Impact of the volume of blood collected by phlebotomy on transfusion requirements in preterm infants with birth weight of less than 1500 g. A quasi-experimental study

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ABSTRACT

Introduction. Anemia is a complication in very low birth weight (VLBW) infants, and lab tests are a predominant risk factor. At least one red blood cell transfusion is given in more than 50 % of cases. Transfusions are associated with a higher risk for infections, intracranial hemorrhage, necrotizing enterocolitis, and bronchopulmonary dysplasia. In 2012, Hospital Italiano de Buenos Aires implemented a strategy to collect a lower blood volume by phlebotomy. The objective of this study was to assess its association with the number of transfusions.

Methods. Before-and-after, quasi-experimental study. The number of transfusions was compared between two groups of VLBW preterm infants with different blood collection volumes. The correlation between the collection volume and the number of transfusions was assessed estimating Spearman's coefficient. A logistic regression model was used to adjust for confounders.

Results. The study included 178 patients with a mean gestational age of 29.4 weeks (standard deviation: 2.7) and a birth weight of 1145 g (875-1345). The baseline red series profile was similar between both groups. The number of transfusions (p = 0.017) and the transfusion volume (p = 0.048) decreased significantly. The correlation coefficient was 0.83. In the multivariate analysis, collection volume and birth weight were associated with a requirement of more than three transfusions.

Conclusion. A lower blood collection volume in VLBW preterm infants is independently associated with fewer transfusion requirements.

Key words: *preterm infant, very low birth weight infant, phlebotomy, anemia, red blood cell transfusion.*

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INTRODUCTION

The major advances made in perinatal management have improved newborn infants' survival and prognosis. These changes have modified the population of patients admitted to the neonatal care unit (NCU), and the sub-group of very low birth weight (VLBW) preterm infants (less than 1500 g) is now larger.[1,2] Given the improved survival of these patients, a great challenge for current neonatology is to achieve their hospital discharge with no sequelae.[1,2]

Among the different complications presented in these patients, the development of anemia is a phenomenon that has been described globally.[3] The depletion of iron deposits, a reduced red blood cell half-life, blood loss due to sample collections, and the inability to increase the level of erythropoietin in response to this context contribute to the development of anemia.[3] Although the latter may be considered the main cause in anemia pathogenesis,[3,4] the multiple lab tests required are a prevalent factor.[5,6]

Preterm patients usually receive red blood cell transfusions (RBCTs) to manage anemia. It has been estimated that more than 50 % of VLBW infants and 90 % of extremely low birth weight infants (less than 1000 g) receive at least one transfusion during their hospitalization. Although transfusions are considered the fastest and most effective treatment, they have been associated with a greater risk for adverse outcomes, such as infection transmission, intraventricular

hemorrhage (IVH), necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia (BPD).[9-12]

Human recombinant erythropoietin has been widely used for the management of this group of patients;[13] however, at present, its routine use is not recommended.[14] A non-pharmacological approach, such as reducing blood loss by phlebotomy, may be perceived as simple and evident, although its safety and effectiveness should be studied.[15] Some publications have reported an adequate correlation between the collection and transfusion volumes and/or a reduced number of RBCTs when collecting smaller blood volumes for lab tests.[5,6,16,17]

In 2012, Hospital Italiano de Buenos Aires implemented a strategy to collect a lower blood volume by phlebotomy for the clinical management of these patients: micro-method for blood count; single sample for blood gases, electrolytes, liver function test, kidney function test; a lower volume required for blood culture collection.

Historically, greater blood volumes were sent to the lab. The hospital's NCU is part of the South American Neonatal Network (Neocosur). It reports information about VLBW infants to a prospective collection database,[2] according to which, 60 % of patients received an average of, at least, three transfusions. The objective of this study was to assess if there was an association between a lower blood volume collected by phlebotomy and the number of RBCTs in VLBW preterm infants.

METHODS

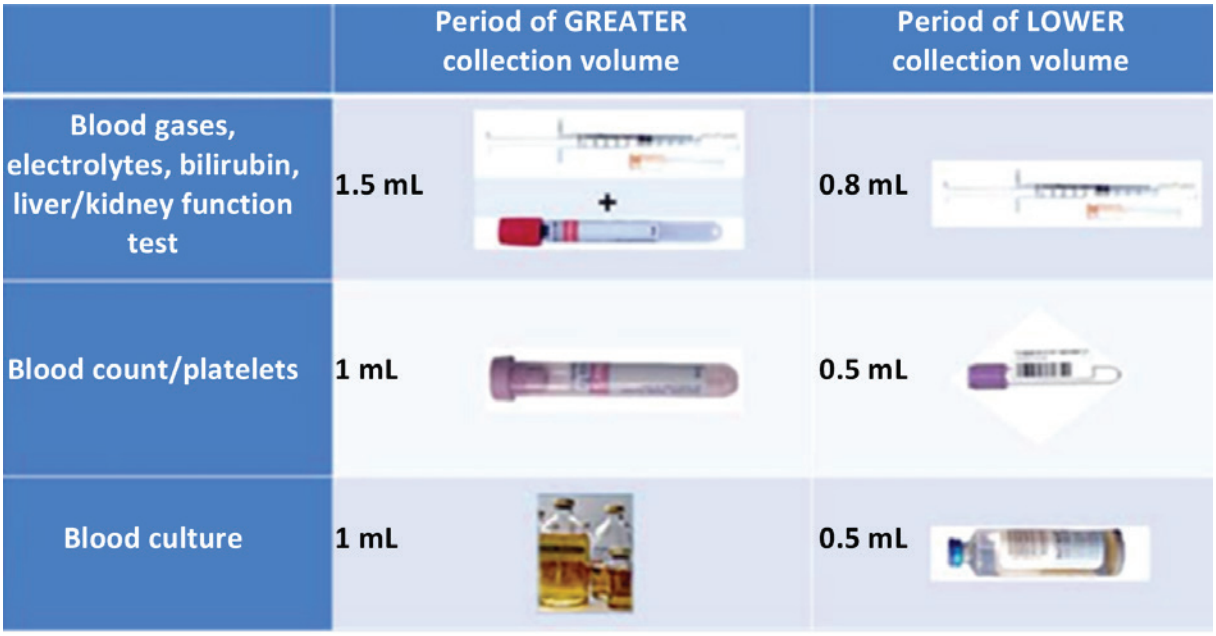
Design: This was a quasi-experimental (before-and-after) study to compare transfusion requirements in a group of VLBW preterm infants, after implementing a strategy for a smaller blood collection volume by phlebotomy, to a group of patients with the same birth weight before such implementation. Infants born between 1/1/2008 and 12/31/2011 were in the group defined as those with a greater collection volume. Infants born between 1/1/2013 and 12/31/2016 were in the other group, defined as those with a lower collection volume. *Figure 1* shows the strategy modification.

Population: Infants born at the hospital with a birth weight < 1500 g were included. Infants referred from another facility, who had congenital malformations, who died in the first month or who were born in 2012 (wash out period, during which both collection modalities may have overlapped) were excluded. Patients were stratified into two groups: <1000 g and 1000-1499 g.

Primary explanatory outcome measure: Blood volume collected by phlebotomy.

Primary outcome variable: Number of RBCTs.

FIGURE 1. *Strategy for a lower collection volume*



Perinatal outcome measures at baseline and related to course: Baseline red series profile (first hemoglobin and hematocrit values) and before each RBCT to compare thresholds, birth weight (BW), gestational age (GA), intrauterine growth restriction (IUGR), sex, antenatal corticosteroids, Neocosur score[18] (likelihood of death), Apgar score, surgical event, confirmed sepsis, confirmed NEC, IVH grade III-IV, moderate/severe BPD, retinopathy of prematurity (ROP), death. Secondary sources (Neocosur collection database) were used for all outcome measures, except for the red series profile (electronic medical record), and data were recorded until hospital discharge.

Statistical analysis: Overall and by stratum. Measures of central tendency and dispersion were described based on distribution. Dichotomous outcome measures were obtained by estimating the percentage of occurrence. A bivariate analysis was performed between both groups comparing continuous outcome measures with the t test or the Mann-Whitney test, and categorical outcome measures, with the χ^2 test or Fisher's test. The correlation between the collection volume and the number of RBCTs was assessed estimating Spearman's coefficient. A multiple linear regression was done initially to adjust for confounders. The statistical assumptions were not met, so the outcome variable was categorized (cut-off point >3 RBCTs as per the clinical criterion and the observation of the upper quartile of the outcome measure distribution) and a logistic regression model was developed. The primary explanatory outcome measure, adjusted for others that were statistically different in the bivariate analysis, and another baseline variable deemed indispensable due to biological plausibility were included. The goodness of fit for this model and its receiver operating characteristic (ROC) curve were assessed. The crude and adjusted odds ratio (OR) with the corresponding 95 % confidence interval (CI) and the p value for each outcome measure were described. A value of $p < 0.05$ was considered statistically significant.

Sample size: It was estimated by stratum because the studied association may have differed. Based on historical data that showed a mean of 0.8 RBCTs per patient (standard deviation [SD]: 0.76), with a reduction to 0.46 RBCTs (SD: 0.5) in a pilot study with a two-tailed $\alpha = 0.05$ and $\beta = 80$ %, 116 patients (58 per group) were required in the upper stratum. In the stratum of smaller patients, based on historical data that showed a mean of 5.5 per patient (SD: 2.8) with a reduction to 3.2 (SD: 2.7), 62 patients (31 per group) were required. Based on the number required per group and stratum, patients were selected by proportional random sampling.

The STATA 13 software was used for statistical analysis. The study was approved by the Ethics Committee for Research Protocols of our hospital.

RESULTS

Figure 2 shows the flow chart of patients. The mean GA was 29.4 weeks (SD: 2.7) and the median BW, 1145 g (25-75 interquartile range [25-75 IQR]: 875-1345). *Table 1* compares the baseline perinatal characteristics between both groups, with no differences observed (except for the Apgar score). The total number of RBCTs was 250 and 171 in each group, respectively

TABLE 1. *Baseline perinatal characteristics*

Outcome measure	Period of greater collection volume n = 89	Period of lower collection volume n = 89	*p* value
GA (weeks), mean ± (SD)	29.1 (2.9)	29.6 (2.6)	0.19*
BW (grams), median (IQR)	1140 (865-1340)	1150 (900-1360)	0.87**
Male sex, n (%)	42 (47.1)	44 (49.4)	0.76†
Apgar score ≤5 at 1 minute, n (%)	20 (22.5 %)	38 (42.7 %)	0.004†
Antenatal corticosteroids, n (%)	80 (89.9 %)	87 (97.7 %)	0.057††
Neocosur score, median (IQR)	0.11 (0.04-0.32)	0.13 (0.05-0.33)	0.51††
IUGR, n (%)	36 (40.4 %)	41 (46 %)	0.45†
Hb at birth, median ± (SD)	15.9 g/dL (1.8)	15.7 g/dL (2.2)	0.52*
Hct at birth, mean ± (SD)	46.9 % (5.3)	47.5 % (6.7)	0.51*

GA: gestational age; BW: birth weight; IUGR: intrauterine growth restriction; Hb: hemoglobin; Hct: hematocrit; SD: standard deviation; IQR: interquartile range.
*t test. **Mann-Whitney. †χ^2. ††Fisher.

(a 31.6 % reduction). Out of the 178 included patients, 100 (56 %) received at least one RBCT: 56 patients corresponding to the period of greater collection volume (63 %) and 44, to the period of lower collection volume (49 %) ($p = 0.07$). In total, 32 % of RBCTs were given to patients with mechanical ventilation and 66 %, to patients receiving some type of oxygen therapy, with no differences observed between groups. No statistical difference was either observed when

FIGURE 2. *Flow chart of patients*

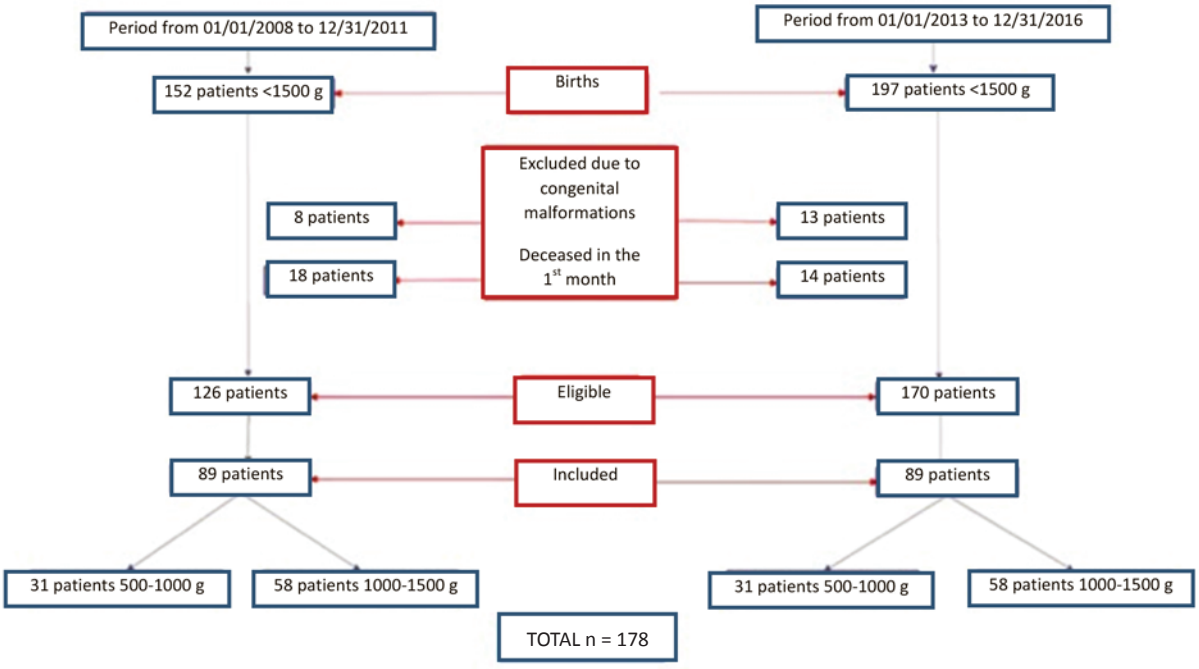
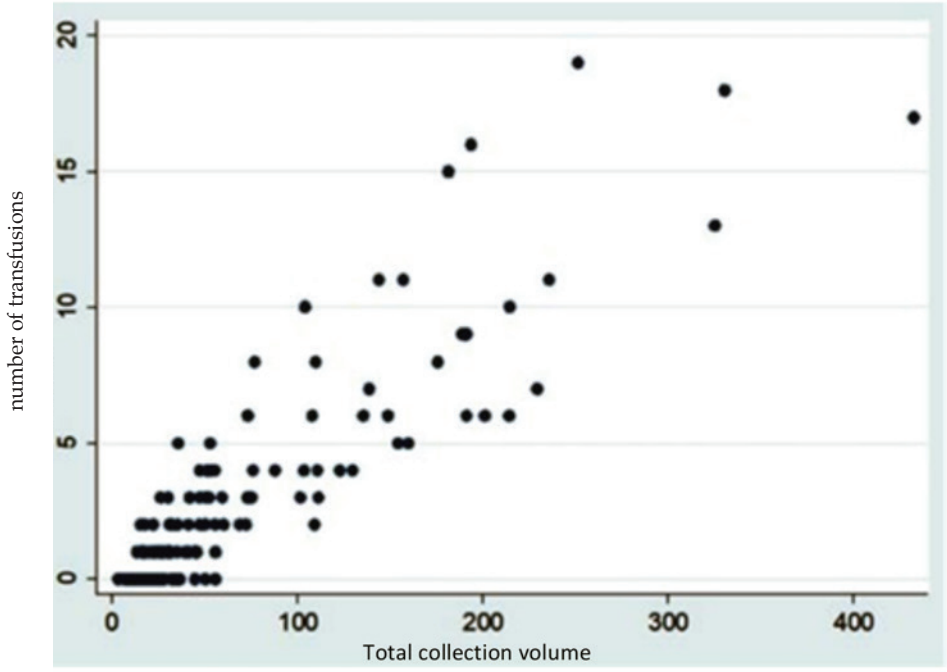


FIGURE 3. *Correlation between total collection volume and number of red blood cell transfusions*



Spearman's coefficient r = 0.83 p = 0.001 n = 178.

comparing pre-transfusion hematocrit levels: 24.8 % versus 24.2 %. *Table 2* compares the characteristics related to patients' course during hospitalization. The number of RBCTs and the transfusion volume decreased significantly. The other characteristics (except for sepsis) related to the course during hospitalization did not show significant differences.

Figure 3 shows the resulting correlation between the collection volume and the number of RBCTs. In the multivariate analysis by logistic regression (*Table 3*), the collection volume and a BW > 1000 g demonstrated a significant association with the outcome variable, regardless of the other outcome measures included. Each blood milliliter collected by phlebotomy was associated with a 6 % higher chance of receiving > 3 RBCTs.

In the stratum of patients with a BW between 1000 g and 1499 g, the number of RBCTs ($p = 0.05$) and the transfusion volume ($p = 0.14$) decreased, but this was not statistically significant. In the stratum of patients with a BW <1000 g, the number of RBCTs ($p = 0.06$) and the transfusion volume ($p = 0.09$) also decreased, and again, this was not statistically significant.

DISCUSSION

This study demonstrates that the group of patients born in the period after implementing the strategy had a lower blood collection volume, less than half, and also required almost one-third fewer RBCTs. Almost 15 % more patients did not receive a RBCT. The perinatal characteristics, such as likelihood of survival and baseline red series profile, were similar in both groups, so they were considered comparable. Regardless of the statistical significance, the results are of great clinical relevance considering that it would be preferable to avoid this practice, but it is however done in more than 50 % of this population.[7,8,19]

It has been established that RBCTs may be beneficial in severely ill preterm infants because they improve organ oxygenation and

TABLE 2. *Characteristics related to course during hospitalization*

Outcome measure	Period of greater collection volume n = 89	Period of lower collection volume n = 89	*p* value
IVH III IV, n (%)	3 (3.4 %)	3 (3.4 %)	1††
BPD O_2 36 weeks, n (%)	18 (20.2 %)	21 (23.6 %)	0.58†
NEC IIa, n (%)	8 (9 %)	9 (10.1 %)	0.8†
ROP, n (%)	18 (20.2 %)	13 (14.6 %)	0.32†
Sepsis, n (%)	31 (34.8 %)	16 (18 %)	0.01†
Surgical event, n (%)	21 (23.6 %)	17 (19.1 %)	0.46†
Death, n (%)	3 (3.4 %)	3 (3.4 %)	1††
Collection volume (mL), median (IQR)	50 (25-109)	21 (15-32)	0.0001*
No. of samples, median (IQR)	60 (29-136)	35 (23-51)	0.0002*
No. of RBCTs, median (IQR)	1 (0-4)	0 (0-2)	0.017*
Transfusion volume (mL), median (IQR)	30 (0-77)	0 (0-43)	0.048*

IVH: intraventricular hemorrhage; BPD: bronchopulmonary dysplasia; NEC: necrotizing enterocolitis; ROP: retinopathy of prematurity; IQR: interquartile range.
†χ^2. ††Fisher. *Mann-Whitney.

TABLE 3. *Multivariate analysis. Variables associated with a requirement of more than three red blood cell transfusions*

Outcome measure	Crude OR (95 % CI)	*p* value	Adjusted OR (95 % CI)	*p* value
Collection volume	1.07 (1.04-1.09)	0.001	1.06 (1.03-1.08)	0.001
Sepsis	26.3 (10.6-65)	0.001	2.18 (0.43-11.1)	0.34
Apgar score ≤ 5 at 1 minute	3.02 (1.46-6.23)	0.003	1.14 (0.23-5.6)	0.87
Weight > 1000 g	0.07 (0.03-0.17)	0.001	0.19 (0.04-0.84)	0.03

n = 178 pseudo-R2 = 0.71.
Model adjustment: Hosmer-Lemeshow test, $p = 0.89$; ROC curve: 0.977, 95% CI (0.957-0.996).
OR: odds ratio; CI: confidence interval.

increase the cardiac output.[19] However, the bibliography about transfusion thresholds and their clinical benefit is controversial.[19] Two studies analyzed the transfusion threshold by randomizing preterm infants to liberal or restrictive RBCTs based on a pre-specified Hb level and ventilatory support. Bell et al., showed that the restrictive group was exposed to a smaller number of RBCTs, although they had a greater incidence of apnea, IVH grade IV or periventricular leukomalacia.[20] On the contrary, Kirplani et al. reported that the restrictive group was also exposed to less RBCTs, but there were no differences in terms of morbidity and mortality,[21] and neurodevelopmental follow-up.[22] Therefore, the evidence suggests that a restrictive policy reduces the number of RBCTs but poses uncertainty in the short- and long-term outcomes, so future, larger, multicenter studies are required.[19,23] In the meantime, at the hospital's NCU, this practice is individualized. In this study, respiratory support and pre-transfusion hematocrit level were similar in both periods, so they do not appear to be related to the lower transfusion requirement after the strategy was implemented.

In addition to inherent risks (inadvertent transfusion of incorrect blood, infections, adverse reactions), RBCTs have been associated with a higher incidence of IVH, BPD, ROP, and NEC.[10-12,24,25]

However, this study found no significant differences in such complications between both groups with different collection volumes and transfusion requirements. Part of the bibliography has described an association between the collection volume by phlebotomy and transfusion requirements.[5,6] Almost half of RBCTs in this population are given in the first 2 weeks of life in the case of more severe cardiorespiratory disease, with a higher blood sample requirement for lab tests.[16] Weekly losses by phlebotomy have been estimated to account for, in average, 10-30 % of blood volume (10 to 25 mL/kg of weight).[16] Considering the BW of the studied population, the average blood volume lost by phlebotomy during hospitalization decreased from 43 mL/kg to 18 mL/kg after the strategy implementation (from 50 % to 25 % of blood volume). Taking into account that most of the other studied outcome measures were similar between both groups, the lower collection volume may be considered to be critically relevant to reduce transfusion requirements.

It is known that patients with a younger GA and a lower BW and those who require resuscitation at birth or suffer severe complications, like sepsis or NEC, receive more RBCTs.[7,19,26] In this study, the group of patients born after the intervention had a lower incidence of sepsis. This may be secondary to the implementation of an Epidemiological Surveillance Program targeted at controlling nosocomial infections.[27] It may be speculated that, due to the lower presence of sepsis, this group received fewer transfusions. However, the multivariate analysis showed that only the collection volume by phlebotomy and BW, regardless of adjustment outcome measures, are associated with transfusion requirements. Instead, the outcome measure "sepsis" lost statistical significance. The group of patients born after the strategy was more depressed at birth, and this is probably by chance, because perinatal practices have improved over time.[1,2] However, this may have resulted in a greater transfusion requirement but, since the opposite occurs, it brings robustness to findings.

This study has limitations and strengths. One of the difficulties when interpreting before-and-after study results is that some health care practices, associated with the primary explanatory outcome measure and the primary outcome variable, may change over time. For example, umbilical cord management at birth. In the case of term infants, delayed clamping has demonstrated to be more beneficial than immediate clamping in relation to the risk for neonatal anemia.[28,29] In preterm infants, although it is still under investigation with some discrepancy,[30] there is also evidence in favor of delayed clamping.[31] When increasing the hematocrit level at birth, transfusion requirements decrease.[31] At Hospital Italiano, although there was no protocol implemented during the study period, clinical practice may have changed towards delayed clamping. However, since no differences were observed in the red series profile at birth, clamping does not appear to have affected the study primary outcome.

Given the observational design of this study, it is not possible to ensure causality between both outcome measures of interest. However, several of Hill's criteria for causation are met,[32] making the observed association even more robust. There is consistency with previous studies,[5,6,16,17] temporality and biological plausibility/gradient observed in the adequate correlation between both outcome measures. In addition, quasi-

experimental studies are considered an option to establish a casual association when clinical trials cannot be performed due to ethical, financial or time constraints.[33] They are pragmatic and, therefore, their results sometimes acquire a greater external validity with a better possibility of generalization.[33] Another limitation was observed when quantifying the primary explanatory outcome measure because the phlebotomy volume was not described prospectively, which accounts for a potential information bias, controlled when obtaining the data of each blood sample collection from the results section of the electronic medical record. Knowing the required amount, it was possible to estimate the total collection volume in milliliters for each patient for their entire hospitalization, whose blood samples were collected uniformly by trained staff. In addition, such data collection (as all other variables) was done by double data entry. Therefore, it is unlikely that, if a systematic error had occurred, it may have modified the primary explanatory outcome measure in opposite directions for both comparison groups. Thus, the magnitude of the association should not have been exaggerated or reduced. Finally, the secondary database used to obtain the other outcome measures was developed prospectively, which warrants data reliability and the eligibility of 100 % of patients who met the inclusion criteria.

To conclude, it is possible to establish that a lower blood collection volume for the clinical management of VLBW preterm infants is independently associated with fewer transfusion requirements, with no impact on the other studied clinical outcome measures. ■

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