Association between blood carboxyhemoglobin level and bronchopulmonary dysplasia in extremely low birthweight infants

Thea Tagliaferro, Rowena Cayabyab, Rangasamy Ramanathan

Keck School of Medicine/ Pediatrics/Division of Neonatology, USC, Los Angeles, California, USA

Correspondence to
Dr Thea Tagliaferro, Keck School of Medicine/ Pediatrics/ Division of Neonatology, USC, Los Angeles, CA 90033, USA; thea.tagliaferro@gmail.com

Accepted 27 July 2021
Published Online First 7 September 2021

To cite: Tagliaferro T, Cayabyab R, Ramanathan R. *J Investig Med* 2022;**70**:68–72.

ABSTRACT

Carboxyhemoglobin (CO-Hb) can be endogenously formed in the presence of oxidative stress and may be elevated in inflammatory lung disease. There is lack of evidence of its relationship with the development of bronchopulmonary dysplasia (BPD) in extremely low birthweight (ELBW) infants. The objective of the study is to evaluate the relationship between blood CO-Hb levels in the first 14 days of life (DOL) in ELBW infants and the development of BPD at 36 weeks postmenstrual age (PMA). This is a retrospective cohort study of 58 ELBW infants born at LAC-USC Medical Center between June 2015 and and June 2019 who survived to 36 weeks PMA. CO-Hb values were collected daily from DOL 1 to DOL 14. BPD definition using the recent 2019 NICHD criteria was used. Multivariate logistic regression was performed to determine the association between blood CO-Hb levels and BPD. Receiver operator curve was used to evaluate the ability of the median fraction of inspired oxygen (FiO_2) level used at DOL 11–14 in discriminating absent to mild BPD versus moderate to severe BPD. 58 ELBW infants were included in the study. 24 (41%) were diagnosed with moderate to severe BPD, while 34 (59%) were diagnosed with no to mild BPD. Severity of BPD was fairly discriminated by FiO_2 at DOL 11–14, but not with CO-Hb levels at any point within the first 14 DOL. The role and mechanism of CO-Hb production in this population need to be further studied.

Significance of this study

What is already known about this subject?

- Oxygen toxicity plays a major role in the development of oxygen radical mediated disorders like bronchopulmonary dysplasia (BPD), retinopathy of prematurity, intraventricular hemorrhage and necrotizing enterocolitis.
- Carboxyhemoglobin (CO-Hb) levels are increased in adults with oxidative stress disorders.
- Heme oxygenase-1 expression is higher in preterm infants compared with term infants and catalyzes heme to generate CO-Hb.

What are the new findings?

- CO-Hb levels in extremely low birthweight infants are higher than the more mature preterm and term infants in the first 2 weeks of life.
- CO-Hb levels during the first 1–14 days of life were not associated with BPD.

How might these results change the focus of research or clinical practice?

- Our findings may provide a rationale to target lower arterial oxygen saturation during the first 2 weeks after birth.
- Further studies should evaluate the impact of targeting lower or higher arterial oxygen saturation during the first 2 weeks of age on CO-Hb levels and the development of BPD and retinopathy of prematurity.

INTRODUCTION

Extreme prematurity faces a high risk of neonatal mortality and, for those who survive, serious neonatal morbidities that are strongly associated with poor long-term outcomes. Bronchopulmonary dysplasia (BPD) remains the most common morbidity of prematurity especially among extremely low birthweight (ELBW) infants. This population is at the highest risk for complications of mechanical ventilation and is therefore predisposed to develop BPD. Over the last 40 years, the definitions, disease phenotypes and risk factors for BPD have changed, but the incidence has remained unchanged or even increased given the increase in survival of the ELBW population.[1] The etiology of BPD is multifactorial, aggravated by oxygen treatment and days on invasive mechanical ventilation. While these risk factors are known to predict later development of BPD, clinical markers readily available to clinicians early after birth that could predict development and severity of BPD are yet to be identified. In adults with inflammatory lung diseases, the level of carboxyhemoglobin (CO-Hb) in the blood is used as a marker of increased response to oxidative stress as it reflects the severity of disease and level of inflammation in the airway and lung in inflammatory pulmonary diseases.[2 3] However, the use of CO-Hb level in the ELBW population to predict severity of oxidative damage in the immature lungs of ELBW infants has not been well studied. CO-Hb is produced by the binding

of hemoglobin and carbon monoxide, which is produced by the catalysis of heme by the inducible form of the enzyme, heme oxygenase (HO-1). The expression of HO-1 in premature infants with respiratory distress syndrome is eightfold higher compared with healthy term and asymptomatic premature infants.[4 5] To our knowledge, in one small study including 25 preterm infants, with 6 infants with moderate to severe BPD, CO-Hb was significantly elevated, and the authors suggested that CO-Hb could be used as a biomarker of oxidative injury and inflammation in the lungs of preterm infants.[6] However, the association with expression of HO-1 and BPD, which is accompanied by an inflammatory process with free radical generation and oxidative stress in the lungs of ELBW infants, has not been elucidated. This study aims to evaluate the relationship between blood CO-Hb levels as an indirect marker of HO-1 expression in the first 14 days of life (DOL) in ELBW infants and the development of BPD at 36 weeks postmenstrual age (PMA).

MATERIALS AND METHODS

This is a retrospective cohort study of all ELBW infants born at LAC-USC Medical Center between June 2015 and June 2019 who survived to 36 weeks PMA. Data were collected from an institutional neonatal database (Neonatal Information Systems 5, Rosemont, Philadelphia). Maternal and neonatal demographics and clinical outcomes were collected. Daily CO-Hb levels during the first 14 DOL, expressed as the percentage of the total hemoglobin, were obtained from capillary, venous or arterial blood gas results and were grouped into three time points: DOL 1–5, DOL 6–10 and DOL 11–14, as previously described.[6] If there were multiple blood gas analyses obtained during the day, the mean value was used for data analysis. GEM 4000 (Instrumentation Laboratory, Bedford, Massachusetts) was used to analyze blood gas and CO-Hb with spectrophotometric measurements following chemical lysing.[7] BPD was defined at 36 weeks PMA as the following: grade 1 (mild): nasal cannula at flow rates <2 L/min; grade 2 (moderate): nasal cannula at flow rates >2 L/min or non-invasive positive airway pressure; and grade 3 (severe): invasive mechanical ventilation.[8] Hemodynamically significant patent ductus arteriosus (PDA) was defined as PDA requiring medical and/or surgical intervention. The infants were grouped into absent or mild BPD and moderate to severe BPD due to the small sample size.

Data analysis

Wilcoxon rank-sum and t-test were used to compare differences in continuous variables as appropriate. Pearson's χ^2 and Fisher's exact tests were used to compare differences in categorical variables. One-way analysis of variance with Bonferroni test was performed to determine differences in the means of CO-Hb levels obtained between DOL 1–5, DOL 6–10 and DOL 11–14 in all ELBW infants. Multivariate logistic regression was used to determine the predictors for the development of moderate to severe BPD. Finally, we used receiver operator curves to evaluate the ability of the median fraction of inspired oxygen (FiO_2) level used at DOL 11–14 in discriminating absent to mild BPD versus moderate to severe BPD. Sensitivity, specificity, positive predictive and negative predictive values were calculated.

Table 1 Maternal and neonatal demographic characteristics

	No to mild BPD (n=34)	Moderate to severe BPD (n=24)	P value
Gestational age (weeks)	25 (24, 26)	26 (25, 26)	0.54
Birth weight (g)	748 (655, 910)	728 (588, 822)	0.13
Pre-eclampsia, n (%)	13 (38)	11 (45)	0.56
Cesarean section, n (%)	27 (79)	20 (83)	0.71
Female sex, n (%)	19 (56)	10 (42)	0.28
Apgar score 1 min	3 (1, 5)	3 (2, 5)	0.72
Apgar score 5 min	7 (6, 7)	6 (3, 7)	0.04

Continuous variables are expressed as median (25th and 75th percentile).
BPD, bronchopulmonary dysplasia.

STATA V.14 was used to perform data analysis. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 58 ELBW infants were included in the study. There were 24 (41%) diagnosed with moderate to severe BPD, while 34 (59%) had absent to mild BPD. There were no statistically significant differences in demographics between the two groups except for the significantly lower Apgar score at 5 min in infants with moderate to severe BPD (table 1). These infants had a higher rate of surfactant use within the first 24 hours, higher FiO_2 from DOL 1–5, 6–10 and 11–14, longer days on invasive mechanical ventilation, and higher rate of postnatal steroid use (table 2). When CO-Hb levels from all infants were grouped into three time points by DOL, the mean CO-Hb levels within and between the three groups were significantly different, particularly CO-Hb levels for CO-Hb levels from DOL 1–5 to DOL 6–10 (−2.1, p=0.048) and from DOL 1–5 to DOL 11–14 (−0.36, p<0.01) (figure 1). The same effect was observed among the absent to mild and moderate to severe BPD groups (table 3, figure 2). CO-Hb level within the first 14 DOL was not associated with BPD (table 4); however, the severity of BPD was significantly associated with FiO_2 requirement at DOL 11–14, after adjusting for days on invasive mechanical ventilation (OR 299.06, 95% CI 1.04 to 85734.01). A cut-off value of 0.43 FiO_2 at DOL 11–14 (median FiO_2 required) correctly classified moderate to severe BPD at 67%, with an area under the curve (AUC) of 75% (95% CI 62 to 87%) (figure 3). Sensitivity, specificity, positive predictive and negative predictive values were as follows: 50%, 79.4%, 63.2% and 69.2%.

DISCUSSION

In this study, the level of FiO_2 at DOL 11–14 but not CO-Hb level in the first 14 DOL of ELBW infants predicted later development of moderate to severe BPD. CO-Hb is an endogenous by-product of red blood cell hemolysis catalyzed by the expression of the enzyme HO-1, known to be increased in pathological conditions related to oxidative stress and inflammatory response. Under physiological conditions, HO-1 regulates several important biological processes as its products exert antioxidant, anti-inflammatory and anti-apoptotic effects in the lungs.[9] However, under oxidative stress and with release of proinflammatory cytokines, the

Table 2 Clinical outcomes of the study population

	No to mild BPD (n=34)	Moderate to severe BPD (n=24)	P value
Intubation at birth, n (%)	9 (27)	11 (46)	0.15
Surfactant during the first 24 hours, n (%)	23 (67)	24 (100)	<0.01
Presence of chorioamnionitis, n (%)	3 (8.8)	3 (12.5)	0.6
Use of postnatal steroids, n (%)	11 (32)	15 (63)	0.02
Invasive MV (days)	9.5 (0, 32)	32 (6, 43)	0.03
PDA, n (%)	13 (38.2)	14 (58.3)	0.13
Average FiO_2 at DOL 1–5	0.24 (0.21, 0.27)	0.32 (25, 47)	<0.01
Average FiO_2 at DOL 6–10	0.25 (0.21, 0.30)	0.34 (25, 45)	<0.01
Average FiO_2 at DOL 11–14	0.26 (0.21, 0.38)	0.42 (0.30, 0.67)	<0.01
FiO_2 at 28 days of life	0.25 (0.22, 0.33)	0.32 (0.30, 0.39)	<0.01
FiO_2 at 36 weeks PMA	0.21	0.25 (0.23, 0.31)	<0.01
Length of stay (days)	97 (78, 112)	121 (107, 143)	<0.01

Continuous variables are expressed as median (25th and 75th percentile).
BPD, bronchopulmonary dysplasia; DOL, day of life; FiO_2, fraction of inspired oxygen; MV, mechanical ventilation; PDA, patent ductus arteriosus; PMA, postmenstrual age.

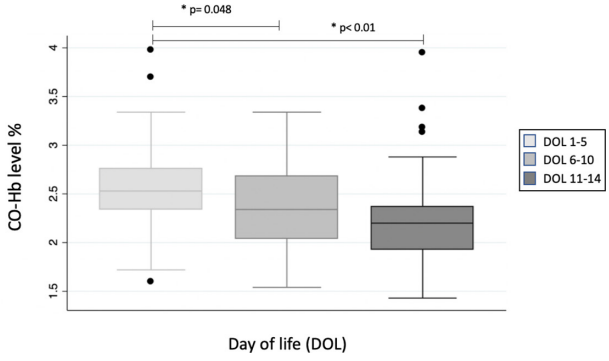

Figure 1 Carboxyhemoglobin (CO-Hb) levels with time (day of life) in the study population.

enzyme action can be upregulated, causing tissue damage with significant oxygen-mediated cytotoxicity. Oxygen radical injury is a common pathogenic mechanism in several neonatal diseases associated with prematurity, including retinopathy of prematurity, BPD, intraventricular hemorrhage and necrotizing enterocolitis.[10] These disorders have a higher incidence in ELBW infants with deficient antioxidant protective systems.[11] Several of these pathological conditions have been shown to be associated with an increased HO-1 expression.[5 12] Thus, it has been used as a possible indicator of cellular stress and injury.[13] Tokuriki *et al*[6] previously showed higher CO-Hb levels during the early postnatal period in infants born <33 weeks of gestation or with a birth weight of <1500 g, who later developed moderate-to-severe BPD at 36 weeks PMA. In this study, a cut-off value of 1% was used to predict moderate to severe BPD.[6] Furthermore, fewer and more mature preterm infants were included compared with our study. Normative levels of CO-Hb in the ELBW population are still not known, although levels of CO-Hb have been shown to be inversely correlated with gestational age.[14] Our results showed that the median CO-Hb values in the first 14 DOL in the ELBW population exceeded 2%, higher than the values found in the study by Tokuriki *et al*.[6] This discrepancy in higher CO-Hb levels found in extremely preterm infants may be due to an additive oxidative stress process induced by inflammation and/or oxygen supplementation relative to the deficient antioxidant defense compared with more mature infants in whom normal concentration of CO-Hb in umbilical cord blood is less than 1.2%.[15]

Despite the increased FiO_2 requirements with increasing DOL, the CO-Hb levels significantly decreased in both groups of infants who developed moderate to severe BPD and those who did not, with higher levels in the first 5 DOL (figures 1 and 2). Previous study reported CO-Hb levels to decrease to a steady value within the first 2 weeks of life following the natural fall of hemoglobin after birth and this was also seen in our study.[16]

Unlike findings in Tokuriki *et al*'s[6] study, none of the CO-Hb levels in the first 14 DOL in our population predicted later development of BPD or its severity. However, FiO_2 at DOL 11–14 had a fair discrimination between absent to mild and moderate to severe BPD with an AUC of 75% (figure 3). The absence of correlation of CO-Hb and BPD in our study might relate to the use of the National Institute of Child Health and Human

Table 3 CO-Hb levels with time (DOL) and severity of BPD

	CO-Hb at DOL 1–5	CO-Hb at DOL 6–10	CO-Hb at DOL 11–14	P value
Overall population	2.58± (0.45)*	2.37± (0.45)	2.22± (0.49)*	<0.01
No to mild BPD (n=34)	2.65± (0.44)**	2.41± (0.42)	2.32± (0.51)**	0.01
Moderate to severe BPD (n=24)	2.49± (0.45)***	2.31±(0.51)	2.07± (0.42)***	0.01

Numbers are expressed as mean± (SD).
*P< 0.01, **P<0.01, ***P<0.01.
BPD, bronchopulmonary dysplasia; CO-Hb, carboxyhemoglobin; DOL, day of life.

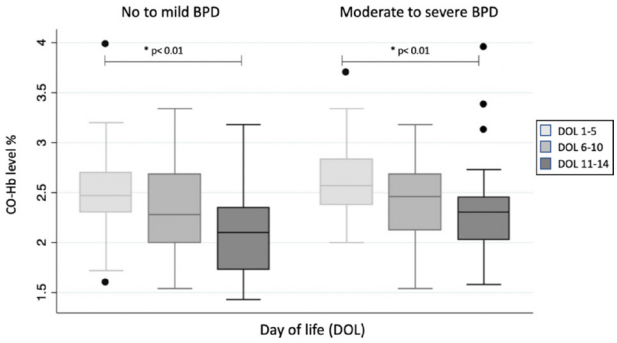

Figure 2 Carboxyhemoglobin (CO-Hb) levels with time (day of life) and bronchopulmonary dysplasia (BPD) severity.

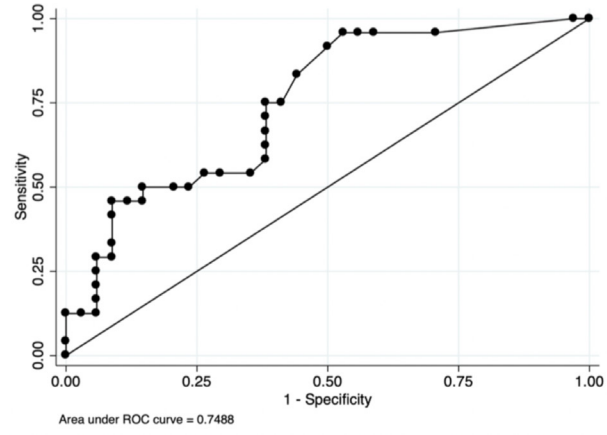

Figure 3 ROC curve for FiO_2 and development of moderate to severe bronchopulmonary dysplasia at days of life 11–14. FiO_2, fraction of inspired oxygen; ROC, receiver operator curve.

Development (NICHD) 2019 BPD definition, which was not used in the previous study that showed a correlation.[8] The 2019 NICHD classifies BPD severity according to the mode of respiratory support at 36 weeks PMA and not on the use of supplemental oxygen before or at 36 weeks PMA. This definition best predicted death after 36 weeks PMA or adverse respiratory and neurodevelopmental outcomes at 18–26 months' corrected age compared with 18 previous BPD definitions.[8] Using a different BPD classification might explain the difference in results compared with other study findings. Another possible reason for the lack of association between CO-Hb and development of BPD may be due to our institutional guideline for lower target arterial oxygen saturation by pulse oximeter (SpO_2) in this population when they are less than 33 weeks PMA.[17 18] The lower SpO_2 targets could have exposed this susceptible group to a decreased risk for oxidative stress. The median FiO_2 required in the first 2 weeks of life in our study was lower than 50% and half of the patients were on non-invasive ventilation (NIV) during this time. In our center, NIV is the primary mode of respiratory support for all premature infants admitted with respiratory distress. By avoiding the inflammatory cascade associated with invasive mechanical ventilation, NIV has been shown to reduce inflammation and the incidence of BPD.[19] Moreover, as hypopharyngeal concentration of FiO_2 may be lower than what is recorded because of the use of NIV, we speculate that lower levels of oxygen delivered may not be sufficient to activate HO-1 and affect levels of CO-Hb.

The current findings raise some questions regarding the clinical utility of CO-Hb as an early predictive marker of BPD development in this vulnerable population. As HO-1 is upregulated by several factors including not only oxidative stress but also inflammatory cytokines, CO-Hb levels in this population might not be specific to inflammatory pulmonary disease, as used in the adult population, rather than being elevated given an extensive inflammatory activation in response to birth, and then follow fetal red blood cell heme catabolism after birth.[16]

There are some limitations to the present study. This is a retrospective study with a small sample size. Moreover, specific oxidative stress markers to hyperoxia were not directly measured. Finally, the presence of fetal hemoglobin (HbF) may have falsely elevated the CO-Hb levels as the absorption spectrum of HbF in spectrophotometry is different from that of HbA1 and interferes with CO-Hb measurement.[20]

In conclusion, CO-Hb level, as a readily accessible biomarker of oxidative damage and ongoing inflammation in the immature lungs of ELBW infants, decreased over time during the first 14 days of age. However, it did not predict subsequent development of moderate to severe BPD. The role and mechanism of CO-Hb production in this population need to be further studied.

Table 4 Relationship of CO-Hb levels by DOL and BPD

	No to mild BPD (n=34)	Moderate to severe BPD (n=24)	P value
CO-Hb at DOL 1–5	2.53± (0.47)	2.66± (0.42)	0.29
CO-Hb at DOL 6–10	2.33± (0.46)	2.43± (0.45)	0.41
CO-Hb at DOL 11–14	2.12± (0.48)	2.36± (0.53)	0.08

Numbers are expressed as mean± (SD).
BPD, bronchopulmonary dysplasia; CO-Hb, carboxyhemoglobin; DOL, day of life.

Acknowledgements We would like to thank Dr Lorayne Barton for her help with data collection.

Contributors TT, RC and RR conceptualized and designed the study, drafted the initial manuscript, reviewed and revised the manuscript. All authors approve the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the Institutional Review Board at LAC-USC Medical Center.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data may be obtained from a third party and are not publicly available. Data were collected from an institutional neonatal database (Neonatal Information Systems (NIS 5), Rosemont, Philadelphia).

ORCID iDs
Thea Tagliaferro http://orcid.org/0000-0002-8834-5614
Rangasamy Ramanathan http://orcid.org/0000-0002-7358-601X

REFERENCES

1 Siffel C, Kistler KD, Lewis JFM, *et al*. Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. *J Matern Fetal Neonatal Med* 2021;34:1721–31.
2 Hara Y, Shinkai M, Kanoh S, *et al*. Arterial carboxyhemoglobin measurement is useful for evaluating pulmonary inflammation in subjects with interstitial lung disease. *Intern Med* 2017;56:621–6.
3 Yasuda H, Yamaya M, Yanai M, *et al*. Increased blood carboxyhaemoglobin concentrations in inflammatory pulmonary diseases. *Thorax* 2002;57:779–83.
4 Farkas I, Maróti Z, Katona M, *et al*. Increased heme oxygenase-1 expression in premature infants with respiratory distress syndrome. *Eur J Pediatr* 2008;167:1379–83.
5 Mumby S, Upton RL, Chen Y, *et al*. Lung heme oxygenase-1 is elevated in acute respiratory distress syndrome. *Crit Care Med* 2004;32:1130–5.
6 Tokuriki S, Okuno T, Ohta G. Carboxyhemoglobin formation in preterm infants is related to the subsequent development of bronchopulmonary dysplasia. *Dis Markers* 2015;2015:620921–8.
7 Bénéteau-Burnat B, Pernet P, Pilon A, *et al*. Evaluation of the GEM premier 4000: a compact blood gas CO-oximeter and electrolyte analyzer for point-of-care and laboratory testing. *Clin Chem Lab Med* 2008;46:271–9.
8 Jensen EA, Dysart K, Gantz MG, *et al*. The diagnosis of bronchopulmonary dysplasia in very preterm infants. An evidence-based approach. *Am J Respir Crit Care Med* 2019;200:751–9.
9 Nath KA. Heme oxygenase-1: a provenance for cytoprotective pathways in the kidney and other tissues. *Kidney Int* 2006;70:432–43.
10 Saugstad OD. Update on oxygen radical disease in neonatology. *Curr Opin Obstet Gynecol* 2001;13:147–53.
11 Perez M, Robbins ME, Revhaug C, *et al*. Oxygen radical disease in the newborn, revisited: oxidative stress and disease in the newborn period. *Free Radic Biol Med* 2019;142:61–72.
12 Maróti Z, Katona M, Orvos H, *et al*. Heme oxygenase-1 expression in premature and mature neonates during the first week of life. *Eur J Pediatr* 2007;166:1033–8.
13 Otterbein LE, Choi AM. Heme oxygenase: colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1029–37.
14 Stark MJ, Clifton VL, Wright IMR. Carbon monoxide is a significant mediator of cardiovascular status following preterm birth. *Pediatrics* 2009;124:277–84.
15 Hengstler K, van 't Sant P, Jira PE. Carboxyhemoglobin in umbilical cord blood and maternal smoking. *J Perinat Med* 2019;47:780–4.
16 McArdle AJ, Webbe J, Sim K, *et al*. Determinants of carboxyhemoglobin levels and relationship with sepsis in a retrospective cohort of preterm neonates. *PLoS One* 2016;11:e0161784.
17 Cayabyab R, Ramanathan R. Retinopathy of prematurity: therapeutic strategies based on pathophysiology. *Neonatology* 2016;109:369–76.
18 Cayabyab R, Arora V, Wertheimer F, *et al*. Graded oxygen saturation targets and retinopathy of prematurity in extremely preterm infants. *Pediatr Res* 2016;80:401–6.
19 Lemyre B, Laughon M, Bose C, *et al*. Early nasal intermittent positive pressure ventilation (NIPPV) versus early nasal continuous positive airway pressure (nCPAP) for preterm infants. *Cochrane Database Syst Rev* 2016;12:Cd005384.
20 Nicholson NA, Karabus CD, Klein M, *et al*. Reduction of neonatal blood eliminates the error induced by oxygen saturation in the spectrophotometric measurement of carboxyhaemoglobin. *Ann Clin Biochem* 1988;25:237–41.