

Table 1 – Risk of bias assessment of cohort studies using the NOS scale.

COHORT STUDIES												
Reference	Selection				Comparability	Outcome			Selection	Comparability	Outcome	TOTAL
	Representative ness of the exposed cohort	Selection of the non-exposed cohort	Ascertain ment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts				
(Alshweki, Perez-Munuzuri et al. 2017) ³⁵	*	*	*	*		*	*	*	4	0	3	7
(Balada, Tebe et al. 2020) ²⁰	*		*	*	**	*	*	*	3	2	3	8
(Bale, Mitra et al. 2014) ⁴¹	*		*	*		*	*	*	3	0	3	6
(Chalak, Sánchez et al. 2014) ¹⁸	*	*	*	*	**	*	*	*	4	2	3	9
(Dehaes, Aggarwal et al. 2014) ³⁰	*		*	*	**	*	*	*	3	2	3	8
(Douglas-Escobar, Yang et al. 2010) ²²	*	*	*	*	*	*	*	*	4	1	3	8
(El-Mazary, Abdel-Aziz et al. 2015) ⁴⁰	*	*	*	*	**				4	2	0	6
(Ennen, Huisman et al. 2011) ¹⁷	*		*		*	*	*	*	2	1	3	6
(Ezgu, Atalay et al. 2002) ³⁹	*	*	*		*	*	*	*	3	1	3	7
(Fredly, Nygaard et al. 2016) ³⁷	*	*	*		**	*	*	*	3	2	3	8
(Haiju, Suyuan et al. 2008) ⁴⁵	*	*	*	*	**	*		*	4	2	2	8
(Jain, Pagano et al. 2017) ²⁷	*	*	*	*	**	*	*	*	4	2	3	9
(Jones, Heep et al. 2018) ³⁴	*	*	*		**	*		*	3	2	2	7
(Locci, Noto et al. 2018) ⁴³	*	*	*	*	**	*	*	*	4	2	3	9
(Lopez-Suarez, Concheiro-Guisan et al. 2019) ²¹	*	*	*	*	**	*	*	*	4	2	3	9
(Maggiotto, Sondhi et al. 2019) ²⁹	*	*	*	*	**	*	*	*	4	2	3	9
(Massaro, Chang et al. 2014) ³⁶	*	*	*	*	**	*	*		4	2	2	8

(Massaro, Chang et al. 2012) ²⁸	*	*	*	*	**	*	*	*	4	2	3	9
(Massaro, Jeromin et al. 2013) ²⁵	*	*	*	*	**	*	*	*	4	2	3	9
(Massaro, Wu et al. 2019) ³²	*	*	*	*		*	*	*	4	0	3	7
(Mitra, Bale et al. 2016) ²⁶		*				*	*		1	0	2	3
(Oh, Perritt et al. 2008) ²³	*	*	*	*	*	*	*	*	4	1	3	8
(Pineiro-Ramos, Nunez-Ramiro et al. 2020) ²⁴	*	*	*	*	**	*	*	*	4	2	3	9
(Ponnusamy, Kapellou et al. 2016) ³¹	*	*	*	*		*	*	*	4	0	3	7
(Saito, Shibasaki et al. 2016) ³³	*	*	*	*		*			4	0	1	5
(Akamatsu, Sugiyama et al. 2019) ¹⁹	*	*	*	*	*	*	*	*	4	1	3	8
(Shaikh, Boudes et al. 2015) ⁴²	*	*	*		**	*	*	*	3	2	3	8
(Sweetman, Onwuneme et al. 2017) ³⁸	*	*	*	*	**	*	*	*	4	2	3	9

Note: the scores represent the sum of the number of stars.

Table 2 – Risk of bias assessment of case-control studies using the NOS scale.

CASE-CONTROL STUDIES												
Reference	Selection				Comparability	Outcome			Selection	Comparability	Outcome	TOTAL
	Is the case definition adequate?	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate				
(Bersani, Ferrari et al. 2019) ⁴⁴	*			*	**	*	*	*	2	2	3	7
(Chouthai, Sobczak et al. 2015) ⁴⁶		*	*	*	**	*		*	3	2	2	7

Note: the scores represent the sum of the number of stars.

Table 3 – Summary of findings of biomarkers using imaging techniques.

Reference	Sample type	Name	Technique	Group	Collection time	Results	Summary	Observations
(Mitra, Bale et al. 2016) ²⁶	Cerebral imaging	Lactate/N-acetylaspartate Δ oxCCO (redox of cytochrome oxidase) Δ HbD (hemoglobin difference)	¹ H MRS NIRS	HIE	During TH			
(Jain, Pagano et al. 2017) ²⁷	Cerebral imaging	CrSO ₂	NIRS	HIE	During TH; <48h	Higher absolute values between 24h and 36h associated to MRI abnormalities (p=0.011) At 30h, increased levels correlated with basal ganglia (p=0.001), white-matter (p=0.012), PLIC (p=0.001) and brain stem (p=0.03)	↑ at 24h-36h	
(Dehaes, Aggarwal et al. 2014) ³⁰	Cerebral imaging	CBV (cerebral blood volume)	FDNIRS–DCS	Control HIE (during TH) HIE (pos TH)	During TH (<72h); Post-TH (<300h)	Increased levels compared to control (p<10 ⁻³) and pos-TH (p<0.01)	↑ at <72h	
		CBF (cerebral blood flow)		Control HIE (during TH) HIE (pos TH)		Decreased levels compared to control (p<0.01) and pos-TH (p<0.01)	↓ at <72h	
		SO ₂ (hemoglobin oxygen saturation)		Control HIE (during TH) HIE (pos TH)		Increased levels compared to control (p=0.014)	↑ at <72h	
		CMRO ₂ (cerebral oxygen metabolism index)		Control HIE (during TH) HIE (pos TH)		Decreased levels compared to control (p<10 ⁻⁵) and pos-TH (p<0.01)	↓ at <72h	
		HbT (total hemoglobin)		Control HIE (during TH)		Increased levels compared to control (p=0.01)	↑ at <72h	
		HbO (oxyhemoglobin)		Control HIE (during TH)		Increased levels compared to control (p<0.01)	↑ at <72h	
(Bale, Mitra et al. 2014) ⁴¹	Cerebral imaging	Thalamic Lactate/N-acetylaspartate	¹ H MRS	HIE	During <144h			Used as a biomarker of severity
		Δ SPO ₂ (systemic oxygen saturation)	NIRS	HIE				
		Δ HbD (oxygenated - deoxygenated hemoglobin)		HIE		Associated to Lac/NAA ratio (p<0.01)		
		Δ HbT (oxygenated + deoxygenated hemoglobin)		HIE				
		Δ oxCCO (redox of cytochrome oxidase)		HIE		Associated to Lac/NAA ratio (p<0.01)		
(Fredly, Nygaard et al. 2016) ³⁷	Cerebral imaging	erebral oxygenation	NIRS	HIE with low CRP levels	24h, 48h, 72h	No statistical differences		
				HIE with high CRP levels	24h, 48h, 72h			

Table 4 – Summary of findings of biomarkers present in urine.

Reference	Sample type	Name	Code	Technique	Group	Collection time	Results	Summary	Observations
Proteins									
(Alshweki, Perez-Munuzuri et al. 2017) ³⁵	Urine	S100B	P04271	ELISA	HIE (favorable outcome)	24h, 48h, 72h	Higher levels at 24h (p=0.031) and 48h (p=0.002)	↑ at 24h ↑ at 48h	Higher levels at 24h, 48h and 72h on infants who died;
					HIE (unfavorable outcome)				Higher levels at 24h, 48h on infants with abnormal PET
(Bersani, Ferrari et al. 2019) ⁴⁴	Urine	S100B	P04271	ILMA	Moderate HIE (with hypothermia)	First void, 4h, 8h, 12h, 16h, 20h, 24h, 36h, 48h, 72h, 96h, 108h, 120h	Peak level at 4h (p<0.05)	↑ at 4h ↑ at 8h ↑ at 12h ↑ at 16h ↑ at 20h ↑ at 24h	Data is also available for groups that were not treated with TH
					Severe HIE (with hypothermia)		Increasing levels from first void, peaking at 16h (p<0.05) Higher levels from 4h to 24h (p<0.001)		
Metabolites									
(Oh, Perritt et al. 2008) ²³	Urine	Lactate/creatinine ratio	-	-	Infants with normal/mild disability Infants with moderate/severe disability or death	6h-24h, 48h-72h	Higher levels at 6h-24h (p<0.001)	↑ at 6h-24h	No statistical differences between control and hypothermia groups
(Locci, Noto et al. 2018) ⁴³	Urine	Lactate	HMDB000190; HMDB0001311	¹ H NMR	Control	<6h	Increased levels compared to healthy controls at <6h	↑ at <6h	Higher lactate levels on non-surviving infants
					HIE	<6h, 48h, 72h, day 30			
		Myo-inositol	HMDB000211		Control	<6h	Increased levels compared to healthy controls at <6h	↑ at <6h	
					HIE	<6h, 48h, 72h, day 30			
		Betaine	HMDB000043		Control	<6h	Increased levels compared to healthy controls at <6h	↑ at <6h	
					HIE	<6h, 48h, 72h, day 30			
		Taurine	HMDB000251		Control	<6h	Increased levels compared to healthy controls at <6h	↑ at <6h	
					HIE	<6h, 48h, 72h, day 30			
		Citrate	HMDB000094		Control	<6h	Decreased levels compared to healthy controls at <6h	↓ at <6h	
					HIE	<6h, 48h, 72h, day 30			
		Acetone	HMDB0001659		Control	<6h	Decreased levels compared to healthy controls at <6h	↓ at <6h	
					HIE	<6h, 48h, 72h, day 30			
		DMA	HMDB0246062		Control	<6h	Decreased levels compared to healthy controls at <6h	↓ at <6h	
					HIE	<6h, 48h, 72h, day 30			
					Control	<6h			

	Glutamine	HMDB0003423; HMDB0000641		HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
	Succinate	HMDB0000254		Control	<6h		
				HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
	Pyruvate	HMDB0000243		Control	<6h		
				HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
	α-Ketoglutarate	HMDB0000208		Control	<6h		
				HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
	N-Acetyl groups	-		Control	<6h		
miRNA	Acetate	HMDB0000042		HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
	Arginine	HMDB0000517; HMDB0003416		Control	<6h		
				HIE	<6h, 48h, 72h, day 30	Decreased levels compared to healthy controls at <6h	↓ at <6h
(Ponnusamy, Kapellou et al. 2016) 31	Urine	RNU6B	GC10P013220	TaqMan miRNA assay	HIE	18h-19h	No comparisons between groups were performed
		Let7b	GC22P046119				
		miR-21	GC17P059841				

Table 5 – Summary of findings of biomarkers present in umbilical cord blood.

Reference	Sample type	Name	Code	Technique	Group	Collection time	Results	Summary	Observations
Proteins									
(Ennen, Huisman et al. 2011) ¹⁷	Cord blood	GFAP	P14136	Electrochemiluminescence Sandwich Immunoassay	Control	At birth	No significant differences	= at 0h	
(Chalak, Sánchez et al. 2014) ¹⁸	Umbilical cord plasma	GFAP	P14136	ELISA	Mild HIE	At birth	Increased levels compared to mild HIE (p=0.001)	↑ at 0h	
					Moderate HIE			↑↑ at 0h	
					Severe HIE				
		UCHL-1	P09936	Mild HIE	At birth	Increased levels compared to mild HIE (p=0.03)	↑ at 0h		
				Moderate HIE			↑↑ at 0h		
				Severe HIE					
	Umbilical cord serum	GFAP	P14136	ELISA	Mild HIE	At birth	Increased levels compared to mild HIE (p<0.05)	↑ at 0h	
					Moderate/severe HIE				
		UCHL-1	P09936		Mild HIE	At birth	No significant differences	= at 0h	
					Moderate/severe HIE				
		IL-1	P01584		Mild HIE	At birth	No significant differences	= at 0h	
					Moderate/severe HIE				
		IL-6	P05231		Mild HIE	At birth	No significant differences	= at 0h	
					Moderate/severe HIE				
		IL-8	P10145		Mild HIE	At birth	No significant differences	= at 0h	
			Moderate/severe HIE						
VEGF		P49767	Mild HIE		At birth	No significant differences	= at 0h		
			Moderate/severe HIE						
	IFN-γ	P01579	Mild HIE	At birth	No significant differences	= at 0h			
			Moderate/severe HIE						
	TNF	P01375	Mild HIE	At birth	No significant differences	= at 0h			
			Moderate/severe HIE						
	RANTES	P13501	Mild HIE	At birth	No significant differences	= at 0h			
			Moderate/severe HIE						
Metabolite									
(Haiju, Suyuan et al. 2008) ⁴⁵	Cord blood	Lactate	HMDB000190	Blood gas and biochemical analysis	Mild HIE	At birth	Increased levels compared to mild HIE (p<0.05)	↑ at 0h	
					Moderate/severe HIE				
Number of cells									
(El-Mazary, Abdel-Aziz et al. 2015) ⁴⁰	Serum	Platelet	-	Chemical analysis	Control	<48h	Decreased levels compared to control (p=0.01)	↓ at <48h	
					Control				
					HIE	<48h	No statistical differences (p=0.1)	= at <48h	
					Mild HIE				

(Haiju, Suyuan et al. 2008) ⁴⁵	Cord blood	Nucleated red blood cells	May-Grunwald-Giemsa stain (cytometer)	Moderate/severe HIE	Increased levels compared to mild HIE (p<0.01)	↑ at 0h
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Table 6 – Summary of findings of biomarkers present in whole-blood.

Reference	Sample type	Name	Code	Technique	Group	Collection time	Results	Summary	Observations
Proteins									
(Maggiotto, Sondhi et al. 2019) ²⁹	Whole blood	GLUT1	P11166	ELISA	Control	6h-76h	Increased levels in pre-TH compared to control groups (p=0.0057)	↑ at pre-TH	GLUT1 was quantified in red blood cells Decreased levels comparing pre-TH and post-TH (p=0.05)
		HIE	Pre-TH; during TH; rewarming; pos-TH						
		GLUT3	P11169		Control	6h-76h	No significant differences	= on all timepoints	GLUT3 was quantified in white blood cells
		HIE	Pre-TH; during TH; rewarming; pos-TH						
Metabolites									
(Bersani, Ferrari et al. 2019) ⁴⁴	Whole blood	Creatinine	HMDB0000562	-	Moderate HIE (with hypothermia)	At birth	No statistical differences (p>0.05)	= on all groups	Data is also available for groups that were not treated with TH
		Severe HIE (with hypothermia)							
		Urea	HMDB0000294	-	Moderate HIE (with hypothermia)	At birth	No statistical differences (p>0.05)	= on all groups	
		Severe HIE (with hypothermia)							
(Jain, Pagano et al. 2017) ²⁷	Whole-blood	Lactate	HMDB0000190	-	HIE (with MRI score 0 or 1) HIE (with MRI score 2) HIE (with MRI score 3)	At admission	No statistical differences (p=0.21)	= on all groups	
RNA									
(Balada, Tebe et al. 2020) ²⁰	Whole-blood	CCR5	GC03P046383	RT-qPCR	Control	6h, 12h, 24h, 48h, 72h, 96h	Decreased levels compared to controls (p<0.001) Lower levels compared to mild HIE (p=0.003)	↓ on all timepoints	Faster decrease on mild HIE compared to moderate/severe HIE (p = 0.074)
		HIE							
		PPARG	GC03P012287		Control	6h, 12h, 24h, 48h, 72h, 96h	Increased levels compared to controls (p=0.002)	↑ on all timepoints	
		HIE							
		MMP9	GC20P046008		Control	6h, 12h, 24h, 48h, 72h, 96h	Increased levels compared to controls (p<0.001); gradual decrease over time Faster decrease on mild infants compared to moderate/severe (p=0.007)	↑ on all timepoints	
		HIE							
		Mild HIE							
		IL-8	GC04P073740		Control	6h, 12h, 24h, 48h, 72h, 96h	Increased levels compared to controls (p=0.004); gradual decrease over time	↑ on all timepoints	
		HIE							

					Severe HIE	6h, 12h	Increased levels compared to mild HIE (p<0.01) and moderate HIE (p<0.01)		
					Control				
					HIE	6h, 12h, 24h, 48h, 72h, 96h	Increased levels compared to controls (p=0.005); gradual decrease over time	↑ on all timepoints	
					Severe HIE	6h, 12h	Increased levels compared to mild HIE (p<0.001) and moderate HIE (p<0.001)		
					Control				
					HIE	6h, 12h, 24h, 48h, 72h, 96h	Increased levels compared to controls (p=0.003); gradual decrease over time	↑ on all timepoints	
miRNA									
(Ponnusamy, Kapellou et al. 2016) ³¹	EDTA-blood	RNU6B	GC10P013220	TaqMan miRNA assay	HIE	18h-19h	No comparisons between groups were performed		
		Let7b	GC22P046119						
		miR-21	GC17P059841						
Number of cells									
(Saito, Shibasaki et al. 2016) ³³	Whole-blood	White-blood cells	-	Flow cytometry	HIE	24h, 48h, 72h, 96h, 120h, 144h, 168h	Negative correlation with CRP levels		
		Neutrophils	-				No statistical differences were found		
		Platelet	-						

Table 7 – Summary of findings of biomarkers present in dried-blood spots.

Reference	Sample type	Name	Code	Technique	Group	Collection time	Results	Summary	Observations	
Proteins										
(Massaro, Wu et al. 2019) ³²	DBS	S100B	P04271	ELISA	HIE	<24h, 48h, 72h, 120h	No relationship with death, severe brain injury (p>0.05) or neurodevelopment impairment (p>0.05)	= comparing the favorable and unfavorable outcome		
		IL-1β	P01584	V-PLEX proinflammatory panel						
		Erythropoietin	P01588	Human EPO base kit						
		IL-6	P05231	V-PLEX proinflammatory panel						HIE (favorable outcome)
		IL-8	P10145							HIE (unfavorable outcome)
										HIE (favorable outcome)
										HIE (unfavorable outcome)
TNF-α	P01375	HIE (favorable outcome)	HIE (unfavorable outcome)							
Metabolite										
(Lopez-Suarez, Concheiro-Guisan et al. 2019) ²¹	DBS	C2	HMDB0240773	ESI-MS/MS	Control	72h	Decreased levels compared to controls (p=0.000)	↓ at 72h		
		C3:1	HMDB0000824		HIE		Increased levels compared to HIE to unfavorable outcome (p=0.001)	↑ at 72h		
					HIE (favorable outcome)		Increased levels compared to HIE to unfavorable outcome (p=0.005)	↑ at 72h		
					HIE (favorable outcome)					
		C4	HMDB0002013		Control		Increased levels compared to controls (p=0.000)	↑ at 72h		
		C4-OH	HMDB0013127		HIE		Decreased levels compared to controls (p=0.005)	↓ at 72h		
					HIE (favorable outcome)		Increased levels compared to HIE to unfavorable outcome (p=0.01)	↑ at 72h		
		C5	HMDB0000378; HMDB0000688		Control		Increased levels compared to controls (p=0.000)	↑ at 72h		
		C5:1	HMDB0002366		HIE					
					Control		Increased levels compared to controls (p=0.04)	↑ at 72h		
					HIE		Increased levels compared to HIE to unfavorable outcome (p=0.004)	↑ at 72h		
		DC5	HMDB0013130		HIE (favorable outcome)					
		C6	HMDB0000756		Control		Increased levels compared to controls (p=0.000)	↑ at 72h		
C6-OH	HMDB0013131	HIE	Increased levels compared to controls (p=0.001)	↑ at 72h						

		HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.031)	↑ at 72h
		Control		
		HIE		
C8	HMDB0000791	HIE (unfavorable outcome)	Increased levels compared to controls (p=0.023)	↑ at 72h
			Increased levels compared to HIE to favorable outcome (p=0.001)	↑ at 72h
C8:1		HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.011)	↑ at 72h
C10		HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.012)	↑ at 72h
C10:1	HMDB0000651	HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.023)	↑ at 72h
C10:2	HMDB0241102	HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.009)	↑ at 72h
C12	HMDB0002250	Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C14		Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C14:1	HMDB0005066	Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C14:2		Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C14:1-OH		HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.032)	↑ at 72h
C14-OH	HMDB0253296	HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.004)	↑ at 72h
C16		Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C16:1	HMDB0000222	Control	Decreased levels compared to controls (p=0.000)	↓ at 72h
		HIE		
C16-OH	HMDB0241520	HIE (unfavorable outcome)	Increased levels compared to HIE to favorable outcome (p=0.048)	↑ at 72h
		Control		
C18	HMDB0000848	HIE	Decreased levels compared to controls (p=0.000)	↓ at 72h
			Increased levels compared to HIE to favorable outcome (p=0.03)	↑ at 72h
C18:1	HMDB0255949	HIE (unfavorable outcome)	Decreased levels compared to controls (p=0.000)	↓ at 72h
		Control		
		HIE		
C18-OH	HMDB0241520	HIE	Decreased levels compared to controls (p=0.028)	↓ at 72h
			Increased levels compared to HIE to favorable outcome (p=0.03)	↑ at 72h
C18:1-OH	HMDB0255949)	HIE (unfavorable outcome)	Increased levels compared to HIE to unfavorable outcome (p=0.001)	↑ at 72h
		HIE (favorable outcome)		

		MeGlut	HMDB0254648		HIE (favorable outcome)		Increased levels compared to HIE to unfavorable outcome (p=0.002)	↑ at 72h
		Free carnitine	-		Control		No significant differences	= on both groups
		Free/total carnitine ratio	-		Control		No significant differences	= on both groups
					HIE		No significant differences	= on both groups
miRNA								
(Ponnusamy, Kapellou et al. 2016) ³¹	DBS	Let7b	GC22P046119	TaqMan miRNA assay	HIE (favorable outcome)	18h-19h	No significant differences	= on both groups
		miR-21	GC17P059841		HIE (unfavorable outcome)		No significant differences	= on both groups
		miR-29b	GC07M130877; GC01M207806		HIE (favorable outcome)		No significant differences	= on both groups
		miR-124	GC08M009903; GC20P063181; GC08P064379		HIE (unfavorable outcome)		No significant differences	= on both groups
		miR-155	GC21P025573		HIE (favorable outcome)		No significant differences	= on both groups
					HIE (unfavorable outcome)		No significant differences	= on both groups
		RNU6B	GC10P013220		HIE		No comparisons between groups were performed	