Comparison of umbilical serum copeptin relative to erythropoietin and S100B as asphyxia biomarkers at birth

Milla Summanen [a,b], Laura Seikku [c], Petri Rahkonen [c,d], Vedran Stefanovic [c], Kari Teramo [c], Sture Andersson [d], Kai Kaila [a,b], Leena Rahkonen [c]

[a] Department of Biosciences, University of Helsinki, Helsinki, Finland; [b] Neuroscience Center, University of Helsinki, Helsinki, Finland; [c] Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; and [d] Children´s Hospital, Helsinki University Hospital, Helsinki, Finland

Short title: Birth asphyxia biomarkers

Keywords: neonate, birth asphyxia, arginine vasopressin (AVP), copeptin, erythropoietin, S100B

Corresponding author: Kai Kaila, Department of Biosciences, Viikinkaari 1, 00790 Helsinki, Finland, tel. +358 40 7256 759, [kai.kaila@helsinki.fi].

Abstract

Background: Birth asphyxia, estimated to account for a million neonatal deaths annually, can cause a wide variety of neurodevelopmental impairments. There is a need to develop new, swift methods to identify those neonates who would benefit from neuroprotective treatments such as hypothermia.

Objectives: To examine the utility of cord serum copeptin, a stable byproduct of arginine vasopressin release, as a biomarker of birth asphyxia based on a comparison with two biomarkers of hypoxia and brain trauma, erythropoietin and S100B.

Methods: The study population consisted of 140 singleton, term neonates; 113 controls and 27 with birth asphyxia (two/three criteria met: umbilical artery pH <7.10, base excess <-12 mmol/l, and 5-minute Apgar score <7). All deliveries were planned vaginal, but 51 neonates were born by emergency cesarean section. Copeptin, S100B, and erythropoietin levels in umbilical artery samples were measured by immunoassays.

Results: Copeptin correlated in the entire study population more strongly with umbilical artery base excess than S100B and erythropoietin, and only copeptin correlated with arterial pH. Furthermore, only copeptin levels were significantly higher in cases of birth asphyxia, and in vaginally born neonates they were found to increase as a function of labor duration. Copeptin was elevated in neonates born via vacuum extraction, whereas erythropoietin levels showed a slight increase after emergency cesarean section.

Conclusions: In this study population, S100B and erythropoietin were not valid biomarkers of birth asphyxia. In contrast, our work suggests that copeptin has high potential to become a routinely used biomarker for acute birth asphyxia and neonatal distress.

Introduction

Birth asphyxia is a severe clinical problem globally, which has been estimated to account for a million of neonatal deaths annually [1]. The neurodevelopmental impairments in individuals who develop neonatal hypoxic-ischemic encephalopathy (HIE) range from minor cognitive problems and sensorimotor defects to cerebral palsy [1, 2]. Methods for identifying those neonates who would benefit from neuroprotective treatments such as therapeutic hypothermia is a major challenge because of the restricted time window of only a few hours for decision making. The increasing risk of HIE associated with enhanced acidemia has been well established. The Apgar scores at 5 minutes are known to correlate with the risk of neurological disability [3]. However, the sensitivity and specificity of arterial cord blood pH values and Apgar scores with regard to outcome following HIE are low [2]. Thus, reliable biomarkers for predicting outcome after birth asphyxia are urgently needed.

A number of studies on blood-borne protein biomarkers in neonates have been published in recent years [4-6]. Among these, erythropoietin (EPO) is a biomarker of chronic hypoxia [7], and high levels of umbilical plasma EPO at birth are associated with an increased risk for adverse outcome [8]. S100B is considered as a biomarker of brain cell damage [9], and its levels are known to rise at the early phase of acute asphyxia [6]. In the present study, we examine the utility of cord serum copeptin, a byproduct of arginine vasopressin (AVP) release, as a biomarker of birth asphyxia based on a comparison with EPO and S100B. The rationale of this approach lies in the fact that a massive surge of AVP release takes place during normal vaginal birth in response to activation of the fetal hypothalamic–pituitary–adrenal (HPA) axis, which is further accentuated by various kinds of stress factors such as infections, intra-uterine growth restriction, and acidosis/birth asphyxia [4, 5, 10, 11]. In contrast to AVP, copeptin is biochemically stable with a much longer half-life, and it is released in an equimolar ratio to AVP, making it an ideal surrogate for AVP measurements [11, 12]. One of the practical advantages of copeptin is that it is widely used as a biomarker of various pathophysiological states like lower respiratory tract infections, septic shock and stroke in emergency departments [13].

Methods

Study design and patients

The serum samples for this retrospective study were collected in the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland between May 2012 and April 2013. The study, approved by the local Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland (105/13/13/03/2012), consisted of 151 singleton births at or beyond 37+0 gestational weeks. Umbilical artery blood samples were collected immediately after birth from 72 neonates with suspected asphyxia, based on a 1-minute Apgar score <4. The 1-minute Apgar score was used only for patient recruitment, to allow midwives to identify neonates suspected of having suffered from birth asphyxia. Umbilical artery cord blood samples from 79 control neonates with a 1-minute Apgar score ≥4 were collected during the five-day work week. Pregnancies complicated by maternal type 1 diabetes (n=1), preeclampsia (n=3), fetal growth restriction (n=4) and Rh immunization (n=1) were excluded. In addition, one neonate was excluded because of chromosomal anomaly and one had mitochondrial disease, leaving a total of 140 neonates for the final analysis. The neonates were divided into two groups, asphyxia and control, for data analysis. The neonate was considered to fulfill the diagnostic criteria for birth asphyxia if two of three criteria were met: umbilical artery pH <7.1, umbilical artery base excess <-12 mmol/l, and a 5-minute Apgar score <7 [14, 15].

Gestational age was defined by fetal crown-rump length measurement at the first trimester ultrasound screening. Deliveries were all planned vaginal. Indications for emergency cesarean sections (ECS) were: fetal distress (n=29), labor dystocia (n=10), prolonged second stage of delivery (n=2), chorionamnionitis (n=1), fetal malpresentation (n=3), unsuccessful vacuum extraction (n=4), and umbilical cord prolapse (n=2). Data on maternal pregnancy characteristics and short-term perinatal outcome were collected from the hospital charts (table 1). Birth weight z-score was defined according to the Finnish population standardized for sex and gestational age [16].

Blood samples from the umbilical artery were used for measurements of pH, base excess (BE), pO_2 and pCO_2 (Radiometer ABL800 Flex blood gas analyzer, Copenhagen, Denmark). Serum samples from the umbilical artery were used for biomarker measurements.

Copeptin measurements

We used a sandwich enzyme-linked immunosorbent assay (ELISA, methodological details in the Supplementary material). The inter-assay variability was 5.8 %, which was estimated by calculating

the CV from eight duplicate samples run on two plates on two separate days. Fifteen serum samples were analyzed with both the ELISA and the BRAHMS copeptin Kryptor assay used in previous publications [4, 5], and a highly significant linear correlation (Pearson $r = 0.9793$, $p < 0.0001$) was found, covering the full range of values obtained using the Kryptor, 6.0 to 4637 pmol/l (median 381.2 pmol/l). This excellent linear correlation, with an R^2 of 0.9591 indicating a congruence of 96 % between the two methods, was used to convert the copeptin concentrations obtained with the ELISA to Kryptor concentrations. This allows for a direct comparison of our data with the already published copeptin results in neonates.

S100B measurements

Serum S100B was measured with electrochemiluminometric immunoassay using Modular e170 analyzer (Roche Diagnostics). The detection range for the S100B assay is 0.005 μg/l and functional sensitivity less than 0.02 μg/l. The intra-assay coefficient of variation (CV) was less than 2.1 % and inter-assay variation better than 6.4 %.

EPO measurements

EPO was measured using a solid-phase chemiluminescent enzyme immunometric assay Immulite 2000 XPI analyser (Siemens Healthcare Diagnostics). The intra-assay CV was 3.6-6.8 %, while the total CV was 6.4-10.3 %. Detection limit was 1.0 IU/l, and functional sensitivity (CV 20 %) was 1.5 IU/l.

Statistical analysis

All three biomarkers were measured from each of the 140 samples included in this study. The following statistical analyses were performed with GraphPad Prism 6 or SPSS 22. Prior to calculating correlations and significances, statistical outliers ($p < 0.01$) for copeptin and S100B (n=1 for each) were excluded from the study population. Correlations between the pH, BE, S100B, EPO and copeptin values from all remaining samples were calculated with the Spearman correlation coefficient. Copeptin, S100B and EPO values between the study groups were compared using the Mann-Whitney U test or the Kruskall-Wallis test, and a receiver operating characteristic (ROC)-curve was drawn to determine the diagnostic accuracy of copeptin to birth asphyxia. The Chi-squared test or Fisher's exact test were used to determine significant differences in the study population.

Results

Maternal and fetal/delivery characteristics

The maternal and fetal/delivery characteristics are shown in table 1. There were no statistically significant differences in any of the maternal or delivery characteristics between the two study groups.

Dependence of biomarker levels on umbilical artery pH and BE

The neonates' median pH, BE, pO_2 and pCO_2 values, which are routinely measured from umbilical arterial cord blood at birth, are shown in table 1. The dependence of the three biomarkers on the blood acid-base parameters are given in figure 1. Only copeptin levels showed a significant correlation with umbilical artery pH ($r = -0.6219$, $p < 0.0001$, fig. 1a). All three biomarkers correlated with umbilical artery BE (fig. 1d-f), with copeptin showing by far the highest correlation coefficient ($r = -0.6372$, $p < 0.0001$).

Biomarker levels and asphyxia

Twenty-seven neonates in the study population belonged to the birth asphyxia group (see Methods for present criteria). Copeptin levels were significantly higher among the neonates in the birth asphyxia group compared to unaffected controls (mean 2450 pmol/l vs 1226 pmol/l, $p < 0.0001$, fig. 2c), whereas no differences in S100B (fig. 2a) or EPO (fig. 2b) levels were found. ROC-curve analysis showed that copeptin concentrations discriminated with moderate accuracy between asphyxia, as defined in this study, and controls: the area under the curve was 0.76 (95%-CI 0.69-0.86, fig. 2d). A cut-off of 1522 pmol/l had a sensitivity of 77 % and a specificity of 70%.

Biomarker levels and delivery mode

All 140 deliveries were planned vaginal, but 51 neonates were ultimately born by ECS. Of the 89 vaginal deliveries, 33 were assisted with vacuum extraction. There were no differences in the delivery mode between the asphyxia and control study groups (table 1). Copeptin levels were higher among the neonates born via vacuum extraction as compared to ECS (mean 2021 pmol/l vs 1190 pmol/l, $p = 0.003$, fig. 3a) or normal vaginal delivery, although the latter difference did not reach statistical significance (mean 2021 pmol/l vs 1362 pmol/l, $p = 0.0522$, fig. 3a). No differences in S100B levels were found between the groups (fig. 3b), whereas EPO levels were higher among neonates born via

ECS compared to normal vaginal delivery or vacuum extraction (means 713.7 U/l, 78.41 U/l, and 61.15 U/l, respectively; p = 0.0002 and p = 0.001, fig. 3c), which might reflect prenatal conditions [7].

Biomarker levels in relation to other variables

S100B levels were higher in male neonates (p = 0.0378), but copeptin and EPO levels did not differ based on the sex of the neonate. All biomarkers showed a correlation with the 5-minute Apgar score, and copeptin and S100B correlated also with the 10-minute Apgar score (table 2). Copeptin and S100B levels did not correlate with gestational age at birth, whereas EPO levels did (r = 0.4513, p < 0.0001). Only copeptin levels correlated significantly with birth weight (r = -0.1713, p = 0.0438). Among the 89 neonates born vaginally, copeptin levels increased as a function of the total duration of labor (r = 0.3267, p = 0.0019) and the duration of the second stage of labor (r = 0.2787, p = 0.0086; table 2). EPO and S100B levels did not correlate with either of these variables.

Discussion

A wide spectrum of adaptive processes in respiratory, cardiovascular, and metabolic functions are triggered at birth [4, 10]. Changes in biomarker concentrations during birth reflect, at least in part, physiological fetal adaptive reactions, such as enhanced activation of the HPA-axis (see below), in response to normal or complicated delivery. Distinct biomarkers have different profiles during fetal and neonatal asphyxia [4, 7, 18]. The S100B level in plasma has been shown to rise at the early phase of acute asphyxia [6, 18] and significantly higher S100B levels have been reported in asphyxiated term neonates with intraventricular hemorrhage (IVH) or with HIE, compared to asphyxiated neonates without IVH/HIE or to apparently healthy neonates [19]. EPO is a biomarker of chronic hypoxia, and increased levels can be detected in fetal plasma and amniotic fluid in various pathological pregnancies [7]. An association of high EPO levels during pregnancy and adverse acute neonatal outcome, such as decreased umbilical cord pH, pO_2 and BE, and increased intensive care unit admission, has also been reported [7, 20]. Furthermore, high levels of umbilical plasma EPO at birth are associated with an increased risk for death or abnormal neurological outcome at two years of age [8].

There are many *a priori* reasons why copeptin might turn out to be a highly useful biomarker of birth asphyxia, and a number of previous observations point in this direction [4, 5, 21]. During birth, the HPA axis shows massive activation, which results in the release of AVP, and this response is further enhanced by various types of stressors. Since AVP is highly unstable with a short half-life of 4-20 minutes [22], assays of this hypothalamic hormone itself are not suitable for clinical use. However, AVP is derived from a larger precursor peptide which contains copeptin, a stable C-terminal fragment with 39 amino acids. Copeptin is released in an equimolar ratio to AVP [23]. In line with the underlying HPA-based mechanisms, copeptin levels in cord blood increase in different stress situations, such as infections and hypoxia, both in term and preterm pregnancies [4, 5, 21]. High copeptin levels at birth are related to acute adverse neonatal outcomes such as IVH [21].

The single most important criterion for diagnosis of birth asphyxia is profound metabolic acidosis [14, 15]. Notably, copeptin levels increase along with decreasing umbilical cord blood pH and BE during normal birth [4], and they are even higher following birth asphyxia [5]. This means that the dynamics of enhanced copeptin plasma concentrations cover a wide range of levels of acidemia from normal to severely abnormal, i.e. those prevailing in birth asphyxia.

In line with the above considerations and data, we demonstrate here that copeptin levels are highly correlated with both arterial cord blood pH and BE (fig. 1). The strong and highly significant dependence of high copeptin levels on negative BE shown presently is of particular interest. In excellent agreement with the above data, copeptin levels turned out to be significantly higher in asphyxiated neonates vs controls in the present study. The sensitivity and specificity parameters in the ROC analysis were not as high as previously shown [5], which is attributable to the present inclusion criteria of the control group based on a 1-minute Apgar score of 4 or higher. In contrast, no difference was seen in S100B and EPO levels (fig. 2), most likely because of the relatively mild asphyxia criteria in the present work.

A further important finding in the present study is that copeptin levels increased as a function of the total duration of labor and on the duration of the second stage of labor, while EPO and S100B levels did not correlate with either variable. This result with copeptin is most likely explained by very recent observations that just a few contractions (most likely acting via transient periods of minor hypoxia

on the fetus) are sufficient to trigger detectable AVP/copeptin [24]. Given the cumulative nature of the copeptin levels with a half-life of 30 minutes [25], the above dependence on labor duration is readily explained. Thus, future work on sequential measurements of copeptin may turn out to be valuable in enhancing the prognostic power of this biomarker.

It has been previously shown that significantly higher copeptin concentrations are observed after vaginal delivery compared to delivery by primary cesarean section [4]. In the present study, the patient cohort included only neonates born by ECS preceded by periods of labor contractions with variable duration. Thus, it is not surprising that no significant difference in copeptin levels was observed between neonates born vaginally or via ECS. However, copeptin levels were higher after vaginal delivery assisted by vacuum extraction when compared to copeptin levels after ECS or normal vaginal delivery, as reported before [4].

To summarize, our study indicates that copeptin has a high potential to become a routine biomarker for neonatal distress and asphyxia. From a (patho)physiological point of view, its advantages are based on the key role of AVP in the adaptations of the fetus to birth. From a practical point of view, it is of much importance that serum copeptin is widely used as a biomarker in adults in emergency departments [13], and therefore this approach can be readily extended to neonatal intensive care units. Future studies are needed to determine whether copeptin concentrations at birth correlate with the severity of HIE, and, more importantly, with long-term neurological outcome following birth asphyxia.

Acknowledgements

The funding of work and materials for blood analyses was provided by Jane and Aatos Erkko Foundation (KK), Sigrid Juselius Foundation (KK) and the Academy of Finland (KK).

References

1 Lee AC, Kozuki N, Blencowe H, Vos T, Bahalim A, Darmstadt GL, Niermeyer S, Ellis M, Robertson NJ, Cousens S, Lawn JE: Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res 2013;74 Suppl 1:50-72.

2 Ahearne CE, Boylan GB, Murray DM: Short and long term prognosis in perinatal asphyxia: An update. World J Clin Pediatr 2016;5:67-74.

3 Dalili H, Nili F, Sheikh M, Hardani AK, Shariat M, Nayeri F: Comparison of the four proposed Apgar scoring systems in the assessment of birth asphyxia and adverse early neurologic outcomes. PLoS One 2015;10:e0122116.

4 Wellmann S, Benzing J, Cippa G, Admaty D, Creutzfeldt R, Mieth RA, Beinder E, Lapaire O, Morgenthaler NG, Haagen U, Szinnai G, Buhrer C, Bucher HU: High copeptin concentrations in umbilical cord blood after vaginal delivery and birth acidosis. J Clin Endocrinol Metab 2010;95:5091-5096.

5 Schlapbach LJ, Frey S, Bigler S, Manh-Nhi C, Aebi C, Nelle M, Nuoffer JM: Copeptin concentration in cord blood in infants with early-onset sepsis, chorioamnionitis and perinatal asphyxia. BMC Pediatr 2011;11:38-2431-11-38.

6 Qian J, Zhou D, Wang YW: Umbilical artery blood S100beta protein: a tool for the early identification of neonatal hypoxic-ischemic encephalopathy. Eur J Pediatr 2009;168:71-77.

7 Teramo KA, Widness JA: Increased fetal plasma and amniotic fluid erythropoietin concentrations: markers of intrauterine hypoxia. Neonatology 2009;95:105-116.

8 Ruth V, Autti-Ramo I, Granstrom ML, Korkman M, Raivio KO: Prediction of perinatal brain damage by cord plasma vasopressin, erythropoietin, and hypoxanthine values. J Pediatr 1988;113:880-885.

9 Beharier O, Kahn J, Shusterman E, Sheiner E: S100B - a potential biomarker for early detection of neonatal brain damage following asphyxia. J Matern Fetal Neonatal Med 2012;25:1523-1528.

10 Lagercrantz H: Sympathoadrenal Mechanisms during Development; in Polin RA (ed): Fetal and Neonatal Physiology. Saunders, WW. Fox, 1998, pp 220-225.

11 Evers KS, Wellman S: Arginine Vasopressin and Copeptin in Perinatology. Front Pediatr 2016;4.

12 Morgenthaler NG, Struck J, Alonso C, Bergmann A: Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 2006;52:112-119.

13 Nickel CH, Bingisser R, Morgenthaler NG: The role of copeptin as a diagnostic and prognostic biomarker for risk stratification in the emergency department. BMC Med 2012;10:7-7015-10-7.

14 Yeh P, Emary K, Impey L: The relationship between umbilical cord arterial pH and serious adverse neonatal outcome: analysis of 51,519 consecutive validated samples. BJOG 2012;119:824-831.

15 Low AJ: Intrapartum fetal asphyxia: definition, diagnosis, and classification. Am J Obstet Gynecol 1997;176:957-959.

16 Pihkala J, Hakala T, Voutilainen P, Raivio K: Characteristics of recent fetal growth curves in Finland. Duodecim 1989;105:1540-1546.

17 Chard T, Hudson CN, Edwards CR, Boyd NR: Release of oxytocin and vasopressin by the human foetus during labour. Nature 1971;234:352-354.

18 Liu F, Yang S, Du Z, Guo Z: Dynamic changes of cerebral-specific proteins in full-term newborns with hypoxic-ischemic encephalopathy. Cell Biochem Biophys 2013;66:389-396.

19 Gazzolo D, Di Iorio R, Marinoni E, Masetti P, Serra G, Giovannini L, Michetti F: S100B protein is increased in asphyxiated term infants developing intraventricular hemorrhage. Crit Care Med 2002;30:1356-1360.

20 Seikku L, Rahkonen L, Tikkanen M, Hamalainen E, Rahkonen P, Andersson S, Teramo K, Paavonen J, Stefanovic V: Amniotic fluid erythropoietin and neonatal outcome in pregnancies complicated by intrauterine growth restriction before 34 gestational weeks. Acta Obstet Gynecol Scand 2015;94:288-294.

21 Benzing J, Wellmann S, Achini F, Letzner J, Burkhardt T, Beinder E, Morgenthaler NG, Haagen U, Bucher HU, Buhrer C, Lapaire O, Szinnai G: Plasma copeptin in preterm infants: a highly sensitive marker of fetal and neonatal stress. J Clin Endocrinol Metab 2011;96:E982-5.

22 Treschan TA, Peters J: The vasopressin system: physiology and clinical strategies. Anesthesiology 2006;105:599-612; quiz 639-40.

23 Morgenthaler NG, Struck J, Jochberger S, Dunser MW: Copeptin: clinical use of a new biomarker. Trends Endocrinol Metab 2008;19:43-49.

24 Wellmann S, Koslowski A, Spanaus K, Zimmermann R, Burkhardt T: Fetal release of copeptin in response to maternal oxytocin administration: a randomized controlled trial. Obstet Gynecol DOI: 10.1097/AOG.0000000000001594.

25 L'Abate P, Wiegert S, Struck J, Wellmann S, Cannizzaro V: Determinants of plasma copeptin: a systematic investigation in a pediatric mechanical ventilation model. Respir Physiol Neurobiol 2013;185:222-227.

Figure legends

Fig. 1 Dependence of copeptin (a, d), S100B (b, e) and EPO levels (c, f) on pH (a-c) and base excess (BE; d-f). P - and r - values for each pair are shown in the respective panels, regression lines were drawn when the correlation was significant.

Fig. 2 Comparisons between the birth asphyxia and control groups. There were no significant differences in S100B (a) or EPO (b) levels between the groups. Copeptin (c) levels were significantly higher in the asphyxia group compared to the control group. The medians and p-values of Mann-Whitney U-test are shown (a-c). (d) ROC-curve for cord serum copeptin concentrations in relation to birth asphyxia. The dotted lines indicate the optimal discriminative cut-off of 1522 pmol/l, resulting in a sensitivity of 77 % and a specificity of 70 %.

Fig. 3 Comparisons between biomarkers and delivery mode. Copeptin (a) levels were significantly higher in neonates born via vacuum extraction compared to emergency cesarean section, whereas no differences in S100B (b) levels were found between groups. EPO (c) levels were significantly higher in neonates born via emergency cesarean section compared to the two other groups. The medians are shown in each panel, and p - values of Kruskal-Wallis test are shown when the difference was significant.

Table 1. Maternal and fetal/delivery characteristics of the study groups					
	Asphyxia (n = 27)		Control (n = 113)		p
Maternal characteristics					
Maternal age, years (mean)	30.9	*SD (5.9)*	31.1	*SD (5.5)*	0.815
Primiparity	15	*(55.6)*	62	*(54.9)*	1.000
In vitro fertilization	2	*(7.4)*	5	*(4.4)*	0.620
Smoking	4	*(14.8)*	9	*(8.0)*	0.277
Obesity (body mass index ≥ 30kg/m^2)	2	*(7.4)*	17	*(15.0)*	0.530
Gestational diabetes	4	*(14.8)*	14	*(12.4)*	0.751
Chronic hypertension	1	*(3.7)*	3	*(2.7)*	0.580
Fetal/delivery characteristics					
Spontaneous vaginal delivery	7	*(25.9)*	49	*(43.4)*	0.257
Vacuum extraction	9	*(33.3)*	24	*(21.2)*	0.309
Emergency cesarean section	11	*(40.7)*	40	*(35.4)*	0.727
Gestational weeks at birth	41.1	*(40.0 - 41.6)*	40.7	*(39.7 - 41.7)*	0.372
Post-term births (≥H42^{+0})	5	*(18.5)*	22	*(19.5)*	1.000
Male	17	*(63.0)*	66	*(58.4)*	0.828
Birth weight (g)	3520	*(3260 - 3945)*	3656	*(3282 - 3991)*	0.499
Relative birth weight (SD)	-0.13	*(-1.02 - 0.62)*	0.02	*(-0.67 - 0.81)*	0.385
5 min Apgar score	6	(4-7)	8	(6-9)	<0.0001
10 min Apgar score	8	(6-9)	9	(8-9.5)	0.0009
Umbilical artery pH	7.03	*(6.97 - 7.08)*	7.21	*(7.14 - 7.30)*	<0.0001
Umbilical artery base excess	-12.7	*(-14.4 - -11.1)*	-5.80	*(-7.85 - -2.85)*	<0.0001
Umbilical artery pO_2 (kPa)	1.9	*(1.1 - 2.8)*	2.4	*(1.7 - 3.3)*	0.029
Umbilical artery pCO_2 (kPa)	10.1	*(9.2 - 12.7)*	7.8	*(6.7 - 9.2)*	<0.0001
Umbilical serum erythropoietin (U/l)	71.7	*(22.2 - 116.0)*	46.1	*(22.2 - 124.5)*	0.683
Umbilical serum S100B (µg/l)	0.33	*(0.19 - 0.63)*	0.31	*(0.24 - 0.45)*	0.712
Umbilical serum copeptin (pmol/l)	2279	*(1476-3144)*	973.7	*(320.9-1961)*	<0.0001
Median (interquartile range) or number (percentage) are shown					

Table 2. Biomarker correlations to other variables									
	Copeptin			**S100B**			**EPO**		
	n	r	p	n	r	p	n	r	p
5 min Apgar score	139	-0.2998	0.0004*	139	-0.2041	0.0159*	140	-0.2914	0.0005*
10 min Apgar score	139	-0.1678	0.0484*	139	-0.2048	0.0156*	140	-0.1380	0.1040
Gestational age	139	0.0644	0.4510	139	-0.0018	0.9836	140	0.4513	<0.0001*
Birth weight	139	-0.1713	0.0438*	139	-0.0235	0.7838	140	-0.0367	0.6672
Total labor duration	88	0.3267	0.0019*	89	0.1247	0.2444	89	0.0656	0.5414
Second stage labor duration	88	0.2787	0.0086*	89	0.1038	0.3332	89	0.0162	0.8802
Significant correlations indicated by an asterisk.									

352

353

354

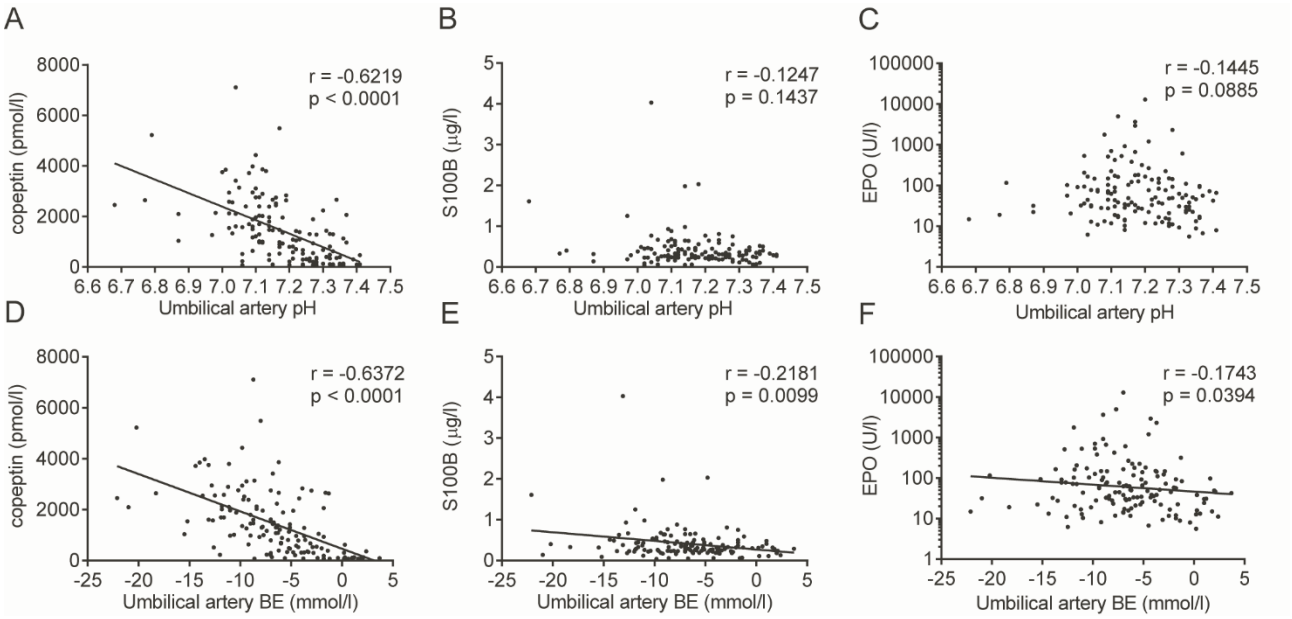
355

356

357

358

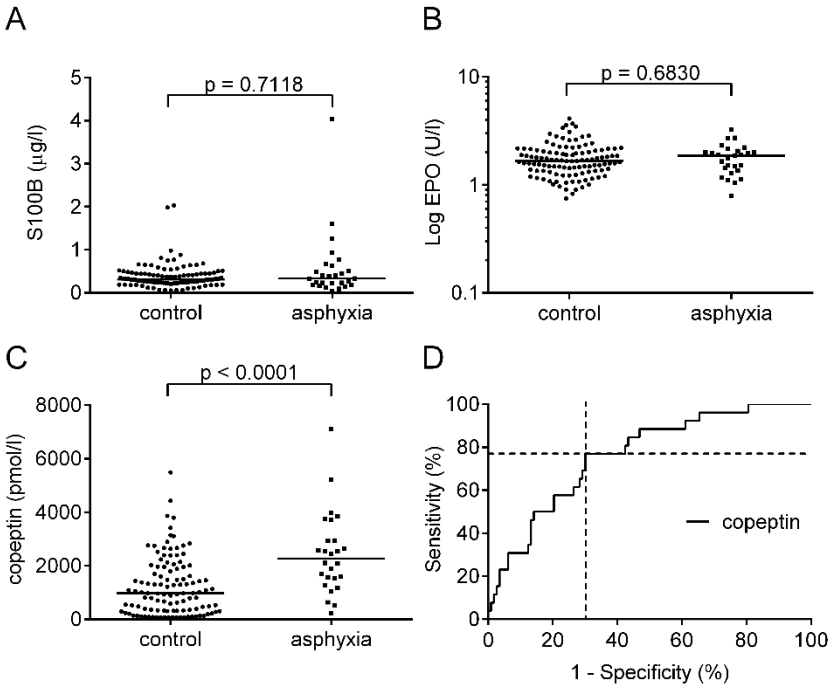
359 Fig. 1



360

361

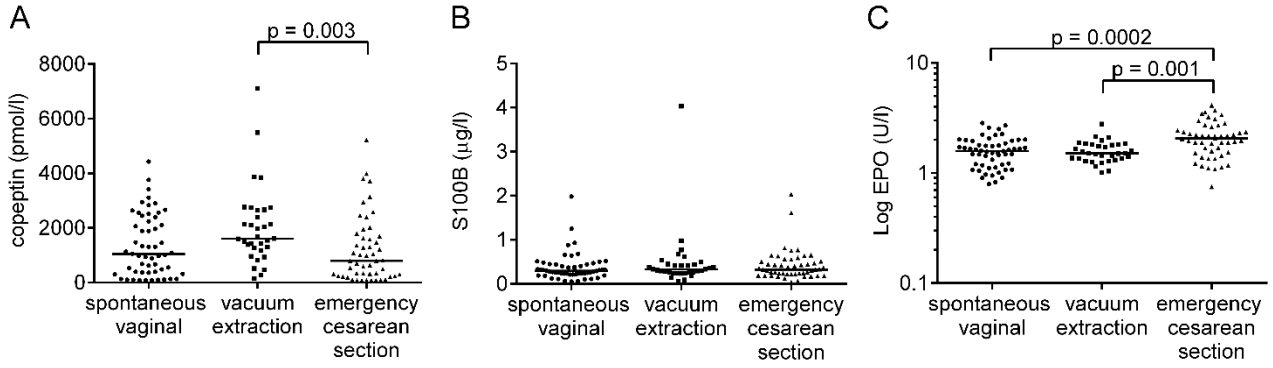
362 Fig. 2



363

364

365 Fig. 3



366

367