Publisher

www.medresearch.in

Biomedical Review-Journal of Basic and Applied Medical Science

2015 Volume 2 Number 3

E-ISSN:2349-3275

P-ISSN:2349-5502

Review Article

Birth Asphyxia

Biomarkers of Birth Asphyxia in Neonates

Rabindran.[1*], Gedam D.[2]

[1*] Rabindran, Consultant Neonatologist, Billroth Hospital, Chennai, Tamil Nadu, India.

[2] D Sharad Gedam, Professor, Department of Pediatrics, L N Medical college, Bhopal, MP, India.

Birth asphyxia is a common cause of neonatal mortality. Identification of severity of asphyxia is vital for prompt management. Biomarkers can be used for timely diagnosis of asphyxia and also helps in prognosis. Serum, CSF, urine, cord blood & magnetic resonance biomarkers for asphyxia in neonates have been studied. Cytokines like IL-6 in CSF, NSE in CSF, Protein S-100b in serum, urine & cord blood, LDH in serum and saliva, CK in serum and urine, cord BDNF, Urinary UA/Cr ratio, GFAP, Glutamate in CSF, PGE2, AST, ALT, Activin A have been studied with varied diagnostic accuracy and feasibility. Research directed towards newer biomarkers like NPBI, Hypoxanthine, total hydroperoxides, AOPP, UCHL-1 & pNFH-1 for early identification of severe asphyxia have shown promising results. Cardiac biomarkers like Troponin T & I, BNP, CK-MB may aid in longterm outcome. NRBC count still remains as the oldest and best-described biomarker of asphyxia. Use of Proton & Nuclear Magnetic Resonance Spectroscopy on day 1 apart from conventional MRI have opened a new era of MR Biomarkers in neonatal asphyxia. Early identification of severity of asphyxia with judicious use of biomarkers can make a huge difference in the management and outcome of birth asphyxia.

Keywords: Birth asphyxia, Biomarkers, Cytokines in birth asphyxia

Corresponding Author	How to Cite this Article	To Browse
Rabindran, Consultant Neonatologist, , Billroth Hospital, Chennai, Tamil Nadu, India. Email: rabindranindia@yahoo.co.in	Rabindran, D Sharad Gedam, Biomarkers of Birth Asphyxia in Neonates. Biomed Rev J Basic Appl Med Sci. 2015;2(3):91-101. Available From https://www.biomedicalreview.in/biomarkers-of-birth-asphyxi-in-neonates-review-article	

Manuscript Received	Review Round 1	Review Round 2	Review Round 3	Accepted
2015-09-02	2015-09-04	2015-09-11	2015-09-18	2015-09-25

Conflict of Interest	Funding	Ethical Approval	Plagiarism X-checker	Note
Nil	Nil	Yes	18%	

Introduction

Birth asphyxia is a common cause of neonatal morbidity and mortality [1, 2]. Similar degrees of insult may completely spare one infant and devastate another, so very sensitive parameters/ Biomarkers are necessary to predict the outcome [3, 4, 5]. Biomarkers are used to identify injury, its extent, timing and likely outcome [6].

Biomarkers in neonatal HIE can be used to (i) determine the need to intervene with therapeutic hypothermia and (ii) for prognosis. Several biomarkers like Brain-specific CK, LDH, Lactate/ Creatinine ratio, GFAP, Uric acid, Hypoxanthine, Glutamate, NSE, Protein-S, BDNF and S100B in the blood or CSF, Urine Lactate/ Creatinine ratio, first urine S100B have been investigated in infants with asphyxia [6-17]. Recently inflammatory cytokines (TNF-alpha, IL-1-beta, IL-6, IL-8) have been implicated in the biochemical pathways leading to hypoxic-ischemic injury [6, 13, 14, 15, 18-27].

Cytokines: Cytokine activation, as manifested by increased IL-1β in the CSF is positively correlated with the severity and outcome of HIE [23, 28]. IL-1 seems to be a better predictor of HIE than TNF-alpha [29]. IL-6 is a pleiotropic cytokine produced from both astrocytes and microglial cells within the CNS [30-32]. CSF and serum IL-6 levels significantly correlated with the degree and outcome of encephalopathy [7, 17, 18, 28, 33]. A pilot study revealed that IL-6 concentrations were 376-fold higher in noninfected HIE infants compared with healthy newborns [34]. Cutoff value 25.9 pg/mL had good predictive values for long term outcome (PPV: 100%; NPV: 86%; sensitivity: 100%) [7]. CSF IL-6 was higher in HIE stage 3 (range, 65-2250 pg/mL) when compared with neonates with HIE stage 0 to 2 (<2 pg/mL) [35].

Neuron Specific Enolase: NSE is released into both CSF and serum after CNS damage [36-40]. Measurements of NSE are rapid, nonexpensive, simple to perform and widely available [41]. CSF NSE levels significantly correlated with the degree and outcome of asphyxia [7, 11- 15, 17]. Following asphyxia, CSF NSE had high predictive values (PPV: 90%; NPV: 55%; sensitivity: 86%; specificity: 64%) for adverse outcome [7]. However Serum NSE had relatively lower values (PPV: 56%; specificity: 67%) for predicting moderate to severe HIE [7, 15].

Serum Protein S-100B: S100B protein is a cytosolic calcium-binding protein [9, 42]. Because of its molecular weight (21 kDa), S100B may be detected in peripheral blood only if the integrity of the blood–brain barrier is disrupted [41]. Its estimation is rapid, nonexpensive and widely available 41]. Elevated levels are reported after asphyxia [15, 38, 43-56]. There was a significant difference in S100B levels between moderate or severe HIE and mild HIE groups [45, 57]. Sensitivity and specificity was higher on day 3 (96.7% and 95% respectively) for predicting asphyxia [57]. A combination of serum protein S-100 and CK-BB had a high PPV (83%) and specificity (95%) for predicting moderate to severe HIE [18].

Urinary S100B: S100B may also be detected in urine following asphyxia [41]. Urinary S100B was significantly increased in infants who experienced early neonatal death following asphyxia [58]. Its concentration in the first urine after birth was significantly higher in patients who had HIE than in controls [48]. The level gradually increased and reached the highest level in the third day of life [57]. Urinary S100b concentrations above 1 mcg/L predicted neonatal death with a sensitivity and specificity of 100%, and concentrations were not affected by renal failure [48, 59].

Cord Blood S100B: Cord blood concentration of S100B has been linked to HIE [17]. Cord blood S100B > 2.02 mg/L had a sensitivity of 87% and a specificity of 88% for predicting the development of moderate or severe HIE [45].

Serum Lactate Dehydrogenase: Excellent diagnostic ability of serum LDH for asphyxia has been reported in literature [60-65]. Raised Serum LDH level in the first six hours predicted the development of HIE between 6-72 hours after birth [16]. At a cut-off value of 2812 IU/L, it had 90% sensitivity, 96.7% specificity, 96.4% PPV & 91% NPV for diagnosis of HIE [66]. In a retrospective study, serum LDH successfully predicted an abnormal mental or psychomotor development index at 18 months of age in neonates with HIE [67].

Salivary Lactate Dehydrogenase: Salivary LDH may rise in neonates with asphyxia because of either hypoxic– ischaemic damage to the salivary glands or leakage from plasma [68, 69].

Measuring salivary LDH provided an early and accurate diagnosis of HIE and could be used as a triage tool [66, 70]. Salivary LDH was significantly higher in the HIE group (median 2578) than in the control group (median 558.5, $p < 0.001$) [66]. At a cut-off value of 894 IU/L, it had 90% sensitivity,73.3% specificity, 77.1% PPV & 88% NPV for the diagnosis of HIE [66].

Creatine Kinase: Serum CK is useful as a screening test and was also predictive of long term outcome [16, 71]. Level > 2860 IU/L in the first six hours of life predicted the development of HIE between 6-72 hours after birth [16]. Urinary CK-BB levels >42 ng/L was predictive of death following asphyxia with a sensitivity of 90.9%, a specificity of 94.9%, PPV of 83.3% & NPV of 97.4% [58].

Brain-Derived Neurotrophic Factor: Newborns who have HIE have higher cord plasma BDNF levels when compared with healthy neonates [72, 73]. BDNF levels at delivery and at 72 hour postnatal were predictors of poor outcome [72].

Urinary Uric Acid / Creatinine Ratio: UA/Cr is a welldefined biomarker in predicting the severity and outcome of HIE [11-15].

Glial Fibrilary Acidic Protein: GFAP is a cytoskeleton intermediate filament protein only released into the blood upon astrocyte death [17]. It has been used as a predictor of mortality or poor neurological outcomes in children [17, 74]. Concentrations of > 0.15 ng/mL during the first 2 days in asphyxiated neonates correlate with abnormal outcome [75].

Glutamate: Glutamate has recently been reported as being present in the CSF of asphyxiated newborns and its level has been shown to correlate with the grade of HIE and outcome [76, 77].

Prostaglandin E2: Acute hypoxia increases the activity of microsomal prostaglandin E synthase–1 in endothelial cells of the BBB and the subsequent release of PGE2 beyond the BBB [78, 79, 80]. PGE2 metabolite levels correlated to a low Apgar score at 5 min ($p < 0.01$) and 10 min ($p < 0.01$), a low pH ($p < 0.001$) and HIE score ($p < 0.05$) [81].

AST, ALT: Serum AST and ALT were higher in infants with moderate and severe asphyxia in comparison with those with no asphyxia [82].

Activin A: Activin A also seems to be a reliable marker of perinatal hypoxia [83].

Newer Biomarkers: Indirect markers of increased FR release and oxidative stress during fetal/ neonatal asphyxia have recently emerged, with reports of increased plasma advance oxidative protein products (AOPP) and non-protein-bound iron (NPBI) in plasma and red cells [84-88]. Hypoxanthine, total hydroperoxides and AOPP levels were found to be significantly higher in cord blood and on 7th day blood samples of hypoxic newborns than controls [83]. Serum UCHL-1 (found in neuronal cell bodies), pNFH-1 (found in white matter brain regions) were noted to be predictive of severe HIE [89].

Cardiac Biomarkers: Cardiac biomarkers may aid in longterm neurodevelopmental outcome prediction following neonatal hypoxic-ischaemia. Biomarkers such as troponin and BNP may aid in outcome prediction [90].

Troponin-T: Newborns with severe HIE have significantly higher serum troponin-T concentrations than other asphyxiated groups (mild to moderate) and healthy neonates on day 1 of life [91, 92]. Troponin-T remains significantly higher in the severely asphyxiated group compared with the mild group on days 3 and 7 [82]. Value > 0.1 microgram / L may predict severity of encephalopathy and mortality [92].

Troponin-I: Cord blood troponin-I levels are significantly higher in infants who sustain a hypoxic insult with or without ensuing encephalopathy compared with normals ($p < 0.0001$) [93-96]. A cut-off value 0.35 microgram / L may predict severity of neonatal hypoxic ischaemia [93, 94]. Cord troponin-I is the marker with highest specificity (86%), sensitivity (88%), NPV (85%), PPV (88%) for prediction of perinatal hypoxia [93]. It is the most sensitive factor for predicting early death [94, 96].

CK-MB: Levels of CK-MB began to rise within the first few hours of life and are significantly higher in moderate and severe grades of HIE compared with mild grades and normal controls within the first 2–4 hour [93].

Urinary Biomarkers: Urine lactate/creatinine is difficult to interpret, because lactate is a global marker of anaerobic metabolism and would not be brain-specific [6]. However Urine S100 is brain-specific [6] & is a very promising marker correlating well with tissue injury in HIE [97, 98].

Nucleated Red Blood Cell Count: NRBC count is one of the oldest and best-described biomarker of asphyxia [57, 99,100]. It was used as an early marker for subsequent neurological impairment in neonatal hypoxia [83,100- 103]. Postnatal NRBCs distinguished between mild and moderate/ severe encephalopathy in normothermic infants but not in infants undergoing therapeutic hypothermia [57,104,105,106]. The increase in NRBCs associated with hypoxia is secondary to raised IL-6 and erythropoietin (EPO) [104,107,108]. The sensitivity and specificity were highest at the first day (96.6% and 100% respectively) [57].

Magnetic Resonance Biomarkers: Meta-analysis suggests that deep gray matter Lac/NAA and Lac/Cr are the most accurate quantitative MR biomarker for prediction of neurodevelopmental outcome after HIE. Thalamic or basal-ganglia lactate/total creatine had sensitivity of 77% and 94% specificity for predicting severe asphyxia [109].

Lactate/choline, NAA/choline, NAA/creatine had sensitivities and specificities of 84% and 81%; 59% and 72%; and 61% and 71% respectively [109].

Proton Magnetic Resonance Spectroscopy: During the first 96 h of life H-MRS could be a useful early prognostic tool in predicting the outcome of asphyxiated neonates. Myo-inositol/ N-Acetyl-Aspartate ratio was found to be the best and time-independent predictor with 85.71% sensitivity and 91.30% specificity [110].

Deep gray matter lactate/N-acetyl aspartate (Lac/NAA) peakarea ratio had 82% sensitivity and 95% specificity for predicting severe asphyxia [110].

Lac/NAA had better diagnostic accuracy than conventional MRI performed at any time during neonatal period. When the MR biomarker was determined at any time in the age range 1 to 30 days, Lac/NAA had a significantly higher specificity than conventional MRI 98% vs 76% [109]. Lac/NAA sensitivity of 86% was comparable to that for conventional MRI of 80% [109].

Nuclear Magnetic Resonance Spectroscopy : When the metabolite profile of Umbilibal Cord Blood was analysed using NMR spectroscopy, a characteristic pattern of raised glycerol + succinate reflecting critical energy failure occurred in those infants with severe encephalopathy and very low voltage EEG ($p < 0.001$) [110].

Conclusion

A therapeutic window exists in the early hours following asphyxia, when intervention can attenuate activation of the neurotoxic cascade that leads to ultimate cell death [111]. Early identification of severity of asphyxia with the use of biomarkers can make a huge difference in the management and outcome of birth asphyxia.

Abbreviation

ALT- Alanine Transaminase

AOPP- Adance Oxidation Protein Products

AST- Aspartate Transaminase

BBB- Blood Brain Barrier

BDNF- Brain Derived Neurotrophic Factor

BNP- Brain Natriuretic Protein

CK- Creatine Kinase

CNS- Central Nervous System

CSF- Cerebro Spinal Fluid

EPO- Erythropoietin

FR- Free Radical

GFAP- Glial Fibrilary Acidic Protein

HIE- Hypoxic Ischemic Encephalopathy

IL- Interleukin

IU/L- International units/litre

KDa- kiloDalton

Lac/Cr - Lactate/Creatinine

Lac/NAA- Lactate/N-acetyl aspartate

LDH- Lactate Dehydrogenase

MRS- Magnetic Resonance Spectroscopy

NAA- N-Acetyl Aspartate

Ng/L- nanogram/litre

Ng/mL- nanogram/millilitre

NPBI- non-protein-bound iron

NPV- Negative Predictive Value

NRBC- NUcleated Red Blood Cell

NSE- Neuron Specific Enolase

PGE2- prostaglandin E2

PPV- POsitive Predictive Value

TNF- Tumour Necrosis Factor

UA/Cr- Uric acid/Creatinine

Reference

01. Gray PH, Tudehope DI, Masel JP, Burns YR, Mohay HA, O'Callaghan MJ, et al. Perinatal hypoxic-ischemic brain injury: prediction of outcome. Dev Med Child Neurol. 1993; 35:965-73.

02. Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: Incidence, clinical course, and outcome in a Swedish population. Acta Paediatr 1995; 84(8):927- 32.DOI: 10.1111/j.1651-2227.1995.tb13794.x.

03. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, Kapellou O, Levene M, Marlow N, Porter E, Thoresen M, Whitelaw A, Brocklehurst P; TOBY Study Group. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med. 2009 Oct 1; 361(14):1349-58. doi: 10.1056/NEJMoa0900854.

04. Pasternak JF. Hypoxic-ischemic brain damage in the term infant. Pediatr Clin North Am 1993; 40:1061-71.

05. Robertson CMT, Finer NN. Long term follow-up of term neonates with perinatal asphyxia. Clin Perinatol 1993; 20:483-90.

06. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain its timing, duration, and outcomes are poorly defined. Injury in term neonatal encephalopathy. Pediatr Neurol 2009; 40:215-226.doi:10.1016/j.pediatrneurol.2008.09.026

07. Tekgul H, Yalaz M, Kutukculer N, Ozbek S, Kose T, Akisu M, Kultursay N, Gokben S. Value of biochemical markers for outcome in term infants with asphyxia. Pediatr Neurol 2004;31 (5) :326-332. DOI: 10.1016/j.pediatrneurol.2004.05.004.

08. Vannucci RC, Perlman JM. Intervention for perinatal hypoxic-ischemic encephalopathy. Pediatrics 1997; 100:1004-14.

01. Fernandez F, Verdu A, Quero J, et al. Cerebrospinal fluid lactate levels in term infants with perinatal hypoxia. Pediatr Neurol 1986;2(1):39-42. DOI: http://dx.doi.org/10.1016/0887-8994 (86)90038-X.

02. Blennow M, Hagberg H, Rosengren L. Glial fibrillary acidic protein in the cerebrospinal fluid: A possible indicator of prognosis in full-term asphyxiated newborn infants. Pediatr Res 1995; 37:260-4.

03. Thornberg E, Thiringer K, Hagberg H, Kjellmer I. Neuron specific enolase in asphyxiated newborns: Association with encephalopathy and cerebral function monitor trace. Arch Dis Child Fetal Neonatal 1995 Jan; 72(1):F39-42.

04. Huang CC, Wang ST, Chang YC, Lin KP, Wu PL. Measurement of the urinary lactate:creatinine ratio for the early identification of newborn infants at risk for hypoxicischemic encephalopathy. N Engl J Med 1999 Jul 29; 341(5):328-35.

05. Bader D, Gozal D, Weinger-Abend M, Berger A, Lanir A. Neonatal urinary uric acid/creatinine ratio as an additional marker of perinatal asphyxia. Eur J Pediatr 1995; 154:747-9.

06. Akisu M, Kultursay N. Value of the urinary uric acid to creatinine ratio in term infants with perinatal asphyxia. Acta Paediatr Jpn 1998 Feb; 40(1):78-81. DOI: 10.1111/j.1442-200X.1998.tb01408.x

07. Nagdyman N, Komen W, Ko HK, Muller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. Pediatr Res 2001; 49:502-6.

08. Karunatilaka DH, Amaratunga GWDS, Perera KDNI, Caldera V. Serum creatine kinase and lactic dehydrogenase levels as useful markers of immediate and long-term outcome of perinatal asphyxia. Sri Lanka Journal of Child Health, 2000; 29: 49-52.

09. Martha Douglas-Escobar, Michael D. Weiss. Neonatal Biomarkers of Brain Injury. NeoReviews Vol.14 No.10 October 2013 e501. doi:10.3389/fneur.2012.00144.

10. Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J. Interleukin-6 in the cerebrospinal fluid

01. after perinatal asphyxia is related to early and late neurological manifestations.Pediatrics 1997 Nov;100(5):789-94. doi: 10.1542/peds.100.5.789.

02. Savman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res 1998 Jun; 43(6):746-51.

03. Oygur N, Sonmez O, Saka O, Yegin O. Predictive value of plasma and cerebrospinal fluid tumor necrosis factor-alpha and interleukin 1 beta concentration on outcome of full term infants with hypoxic ischemic encephalopathy. Arch Dis Child Fetal Neonatal 1998 Nov; 79(3):F190-3.

04. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM.Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics 2002 Oct; 110(4):673-80.

05. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S,Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol 2009 Mar; 40(3): 215–26.doi: 10.1016/j.pediatrneurol.2008.09.026.

06. H. Aly, M.T. Khashaba, M. El-Ayouty, O. El-Sayed and B.M. Hasanein, IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy, Brain Dev 2006, Apr;28(3):178-82.

07. Minami M, Kuraishi Y, Yabuuchi K, Yamazaki A, Satoh M. Induction of interleukin-1beta mRNA in rat brain after transient forebrain ischemia. J Neurochem. 1992; 58:390 –392.

08. Hagberg H, Gilland E, Bona E, Hanson LA, HahinZoric M, Blennow M, Holst M, McRae A, Söder O. Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxiaischemia in neonatal rats. Pediatr Res. 1996 Oct;40(4):603-609.

09. Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC, Feuerstein GZ. Tumor necrosis factoralpha expression in ischemic neurons. Stroke. 1994 Jul; 25(7):1481-8.

10. Szaflarski J, Burtrum D, Silverstein FS. Cerebral hypoxia-ischemia stimulates cytokine gene expression in perinatal rats. Stroke. 1995 Jun; 26(6):1093-100.

01. Mohamed T. Khashabaa, Basma O. Shoumana, Bothina M. Hasaneina, Ali A. Shaltouta , Hala M. AlMarsafawya , Mohamed M. Abdel-Azizb , Tahmina Ahmadc, Hany Alyc. Interleukin-1β , interleukin-6, and tumor necrosis factor-α in the cerebrospinal fluid of term infants with hypoxic-ischemic encephalopathy after postnatal treatment with magnesium sulfate. Journal of Pediatric Neurology 2011;9(3):299–304 .DOI 10.3233/JPN-2011-0501.\

02. Ozdemir A, Oygür N, Gültekin M, Copkun M, Yegin O. Neonatal tumor necrosis factor, interleukin-1alpha, interleukin-1B and interleukin-6 response to Infection. Am J Perinatol 1994; 11:282-4

03. Selmaj KW, Farooq M, Norton WT, Raine CS, Brosnan CF. Proliferation of astrocytes in vitro in response to cytokines: A primary role for tumor necrosis factor. J Immunol 1990 Jan 1; 144(1):129-35.

04. Hama T, Miyamoto M, Tsukui H, Nishio C, Hatanaka H.Interleukin 6 as a neurotrophic factor for promoting the survival of cultured basal forebrain cholinergic neurons from postnatal rats. Neurosci Lett. 1989 Oct 9; 104(3):340-4.

05. Maeda Y, Matsumoto M, Hori O, et al. Hypoxia/reoxygenation-mediated induction of astrocyte interleukin-6: A paracrine mechanism potentially enhancing neuron survival. J Exp Med 1994; 180:2297- 308.

06. Ancel AM, Alix-AG, Salcedo DP, Cabanas F, Valcarce M, Quero J. Interleukin-6 in the cerebral fluid after perinatal asphyxia is related to early and late neurologic manifestations. Pediatrics 1997; 100:789-94.

07. Chiesa C, Pellegrini G, Panero A, T. De Luca, M. Assumma, F. Signore, L. Pacifico. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest. 2003 Mar; 33(4):352–358.DOI: 10.1046/j.1365- 2362.2003.01136.x.

08. Ana Martın-Ancel, Alfredo Garcıa-Alix, Dora Pascual-Salcedo, Fernando Cabanas, Manuel Valcarce, Jose Quero.Interleukin-6 in the Cerebrospinal Fluid After Perinatal Asphyxia Is Related to Early and Late Neurological Manifestations. Pediatrics 1997; 100:789 – 794.

01. De Praeter C, Vanhaesebrouck P, Govaert P, et al.Creatine Kinase Isoenzyme BB concentrations in the cerebrospinal fluid of newborns. Pediatrics 1991; 88:1204-10.

02. Fernandez F, Verdu A, Quero J. A serum CPK-BB Isoenzyme in the assessment of brain damage in asphyctic term infants. Acta Paediatr Scand. 1987 Nov; 76(6):914-8.

03. Celtik C, Acunas B, Oner N, Pala O. Neuron-specific enolase as a marker of the severity and outcome of hypoxic ischemicbencephalopathy. Brain Dev 2004 Sep; 26(6):398-402.

04. Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J. Neuron-specific enolase and myelin basic protein: relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term infants. Pediatrics. 1994 Feb; 93(2):234-40.

05. Persson L, Hardemark HG, Gustafsson J, Rundstrom G, Mendel-Hartvig I, Esscher T, Pahlman S. S-100 protein and neuron-specific enolase on cerebrospinal fluid and serum: Markers of cell damage in human central nervous system. Stroke 1987 Sep-Oct; 18(5):911-8.

06. Aniko Roka, Dorottya Kelen, Jozsef Halasz, Gabriella Beko, Denis Azzopardi, Miklos Szabo. Serum S100B and neuron-specific enolase levels in normothermic and hypothermic infants after perinatal asphyxia. Acta Pædiatrica. DOI:10.1111/j.1651-2227.2011.02480.x

07. Heizmann CW. Calcium binding S100 proteins in the central nervous system. Neurochem Res 1999 Sep; 24(9):1097-100.

08. Gazzolo D, Vinesi P, Marinoni E, Di Iorio R, Marras M, Lituania M, Pierluigi Bruschettini, Fabrizio Michetti. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. Clin Chem 2000 Jul; 46(7): 998–1000.

09. Gazzolo D, Di Iorio R, Marinoni E, Masetti P, Serra G, Giovannini L, et al. S100B protein is increased in asphyxiated term infants developing intraventricular haemorrhage. Crit Care Med 2002; 30: 1356–60.

10. Qian J, Zhou D, Wang YW. Umbilical artery blood S100 beta protein: a tool for the early identification of neonatal hypoxic-ischemic

01. encephalopathy. Eur J Pediatr. 2009 Jan; 168(1):71-7. doi: 10.1007/s00431-008-0711-4.

02. Murabayashi M, Minato M, Okuhata Y, Makimoto M, Hosono S, Masaoka N, et al. Kinetics of serum S100B in newborns with intracranial lesions. Pediatr Int 2008; 50: 17–22.

03. Giuseppe D, Sergio C, Pasqua B, Giovanni LV, Salvatore C, Frigiola A, Petra H, Maurizio A. Perinatal asphyxia in preterm neonates leads to serum changes in protein S-100 and neuron specific enolase. Curr Neurovasc Res 2009 May;6(2):110-6.

04. Gazzolo D, Frigiola A, Bashir M, Iskander I, Mufeed H, Aboulgar H, Venturini P, Marras M, Serra G, Frulio R, Michetti F, Petraglia F, Abella R, Florio P. Diagnostic accuracy of S100B urinary testing at birth in full-term asphyxiated newborns to predict neonatal death. PLoS One. 2009; 4(2):e4298. doi: 10.1371/journal.pone.0004298.

05. Michetti F, Gazzolo D. S100B protein in biological fluids: a tool for perinatal medicine. Clin Chem 2002 Dec; 48(12):2097-104.

06. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, Serpero L, Diego Gazzolo. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem 2012; 120: 644– 59.DOI: 10.1111/j.1471-4159.2011.07612.x.

07. Florio P, Abella R, Marinoni E, Di Iorio R, Li Volti G, Galvano F, Pongiglione G, Frigiola A, Pinzauti S, Petraglia F, Gazzolo D. Biochemical markers of perinatal brain damage. Front Biosci (Schol Ed) 2010 Jan 1; 2:47- 72. doi: 10.2741/s45.

08. Gazzolo D, Abella R, Marinoni E, di Iorio R, Li Volti G, Galvano F, et al. New markers of neonatal neurology. J Matern Fetal Neonatal Med 2009; 22: 09/2009; 22 Suppl 3:57-61. DOI: 10.1080/14767050903181468

09. Gazzolo D, Bruschettini M, Lituania M, Serra G, Bonacci W, Michetti F. Increased urinary S100B protein as an early indicator of intraventricular hemorrhage in preterm infants: correlation with the grade of hemorrhage. Clin Chem. 2001 Oct; 47(10):1836-8.

10. Gazzolo D, Marinoni E, Di Iorio R, Bruschettini M, Kornacka M, Lituania M, et al. Measurement of urinary S100B protein concentrations for the

01. early identification of brain damage in asphyxiated full-term infants. Arch Pediatr Adolesc Med. 2003;157(12):1163-1168. doi:10.1001/archpedi.157.12.1163.

02. Bashir M, Frigiola A, Iskander I, Said HM, Aboulgar H, Frulio R, Bruschettini P, Michetti F, Florio P, Pinzauti S, Abella R, Mussap M, Gazzolo D. Urinary S100A1B and S100BB to predict hypoxic ischemic encephalopathy at term. Front Biosci (Elite Ed). 2009 Jun 1;1:560-7.

03. K. Thorngren-Jerneck, C. Alling, A. Herbst, I. AmerWahlin, K. Marsal. S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy;Pediatr Res (2004) 55, 406–412; doi:10.1203/01.PDR.0000106806.75086.D3.

04. Heba S. Elmahdy, Abed Elrahman Elmashad, Adel A. Hagaga, Sahar Hamouda. Serum protein S100B and nucleated red blood cell counts as early markers of cerebral damage in neonates with hypoxic ischemic encephalopathy. Journal of Pediatric Neurology 7 (2009) 337–343. DOI 10.3233/JPN-2009-0332.

05. Francesco M Risso, Laura D Serpero, Luc JI Zimmermann, Antonio WD Gavilanes, Rosanna Frulio, Fabrizio Michetti, Pasquale Florio, Moataza Bashir, Iman Iskander, Hala Mufeed, Hanna Aboulgar, Diego Gazzolo. Urine S100 BB and A1B dimers are valuable predictors of adverse outcome in full-term asphyxiated infants. Acta Pædiatrica .DOI:10.1111/apa.12343.

06. Risso FM, Serpero LD, Zimmermann LJ, Gavilanes AW, Frulio R, Michetti F, Florio P, Bashir M, Iskander I, Mufeed H, Aboulgar H, Gazzolo D. Perinatal asphyxia:kidney failure does not affect S100b urine concentrations. Clin Chim Acta. 2012 Jan 18; 413(1-2):150-3. doi: 10.1016/j.cca.2011.09.011.

07. Barberi I, Calabro MP, Cordaro S, Gitto E, Sottile A, Prudente D, Bertuccio G, Consolo S. Myocardial ischaemia in neonates with perinatal asphyxia. Electrocardiographic, echocardiographic and enzymatic correlations. Eur J Pediatr. 1999 Sep; 158(9):742-7.

08. Gunes T, Ozturk MA, Koklu SM, Narin N, Koklu E. Troponin- T levels in perinatally asphyxiated infants during the first 15 days of life. Acta Paediatr. 2005 Nov; 94(11):1638-43.

01. Roka A, Vasarhelyi B, Bodrogi E, Machay T, Szabo M. Changes in laboratory parameters indicating cell necrosis and organ dysfunction in asphyxiated neonates on moderate systemic hypothermia. Acta Paediatr. 2007 Aug; 96(8):1118-21.

02. Reddy S, Dutta S, Narang A. Evaluation of lactate dehydrogenase, creatine kinase and hepatic enzymes for the retrospective diagnosis of perinatal asphyxia among sick neonates. Indian Pediatr. 2008 Feb; 45(2):144-7.

03. Karlsson M, Wiberg-Itzel E, Chakkarapani E, Blennow M, Winbladh B, Thoresen M. Lactate dehydrogenase predicts hypoxic ischaemic encephalopathy in newborn infants: a preliminary study. Acta Paediatr. 2010 Aug; 99(8):1139-44. doi: 10.1111/j.1651-2227.2010.01802.x.

04. Fernandez F, Quero J, Verdii A, Ferreiros MC, Daimiel E, Roche MC. LDH isoenzymes in CSF in the diagnosis of neonatal brain damage. Acta Neurol Scand. 1986 Jul;74(1):30-3.

05. Akshay Mehta, Deepak Chawla, Jasbinder Kaur, Vidushi Mahajan, Vishal Guglani. Salivary lactate dehydrogenase levels can provide early diagnosis of hypoxic–ischaemic encephalopathy in neonates with birth asphyxia. Acta Pædiatrica. 2015 104(6): e236–e240. DOI:10.1111/apa.12964.

06. Thoresen M, Liu X, Jary S, Brown E, Sabir H, Stone J, Cowan F, Karlsson M. Lactate dehydrogenase in hypothermia-treated newborn infants with hypoxicischaemic encephalopathy. Acta Paediatr. 2012 Oct; 101(10):1038-44. doi: 10.1111/j.1651- 2227.2012.02778.x.

07. Aps JK, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. Forensic Sci Int. 2005 Jun 10; 150(2-3):119-31.

08. Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc. 2006 Mar; 137(3):322-9.

09. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD,Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet. 2005 Feb 19-25; 365(9460):663-70.

01. De Praeter C, Vanhaesebrouck P, Govaert P, Delanghe J, Leroy J. Creatine kinase isoenzyme BB concentrations in the cerebrospinal fluid of newborns: relationship to short term outcome. Pediatrics. 1991 Dec; 88(6):1204-10.

02. S Atef, G Gad, S Imam, M Shawky. Cord Blood Brain Derived Neurotrophic Factor: Diagnostic and Prognostic Marker in Fullterm Newborns with Perinatal Asphyxia; Arch Dis Child 2012; 97: A314 doi: 10.1136/archdischild- 2012-302724.1094.

03. Imam SS, Gad GI, Atef SH, Shawky MA. Cord blood brain derived neurotrophic factor: diagnostic and prognostic marker in full-term newborns with perinatal asphyxia. Pak J Biol Sci. 2009 Dec 1; 12(23):1498-504.

04. Bembea MM, Savage W, Strouse JJ, Schwartz JM, Graham E, Thompson CB, Everett A.. Glial fibrillary acidic protein as a brain injury biomarker in children undergoing extracorporeal membrane oxygenation.Pediatr Crit Care Med. 2011 Sep; 12(5):572-9. doi: 10.1097/PCC.0b013e3181fe3ec7.

05. Ennen CS, Huisman TA, Savage WJ, Northington FJ, Jennings JM, Everett AD, Graham EM. Glial fibrillary acidic protein as a biomarker for neonatal hypoxicischemic encephalopathy treated with whole-body cooling. Am J Obstet Gynecol. 2011 Sep; 205(3):251.e1-7. doi: 10.1016/j.ajog.2011.06.025.

06. Riikonen R, Kero P, Simell 0. Excitatory amino acids in cerebrospinal fluid in neonatal asphyxia. Pediatr Neurol. 1992 Jan-Feb;8(1):37-40. doi: 10.1016/0887- 8994(92)90050-9.

07. Hagberg H, Thornberg E, Blennow M, Kjellmer I, Lagercrantz H, Thiringer K, et al. Excitatory amino acids in the cerebrospinal fluid of asphyxiated infants: Relationship to hypoxic-ischemic encephalopathy. ActaPaediatr., 1993; 82: 925-9.

08. Ek M, Engblom D, Saha S, Blomqvist A, Jakobsson PJ, Ericsson-Dahlstrand A. Inflammatory response: pathway across the blood-brain barrier. Nature. 2001 Mar 22; 410(6827):430-1.

09. Herlenius E. An inflammatory pathway to apnea and autonomic dysregulation. Respir Physiol Neurobiol. 2011 Sep 30; 178(3):449-57. doi: 10.1016/j.resp.2011.06.026.

01. Hofstetter AO, Saha S, Siljehav V, Jakobsson PJ, Herlenius E. The induced prostaglandin E2 pathway is a key regulator of the respiratory response to infection and hypoxia in neonates. Proc Natl Acad Sci U S A. 2007. Jun 5; 104(23):9894-9.

02. Lars Bjerk, Kristin Leifsdottir, Sipra Saha, Eric Herlenius. PGE2 – metabolite levels in CSF correlate to HIE score and outcome after perinatal asphyxia. Acta Pædiatrica 2013 Nov 102(11); 1041–1047. DOI:10.1111/apa.12361.

03. Gunes T, Ozturk MA, Koklu SM, Narin N, Koklu E. Troponin-T levels in perinatally asphyxiated infants during the first 15 days of life. Acta Paediatr. 2005 Nov; 94(11):1638-43.

04. Perrone S, Bracci R, Buonocore G. New biomarkers of fetal-neonatal hypoxic stress. Acta. Paediatr Suppl 2002; 438:135-138.

05. S Perrone et al.post asphyxial reperfusion injury of the newborns. Pediatrics 1996; 98: 883–9

06. Buonocore G, Zani S, Sargentini I, Gioia D, Signorini C, Bracci R. Hypoxia-induced free iron release in the red cells of newborn infants. Acta Paediatr. 1998 Jan;87(1):77-81.

07. Buonocore G, Perrone S, Longini M, Terzuoli L, Bracci R. Total hydroperoxide and advanced oxidation protein products in preterm hypoxic babies. Pediatr Res. 2000 Feb; 47(2):221-4.

08. Buonocore G, Perrone S, Carlini M, Bagnoli F, Gatti MG,Paffetti P, et al. Non protein bound iron plasma levels in critically ill newborn. Biol Neonate 2001; 80: 93-4.

09. Bracci R, Perrone S, Buonocore G. Red blood cell involvement in fetal/neonatal hypoxia. Biol Neonate 2001; 79: 210–12.DOI:10.1159/000047093.

10. Douglas-Escobar M1, Yang C, Bennett J, Shuster J, Theriaque D, Leibovici A, Kays D, Zheng T, Rossignol C, Shaw G, Weiss MD. A pilot study of novel biomarkers in neonates with hypoxic-ischemic encephalopathy. Pediatr Res. 2010 Dec; 68(6):531-6. doi: 10.1203/PDR.0b013e3181f85a03.

11. D Sweetman, K Armstrong, JFA Murphy, EJ Molloy .Cardiac biomarkers in neonatal hypoxic ischaemia. Acta Pædiatrica. 2012 101, pp. 338–343. DOI:10.1111/j.1651- 2227.2011.02539.x

01. Costa S, Zecca E, De Rosa G, De Luca D, Barbato G, Pardeo M, Romagnoli C. Is serum troponin T a useful marker of myocardialdamage in newborn infants with perinatal asphyxia? Acta Paediatr. 2007 Feb; 96(2):181-4.

02. Boo NY, Hafidz H, Nawawi HM, Cheah FC, Fadzil YJ, Abdul Aziz BB, Ismail Z. Comparison of serum cardiac troponin T and creatine kinase MB isoenzyme mass concentrations in asphyxiated term infants during the first 48 hours of life. J Paediatr Child Health. 2005 Jul; 41(7):331-7.

03. Turker G, Babaoglu K, Duman C, Gokalp A, Zengin E, Arisoy AE. The effect of blood gas and Apgar score on cord blood cardiac Troponin I. J Matern Fetal Neonatal Med. 2004 Nov; 16(5):315-9.

04. Turker G, Babaoglu K, Gokalp AS, Sarper N, Zengin E, Arisoy AE. Cord blood cardiac Troponin I as an early predictor of short-term outcome in perinatal hypoxia. Biol Neonate. 2004; 86(2):131-7.

05. Trevisanuto D, Picco G, Golin R, Doglioni N, Altinier S, Zaninotto M, Zanardo V. Cardiac Troponin I in asphyxiated neonates. Biol Neonate. 2006; 89(3):190-3.

06. Kanik E, Ozer EA, Bakiler AR, Aydinlioglu H, Dorak C, Dogrusoz B, Kanik A, Yaprak I. Assessment of myocardial dysfunction in neonates with hypoxicischemic encephalopathy: is it a significant predictor of mortality? J Matern Fetal Neonatal Med. 2009 Mar; 22(3):239-42. doi: 10.1080/14767050802430834.

07. Kecskes Z, Dunster KR, Colditz PB. NSE and S100 after hypoxia in the newborn pig. Pediatr Res. 2005 Nov; 58(5):953-7.

08. Fujii EY, Kozuki M, Mu J, Ino Y, Ushioda N, Tomimatsu T, Fukuda H, Kanzaki T, Nakayama M, Murata Y. Correlation of neuron-specific enolase and S100B with histological cerebral damage in fetal sheep after severe asphyxia. Brain Res. 2004 Aug 20; 1018(1):136-40.

09. Anderson GW. Studies on the nucleated red cell count in the chorionic capillaries and the cord blood of various ages of pregnancy. Am J Obstet Gynecol 1942; 42: 1–14?

10. Ghosh B, Mittal S, Kumar

01. S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. Int J Gynaecol Obstet. 2003 Jun; 81(3):267-71.

02. J.P. Phelan, M.O. Ahn, L.M. Korst and G.I. Martin, Nucleated red blood cells: a marker for fetal asphyxia? Am J Obstet Gynecol. 1995 Nov; 173(5):1380-4.

03. U. Vatansever, B. Acunaş B, Demir M, Karasalihoglu S, Ekuklu G, Ener S, Pala O. Nucleated red blood cell counts and erythropoietin levels in high-risk neonates, Pediatr Int. 2002 Dec;44(6):590-5.

04. L.M. Korst, J.P. Phelan, M.O. Ahn and G.I. Martin, Nucleated red blood cells: an update on the marker for fetal asphyxia. Am J Obstet Gynecol. 1996 Oct; 175(4 Pt 1):843-6.

05. Walsh BH1, Boylan GB, Dempsey EM, Murray DM. Association of nucleated red blood cells and severity of encephalopathy in normothermic and hypothermic infants. Acta Paediatr. 2013 Feb; 102(2):e64-7. doi: 10.1111/apa.12086.

06. Walsh BH, Boylan GB, Murray DM. Nucleated red blood cells and early EEG: predicting Sarnat stage and two year outcome. Early Hum Dev. 2011 May; 87(5):335- 9. doi: 10.1016/j.earlhumdev.2011.01.041.

07. Haiju Z, Suyuan H, Xiufang F, Lu Y, Sun R. The combined detection of umbilical cord nucleated red blood cells and lactate: early prediction of neonatal hypoxic ischemic encephalopathy. J Perinat Med. 2008; 36(3):240- 7. doi: 10.1515/JPM.2008.035.

08. Ferber A, Fridel Z, Weissmann-Brenner A, Minior VK, Divon MY.Are elevated fetal nucleated red blood cell counts an indirect reflection of enhanced erythropoietin activity? Am J Obstet Gynecol. 2004 May; 190(5):1473-5.

09. G. Buonocore, S. Perrone, D. Gioia , Gatti MG, Massafra C, Agosta R, Bracci R. Nucleated red blood cell count at birth as an index of perinatal brain damage; Am J Obstet Gynecol. 1999 Dec;181(6):1500-5..

10. Sudhin Thayyil, Manigandan Chandrasekaran, Andrew Taylor, Alan Bainbridge, Ernest B. Cady, FInstP, W. K. Kling Chong, Shahed Murad, Rumana Z. Omar, Nicola J. Robertson. Cerebral Magnetic Resonance Biomarkers

01. in Neonatal Encephalopathy: A Metaanalysis.Pediatrics 2010 Jan; 125:e382–e395. doi: 10.1542/peds.2009-1046

02. H Barta, A Jermendy, M Kolossvary, G Rudas, M Szabo. Prognostic Performance of Proton of life in asphyxiated neonates. 10.1136/archdischild-2014- 307384.408

03. Gluckman P D, Williams C E. When and why do brain cells die? Dev Med Child Neurol. 1992 Nov; 34(11):1010-4.