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Research Paper

Plasma metabolite score correlates with Hypoxia time in a newly born piglet model for asphyxia

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ABSTRACT

Hypoxic-ischemic encephalopathy (HIE) secondary to perinatal asphyxia is a leading cause of mortality and acquired long-term neurologic co-morbidities in the neonate. The most successful intervention for the treatment of moderate to severe HIE is moderate whole body hypothermia initiated within 6 h from birth. The objective and prompt identification of infants who are at risk of developing moderate to severe HIE in the critical first hours still remains a challenge. This work proposes a metabolite score calculated based on the relative intensities of three metabolites (choline, 6,8-dihydroxypurine and hypoxanthine) that showed maximum correlation with hypoxia time in a consolidated piglet model for neonatal hypoxia-ischemia. The metabolite score's performance as a biomarker for perinatal hypoxia and its usefulness for clinical grading and decision making have been assessed and compared to the performance of lactate which is currently considered the gold standard. For plasma samples withdrawn before and directly after a hypoxic insult, the metabolite score performed similar to lactate. However, it provided an enhanced predictive capacity at 2 h after resuscitation. The present study evidences the usefulness of the metabolite score for improving the early assessment of the severity of the hypoxic insult based on serial determinations in a minimally invasive biofluid. The applicability of the metabolite score for clinical diagnosis and patient stratification for hypothermia treatment has to be confirmed in multicenter trials involving newborns suffering from HIE.

1. Introduction

Perinatal asphyxia is characterized by intermittent periods of hypoxia ischemia that, if prolonged and intense enough, may cause irreversible damage to oxy-regulatory tissues such as brain [1]. The resulting hypoxic-ischemic injury evolves over time. Hence, the primary phase corresponding to tissue hypoxia is followed by a partial recovery upon reoxygenation/reperfusion (secondary phase). Along both these periods a precise sequence of pathophysiologic events leading to specific injuries is set in motion [2,3]. Hypoxic-ischemic encephalopathy (HIE) secondary to perinatal asphyxia is a leading cause of mortality and acquired long-term neurologic co-morbidities in both, the late preterm and term neonate with its overall incidence varying notably [4].

Clinical management of HIE patients is strongly affected by the perceived prognosis. To date, moderate whole body hypothermia is, together with air resuscitation, the most successful intervention for the treatment of moderate to severe HIE. Yet, the therapeutic window for initiating treatment is limited to 6 h from birth [4]. To make matters worse, the clinical severity of HIE varies over time after the insult, hampering an accurate assessment for diagnosis especially in the first hours after birth [1]. Currently, the diagnosis of an asphyctic process that evolves to HIE relies on prenatal clinical information (sentinel events), postnatal clinical evaluation including serial Apgar scores and neurological assessment, and cord blood gas analysis reflecting increased lactate levels and metabolic acidosis [5]. At a later time point amplitude-integrated (aEEG) or multichannel electroencephalography (mchEEG) and brain magnetic resonance imaging (MRI) further

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confirm the diagnosis and the degree of severity [1,2].

Metabolomic analysis of biofluids and tissues is becoming an increasingly popular field of research in neonatal medicine [6]. To date, literature reports a very limited number of studies on HIE involving human subjects. In a targeted LC-MS approach Walsh et al. [7] found changes in umbilical cord serum levels of acylcarnitines, glycerophospholipids and aminoacids in newborns with HIE. Reinke et al. studied umbilical cord serum from newborns suffering from asphyxia and HIE employing NMR [8] and established a correlation of their findings with clinical outcomes at 3 years of life in the same cohort [9].

Animal studies seeking for novel biomarkers capable of providing improved diagnostic power have been carried out [10,11]. Changes in retina and choroid tissues of piglets during hypoxia were studied [12,13]. With the aim of discovering early biomarkers, Solberg et al. [13] performed an untargeted metabolomic study in retinal tissue samples from a piglet model of perinatal asphyxia. After the hypoxic insult, elevated levels of the limiting intermediate compound in the major pathway of phosphatidyl-choline biosynthesis [14] (i.e. CDP-choline) were found with its concentrations correlating with the duration of retinal hypoxia. Supported by the observations in neuronal tissue, follow-up studies in minimal-invasively obtained biofluids were carried out revealing a set of 21 metabolites which showed significant changes in a liquid chromatography-time-of-flight-mass spectrometry (LC-TOF-MS) untargeted metabolomics study on plasma samples from piglets subjected to hypoxia and reoxygenation in comparison to a non-asphyxiated control group [15].

In this context, the present work proposes a metabolite score as an estimate of the duration and intensity of hypoxia based on LC-TOF-MS data from an untargeted metabolomics study on plasma samples from piglets subjected to hypoxia and reoxygenation conducted previously [15]. The metabolite score involves plasma metabolites that showed maximum correlation with the duration of hypoxia in a piglet model. With the introduction of the metabolite score we strive after a tool for a user-independent, accurate grading thereby aiding to stratify newborns suffering from HIE who are most likely to benefit from early, moderate therapeutic hypothermia and/or predicting outcomes.

2. Material and methods

2.1. Piglet model for neonatal hypoxia-ischemia

The animal study was carried out at Oslo University Hospital (Norway) and the Norwegian Council for Animal Research approved the experimental protocol (approval number 3399). Animals were cared for and handled in accordance with the European Guidelines for Use of Experimental Animals by scientists certified by the Federation of European Laboratory Animals Science Association.

Fig. 1 illustrates the experimental study design. For details on the animal experiment, the reader is referred to Solberg et al. [15]. In short, 32 newborn Noroc (LyxLD) pigs aged between 12 h and 36 h, with hemoglobin (Hb) levels > 5 g/dL and in good general conditions were included in the study. Piglets were orally anesthetized, intubated, ventilated and surgically prepared [16]. After 60 min of stabilization, the piglets were randomized either to the intervention group (n=26) or the control group (n=6). Hypoxemia and subsequently hypoxia-ischemia were achieved by ventilation with a gas mixture of 8% O_2 in N_2 until either the mean arterial blood pressure (MABP) decreased to < 20 mm Hg or the base excess (BE) reached -20 mM L^{-1}. CO_2 was added during hypoxemia aiming at a $PaCO_2$ of 8.0–9.5 kPa (60–71.3 mmHg) in order to imitate perinatal asphyxia. After 30 min of reoxygenation, all animals were observed for 9 h receiving room air with continuous surveillance of blood pressure, saturation, pulse, temperature, blood gas measurements and lactate in whole blood samples taken directly from the arterial line and automatically drawn into an ABL 800 FLEX (Radiometer, Copenhagen, Denmark). At the end of the observation time, the animals were given an overdose of pentobarbital (150 mg/kg IV). The control group underwent the same procedures (i.e. anesthesia, surgery, ventilation and sample collection) and observation times, but was not exposed to hypoxia and reoxygenation.

2.2. Plasma sample collection, preparation and LC-TOFMS analysis

Blood samples were taken in ethylene-diamine-tetraacetic acid Vacutainer blood collection tubes before start of hypoxia (t_0), at the end of hypoxia (t_1) and 120 min after end of hypoxia (t_2) and at the corresponding time points for the control group (see Fig. 1). Plasma was obtained immediately after sampling by centrifugation of the whole blood samples at 2000*g* for 10 min at 4 °C. Plasma samples were stored at −80 °C until analysis.

After thawing plasma samples on ice, 150 µL of cold (4 °C) acetonitrile were added to 50 µL of plasma, followed by homogenizing on a Vortex mixer. Samples were centrifuged at a speed of 10000*g* at 4 °C during 10 min 25 µL of supernatant were added to 100 µL of IS solution (5 µM Phe-D_5 and 10 µM Meth-D_3 in H_2O, 0.1% v/v formic acid). 100 µL aliquots of sample extracts were transferred into 200 µL capped glass vials and placed in the refrigerated auto-sampler compartment.

Metabolomic profiling of the plasma extracts was performed on a 1200 RRLC Series Agilent chromatograph (Palo Alto, CA., USA) using a Zorbax SB-C8 (3×150 mm, 3.5 µm, Agilent) column coupled to a 5600-TripleTOF MS spectrometer (ABSciex, Framingham, MA, USA) operating in the positive ionization mode (ESI^+). Peak tables were generated employing the XCMS software [17]. Detailed information on the LC-TOFMS metabolic profiling can be found elsewhere [15].

2.3. Data analysis

Data analysis was carried out in Matlab 2015a (The Mathworks, Natick, MA, USA) using built-in as well as in-house written functions and the PLS Toolbox 8.0 from Eigenvector Research Inc. (Wenatchee, WA, USA). Data for Partial Least Squares regression (PLS) models were autoscaled and venetian blinds cross validation (CV) with 5 data splits was employed. Receiver Operating Characteristic (ROC) curves were calculated using the Biomarker Analysis module available on the MetaboAnalyst platform [18]. Missing values were estimated using the k-nearest neighbors algorithm. Data were used without further normalization, transformation or scaling.

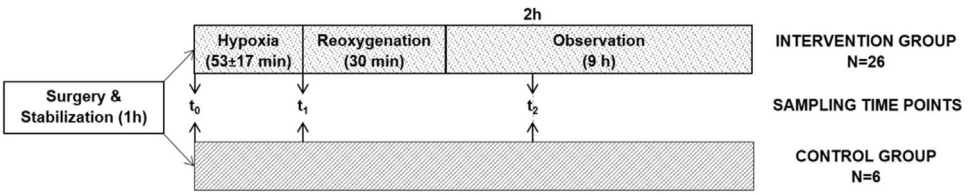

Fig. 1. Overview of the study design.

Table 1
Physiological parameters of piglets in the control and intervention groups at different time points.

Parameter	Time point	Control group	Intervention group	p
Weight [g]		1810 (± 173)	1889 (± 127)	p=0.2
Age [h]		28.7 (± 3)	25.6 (± 4)	p=0.12
Gender [male/female]		3/3	12/14	p=1
Hypoxia Time [min]		0	53.4 (± 17)	p < 0.01
Hb [g 100 mL^{-1}]	t_0	7.2 (± 1.0)	7.3 (± 1.1)	p=0.8
pH	t_0	7.41(± 0.04)	7.44 (± 0.07)	p=0.9
	t_1	7.42 (± 0.03)	6.86 (± 0.07)	p < 0.01
	t_2	7.46(± 0.03)	7.39 (± 0.08)	p=0.8
BE [mmol L^{-1}]	t_0	2.25 (± 2.8)	1.89 (± 3.4)	p=0.4
	t_1	1.98 (± 2.7)	−19.3 (± 2.2)	p < 0.01
	t_2	2.33 (± 2.6)	−0.4 (± 4.2)	p=0.13
MABP [mm Hg]	t_0	49.4 (± 4.9)	54.4 (± 8.1)	p=0.8
	t_1	48.2 (± 7.3)	22.7 (± 8.1)	p=0.02
	t_2	46.7 (± 6.2)	47.5 (± 11.3)	p=0.9
Heart rate [beats min^{-1}]	t_0	147 (± 11)	144 (± 26)	p=0.9
	t_1	156 (± 20)	160 (± 47)	p=1
pO_2 [kPa]	t_0	9.9 (1.0)	10.6 (1.7)	p=0.4
	t_1	10.3 (0.8)	5.0 (0. 6)	p < 0.01
pCO_2 [kPa]	t_0	5.4 (0.5)	5.2 (0.8)	p=0.7
	t_1	5.1 (0.3)	9.3 (1.1)	p < 0.01

Values are presented as mean (± s). Hb=hemoglobin; BE=base excess, MABP=mean arterial blood pressure; pO_2=partial O_2 pressure; pCO_2=partial CO_2 pressure

3. Results

3.1. Piglet Cohort characterization

Table 1 shows physiological parameters monitored during the experiment. No differences between control and intervention group were found when comparing the basic physiologic and clinical parameters at the beginning of the experiment (t_0). However, after 53.4 (± 17) min of intense hypoxia, the intervention group showed significantly lower pH, BE and MAPB, while no differences in heart rate were evidenced (t_1). Resuscitation with room air in the intervention group rapidly improved clinical variables and 2 h after resuscitation (t_2) no significant differences were found when comparing the parameters listed in Table 1.

3.2. Plasma metabolite score for hypoxia time prediction

In a recent study Solberg et al. [15] identified 46 differentiating variables out of a total of 452 detected features after data cleaning from LC-TOF-MS data in a metabolomics study focusing on the changes observed in the plasma metabolome of newly born piglets when subjected to postnatal hypoxia and resuscitation with air. In the present study, the set of 46 differentiating variables was used to model the hypoxia time of each piglet by Partial Least Squares (PLS) regression. The calibration set included metabolic levels from the analysis of plasma samples from 17 animals in the intervention group, withdrawn at t_0 and t_1. One data point from t_1 from the calibration data set was excluded due to unusual residuals [15]. Using 6 latent variables (LVs), root-mean-square-errors of calibration (RMSEC) and cross validation (RMSECV) of 4.3 and 10.8 min, respectively, were obtained.

From this model, three variables with importance in projection (VIP) scores > 2 and a putatively identified metabolite annotation (i.e. choline, 6,8-dihydroxypurine and hypoxanthine) were selected as candidate biomarkers for perinatal asphyxia. The profiles of the selected metabolites over time are shown in Fig. 2. Significant changes were detected immediately after hypoxia at t_1 as compared to t_0 in both the calibration and validation data sets (Wilcoxon rank sum, p-value < 0.05). 2 h after reoxygenation (t_2) statistically significant changes (Wilcoxon rank sum, p-value < 0.05) persisted for 6,8-dihydroxypurine, whereas for choline, hypoxanthine and lactate no significant differences in comparison to t_0 were detected. As expected, metabolite levels of the control group remained unchanged at t_1 and t_2 in comparison to t_0 (Wilcoxon rank sum, p-value > 0.05).

Then, a second PLS model was constructed in order to calculate a metabolite score using levels of choline, 6,8-dihydroxypurine and hypoxanthine and 1 LV achieving RMSEC and RMSECV of 9.9 min and 10.9 min, respectively. Predicted against measured hypoxia times for the calibration data are depicted in Fig. 3 (top). The PLS regression model was applied to the validation data set for the estimation of its predictive performance. This data set was not in any way used for model development or metabolite selection and thus, provided external figures of merit. The validation data set included: i) samples from 6 piglets of the intervention group collected at t_0, t_1 and t_2, ii) samples collected at t_2 from piglets included in the calibration data set and iii) samples from control animals collected at t_0, t_1 and t_2. Three piglets from the intervention group were excluded from data analysis due to abnormal clinical observations during the experiment [15]. Coefficients of determination (R^2) for calibration and validation data (intervention group) at t_0 and t_1 were ≥0.9 in both cases indicating a strong correlation between the PLS predicted hypoxia time and the measured hypoxia time. For samples from the intervention group at t_0 and t_1 a root-mean-square error of prediction (RMSEP) of 19.1 min was obtained, while at t_2 it increased to 39.3 min. For control animals a RMSEP of 2.3 min was obtained using the metabolite score.

Fig. 3 (bottom) shows results of linear regression models calculated to assess the association between plasmatic lactate concentrations and hypoxia time for the same individuals. Again, high R^2 (> 0.8) were obtained for samples from the intervention group at t_0 and t_1 in the calibration and validation data sets. The RMSEP for the validation data at t_0 and t_1 was 14.2 min, slightly lower to that provided by the abovementioned metabolite score. However, lactate levels at t_2 showed a significant increase in the RMSEP to 60 min. For control animals a RMSEP of 9.7 min was obtained.

Furthermore choline, 6,8-dihydroxypurine and hypoxanthine levels were combined with lactate concentrations with the aim of further improving the prediction performance of the metabolite score. However, including lactate did not help to improve the model's performance (data not shown).

3.3. Diagnostic capacity of the studied biomarkers

The performance of both, the PLS-based metabolite score and lactate levels for classification of piglets that had suffered from hypoxia-ischemia was assessed using ROC curve analysis. The constructed ROC curves were used to select the optimal cut-off level for the classification of samples included in the external validation data set. Under the tested experimental conditions, the metabolite score and the lactate concentration provided sensitivity and specificity values equal to 1 for the calibration data, with cut-off values of 15.8 min for the metabolite score and 9 mM for the lactate concentration. Besides, both variables correctly classified t_0 and t_1 as well as control samples included in the validation set, with the exception of one sample. However, lactate levels misclassified samples collected at t_2 from asphyxiated piglets and they were assigned to the non-asphyxiated class. In case of the metabolite score, 56.3% of the asphyxiated samples were correctly classified. These results are represented in Fig. 4.

4. Discussion

The prompt identification of infants who are at higher risk of developing moderate to severe HIE in the first critical hours is essential. Tools are needed for helping to guide clinical decision-

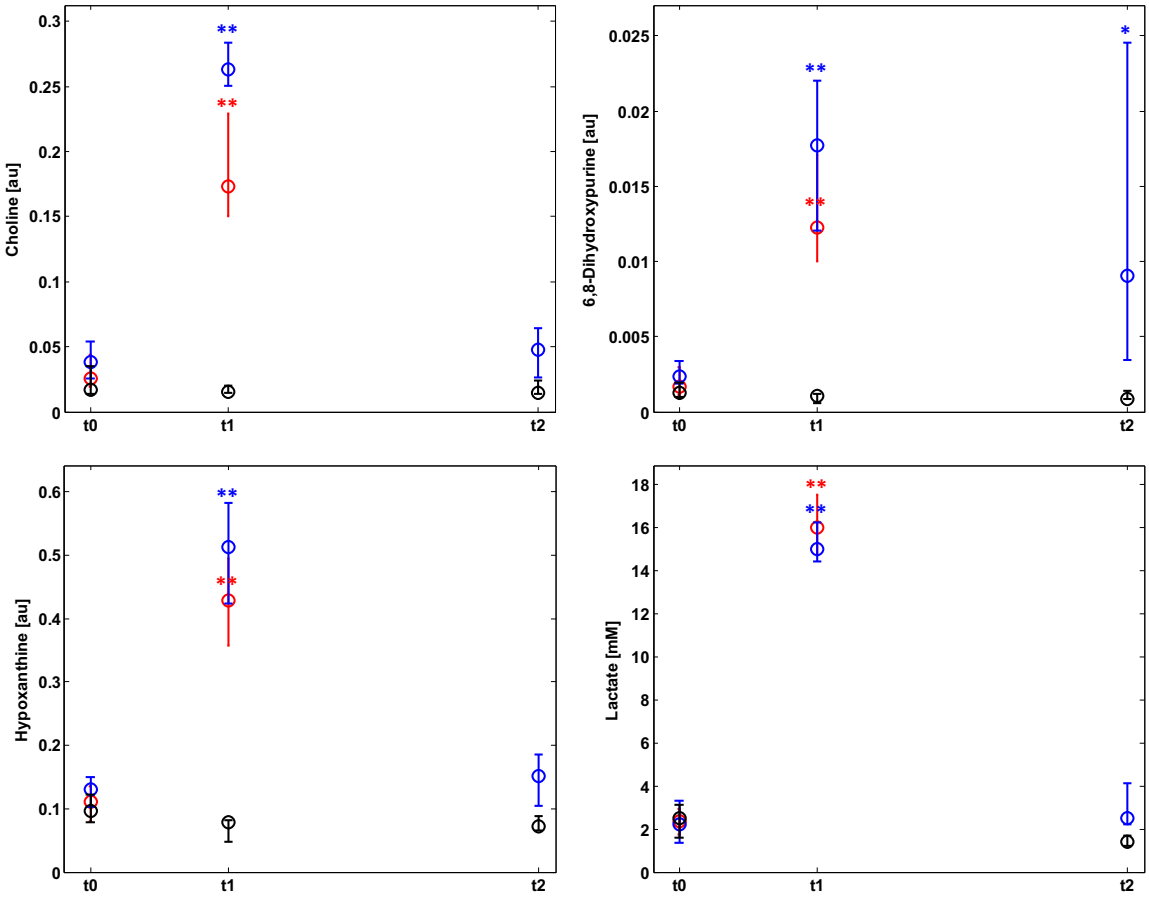

Fig. 2. Plasma metabolite levels of choline, hypoxanthine, 6,8-dihydroxypurine and lactate. Note: circles represent median concentrations and error bars depict interquartile ranges (IQRs); red: calibration data set; blue: validation data set; black: controls; no lactate values were available for the control group at t_1; *= p-vale < 0.05 (Wilcoxon rank sum) for comparing t_1 or t_2 to the corresponding data points at t_0; **= p-value < 0.01 (Wilcoxon rank sum) for comparing t_1 or t_2 to the corresponding data points at t_0. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

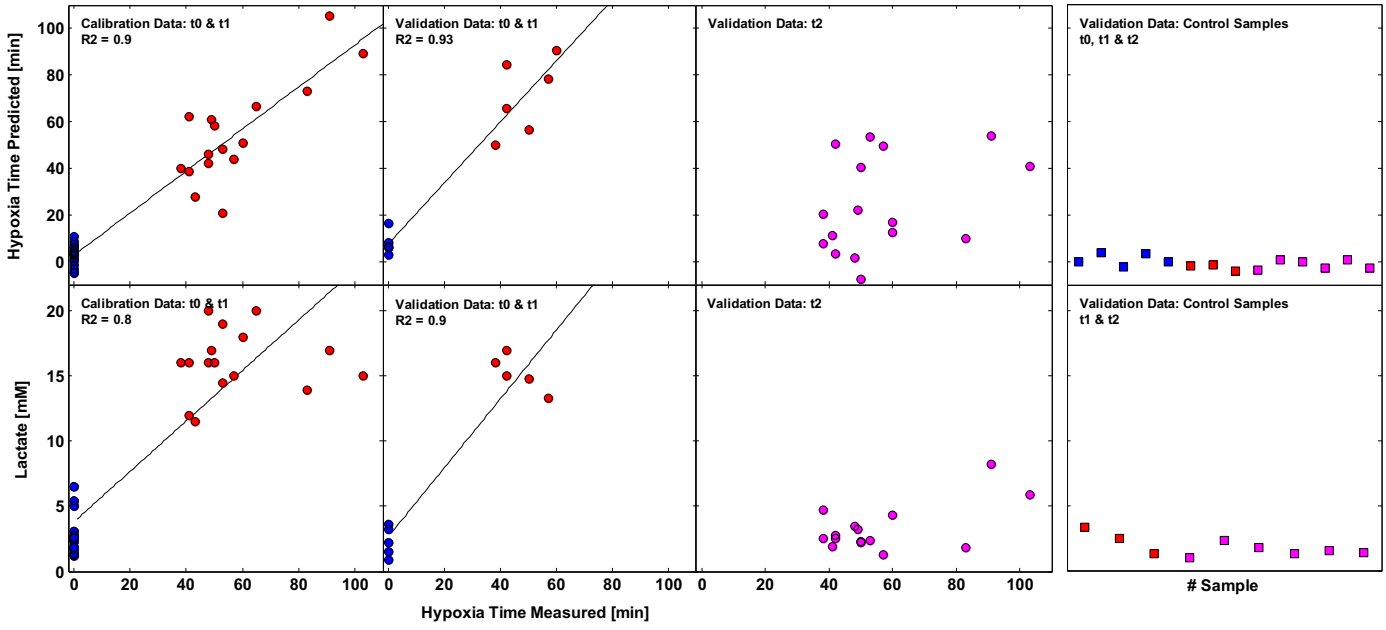

Fig. 3. PLS predicted vs. measured hypoxia time (top) and lactate vs. measured hypoxia time (bottom). Note: circles=intervention group; squares=control group; blue=t_0; red=t_1; magenta=t_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

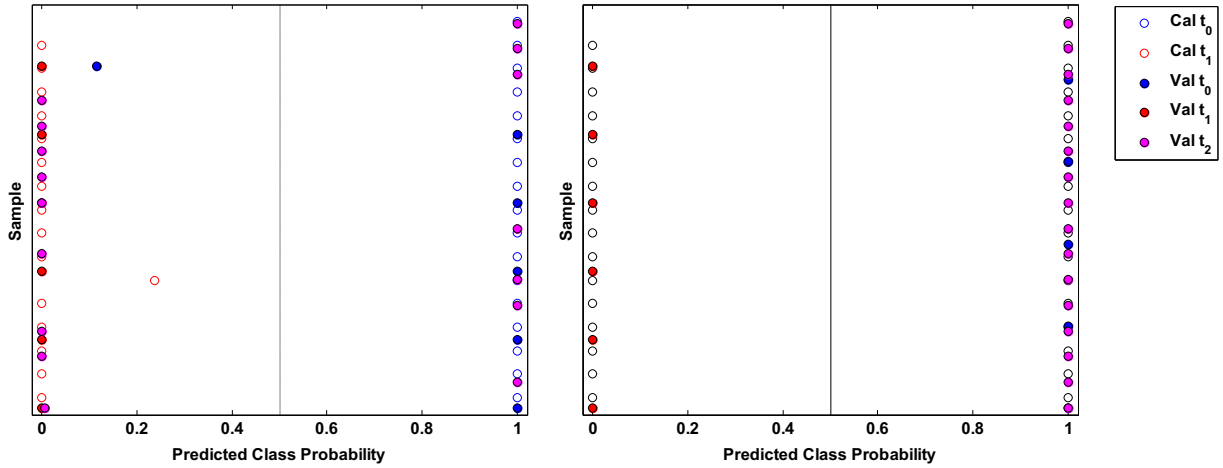

Fig. 4. ROC predictions using the metabolite score (left) or lactate (right). Note: Cal=calibration data set; Val=validation data set; Predicted Class Probability=1: non-asphyxiated class; Predicted Class Probability=0: asphyxiated class.

making and establish a prognosis. A clear association between parameters such as umbilical cord blood pH and neonatal outcomes have been reported [19]. However, Table 1 evidences that the typically recorded physiologic variables generally employed return to normal values during resuscitation and although they may reflect the intensity they do not inform of the duration of the insult. Consequently, they lack overall predictive capacity. Brain MRI and mchEEG are powerful and they have been validated in clinical studies for the diagnosis of HIE, but they are not readily available at all centers and therefore they cannot be employed within the first six hours of life when decision upon using therapeutic hypothermia has to be taken. For these reasons, there is an ongoing interest in the discovery of metabolic markers to objectively evaluate the degree of brain damage at an early point in time. Several novel biochemical markers including proteins apparently specific for neuronal tissue (creatine kinase brain band, protein S100B, neuron-specific enolase) and proteins involved in the pathogenesis of traumatic brain injury (e.g. glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase L1, phosphorylated axonal neurofilament heavy chain) as well as circulating pro-inflammatory cytokines (interleukin 1β and 6) and circulating mRNAs, among others, have been proposed [1,2,9,20,21]. Up to now, in most cases their usefulness has only been shown in pilot studies and none has entered routine clinical use [21,22]. Furthermore, studies on the correlation of the reported biomarkers with long term outcomes are lacking [1] and issues about their specificity have been raised [21].

In a very recent study by our group [15], a set of 46 metabolic variables capable of differentiating plasma samples from piglets before and after a hypoxic insult was identified. Putative metabolite assignment showed the implication of several metabolic pathways and metabolite classes including the phospholipid biosynthesis and purine catabolism revealing the multifactorial character of HIE. In the present study, the correlation of the previously selected differentiating metabolites with the time of hypoxia was investigated. This is of practical importance as the duration and intensity of hypoxia was found to be directly related to the degree of brain damage in perinatal asphyxia [23]. In the clinical setting, the exact duration of hypoxia is unknown because most of it elapses *in utero*. However, in a controlled animal experiment this parameter is easily accessible. Our study revealed the correlation of a metabolite score based on the relative intensities of choline, 6,8-dihydroxypurine and hypoxanthine with hypoxia time in a consolidated newborn piglet model of hypoxia (see Fig. 3). In the brain, choline is incorporated into phosphatidylcholine following the cytidine 5-diphosphocholine pathway [24]. In foregoing animal studies increased choline levels in brain tissue after hypoxia-ischemia in comparison to sham-controls have been found in *in vivo* mouse [25] and rat models [26]. In addition, a decrease of choline in brain tissue after oxygen-glucose deprivation has been reported in an *ex vivo* rat model comparing hypothermia and normothermia groups [27]. On the other hand, choline's function as a part of the neurotransmitter acetylcholine is widely known [28,29]. However, in preceding studies elevated CDP-choline levels in retinal tissue were observed supporting the alteration of the Kennedy pathway rather than the formation of acetylcholine as acetylcholine levels remained unaffected during hypoxia-reoxygenation in the studied neuronal tissue [13]. Recently it was shown that the combination of lactate with choline levels and related metabolites in plasma provided improved class prediction performance for the differentiation between a control and a hypoxia exposed group as well as improved prediction of the duration of hypoxia in an animal model of perinatal asphyxia-reoxygenation [30]. Hence, the evolution of the plasmatic profiles of choline could potentially be related with the alteration of the metabolic pathways (e.g. Kennedy pathway) together with the disturbance of the function of the blood brain barrier (BBB), which has been reported during HI insults in neonates [31]. This is also supported by a recent study on neonatal mouse brain, which revealed the transient opening of the BBB within early hours after the insult [32]. Hypoxanthine has been widely acknowledged as a hallmark for hypoxia. Hypoxanthine is a breakdown product of ATP and oxygen is needed for a further conversion into uric acid *via* xanthine. It has furthermore been described to be a free radical generator and hence it plays a key role in oxidative stress-associated diseases of the newborn [23,33]. The diagnostic value of hypoxanthine has been discovered decades ago and tested in animal and clinical studies ever since. Yet, it still has not replaced lactate as the gold standard of biochemical biomarkers for hypoxia. Big-scale multicenter clinical studies corroborating it usefulness in the clinical practice are still lacking [34].

In this study, results obtained using the metabolite score involving the determination of choline, 6,8,-dihydroxypurine and hypoxanthine are compared to those obtained using lactate levels, which are currently considered the gold standard for assessing asphyxia in the clinics. Strong correlations with the measured hypoxia time were found for both lactate ($R^2 \geq 0.8$) and the established metabolite score ($R^2 \geq 0.9$), in both calibration and external validation data sets (see Fig. 3). The metabolite score accurately predicted hypoxia times for control samples with a RMSEP of 2.3 min, whereas this parameter was more than quadruplicated when using lactate. Although lactate slightly outperformed the metabolite score in terms of prediction error at t_1 with RMSEPs of 14.2 and 19.1 min, respectively, at t_2 lactate values had almost returned to initial levels observed before hypoxia (t_0) precluding the accurate prediction of hypoxia time in samples withdrawn two hours after resuscitation.

In view of the clinical applicability as valuable biomarkers of these

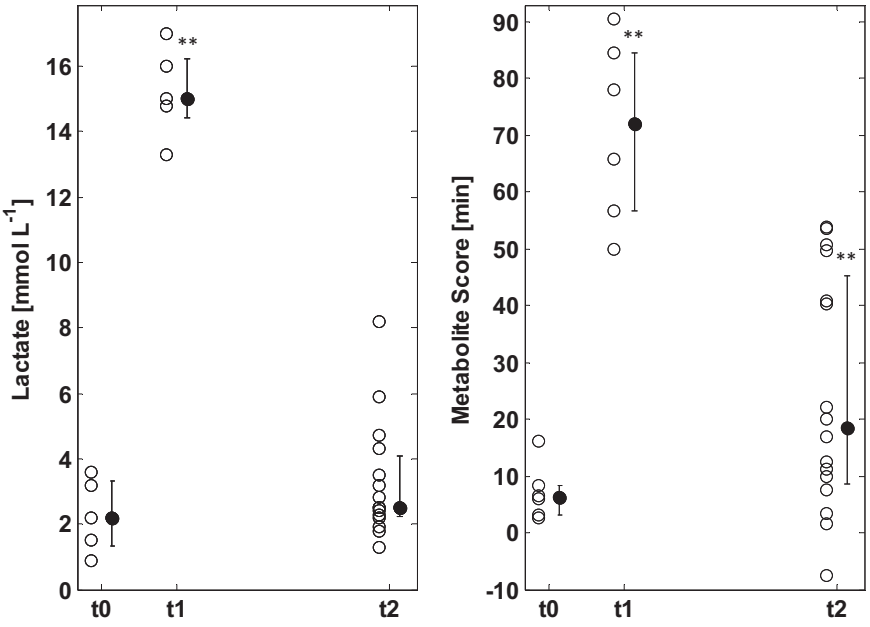

Fig. 5. Lactate concentrations (left) and PLS predicted metabolite scores (right) of the validation data at different sample collection time points. Note: open circles represent each measured value; solid circles represent median values; error bars represent IQRs; **=p-value < 0.01 (Wilcoxon rank sum) for comparing t_1 or t_2 to the corresponding data points at t_0 after elimination of outliers defined as data points outside 1.5 times the IQR.

metabolites, the time course is of great interest (see Fig. 2). The selection of the most appropriate therapeutic strategy for newborns with HIE is limited by the window of six hours from birth for initiating therapeutic hypothermia. Here, the metabolite score based on the relative intensities of choline, 6,8-dihydroxypurine and hypoxanthine in plasma samples was tested for its diagnostic capacity and compared to the performance of lactate as a biomarker in terms of patient stratification into HIE or control groups. Both lactate and the metabolite score performed as ideal biomarkers in this experimental study with extreme conditions when considering samples withdrawn before (t_0) and directly after hypoxia (t_1). Also for control animals, both approaches provided correct class assignments. However, it has to be remarked that the proposed metabolite score allowed covering a longer time period after the hypoxic insult. Results obtained showed that in 56.3% of the samples collected 2 h after reoxygenation (t_2), a clear alteration in the levels of the three selected metabolites persisted, whereas lactate levels provided no discrimination among samples withdrawn before (t_0) and 2 h after hypoxia (t_2). This indicates that the proposed metabolite score could potentially be applied to carry out determinations in a serial fashion covering the first hours of life, thereby aiding clinical decision-making together with routinely employed diagnostic tools in the delivery room. This is illustrated by the results presented in Fig. 5, where it can clearly be seen that at t_1 both lactate and the metabolite score show a marked change as compared to t_0. Instead, at t_2, lactate determinations are not useful to guide clinical decisions anymore whereas the metabolite score remains elevated as compared to t_0.

The preliminary results indicate that the metabolite score might be potentially extrapolated to the stratification of newborn infants with HIE within the first six hours of life for receiving therapeutic hypothermia and/or predicting outcomes. Future animal studies including hypothermia treatment will focus on the application of the metabolite score for an early assessment of the risk of brain injury and for the identification of cases with a good prognosis of benefiting from therapeutic hypothermia. Although the exact hypoxia time is not a measureable variable in the clinic, the metabolite score based on the measurement of choline, hypoxanthine and 6,8-dihydroxypurine in plasma might potentially correlate with the duration and/or intensity of hypoxia. Findings described in the present work will have to be validated in clinical studies involving the analysis of plasma samples from newborns suffering from HIE. Although in this study metabolite levels were determined employing sophisticated analytical platforms it is realistic to count on the development of point-of-care devices for the determination of metabolic markers, once established their effectiveness, similar to other portable devices used in the clinic on a daily basis for glucose, pH and lactate measurements.

5. Conclusions

To summarize, the present study showed the potential of a plasmatic metabolite score involving the determination of choline, 6,8-dihydroxypurine and hypoxanthine for estimating the duration of hypoxia. The metabolite score performed similar to lactate for samples withdrawn before (t_0) and directly after a hypoxic insult (t_1) and provided an enhanced predictive capacity at 2 h after resuscitation (t_2). Consequently, at the later time point (t_2) the metabolite score was able to improve the diagnostic capacity as compared to lactate. The applicability of the metabolite score for clinical diagnosis and patient stratification for hypothermia treatment has to be confirmed in multicenter trials involving the analysis of plasma samples from newborns suffering from HIE. These studies will also assess the correlation with long-term neurodevelopmental outcomes.

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