Cord blood S100B: reference ranges and interest for early identification of newborns with brain injury

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Short title: Interest of S100B in cord blood

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ABSTRACT (244 words)

Background: Neurological complications are common in the premature and full-term neonates admitted to the intensive care unit, but the diagnosis of these complications is often difficult to make. S100B protein, measured in cord blood, may represent a valuable tool to better identify patients at risk of brain injury.

Methods: As a first step, we established S100B cord blood serum reference intervals from 183 preterm and 200 full-term neonates. We then measured cord blood serum S100B to identify neurological complications in 272 neonates hospitalized at the neonatal intensive care unit (NICU). Diagnosis of brain injury relied on imaging examination.

Results: The 95th percentiles of S100B concentration in cord blood were established as 1.21 µg/L for the 383 neonates, 0.96 µg/L for full-term neonates, and 1.36 µg/L for premature neonates. Among the 272 neonates hospitalized at the NICU, 11 presented neurological complications. Using 1.27 µg/L as the optimal sensitivity/specificity threshold, S100B differentiate neonates with and without neurological complications with a sensitivity of 45.5% (95%CI: 16.7-76.6) and a specificity of 88.9% (95%CI: 84.4-92.4) (p=0.006). In combination with arterial pH (<7.25), sensitivity increased to 90.9% (95%CI: 58.7-99.8), while specificity was 51.2% (95%CI: 44.8-57.7). The sensitivity is significantly (p=0.03) increased in comparison to S100B alone. The specificity is significantly higher with S100B only than with pH + S100B (p<0.001).

Conclusions: Cord blood S100B protein, in combination with arterial cord blood pH, has the potential to help clinicians to detect at birth neurological complications in neonates hospitalized in a NCIU.

Keywords: S100B, cord blood, reference ranges, neurological complications, brain injury

LIST OF ABBREVIATIONS:

AUC: Area Under Curve;
CI: confidence interval;
GDM: gestational diabetes mellitus
GFAP: Glial Fibrillary Acidic Protein ;
GH: gestational hypertension
HDP: hypertensive disorders of pregnancy
HIE: Hypoxic Ischaemic Encephalopathy;
IQR: interquartile range;
IUGR: intrauterine growth restriction
IVH: intraventricular haemorrhage;
MRI: magnetic resonance imaging;
NICU: Neonatal Intensive Care Unit;
PTB: Preterm birth;
PVL: periventricular leukomalacia;
ROC: receiver operating characteristic;
SD: Standard Deviation;
UCHL1: Ubiquitin carboxy-terminal hydrolase L1.

INTRODUCTION

Preterm birth (PTB) includes all births before 37 completed weeks of gestation (1) and represents 12% of all births (2). Complications of preterm birth represent the leading cause of death in children younger than 5 years of age, accounting for approximately 16% of all deaths, and 35% of deaths among newborn babies (3). Neurological complications, particularly periventricular leukomalacia (PVL), intraventricular haemorrhage (IVH), cerebral palsy, and hypoxic ischaemic encephalopathy (HIE) are common in the premature infant (4)(5), but the diagnosis of these complications is often difficult to establish and rely essentially on clinical observation, arterial pH, and imaging (6)(7)(8). Neurological complications can also be diagnosed in full-term neonates (9). In this context, serum biomarkers, measured in cord blood, represent a potentially useful tool to improve the identification of patients at risk of neurological complications that would need immediate diagnostic measures such as imaging.

S100B protein, a small dimeric cytosolic protein (21 kDa) consisting of ββ or αβ dimers, is well-established as a sensitive traumatic brain injury biomarker and is therefore a relevant candidate. It is one of the calcium-binding proteins found in glial cells involved in a variety of intracellular and extracellular regulatory activities (10)(11). Following the onset of cerebral lesions, S100B is immediately released from damaged glial cells into the circulation and subsequently eliminated by the kidneys, exhibiting a short half-life of about 30–100 minutes (12)(13)(14). In premature babies, umbilical serum S100B levels were associated with PVL and IVH (13) and cerebral palsy (14). The interest of S100B in detecting HIE has been studied in few small cohorts but the results showed discrepancies (15)(16). S100B protein has been described as a good clinical marker for detecting subdural hematomas in the context of mild traumatic brain injury (17). Of note, serum S100B concentration is high at birth and physiologically decreases during the first years of life with a sex-related change and in correlation with height growth velocity (18). In 58 children (30 terms and 28 premature infants), cord blood S100B concentration was found to be inversely correlated with gestational age at birth (19). Establishing S100B serum

concentration reference values in children has been shown to help the management of mild traumatic brain injury (20). However, S100B reference values in cord blood remain to be determined.

The aim of this work is twofold: 1) to establish reference intervals of S100B in cord blood in premature and term neonates and 2) to measure S100B concentration in cord blood as a mean to screen for neurological complications in neonates admitted at the neonatal intensive care unit (NICU).

MATERIALS AND METHODS

Study design and patients

This is a study of the performance of S100B protein for screening and identifying neonates at risk of neurological complications hospitalized in a NICU, combining biological data and serum cord blood measurement of S100B from an already constituted prospective biobank for which arterial cord blood was collected. At first, we established cord blood serum S100B reference intervals from preterm and full-term infants without neurological complications. Our project took advantage of a research program funded by the CIHR Institute of Human Development, Child and Youth Health Initiative (Grant Number: NRFHPG-78880). An extensive clinical database (environmental, psychosocial, socio-demographic and anthropometric, clinical personal and family history data) was developed with a comprehensive biobank (blood samples including umbilical cord) from pregnant women recruited prospectively from the first trimester/early second trimester and followed during pregnancy. The biobank includes samples from 7,866 pregnant women recruited at the CHU de Québec between April 2005 and March 2010 and followed during pregnancy until delivery (21). Participants gave their informed written consent and the study was approved by the Ethics Committee of the CHU de Québec (initial approval date: November 9, 2004, project 5-04-10-01 [95.05.17], SC12-01-159). All neonates who were hospitalized in a NICU for which an umbilical cord serum sample stored in the biobank described below were included (case group). This cohort includes 272 babies (3.5% of the total cohort) hospitalized in a NICU: 84 premature infants

and 188 full-term infants. To establish S100B reference values in cord blood sera from term infants, we selected 200 babies with equal proportions of gender (girls / boys) and mode of delivery (vaginal / caesarean). For these 200 babies, inclusion criteria were: birth after 37 weeks of pregnancy, APGAR score at 1 or 5 min > 8, duration of labor < 15 hours (a known criterion for increasing serum S100B level (22)), no pregnancy and delivery complications (hypertensive disorders, gestational diabetes mellitus, abruptio placentae, intrauterine growth restriction, chorioamnionitis, NICU hospitalization). To establish S100B reference values in cord blood sera from premature infants (i.e. delivery < 37 weeks gestation), all neonates who were premature without neurological complications and had not been hospitalized in a NICU for which an umbilical cord serum sample was stored in the biobank were also included. This group included 183 preterm infants. In total, S100B protein was assayed on 655 samples (272 cases hospitalized in a NICU and 383 controls for reference ranges).

Data collection

The data collected were: gestational age at birth, maternal age, smoking during pregnancy, hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM), abruptio placentae, intrauterine growth restriction (IUGR), multiple pregnancies, chorioamnionitis, mode of delivery, use of instrument (forceps, suction cup) during vaginal delivery, gender of newborn, birthweight and length of newborn, head circumference, APGAR scores and blood gas analysis results. Diagnosis of brain injury relied on imaging examination. Diagnosis of hypertensive disorders of pregnancy (HDP) was made by a senior obstetrician according to the classification of the Society of Obstetricians and Gynaecologists of Canada based on information retrieved from medical records, which includes gestational hypertension (GH) and preeclampsia. GH was defined as de novo hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) after 20 weeks of pregnancy. Preeclampsia was defined as GH with proteinuria (≥300 mg in a 24-hour urine collection or ≥2+ on dipstick in a random sample) or pre-existing hypertension and new or worsening proteinuria. Gestational diabetes mellitus (GDM) diagnosis was

established according to the Canadian Diabetes Association 2013 Clinical Practice Guidelines (23). IUGR was defined as birth weight and/or birth length below the 10th percentile for gestational age (24). To check for brain injury, cerebral ultrasound scanning was performed on newborns clinically suspected of brain injury (seizures …) within 72 h of birth, and the scan was repeated serially at 7-d intervals until hospital discharge. If cranial ultrasound showed no structural abnormalities but suspected intracranial lesions, brain magnetic resonance imaging (MRI) examination was performed to confirm the diagnosis of brain injury. Experienced radiologists in neonatal imaging interpreted the neonatal scans. Brain injury included PVL, IVH, HIE, subdural haematoma, stroke, oedema and hydrocephalus. The classification of IVH was as follows: grade 1 (bleeding confined to periventricular area); grade 2 (intraventricular bleeding with 10-50% of ventricular area); grade 3 (intraventricular bleeding with >50% of ventricular area or distended ventricule); grade 4 (periventricular hemorrhagic infarction) (25). HIE was diagnosed by MRI by either white matter injury extending to the cortical areas or deep gray nuclei injury in the basal ganglia or thalamus, or involvement in both areas (26). Neonates with suspect HIE were classified into the 3 categories according to Sarnat stage (mild, moderate, and severe)(27). PVL refers to necrosis of cerebral white matter (8).

Serum S100B measurement

Arterial cord blood samples were processed to serum, aliquoted and deep frozen at −80 °C within 2 hours of sampling until assayed. We determined serum S100B concentrations by electro-chemiluminescence immunoassay on a Roche Diagnostics Cobas e602® instrument (Roche Diagnostics, Laval, Canada). The S100B calibration curve is linear up to 39 μg/L, and the coefficient of variation for duplicates across the entire concentration range for calibrators and samples was 3.1%. The lower limit of detection of the assay was set at 0.005 μg/L, as given in the manufacturer's instructions. As S100B measurement has been performed retrospectively, the results of S100B had no effect on the clinical management of the neonates.

Statistics

Sample size for determination of S100B reference values was calculated according to Gazzolo *et al.* (19) which reports standard-deviation (SD) of cord blood S100B concentrations ranging between 0.35 and 0.64 µg/L. Using this SD, it was considered that at least 350 patients were needed to highlight an effect-size greater than 0.3 (i.e. a between-groups difference around 0.10 for SD=0.35) for a two-sided type I error of 5% and a statistical power greater than 80%.

The categorical variables were presented as numbers and associated percentages, whereas the continuous variables were expressed as mean ±standard deviations, or median and interquartile range (IQR) and range (minimum – maximum). The normality assumption was assessed graphically and using the Shapiro-Wilk's test. For the control group used to establish reference ranges, the 95^{th} percentile was also calculated. To study the relationships between quantitative parameters, correlation coefficients (Pearson or Spearman according to the statistical distribution) were calculated. The comparisons between groups (case versus control group) were carried out using the Chi-squared test or Fisher's exact test when appropriate, for categorical data, and using the Student's t-test or the Mann-Whitney's test when the assumptions of t-test were not met for continuous data. In control group, the comparisons concerning S100B according to gestational age, gender of neonate, mode of delivery, smoking during pregnancy and presence of neonatal jaundice were performed with same inferential statistical tests. Multivariable analysis was carried out using multiple linear regression with logarithmic S100B as the dependent outcome and the following covariates: IUGR, GDM, HDP, maternal age, use of instrument during delivery, parity, gestational age at birth, gender, mode of delivery, placenta abruptio, smoking during pregnancy and neonatal jaundice. For the comparison between patients with and without brain injuries, the best threshold of S100B and arterial pH was determined by ROC curve analysis estimating usual Youden and Liu indexes. Area under the curve (AUC), sensitivity, specificity, positive and negative values were calculated and presented with 95% confidence intervals (95%CI). The results were

graphically presented with forest-plots. Sensitivity and specificity of S100B and arterial pH were compared using McNemar's test for paired proportions. Then, the combination of S100B and arterial pH was developed, studying these two parameters as continuous or binary variables according to cut-off determined as described before. To conclude, multivariable analysis using exact logistic regression model was performed to study the association between brain injuries and the combination of S100B and arterial pH (S100B+pH) adjusting for covariates fixed according to univariate results and to their clinical relevance: smoking during pregnancy, gestational age at birth, mode of delivery, GDM, HDP, IUGR, multiple pregnancy, chorioamnionitis, maternal age and placenta abruptio. Attention was paid on the study of interactions between significant factors and on the presence of multicollinearity. The model was validated by a two-step bootstrapping process. For each step, bootstrap samples with replacements (n=1000) were generated from the training set. In the first phase, the percentage of models including each initial variable was determined by usual stepwise approach. Then, in the second phase, parameters of generalized linear regression (exact logistic regression) of the final model were independently estimated. The bootstrap estimates associated to each covariate regression coefficient, and their associated standard errors, were finally averaged from replicates. Statistical analyses were carried out using STATA software (version 12, StataCorp, College Station, US). A two-sided p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Patients and serum S100B median concentrations

In the case group, 272 neonates who were hospitalized in a NICU, included 84 premature infants and 188 full-term infants. In control group (to establish S100B reference values for cord blood sera), 383 neonates were selected: 200 full-term neonates and 183 preterm infants (Table 1). The proportion of neonates from multiple pregnancies was significantly higher in the case group than in the control group

(14% and 4.4% respectively, p<0.0001)(Table 1). Cases weighed significantly less than neonates from the control group (3068 g (min: 660, max: 5118, IQR: 2537-3528) and 3155 g (min: 1856, max: 4676, IQR: 2719-3548) respectively, p=0.02)(Table 1). The median head circumference was significantly (p=0.002) smaller among cases (33.5 (min: 26, max: 39, IQR: 32-34.6)) than controls (34 (min: 28, max: 49, IQR: 33-35))(Table 1). Arterial and venous pH were significantly (p=0.002 and 0.003 respectively) lower in cases (7.27 and 7.33 respectively) than in controls (7.29 and 7.35 respectively)(Table 1). The case group median concentration of S100B was 0.51 µg/L (min: 0.16, max: 11.48, IQR: 0.36-0.82), while the control group median concentration of S100B was 0.37 µg/L (min: 0.08, max: 5.64, IQR: 0.29-0.54). The differences between these 2 groups were statistically significant (p<0.001)(Table 1).

Reference ranges for S100B cord blood serum in control group

The control group median concentration of S100B was 0.37 µg/L (min: 0.08, max: 5.64, IQR: 0.29-0.54)(Table 1). There were no significant differences in S100B median concentration according to gender of neonate (p=0.66), smoking during pregnancy (p=0.87), presence of neonatal icteria (p=0.07) and parity (p=0.67)(Table2). With multivariable analysis, the parity has a significant impact on the S100B concentration (p=0.04)(Table 2). Cord blood S100B concentration was found to be inversely correlated with gestational age at delivery (r=-0.22, p<0.001) and was significantly higher in premature neonates than in full-term neonates (0.42 and 0.34 µg/L respectively, p<0.001))(Table 2). In premature neonates, there was no significantly difference (p = 0.11) of S100B concentration between gestational age subgroups (34-35 weeks (n = 13), 35-36 weeks (n = 35), 36-37 weeks (n = 135)). S100B median concentration was significantly different according to the mode of delivery being greater vaginal delivery (p<0.001)(Table 2). With multivariable analysis, the effect of the delivery mode on the S100B concentration is no longer significant mainly because of the criterion “use of instrument”. Indeed, S100B median concentration was significantly different according to the use of instrument (forceps, suction cup)

during vaginal delivery (p<0.001)(Table 2). The maximum normal serum S100B reference level (95th percentile) was 1.21 µg/L for the whole control group, 0.96 µg/L for the full-term subgroup and 1.36 µg/L for the premature subgroup (Table 2).

S100B, arterial pH and neurological complications in case group

In the case group, 16 neonates (5.9%) presented a neurological complication (Table 1). The 5 patients with IVH were all with a grade 1 and all evolved satisfactorily without interventions and were promptly discharge from the NICU. For the following analyses, they were removed from the subgroup of cases with neurological complications.

The best threshold using Youden and Liu indexes was >1.27 µg/L for S100B and <7.25 for arterial pH.

Using a threshold of 1.27 µg/L, we observed a significant capacity (p=0.006) of S100B to differentiate neonates with and without neurological complications with an AUC of 0.67 (95%CI: 0.52-0.83)(Figure 1), a sensitivity of 45.5% (95%CI: 16.7-76.6), a specificity of 88.9% (95%CI: 84.4-92.4), and a negative predictive value of 97.5% (95%CI: 94.6-99.1)(Figure 2).

Using a threshold of 7.25, we observed a significant capacity (p=0.046) of arterial pH to differentiate neonates with and without neurological complications with an AUC of 0.68 (95%CI: 0.55-0.82)(Figure 1), a sensitivity of 80% (95%CI: 44.4-97.5), a specificity of 56.6% (95%CI: 52.1-62.9), and a negative predictive value of 98.6% (95%CI: 94.9-99.8)(Figure 2).

After combining S100B and arterial pH, we observed a significant capacity (p=0.01) of the bivariate model to differentiate neonates with and without neurological complications with an AUC of 0.76 (95%CI: 0.62-0.90)(Figure 1), a sensitivity of 90.9% (95%CI: 58.7-99.8), a specificity of 51.2% (95%CI: 44.8-57.7), a negative predictive value of 99.4% (95%CI: 95.7-100)(Figure 2). The sensitivity is significantly (p=0.03) increased in comparison to S100B alone, but not to arterial pH, while the specificity is significantly higher with S100B alone than with pH alone (p<0.001) or pH + S100B

(p<0.001)(Figure 2). The diagnostic performance of the S100B seems excellent for diagnosing periventricular leukomalacia with values at 1.67 and 11.48 µg/L (Table 3).

Exact logistic regression multivariable analysis applying a bootstrap procedure confirmed the association between brain injuries and the combination of S100B and arterial pH in cord blood, independently from smoking during pregnancy, gestational age birth, mode of delivery, GDM, HDP, IUGR, multiple pregnancy, chorioamnionitis, maternal age and placenta abruptio (p=0.03).

DISCUSSION

Our prospective study, based on 655 neonates, demonstrates the potential interest of measuring S100B protein cord blood concentrations for the management of neonates hospitalized in a NCIU. This is the first study to report reference ranges for serum S100B protein in cord blood that, as of yet, is the largest published population of healthy neonates.

In the 383 neonates from the control group, the 95th percentile of S100B concentration in cord blood was established as 1.21 µg/L, which is higher than the 95th percentile S100B values reported for children aged under 4 months (0.51 µg/L) using the same technology (28). As was previously observed, in a cohort of 58 children (19), cord blood S100B concentration was found to be inversely correlated with gestational age at birth. The serum S100B reference levels (95th percentile) were derived for 2 gestational age groups: 0.96 µg/L for full-term neonates (≥ 37 weeks); 1.36 µg/L for premature neonates (< 37 weeks). We confirmed an effect of the mode of delivery on S100B serum concentrations as previously described by Schulpis *et al.* (22). However, unlike this previous study, we showed that this difference is found even when the duration of labor is <15 hours. The evidence of this effect is probably explained by the size of our cohort (146 caesarean deliveries and 237 vaginal deliveries in our cohort against 22 and 20 respectively by Schulpis *et al*). After multivariate analysis, this effect is probably more related to the use of instruments (forceps, suction cup) during vaginal delivery than to the delivery mode itself. Indeed, we

have highlighted a significant link between the use of instrument and the elevation of S100B serum concentrations. We did not find any impact of neonatal jaundice or maternal smoking on the S100B concentration in the cord blood but we observed an impact of parity. Furthermore, we did not find any association with smoking, which contradicts a previous study (29). The effect of gender was not observed. Gazzolo *et al.* highlighted a sex-related change of pediatrics concentrations of S100B protein in blood in a large cohort of 1004 children aged 0-15 years. Note, however, that this difference was not significant in the subgroup of children aged 0 to 2 years (18). A sex-related change of 0.01 µg/L was also observed in maternal blood in third trimester (30).

Among the 272 neonates from the case group necessitating admission at the NICU, the threshold combining the best compromise of sensitivity / specificity to differentiate neonates with and without neurological complications was 1.27 µg/L for S100B, which validates our upper reference value of 1.21 µg/L determined from the control group. The sensitivity of 45.5% is significantly increased to 90.9% when combining S100B to arterial cord blood pH which is already routinely determined. The only case of a false negative result, which showed a slight subdural hematoma of the cerebellum, did not present any aggravation during hospitalization. A link between high S100B and decreased pH in cord blood has recently been observed in another context (31). We demonstrate here a complementarity of these two biological parameters. The S100B protein alone is however more specific, which is understandable in view of its cellular origin (10). However, Roche Diagnostics instruments dose both of ββ or αβ dimers and only ββ dimer is strongly expressed by the nervous system (32). In view of these results, one could consider an algorithm using cord blood S100B coupled to the arterial cord blood pH to improve sensitivity and to reduce the occurrence of false positive results. S100B appears to be an interesting candidate predictor of PVL at birth. For the other complications (hydrocephalus, HIE, subdural haematoma, stroke, oedema), sensitivity can be improved at birth, i.e. potentially 1-2 days before

neurological complication, after combination with arterial pH, or by serial measurements over time or at the onset of clinical symptoms as described in others studies (33). The latter has yet to be demonstrated. The low number of neurological complications and their heterogeneity represent a constraint to the generalization of our study and need to be confirmed. However, exact logistic regression multivariable analysis applying a bootstrap procedure confirmed the association between brain injuries and the combination of S100B and arterial pH in cord blood and is a strength of our work.

The diagnostic performance of S100B could possibly be further improved by combining S100B with other potential biomarkers of brain injury, namely glial fibrillary acidic protein (GFAP) or ubiquitin c-terminal hydrolase-L1 (UCH-L1). The interest of GFAP and UCHL1 in detecting HIE has been studied in small cohorts of children but the results were discrepant (34)(35)(36).

In conclusion, we provided reference values of S100B protein in cord blood and showed that the diagnostic performance of S100B in combination with arterial cord blood pH has the potential to help clinicians to predict at birth the occurrence of neurological complication in at-risk neonates hospitalized in a NCIU.

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3 **TABLE 1**

Characteristics		Case group	Control group	p
n (%)		272 (100)	383 (100)	/
Sex ratio M (%) / F (%)		148 (54.4) / 124 (45.6)	206 (53.8) / 177 (46.2)	0.87
Mean age of mothers (SD) in years		30.6 (4.2)	30.3 (4.7)	0.36
Neonates with multiple pregnancies (%)		38 (14)	17 (4.4)	<0.001
Cesarean (%)		89 (32.7)	146 (38.1)	0.16
Distribution by gestational age at birth	Full-term neonates ≥ 37 weeks (%)	188 (69.1)	200 (52.2)	<0.001
	Premature neonates 34-37 weeks (%)	63 (23.2)	183 (47.8)	
	Premature neonates < 34 weeks (%)	21 (7.7)	0	
Anthropometric characteristics of neonates	Median of weight (min, max, IQR) in g	3068 (660, 5118, 2537-3528)	3155 (1856, 4676, 2719-3548)	0.02
	Median of size (min, max, IQR) in cm	50 (30.5, 59, 47-53)	50 (32, 58, 48-52)	0.18
	Median of head circumference in cm	33.5 (26, 39, 32-34.6)	34 (28, 49, 33-35)	0.002
APGAR 5 minutes	0 to 7 (%)	45 (16.5)	1 (0)	<0.001
	8 to 10 (%)	227 (83.5)	382 (100)	
Neonates with one or more neurological complications (%)		16 (5.9)	0	<0.001
Brain injuries findings	IVH grade 1	5	0	/
	PVL	2	0	/
	HIE Sarnat 1	1	0	/
	Subdural haematoma	5	0	/
	Stroke	2	0	/
	Oedema	3	0	/
	Hydrocephalus	2	0	/
Biological data	Median of arterial pH (min, max, IQR)	7.27 (7.02, 7.52, 7.21-7.31)	7.29 (7.03, 7.54, 7.24-7.32)	0.002
	Median of venous pH (min, max, IQR)	7.33 (7.08, 7.47, 7.29-7.37)	7.35 (7.09, 7.54, 7.31-7.37)	0.003
	Median of cord blood S100B (min, max, IQR) in µg/L	0.51 (0.16, 11.48, 0.36-0.82)	0.37 (0.08, 5.64, 0.29-0.54)	<0.001

4

TABLE 2

Group		n	S100B in µg/L			p^1	p^2
			Median	IQR	95th percentile		
Total		383	0.37	0.29-0.54	1.21	/	/
According to gestationnel age	Full-term neonates ≥ 37 weeks	200	0.34	0.26-0.45	0.96	<0.001	0.02
	Premature neonates < 37 weeks	183	0.42	0.32-0.62	1.36		
According to gender of neonate	Female	177	0.37	0.31-0.54	1.17	0.66	0.53
	Male	206	0.37	0.28-0.54	1.27		
According to the mode of delivery	Cesarean delivery	146	0.34	0.25-0.44	0.83	<0.001	0.54
	Vaginal delivery	237	0.40	0.32-0.59	1.27		
According to smoking during pregnancy	Non-smoking mother	319	0.37	0.29-0.54	1.26	0.87	0.05
	Smoking mother	50	0.36	0.28-0.54	1.17		
According to the presence of neonatal jaundice	Absence	305	0.36	0.28-0.51	1.16	0.07	0.70
	Presence	55	0.42	0.30-0.65	1.17		
According to parity	Nulliparity	173	0.40	0.29-0.56	1.09	0.67	0.04
	Multiparity	210	0.36	0.28-0.53	1.27		
According to the use of instrument (forceps, suction cup) during vaginal delivery	No use	190	0.37	0.30-0.54	0.57	<0.001	0.002
	Use	30	0.55	0.44-0.94	1.02		

TABLE 3

Patient	Sexe	Gestational age at delivery	Mode of delivery	Brain injuries						S100B (µg/L)	Arterial pH
				PVL	Hydrocephalus	HIE	Subdural haematoma	Stroke	Oedema		
1	M	37.29	vaginal	0	1	0	1	0	0	0.20	7.24
2	F	39.71	vaginal	0	0	1	0	0	1	0.45	7.23
3	M	38	vaginal	0	0	0	1	0	0	0.48	7.35
4	F	41.14	cesarean	0	0	0	0	1	1	0.57	7.25
5	F	40.86	vaginal	0	0	0	1	0	0	0.59	7.12
6	M	38.86	vaginal	0	0	0	1	0	0	0.67	7.08
7	M	40.71	cesarean	0	0	0	0	0	1	1.27	7.02
8	F	40.57	vaginal	0	1	0	0	0	0	1.34	7.28
9	M	33.29	vaginal	1	0	0	0	0	0	1.36	/
10	M	40	vaginal	0	0	0	0	1	0	1.67	7.06
11	F	39	vaginal	1	0	0	1	0	0	11.48	7.21

FIGURES AND TABLES LEGEND

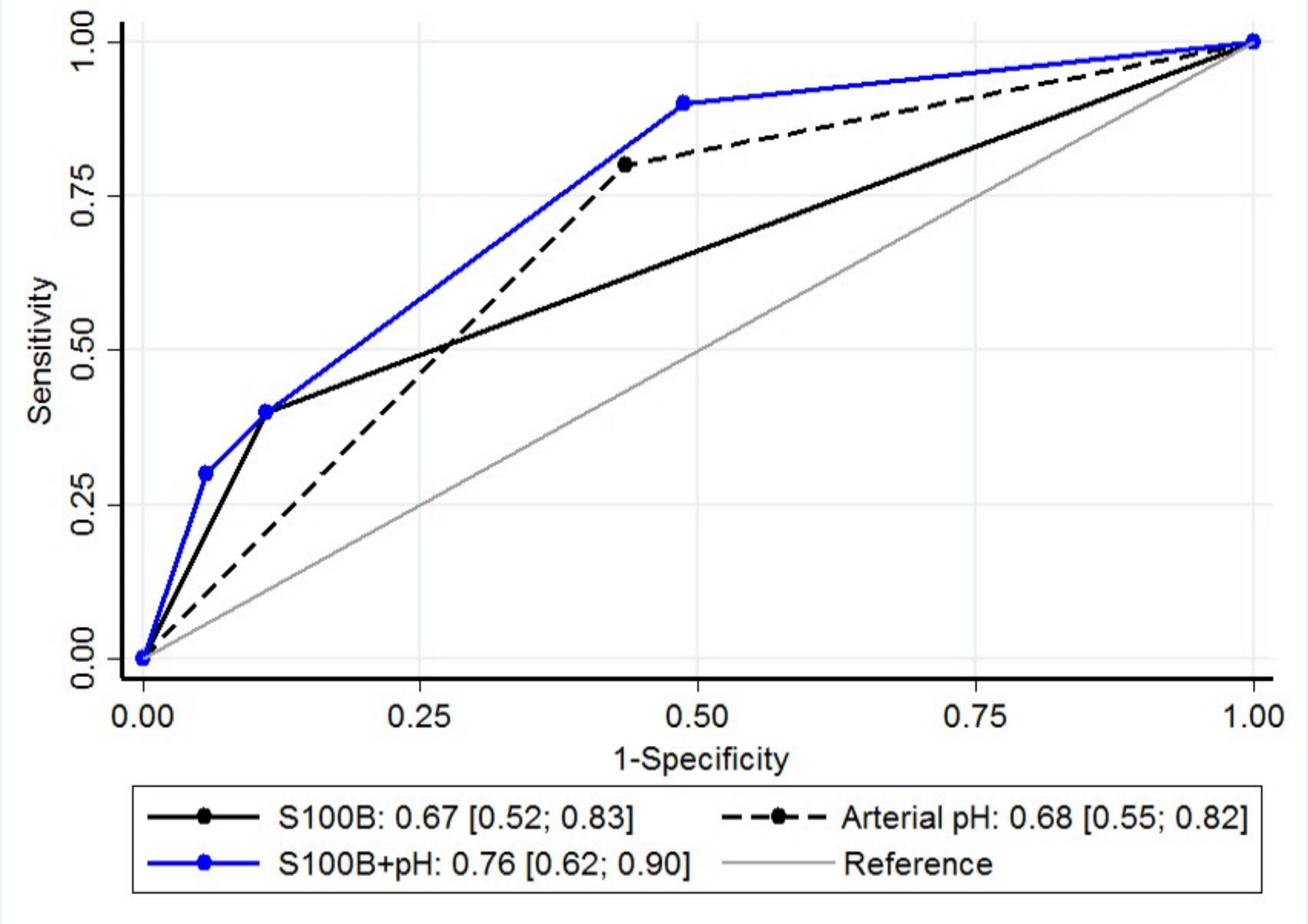
Table 1: Clinical and biological characteristics of the cohort. All neonates from the case group were hospitalized in a neonatal intensive care unit. A two-sided p value of less than 0.05 was considered to indicate statistical significance. IVH: intraventricular haemorrhage; HIE: hypoxic ischaemic encephalopathy; IQR: interquartile range; PVL: periventricular leukomalacia.

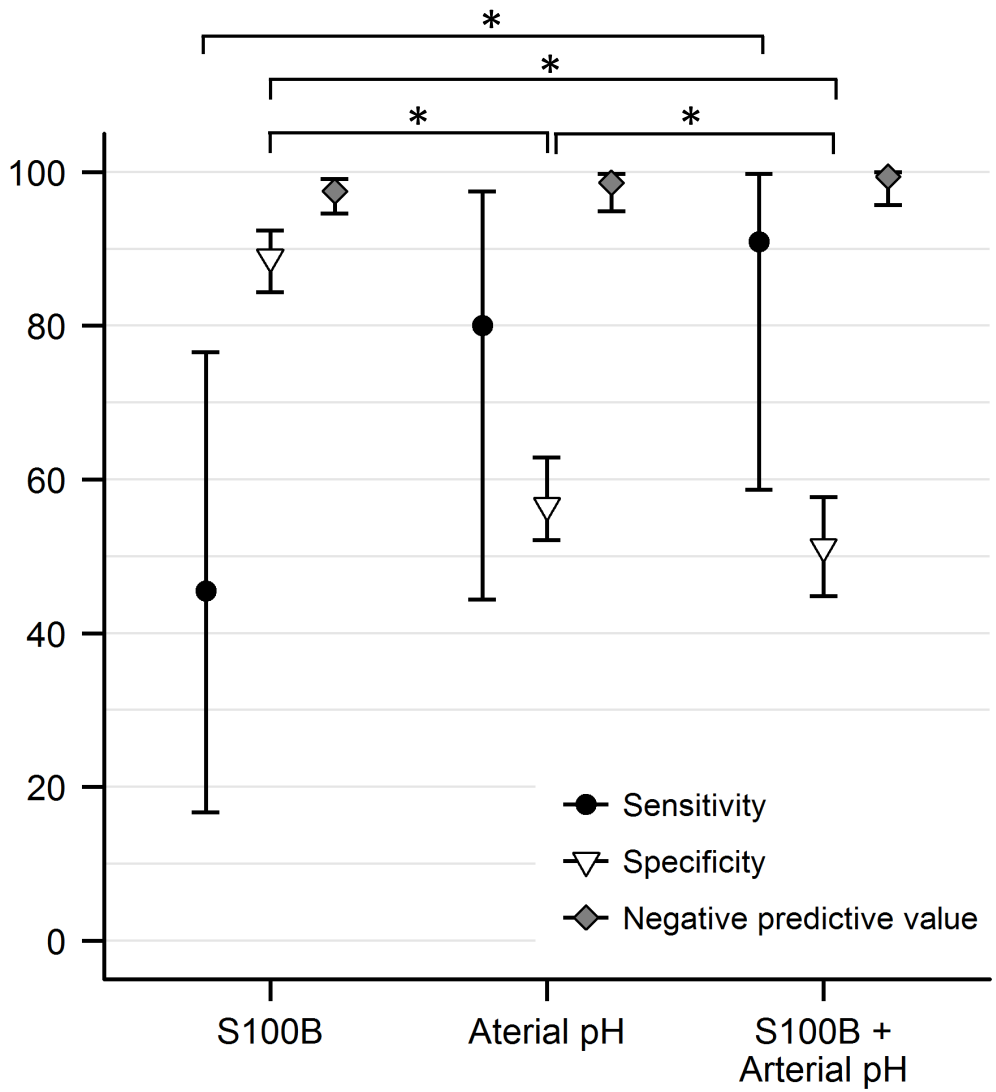
Table 2: Cord blood S100B levels and distribution in neonates from control group. Serum S100B concentrations are shown according to different condition defined by the number of samples (n), median, interquartile range (IQR) and 95th percentile. p^1: Mann and Whitney test; p^2: Multivariate analysis with intrauterine growth restriction, gestational diabetes mellitus, hypertensive disorders of pregnancy, maternal age, use of instrument during delivery, parity, gestational age at birth, gender, mode of delivery, placenta abruptio, smoking during pregnancy and neonatal jaundice. A two-sided p value of less than 0.05 was considered to indicate statistical significance.

Figure 1: ROC curve of S100B (> 1.27 µg/L), arterial pH (< 7.25) and S100B+pH (> 1.27 µg/L and/or < 7.25 respectively) measurements in cord blood for the discrimination of neurological complications in neonates hospitalized in a neonatal intensive care unit (case group). The areas under the curve are expressed with 95% confidence intervals.

Figure 2: Forest plot showing sensitivity, specificity and negative predictive value of S100B (> 1.27 µg/L), arterial pH (< 7.25) and S100B+pH (> 1.27 µg/L and/or < 7.25 respectively) measurements in cord blood for the discrimination of neurological complications in neonates hospitalized in a neonatal intensive care unit (case group). *: $p<0.05$.

Table 3: S100B and arterial pH values of eleven neonates with neurological complications. IVH: intraventricular haemorrhage; HIE: hypoxic ischaemic encephalopathy; PVL: periventricular leukomalacia.


Sensitivity
0.00
0.25
0.50
0.75
1.00
1-Specificity
0.00
0.25
0.50
0.75
1.00
S100B: 0.67 [0.52; 0.83]
Arterial pH: 0.68 [0.55; 0.82]
S100B+pH: 0.76 [0.62; 0.90]
Reference


*
*
*
*
100
80
60
40
20
0
Sensitivity
Specificity
Negative predictive value
S100B
Aterial pH
S100B +
Arterial pH