Red blood cell transfusions can induce proinflammatory cytokines in preterm infants

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BACKGROUND: The risk of developing red blood cell (RBC) transfusion–associated necrotizing enterocolitis (TANEC) in preterm infants has recently been emphasized. Our aim was to assess changes in cytokine serum levels after RBC transfusions in a cohort of very preterm infants to evaluate their possible proinflammatory effect.

STUDY DESIGN AND METHODS: We carried out a prospective observational study. One transfusion event was studied in infants less than 32 weeks' gestation and more than 7 days old (n = 20) admitted to a tertiary neonatal intensive care unit. Interleukin (IL)−1β, IL-6, IL-8, tumor necrosis factor-α, interferon-γ (IFN-γ), IL-17, monocyte chemoattractant protein-1 (MCP-1), interferon-γ–induced protein 10 (IP-10), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule serum levels were measured in enrolled patients within 120 minutes before (T_0) the RBC transfusion and then within 120 minutes (T_1), 12 ± 3 hours (T_2), 24 ± 6 hours (T_3), and 48 ± 6 hours (T_4) after the end of RBC transfusion.

RESULTS: Infants received 19.8 ± 3.0 mL of RBCs at the mean age of 50 ± 18 days. Their hematocrit level increased from 24.1 ± 1.2% to 39.4 ± 2.9%. IL-1β, IL-8, IFN-γ, IL-17, MCP-1, IP-10, and ICAM-1 increased significantly after RBC transfusions.

CONCLUSION: Proinflammatory cytokines are increased after RBC transfusion. These findings may contribute to explaining the pathogenesis of TANEC and suggest the opportunity of adopting wise transfusion guidelines that would help to avoid detrimental risks of transfusion-related immunomodulation and of undertransfusion.

Preterm infants are at high risk of becoming rapidly anemic due to a combination of frequent laboratory blood sampling and their immature hematopoietic system.[1,2] Therefore, from 80% to 90% of extremely low birthweight infants receive one or more transfusions of red blood cells (RBCs).[3,4] The risks and benefits of RBC transfusions for preterm infants remain unclear; guidelines for transfusing RBCs are controversial and practices vary greatly. Studies on liberal[5] or restrictive[6] policies of RBC transfusions have provided inconclusive and contradictory results, although one meta-analysis suggests that restrictive RBC transfusion may be utilized without increasing short-term neonatal morbidities.[4]

It has been reported that RBC transfusions can increase the risk of developing multifactorial disease such

ABBREVIATIONS: BPD = bronchopulmonary dysplasia; ICAM-1 = intracellular adhesion molecule-1; IP-10 = interferon-γ–induced protein 10; IQR = interquartile range; MPC-1 = monocyte chemoattractant protein-1; NEC = necrotizing enterocolitis; NTBI = non–transferrin-bound iron; ROP = retinopathy of prematurity; TANEC = transfusion–associated necrotizing enterocolitis; TRIM = transfusion-related immunomodulation; VCAM = vascular cell adhesion molecule.

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as bronchopulmonary dysplasia (BPD)[7-9] and retinopathy of prematurity (ROP).[10,11] However, their pathogenetic role has been mainly emphasized for necrotizing enterocolitis (NEC), [12,13] which is the most common neonatal gastrointestinal complication in preterm infants.[14] In fact, several cases of NEC develop after RBC transfusions,[15-18] and 25% to 30% of these occur within 48 hours after the transfusion.[13,19] It has been reported that RBC transfusions increase significantly (odds ratio, 2.3; 95% confidence interval, 1.2-4.2) the risk of developing transfusion associated-NEC (TANEC),[20] while a strict policy of withholding feeds during RBC transfusions seems to decrease it.[21] Another article suggests that severe anemia, but not RBC transfusion, was associated with an increased risk of NEC.[22]

Despite these findings, the pathogenesis of TANEC is unknown. Previous studies have investigated the possible inflammatory effect of RBC transfusions mediated by the increase in proinflammatory cytokine serum values[23,24] and iron[25] in transfused preterm infants, but their results were contradictory and inconclusive.

In light of previous data, we have hypothesized that RBC transfusions can induce a proinflammatory response in treated preterm infants and to test this hypothesis we assessed changes in serum levels of interleukin (IL)1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α), interferon-γ (IFN- γ), IL-17, monocyte chemoattractant protein-1 (MCP-1), IFN-γ–induced protein 10 (IP-10), intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule (VCAM) during the 48 hours after RBC transfusions in a cohort of very preterm infants.

MATERIALS AND METHODS

Patient population

This prospective center-based study was carried out at the neonatal intensive care unit of Careggi University Hospital of Florence. The study was approved by the Tuscany pediatric ethics committee. Infants with gestational age of less than 32 weeks and postnatal age of more than 7 days were enrolled in the study if they needed RBC transfusions according to the guidelines of the Italian Society of Neonatology[26] and after parental informed consent was given. Exclusion criteria were major congenital malformations, inherited metabolic diseases, previous RBC transfusions during the 4 weeks before the study, and suspected or blood culture–proven sepsis or steroidal treatment during the 7 days before and the 2 days after the transfusion (because these conditions could affect the cytokine profile).

Study design

Serum levels of IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-17, MCP-1, IP-10, ICAM-1, and VCAM were measured in enrolled patients within 120 minutes before (T_0) the RBC transfusion, and then within 120 minutes (T_1), 12 ± 3 hours (T_2), 24 ± 6 hours (T_3), and 48 ± 6 hours after the end of RBC transfusion. Cytokines were determined in whole blood samples (150 μL) collected in tube from heel puncture, or in syringe from vein puncture, contemporary to sampling for routine blood gas analyses or other biochemical tests as to avoid further withdrawals. Whole blood samples were centrifuged at $112 \times g$ at room temperature for 15 minutes. The supernatant was then collected and stored at -80°C until measurements were made using Luminex technology (Bio-Plex 200 system, Bio-Rad) according to the manufacturer's instructions. The concentration of IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-17, MCP-1, and IP-10 was expressed as pg/mL, while that of ICAM-1 and VCAM was expressed as ng/mL. The same method was used for measuring studied cytokines in all RBC donor units.

RBC transfusion procedure

RBCs were transfused via a peripheral vein at the infusion rate of 5 mL/kg/hr and to avoid fluid overload the enteral and/or parenteral nutrition was generally decreased to 50%, without changes during the transfusion. The volume of RBCs needed by each patient was calculated using the formula $80 \times$ weight (kg) $\times$ (desired hematocrit [Hct] – current Hct)/Hct of donor unit.

In each case adult leukoreduced RBCs stored in SAG-M (adenine, 0.169 g/L; glucose, 9.0 g/L; mannitol, 5.25 g/L; sodium chloride, 8.77 g/L) without further concentration by centrifugation, less than 1 week old, and within 2 hours of irradiation were used. Within 15 minutes before the transfusion every RBC unit was studied to evaluate its hemoglobin and Hct (ABL 735, Radiometer).

Clinical data

The following variables were recorded for each patient: gestational age, birthweight, sex, antenatal steroids treatment, type of delivery, venous umbilical pH, Apgar score at 5 minutes, patients' age at transfusion, pre- and post-transfusion Hct, RBC transfused volume, age at transfusion, occurrence of respiratory distress syndrome, occurrence of noninvasive ventilation (high flow nasal cannula, nasal continuous airway pressure, nasal intermittent mandatory ventilation) and mechanical ventilation (patient-triggered ventilation, high-frequency oscillatory ventilation), occurrence of patent ductus arteriosus, intraventricular hemorrhage, periventricular leukomalacia, BPD, NEC, ROP, sepsis, and length of hospital stay. Intraventricular hemorrhage was diagnosed and staged according to the classification of Papile and colleagues,[27] BPD was defined as oxygen requirements at 36 weeks of postconceptional age,[28] NEC was diagnosed and staged according to Bell's criteria,[29] ROP was diagnosed and staged according to the current international

classification,[30] and sepsis was diagnosed when patients developed clinical signs and symptoms associated with a positive blood culture.

TABLE 1. Clinical characteristics of infants*

Clinical characteristics	n = 20
Gestational age (weeks)	27.7 ± 2.4
Birthweight (g)	889 ± 147
Male sex	10 (50)
Antenatal steroids	18 (90)
Cesarean section	19 (95)
Venous umbilical pH	7.30 ± 0.09
Apgar score at 5 min	8 (8-9)
RDS	10 (50)
Noninvasive support	17 (85)
Mechanical ventilation	6 (30)
PDA	12 (60)
IVH	2 (10)
Grade ≥ 3	0 (0)
PVL	0 (0)
BPD	5 (25)
NEC	0 (0)
ROP	2 (10)
Sepsis	6 (30)
Mortality	0 (0)
Hospital stay (day)	76 ± 25
Respiratory support at enrollment	5 (25)
N-IMV	0 (0)
NCPAP	4 (20)
HFNC	1 (5)
FiO_2 at enrollment	0.22 ± 0.01

* Data are reported as mean ± (SD) or rate (%).
HFNC = high-flow nasal cannula; IVH = intraventricular hemorrhage; NCPAP = nasal continuous airway pressure; NEC = necrotizing enterocolitis; N-IMV = noninvasive mechanical ventilation; PDA = patent ductus arteriosus; PVL = periventricular leukomalacia; RDS = respiratory distress syndrome.

Statistical analysis

In planning our study, we calculated that a sample size of at least 18 infants was required to detect a significant increase of IL-1β from 0.75 ± 0.75 to 0.95 ± 0.75 (27%) after the conclusion of the RBC transfusion (T_1) with 80% power at 0.05 level. Patients' clinical characteristics were described as mean and standard deviation (SD) or rate and percentage and cytokine values as median and interquartile range (IQR). Changes in cytokine pretransfusion and posttransfusion serum levels were assessed by using the Wilcoxon signed rank test as they were not normally distributed. All statistical tests were two-sided, and p values of not more than 0.05 were considered to be significant.

In addition, to investigate the possibility that effects of RBC transfusion on cytokine serum levels could be affected by patients' gestational age and anemia severity groups of infants who were born at less than or 28 or more weeks of gestation, who were transfused at Hct level of less than or 24% or more, and who had a posttransfusional increase of Hct level of less than or 15% or more were compared.

RESULTS

We studied 20 preterm infants who received 19.8 ± 3.0 mL of RBCs at the mean age of 50 ± 18 days. Their Hct level increased from $24.1 \pm 1.2\%$ to $39.4 \pm 2.9\%$. The mean age of RBC unit was 3.2 ± 0.9 days. The baseline clinical characteristics of infants are reported in Table 1.

We found a significant increase in IL-1β from baseline to T_3 value ($p = 0.04$); IL-8 from baseline to T_1 value

TABLE 2. Serum concentrations of cytokines and adhesion molecules after RBC transfusion*

Cytokine or adhesion molecule	T_0	T_1	T_2	T_3	T_4
IL-1β (pg/mL)	0.72 (0.63-1.30)	0.91 (0.63-1.34)	0.89 (0.58-1.58)	1.05† (0.80-1.52)	0.93 (0.63-1.32)
IL-6 (pg/mL)	1.99 (1.36-4.24)	2.45 (1.37-4.40)	2.27 (1.13-3.87)	2.87 (1.67-4.66)	2.13 (0.98-4.34)
IL-8 (pg/mL)	14.06 (7.03-24.10)	14.61† (8.35-26.91)	11.08 (6.93-21.50)	19.18 (12.03-29.80)	11.32 (8.05-26.30)
TNF-α (pg/mL)	3.68 (1.53-5.36)	3.83 (1.94-5.52)	3.36 (1.87-4.95)	4.17 (1.98-4.96)	3.94 (1.60-5.77)
IFN-γ (pg/mL)	9.39 (4.97-1.95)	10.52 (5.98-14.81)	11.09† (5.20-12.67)	11.66‡ (9.04-17.12)	13.38† (8.54-16.54)
IL-17 (pg/mL)	3.06 (2.68-4.01)	4.53† (3.94-6.18)	4.72† (3.37-5.68)	4.09† (2.98-5.39)	5.11‡ (3.29-7.40)
MCP-1 (pg/mL)	285.33 (204.97-330.31)	432.19‡ (385.43-488.06)	441.52‡ (306.41-611.75)	481.49‡ (363.92-617.27)	473.03‡ (363.32-570.28)
IP-10 (pg/mL)	102.71 (70.56-151.93)	109.20† (101.37-147.90)	109.21† (86.59-268.40)	115.14† (83.41-256.89)	140.14† (103.20-206.93)
ICAM (ng/mL)	492,92 (281.27-901.51)	388.79† (245.28-771.49)	392.32 (204.95-970.22)	378.28 (226.74-1009.91)	417.79 (248.99-918.84)
VCAM (ng/mL)	969.92 (604.99-2561.74)	1066.77 (517.31-1867.49)	1120.33 (604.99-2471.21)	1469.00 (564.27-2636.81)	1661.43 (692.45-2286.18)

* Data are reported as median (IQR).
† $p < 0.05$.
‡ $p < 0.001$.

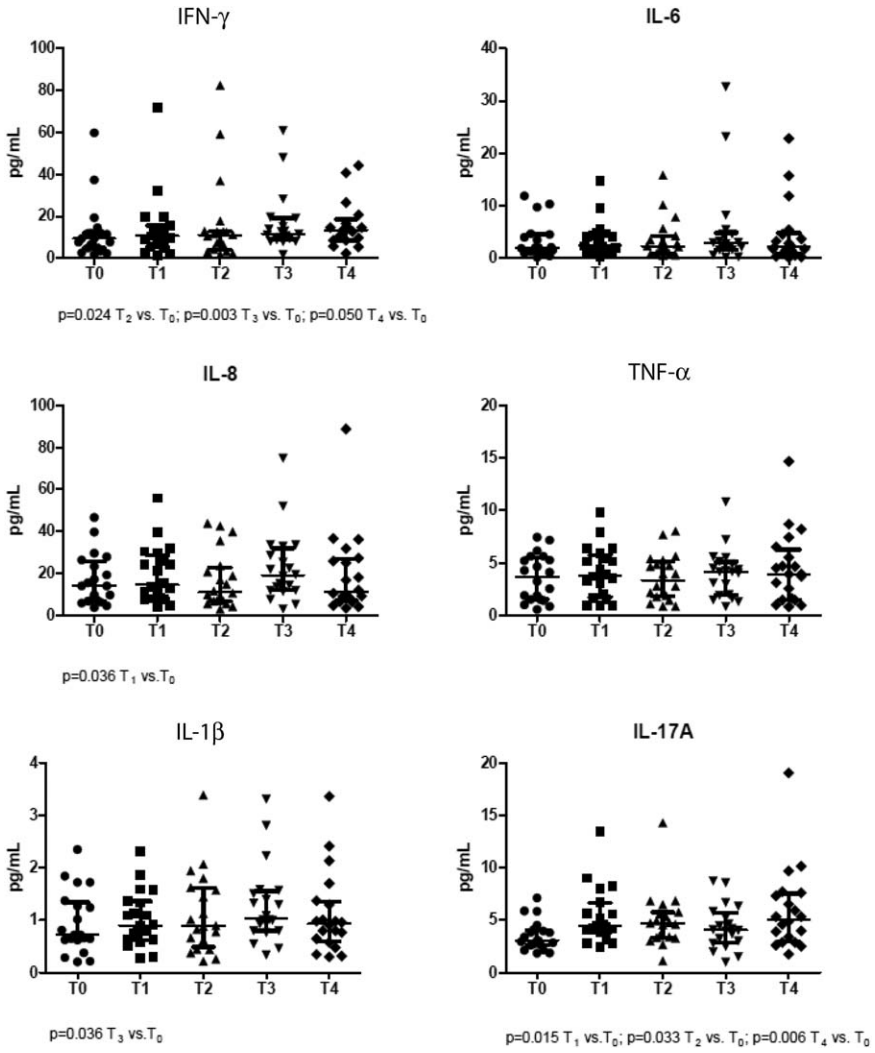

Fig. 1. Changes of cytokine serum level after RBC transfusion. Median (IQR).

($p = 0.04$); IFN-γ from baseline to T_2 ($p = 0.02$), T_3 ($p < 0.001$), and T_4 ($p = 0.05$); IL-17 from baseline to T_1 ($p = 0.02$), T_3 ($p = 0.03$), and T_4 ($p < 0.001$); MCP-1 from baseline to T_1 ($p < 0.001$), T_2 ($p < 0.001$), T_3 ($p < 0.001$), and T_4 ($p < 0.001$); and IP-10 from baseline to T_1 ($p < 0.01$), T_2 ($p = 0.02$), T_3 ($p < 0.01$), and T_4 ($p < 0.01$; Table 2, Figs. 1 and 2). Changes of cytokine serum levels were similar between groups of infants who were born at less than or 28 or more weeks of gestation, who were transfused at Hct level of less than or 24% or more, and who had a posttransfusional increase of Hct level of less than or 15% or more (unreported data). None of the studied cytokines were found in RBC donor units.

DISCUSSION

In this study we evaluated changes in serum levels of IL-1β, IL-6, IL-8, TNF-α, IFN-γ, IL-17, MCP-1, IP-10, ICAM-1, and VCAM after RBC transfusions in preterm infants to assess their potential proinflammatory effect and their possible pathogenetic contribution to the development of TANEC during the 48 hours after transfusions. We found that IL-1β increased 24 hours after transfusions; IL-8 and ICAM-1 increased 2 hours after transfusions; while IFN-γ, IL-17, MCP-1, and IP-10 were increased throughout the 48-hour study period after transfusions. Thus, our findings can contribute to explain the pathogenesis of TANEC, although the correlation between RBC transfusions and NEC is still debated,[20-22] but also the reported association between RBC transfusions and the development of BPD[7-9] and ROP.[10,11]

Our results are in agreement with Keir and colleagues[24] regarding the increase in IL-1β, IL-8, MCP-1, and ICAM-1, but are in disagreement in terms of our unchanged value of TNF-α and increased value of IFN-γ. Similarly, our results are in agreement with Locke and coworkers[23] in regard to unchanged values of IL-6 and TNF-α, but are in disagreement regarding the increase in

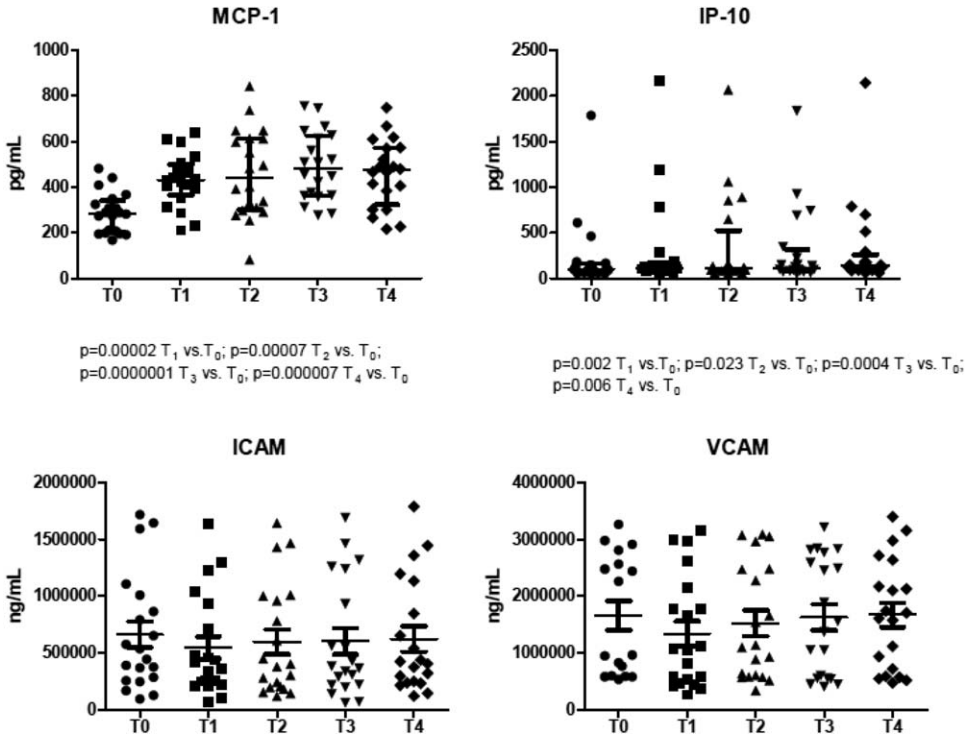

Fig. 2. Changes of MCP-1, IP-10, ICAM-1, and VCAM serum level after RBC transfusion. Median (IQR).

IL-1β observed in our study. These differences can be explained by several factors, such as the different study period duration. We measured cytokine serum level four times during the 48 hours after RBC transfusion, while in previous studies they were evaluated only once after 1[23] or 2 to 4[24] hours. Second, we did not find any cytokines in RBC donor units similarly to Locke and colleagues[23] and in disagreement with Keir and colleagues.[24] These findings might depend on the different age of RBC units at transfusion that was shorter in our study (3.2 ± 0.9 days) than in the study by Keir and coworkers (median age, 23 days; range, 6-33 days), although they did not find any correlation between cytokine level and transfusion pack age.[24] Third, the blood pack store additive solution was similar to ours in the study by Locke and coworkers (AS-5 Optisol, Terumo Corp.),[23] but unreported in the study by Keir and coworkers.[24] Fourth, there are different methods of cytokine measurement, the one in the study by Keir and coworkers being similar to ours (Luminex technology),[24] but different in the study by Locke and coworkers (sandwich immunoassay technique).[23]

The mechanisms by which RBC transfusion can induce an increase in cytokine serum level can be explained by the transfusion-related immunomodulation (TRIM). In the clinical setting of an underlying inflammatory condition, such as prematurity, RBC transfusions may trigger immune cell activation and induce proinflammatory effects on endothelial, epithelial, and innate immune system cells,[24] thus promoting cytokine release. In fact, although leukoreduction removes 99.9% of white blood cells from transfusion units limiting TRIM,[31] a significant residual capacity for an immunomodulatory effect might be mediated by bioactive substances, such as non–transferrin-bound iron (NTBI) and unbound free heme that continue to accumulate over time in microparticle and supernatant fractions, even after leukoreduction.[24,32] It has been recently demonstrated that NTBI is detectable in the transfusion packs in correlation with their age, and its level is higher after transfusion and is associated with an increase in oxidative stress in preterm infants.[25] It is noteworthy that NTBI oxidative stress can promote inflammation through the up regulation of IL-1β, IL-6, and IL-8.[33,34] Moreover, free heme has also been found to induce oxidative stress and the release of IL-8[35] and ICAM-1.[36] Another underestimated potential mechanism by which RBC transfusion can promote an increase in cytokine serum level in transfused preterm infants is the associated great variability in splanchnic tissue oxygenation that has been recently reported[37] and that might result in the production of free radicals and proinflammatory cytokines triggered by ischemia–reperfusion injury of intestinal tissue.

Increased serum levels of IL-1β, IL-8, IFN-γ, MCP-1, and IP-10 have been reported in preterm infants with NEC[38-40] and/or sepsis,[41] while ICAM-1 and IL-17 have never been studied in these clinical conditions. Nonetheless, our findings confirm[24] that proinflammatory cytokine concentrations can increase also in infants who

receive RBC transfusions, although serum levels of IL-1β,[38,39,42] IL-8,[40,41] IFN-γ,[38,40] MCP-1,[38] and IP-10[41] are lower than those reported in infants with NEC or sepsis. This might be partially due to the different methods used for cytokines assessment,[38-42] but, in our opinion, it suggests that RBC transfusions can exert a proinflammatory action less powerful than that induced by sepsis or NEC. However, the combination of the contemporary and rather prolonged increase in many proinflammatory cytokines induced by RBC transfusions and in association with other risk factors such as prematurity, abnormal gut microbiota, and inadequate gastrointestinal perfusion can help explain why several cases of TANEC have developed after RBC transfusions[13-16] and why 25% to 30% of these cases occur within 48 hours of the transfusion.[17,18]

Strategies for limiting TRIM and detrimental proinflammatory effects of RBC transfusions have been investigated, but the use of leukoreduction has given contradictory clinical results.[33] The recent ARIPI study failed to demonstrate improved outcomes from the use of fresh RBCs ($\leq$7 days old) in very low birthweight infants,[43] and a restrictive RBC transfusion policy has not been found to decrease the risk of TANEC.[44] Thus, care and caution in prescribing RBC transfusions following shared guidelines seems to be the most powerful approach to create benefits without detrimental effects from RBC transfusional therapy.

The strengths of our study are that our patients were investigated for 48 hours after RBC transfusion, which is the riskiest period for TANEC development. Also, our patients did not present contemporary potential proinflammatory confounding factors, such as infections or mechanical ventilation. A limitation was that the use of fresh RBC packs could have attenuated the cytokine up regulation that might be more relevant when packs older than ours are used.[24-26] Moreover, the population size might not allow to demonstrate a different effect of RBC transfusions in more immature infants and in infants with more severe anemia.

In conclusion, we found that RBC transfusions are associated with an increase in IL-1β, IL-8, IFN-γ, IL-17, MCP-1, IP-10, and ICAM-1 during the 48 hours after transfusions. These findings can contribute to an explanation of the pathogenesis of TANEC and suggest that physicians should adopt wise transfusion guidelines which would allow them to avoid the detrimental risks of either TRIM or undertransfusion.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Strauss RG. Anaemia of prematurity: pathophysiology & treatment. Blood Rev 2010;24:221-5.
2. Widness JA. Pathophysiology of anemia during the neonatal period, including anemia of prematurity. NeoReviews 2008; 9:e520.
3. Maier RJ, Sonntag J, Walka MM, et al. Changing practices of red blood cell transfusions in infants with birth weights less than 1000 g. J Pediatr 2000;136:220-4.
4. Keir AK, Yang J, Harrison A, et al. Temporal changes in blood product usage in preterm neonates born at less than 30 weeks' gestation in Canada. Transfusion 2015;55:1340-6.
5. Kirpalani H, Whyte RK, Andersen C, 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.
6. Bell EF, Strauss RG, Widness JA, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics 2005;115:1685-91.
7. Zhang Z, Huang X, Lu H. Association between red blood cell transfusion and bronchopulmonary dysplasia in preterm infants. Sci Rep 2014;4:4340.
8. Cooke RW, Drury JA, Yoxall CW, et al. Blood transfusion and chronic lung disease in preterm infants. Eur J Pediatr 1997; 156:47-50.
9. Valieva OA, Strandjord TP, Mayock DE, et al. Effects of transfusions in extremely low birth weight infants: a retrospective study. J Pediatr 2009;55:331-7.
10. Inder TE, Clemett RS, Austin NC, et al. 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.
11. Dani C, Reali MF, Bertini G, et al. The role of blood transfusions and iron intake on retinopathy of prematurity. Early Hum Dev 2001;62:57-63.
12. Singh R, Bhavesh MS, Frantz ID 3rd. Necrotizing enterocolitis and the role of anemia of prematurity. Semin Perinatol 2012;36:277-82.
13. Christensen RD, Lambert DK, Henry E, et al. Is "transfusion associated necrotizing enterocolitis" an authentic pathogenic entity? Transfusion 2010;50:1106-12.
14. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364:255-64.
15. Mally P, Golombek SG, Mishra R, et al. Association of necrotizing enterocolitis with elective packed red blood cell transfusions in stable, growing, premature neonates. Am J Perinatol 2006;23:451-8.
16. Christensen RD, Wiedmeier SE, Baer VL, et al. Antecedents of Bell stage III necrotizing enterocolitis. J Perinatol 2010;30: 54-7.
17. Josephson CD, Wesolowski A, Bao G, et al. Do red cell transfusions increase the risk of necrotizing enterocolitis in premature infants? J Pediatr 2010;157:972-8.
18. Singh R, Visintainer PF, Frantz ID 3rd, et al. Association of necrotizing enterocolitis with anemia and packed red blood cell transfusions in preterm infants. J Perinatol 2011;31:176-82.
19. Blau J, Calo JM, Dozor D, et al. 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.
20. Paul DA, Mackley A, Novitsky A, et al. Increased odds of necrotizing enterocolitis after transfusion of red blood cells in premature infants. Pediatrics 2011;127:635-41.
21. El-Dib M, Narang S, Lee E, et al. Red blood cell transfusion, feeding and necrotizing enterocolitis in preterm infants. J Perinatol 2011;31:183-7.
22. Patel RM, Knezevic A, Shenvi N, et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very low-birth-weight infants. JAMA 2016;315:889-97.
23. Locke R, Paul D, Touch S, et al. Cytokine load in prestorage leukoreduced PRBC transfusions in premature infants. J Perinatol 2005;25:526-30.
24. Keir AK, McPhee AJ, Andersen CC, et al. Plasma cytokines and markers of endothelial activation increase after packed red blood cell transfusion in the preterm infant. Pediatr Res 2013;73:75-9.
25. Stark MJ, Keir AK, Andersen CC. Does non-transferrin bound iron contribute to transfusion related immune-modulation in preterms? Arch Dis Child Fetal Neonatal Ed 2013;98:F424-9.
26. Motta M, Casadei AM, Casani A, et al. Raccomandazioni per la terapia trasfusionale in neonatologia [Internet]. [cited 2016 Jul 31]. Available at: http://www.neonatologia.it/upload/103.pdf.
27. Papile LS, Burstein J, Burstein R, et al. Incidence and evolution of the sub-ependymal intraventricular hemorrhage: a study of infants weighing less than 1500 grams. J Pediatr 1978;92:529-34.
28. Shennan AT, Dunn MS, Ohlsson A, et al. Abnormal pulmonary outcomes in premature infants: prediction from oxygen requirement in the neonatal period. Pediatrics 1988;82:527-32.
29. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging. Ann Surg 1978;187:1-12.
30. International Committee for the Classification of the Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol 2005; 123:991-9.
31. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. BMC Blood Disord 2004;4:6.
32. Watkins TR, Rubenfeld GD, Martin TR, et al. Effects of leukoreduced blood on acute lung injury after trauma: a randomized controlled trial. Crit Care Med 2008;36:1493-9.
33. Hod EA, Spitalnik SL. Stored red blood cell transfusions: iron, inflammation, immunity, and infection. Transfus Clin Biol 2012;19:84-9.
34. Hod EA, Zhang N, Sokol SA, et al. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood 2010;115: 4284-92.
35. Cosgrove S, Chotirmall SH, Greene CM, et al. Pulmonary proteases in the cystic fibrosis lung induce interleukin 8 expression from bronchial epithelial cells via a heme/meprin/epidermal growth factor receptor/Toll-like receptor pathway. J Biol Chem 2011;286:7692-704.
36. Wagener FA, Feldman E, de Witte T, et al. Heme induces the expression of adhesion molecules ICAM-1, VCAM-1, and E selectin in vascular endothelial cells. Proc Soc Exp Biol Med 1997;216:456-63.
37. Bailey SM, Hendricks-Muñoz KD, Mally PV. Variability in splanchnic tissue oxygenation during preterm red blood cell transfusion given for symptomatic anaemia may reveal a potential mechanism of transfusion-related acute gut injury. Blood Transfus 2015;13:429-34.
38. Maheshwari A, Schelonka RL, Dimmitt RA, et al. Cytokines associated with necrotizing enterocolitis in extremely-low-birth-weight infants. Pediatr Res 2014;76:100-8.
39. Benkoe T, Baumann S, Weninger M, et al. Comprehensive evaluation of 11 cytokines in premature infants with surgical necrotizing enterocolitis. PLoS One 2013;8:e58720.
40. Lodha A, Asztalos E, Moore AM. Cytokine levels in neonatal necrotizing enterocolitis and long-term growth and neurodevelopment. Acta Paediatr 2010;99:338-43.
41. Ng PC, Li K, Chui KM, et al. IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants. Pediatr Res 2007;61:93-8.
42. Sharma R, Tepas JJ 3rd, Hudak ML, et al. Neonatal gut barrier and multiple organ failure: role of endotoxin and proinflammatory cytokines in sepsis and necrotizing enterocolitis. J Pediatr Surg 2007;42:454-61.
43. Fergusson DA, Hébert P, Hogan DL, et al. Effect of fresh red blood cell transfusions on clinical outcomes in premature, very low-birth-weight infants: the ARIPI randomized trial. JAMA 2012;308:1443-51.
44. Ibrahim M, Ho SK, Yeo CL. Restrictive versus liberal red blood cell transfusion thresholds in very low birth weight infants: a systematic review and meta-analysis. J Paediatr Child Health 2014;50:122-30.