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Research Article

Early Prediction of Hypoxic-Ischemic Brain Injury by a New Panel of Biomarkers in a Population of Term Newborns

Simona Negro,[1] Manon J. N. L. Benders,[2,3,4] Maria Luisa Tataranno,[2] Caterina Coviello,[5] Linda S. de Vries,[2,4] Frank van Bel,[2,4] Floris Groenendaal,[2,4] Mariangela Longini,[1] Fabrizio Proietti,[1] Elisa Belvisi,[1] Giuseppe Buonocore,[1] and Serafina Perrone[1]

[1]*Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy*
[2]*Department of Neonatology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, Netherlands*
[3]*Centre for the Developing Brain, King's College, London, UK*
[4]*Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, Netherlands*
[5]*Division of Neonatology, Careggi University Hospital of Florence, Firenze, Italy*

Correspondence should be addressed to Serafina Perrone; saraspv@yahoo.it

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This research paper is aimed at evaluating the predictive role of a default panel of oxidative stress (OS) biomarkers for the early identification of infants at high risk of HIE and their validation through the correlation with MRI findings. A multicenter prospective observational study was performed between March 2012 and April 2015 in two European tertiary NICUs. Eighty-four term infants at risk for HIE (pH < 7, BE < −13 mmol/L, and 5′ Apgar < 5) were enrolled. Three were excluded for chromosomal abnormalities and one due to lack of blood samples. The final population was divided according to the severity of perinatal hypoxia into 2 groups: mild/moderate HIE and severe HIE. Advanced oxidation protein products (AOPP), non-protein-bound iron (NPBI), and F2-isoprostanes (F2-IsoPs) were measured in blood samples at P1 (4–6 hours), P2 (24–72 hours), and P3 (5 days), in both groups. MRIs were scored for the severity of brain injury, using a modified Barkovich score. The mean GA was 39.8 weeks (SD 1.4) and the mean birth weight 3538 grams (SD 660); 37 were females and 43 males. Significantly lower 5′ Apgar score, pH, and BE and higher Thompson score were found in group II compared to group I at birth. Group II showed significantly higher AOPP and NPBI levels than group I (mean (SD) AOPP: 15.7 (15.5) versus 34.1 (39.2), $p = 0.033$; NPBI 1.1 (2.5) versus 3.9 (4.4), $p = 0.013$) soon after birth (P1). No differences were observed in OS biomarker levels between the two groups at P2 and P3. A regression model, including adjustment for hypothermia treatment, gender, and time after birth, showed that AOPP levels and male gender were both risk factors for higher brain damage scores (AOPP: OR 3.6, 95% CI (1.1–12.2) and gender: OR 5.6, 95% CI (1.2–25.7), resp.). Newborns with severe asphyxia showed higher OS than those with mild asphyxia at birth. AOPP are significantly associated with the severity of brain injury assessed by MRI, especially in males.

1. Introduction

Birth asphyxia is largely recognized as the most frequent cause of acute interruption of oxygen to the fetus and the most common cause of brain damage [1]. Currently, despite the advances offered by therapeutic hypothermia in terms of neuroprotection, the improvements on long-term neurological outcome remain modest [2–4]. Twenty to fifty percent of asphyxiated infants who develop HIE die in the neonatal period, and about twenty-five percent of survivors will develop neurological disabilities, such as cerebral palsy, cognitive deficits, learning disorders, sensory disruption, and neuropsychiatric problems [5]. Therefore, one of the most important goals in the approach to patients with HIE remains actually to determine the exact period in which the effects of potential damaging factors occur [1, 2, 5, 6]. Several methods are now available for detecting the type and timing of brain damage: conventional prenatal tests, such as fetal

cardiotocography; ultrasound; Doppler and amniotic fluid examination neuroimaging; aEEG; NIRS; and determination of numerous currently available biomarkers. Each provides information about different expressions of brain injury and has some limitations [1, 7]. MRI is the gold standard for the early evaluation of brain injury after HIE, including not only traditional neuroimaging methods but also advanced imaging techniques (DWI, ^{1}H-MRS, and ASL) [8–11]. In this context, the use of specific biomarkers that will increase within the first hours of life in hypoxic-ischemic neonates may help in the early diagnosis of HIE and promptly identify neonates who may qualify for neuroprotection. Oxidative stress is involved in the mechanisms of hypoxic-ischemic and inflammatory brain injury, although the relationship between brain damage and OS is very complex and not entirely clear [12–15]. The pathophysiological process that leads to the development of brain lesions is in fact characterized by the combination of several mechanisms, either exogenous or endogenous (hypoxia, ischemia, ischemia-reperfusion, hyperoxia, inflammation, and mitochondrial damage), whose effect on cell biology and on oxidative metabolism varies according to the severity and duration of the insult [16]. Furthermore, certain brain areas are particularly rich in iron, released by cells damaged during hypoxia, which may catalyze, through the Fenton reaction, the formation of hydroxyl radicals and nitroperoxide and so make the central nervous system more susceptible to the attack of the reactive species [17]. In addition, the brain of a full-term baby, being rich in polyunsaturated fatty acids and low in antioxidants, is particularly vulnerable to the free radical attack [18]. Increased oxidative stress in hypoxic fetuses and neonates has been detected by assaying several biomarkers: products of lipid peroxidation in expired air, serum malondialdehyde reaction, serum isoprostanes, serum total hydroperoxides, advanced oxidative protein products, and increased NPBI in serum [18–20]. Despite extensive research in the field over the last few years, no such biomarker has been validated in clinical practice so far. So the aim of our study was to evaluate the predictive role of a default panel of OS biomarkers for the early identification of infants at high risk of hypoxic-ischemic brain injury and their validation through the correlation with MRI.

2. Methods

2.1. Subjects. Eighty-four term subjects, born between March 2012 and April 2015, with clinical and biochemical signs of HIE, admitted to two European tertiary NICUs as part of a multicenter prospective observational study, were consecutively enrolled. The inclusion criteria were the presence of perinatal asphyxia defined as at least three of the following criteria: (1) late decelerations on fetal monitoring or meconium staining; (2) delayed onset of respiration, resuscitation, or ventilation of at least 10 min; (3) Apgar scores < 5 at 5 minutes; (4) arterial cord blood pH < 7.1 with a base deficit > 16 mmol/L or serum lactate > 10 mmol/L; (5) multiorgan failure, followed by symptoms of encephalopathy, such as altered alertness, abnormal tone, feeding difficulties, or seizures demonstrated by a Thompson score ≥ 7 and/or abnormal brain activity by aEEG; and (6) hypothermia treatment started within 6 h after birth [4, 7, 21]. HIE was classified as mild (grade I), moderate (grade II), or severe (grade III) according to the criteria described by H.B. Sarnat and M.S. Sarnat [22]. The clinical evaluation of encephalopathy took place 24 and 48 hours after birth. Babies with major congenital abnormalities, brain malformations, central nervous system infections, and inborn errors of metabolism were excluded. As soon as possible after admission to the Neonatal Intensive Care, all enrolled children were subjected to the routine checks, including a blood gas analysis, and were started on aEEG and NIRS monitoring. Hypothermia was initiated within 6 h after birth, lasted for 72 h, and was aimed for a rectally measured body temperature of 33.5°C. Seventy-two hours after starting hypothermia, subsequent rewarming at 0.5°C per hour was performed. Body temperature (°C), heart rate, arterial blood pressure, and arterial oxygen saturation (SaO_2) were monitored simultaneously with NIRS and aEEG parameters, and their recorded values were all stored on a personal computer for off-line analysis (software: Poly 5, Inspektor Research Systems, Amsterdam, the Netherlands). All clinical and demographic data were collected from the hospital records. The study was approved by the medical ethical review board of the two respective university hospitals, with written informed parental consent, obtained according to the Declaration of Helsinki.

Table 1: Scoring system for brain injury seen on MRI scans.

Score	Description
Basal ganglia and thalamus	
0	Normal
1	Abnormal signal in the thalamus
2	Abnormal signal in the thalamus and lentiform nucleus
3	Abnormal signal in the thalamus, lentiform nucleus, and perirolandic cortex
4	More extensive involvement
Watershed areas	
0	Normal
1	Single focal infarction
2	Abnormal signal in the anterior or posterior watershed white matter
3	Abnormal signal in the anterior or posterior watershed cortex and white matter
4	Abnormal signal in both anterior and posterior watershed zones
5	More extensive cortical involvement
Posterior limb of the internal capsule	
0	Myelination present
1	Myelination present but impaired
2	Myelination absent

2.2. Oxidative Stress Methodology. Advanced oxidation protein products (AOPP), F2-isoprostanes (F2-IsoPs), and non-protein-bound iron (NPBI) were all measured in blood samples, taken during routine tests and only after obtaining

Table 2: Clinical and biochemical signs of hypoxic-ischemic encephalopathy reported by groups.

	Mild-to-moderate HIE group ($n = 21$)	Severe HIE group ($n = 59$)	p
Median Apgar 1 min (IR)	2 (1–4)	1 (0–2)	NS
Median Apgar 5 min (IR)	4 (4-5)	3 (1–5)	0.002
Median Apgar 10 min (IR)	7 (5–7)	6 (3–7)	0.011
Mean umbilical pH (SD)	7.0 (0.1)	7.0 (0.2)	NS
Mean umbilical BE (mmol/L) (SD)	−13.7 (6.2)	−13.8 (8.3)	NS
Mean pH at admission (15 min–6 h of life) (SD)	7.1 (0.1)	6.9 (0.2)	0.043
Mean BE at admission (15 min–6 h of life, mmol/L) (SD)	−11.1 (6.7)	−16.3 (7.7)	0.013
Mean lactate at admission (15 min–6 h of life, mmol/L) (SD)	13.6 (5.2)	15.4 (7.8)	NS
Mean PNA at MRI (days) (SD)	3 (2)	6 (4)	NS
Median Thompson score (1 h of life) (IR)	4 (2–5)	9 (7–13)	0.000
Seizures n (%)	2 (9)	27 (54)	0.001

IR: interquartile range; SD: standard deviation. NS: not statistically significant.

the parents' written consent, at P1 (4–6 h after birth), P2 (24–72 h after birth), and P3 (5 days after birth). For each blood sample, 2.5 ml of blood was collected: 1.3 ml in EDTA (ethylenediaminetetraacetic acid) tubes and 1.2 ml in two test tubes (0.6 ml each) containing heparin. Each of these samples was immediately centrifuged (Prog 1, RTM 1500, T 4°C, 10 min) to remove cells and obtain the supernatant, which was then separated into five different microtest tubes, one of which contains BHT (butylated hydroxytoluene), and stored at −80°C. The obtained samples were subsequently analyzed to measure OS biomarkers. AOPP and F2-IsoPs were detected as markers of protein and lipid OS-induced injury, respectively, by the method of Witko-Sarsat et al., using spectrophotometry on a microplate reader, and isoprostanes were detected according to the LC-MS/MS methodology described by Casetta et al. [23, 24]. The AOPP were calibrated with chloramine-T solutions that absorb at 340 nm in the presence of potassium iodide. In test wells, 200 μL of plasma diluted at 1 : 5 in phosphate-buffered saline solution (PBS) was distributed on a 96-well microtiter plate, and 20 μL of acetic acid was added. In standard wells, 10 microliters of 1.16 M potassium iodide was added to 200 μL of chloramine-T solutions followed by 20 μL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm on the microplate reader against a blank containing 200 μL of PBS, 10 μL of potassium iodide, and 20 μL of acetic acid. Because the absorbance of chloramine-T at 340 nm is linear up to 100 μmol/L, AOPP were expressed as μmol/L of chloramine-T equivalents. NPBI was detected as a marker of OS potential risk, by HPLC using the method described by Paffetti et al. [25].

2.3. MRI Scoring. Depending on their clinical condition, infants underwent MRI at a postnatal age of 5 ± 3 days. Intravenous sedation was continued during the MR examination for infants who had an intravenous line placed; others received an oral sedation with chloral hydrate (50–60 mg/kg). Infants were wrapped into a vacuum cushion to minimize motion, and earmuffs (EM's 4 Kids, Everton Park, Australia) were used for hearing protection. Respiratory rate (Philips Medical Systems, Best, the Netherlands), heart rate, and transcutaneous oxygen saturation (Nonin Pulse Oxymetry, Nonin Medical, Plymouth, MN) were monitored during MR imaging, and a neonatologist was present throughout the examination. The severity of brain injury was assessed by using conventional axial T1- and T2-weighted spin-echo sequences, DWI, and ADC maps. MRIs were reviewed retrospectively by two expert investigators (LV and FG) who were blinded to the infant's outcome. Injury was scored for the basal ganglia and thalami in combination with cortical involvement, the watershed areas, and the posterior limb of the internal capsule, by using the modified Barkovich score (Table 1), ranging from 0 (no damage) to 11 (massive brain damage), described previously as being predictive for neurodevelopmental outcomes after HIE [21, 22].

2.4. Statistical Analysis. Descriptive and inferential analyses were performed using the SPSS v23 for Windows statistical package (SPSS Inc., Chicago, IL, USA). Data are presented as mean and SD or median and interquartile range (IR) for descriptive analysis of continuous variables, whereas for categorical variables, the absolute frequencies are reported. A logarithmic transformation was performed for the variables that were not parametrically distributed. The independent t-test and the Mann–Whitney U test were used, where appropriate, to make comparisons regarding all patient characteristics, OS biomarker measurements at each time point, and gender differences. Pearson correlation and scatter dot plot were used, respectively, to examine and visualize the relationship between OS biomarkers and MRI score for each period of interest. A longitudinal model was built to analyze the association between OS biomarkers and the brain damage measured through the Barkovich score. Gender, treatment with hypothermia, and time of life (corresponding to the selected periods of blood sample collection) were introduced into the model as confounding factors. ROC curve was performed to find an MRI score cut-off able to discriminate between newborns with a major risk to die and newborns with a good outcome. A multivariable logistic regression model was then built to verify if the increased level of OS biomarkers may be a risk factor for

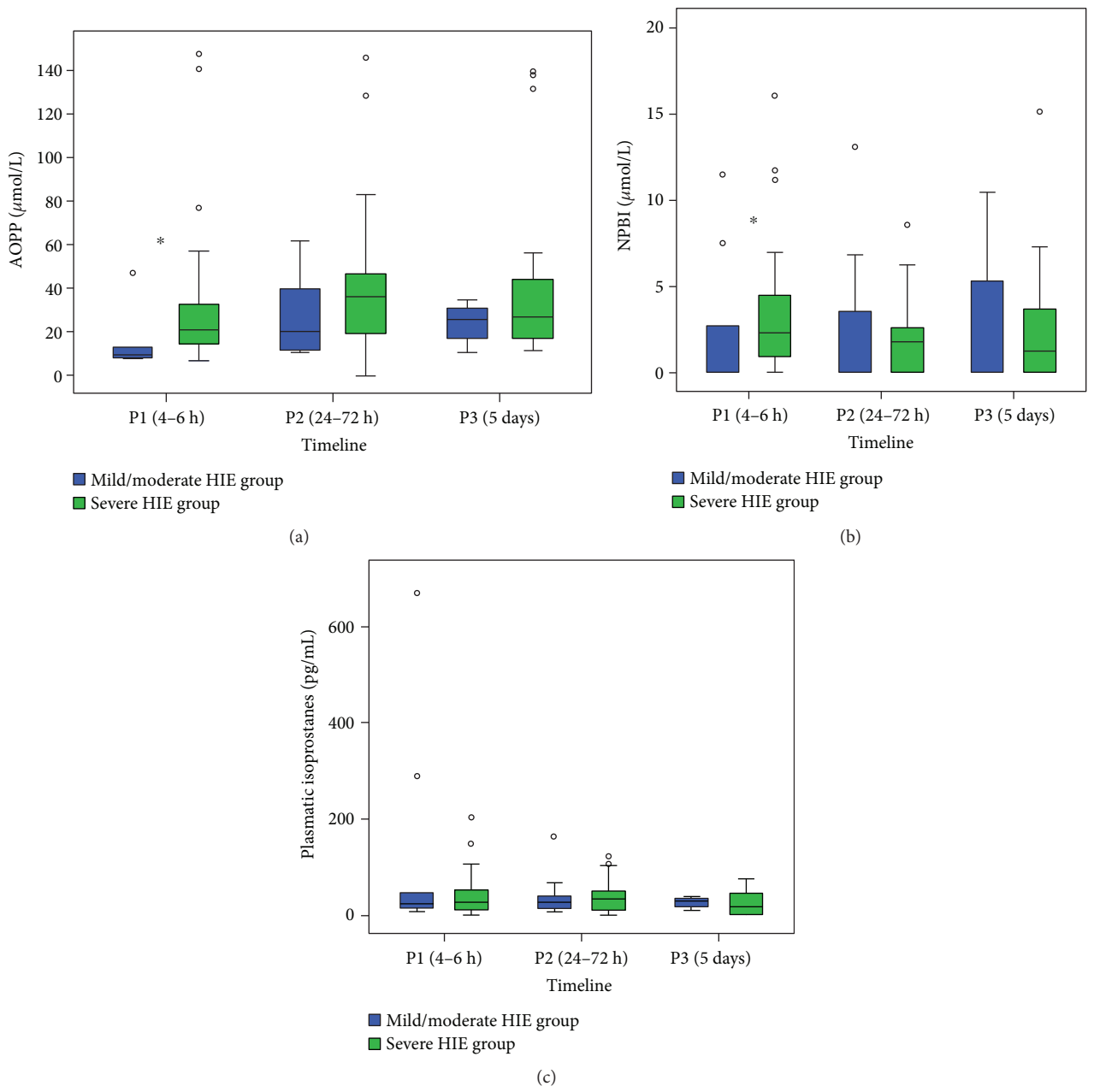

Figure 1: Relationship between OS biomarkers and the grade of perinatal hypoxia reported over time. Comparison of AOPP levels (μmol/L) (a), NPBI levels (μmol/L) (b), and IsoP levels (pg/mL) (c), between each group in the first 5 days of life. AOPP: advanced oxidation protein products; NPBI: non-protein-bound iron; ○: outliers. $^{*}p < 0.05$.

neurological damage, measured using MRI. The MRI score was introduced into the model as a dichotomic dependent variable, using the ROC curve cut-off; gender, treatment with hypothermia, and time of life were also considered in the model as covariates together with each biomarker. A p value < 0.05 was considered statistically significant.

3. Results

Out of eighty-four enrolled patients, three were excluded for chromosomal abnormalities and one due to lack of blood samples. So the final population consisted of eighty infants with a mean gestational age of 39.8 weeks (SD 1.4) and a mean birth weight of 3538 grams (SD 660); 37 were females and 43 males. Twenty newborns at risk for HIE were classified with mild (Sarnat I), 1 was classified with moderate (Sarnat II), and 59 were classified with severe signs of HIE (Sarnat III). The mild group was not considered for hypothermia, while the severe group was considered eligible for hypothermia. The moderate one was eligible for hypothermia treatment; however, he was born in a peripheral hospital and arrived too late (thus later than 6 hours after birth) to perform it. Newborns were then divided according to the severity of HIE into two groups: mild/moderate HIE (not eligible/late for the treatment with hypothermia, $n = 21$) and severe HIE (eligible for hypothermia treatment, $n = 59$).

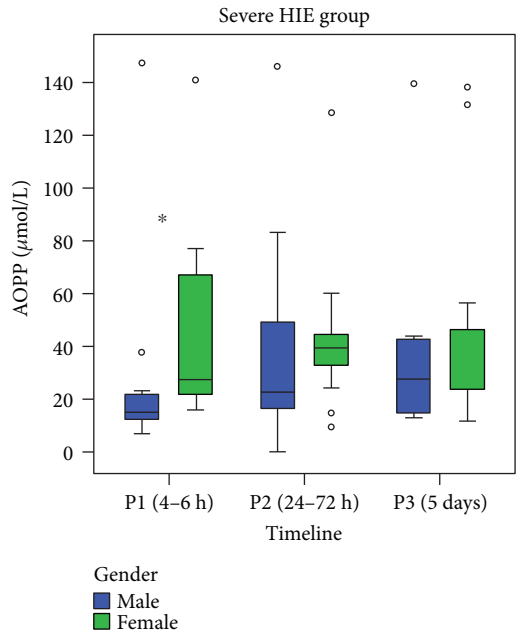

Figure 2: Relationship between AOPP levels (μmol/L) and the grade of perinatal hypoxia over time, reported by gender. AOPP: advanced oxidation protein products; ∘: outliers. $^{*}p < 0.05$.

Clinical and biochemical signs of HIE for each group are reported in Table 2. None of the infants of the mild group showed signs of progression to moderate or severe HIE.

3.1. Relationship between OS Biomarkers and the Grade of HIE—Comparison between the Two Groups over Time. The mild/moderate HIE group showed significantly lower AOPP and NPBI levels than the severe HIE group at time P1 (AOPP median (IR): 9.4 (13.6) versus 18.6 (19.5) μmol/L, $p = 0.033$ and NPBI median (IR): 0.0 (3.8) versus 2.4 (3.3) μmol/L, $p = 0.013$, resp.) (Figures 1(a) and 1(b)). No differences were observed in AOPP and NPBI levels in P2 and P3 (Figures 1(a) and 1(b)). No other differences were observed in F2-IsoP levels between the two groups (Figure 1(c)).

The severe HIE group showed also significantly lower AOPP levels in males than in females at time P1 (AOPP median (IR): 14.8 (10) versus 27.6 (50.7) μmol/L, $p = 0.013$; Figure 2). No differences in AOPP levels were observed between males and females in P2 and P3. No differences in NPBI and IsoP levels were found between males and females at any time.

3.2. MRI Score and Survival. Out of eighty enrolled patients, fifty-six (70%) underwent MRI at a postnatal age of 5 ± 3 days: respectively, 5 of 21 (23%) of the mild/moderate asphyxia group and 51 of 59 (86%) of the severe asphyxia group, according to their clinical condition. Each MRI score and the corresponding outcome are reported in Table 3.

The ROC curve discriminating newborns with a major risk to die versus newborns surviving without impairments was set at an MRI score value of 4.5 (100% sensitivity, 97.7% specificity, $p = 0.0001$; Figure 3). The MRI score ranging from 0 to 4.5 showed 100% sensitivity and 100% of true negative fraction for a good neurological outcome. Conversely, 100% of poor neurodevelopmental outcome was observed for MRI score values > 8.5. The MRI score plotted curve indicated 4.5 as the best predictive threshold with a sensitivity of 100% (95% CI 63.06–100) and a specificity of 97.7% (95% CI 88.2–99.9).

3.3. Relationship between OS Biomarkers and the Severity of Brain Injury Assessed with MRI. The longitudinal multivariable model, adjusted for confounding factors, showed a significant independent association between AOPP levels (ln transformation) and MRI scores ($p = 0.006$, $B = 1.301$, CI 95% 0.38–2.22; Figure 4(a)), indicating that the increase of protein peroxidation levels during the first five days of life is associated with severe brain damage. In particular, males showed an increased risk of oxidative neurological damage, as comes to light from the significant correlation between AOPP and brain damage in males ($p = 0.005$, $r = 0.465$; Figure 4(b)). Using the ROC curve cut-off, a multivariable logistic regression model was performed, taking the MRI score as a dichotomic dependent variable. The last model confirmed that AOPP levels and male gender were both risk factors for more severe brain damage (AOPP: OR = 3.6, 95% CI 1.1–12.2 and gender: OR = 5.6, 95% CI 1.2–25.7, resp.). In detail, the increase of an AOPP logarithmic scale of 1 increases 3.6 times the risk of brain damage. As for the other two biomarkers, both regression models showed a negative association between the levels of NPBI (ln) and MRI score (linear model: $B = -0.94$, 95% CI -1.69 to -0.18; logistic model: OR = 0.20, 95% CI 0.06–0.71). No statistically significant association was found between the plasma levels of IsoPs and MRI score.

4. Discussion

Early objective diagnosis of brain injury is important for prognostication and decision-making in term newborns with HIE. Our study illustrates that infants who have suffered from severe birth asphyxia show increased OS levels compared with those who have had mild or moderate asphyxia. In particular, the increase of OS levels in the perinatal period was highlighted by a higher accumulation of plasmatic levels of AOPP and the index of membrane protein oxidative damage. The high levels of AOPP were significantly associated with an increase in brain damage, quantified with MRI. In our study, males, despite lower levels of OS at birth, were at the greatest risk of developing brain injury, showing an increased susceptibility to oxidative stress damage. The newborn brain is particularly susceptible to oxidative damage, both for the reduced antioxidant capacity and for the high consumption of oxygen and the high concentration of lipids and chemically reactive species, such as free iron [26, 27]. Free radicals, which are continuously produced in the course of the common cellular metabolic processes, greatly increase, especially at the cerebral level, after a hypoxic-ischemic event. Asphyxia and acidosis result in fact in the release of free iron in plasma, and free iron itself is responsible for further free radical formation, such as isoprostanes and AOPP. The neonatal brain, being particularly rich in polyunsaturated fatty

Table 3: MRI scores and outcome reported by groups.

Group	MRI score	Outcome
Mild/Moderate HIE		
1	7	Survived
2	1	Survived
3	1	Survived
4	0	Survived
5	0	Survived
Severe HIE		
1	11	Died
2	11	Died
3	10	Died
4	7	Survived
5	6	Died
6	5	Died
7	5	Died
8	5	Died
9	5	Died
10	5	Died
11	4	Survived
12	4	Survived
13	3	Survived
14	3	Survived
15	1	Survived
16	1	Survived
17	1	Survived
18	1	Survived
19	1	Survived
20	1	Survived
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41	0	Survived
42	0	Survived
43	0	Survived
44	0	Survived
45	0	Survived
46	0	Survived
47	0	Survived
48	0	Survived
49	0	Survived
50	0	Survived
51	0	Survived

acids, is particularly vulnerable to lipid peroxidation during asphyxia, making the immature myelin particularly susceptible to free radical attack [28]. The OS derived from the overproduction of free radicals dominates the lack of antioxidant mechanisms, including reduced activity of superoxide dismutase, catalase, and glutathione peroxidase (GPX), and it was thus involved in the pathogenesis of hypoxic-ischemic injury [29–31]. The increased production of GPX detected in the cerebrospinal fluid of neonates with HIE by Vasiljević et al. suggests that this is an active mechanism of response to oxidative stress induced by hypoxia-ischemia. Moreover, the same authors found that increased GPX activity correlates with the severity of the insult and hypoxic brain damage [31]. After HIE, the increase in OS occurs especially during the reperfusion and reoxygenation phase. In this phase, the free iron release and the activation of some prooxidant enzymes (such as the nitric oxide synthese, cyclooxygenase, lipooxygenase, and xanthine oxidase) determine a chain reaction, culminating in the production of free radicals, such as the highly toxic peroxynitrite, which cause cell damage through the peroxidation of membrane lipids and damage to DNA and proteins [32, 33]. The cascade activation of all these mechanisms leads to endothelial damage, with increased capillary permeability and therefore cytotoxic edema; soon after, the activation of caspases 3 and 9 causes the activation of apoptotic mechanisms [28, 29, 32, 34–36]. The higher the plasma AOPP, end products of protein peroxidation, and thus index of free radical damage, the more severe was the perinatal hypoxia-ischemia, confirming what has so far reported in the literature and emphasizing how proteins are the first target of toxic action of free radicals. An increase of carbonyl groups and expression of protein oxidation has been widely demonstrated and observed from the earliest 3 hours after the advent of hypoxia-ischemia [18, 37, 38]. The altered protein molecules act as a trap for free radicals, which further start chain reactions, worsening the damage. Often, this type of reactions is catalyzed by transition metals such as iron and copper, which enter the Fenton reaction [39]. Iron, in particular, is released from ferritin, although it may also derive from transferrin, hemoglobin, myoglobin, lactoferrin, and cytochromes. During hypoxia and subsequent reoxygenation, iron is moved from storage

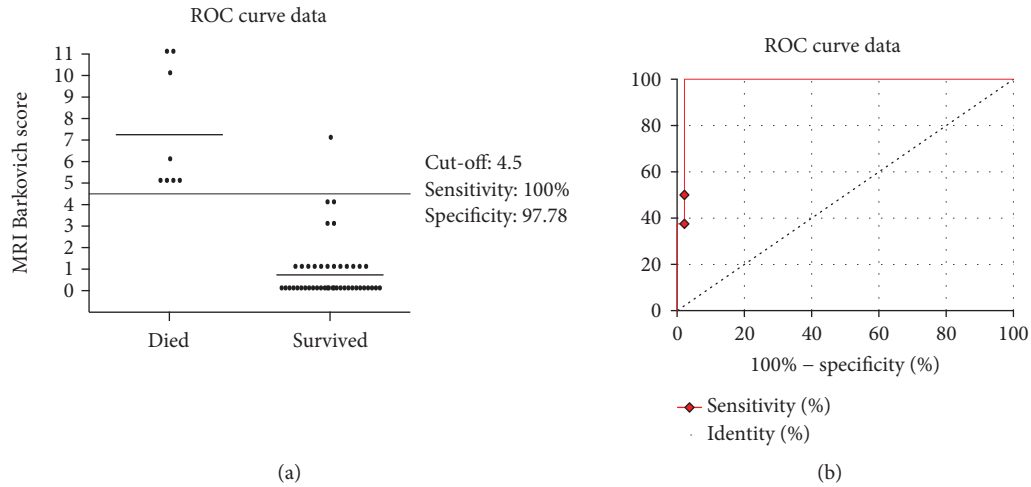

Figure 3: ROC curve analysis for the MRI score. The MRI score plotted curve indicated 4.5 as the best predictive threshold with a sensitivity of 100% (95% CI 63.06–100) and a specificity of 97.7% (95% CI 88.2–99.9). ROC curve discriminate newborns with a major risk to die from newborns surviving without impairments.

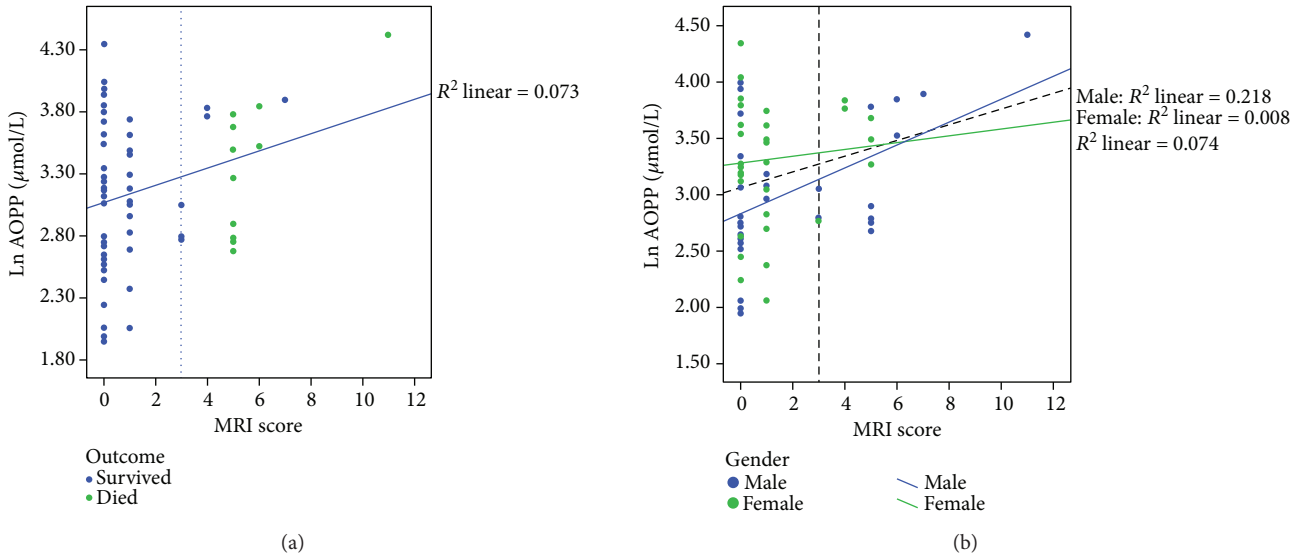

Figure 4: Scatter dot plots illustrate the relationship between the AOPP level (ln scale) and MRI score, reported, respectively, by short-term outcome (a) and by gender (b).

sites and converted to a form detectable in the plasma and extracellular space, so called "free iron" or NPBI. The increase in NPBI levels exposes proteins and membrane lipids to the attack of free radicals and thus to oxidative damage [37, 40]. In the literature, several studies have reported the role of free iron in the post anoxic oxidative damage. Ciccoli et al. have shown that erythrocytes of hypoxic infants released more iron than those of normoxic adults [41]. Buonocore et al. reported that the plasma level of NPBI is highly predictive of intrauterine suffering and brain damage [42]. In our study, NPBI levels were significantly higher in infants with severe perinatal asphyxia in the first six hours of life, while free iron plasma levels in this group of infants showed a downward trend, probably linked to the effect of hypothermia. Numerous studies in fact have shown that therapeutic hypothermia is effective not only in reducing brain damage and improving long-term outcomes but also in reducing levels of reactive oxygen species responsible for oxidative damage [38, 43]. As overproduction of free iron is known to be a consequence of hypoxia-induced acidosis, we hypothesized that the recovery of cellular metabolism during hypothermia will lead to a reduction of triggers of free iron release; meanwhile, AOPP and isoprostanes and expression of oxidative cellular damage persist to be increased in plasma.

Despite the significant difference highlighted in the levels of AOPP between boys and girls at birth, our study confirms what is already well known that there is a higher male susceptibility to brain oxidative damage. A recent European analysis on 4500 children with cerebral palsy revealed in fact that incidence of cerebral palsy is 30 times higher in males than in

females [44]. These gender differences in the immature brain seem to be related to intrinsic chromosomal and hormonal differences. The protective role of estrogen against OS and thus the fact that most males are susceptible to oxidative damage have been reported by numerous studies [45–47]. Giordano et al. demonstrated in an animal model that estrogens modulate the cerebral expression of some antioxidant enzymes (paraoxonase 1 and 2 (PON1 and PON2)), increasing in this way the resistance of female neurons to oxidative damage [46]. Furthermore, although the neuroprotective effect of estrogen is well known, the absence of the protective effect of estradiol in some cells of PON2-knockout mice ($PON2^{-/-}$) suggests that other mechanisms of neuroprotection related to female hormones may come into action against free radical damage [45, 48]. The presence of differences in the OS levels between males and females and their relationships with the hormonal status have also been emphasized by another study by Minghetti et al, in which males have a higher lipid peroxidation and reduced antioxidant capacity than females, contributing to the concept of male disadvantage in respect of the damage caused by free radicals [47]. Despite the advent of new studies and research projects, the exact mechanisms producing these gender differences are still largely unknown.

The role of oxidative stress in newborn morbidity with respect to the increased risk of free radical damage in these babies is growing. However, challenges remain in the early identification of infants at risk for neonatal encephalopathy, determination of timing and extent of hypoxic-ischemic brain injury, and optimal management and treatment [49]. Despite ongoing limits such as the need of a specific kit and specific instruments to measure oxidative stress biomarkers consequently associated with the need of an expert laboratory team and the high cost, making the process of reaching the goal slower, researchers are currently working to develop a biomarker panel, which can become useful to the clinicians as a point of care.

Literature on brain imaging of asphyxiated newborns typically uses MRI standard imaging techniques, performed following hypothermia at the end of the first week of life, to define the presence and extent of brain injury in these newborns and to provide a prognosis for subsequent neurological impairment [50]. DWI allows recognition of structural brain abnormalities during the first week, which only become clearly visible on conventional imaging by days 7–10 of life [51, 52].

The presence of an association between biomarkers of oxidative stress, measured in the first hours of life, and brain damage successfully evaluated through neuroimaging emphasizes the possibility of early identification of newborns at greater risk of brain damage and also underlines the validity of the AOPP, as products of OS damage in the plasma and therefore as biomarkers of neuronal damage. Knowing also that, after a hypoxic-ischemic insult, cellular damage on energy substrates continues to evolve over the first 12–48 hours, we suggest that the introduction of new neuroprotective strategies and antioxidants in such an early stage of life could change the long-term outcome of these infants.

Abbreviations

ADC: Apparent diffusion coefficient
aEEG: Amplitude integrated electroencephalography
AOPP: Advanced oxidation protein products
ASL: Arterial spin labeling
DWI: Diffusion-weighted imaging
F2-IsoPs: F2-isoprostanes
GPX: Glutathione peroxidase
HIE: Hypoxic-ischemic encephalopathy
^{1}H-MRS: Hydrogen magnetic resonance spectroscopy
HPLC: High-performance liquid chromatography
LC-MS/MS: Liquid chromatography tandem mass spectrometry
NIRS: Near-infrared spectroscopy
NPBI: Non-protein-bound iron
OS: Oxidative stress
PON1: Paraoxonase 1
PON2: Paraoxonase 2.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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