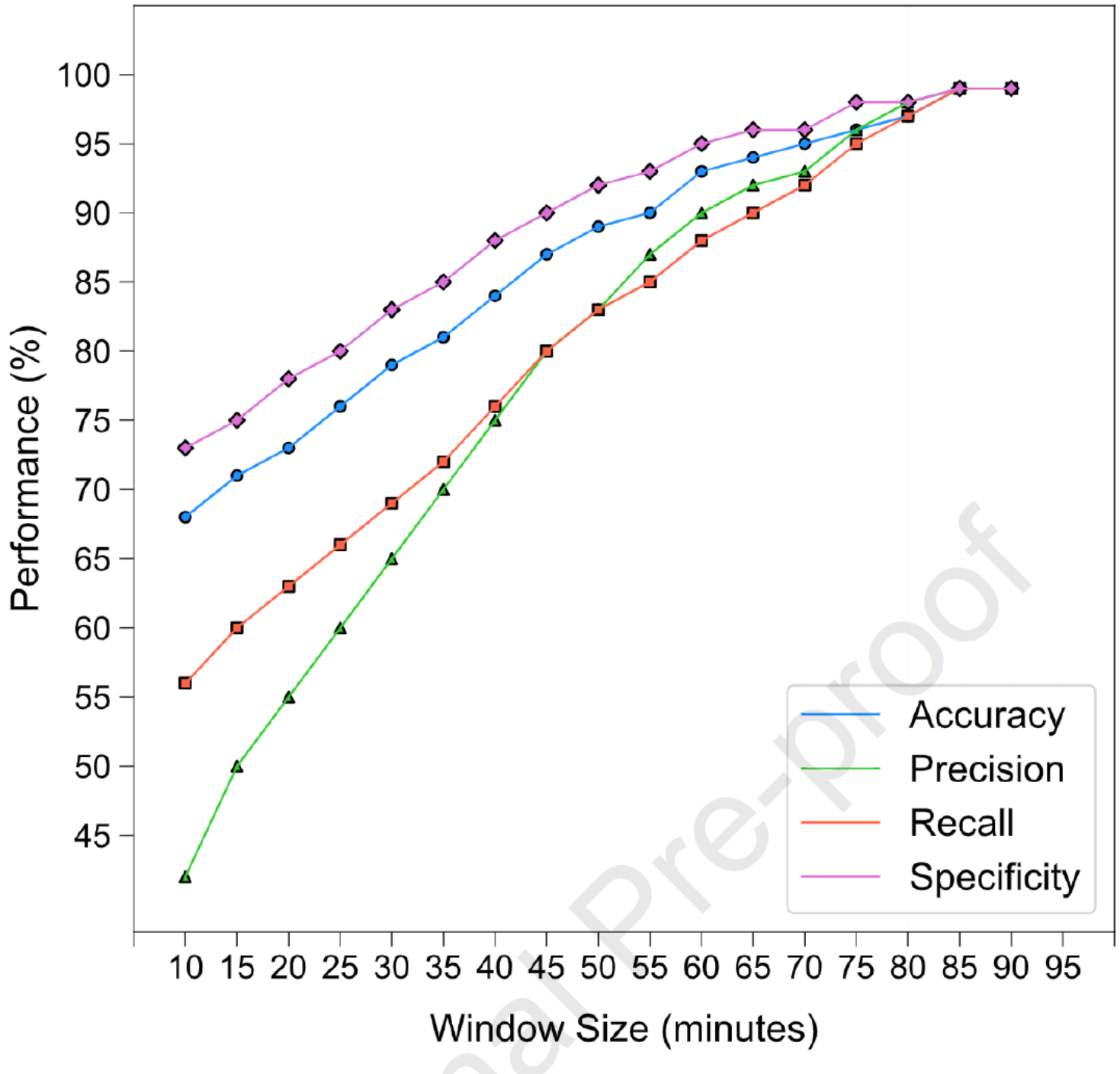
- Few partitions to isolate a node = “abnormal”

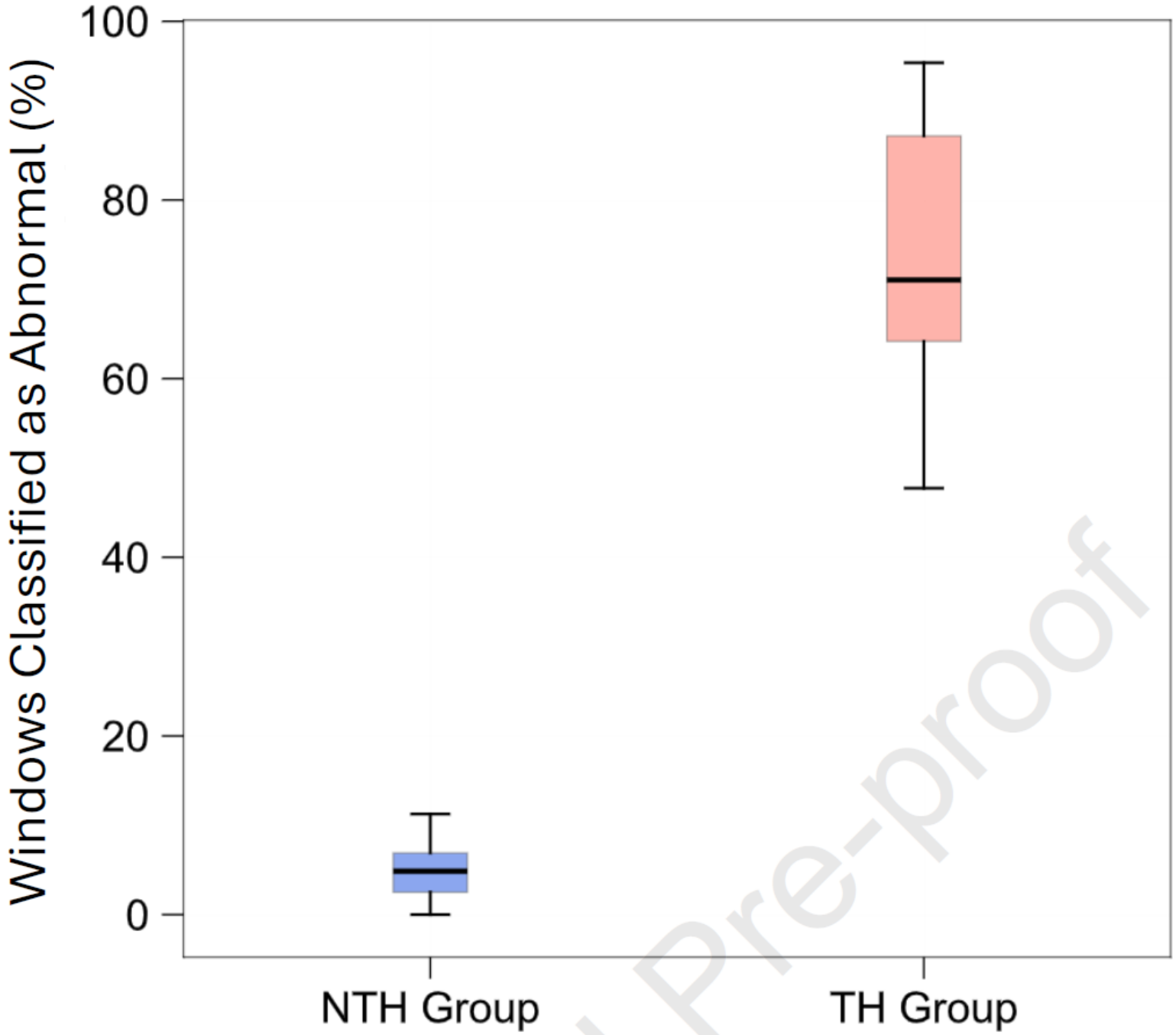
Isolation Forest Demo



Machine Learning Methods

- Random Forest is used to reduce *variance* or *overfitting*
  - Overfitting – algorithm is "too good" at interpreting its training set, so much so that its predictive value is no longer applicable to new unseen datasets
- Rather than having one decision tree classifying tracing as abnormal or normal, Random Forest has *many* trees make the classification based on *random* features of the tracing
- Final prediction is the aggregate of the decision of these many trees


Performance (%)
100
95
90
85
80
75
70
65
60
55
50
45
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
Window Size (minutes)
Accuracy
Precision
Recall
Specificity


Windows Classified as Abnormal (%)
100
80
60
40
20
0
NTH Group
TH Group

Conclusion/Next Steps

- Takeaway: This model accurately identified neonates that required cooling based on FHRT alone, from a cohort with equivalent risk factors and FHRT by usual classification
- Further study of this ML model on larger cohorts, multi-center
- Eventual work on establishing thresholds for the model in real-time interpretation of tracing
- Taking outcomes a step further – can model predict severity of HIE on MRI or seizure activity based on tracing?

IV. Summary-The Future

V. Summary


STAR WARS
EPISODE III
REVENGE OF THE SITH
MASTER YODA

Neonate >36 weeks with a diagnosis of HIE

Systemic supportive Care

Stratify Neonate Into Phenotypes
- Neuro exam
- aEEG/vEEG
- Cerebral Oximetry
- Biomarker (Blood and Digital)

Mild

Supportive Care
?Hypothermia
?Pharmacologic Intervention

Moderate

0-24 hours
- Hypothermia
- Pharmacologic Agents Based on Phenotypes

Re-Stratify Neonate
- Neuro exam
- aEEG/vEEG
- Cerebral Oximetry
- Biomarker (Blood and Digital)
- MRI with DWI

→ Machine Learning

Moderate

Supportive Care

Continue Hypothermia For 72 hours

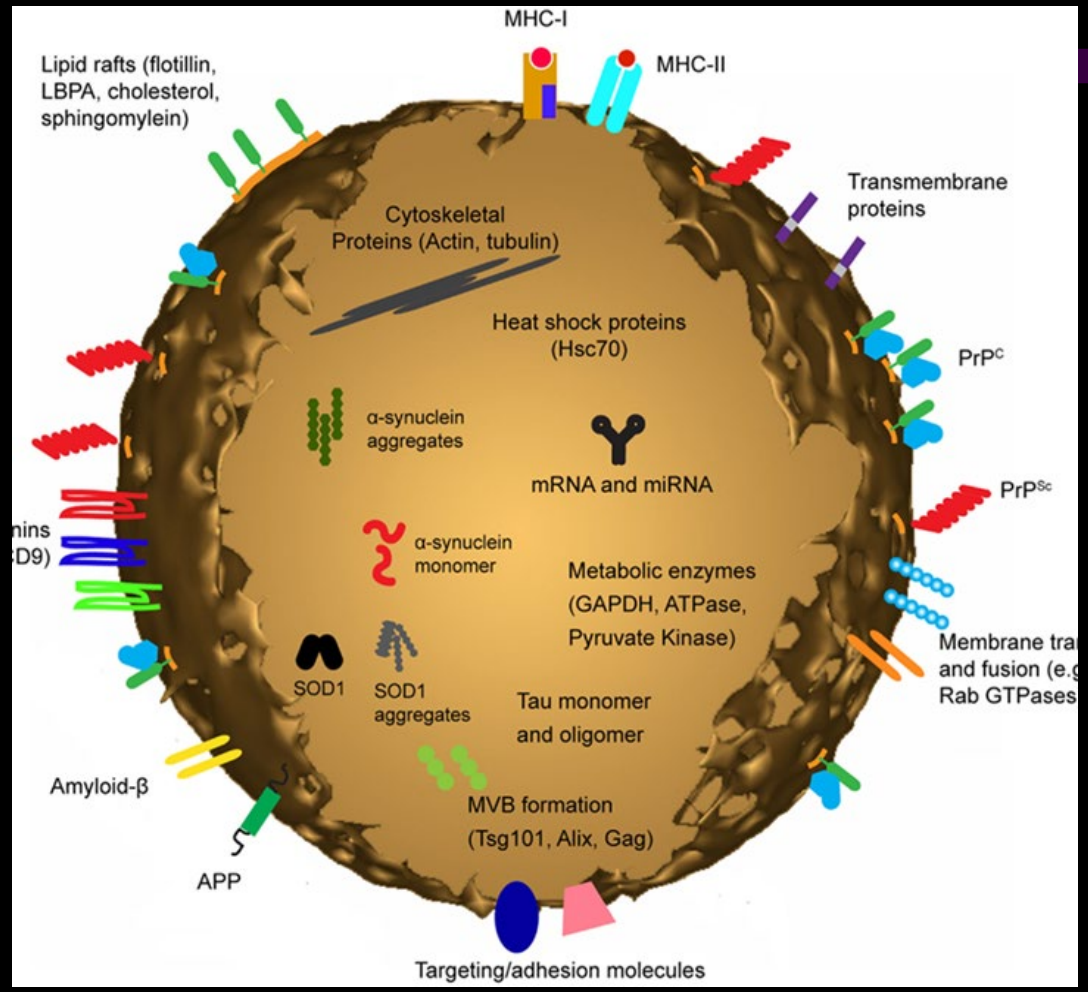
Severe

Continue pharmacologic intervention
Stem Cell Therapy at 7-10 days?

Machine Learning Methods

- Tracings analyzed using a sliding time window with 50% overlap
- Isolation Forest used to identify windows that are anomalous or “abnormal”
- Random Forest then used to predict these abnormal labels
- Percentage of windows for each subject labeled abnormal calculated

Enriched Markers In Neuronal ECVs



- More likely
  - Transmembrane proteins
  - Cytoskeletal Proteins
  - miRNAs
  - siRNAs
  - Lipids
- Less Abundant
  - DNA/mRNA
  - Mitochondria
  - Cytosol proteins (variable)

Bellingham et al. Front Physiol 2012

Digital Biomarkers

- We built a Long-Short Term Memory (LSTM) model to predict no-to-mild versus moderate-to-severe brain injury on MRI, based on the time sequence biomarker features.
- LSTM is a specialized type of recurrent neural network (RNN)

Neonatal Outcomes

	Control Group (n = 22)	Study Group (n = 22)	P value
APGAR scoring			
1-minute APGAR	2.5 [2,5]	2 [1,3]	0.0337
5-minute APGAR	7 [5,8]	4.5 [3,6]	0.0331
10-minute APGAR	7.5 [7,8.5]	6 [3,7]	0.0124
Umbilical cord blood			
pH	7 ± 0.1	7 ± 0.1	0.57
Base deficit	15 ± 3.8	16.6 ± 4.4	0.27
Need for mechanical/assisted ventilation	8 (36.4)	12 (54.6)	0.23
Need for chest compressions	3 (13.6)	1 (4.6)	0.61
Sarnat score	0 [0,0]	2 [2,3]	<0.0001
0 (no encephalopathy)	21 (95.5)	1 (0.05)	
1 (mild encephalopathy)	0	0	
2 (moderate encephalopathy)	1 (0.05)	14 (0.64)	
3 (severe encephalopathy)	0	7 (0.32)	
Seizures during admission	0	8 (36.4)	0.0036
Initial labs			
Lactate	4.3	9.9 ± 5.8	0.11
AST	59.2 ± 23.9	121.9 ± 119.3	0.02
ALT	15.8 ± 11.3	36.4 ± 37.4	0.02
Troponin	82.1 ± 150.6	90.9 ± 150.5	0.48
Creatinine	0.7 ± 0.3	0.8 ± 0.3	0.22
NICU length of stay (days)	3 [2,6]	9 [7,20]	0.0009

Free circulating versus ECV Biomarkers

- Head-to-head comparison between EV miRNAs and circulating miRNAs in the same set of biologic samples (n=35).
- Investigated a direct comparison of 4 miRNAs associated with disease severity in neonatal HIE in free circulating compared with central nervous system (CNS) EV contained forms.
  - miR-197-3p
  - miR-342-3p
  - miR-150-5p
  - miR-328-3p.
- Predictive modeling was performed using logistic regression with lasso penalty to evaluate the combined predictive value of the four miRNAs.

Maternal Demographics & Baseline Characteristics

	Control Group (n = 22)	Study Group (n = 22)	P value
Age (years)	28.5 [25.75;30.75]	29 [23.75;31.25]	0.89
Number of prior deliveries	0 [0;1]	0 [0;1]	0.69
Race/ethnicity			0.15
White	15 (68.2)	14 (63.6)	
Black	6 (27.7)	4 (18.2)	
Hispanic	0	4 (18.2)	
Asian	1 (4.55)	0	
Insurance Status			0.76
Private	12 (54.6)	10 (45.5)	
Public/self-pay	10 (45.5)	12 (54.6)	
Preexisting medical conditions			
Chronic hypertension	3 (13.6)	4 (18.2)	1.00
Type 1 diabetes mellitus	0	0	
Type 2 diabetes mellitus	1 (4.6)	1 (4.6)	1.00
Pregnancy complications			
Gestational hypertension/preeclampsia	4 (18.2)	4 (18.2)	1.00
Gestational diabetes	1 (4.6)	1 (4.6)	1.00
Substance use			
Tobacco	2 (9.1)	4 (18.2)	0.66
Alcohol	1 (4.6)	1 (4.6)	1.00
Illicit drugs	2 (9.1)	3 (13.6)	1.00
Gestational age at first prenatal visit (weeks)			0.09
<14w0d	19 (86.4)	16 (72.7)	
14w0d-27w6d	1 (4.6)	6 (27.3)	
28w0d-42w0d	1 (4.6)	0	
No prenatal care	1 (4.6)	0	
Location of prenatal care			1.00
UF Health Shands	16 (72.7)	17 (77.3)	
Outside facility	5 (22.7)	5 (22.7)	
No prenatal care	1 (4.6)	0	
Optimal pregnancy dating	19 (86.4)	21 (95.5)	0.61

Delivery Summary

	Control Group (n = 22)	Study Group (n = 22)	P value
Clinical presentation			0.54
Induction	12 (54.5)	15 (68.2)	
Spontaneous labor	10 (45.5)	7 (31.8)	
Mode of delivery			0.35
Spontaneous vaginal delivery	12 (54.6)	13 (59.1)	
Operative vaginal delivery	2 (9.1)	0	
NRFHT	2 (9.1)	0	
Dystocia	0	0	
Cesarean delivery	8 (36.4)	9 (40.9)	
NRFHT	6 (27.3)	9 (40.9)	
Dystocia	2 (9.1)	0	
Intrapartum complications			
Meconium stained fluid	6 (27.3)	7 (31.8)	1.00
Chorioamnionitis	7 (31.8)	6 (27.3)	1.00
Pharmacologic agents			
Inductions	12 (54.5)	15 (68.2)	0.25
Misoprostol + pitocin	4 (18.2)	9 (40.9)	
Pitocin alone	8 (36.3)	6 (40)	
Maximum dose of pitocin (milliunits/min)	17 ± 9.8	15.2 ± 7.9	0.64
Duration of maximum pitocin dose administration (hours)	5.8 ± 7.8	3.4 ± 4.8	0.22
Maternal vital signs			
Maximum SBP (mmHg)	150 ± 15.7	153.1 ± 17.4	0.48
Minimum SBP (mmHg)	103.8 ± 11.3	107.4 ± 10.2	0.26
Maximum DBP (mmHg)	94.7 ± 12.8	91.8 ± 12.3	0.64
Minimum DBP (mmHg)	55.6 ± 6.6	56.1 ± 6	0.77
Maximum temperature (Celsius)	37.5 ± 0.6	37.4 ± 0.8	0.57
Minimum temperature (Celsius)	36.4 ± 0.4	36.6 ± 0.3	0.22
Placental pathology categorization			
Acute placental inflammation	10 (45.5)	14 (63.6)	0.36
Chronic placental inflammation	0	0	
Maternal vascular malperfusion	2 (9.1)	4 (18.2)	0.66
Fetal vascular malperfusion	0	0	
No significant findings	3 (13.6)	6 (27.3)	0.46
Pathology not available	9 (40.9)	1 (4.6)	0.009

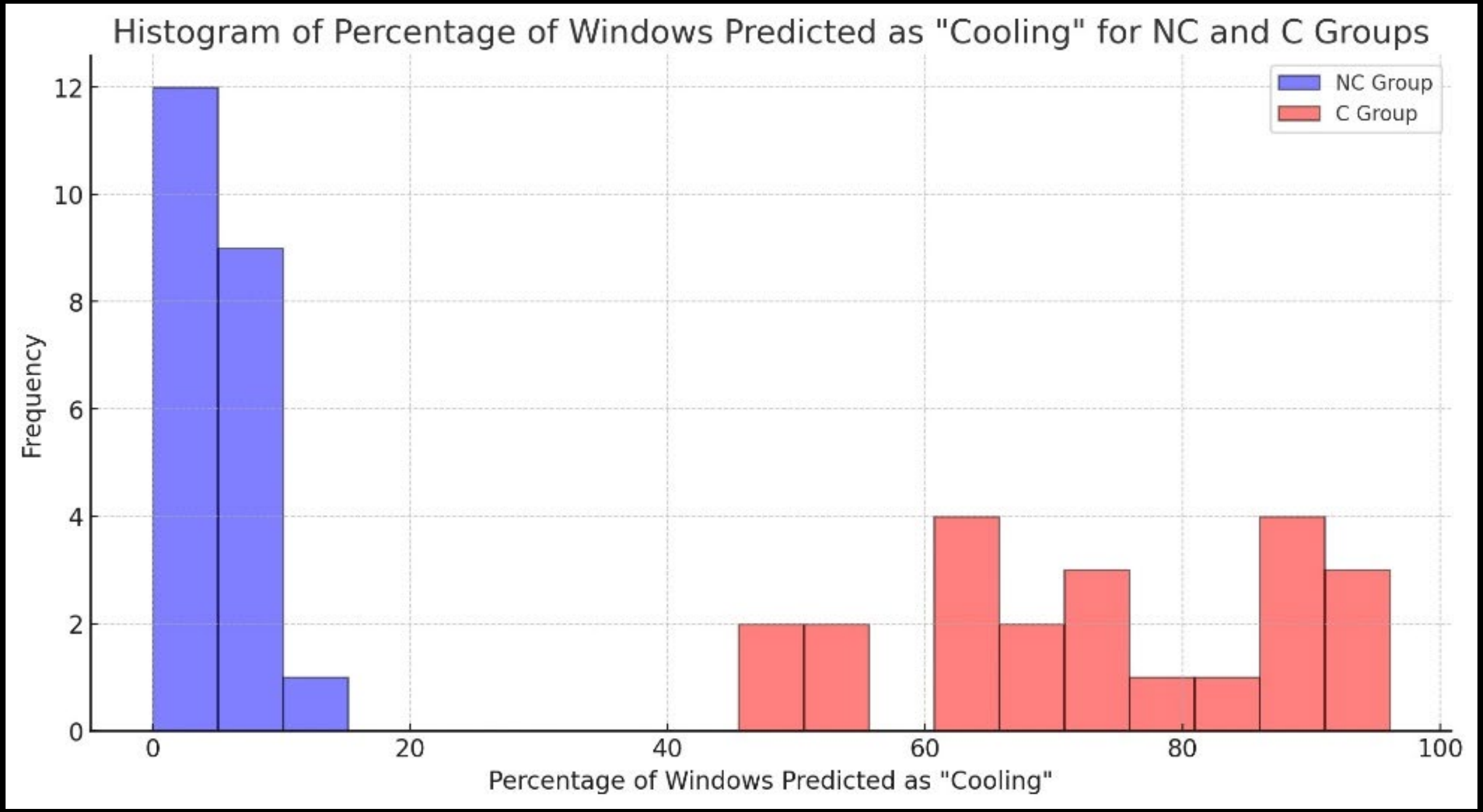

SEMINAR
PREVENTIVE MEDICINE
"WELL, THEN — TWO APPLES A DAY."

Methods

- Infants were then divided into two groups:
  - Low cord pH group that did not meet NICHD criteria for therapeutic hypothermia
  - Low cord pH group that met NICHD criteria and underwent therapeutic hypothermia
    - Gestational age ≥ 36+0 weeks
    - Birth weight ≥ 1.8 kg
    - ≤ 6 hours of age
    - Evidence of moderate-severe encephalopathy based on modified Sarnat exam OR seizure

Neonatal Demographics & Baseline Characteristics

	Control Group (n = 22)	Study Group (n = 22)	P value
Gestational age at time of delivery (weeks)	39.2 [37.8;40.7]	39.4 [37.6;40.0]	0.87
Birthweight (grams)	3335 [2987;3700]	3590 [2980;3855]	0.28
Small for gestational age	5 (22.7)	2 (9.1)	0.22
Sex			0.13
Female	14 (63.6)	8 (36.4)	
Male	8 (36.4)	14 (63.6)	


Histogram of Percentage of Windows Predicted as "Cooling" for NC and C Groups
NC Group
C Group
Frequency
0
2
4
6
8
10
12
0
20
40
60
80
100
Percentage of Windows Predicted as "Cooling"