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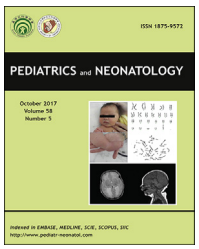

Original Article

Determination of tissue hypoxia by physicochemical approach in premature anemia

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Key Words
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Background: Anemia is a common problem in premature infants and its most rapid and effective therapy is erythrocyte transfusion. However, owing to inherent risks of transfusion in this population, transfusions should be administered only when adequate oxygen delivery to tissues is impaired. The aim of this study was to determine tissue acid levels using Stewart method in an attempt to evaluate the tissue oxygenation level and thereby the accuracy of transfusion timing.
Methods: This study included 47 infants delivered at gestational age below 34 weeks who required erythrocyte transfusion for premature anemia. Strong ion gap (SIG), unmeasurable anions (UMA), tissue acid levels (TA), and Cl/Na ratios were calculated before and after transfusion.
Results: The mean birth weight and gestational age of the study population were 1210 $\pm$ 365 g and 29.2 $\pm$ 2.7 weeks, respectively. Tissue acid levels were increased (TA $\geq$ 4) and tissue hypoxia developed in 10 (16.6%) of 60 erythrocyte transfusions administered according to the restrictive transfusion approach. The patients were divided into two groups according to tissue acid levels as low ($<$4) and high ($\geq$4) tissue acid groups. The group with tissue hypoxia (TA $\geq$ 4) had significantly higher UMA levels but a significantly lower Cl/Na ratio; and UMA levels decreased and Cl/Na ratio increased after transfusion in this group. Tissue hypoxia secondary to anemia was shown to be improved by erythrocyte transfusion.
Conclusion: The results of the present study suggest that the determination of the level of tissue hypoxia by the Stewart approach may be an alternative to restrictive transfusion guidelines for timing of transfusion in premature anemia. It also showed that a low Cl/Na ratio can be used as a simple marker of tissue hypoxia.

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1. Introduction

Anemia is a common problem in premature infants and its most rapid and effective therapy is erythrocyte transfusion. However, owing to the inherent risks of transfusion in this population, many centers have introduced more restrictive policies. Erythrocyte transfusion is performed when the level of anemia becomes symptomatic or is thought to compromise adequate oxygen delivery to tissues. Nevertheless, diagnosis of tissue hypoxia in newborns is difficult and controversial. The aim of this study was to determine tissue acid levels (TA) by the Stewart method before and after transfusion in an attempt to evaluate the state of tissue oxygenation and therefore the adequacy of transfusion timing in preterm infants.

The Stewart physiochemical method was described in 1981 and, unlike the Henderson—Hasselbach method it is based on the theory that HCO3 does not determine plasma pH.[1—4] Independent variables responsible for plasma pH are arterial partial carbon dioxide pressure (PaCO2), strong ion difference (SID), and nonvolatile weak acids (Atots). SID is the difference between strong cations and strong anions dissolved in plasma, and calculated with the formula SID = (Na + K + Ca + Mg) − (Cl + lactate). Atots are the sum of bound or dissolved acids in plasma; it consists of albumin and phosphate (P) concentrations and it is equal to anion gap under normal conditions. SID is elevated in pathological conditions where unmeasurable anions (UMA) such as lactate, ketoacids, and sulfate are increased. In that case, effective SID (SIDe) is calculated with the formula SIDe = HCO3 + (0.28 × Albumin) + (1.8 × P). The difference between SID and SIDe is equal to strong ion gap (SIG) and reflects UMA. TA are equal to the sum of lactate and UMA levels.

The Stewart method accurately quantifies the individual components of acid—base balance and allows the clinician to understand the pathogenesis of acid—base alterations in critically ill patients.[3,5—8] Respiratory disorders are induced as a consequence of a change in PaCO2, whereas metabolic disorders are always due to an alteration in either SID or Atot. When SID narrows, either by a relative decrease in cations (e.g., hyponatremia) or a relative increase in anions (e.g., hyperchloremia), metabolic acidosis develops. A relative increase in cations or a relative decrease in anions widens SID and causes metabolic alkalosis. For example, furosamide causes greater renal loss of Cl than Na, leading to increased SID and metabolic alkalosis. Likewise, increased Atot concentration (e.g., hyperphosphatemia) leads to metabolic acidosis and decreased Atot concentration (e.g., hypoalbuminemia) leads to metabolic alkalosis. In addition, various UMA generated in pathologic conditions can change SID. For example, tissue acids generated in peripheral tissues during hypoxia lead to metabolic acidosis. The potential advantage of the Stewart method is direct quantification of SIG, UMA, and TA.[3,5—8] However, the analysis of acid—base equilibrium by Stewart method requires multiple arithmetic calculations. Cl/Na ratio is a simpler method used to assess the presence of tissue acidosis.[6,8—12] It is based on the hypothesis that, because SIG ions are negatively charged, other plasma anions (Cl and albumin) must fall during tissue acidosis to maintain electroneutrality if the cations (Na and K) remain constant. Therefore, metabolic acidosis and a low Cl/Na ratio (<0.75) indicate SIG elevation, whereas a high Cl/Na ratio (>0.79) indicates acidosis caused by hyperchloremia. A normal Cl/Na ratio (0.75—0.79) in conjunction with metabolic acidosis suggests mixed type metabolic acidosis where hyperchloremia and SIG increases occur simultaneously.

2. Materials and methods

This study was conducted at Başkent University, Faculty of Medicine, Neonatal Intensive Care Unit between February 1, 2010 and July 31, 2012. This study was approved by our University Institutional Review Board (Project no: KA11/124). Forty-seven infants with a gestational age below 34 weeks who needed erythrocyte transfusion for anemia were enrolled. Infants with sepsis, patent ductus arteriosus, necrotizing enterocolitis, or major congenital anomalies were excluded. Erythrocyte transfusions were given according to restrictive transfusion guidelines used in our neonatal intensive care unit (Table 1). Transfusions consisted of packed red blood cells with a storage life of less than 10 days which were passed through leukocyte removal filter and irradiated. Erythrocyte transfusions were administered at a volume of 15 mL/kg and infusions lasted at least 2 hours.

In the present study, blood samples of 2.5 mL were obtained before and 12 hours after erythrocyte transfusion. Samples were analyzed for complete blood count, complete blood chemistry, and blood gas analysis. Concentrations of Na, K, Cl, Mg, and P were measured by an automated ion-specific electrode and albumin by bromocresol green dye binding. Blood gas analysis was performed using a GEM premier 3000 automatic blood gas analyzer (Instrumentation Laboratory, Bedford, MA 01730, U.S.A) and included the

Table 1 Transfusion guidelines.

Transfusions based upon hemoglobin or hematocrit triggers can be considered for the following clinical settings:

(1) infants connected to a conventional ventilator who require mechanical ventilatory support and have a hematocrit level less than 35%;

(2) infants who do not require mechanical ventilatory support but have a hematocrit level less than 30% and one or more of the following criteria: tachycardia lasting for more than 24 hours (peak heart rate >180 bpm), tachypnea lasting for more than 24 hours (respiratory rate > 60 breaths/min), twofold increase in oxygen requirement in the past 48 hours, metabolic acidosis as evidenced by pH: 7.20, weight increase less than 10 g/kg per day despite being fed on a 120 kcal/kg per day diet in the previous 4 days;

(3) preterm infants who have a hematocrit level less than 30% and required a serious surgical procedure in the past 72 hours;

(4) asymptomatic preterm infants who have a hematocrit level less than 25%.

bpm = beats per minute.

parameters of lactate, pH, base excess (BEecf), actual HCO3 (actHCO), and İonized Calcium (iCa). Pre- and post-transfusion SID, SIDe, SIG, UMA, TA, and Cl/Na ratio were calculated using the aforementioned formulae.

The participants were divided into two groups according to TA; low (<4) and high (≥4) tissue acid groups. Pre- and post-transfusion markers of tissue hypoxia were calculated in both groups. Next, the changes between pre- and post-transfusion values were calculated in the two groups and compared with each other.

All statistical analyses were performed with SPSS, Version 17 (SPSS Inc, Chicago, IL, USA) software package. Descriptive statistics included mean ± standard deviation (SD), median, minimum, and maximum for continuous variables; and number and percentage (%) for categorical variables. After testing for normality and homogeneity of variances by Shapiro–Wilk and Levene tests respectively, paired groups meeting the criteria for normal distribution were compared with Student *t* test; Mann–Whitney U-test was used for paired groups that did not meet the requirements for a normal distribution. After confirming that the variables met the criteria for the Mauchly test for sphericity, which is a prerequisite for repeated measures analysis of variance, repeated measures were compared using dependent samples *t* test or Wilcoxon test. Significance level was set at $p < 0.05$ for all comparisons.

3. Results

This study enrolled a total of 47 participants, of whom 23 (49%) were male and 24 (51%) were female. Study participants received a total of 60 erythrocyte transfusions during the study period. The mean birth weight and the mean gestational age were 1210 ± 365 g and 29.2 ± 2.7 weeks, respectively.

Increased TA (TA ≥ 4) as an evidence of tissue hypoxia were present before transfusion in 10 of 60 erythrocyte transfusions (16.6%).

The participants were divided into two groups according to TA as low (<4) and high (≥4) tissue acid groups. Pre-treatment and posttreatment Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) levels did not show any significant differences between these groups ($p > 0.05$).

The intragroup analysis of the group with tissue hypoxia (TA ≥ 4) revealed that, UMA and TA were significantly reduced ($p < 0.05$, Table 2) and Cl/Na ratio was increased after transfusion but this was not statistically significant ($p = 0.433$, Table 2). The two groups had no significant differences with respect to changes between pre- and post-transfusion blood gas parameters (pH, PO2, PCO2, HCO3act, BEecf, lactate; $p > 0.05$, Table 2). The two groups were also compared for the change between pre-and post-transfusion UMA, TA, and Cl/Na ratio values. The differences between the quantity of changes in UMA, TA, Cl/Na ratio values were statistically significant ($p < 0.05$, Table 2).

Figures 1, 2, and 3 show the error bars of pre- and post-transfusion changes of lactate, UMA, and Cl/Na ratio levels in the two groups, respectively (Figure 1, 2, and 3). Lactate levels were higher in the group with tissue hypoxia (TA ≥ 4) and decreased following transfusion, but change in lactate levels between pre- and post-transfusion measurements was not significantly different between the TA < 4 and TA ≥ 4 groups

Table 2 Comparison of blood gas values and hypoxia markers between the groups with low versus high tissue acid levels.

	TA < 4 (n = 50)	p^a	TA ≥ 4 (n = 10)	p^b	p
Pre-pH	7.36 ± 0.06	0.025	7.40 ± 0.06	0.242	0.715
Post-pH	7.39 ± 0.06		7.37 ± 0.08		
Pre-pCO2 (mmHg)	41.9 ± 9.4	0.408	35.2 ± 7.2	0.063	0.363
Post-pCO2	40.6 ± 11.8		41.7 ± 9.2		
Pre-pO2 (mmHg)	40.7 ± 22.9	0.012	56.7 ± 24.3	0.089	0.626
Post-pO2	51.0 ± 25.0		41.3 ± 10.6		
Pre-HCO_3act (mmol/L)	24.0 ± 4.0	0.358	21.6 ± 2.4	0.052	0.241
Post-HCO_3act	24.5 ± 4.4		24.0 ± 2.9		
Pre-BEecf (mmol/L)	−1.1 ± 4.1	0.040	−3.0 ± 2.5	0.008	0.473
Post-BEecf	−0.1 ± 4.3		−0.1 ± 3.1		
Prelactate (mmol/L)	1.6 ± 0.6	0.409	2.4 ± 1.9	0.190	0.073
Postlactate	1.8 ± 0.8		1.9 ± 0.5		
Pre-SID	35.4 ± 3.7	0.002	39.7 ± 5.5	0.588	0.011
Post-SID	37.1 ± 3.3		39.1 ± 6.2		
Pre-SIDe	35.7 ± 4.2	0.168	32.5 ± 2.6	0.033	0.066
Post-SIDe	36.5 ± 4.2		35.1 ± 2.4		
Pre-UMA	−2.0 ± 2.9	0.186	4.7 ± 4.5	0.051	0.001
Post-UMA	−1.2 ± 3.8		2.0 ± 5.0		
Pre-TA	−0.3 ± 2.7	0.124	7.2 ± 6.0	0.022	0.001
Post-TA	0.5 ± 3.8		3.9 ± 5.0		
Pre-Cl/Na	0.78 ± 0.02	0.003	0.74 ± 0.03	0.433	0.005
Post-Cl/Na	0.77 ± 0.02		0.75 ± 0.04		

p^a and p^b are significance of the intragroup changes.
p Significance of the intergroup changes.
BEecf = base excess; HCO_3act = actual HCO_3; SID = strong ion difference; TA = tissue acid level; UMA = unmeasurable anions.

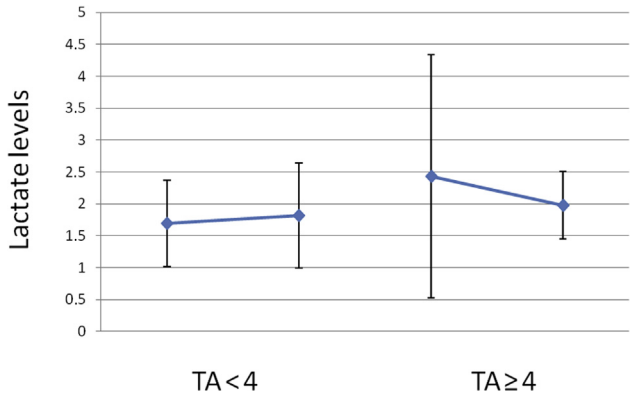

Figure 1 The error bars of pre- and posttransfusion changes of lactate ratio levels between the two groups. TA = tissue acid levels.

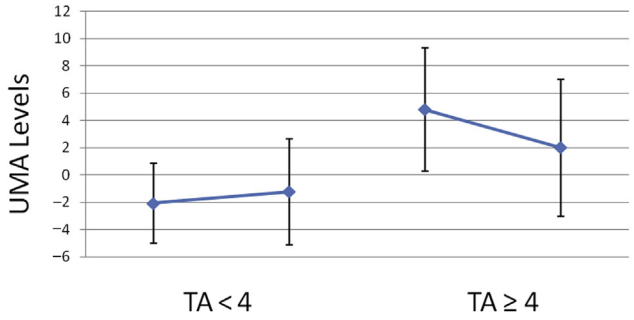

Figure 2 The error bars of pre- and posttransfusion changes of UMA ratio levels between the two groups. TA = tissue acid levels; UMA = unmeasurable anions.

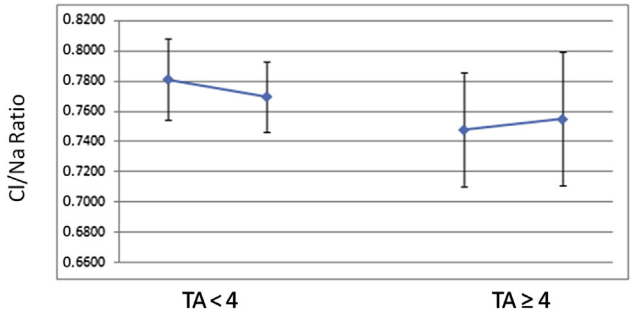

Figure 3 The error bars of pre- and posttransfusion changes of Cl/Na ratio levels between the two groups. TA = tissue acid levels.

($p = 0.073$; Figure 1). However, UMA levels were higher and decreased following transfusion and Cl/Na ratio levels were lower and increased following transfusion in the group with tissue hypoxia (TA ≥ 4; Figures 2 and 3). Changes in UMA and Cl/Na ratio levels between pre- and post-transfusion measurements were significantly different between the TA < 4 and TA ≥ 4 groups ($p = 0.001$ and $p = 0.005$, respectively).

4. Discussion

Erythrocyte transfusion is a treatment that effectively and rapidly corrects oxygen supply to body tissues in premature anemia. However, the optimal hemoglobin threshold for erythrocyte transfusion is unknown in premature infants. To date, two large-scale randomized controlled studies (Iowa and The Premature Infants in Need of Transfusion [PINT] studies) compared the impact of restricted and liberal transfusion approaches in premature anemia.[13,14] The Iowa study determined that apnea attacks, parenchymal brain hemorrhage, and periventricular leukomalacia were more common in patients assigned to restricted transfusion protocol.[13] The PINT study, however, failed to show any significant difference between restricted and liberal transfusion protocols with respect to death, premature retinopathy, bronchopulmonary dysplasia, and brain injury.[14] In the continuation study of the PINT study, Whyte et al[15] failed to show any significant difference in death or marked disability (Bayley Mental Developmental Index <2 SD, cerebral palsy, or blindness, or deafness) between the two groups at corrected 18–21 months. However, a *post hoc* analysis demonstrated that the number of patients with Bayley Mental Developmental Index <1 SD was significantly greater in the restricted transfusion group.[15] These trials suggested that hemoglobin concentration alone is not a reliable marker for transfusion decision in premature infants. Our study is the first to evaluate tissue hypoxia by the Stewart method in anemic preterm infants who received erythrocyte transfusion according to restricted transfusion guidelines. The advantage of the Stewart method is the ability to measure SIG, UMA, and TA. The presence of UMA in plasma provides evidence for tissue hypoxia. Lactate, which constitutes the other component of tissue acids, is known to be a useful marker of disease severity and mortality.[16–18] However, the earliest marker of acidosis at tissue level is an elevated level of unmeasurable tissue acids (UMA). Impaired tissue perfusion and metabolic acidosis secondary to tissue hypoxia can result from elevated UMA levels alone, without a necessarily elevated lactate level. Durward et al[9] evaluated tissue acidosis from blood samples obtained at admission and 24 hours later in patients admitted to a Pediatric Intensive Care Unit. They detected elevated UMA levels in 52.2% of patients with metabolic acidosis. Although there was an elevated lactate level in 47.5% of patients who also had elevated UMA level, isolated lactate elevation was detected in only 9.6% of patients with metabolic acidosis. Likewise, Murray et al[19] examined infants operated on for congenital heart disease, for tissue acidosis at the postoperative period. They obtained a total of 150 samples and detected acidosis in 60 samples. From among 60 samples with tissue acidosis, there was UMA elevation alone in 44 samples, lactate elevation alone in 6 and both UMA and lactate elevation in 10.

Following the introduction of the measurement of UMAs produced by impaired tissue perfusion and tissue hypoxia by strong ion theory, the correlation between SIG and mortality has also been explored. Balasubramanyan et al[20] investigated the correlation between BEecf, anion gap, lactate, and SIG levels and mortality in patients admitted to the Pediatric Intensive Care Unit.[20] They concluded that increased SIG level had a stronger correlation with mortality compared with BEecf, anion gap, and lactate levels. Further, Durward et al[21] studied the prognostic value of SIG level measured within the first 24 hours after cardiopulmonary bypass

surgery in infants. The authors reported that an increased SIG level was superior to lactate for predicting mortality.

Our study revealed that TA increased and tissue acidosis developed prior to transfusion in 16.6% of anemic preterm infants. Although there was no significant difference between the groups with and without tissue hypoxia with respect to change in lactate level between pre- and post-transfusion measurements, lactate level was greater and reduced following transfusion in the group with tissue hypoxia. UMA levels, however, were significantly greater before transfusion and reduced after it in the group with tissue hypoxia. Thus, we concluded that anemia-induced tissue hypoxia was corrected by erythrocyte transfusion.

Our study demonstrated that tissue hypoxia developed despite the absence of any clinical signs or a blood gas parameter showing metabolic acidosis in 16.6% of anemic preterm infants. Hence, we suggest that signs of tissue hypoxia should be sought for transfusion decisions in anemic preterm infants. As the Stewart method requires multiple arithmetic calculations to determine TA, Cl/Na ratio can also be used as a simpler alternative for determining tissue hypoxia. A low Cl/Na ratio (<0.75) indicates increased levels of UMAs. Durward et al[9] analyzed 540 blood samples taken at admission and 24 hours later from 282 patients admitted to a Pediatric Intensive Care Unit. They reported that a low Cl/Na ratio was a good marker of increased TA (PPV 88%) whereas a high Cl/Na ratio (>0.79) was suggestive of the absence of such an increase (PPV 81%). Further, Kurt et al[6] analyzed 105 blood samples in 59 critically ill newborns with metabolic acidosis. They reported a negative correlation between Cl/Na ratio and SID, corrected anion gap, UMA, and TA. Our study also demonstrated a reduced Cl/Na ratio in the group with tissue hypoxia, which increased after erythrocyte transfusions. Change in Cl/Na ratio levels between pre- and post-transfusion measurements was significantly different between the TA < 4 and TA ≥ 4 groups ($p = 0.005$). Therefore, a low Cl/Na ratio was considered as a candidate marker for tissue hypoxia in anemic preterm infants.

In conclusion, the results of the present study suggest that the determination of the level of tissue hypoxia by the Stewart approach may be an alternative to restrictive transfusion guidelines for timing of transfusion in premature anemia. The study also showed that a low Cl/Na ratio could be used as a simple marker of tissue hypoxia.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Stewart PA. Modern quantitative acid—base chemistry. *Can J Physiol Pharmacol* 1983;**61**:1444—61.
2. Figge J, Mydosh T, Fencl V. Serum proteins and acid—base equilibria: a follow-up. *J Lab Clin Med* 1992;**120**:713—9.
3. Kellum JA. Disorders of acid—base balance. *Crit Care Med* 2007;**35**:2630—6.
4. Gomez H, Kellum JA. Understanding acid base disorders. *Crit Care Clin* 2015;**31**:849—60.
5. Rastegar A. Clinical utility of Stewart's method in diagnosis and management of acid—base disorders. *Clin J Am Soc Nephrol* 2009;**4**:1267—74.
6. Kurt A, Ecevit A, Ozkiraz S, Ince DA, Akcan AB, Tarcan A. The use of chloride—sodium ratio in the evaluation of metabolic acidosis in critically ill neonates. *Eur J Pediatr* 2012;**171**:963—9.
7. Kishen R, Honoré PM, Jacobs R, Joannes-Boyau O, De Waele E, De Regt J, et al. Facing acid—base disorders in the third millennium —the Stewart approach revisited. *Int J Nephrol Renovasc Dis* 2014;**7**:209—17.
8. Szrama J, Smuszkiewicz P. An acid—base disorders analysis with the use of the Stewart approach in patients with sepsis treated in an intensive care unit. *Anaesthesiol Intensive Ther* 2016;**48**:180—4.
9. Durward A, Skellett S, Mayer A, Taylor D, Tibby SM, Murdoch IA. The value of the chloride:sodium ratio in differentiating the aetiology of metabolic acidosis. *Intensive Care Med* 2001;**27**:828—35.
10. Moviat M, van Haren F, van der Hoeven H. Conventional or physicochemical approach in intensive care unit patients with metabolic acidosis. *Crit Care* 2003;**7**:R41—5.
11. Badr A, Nightingale P. An alternative approach to acid—base abnormalities in critically ill patients. *Contin Educ Anaesth Crit Care Pain* 2007;**7**:107—11.
12. Nagaoka D, Nassar Junior AP, Maciel AT, Taniguchi LU, Noritomi DT, Azevedo LC, et al. The use of sodium-chloride difference and chloride—sodium ratio as strong ion difference surrogates in the evaluation of metabolic acidosis in critically ill patients. *J Crit Care* 2010;**25**:525—31.
13. Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. *Pediatrics* 2005;**115**:1685—91.
14. Kirpalani H, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, et al. The premature infants in need of transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. *J Pediatr* 2006;**149**:301—7.
15. Whyte RK, Kirpalani H, Asztalos EV, Andersen C, Blajchman M, Heddle N, et al. Neurodevelopmental outcome of extremely low birth weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. *Pediatrics* 2009;**123**:207—13.
16. Grayck EN, Meliones JN, Kern FH, Hansell DR, Ungerleider RM, Greeley WJ. Elevated serum lactate correlates with intracranial hemorrhage in neonates treated with extracorporeal life support. *Pediatrics* 1995;**96**:914—7.
17. Cheung PY, Etches PC, Weardon M, Reynolds A, Finer NN, Robertson CM. Use of plasma lactate to predict early mortality and adverse outcome after neonatal extracorporeal membrane oxygenation: a prospective cohort in early childhood. *Crit Care Med* 2002;**30**:2135—9.
18. Kalyanaraman M, DeCampli WM, Campbell AI, Bhalala U, Harmon TG, Sandiford P, et al. Serial blood lactate levels as a predictor of mortality in children after cardiopulmonary bypass surgery. *Pediatr Crit Care Med* 2008;**9**:285—8.
19. Murray DM, Olhsson V, Fraser JI. Defining acidosis in postoperative cardiac patients using Stewart's method of strong ion difference. *Pediatr Crit Care Med* 2004;**5**:240—5.
20. Balasubramanyan N, Havens PL, Hoffman GM. Unmeasured anions identified by the Fencl—Stewart method predict mortality better than base excess, anion gap, and lactate in patients in the pediatric intensive care unit. *Crit Care Med* 1999;**27**:1577—81.
21. Durward A, Tibby SM, Skellett S, Austin C, Anderson D, Murdoch IA. The strong ion gap predicts mortality in children following cardiopulmonary bypass surgery. *Pediatr Crit Care Med* 2005;**6**:281—5.