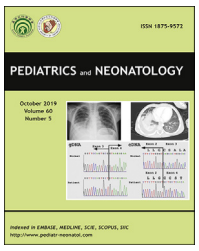
Pediatrics and Neonatology (2019) 60, 537–542

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



Original Article

Effect of packed red blood cell transfusion on IL-8 and sICAM-1 in premature neonates at different postnatal ages

Lamiaa Mohsen [a,f,1], Hala Youssef [b], Hala Abdelrahman [c], Rabab El Hawary [c], Abeer Selim [b,*], Safwat Aly [d], Mustafa Nada [b], Hany Aly [e]

[a] Department of Neonatology, Faculty of Medicine, Cairo University Hospitals, Egypt
[b] Pediatrics Department, Medical Research Division and Medical Research Centre of Excellence (MRCE), National Research Centre, Cairo, Egypt
[c] Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt
[d] Department of Pediatric Cardiology, Hospital for Sick Children, Toronto, Canada
[e] Department of Neonatology, Cleveland Clinic Children's Hospital, USA
[f] Faculty of Medicine, New Giza University, Cairo, Egypt

Received Mar 2, 2018; received in revised form Aug 22, 2018; accepted Jan 22, 2019
Available online 30 January 2019

Key Words
blood transfusion;
cytokines;
interleukin 8;
preterm;
transfusion-related immunomodulation

Background: Transfusion-related immunomodulation (TRIM) has been described in adults; however, its existence in neonates is not confirmed. The generation of TRIM is attributed to increased concentrations of IL-8, sICAM-1 and other pro-inflammatory cytokines. This study aimed to monitor changes in IL-8, sICAM-1 as markers for TRIM in premature infants at different postnatal ages.

Methods: Preterm infants with a gestational age between 28 and 32 weeks who were receiving PRBC transfusion during the first 28 days of life were included in the study. Infants were stratified into two groups according to their postnatal age: Group 1 with postnatal ages of (0–14) days and Group 2 of (15–28) days. The concentrations of IL-8 and sICAM-1 were measured by enzyme-linked immunosorbent assay (ELISA) before transfusion, 6 h after the end of transfusion and in the donor's PRBCs bag immediately before infusion into the baby.

Results: IL-8 concentration in the PRBCs bags correlated with post-transfusion level in Group 2 ($r = 0.59$, $p = 0.002$) but not in Group 1 ($r = 0.39$, $p = 0.06$). sICAM-1 concentration in the bag correlated with infants'concentrations in neither group. In Group 1, pre-transfusion

* Corresponding author. Pediatrics Department, Medical Research Division and Medical Research Centre of Excellence (MRCE), National Research Centre, 33 El Buhouth street, Dokki, Giza, Cairo, Egypt.
E-mail addresses: Lamiaa7777@yahoo.com, Lmohsen@ngu.edu.eg (L. Mohsen), dr.hala_youssef@hotmail.com (H. Youssef), halahelmi@hotmail.com (H. Abdelrahman), rabab_elhawary@hotmail.com (R. El Hawary), abeerselim2004@yahoo.com, as.moustafa@nrc.sci.eg (A. Selim), safwataly36@gmail.com (S. Aly), dr.mostafa.abdelkader@hotmail.com (M. Nada).
[1] Pediatrics & Neonatology Department, Faculty Of Medicine, Cairo University, Faculty of Medicine, New Giza University, Cairo, Egypt.

https://doi.org/10.1016/j.pedneo.2019.01.010

concentrations of both cytokines (IL-8 and sICAM-1) did not correlate whereas post-transfusion concentrations did correlate ($r = -0.09$, $p = 0.68$ and $r = 0.4$, $p = 0.05$ respectively). In Group 2, the concentrations of both cytokines did not correlate with each other during pre-transfusion ($r = 0.11$, $p = 0.58$) as well as post-transfusion ($r = 0.12$, $p = 0.56$). There was no significant increase in either cytokines after transfusion in each group.
Conclusion: This study showed positive correlation between IL-8 concentration in the transfusion bag and post transfusion in Group 2 infants which could be attributed to passive transmission from the bags. This study does not support an immune modulatory effect for packed RBC in preterm infants.

1. Introduction

Blood transfusion is a common intervention in premature infants. More than 80% of very low birth weight infants (VLBW) receive at least one transfusion during their hospital stay.[1,2] Complications related to transfusions in premature infants are mostly non-infectious.[3] Premature infants receiving blood transfusion are at increased risk of chronic lung disease, retinopathy of prematurity, and necrotizing enterocolitis (NEC).[4,5] The incidence and severity of these complications are correlated with the number and volume of packed red blood cell (PRBC) transfusions received.[4,6]

Transfusion-related immunomodulation (TRIM) has been described in adults and older children; however, its role in causing morbidities in premature infants is not clear. With the increase in identification of such complications, the need for implementation of blood conservation practices has become critical.[7]

TRIM is more potent when whole blood, rather than PRBC, is transfused. Thus, it is believed to be related to the infusion of allogeneic leukocytes,[8] platelet, plasma, or other micro particles.[9] As TRIM can also occur after the administration of plasma and leuko-reduced products, previous studies related TRIM to allogenic plasma which is present in the transfused products, including contaminating mediators that have been built into these components during the storage period.[8,10]

The endothelium plays an essential role in facilitating tissue response to inflammatory stimuli. Activation of endothelial cells during inflammatory responses is typically induced by pro-inflammatory cytokines, resulting in leukocyte recruitment at the sites of cellular damage.[11] The process of leukocyte recruitment occurs through the expression of several adhesion molecules and chemokines, such as macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule-1 (sICAM-1), and IL-8. MIF arrests leukocyte rolling.[12] sICAM-1 promotes leukocyte adhesion,[11,13] and IL-8 helps leukocyte emigration from the vasculature.[14] Both MIF and sICAM-1 were found to be increased post-transfusion while MIF was reported to be acutely raised in preterm infants with necrotizing enterocolitis.[15] The increased concentrations of these factors may contribute to the generation of TRIM.

The aim in this prospective study on preterm infants was to assess the impact of PRBC transfusion on IL-8, which is a pro-inflammatory cytokine and on sICAM-1, which is a marker of endothelial activation, both of which are essential factors for the generation of TRIM. We hypothesized that immunological impact of PRBC might not exist in the first two weeks of life, but would occur after two weeks.

2. Methods

2.1. Study population

This prospective observational study was conducted at Cairo University Children's Hospital, Cairo, Egypt. The study was approved by the Institutional Review Board (IRB) of the Children's Research Center. Parental consent was obtained before enrollment. The study included preterm infants with gestational age between 28 and 32 weeks who were to receive PRBC transfusion during their first weeks of life. Infants were excluded from the study if they had gestational age <28 or >32 weeks, major congenital anomalies, and clinical or culture-proven sepsis at the time of transfusion. Since immune responses may differ according to postnatal age, equal numbers of infants were recruited in two groups: Group 1 with postnatal ages of (0—14) days and Group 2 of (15—28) days.

2.2. Study design

2.2.1. Data collection

We collected demographic data including birth weight (BW), gestational age (GA), sex and maternal age. We also collected clinical data including mode of delivery, Apgar scores, duration of respiratory support, culture-proven sepsis, bronchopulmonary dysplasia (BPD): defined as oxygen requirement or dependent at 36 weeks postmenstrual age, retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), length of hospital stay and mortality.

2.3. Packed red blood cell transfusion

The decision to transfuse was made by the attending neonatologist according to a standardized transfusion algorithm.[16] Babies were transfused for hematocrit $<35\%$ if symptomatic or if birth weight <1200 g in the first 2 weeks of

life regardless of their symptoms. These symptoms included the need for respiratory support or unexplained tachycardia. Asymptomatic infants or infants <1200 g beyond the first 2 weeks of life were transfused if hematocrit <30% based on an individualized approach, which considered the reticulocytic count, post-menstrual age, last time of transfusion, availability of the same donor blood and/or poor sucking. The transfused RBCs were O Rh negative in blood type, stored in citrate-phosphate-dextrose-adenine-1. A volume of 15 ml/kg was given over a period of 4 h.

2.4. Blood sampling

Three blood samples were obtained for each subject: one from the infant before transfusion, one from the donor's PRBC bag immediately before infusion, and the last one from the infant 6 h after the end of transfusion. Samples were collected by clean venipuncture from patients under sterile conditions using acceptable medical techniques to avoid hemolysis in citrated vacutainers, and the plasma was preserved in aliquots at −80 °C until analyzed.

2.5. IL-8 and sICAM-1 assays

The concentrations of IL-8 (Assaymax EI1008-1, USA) and sICAM-1 (Invitrogen #KHS5411, USA) were measured using commercially available enzyme-linked immunosorbent assay (ELISA).

2.6. Statistical method

Data were described in mean, standard deviation, median and range. Comparison between the study groups was done using Mann—Whitney U test for independent samples. Within group comparison between pre- and post-transfusion values were done using Wilcoxon signed rank test for paired samples. Correlations between various variables were done using Pearson correlation coefficient for linear relation in normally distributed variables and Spearman rank correlation coefficient for non-normal variables/non-linear monotonic relation. *P* values of 0.05 were considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

3. Results

Fifty premature infants who received PRBC transfusion were included in this study. Their demographic and clinical characteristics are presented in Table 1.

The median IL-8 concentration in Group 1 PRBC bags was 23.5 pg/ml, and in Group 2 bags was 36.5 pg/ml, whereas for sICAM-1 the median concentration was 190 ng/ml in Group 1 bags and 140 ng/ml in Group 2 bags. There was no statistically significant relationship between the cytokine concentrations and the age of the transfused red cells in either group. For IL-8 and age of RBC results were as follows: ($r = -0.023$, $p = 0.29$ and $r = -0.18$, $p = 0.4$ in Groups 1 and 2 respectively, whereas for sICAM-1 and age of RBC results were: $r = 0.12$, $p = 0.67$ and $r = 0.25$, $p = 0.37$, respectively). There was no significant change in pre- and post-transfusion concentrations of cytokines. Fig. 1 illustrates pre- and post-transfusion concentrations of IL-8 and sICAM-1 in both groups.

IL-8 concentration in the PRBC bags correlated significantly with post-transfusion concentration IL-8 in Group 2 ($r = 0.59$, $p = 0.002$); however, this correlation was not significant in Group 1 ($r = 0.39$, $p = 0.06$) and it was not significant for sICAM-1 in both groups ($r = -0.32$, $p = 0.24$ and $r = 0.33$, $p = 0.23$ in groups 1 and 2, respectively).

Pre- and post-transfusion concentrations for IL-8 did not correlate in Group 1 or 2 ($r = -0.09$, $p = 0.68$ and $r = 0.11$, $p = 0.58$ respectively). Pre- and post-transfusion concentrations for sICAM-1 correlated in Group 1 ($r = 0.4$, $p = 0.05$) but not in Group 2 ($r = 0.12$, $p = 0.56$). Correlations of IL-8 and sICAM-1 with clinical characteristics are presented in Table 2.

There was a negative correlation between the post-transfusion levels of IL-8 and IVH in all neonates ($r = -0.34$, $p = 0.02$).

Table 1 Demographics and clinical characteristics of the study population (n = 50).

	Group 1 (n = 25)	Group 2 (n = 25)	p value
GA (weeks)	29.5 ± 1.6	29.9 ± 1.5	0.36
Postnatal age (days)	7.4 ± 4.2	22.9 ± 4.6	0.009
Weight (grams)	1160 ± 233	1331 ± 228	0.01
Sex: Male (%)	8 (32)	12 (48)	0.25
Mode of ventilation:			
Room air	8 (32)	14 (56)	0.09
Nasal cannula with oxygen	4 (16)	4 (16)	1
Continuous positive airway pressure	1 (4)	1 (4)	1
Conventional ventilation	12 (48)	6 (24)	0.07
Feeding at time of transfusion	12 (48)	20 (80)	0.02
Complications:			
Pulmonary hemorrhage	3 (12)	0 (0)	0.07
Intraventricular hemorrhage	3 (12)	0 (0)	0.07
Pneumothorax	4 (16)	2 (8)	0.38
Necrotizing enterocolitis	0 (0)	0 (0)	NA

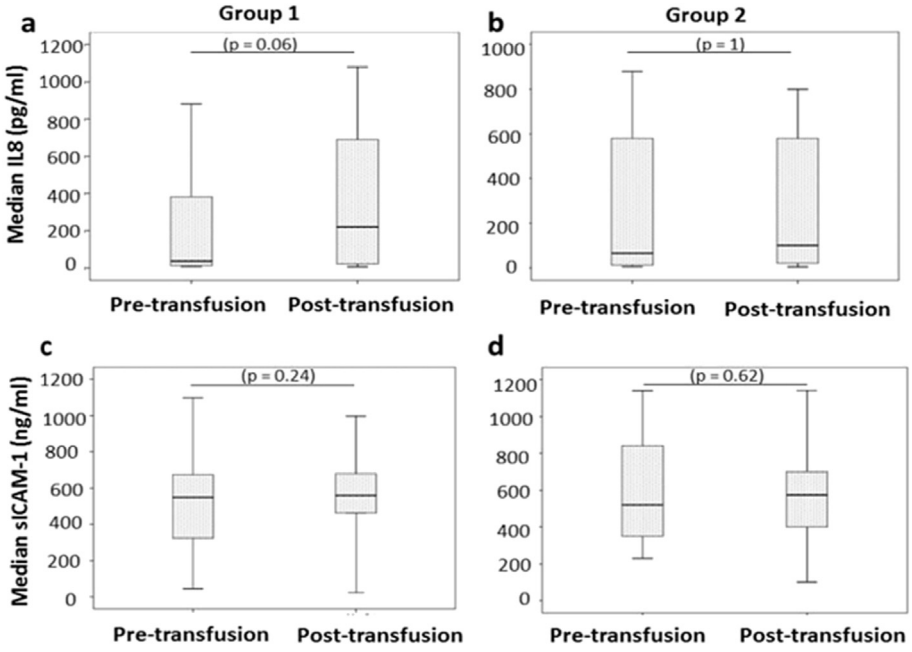


Figure 1 Median pre-transfusion and post-transfusion levels of IL8 and sICAM-1 in Group 1 and 2 neonates.

Table 2 Correlations of IL8 and sICAM-1 measured pre- and post-transfusion with clinical variables (n = 50).

	IL8		sICAM-1	
	Group 1	Group 2	Group 1	Group 2
Gestational age (pre-transfusion)	r = −0.2, p = 0.33	r = −0.05, p = 0.83	r = −0.29, p = 0.17	r = −0.27, p = 0.2
Gestational age (post-transfusion)	r = −0.36, p = 0.08	r = −0.41, p = 0.04	r = −0.32, p = 0.12	r = −0.4, p = 0.05
NPO status (pre-transfusion)	r = 0.02, p = 0.94	r = −0.43, p = 0.03	r = 0.4, p = 0.05	r = 0.01, p = 0.97
NPO status (post-transfusion)	r = 0.06, p = 0.79	r = −0.26, p = 0.26	r = 0.44, p = 0.03	r = 0.14, p = 0.49
Mortality (pre-transfusion)	r = −0.08, p = 0.57		r = 0.25, p = 0.08	
Mortality (post-transfusion)	r = 0.04, p = 0.77		r = 0.29, p = 0.04	

4. Discussion

This study demonstrated no change in IL-8 and sICAM-1 concentrations after PRBC transfusions. IL-8 concentrations post-transfusion correlated with IL-8 concentration in the donor bag in Group 2. Feeding status correlated significantly with both cytokines in the two groups. Infants who were kept NPO had greater concentrations of both factors. Infants with intraventricular hemorrhage (IVH) had less IL-8 measured post-transfusion.

The median pre-transfusion concentration of IL-8 was 35 pg/ml in Group 1 neonates and 65 pg/ml in Group 2 neonates. These concentrations were greater than previously reported concentrations by Lusyati et al.[17] However, the reported concentrations in the current study were in line with those of Apostolou et al.,[18] who reported a median sICAM-1 level in preterm neonates of 469.6 ng/ml and in full term neonates one of 353.4 ng/ml. They concluded that prematurity resulted in increased serum concentration of sICAM1.

In Group 1 neonates the post-transfusion concentrations of IL-8 tended to exceed the pre-transfusion concentrations although this was not statistically significant (p = 0.06). Keir et al.[19] reported a significant increase in post-transfusion levels of IL-8 and sICAM-1 following packed RBC exposure.

In our study, there was no significant difference in the pre-transfusion levels of both cytokines between the two neonatal groups (p = 0. 61 for IL-8 and p = 0.59 for sICAM-1) or in post-transfusion levels (p = 0.33 for IL-8 and 0.67 for sICAM). The assessment of the IL-8 and sICAM-1 concentrations in the blood bag supernatants showed a great variation in different donor bag. This is in agreement to the study by Dean et al.,[20] who found that the concentration of biological mediators varied greatly between individual PRBC units. They reported a donor-donor variation in the level of sICAM-1 that could reach up to 10 fold difference between PRBC units.

Correlation studies between the age of PRBC bags and IL-8 and sICAM-1 levels were not statistically significant in Groups 1 and 2 neonates. This lack of relationship could be attributed to the use of fresh PRBC that were all subject to less than 5 days of storage. This was in agreement to Keir et al.,[19] who confirmed that the level of mediators (IL-8 and sICAM) showed no significant relationship with the age of the leukoreduced PRBC transfused. Other studies showed progressive accumulation of IL-8 during storage of non

leukoreduced PRBC, reaching the highest level at day 42 of storage.[21]

There was a positive correlation between the post-transfusion level of IL-8 and its level in the PRBC bags. This correlation was of statistical significance in Group 2 neonates ($p = 0.002$) and was not significant in Group 1 neonates ($p = 0.06$). This finding was consistent with other studies that showed that the supernatant from non-leukoreduced PRBC induced the expression of CD11b on PMNs, priming PMNs to release IL-8.[21] However, the correlation between post transfusion level of sICAM-1 and its level in blood bags was not statistically significant in Groups 1 and 2 neonates.

There was a significant correlation between post-transfusion levels of IL-8 and sICAM-1 in Group 1 neonates ($p = 0.05$), but not in Group 2 neonates ($p = 0.56$). Regarding the correlations between concentrations of cytokines and the type of feeding, a positive correlation was found between TPN and each of pre-transfusion level of sICAM-1 ($p = 0.003$) and post-transfusion level of sICAM-1 ($p = 0.02$) in Group 1. However, this correlation was not significant in Group 2 neonates. Forty percent of neonates in Group 1 were on TPN while only 16% of neonates in Group 2 were on TPN. This was in agreement with Ooi et al.,[22] who stated that adequate nutrition through TPN may augment immune function, and there was significant increase of mitogen-stimulated lymphocyte proliferation and sICAM-1 level after TPN supply. On the other hand, there was a negative correlation between TPN and pre-transfusion level of IL-8 ($p = 0.03$) in Group 2 and this was in contradiction with the results of Lavoie et al.,[23] who linked inflammatory responses to parenteral nutrition.

Correlation studies between the post-natal age of neonates and pre- and post-transfusion levels of IL-8 and sICAM-1 were non-significant in the two neonatal groups. On the other hand, this study showed a negative correlation between gestational age and post-transfusion levels of IL-8 in Group 2 neonates ($p = 0.04$). This was consistent with Huang et al.,[24] who reported a negative relationship between IL-8 and gestational age of prematurity.

There was a positive correlation between the post-transfusion levels of sICAM-1 and mortality in all neonates ($p = 0.04$). This finding implies that endothelial cell injury as evidenced by increased sICAM-1 contributes to the pathogenesis that leads to death. For example, lack of endothelial integrity and capillary leak are not uncommon in infants with sepsis who subsequently die. There is a tendency among clinicians to follow a conservative strategy that minimizes transfusions as reports showed increased mortalities in infants who received PRBC transfusions.[25] However, this notion is unfounded in premature infants because transfusion could only be a marker of illness; infants who receive transfusions are usually the sickest. Previous clinical trials did not show increased mortality with transfusion. In fact, morbidities were significantly less in infants randomized to receive transfusions.[1] In addition, the current study did not show increased immune-related response to transfusions.

5. Conclusion

The positive correlation between the bag IL-8 concentration and the post-transfusion concentration in preterm neonates receiving PRBC could be attributed to passive transmission from the bags. It is not evident in this study that there is an immune modulatory effect of packed RBCs; however, further studies with larger sample size that assess other endothelial markers and cytokines should be conducted in order to exclude the PRBC TRIM.

Financial support

The authors declare that they did noy receive any financial support from agencies or others.

Conflict of interest statement

The authors declare that there was no conflict of interest.

Acknowledgment

The authors thank all the patients who participated in the study and their parents.

References

1. 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.
2. Ohls RK. Transfusions in the preterm infant. *Neoreviews* 2007; **8**:377–86.
3. AuBuchon JP, Birkmeyer JD, Busch MP. Safety of the blood supply in the United States: opportunities and controversies. *Ann Intern Med* 1997;**127**:904–9.
4. Cooke RW, Drury JA, Yoxall CW, James C. Blood transfusion and chronic lung disease in preterm infants. *Eur J Pediatr* 1997; **156**:47–50.
5. Blau J, Calo JM, Dozor D, Sutton M, Alpan G, La Gamma EF. Transfusion related acute gut injury: necrotizing enterocolitis in very low birth weight neonates after packed red blood cell transfusion. *J Pediatr* 2011;**158**:403–9.
6. Inder TE, Clemett RS, Austin NC, Graham P, Darlow BA. High iron status in very low birth weight infants is associated with an increased risk of retinopathy of prematurity. *J Pediatr* 1997; **131**:541–4.
7. Gernsheimer T. Transfusion therapy: blood components and transfusion complications. In: Irwin R, Rippe J, editors. *Intensive care medicine*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2003. p. 278–83.
8. Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. *Blood* 1994;**84**:1703–21.
9. Saas P, Angelot F, Bardiaux L, Seilles E, Garnache-Ottou F, Perruche S. Phosphatidylserine-expressing cell by-products in transfusion: a pro-inflammatory or an anti-inflammatory effect? *Transfus Clin Biol* 2012;**19**:90–7.
10. Kirkley SA, Cowles J, Pellegrini VD Jr, Harris CM, Boyd AD, Blumberg N. Cytokine secretion after allogeneic or autologous blood transfusion. *Lancet* 1995;**345**:527.
11. Petri B, Phillipson M, Kubes P. The physiology of leukocyte recruitment: an in vivo perspective. *J Immunol* 2008;**180**: 6439–46.
12. Cheng Q, McKeown SJ, Santos L, Santiago FS, Khachigian LM, Morand EF, et al. Macrophage migration inhibitory factor increases leukocyte-endothelial interactions in human

endothelial cells via promotion of expression of adhesion molecules. *J Immunol* 2010;**185**:1238—47.
13. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;**83**:2008—17.
14. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. *Microcirculation* 2009;**16**:31—42.
15. Ren Y, Lin CL, Li Z, Chen XY, Huang X, Lui V, et al. Up-regulation of macrophage migration inhibitory factor in infants with acute neonatal necrotizing enterocolitis. *Histopathology* 2005;**46**:659—67.
16. Shannon KM, Keith JF 3rd, Mentzer WC, Ehrenkranz RA, Brown MS, Widness JA, et al. Recombinant human erythropoietin stimulates erythropoiesis and reduces erythrocyte transfusions in very low birth weight preterm infants. *Pediatrics* 1995;**95**:1—8.
17. Lusyati S, Hulzebos CV, Zandvoort J, Sauer PJ. Levels of 25 cytokines in the first seven days of life in newborn infants. *BMC Res Notes* 2013;**6**:547.
18. Apostolou M, Dimitriou H, Kaleyias J, Perdikogianni C, Stiakaki E, Castalos C, et al. Levels of soluble ICAM-1 in premature and full-term neonates with infection. *Mediators inflamm* 2002;**11**:95—8.
19. Keir AK, McPhee AJ, Andersen CC, Stark MJ. Plasma cytokines and markers of endothelial activation increase after packed red blood cell transfusion in the preterm infant. *Pediatr Res* 2013;**73**:75—9.
20. Dean MM, Samson LD, Rooks K, Fryk J, Baidya S, Flower RL. Donor variation in biological mediators during storage of packed red blood cells. *Blood* 2013;**122**:3655.
21. Sparrow RL, Patton KA. Supernatant from stored red blood cell primes inflammatory cells: influence of prestorage white cell reduction. *Transfusion* 2004;**44**:722—30.
22. Ooi SE, Chen GW, Chou CT. Adequate nourishment through total parenteral nutrition treatment may augment immune function in patients with colon cancer. *Arch Med Res* 2004;**35**:289—93.
23. Lavoie PM, Lavoie JC, Watson C, Rouleau T, Chang BA, Chessex P. Inflammatory response in preterm infants is induced early in life by oxygen and modulated by total parenteral nutrition. *Pediatr Res* 2010;**68**:248—51.
24. Huang HC, Tai FY, Wang FS, Liu CA, Hsu TY, Ou CY, et al. Correlation of augmented IL-8 production to premature chronic lung disease: implication of posttranscriptional regulation. *Pediatr Res* 2005;**58**:216—21.
25. Dos Santos AM, Guinsburg R, de Almedia MF, Procianoy RS, Leone CR, Marba ST, et al. Red blood cell transfusions are independently associated with intra-hospital mortality in very low birth weight preterm infants. *J Pediatr* 2011;**159**:371—6.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2019.01.010.