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Original article

Red blood cells transfusions in very low birth weight neonates

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Abstract

Introduction: Very low birth weight preterm infants are associated with a greater risk of morbidity and mortality and are more susceptible to therapy with red blood cells transfusions. The aim of this study is to analyse the frequency and factors associated with red blood cells transfusions in very low birth weight infants.

Material and methods: This retrospective study included neonates with very low birth weight admitted between November 1, 2011 and October 31, 2015. Demographic, perinatal and clinical data during hospitalization were obtained through medical records. Statistical tests were used to compare neonates with and without need for red blood cells transfusion and multivariate regression analysis to find predictor factors.

Results: Seventy-nine patients were studied, median birth weight of 1,190 grams and mean gestational age of 29 ± 2 weeks. Forty-nine (62%) received transfusion support with red blood cells. Higher need for red blood cells transfusions was significantly associated with low birth weight (OR = 0.99, 95% CI 0.990-0.999) and total millilitres of phlebotomy losses (OR = 1.17, 95% CI 1.07-1.28). Birth weight (B = -0.01, 95% CI -0.008 to -0.003), hemoglobin at admission (B = -0.33, 95% CI -0.53 to -0.13) and sepsis (B = 1.85, 95% CI 0.72-2.98) were predictive factors for the number of red blood cells transfusions. Regarding the treatment with erythropoietin, there were no differences for all outcomes.

Conclusions: Phlebotomy losses are one of the major factors for the need of transfusion in preterm infants, and therefore sampling should be minimized to the maximum.

Keywords

Infant, very low birth weight, premature birth, anemia, blood transfusion.

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Introduction

Right after birth, all neonates suffer a decline in their number of circulating red blood cells. In term and stable infants, this postnatal fall is well tolerated and asymptomatic, with the hemoglobin values reaching a minimum by 8 to 12 weeks of age that is said to rarely go below 9.5-11 g/dl [1, 2]. Premature infants, in contrast, are more susceptible to this decline and anemia is more severe and settles more rapidly than in term infants. By 4-6 weeks of age, hemoglobin tends to fall to values between 7 and 8 g/dL, being the lowest values found in the tinier and less mature newborns [3]. This state is often associated with clinical symptoms and signs such as tachycardia, tachypnea, higher oxygen requirement, higher frequency of apnea and bradycardia, pallor and poor weight gain [4]. Due to these symptoms and its consequences, anemia is not accepted as a benign event and is referred to as "anemia of prematurity", with its degree and severity apparently determined by the gestational age and also by a combination of multiple physiologic and non-physiologic factors. As physiologic factors able to condition this post-birth fall we have an ineffective erythropoiesis, an accelerated catabolism, shortened red blood cells lifespan (in the most immature infants they may only survive 35 to 50 days), high extra-uterine growth rate, great rise of volemia and the insufficient iron storage, as the bulk passage of iron only happens in the third trimester of gestation [1, 2, 5].

Besides facing an anemia of prematurity that requires correction, sepsis and its oxidative hemolysis and acute and/or iatrogenic blood losses are other factors that require therapeutic measures to maintain and improve oxygen availability to all tissues in the infant. Red blood cells transfusions remain the mainstay of therapy for all these occurrences. Inside the hospital and neonatal care units, close to 80% of very low and more than 90% of extremely low birth weight infants are exposed to transfusions and, among these, almost 50% are given during the first two weeks after birth [3, 6, 7].

The risk associated with red blood cells transfusion is greatly lower today due to adoption of single-donor and small volume pack units that can be stored up to 35 days, with the donor plasma replaced by anticoagulant additive solutions and with the use of additives such as mannitol and glucose [8]. However, choosing transfusion as treatment is still important for its well-known risk of infection by transmission of virus such as hepatitis virus, cytomegalovirus, Epstein-Barr and human immunodeficiency virus, as well as risk of transmission of emerging agents, volume overloading, electrolyte imbalances, higher exposure to toxins and serious complications, such as bronchopulmonary dysplasia [5, 9].

The group of infants with very low birth weight, now able to survive in a high percentage, due to great technological advances and creation of Neonatal Intensive Care Units, requires close monitorization and frequent diagnostic and therapeutic laboratory assessment through blood sampling. These blood losses by phlebotomy remain a key factor to determine infants' transfusion needs as the volume of blood taken correlates directly with the volume transfused. To avoid overdrawing these overly immature preterm infants by controlling the volumes removed with each sample shows up as one of the current and more invested goals to prevent and control anemia of prematurity [8, 10].

Taking into account the diminished levels of erythropoietin after birth, its use as an alternative to transfusion has been extensively studied but trials showed unsatisfying results [11, 12]. Other promising alternatives remain unclear or unable to eliminate the need for transfusion and, besides the shortage of alternative therapies, there also are not satisfactory clinical and laboratory parameters to guide the transfusion moment, neither globally accepted protocols [13].

Regarding the Portuguese situation, available studies regarding anemia of prematurity and its treatment with transfusion date from late nineties and there is just a single one-year retrospective study that aimed to evaluate the effect of treatment with recombinant erythropoietin over the need and numbers of transfusions of very low birth weight neonates [14-16]. More recent and updated studies are missing.

Objectives

This study proposes 1) to analyse the need of red blood cells transfusions as therapy in very low birth weight preterm infants and 2) to identify factors and morbidities associated with this choice of treatment.

Methods

This is a retrospective study of very low birth weight preterm infants admitted to a Neonatal Intensive Care Unit of a public university and tertiary-care hospital during a 4-year period (between November 1, 2011 and October 31, 2015). We included in the present study the neonates weighing less than 1,500 grams. Those who had death as outcome or were transferred to other medical departments or hospitals were excluded.

All data regarding their demographic, delivery, admission and clinical evolution in the unit were collected from analysis of hospital electronic database and neonates medical reports. Demographic data included gender, gestational age at birth (taken from neonatal assessment), birth weight, type of delivery, Apgar score at first and fifth minutes and need for resuscitation within the delivery room, using ventilation with bag, mask or endotracheal intubation, chest compressions or medications. From the hospitalization the following data were included: length of stay, need and duration of ventilator support or oxygen therapy, use and duration of parenteral nutrition and use of umbilical catheters and vasoactive drugs. Morbidities such as bronchopulmonary dysplasia, sepsis, patency of ductus arteriosus, major congenital cardiopathies, surgical interventions, pulmonary and/or intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis and retinopathy of prematurity were also collected. Treatment with recombinant erythropoietin, iron supplementation, hemoglobin values at admission and pre-transfusion, total volume blood losses by phlebotomy during hospitalization and all transfusion-related data were analysed.

Bronchopulmonary dysplasia was diagnosed when supplementary oxygen need persisted at least at the first 28 days after delivery, according to the National Institutes of Health Consensus [17]. Sepsis was diagnosed if systemic signs were corroborated by a positive blood culture. Patency of ductus arteriosus was considered after echocardiography with Doppler and/or need for therapy [18]. Major congenital cardiopathy considered all malformations with actual and potential functional importance, which may require specific medical or surgical treatment during and after hospitalization [19]. Intraventricular hemorrhage was diagnosed if routine transfontanellar ultrasound showed any bleeding associated with ventricular dilatation (grade 2-3) and/or evidence of bleeding on parenchyma (grade 4) [20]. Periventricular leukomalacia was considered in accordance to de Vries' classification [21]. Retinopathy of prematurity was routinely evaluated and graded by ophthalmologists according to the International Committee Classification [22]. Necrotizing enterocolitis diagnosis followed the Bell's staging criteria [23].

The transfusion guidelines followed the Portuguese guidelines proposed in the Anemia of Prematurity Consensus, revised in 2013 by the Portuguese Neonatology Society [1]. Red blood cells transfusions are indicated when 1) hemoglobin values are lower than 10 g/dl or hematocrit below 30%, if associated with moderate or significant need of mechanical ventilation (MAP above 8, or 14 with FiO_2 higher than 0.4). 2) If with mild ventilator support, neonates should be transfused if hemoglobin or hematocrit values are below 8 and 25%, respectively. 3) Neonates with oxygen dependency associated with tachycardia or tachypnea, acute metabolic acidosis, a surgery between 72 hours and doubled oxygen therapy needs over the last 48 hours, are indicated for transfusion if their hemoglobin or hematocrit are lower than 7 and 20%, respectively. 4) Asymptomatic neonates shall receive transfusion support if their hemoglobin level is below 6 or hematocrit is lower than 18%. The transfusion volumes recommended by the guidelines range between 10 and 20 ml/kg, for a 2 to 4 hours perfusion.

Treatment with recombinant erythropoietin was routinely used in this Neonatal Intensive Care Unit until May 2013, being administered to all neonates below 32 weeks and/or 1,500 grams. The first injection was given subcutaneously between days 14 and 17 of life at an initial dose of 250 UI/kg three times a week and for a total of 6 weeks [24]. All infants received daily iron supplementation after 2 postnatal weeks: 1 mg/kg intravenously and then 4-6 mg/kg per oral solution [24]. The protocol for iron supplementation suffered no modifications during the period of the study. Total volumes of phlebotomy losses during hospitalization were estimated through the following equation: number of blood cell counts (0.5 ml) + number of biochemical tests (1.1 ml) + number of blood cultures (1 ml) + number of coagulation studies (1 ml) + blood gas tests (0.3 ml).

Data compilation and statistical analysis were performed by using SPSS® 23 (IBM®, New York,

USA). Categorical variables were characterized by absolute and relative frequencies and continuous variables by mean (± standard deviation) or median (minimum-maximum values), if they had symmetric or asymmetric distribution, respectively. To compare categorical variables we used chi-square or Fisher's exact test, the latest one for contingency tables 2 × 2 when expected values were less than 5. To compare continuous variables we used parametric tests (independent t test) or non-parametric tests (Mann-Whitney U test) if they had symmetric or asymmetric distribution, respectively. A multivariate analysis by linear regression and logistic regression was performed to evaluate predictive factors of red blood cells transfusion need and of treatment with erythropoietin. A p-value below 0.05 was considered significant.

The study protocol was approved by the Research Ethics Committee of this hospital.

Results

During the study period, a total of 211 neonates with very low birth weight were admitted to this Neonatal Intensive Care Unit. From these, 79 neonates (42 male, 37 female) were eligible. The median birth weight was 1,190 grams (range: 500-1,500 grams) and the mean gestational age was 29 (± 2) weeks. Most births were done resorting to caesarean section (81%). Demographic characteristics of neonates and/or related to red blood cells transfusion are summarized in **Tab. 1**.

Forty-nine (62%) of these neonates had to receive transfusion support with red blood cells packs during hospital stay. From the former group, 22 (45%) of these neonates only needed a single red blood cells transfusion, whereas 16 (33%) needed two to five transfusions and 11 (22%) over five red blood cells packs, for a maximum of sixteen. In total, there were 178 red blood cells transfusions prescribed during the study period for a mean volume of 14.41 ml/kg per transfusion.

Comparing the transfusion and non-transfusion group, the former group had a significantly lower birth weight (1,000 versus 1,330 grams, $p < 0.001$) and gestational age (mean 28.74 versus 30.73 weeks, $p < 0.001$). There were no differences between the two groups regarding gender, Apgar score at first and fifth minutes and need for neonatal resuscitation.

Tab. 2 shows a comparison of the clinical characteristics and outcomes during hospitalization for neonates who received or not red blood cells transfusions. There was a significant higher frequency of sepsis (45% versus 3%, $p < 0.001$), retinopathy of prematurity equal or above stage 2 (30% versus 7%, $p = 0.015$), surgical interventions (37% versus 10%, $p = 0.01$) and bronchopulmonary dysplasia (53% versus 3%, $p < 0.001$) in the transfusion group when compared to the non-transfusion one. The same pattern was found with the need for ventilator support and oxygen supplementation: transfused neonates used more mechanical ventilation (98% versus 77%, $p = 0.004$) and during more days (median 44 versus 12 days, $p < 0.001$); they also required more oxygen right at admission, after 28 days of life and at 36 weeks of gestational age (74% versus 40%, $p = 0.003$, 49% versus 3%, $p < 0.001$ and 27% versus 0, $p < 0.001$, respectively). Neonates subjected to red blood cells transfusion also stayed longer in the intensive care unit (median 70 versus 38 days, $p < 0.001$), had more days of parenteral nutrition (median 22 versus

Table 1. Demographic data of the neonates with or without red blood cells transfusion.

	Total (n = 79)	Transfusion group (n = 49)	Non-transfusion group (n = 30)	p-value
Gestational age (weeks), mean (± SD)	29.31 (± 2.37)	28.74 (± 2.17)	30.73 (± 2.2)	< 0.001[a]
Birth weight (grams), median (min-max)	1,190 (500-1,500)	1,000 (500-1,500)	1,330 (696-1,475)	< 0.001[b]
Gender, n (%)				
Male	42 (53)	26 (53)	16 (53)	0.981[c]
Delivery, n (%)				
Caesarean section	63 (81)	39 (80)	24 (87)	0.732[c]
Apgar score at first minute < 7, n (%)	38 (47)	26 (53)	12 (40)	0.259[c]
Apgar score at fifth minute < 7, n (%)	10 (12)	8 (16)	2 (7)	0.303[d]
Neonatal resuscitation, n (%)[e]	42 (53)	28 (57)	14 (47)	0.365[c]

[a]Independent t test; [b]Mann-Whitney U test; [c]Chi-square test; [d]Fisher's exact test.
[e]Neonatal resuscitation: need for bag and mask ventilation and/or endotracheal intubation, chest compressions, medications.

Table 2. Clinical data and morbidities for neonates with and without red blood cells transfusion.

	Total (n = 79)	Transfusion group (n = 49)	Non-transfusion group (n = 30)	p-value
Hemoglobin at admission (g/dl), mean (± SD)	17.06 (± 2.79)	16.37 (± 2.92)	18.22 (± 2)	0.004[a]
Total phlebotomy volume (ml), median (min-max)	20.6 (2.2-101)	30.1 (10.9-101)	13.05 (2.2-38.1)	< 0.001[b]
Mechanical ventilation, n (%)	71 (90)	48 (98)	23 (77)	0.004[d]
Duration of mechanical ventilation (days), median (min-max)	34 (0-112)	44 (0-112)	12 (0-52)	< 0.001[b]
Oxygen supplementation at admission, n (%)	48 (61)	36 (74)	12 (40)	0.003[c]
Oxygen supplementation at 28 days of life, n (%)	25 (32)	24 (49)	1 (3)	< 0.001[d]
Oxygen supplementation at 36 weeks, n (%)	13 (17)	13 (27)	0	0.001[d]
Parenteral nutrition, n (%)	77 (98)	48 (98)	29 (97)	0.999[d]
Parenteral nutrition (days), median (min-max)	18 (0-53)	22 (0-53)	12.5 (0-22)	< 0.001[b]
Umbilical catheter, n (%)	25 (32)	20 (41)	5 (17)	0.025[c]
Vasopressor support, n (%)	7 (9)	7 (14)	0	0.040[d]
Recombinant erythropoietin, n (%)	21 (27)	16 (53)	5 (17)	0.119[c]
Duration of treatment with recombinant erythropoietin (days), median (min-max)	43 (15-53)	43 (23-53)	30 (15-47)	0.313[b]
Iron supplementation, n (%)	76 (96)	47 (96)	29 (97)	0.999[c]
Platelets transfusions, n (%)	10 (13)	8 (16)	2 (7)	0.303[d]
Surgery, n (%)	21 (27)	18 (37)	3 (10)	0.010[d]
Sepsis, n (%)	23 (29)	22 (45)	1 (3)	< 0.001[d]
Patent ductus arteriosus, n (%)	28 (35)	20 (41)	8 (27)	0.202[c]
Major congenital cardiopathy, n (%)	4 (5)	4 (8)	0	0.292[d]
Intraventricular hemorrhage (≥ grade 3), n (%)	4 (5)	4 (8)	0	0.091[d]
Cystic periventricular leukomalacia, n (%)	7 (9)	6 (12)	1 (3)	0.243[d]
Necrotizing enterocolitis (≥ grade 2), n (%)	5 (6.3)	5 (10.2)	0	0.151[d]
Retinopathy of prematurity (≥ grade 2), n (%)	12 (15)	10 (30)	2 (7)	0.015[c]
Bronchopulmonary dysplasia, n (%)	27 (34)	26 (53)	1 (3)	< 0.001[d]
Length of stay (days), median (min-max)	53 (1-148)	70 (1-148)	38 (3-70)	< 0.001[b]

[a]Independent t test; [b]Mann-Whitney U test; [c]Chi-square test; [d]Fisher's exact test.

12.5 days, $p < 0.001$), required more frequently use of umbilical catheters (41% versus 17%, $p = 0.025$) and had more conditions that demanded vasopressor support (14% versus 0, $p = 0.04$) when compared to the other group. Necrotizing enterocolitis, major congenital cardiopathies, patent ductus arteriosus, intraventricular hemorrhage above grade 3 and periventricular leukomalacia weren't statistically different between the two groups.

Values of hemoglobin at admission were significantly lower in the transfusion group than in the non-transfusion group: mean 16 g/dl (± 3) against a mean of 18 g/dl (± 2) in the second group ($p = 0.004$). There also were greater and statistically significant total phlebotomy losses in the transfused neonates: median 30 ml versus 13 ml in the non-transfused ones ($p < 0.001$). From all of study neonates, 76 (96%) received iron supplementation.

Twenty-one (27%) neonates received treatment with recombinant erythropoietin for a median of 43 days (range: 15-53) and there were no differences between the transfusion and non-transfusion groups. A multivariate analysis evaluating the treatment with erythropoietin did not show statistically significant associations with clinical outcomes and number of red blood cells transfusions.

Tab. 3 and **Tab. 4** show the multivariate analysis. A multivariate analysis by logistic regression revealed that birth weight (OR = 0.99, 95% CI 0.990-0.999, $p = 0.001$) and total volume of phlebotomy losses (OR = 1.17, 95% CI 1.07-1.28, $p = 0.001$) are predictive factors for the red blood cells transfusion need. A multivariate analysis by linear regression showed that birth weight (B = -0.01, 95% CI -0.008 to -0.003, $p < 0.001$), hemoglobin at admission (B = -0.33, 95% CI -0.53

Table 3. Multivariate analysis by logistic regression of red blood cells transfusion need in very low birth weight neonates.

	OR	95% CI	p-value
Birth weight (grams)	0.99	0.990-0.999	0.001
Gestational age (weeks)	1.21	0.80-1.84	0.358
Hemoglobin at admission (g/dl)	0.75	0.55-1.04	0.082
Total volume phlebotomy losses (ml)	1.17	1.07-1.28	0.001
Apgar score at fifth minute < 7	1.17	0.13-10.67	0.892

Table 4. Multivariate analysis by linear regression for number of red blood cells transfusions in very low birth weight infants.

	B	95% CI	p-value
Birth weight (grams)	-0.01	-0.008 to -0.003	< 0.001
Gestational age (weeks)	-0.09	-0.37 to -0.20	0.548
Hemoglobin at admission (g/dl)	-0.33	-0.53 to -0.13	0.002
Sepsis	1.85	0.72 to 2.98	0.002

to -0.13, p = 0.002) and sepsis (B = 1.85, 95% CI 0.72-2.98, p = 0.002) were predictive factors for the number of red blood cells transfusions.

Discussion

This study showed that the majority of preterm infants during the study period received support with red blood cells transfusions and there was a higher prevalence of these in the smallest and more premature infants. It also showed that 1) surgeries, sepsis, retinopathy of prematurity and bronchopulmonary dysplasia were more frequent in the transfusion group; 2) preterm infants who received transfusion support stayed more days with mechanical ventilation and with parenteral nutrition, and had a higher request for oxygen supplementation, vasopressor support and umbilical catheters; 3) lower birth weight and higher phlebotomy losses were associated with greater chance of requiring a red blood cells transfusion during the hospital stay; 4) higher birth weight and higher hemoglobin at admission were predictors for a lower need of further red blood cells transfusions, while sepsis events were significantly associated with need for additional transfusions.

The prevalence of transfusion with red blood cells in the infants of our sample was 62%, a percentage that is in accord with the range found in literature for neonates born with very low birth weight [25-27]. The same studies are also in accord with the present one as they showed infants with lower birth weight and lower gestational age as more frequently subjected to transfusion support with red blood cells [25-28]. Not considering weight as criterion, a study found a relatively lower (21%) prevalence of transfusion among neonates [7].

The use of mechanical ventilation and the need for oxygen supplementation, from admission to 36 weeks of gestational age were more frequent among the transfusion group. This supports and is in accordance with the transfusion guidelines operating in the studied Neonatal Intensive Care Unit, and other existent and restrictive guidelines [29], which take into account the ventilator support prior to the decision to transfuse.

Among literature, maintaining parenteral feeding for a longer period through a central line was associated with a higher risk for red blood cells transfusions, as this delay may play a role in the incidence of sepsis and consequently in the number of phlebotomies and red blood cells transfusions [25]. The duration of parenteral nutrition was significantly longer in the transfused group in the present study (median 22 versus 12.5 days) but there was no significant association between longer parenteral feeding and higher need of transfusion.

Other clinical factors such as use of vasopressor support and use of umbilical catheter also were more frequent among the preterm infants in the transfusion group. The umbilical catheters are particularly used in smaller and premature infants with a more severe clinical status and are important because their use facilitate higher iatrogenic blood losses [26].

Starting from the nineties, investigators evaluated possible advantages of more restrictive guidelines in opposition to the liberal and "hemoglobin-centered" ones used until then. A large and randomized trial titled "The Premature Infants in Need of Transfusion", which studied the morbidity and mortality of two groups of extremely low birth weight infants at lower and higher hemoglobin thresholds for transfusion decision, didn't show any association between the transfusion guidelines and incidence of intraventricular hemorrhage [28]. On the other hand, another single-centre and randomized trial found a greater incidence of neurologic events when combining intraventricular hemorrhage above grade four and periventricular leukomalacia

[29]. Following the later, more recent studies have also found an association between intraventricular haemorrhage of higher grades and red blood cells transfusion, and with the volume transfused [26, 27]. The dilemma still remains over other potential short- and long-term effects in the neurodevelopment of these small infants with more restrictive guidelines but nowadays tendency is to favour restrictive over liberal guidelines [30]. Confronting these results with our study, we found no differences between our two groups and no association with need for red blood cells transfusion upon the diagnosis of intraventricular hemorrhage equal or above grade 3 or periventricular leukomalacia, probably due to the exclusion criteria of the preterm infants in our study.

Other clinical complications such as sepsis, retinopathy of prematurity (≥ grade 2) and bronchopulmonary dysplasia were diagnosed more frequently, and in a statistically significant way, in the group that required transfusion with red blood cells. Consequently, in infants with lower birth weight, some literature supports a correlation between these morbidities and higher transfusion demands [31]. This association with red blood cells transfusions is also involved in necrotizing enterocolitis but this association is not yet clear due to its multifactorial nature [31, 32]. A recent prospective and cohort study found necrotizing enterocolitis to be associated with greater anemia severity in extremely low birth weight infants, rather than with red blood cells transfusion [33]. Regarding this complication, our study did not find any differences between the two groups.

Physiologically, erythropoietin is known to be the greatest stimulator of red blood cells production and is synthesized as a response to tissue hypoxia and anemia but, in preterm infants, this mediator has an inadequate response that leads to a lower red blood cells count and haematocrit level right after birth [3, 5]. A study, comparing infants before and after discontinuation of treatment with recombinant erythropoietin, showed no significant modifications in the number of transfused neonates, nor in the number of transfusions after the fifteenth day of life [34]. The updated *Cochrane* review showed that late treatment with erythropoietin reduced the use of one or more red blood cells transfusions (RR = 0.71, 95% CI 0.64-0.79) [11]. Our study is in accordance with the former, as the treatment with erythropoietin showed no significant association with number of transfusions, as well as with other morbidities.

As treatment with erythropoietin could only show modest results when confronted with more restrictive transfusion guidelines, it is understandable that many facilities, just like the one in this study, stopped using recombinant erythropoietin in their routine. Authors defend that the investigation shall majorly focus on strategies to minimize the risk of transfusion during the infants' first week of life [11].

Preterm infants subjected to red blood cells transfusions also stayed for a longer period in the Neonatal Intensive Care Unit. This was to be expected since these infants were significantly smaller and more premature than the ones in the no transfusion group.

A higher weight at birth was associated with a lower possibility of recurring to treatment with red blood cells during the hospital stay. This finding corroborates birth weight as one of the greatest predictors for morbidity and mortality in the immature infants and is consistent with the literature that states these smaller infants as having higher transfusion requirements [25, 32]. Fabres et al. stated that preterm infants above 1,200 grams were at lower risk of requiring a red blood cells transfusion [27].

Our results showed total volume phlebotomy losses, in millilitres, as statistically higher in the transfusion group and as one of the factors statistically associated with the need for red blood cells transfusions: per one millilitre of blood taken from the infant, there was a 1.17-fold greater chance of needing a transfusion, when adjusted for birth weight and gestational age. This significant finding is in accord with the literature [25, 34]. Widness, who took into account six studies with infants below 1,500 grams, estimated a mean of 16.5 ml/kg for weekly phlebotomy losses, being the highest value 36.7 ml/kg [3]. In our study the mean for the total volume losses during hospital stay, adjusted for birth weight, was 24.7 ml/kg and, if only referring to the transfusion group, this number rose up to 36.4 ml/kg. Both numbers are within the range referenced in the literature, as more recent studies stated monthly mean losses by phlebotomy between 15.1 and 63 ml/kg in very low birth weight infants [8, 9, 25, 34].

A simulation model, together with restrictive transfusion guidelines, predicted a reduction in the average number of red blood cells transfusions by approximately 48% with a hundred percent reduction in phlebotomy losses [35]. A study from the same author even reported that only

one-third of the sampled blood is effectively used for analysis, while the remaining is discarded. This discrepancy can partly be clinically justified if we accept that these samples can be used to make a reanalysis if necessary [9]. These findings and hypothesis solidify a clear consensus in the literature for the major role of phlebotomy losses in the postnatal hemoglobin decrease and emphasize the importance of controlling and reducing these in order to minimize the use of red blood cells transfusions in preterm infants. A cautious request for laboratory tests in association with microsampling capillary techniques through micro collection tubes, early removal of central lines and *ex vivo* bedside monitors and point-of-care analysers showed promising results. The later devices showed decreases in phlebotomy losses and number of red blood cells transfusions to 22-30% and 32-43%, respectively [25]. Even so, further studies are needed to optimize its cost and technical and clinical impact [8].

Another promising measure to avoid over-exposure in preterm infants lies on placental transfusion by delaying cord clamping and milking of the umbilical cord. Cord clamping, especially if delayed, can provide 30% of additional blood volume and 60% more red blood cells at birth time [36]. This procedure was formally introduced in the clinical practice during 2010 and upon the publication of the Guidelines for Resuscitation. These recommended cord clamping after at least one minute if the newborn was found to be clinically stable [37], but the perfect timing for this procedure is still under debate among the medical community. A systematic review, including preterm infants' data from fifteen trials, showed that infants with a 30 to 120 seconds delay to cord clamp had lower risk of developing short-term complications as intraventricular hemorrhage and necrotizing enterocolitis and had less need for inotropic support. They also have shown a reduction in the number of red blood cells transfusions [38]. Another single-centre and prospective study corroborated the reduction of the need for red blood cells transfusions in the delayed cord clamping group (between 60 and 75 seconds) but found no associations with death and other neonatal complications [39]. Milking of the umbilical cord towards the infant, just before clamping, can retrieve up to 20 millilitres of placental blood. One small study stated that this procedure had beneficial effects over the hemoglobin value after birth and over the overall need of red blood cells transfusions [25, 38]. In this study, when adjusted for the birth weight and gestational age, hemoglobin at admission failed to have any statistically significance over the need of transfusion during the infants hospital stay. We believe this can be partially justified by the low amplitude of the hemoglobin values among our study sample. Even so, we found that an increase of one g/dl in the initial hemoglobin value was associated with a decrease in the request for further red blood cells transfusions (B = -0.33, 95% CI -0.53 to -0.13).

Preterm infants, especially very low birth weight ones, are more susceptible to sepsis. Within this group, the sepsis events range between 11 and 46% [40]. Sepsis is mostly acquired by premature infants, who require longer hospitalizations, longer days with parenteral feeding and central lines and more ventilator support [41]. In this study, sepsis was significantly more frequent among the transfusion group: 22 out of a total 23 diagnosis; and was associated with a 1.85 increase in the need of an additional transfusion per each new event (B = 1.85, 95% CI 0.72-2.98). Among the literature, additionally to the great improvement in the neonatal care and creation of specialized Neonatal Intensive Care Units, an early and exclusive breastfeeding is stated as a crucial mean of prevention against sepsis and, consequently, against neonatal mortality and posterior brain damage [41].

There are some limitations to our study. Firstly, due to its observational and retrospective nature, some bias may exist, including misclassification bias. Secondly, this study is based on data from an electronic database and medical records of a single Neonatal Intensive Care Unit, which compromises the number of patients enrolled and implies that there may have been unmeasured values and missing data that could have changed the found results. As we solely estimated the preterm infants' phlebotomy losses, through electronic data and a mathematical formula, there may be an underestimation of the volumes taken from the neonates. Lastly, as the transfusion and erythropoietin guidelines adopted in our country and centre were revised and modified during 2013, it could turn into a confounding factor.

Conclusion

In this study, 62% of very low birth weight infants were exposed to red blood cells transfusions

during their stay in the Neonatal Intensive Care Unit. The smallest and more premature infants were more frequently transfused. Lower birth weight and higher volume phlebotomy losses were associated with a greater need of red blood cells transfusions during their stay. Taking the side of previous results, our study also strengthens phlebotomy losses as one of the major factors regarding the need to transfuse. Thus, it is important to reduce the amount of blood taken by a judicious and less frequent request for laboratory tests and by the use of capillary microsampling methods. We showed that a higher number of sepsis events was associated with higher exposure and number of red blood cells transfusions. Regarding the treatment with recombinant erythropoietin, this study showed no significant modifications in the need and number of transfusions for the very low birth weight infants after its discontinuation, results that support its cessation in this unit.

Further prospective and randomized studies, which simultaneously rigorously control phlebotomy losses, apply restrictive criteria to red blood cells transfusions and objectively account symptoms and signs, such as apnea, tachycardia and weight gain, can be important to reduce the number of red blood cells transfusions administered to very low birth weight infants.

Declaration of interest

The Authors state there have been no conflicts of interest and received no payment to perform this work. No subsidies or grants contributed to this work.

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