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Reference percentiles for paired arterial and venous umbilical cord blood gases: An indirect nonparametric approach

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ABSTRACT

Background: Reference intervals for arterial and venous umbilical cord blood gas (UCBG) parameters are scarce, are mainly focused on pH, pO2, pCO2 and base deficit, and are usually assessed using parametric tests, despite a generally skewed data distribution. Here, the purpose is to determine reference percentiles for nine parameters of concomitant arterial and venous UCBG (CAV-UCBG) from neonates at birth, using nonparametric tests.

Methods: Results of CAV-UCBG, assayed over a 4.5-year period, were extracted from a hospital laboratory database for pH, pCO2, pO2, oxygen saturation, concentration of total oxygen, total carbon dioxide, hydrogen carbonate, total haemoglobin, and acid-base excess. Exclusion criteria were: a venous–arterial pH difference < 0.02, an arterial–venous pCO2 < 0.7 kPa, and a venous pCO2 < 2.9 kPa. Nonparametric bivariate kernel density estimations were used for the selection of plots within the 95% percentile surface of the pCO2-to-pH relationship (NBKDE-95P). Outliers from skewed data were removed using an adjusted-Tukey method, and percentiles were calculated according to the CLSI EP28-A3 nonparametric method.

Results: Overall, 31% (5033/16164) of CAV-UCBG were discarded using the three exclusion criteria. Then, 6% (670/11131) of CAV-UCBG were excluded from the NBKDE-95P, and 0.1 to 3.5% outliers were subsequently removed. Depending on the parameter, the 2.5th and 97.5th percentiles from the whole group were similar or slightly narrower compared to reference intervals from other studies, while those from female and male neonates did not differ substantially.

Conclusions: Using an indirect nonparametric approach, this study proposes new percentiles for parameters from concomitant arterial and venous umbilical cord blood gases.

1. Introduction

During pregnancy, the foetus receives oxygen and nutrients from the mother's arterial blood through the umbilical vein, by diffusion across the placenta. The deoxygenated foetal blood gets back to the placenta via two umbilical arteries, for removal of carbon dioxide (CO2) and metabolic waste from the maternal circulation by the lungs and kidneys. Hence, at birth, the venous umbilical cord blood (UCB) reflects the maternal-placental acid-base status and is richer in oxygen than the arterial UCB, which is richer in CO2, and with a lower pH reflecting the neonatal acid-base status. The composition of venous UCB is closer to that of adult arterial blood than that of arterial UCB, even though pH and partial pressure of oxygen (pO2) levels from both arterial and venous UCB are much lower [1]. Umbilical cord blood gas (UCBG) and acid-base assessment are considered as the most objective and accurate way to explore the foetal metabolic status at birth. Mainly, it reveals foetal exposure to hypoxia and associated metabolic acidosis that may potentially lead to encephalopathy and cerebral palsy, notably at arterial pH levels < 7 with a base deficit ≥12 mmol/L [2,3]. As recommended by the American College of Obstetricians and Gynecologists (ACOG), an arterial UCBG analysis is required at birth. When possible, its paired venous sample should also be analysed, especially for caesarean delivery for foetal risk, low 5-minute Apgar score, severe growth restriction, abnormal foetal heart rate tracing, maternal thyroid disease, intrapartum fever, and multifoetal gestations [3]. These recommendations differ between countries, some of which recommend

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umbilical blood sampling for all deliveries [4].

Given the importance of UCBG at birth, some studies have proposed reference intervals for arterial and venous parameters, and mainly for pH, pCO2, bicarbonate, acid-base excess (ABE), and pO2 [4–6]. However, these studies did not take into consideration the need for validation of paired arterial and venous UCBG, brought to light by Westgate et al. in 1994, whose observational study showed that only ~75% of supposedly paired samples had validated pH and pCO2 data from both an artery and the vein, i.e. ~25% of erroneous values [7]. Some studies have used the Westgate et al. data as quality indicators [8–10], but most of them have ignored the importance of such validation. In 2010, Kro et al. have considered this requirement for the establishment of percentiles of arterial pCO2, determined for specific values of arterial pH based on nearly 27000 neonates from two trials of foetal monitoring and from a Swedish hospital database, all selected from pregnancies of at least 36 weeks of gestation, regardless of birth weight, Apgar score, or type of delivery [11]. Kro et al. have determined the 2.5th, 5th, 50th, 95th, and 97.5th percentiles of umbilical cord artery pCO2 for specified pH values, which they proposed as an identification tool for cases with erroneously low pCO2 values, to avoid an incorrect interpretation of new-born acid-base status. To this end, they selected results from paired arterial and venous cord pH and pCO2 samples, from which they established three exclusion criteria: (1) a venous-arterial pH difference < 0.02 corresponding to the 5th percentile for pregnancies of at least 36 weeks of gestation, (2) an arterial-venous pCO2 difference < 0.7 kPa corresponding to the 10th percentile of the population with 38–40 weeks of gestation, and (3) a venous umbilical cord pCO2 < 2.9 kPa, given as the lower limit of the maternal mean arterial pCO2 during labour [12]. Applying these criteria for the selection of an arterial pCO2 considered as being within the normal limits according to pH, they concluded that acid-base values were incorrect or unreliable in one out of seven cases, thus recommending their tool for error checking in clinical practice. So far, although these criteria have been used and/or cited in some studies [13–15], they have never served as a basis for the determination of UCBG percentiles. Furthermore, Kro et al. focused on pH, pCO2 and base deficit only, from which percentiles were calculated based on a fractional polynomial regression model rather than non-parametric methods, which are more appropriate for skewed data. Moreover, they did not provide specifications regarding their outlier removal strategy, which may have an impact on final reference intervals [16].

The aim of the present study is therefore to determine the percentiles for nine arterial and venous parameters of CAV-UCBG from female and male neonates at birth, based on the three exclusion criteria from Kro et al. [11], and using nonparametric methods for the selection of pH-specific pCO2 values, for outlier removal from skewed distribution, and for CLSI EP28-A3-based percentile calculation.

2. Material and methods

2.1. Data extraction and assays

Concomitant arterial and venous UCBG (CAV-UCBG) results from the biochemistry laboratory (Lyon East Hospital, Hospices Civils de Lyon, France) were extracted from the Laboratory Information System (GLIMS® Software, MIPS-CliniSys, Chertsey, Surrey, UK) over a 4.5-year period (January 2014 to June 2018). The following parameters were measured (*ms*) or derived (*dv*): arterial and venous pH (*ms*), partial pressure of carbon dioxide (pCO2, *ms*), oxygen saturation (sO2, *ms*), partial pressure of oxygen (pO2, *ms*), concentration of total oxygen (ctO2, *dv*), total carbon dioxide (*c*tCO2, *dv*), hydrogen carbonate ($cHCO3^-$, *dv*), total haemoglobin (*c*tHb, *ms*), and acid-base excess (ABE, *dv*).

Over the study period, all measurements were assessed using three ABL800Flex® blood gas analyzers (Radiometer Medical ApS, Denmark), two of which were replaced in April 2018. The imprecision (coefficient of variation, CV%), inaccuracy (Bias%), and total error (TE% = |Bias%| + 1.65 × CV%) were determined for three quality control levels from Radiometer, assayed over a 5-month period. Measurement uncertainties (MUs, expanded with the coverage factor $k = 2$) were determined for measured parameters, from 2014 to 2018, according to the external quality assurance (EQA) program from ProBioQual (Lyon, France), which use the long-term MUs method [17,18], applicable to parameters with at least 8 monthly reports, as recommended by the NordTest [19]. An inter-analyzer comparison of measured parameters from thirty blood gases (2/3 arterial, 1/3 venous) was assessed using the Passing-Bablok regression [20], with the Cusum test for linearity (linear relationship if $P > 0.1$).

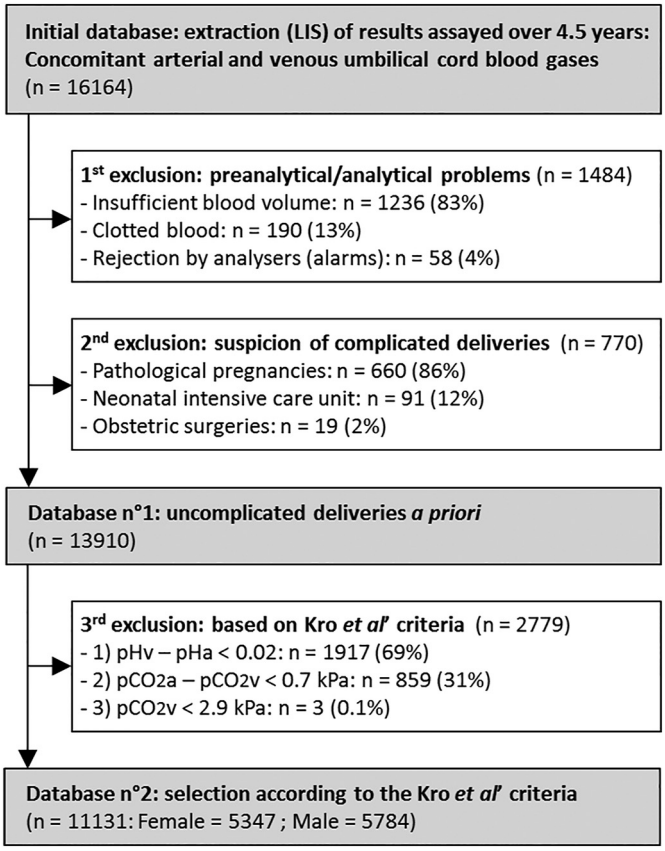

Fig. 1. From the initial data extraction of concomitant arterial and venous umbilical cord blood gases, nearly 9% were excluded because of preanalytical and/or analytical problems, 5% because of suspicion of complicated delivery, and 17% since they did not meet the Kro et al. selection criteria [11]. Abbreviations: LIS: laboratory information system; pCO2: arterial (pCO2a) and venous partial pressure of carbon dioxide (pCO2v); pH: arterial (pHa) and venous pH (pHv).

2.2. Data selection

The first exclusion steps are depicted in Fig. 1. From a total of 16164 CAV-UCBG, 1484 (~9%) were excluded because of preanalytical and/or analytical problems. Then, nearly 5% of CAV-UCBG from units of pathological pregnancies, neonatal intensive care, and obstetric surgery were discarded because of possible complicated deliveries. From the 13910 remaining CAV-UCBG, 2779 (~20%) were excluded according to the three criteria from Kro et al., i.e. excluded if 1) venous–arterial pH difference (pHvad) < 0.02, 2) arterial–venous pCO2 difference (pCO2avd) < 0.7 kPa, and 3) venous pCO2 < 2.9 kPa [11], resulting in a total of 11131 CAV-UCBG, from ~48% of female neonates, and ~52% of male neonates. The second part of the selection is depicted in Fig. 2. Since both arterial and venous pH and pCO2 distributions were skewed,

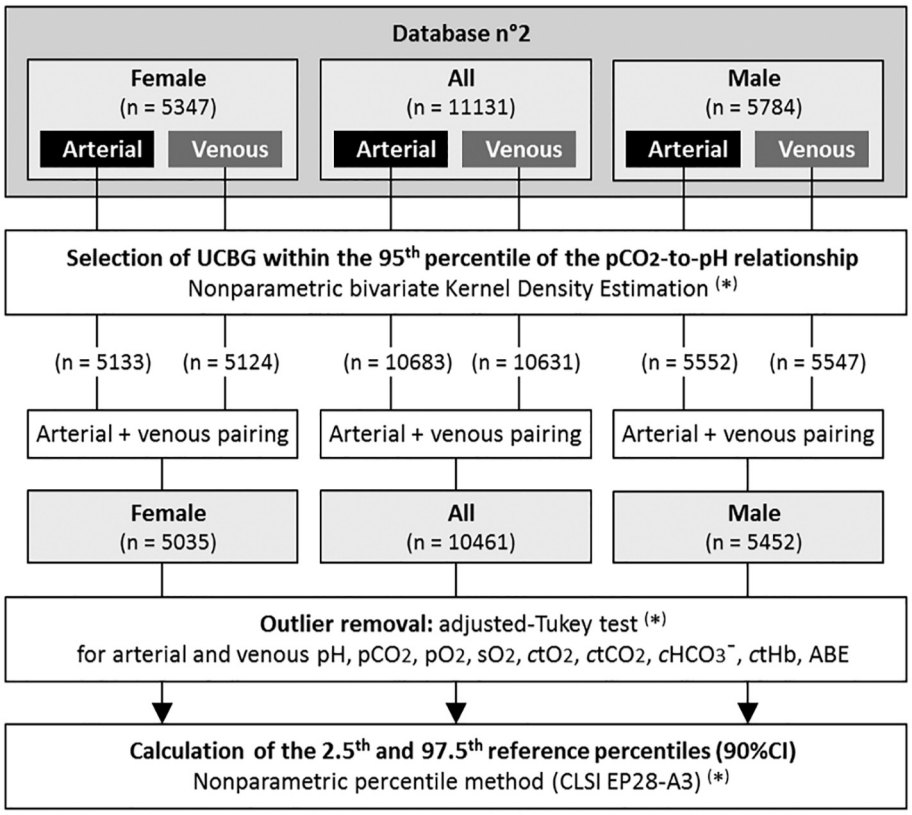

Fig. 2. Arterial and venous concomitant UCBG were selected within the 95% percentile surface of the pCO2-to-pH relationship, for the whole group, female neonates group, and male neonates group, separately. Then, the selected arterial and venous UCBG were paired, for each group. Outliers from data distributions were removed using the adjusted-Tukey test, for the nine arterial and venous UCBG parameters, within each group. Finally, the 2.5th and 97.5th percentiles were calculated for these parameters, using the CLSI EP28-A3 nonparametric method. (*) Kolmogorov–Smirnov test for normal distribution: rejected.

we used a nonparametric bivariate kernel density estimation for the selection of plots within the 95% percentile surface of the pH-to-pCO2 relationship (NBKDE-95P) [21,22], applied separately on arterial and venous data, for the whole, female, and male neonate groups. Then, after exclusion of points outside of the NBKDE-95P, selected arterial and venous UCBG were paired, within each group. The NBKDE-95P smoothed surfaces were assessed using the nonparametric density option from JMP®7.0 software (SAS Institute, Cary, NC, USA), which provides quantile contour lines in 5% intervals.

2.3. Outlier removal and percentiles calculation

Before each of the three steps from the second data selection (Fig. 2), we used a Kolmogorov-Smirnov test (K-S test) for normal distribution, considering a P value < 0.05 as rejected. The data resulting from the NBKDE-95P-based selection were not normally distributed, regardless of the parameters (arterial or venous) or group (whole, female or male neonates); we therefore used the adjusted-Tukey test, as done elsewhere for outlier removal from skewed data [23]. Briefly, this method multiplies the lower (Q1) and upper quartiles (Q3) from the interquartile range (IQR) by a factor based on the medcouple measure of skewness (MC) [24], for the removal of values outside the [Q1–1.5*e^{-4MC}*IQR; Q3 + 1.5*e^{3MC}*IQR] interval limits, which correspond to the skewness-adjusted lower and upper whiskers of the boxplot [25,26]. The adjusted-Tukey test was assessed using the *adjboxStats* tool from the *Robustbase* package (version 0.93-3) on R software (version 3.5.1, R Foundation, Vienna, Austria) [27].

After outlier removal, all data remained not normally distributed, even after Box-Cox transformation; we therefore calculated the 2.5th, 50th, and 97.5th percentiles according to the CLSI EP28-A3 nonparametric method [28], using the MedCalc®14.0 software (MedCalc, Ostend, Belgium). The 2.5th and 97.5th percentiles were provided along with their 90% confidence intervals (90%CI), defined by the rank of the lower limit nq – (1.645√(nq(1–q))) and the rank of the upper limit 1 + nq + (1.645√(nq(1–q))), wherein n is the sample size, and q is the quantile (i.e. 0.025 for the lower reference limit, and 0.975 for the higher reference limit) [29].

3. Results

3.1. Analytical performances

The total errors (TEs) of pH, pCO2, pO2, sO2 and ctHb, along with the long-term measurement uncertainties (LTMUs) are provided in Table 1. The TEs were within the allowable limits criteria from Ricos [30], Clinical Laboratory Improvement Amendments (CLIA) [31] and/or Royal College of Pathologists of Australasia (RCPA) [32], for the three analyzers. Based on EQA evaluations from 2014 to 2018, the expanded LTMUs were, for the first, second and third analyzer, respectively: pH: 0.1, 0.1, 0.1%; pCO2: 5.6, 6.3, 4.7%; pO2: 6.3, 4.8, 5.6%; ctHb: 1.3, 1.2, 1.1%, which were within the allowable TE% from Ricos, CLIA and/or RCPA. Overall, the three analyzers provided comparable results for the measured parameters, with slopes not deviating significantly from 1, and with linearity (Supplemental Table 1).

3.2. Reference percentiles

Overall, ~31% (5033/16164) of CAV-UCBG were discarded after the three exclusion steps, resulting in 11131 CAV-UCBG (Fig. 1). The arterial and venous pCO2-to-pH relationships before the NBKDE-95P-based selection are depicted in Fig. 3A and B, and those after in Fig. 3C and D. As expected, given the skewed pCO2 and pH data, the four graphs display an asymmetric density of plots. Most points outside of the area are located in the most acidic pH range with a higher pCO2, and in a more marked way for arterial UCBG. Considering the whole group, 448 arterial UCBG and 500 venous UCBG were outside of the NBKDE-95P, resulting in 10683 and 10631 arterial and venous UCBG, respectively (Fig. 2). Then, since arterial and venous NBKDE-95P

Table 1
Total error and long-term measurement uncertainty of blood gas parameters measured on the three ABL800®Flex analyzers.

Measured parameter	ABL no.	Total error[a]									Long-term measurement uncertainty[b]			Allowable TE%			Conclusion
		QC Low			QC Medium			QC High			N_{EQA}[d]	Median	MU%	Ricos	CLIA[e]	RCPA[e]	
		N[c]	Mean	TE%	N[c]	Mean	TE%	N[c]	Mean	TE%					Max/Min	Max/Min	
pH	1	60	6.808	0.07	84	7.397	0.10	88	7.565	0.02	37	7.380	0.1	na	0.6/0.5	0.6/0.5	Acceptable
	2	72	6.810	0.10	101	7.396	0.09	101	7.567	0.05	35	7.379	0.1	na	0.6/0.5	0.6/0.5	Acceptable
	3	80	6.807	0.11	103	7.396	0.04	110	7.566	0.03	37	7.381	0.1	na	0.6/0.5	0.6/0.5	Acceptable
pCO2 (kPa)	1	87	2.95	4.92	84	5.38	4.36	62	12.74	2.59	37	5.30	5.6	5.7	23/8	9/8	Acceptable
	2	101	2.87	0.66	101	5.23	0.10	72	12.60	0.88	37	5.37	6.3	5.7	23/8	9/8	Acceptable
	3	110	2.97	5.00	103	5.22	0.23	80	12.61	1.48	37	5.28	4.7	5.7	23/8	9/8	Acceptable
pO2 (kPa)	1	89	9.49	4.91	84	13.28	2.31	56	18.98	1.05	37	16.3	6.3	na	9/4	7/5	Acceptable
	2	101	9.28	2.79	101	13.28	2.31	74	19.09	1.61	36	16.5	4.8	na	9/4	7/5	Acceptable
	3	110	9.49	4.91	103	13.43	3.39	78	19.11	1.71	37	16.5	5.6	na	9/4	7/5	Acceptable
sO2 (%)	1	56	50.0	0.66	89	70.2	0.47	84	97.3	0.55	4	97.8	nd	na	na	na	Acceptable
	2	74	50.0	0.66	101	70.2	0.47	101	97.1	0.34	4	100.0	nd	na	na	na	Acceptable
	3	78	50.0	0.66	110	70.2	0.47	102	97.2	0.44	3	97.9	nd	na	na	na	Acceptable
*c*tHb (g/L)	1	56	82.5	2.49	84	129.0	2.08	89	192.3	1.25	8	135.0	1.3	4.2	7	4/3	Acceptable
	2	74	82.5	2.49	101	129.4	2.39	101	193.4	1.82	8	135.0	1.2	4.2	7	4/3	Acceptable
	3	78	82.2	2.13	103	128.6	1.77	110	192.4	1.30	8	140.0	1.1	4.2	7	4/3	Acceptable

Abbreviations: na: not available; nd: not determined; pH: acidity or alkalinity; pCO2: partial pressure of carbon dioxide; pO2: partial pressure of oxygen; sO2: oxygen saturation; *c*tHb: concentration of total haemoglobin in blood.

[a] Total error (TE%) of the three ABL800®Flex analyzers, calculated as TE% = |Bias%| + 1.65 × CV%, with Bias% (inaccuracy) and Coefficient of variation (CV%, imprecision), determined over a 5-month period, using three levels of quality control (QC) from Radiometer.

[b] Long-term measurement uncertainty (MU%, expanded with the coverage factor k =2) determined from 2014 to 2018, according to the external quality assurance (EQA) program from ProBioQual (Lyon, France) [17,18]

[c] Number of QC values from our laboratory, cumulated over a 5-month period.

[d] Number of EQA evaluations over the study period (2014 to 2018).

[e] Allowable TE% based on the CLIA and RCPA criteria, considering the mean of the lower QC if needed (Max%), and the mean of the higher QC (Min%).

selections were assessed separately, both types of UCBG were paired, leading to 10461 CAV-UCBG. In the same way, the NBKDE-95P-based selection applied on arterial and venous UCBG from female and male neonates resulted in 5035 and 5452 CAV-UCBG, respectively. Overall, the NBKDE-95P-based selection and subsequent arterial and venous pairing excluded ~6% of UCBG from the whole group, as well as from the female and male neonates groups. Thereafter, the adjusted-Tukey-based method led to outlier removal, in proportions ranging from 0.1% (arterial *c*$HCO3^-$, whole group, n = 14/10461) to 3.7% (arterial pO2, male neonates group, n = 202/5452) (Table 2). Reference percentiles of CAV-UCBG parameters are provided in Table 2. Overall, the percentiles were higher in arterial blood for pCO2, *c*tCO2, *c*$HCO3^-$, and slightly higher for ABE, while other percentiles were higher for venous pH, pO2, sO2, *c*tO2, except for *c*tHb which was similar in arterial and venous umbilical blood. Regardless of the UCBG parameter, arterial and venous percentiles did not differ substantially between female and male neonates. As expected given the high volume of data, the 90%CIs are, on the whole, very narrow for most of arterial and venous parameters (Supplemental Table 2).

4. Discussion

This study proposes new reference percentiles for nine arterial and venous UCBG parameters, for the first time determined using non-parametric tests. The study shows that percentiles from female and male neonates are close, supporting the use of common reference intervals.

Many reference values have been proposed for arterial pH (pHa) from UCBG, with low limits ranging from 7.03 [33,34] to 7.18 [35,36], and high limits ranging from ~7.36 [1,37] to 7.43 [38,39], regardless of selection criteria. In accordance, our 2.5th percentile pHa at 7.14 is within the expected ranges, keeping in mind that the probability of intrapartum hypoxia-related neonatal encephalopathy increases for a pHa < 7.0, but decreases progressively up to 7.20, the pHa threshold for which this likelihood is admitted as null [40]. Our 97.5th percentile pHa of 7.36 is rather low; however, it is close to that of 7.38 from Helwig et al. [41] and Westgate et al. [7], who considered –like us– its left-skewed distribution, while most of studies used two standard deviation (SD) above the mean as the upper limit. Regarding venous pH, our percentile range of 7.24 to 7.43 is slightly narrower than those published, which range from 7.13–7.25 [34,35] to 7.44–7.49 [34,42]. This could be due, at least in part, to our NBKDE selection, which excluded more CAV-UCBG with extreme pH than traditional mean ± 2SD or percentiles-based models. In this way, Kro et al. found a 2.5th–97.5th pHa and pHv ranges of 7.06–7.37 and 7.17–7.45, but their fractional polynomial regression model used for the pCO2-to-pH selection did not exclude extreme pH, with the exception of pHa < 6.86 and > 7.44, on the argument that the number of values was too low for percentile calculation [11]. Besides a pH < 7.0, a base deficit ≥12 mmol/L is the other essential criterion for suspecting a hypoxia-associated metabolic acidosis [1,2]. In our study, the arterial base deficit (BDa) 2.5th percentile of –6.9 mmol/L is higher than the usual cut-offs, which range from –9.0 [1,39,42,43] to –10.0 mmol/L [7,11,41]; the lowest published BDa reaching –12 mmol/L [36,38]. Besides differences in the selection criteria of populations, such discrepancies between BDs may partly be due to the different calculation formulae, which mostly rely on pH, pCO2, $HCO3^-$, and/or *c*tHb, depending on the chosen blood gas analyzer [44]. Our venous BD 2.5th percentile at –7.0 mmol/L was, however, nearer to the limits from other studies, which range from approximatively –7 to –9 mmol/L [1,36,41,43]. It could be argued that pH does not optimally estimate the foetus' exposure to hypoxia since its logarithmic term does not reflect acid accumulation in a linear manner, unlike BD, which advantageously takes into account the variation in pCO2 [45]. On the other hand, besides its dependence on calculation algorithms, BD is also impacted by the choice of foetal fluid compartment and by gestational age, which may lead to false-positive or –negative diagnoses of cord blood acidosis [44]. Nevertheless, acidosis during labour results from both respiratory and metabolic components, making pH and BD measurement in CAV-UCBG necessary for the evaluation of the foetus' exposure to hypoxia; the present percentiles may potentially be used as reference values, at least by laboratories equipped with Radiometer ABL800® analyzers.

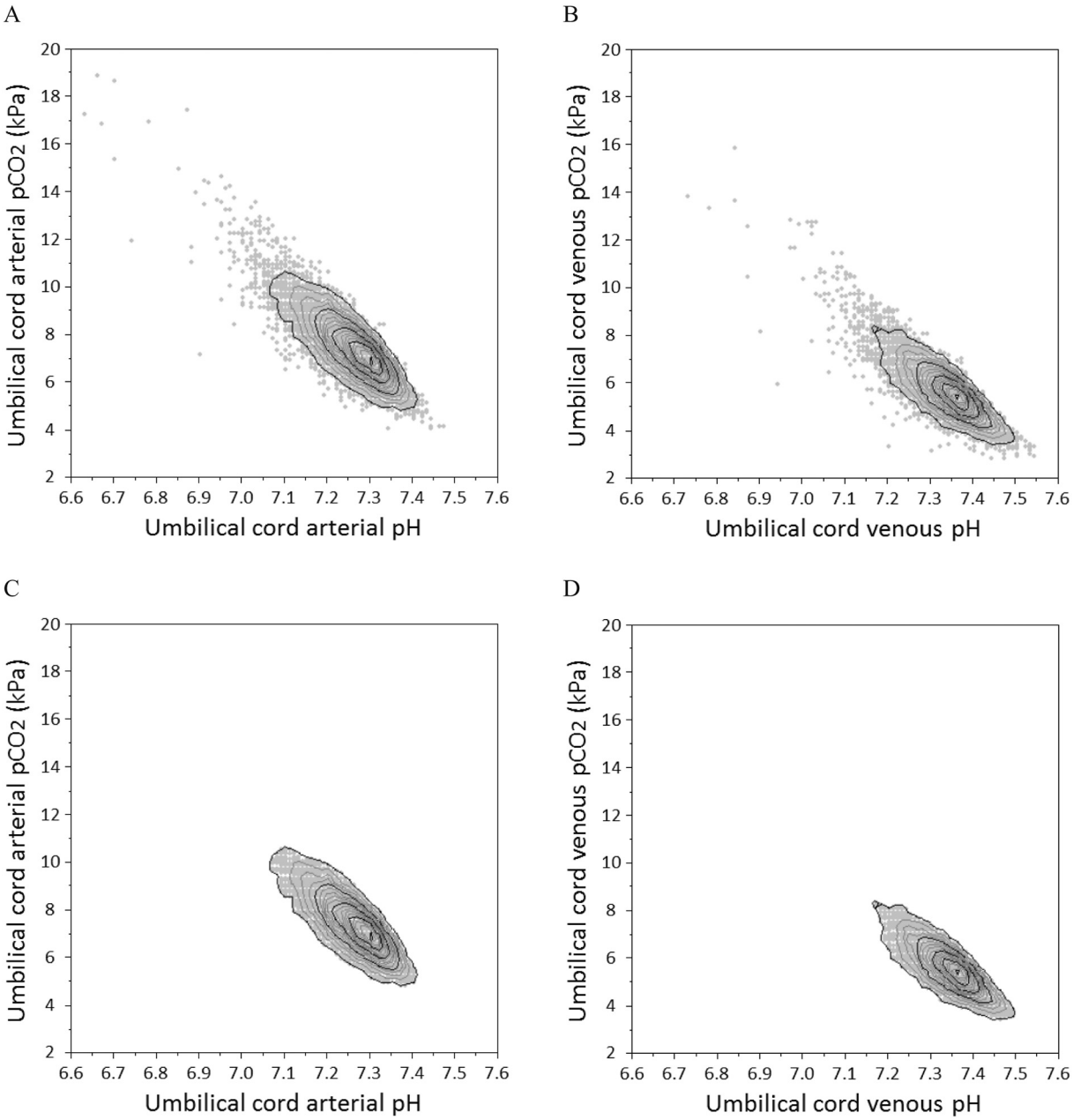

Fig. 3. Scatter plots of the pCO2-to-pH relationship for the whole arterial (A) and venous (B) concomitant umbilical cord blood gases (UCBG) (total n = 11131). The grey smoothed contour lines represent, in 5% intervals, the quantiles of the nonparametric bivariate kernel density estimations (NBKDE). From inside to outside respectively, the black smoothed contour lines represent the 5th, 25th, 50th, 75th, and 95th quantiles of the NBKDE. Plots out of the NBKDE 95th percentile surface were further removed, resulting in 10683 arterial (C) and 10631 venous UCBG (D).

Foetal oxidative metabolism results in the production of large amounts of carbon dioxide, which is eliminated across the placental barrier through gas diffusion. The pCO2 is therefore higher in arterial than in venous UCBG. Our 2.5th–97.5th percentile range of arterial pCO2 5.6–9.5 kPa is slightly narrower, but in accordance with that of Westgate et al. (4.9–10.7 kPa) [7], Kro et al. (5.2–10.5 kPa) [11], White et al. (5.6–9.8 kPa, 5th–95th percentile) [1], and Thorp et al. (5.2–9.8 kPa, mean ± 2SD) [42]. It was also the case for our venous pCO2 percentile range 4.2–7.1 kPa, when compared to the same studies: Westgate et al. (3.5–7.9 kPa) [7], Kro et al. (3.7–7.5 kPa) [11], White et al. (3.8–7.1 kPa) [1], and Thorp et al. (4.1–7.6 kPa) [42]. Focusing on arterial pO2, our 2.5th percentile of 1.2 kPa is higher than the low limits from other studies, which ranged from 0.3 [46] to 0.9 kPa [36], suggesting that our nonparametric selection model was more prone to discard neonates with slight arterial hypoxia. Our 97.5th percentile of 4.0 kPa was more in accordance with the high limits from other studies ranging from 3.3 [47] to 5.8 kPa [39], most of them ranging from 3.9 to 4.2 kPa [35,36,41,42]. Our venous pO2 percentiles 2.3–5.5 kPa were in accordance with those of Kro et al. (2.2–5.3 kPa) [11] and Yeomans et al. (2.3–5.5 kPa, mean ± 2SD) [35], even though slightly narrower than ranges from other studies like Riley and Johnson (1.8–5.8 kPa, mean ± 2SD) [46] or Dickinson et al (1.3–6.5 kPa, mean ± 2SD) [43]. Our arterial (4.0 kPa) and venous (5.5 kPa) pO2 upper percentiles are therefore globally in accordance with those from the studies series elsewhere reported at ~4.2 kPa and ~5.8 kP (mean + 2SD) [45]. It must be remembered, however, that maternal oxygen supplementation during delivery can raise the cord arterial pO2 up to 5.0 kPa [48,49], and that values above this threshold are likely to result from the presence of air bubbles in the sample.

Overall, as compared to previous studies, summarized by Thorp

Table 2
Percentiles of blood gas parameters from concomitant arterial (A) and venous (B) umbilical cord blood.

Parameter	All neonates (Ni = 10461)					Female neonates (Ni = 5035)					Male neonates (Ni = 5452)				
	Nf	(%RO)	2.5P	50P	97.5P	Nf	(%RO)	2.5P	50P	97.5P	Nf	(%RO)	2.5P	50P	97.5P
A. Arterial UCBG															
pH	10444	(0.2)	7.14	7.27	7.36	4951	(1.7)	7.14	7.27	7.35	5356	(1.8)	7.14	7.27	7.35
pCO2 (kPa)	10416	(0.4)	5.6	7.3	9.5	4993	(0.8)	5.7	7.2	9.5	5416	(0.7)	5.6	7.3	9.5
pO2 (kPa)	10095	(3.5)	1.2	2.3	4.0	4868	(3.3)	1.3	2.3	4.0	5250	(3.7)	1.2	2.3	4.0
sO2 (%)	10441	(0.2)	8.0	28.3	64.9	5025	(0.2)	8.1	28.5	64.9	5443	(0.2)	7.9	28.1	65.6
*c*tO2 (mmol/L)	10432	(0.3)	0.8	2.8	6.4	5024	(0.2)	0.8	2.8	6.3	5435	(0.3)	0.8	2.8	6.4
*c*tCO2 (mmol/L)	10417	(0.4)	21.6	25.8	29.4	4996	(0.8)	21.6	25.8	29.2	5432	(0.4)	21.4	25.8	29.5
*c*HCO3$^-$ (mmol/L)	10447	(0.1)	20.0	24.1	27.6	4995	(0.8)	20.1	24.2	27.4	5443	(0.2)	19.8	24.1	27.7
*c*tHb (g/L)	10338	(1.2)	131	162	192	4980	(1.1)	131	161	190	5383	(1.3)	131	163	192
ABE (mmol/L)	10396	(0.6)	−6.9	−1.8	+1.8	4987	(1.0)	−6.7	−1.7	+1.6	5443	(0.2)	−7.1	−1.9	+1.9
B. Venous UCBG															
pH	10190	(2.6)	7.24	7.35	7.43	4928	(2.1)	7.25	7.35	7.44	5307	(2.7)	7.22	7.35	7.42
pCO2 (kPa)	10317	(1.4)	4.2	5.5	7.1	4972	(1.3)	4.1	5.5	7.1	5330	(2.2)	4.2	5.5	7.1
pO2 (kPa)	10235	(2.2)	2.3	3.7	5.5	4966	(1.4)	2.2	3.8	5.5	5350	(1.9)	2.2	3.7	5.5
sO2 (%)	10432	(0.3)	24.2	61.0	86.5	5013	(0.4)	24.2	61.9	86.7	5439	(0.2)	23.7	60.3	86.3
*c*tO2 (mmol/L)	10380	(0.8)	2.4	5.9	8.4	5004	(0.6)	2.5	5.9	8.4	5398	(1.0)	2.4	5.8	8.4
*c*tCO2 (mmol/L)	10426	(0.3)	19.3	23.4	26.8	5024	(0.2)	19.4	23.4	26.8	5434	(0.3)	19.2	23.4	26.8
*c*HCO3$^-$ (mmol/L)	10427	(0.3)	18.2	22.1	25.3	5020	(0.3)	18.3	22.1	25.3	5425	(0.5)	18.1	22.1	25.3
*c*tHb (g/L)	10359	(1.0)	130	161	193	4990	(0.9)	130	160	191	5377	(1.4)	129	163	193
ABE (mmol/L)	10402	(0.6)	−7.0	−2.6	+0.6	5009	(0.5)	−6.9	−2.6	+0.5	5431	(0.4)	−7.2	−2.7	+0.6

After outlier removal using the adjusted-Tukey test, percentile values were calculated using the CLSI EP28-A3 nonparametric method, for each UCBG parameter. For each group, the volume of data before (initial number, Ni) and after outlier removal (final number, Nf) is provided. Abbreviations (*dv*: derived parameters; *ms*: measured parameters): ABE: acid-base excess (*dv*); *c*HCO3$^-$: concentration of hydrogen carbonate (*dv*); *c*tCO2: concentration of total carbon dioxide (*dv*); *c*tHb: concentration of total haemoglobin in blood (*ms*); *c*tO2: concentration of total oxygen (*dv*); pCO2: partial pressure of carbon dioxide (*ms*); pH: acidity or alkalinity (*ms*); pO2: partial pressure of oxygen (*ms*); sO2: oxygen saturation (*ms*). %RO: percentage of removed outliers; 2.5P, 50P, 97.5P: 2.5th, 50th and 97.5th percentile.

et al. [5], the present arterial 2.5th–97.5th percentiles are similar for pH, pCO2, pO2, and ABE. They are also roughly concordant with the arterial decision levels from Huch et al. [50], adapted by Brouillette et al. [6], proposed as pH > 7.06, pCO2 < 9.33 kPa, pO2 > 1.33 kPa, and base deficit < 15 mmol/L. Regarding venous UCBG parameters, our 2.5th–97.5th percentile ranges are also in agreement with the reported decisional limits, since above pH 7.14 and pO2 1.6 kPa, and below pCO2 7.46 kPa and base deficit 13 mmol/L [6,50]. In our study, as expected, venous pH percentiles are higher by almost one-tenth of a unit compared to those of arterial pH. Applying the CLSI nonparametric method to our data, the 2.5th–97.5th percentiles of pHvad were 0.03–0.16 (median: 0.07, min-max: 0.02–0.32, n = 10188), which is a normal range with regards to the small pHvad (≤0.06) and large pHvad (≥0.30) associated with placental abruptions and cord prolapses, respectively, in acidaemic neonates with an arterial umbilical pH < 7.05 [51]. The other parameters have scarcely been assessed in UCBG-based studies and, to our knowledge, no reference percentile has yet been proposed.

From a methodological standpoint, drawing on the work from Westgate et al. [7], Kro et al. developed pH-specific percentiles of arterial pCO2 based on a large set of neonates from European centres, in order to provide an applicable tool for identifying cases with erroneously low pCO2 values [11]. They validated the paired arterial and venous umbilical cord blood samples based on three exclusion criteria of arteriovenous pH and pCO2 differences. Here, we applied these criteria to select paired arterial and venous UCBG. Since cord pH and pCO2 distributions were clearly skewed, Kro et al. fitted the data using a fractional polynomial regression model, after minimizing the variances (inter-individual, inter-clinic, and inter-trial) through a sophisticated multilevel modelling, while power transforming arterial pCO2 values to reach normality. However, their scatter plot displayed a substantial proportion of cases respectively below and above the 2.5th and 97.5th percentile pH-to-pCO2 polynomial curves, which may have been wrongly discarded. Moreover, they removed cases with pH < 6.86 and > 7.44 in an arbitrary way, arguing their small numbers for percentile calculations. Given these potential selection biases, the elliptical aspect of the scatter plot, and the skewed distribution of pH and pCO2 values, we chose the nonparametric bivariate KDE strategy, which allows a 360° multidirectional selection of cases within a given percentile surface. Then, as required for skewed distribution, we removed outliers for each UCBG parameter using the adjusted-Tukey method [25], before calculating the percentiles using the CSLI EP28-A3 nonparametric method, as appropriate [28]. In our study, and at every stage, the data were not normally distributed, even after Box-Cox transformation, leading us to use nonparametric tests, as recommended [28]. It is known that different statistical methods may provide different reference values, which depend, among others, on the sample size and the analyte distribution [52]. Given that larger sample sizes provide more precise estimates [52,53], the use of parametric tests could lead to similar results in our study. Nevertheless, this assumption is more uncertain in the particular context of bivariate pCO2-to-pH relationship of UCBG. Since both pH and pCO2 distributions were skewed in arterial and venous UCBG, we used the NBKDE-95P model rather than the density ellipse for bivariate normal distribution. Compared to other studies, our nonparametric approach globally led to similar or slightly narrower reference intervals. However, most studies have determined their reference values using mean ± 2SD [35,37,39,42,43,47], and others used medians and percentiles [1,7,11,41], but few report having tested for distribution normality of UCBG data prior to this determination [36,41]. Furthermore, to the best of our knowledge, no study has yet applied outlier removal to UCBG data, and none has mentioned having used the CLSI nonparametric percentile method to determine their reference intervals.

5. Limitations

We used the pHvad < 0.02 and pCO2avd < 0.7 kPa criteria from the Kro et al. study [11] rather than defining our own criteria based on our own population. Applying these criteria to our population, a greater percentage of results were excluded (~24%, i.e. 11131/14680, not considering CAV-UCBG from the first preanalytical/analytical exclusion step) compared to the Kro et al. study (~14%). However, our 24% excluded paired values is close to the 20% rate from White et al. (12345/15443 of paired accurate samples based on a pHvad < 0.022

and a pCO2avd < 0.7 kPa) [1], and even closer to the 25% rate from Westgate et al., who used a pHvad < 0.02 and a pCO2avd < 0.5 kPa as exclusion criteria [7]. We chose the criteria from Kro et al. since they were determined on a population more than twice as large as ours, which moreover included neonates of gestational age of at least 36 weeks.

The other main limitation is that our study relies exclusively on UCBG data; it did not include gestational age, birth weight, Apgar score, or other obstetric information. In particular, even though we discarded UCBG from complicated deliveries *a priori*, we did not exclude those from preterm neonates. The preterm birth (< 37 weeks of gestation) worldwide rate is ~11%, while the French rate is ~7.4%, among which 85% are moderate to late preterm births (32–36 weeks) [54,55]. Considering these rates and our highly selective model, it is likely that a substantial proportion of CAV-UCBG from preterm neonates has been excluded; the remaining proportion having probably a minor impact on the final percentiles. In support of this assumption, our arterial and venous pH, pO2, and pCO2 medians were close to the means of term singletons, and to those of a group including all gestations, regardless of delivery type [8,41,42,46], all of which are also close to those of preterm neonates (24–36 weeks of gestation) [43], as summarized by Armstrong et al. [45]. Furthermore, Kro et al. also did not include the gestational age in their final analysis since it did not improve the goodness of fit to the model [11]. Even though they are not based on clinical data, UCBG data are numerous and easily available in hospital groups, allowing reliable percentile estimates [56]. Although debated for a long time [57], indirect approaches for the establishment of reference intervals from hospitalized patients are still used [58,59] since they remain less tedious, long, expensive, and administratively complicated than doing so from healthy subjects, and particularly from neonates.

Beyond providing percentiles that are potentially usable as references, the present study proposes a new indirect nonparametric approach for skewed data like those from umbilical cord blood gases.

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Declaration of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.02.014.

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