Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

Complications and blood transfusions in newborns are associated with a higher milliliters per kilogram ratio of blood extracted for laboratory studies

Lorena Salazar-Cavazos[1], Jesus J. Solis-Flores[1], Carmen L. de Hoyos-Ramón[1], Marco A. Flores-Heredia[1], Luis G. Martínez-Valades[1], and Lucas A. Garza-Garza[2]

[1]Departamento de Neonatología, Hospital Conchita; [2]Departamento de Oftalmología, Tec de Monterrey. Monterrey, Nuevo León, México

Abstract

Background: *Phlebotomy-associated blood loss is a clinical concern in term and pre-term newborns. Previous reports have associated a higher volume of phlebotomy associated blood loss to a more pre-term status and to the need for blood transfusions. The present study was undertaken to evaluate the amount of phlebotomy-associated blood loss and its associations.* **Methods:** *Retrospective, observational, and analytical study conducted through a retrospective chart review. The primary objective was to determine the association between the presence of the endpoint "any complications" and the mean phlebotomy-associated blood loss in milliliters/kilograms (mL/kg). Blood loss was quantified using the corresponding documentation sheet in the clinical record, as reported by the nursery service.* **Results:** *176 patients were included in the present study. Male: female ratio was 1.67:1.00. Mean gestational weeks was 34.2 with a standard deviation of 3.7. Patients meeting the composite endpoint "any complications" had a higher mean mL/kg of phlebotomy-associated blood loss than those who did meet it (10.93 vs. 2.91, $p < 0.001$). In addition, patients requiring blood transfusions had higher mL/kg of phlebotomy-associated blood loss than those who did not (21.16 vs. 4.21, $p < 0.001$). Finally, more pre-term status was significantly associated with a higher phlebotomy-associated blood loss ($p < 0.001$).* **Conclusions:** *The presence of any complications, more pre-term status and need for blood transfusions was significantly associated with a higher mL/kg phlebotomy-associated blood loss. Bigger, prospective studies controlling other variables and temporality are needed to fully grasp the clinical consequences of a high mL/kg phlebotomy-associated blood loss.*

Keywords: *Pre-term. Laboratory studies. Blood extraction. Mililiters/kilograms. Blood transfusions.*

Las complicaciones y las transfusiones de sangre en los recién nacidos se asocian a una mayor proporción de mililitros por kilogramo de sangre extraída para estudios de laboratorio

Resumen

Introducción: *La pérdida de sangre asociada a la flebotomía es una preocupación clínica en los recién nacidos a término y prematuros. Informes previos han asociado un mayor volumen de pérdida de sangre asociada a la flebotomía con un estado más prematuro y con la necesidad de transfusiones de sangre. El presente estudio se realizó para evaluar la cantidad de pérdida de sangre asociada a la flebotomía y sus asociaciones.* **Métodos:** *Estudio retrospectivo, observacional y analítico realizado por*

***Correspondence:**
Lorena Salazar-Cavazos
E-mail: lorena.salazar.cavazos@gmail.com

Date of reception: 20-11-2024
Date of acceptance: 27-01-2025
DOI: 10.24875/BMHIM.24000148

Available online: 04-09-2025
Bol Med Hosp Infant Mex. 2025;82(5):289-294
www.bmhim.com

medio de una revisión retrospectiva de historias clínicas. El objetivo principal fue determinar la asociación entre la presencia del objetivo compuesto «cualquier complicación» y la pérdida de sangre media asociada a la flebotomía en mL/kg. La pérdida de sangre se cuantificó usando la hoja correspondiente en el expediente clínico documentada por el servicio de enfermería. **Resultados:** *Se incluyeron 176 pacientes en el presente estudio. La relación hombre: mujer fue de 1.67:1.00. La media de semanas de gestación fue de 34.2, con una desviación estándar de 3.7. Los pacientes que cumplieron con el criterio de valoración compuesto «cualquier complicación» tuvieron una media mayor de mL/kg de pérdida de sangre asociada a la flebotomía que aquellos que sí lo cumplieron (10.93 vs. 2.91; p < 0.001). Además, los pacientes que requirieron transfusiones de sangre tuvieron una mayor pérdida de sangre asociada a la flebotomía en mL/kg que aquellos que no las requirieron (21.16 vs. 4.21; p < 0.001). Finalmente, un mayor estado de prematuridad se asoció significativamente con una mayor pérdida de sangre asociada a la flebotomía (p < 0.001).* **Conclusiones:** *La presencia de cualquier complicación, un mayor estado de prematuridad y la necesidad de transfusiones de sangre se asociaron significativamente con una mayor pérdida de sangre asociada a la flebotomía en mL/kg. Se necesitan estudios prospectivos más amplios que controlen otras variables y la temporalidad para comprender plenamente las consecuencias clínicas de una alta pérdida de sangre asociada a la flebotomía en mL/kg.*

Palabras clave: *Prematuro. Estudios de laboratorio. Extracción de sangre. Mililitros/kilogramos. Transfusiones de sangre.*

Introduction

In the pre-term newborn (PN) and term newborn (TN) phlebotomy-associated blood loss (PABL) is a common concern in the neonatal intensive care unit (NICU)[1-4], as it may participate in the development of anemia[1-4]. Anemia is more common, severe, and prolonged in the PN than in its term counterpart (TN) and commonly requires at least one blood transfusion as treatment[5]. The severity and duration of anemia in the PN is associated with faster-dwindling iron deposits, a lower half-life of red blood cells and a temporary inability to raise the circulating erythropoietin levels[5]. As this can lead to a need for blood transfusion, which is not free of risks[6-8], a strict vigilance of the PABL in milliliters/kilograms (mL/kg) extracted is of the utmost importance. Some of the risks associated to blood transfusions in PNs include hemolytic transfusional reactions, alloimmunization, volume overload, allergic reactions; iron excess, transfusion-associated infections, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD) and increased risk of retinopathy of pre-maturity[6-8]. Previous guidelines have marked safe thresholds for PABL[9,10]. Still, previous reports have found that PABL can vary between 16 and 65 mL/kg in the first 2 weeks of life in PNs[11-13]. To the authors' best knowledge, no previous study has reported the PABL in PNs and TNs in Mexico. Therefore, it is the objective of the present study to describe the mL/kg PABL and its associations in a group of patients from northern Mexico.

Methods

The present study has an observational, retrospective and analytical design. It was undertaken in the NICU of a tertiary academic center in northern Mexico. The present study was waived from being presented to the local ethics committee (Hospital de Ginecología y Obstetricia de Monterrey (Hospital Ginequito [HG]) internal ethics committee) due to its retrospective and observational nature. In addition, the present study adhered to the tennets present in the Declaration of Helsinki and no identifiable information from any patient was included. PN and TN born in HG who entered the NICU of any gender and age in 2022 and 2023 were included. The only two exclusion criteria were an incomplete clinical record and the transfer to another NICU outside the study's center. Physical and electronic clinical records of included patients were reviewed for sociodemographic data, clinical characteristics and PABL, which was reported as milliliters and as mL/kg. PABL was measured daily and documented in the fluid specifications sheet by the nursery staff, which is present in every clinical record of the study's center. The total PABL for each patient in their full hospitalization was used as the final measure. Due to the retrospective nature of the present study, no study's investigator was present at the time of the PABL quantification. As the fluid specifications sheet did not report the PABL divided between peripheral and central catheters, the amount provided by each is not available. As is inherent to retrospective studies, this data recollection method is imperfect and is prone to retrospective biases. Term status was classified as: Term ≥ 37 weeks of gestation (WG), Late pre-term 34-36 WG, Moderate pre-term 32-33.6 WG, Very pre-term 28-31.6 WG, and Extreme pre-term < 28 WG, in accordance to previous reports[14]. Data were emptied into an Excel spreadsheet (Microsoft Office Excel, Microsoft, USA) without any identifying data. The primary objective was to determine if the

Table 1. Descriptive statistics of the full sample

Variable	Result (n = 176)
Maternal age‡	31.39 (5.157)
Newborn gender*	31.40
Male	110 (62.5)
Female	66 (37.5)
Male: Female ratio	1.67:1.00
Prenatal steroid scheme*	
Complete	22 (12.5)
Incomplete	30 (17.0)
None	123 (69.9)
Birth*	
Labor	2 (1.1)
Accidental birth	1 (0.6)
Cesarean section	173 (98.3)
Primary diagnosis*	
Preterm	91 (51.7)
Neonatal sepsis	65 (36.9)
Other	20 (11.3)
Gestational weeks‡	34.244 (3.7564)
OMS term classification*	
Term ≥ 37 WG	58 (33.0)
Late preterm 34-36 WG	56 (31.8)
Moderate preterm 32-33.6 WG	19 (10.8)
Very preterm 28-31.6 WG	32 (18.2)
Extreme preterm < 28 WG	11 (6.3)
Birth weight‡	2282.34 (928.452)
Apgar 1‡	8.20 (1.293)
Apgar 5‡	9.29 (.973)
Silverman 1‡	0.28 (0.777)
Silverman 2‡	1.64 (1.473)
Size (centimeters)‡	45.037 (6.3558)
Cephalic perimeter‡	31.509 (4.0888)
Need for antibiotics*	149 (84.7)
Need for antifungals*	25 (14.2)
Need for amine therapy*	19 (10.8)
Retinopathy of preterm*	8 (4.5)
Necrotizing enterocolitis*	19 (10.8)
Bronchopulmonar dysplasia*	8 (4.5)
Intraventricular hemorraghe*	8 (4.5)
Need for surgery*	21 (11.9)
Laboratory values‡	
Basal hemoglobin	16.2480 (2.61141)
Basal hematocrit	47.5378 (8.62823)
Last hemoglobin	14.2035 (2.99189)
Last hematocrit	41.3594 (9.76327)

(*Continues*)

Table 1. Descriptive statistics of the full sample (*continued*)

Variable	Result (n = 176)
Blood tranfusions*	32 (18.2)
Number of transfusions in all patients‡	26 (0.623)
Number of transfusions only in patients needing transfusions‡	1.44 (0.669)
Hematocrit during transfusion‡	27.3875%
Phlebotomy-associated blood-loss in milliters‡	11.2153 (10.12978)
Phlebotomy-associated blood-loss in milliters/kilogram‡	7.3177 (10.11590)
Any complication*§	96 (54.5)
Phlebotomy-associated blood-loss in milliters/kilogram according to preterm classification‡	
Term ≥ 37 WG	2.9777 (3.28885)
Late preterm 34-36 WG	3.2653 (2.22132)
Moderate preterm 32-33.6 WG	8.6901 (11.89036)
Very preterm 28-31.6 WG	17.0702 (13.78024)
Extreme preterm < 28 WG	19.7224 (13.12802)
Blood transfusions per preterm classification*	
Term ≥ 37 WG	7 (12.1)
Late preterm 34-36 WG	2 (3.6)
Moderate preterm 32-33.6 WG	2 (10.5)
Very preterm 28-31.6 WG	14 (43.8)
Extreme preterm < 28 WG	7 (63.6)

*Absolute number and percentage.
‡Average and standard deviation.
§Any complication includes: death, sepsis, need for surgery, need for vasoactive drugs administration, intraventricular hemorraghe in any degree, bronchopulmonar dysplasia or necrotizing enterocolitis.
WG: Weeks of gestation.

mL/kg PABL was different in the composite endpoint "presence of any complication" (death, sepsis, need for surgery, need for vasoactive drugs administration, IVH in any degree, BPD or NEC) than in its absence. Additional secondary objectives included the association between pre-term staging and PABL, transfusions and PABL, pre-term staging and complications and transfusions and pre-term staging.

Statistical analysis

Data from the Excel spreadsheet were transposed into the Statistical Package for the Social Sciences (SPSS) sheet (SPSS statistics, IBM, Addison). Numerical variables were described with central tendency and dispersion measures and categorical variables with absolute numbers and percentages. The association between non-related categorical variables

Table 2. Difference in phlebotomy associated blood-loss in milliliters, milliliters/kilogram, initial hemoglobin, final hemoglobin, initial hematocrit and final hematocrit according first to the presence or absence of any complications and then to need for blood transfusions

Variable	Any complication	No complication	p
Phlebotomy associated blood-loss in milliliters*	15.2474	6.3769	< 0.001
Phlebotomy associated blood-loss in milliliters/kilogram*	10.9398	2.9162	< 0.001
Basal hemoglobin*	16.0359	16.5024	0.239
Basal hematocrit*	46.8162	48.4038	0.225
Last hemoglobin*	13.1723	15.4410	< 0.001
Last hematocrit*	38.5788	44.6963	< 0.001
Variable	**Patients requiring blood transfusions**	**Patients not requiring blood transfusions**	**p**
Phlebotomy associated blood-loss in milliliters*	24.7172	8.2149	< 0.001
Phlebotomy associated blood-loss in milliliters/kilogram*	21.1669	4.2186	< 0.001

*Expressed as average.

was examined with the Chi square test. The difference in numerical variables divided by 2 and more than 2 groups was analyzed with the Student-t and one-way ANOVA tests, respectively. A p value lower than 0.05 was considered statistically significant.

Results

We included 176 patients in the present study. The descriptive statistics for the full sample are depicted in table 1. The mean of maternal age was 31.39 with a standard deviation (SD) of 5.1 years. A slight preponderance of male newborns was detected (male: female ratio of 1.67:1.00). Most newborns included in the present study were born through Cesarean section (98.3%) and their primary diagnosis for entering the NICU were pre-term in 51.7%, neonatal sepsis in 36.9% and others in 11.4%. The mean of gestational weeks in the full sample was 34.2 with a SD of 3.7. The most common term classifications were term in 33.0% and late pre-term in 31.8%. The mean PABL (mL/kg) in the full sample was 7.31 with an SD 10.11 and the presence of any complication was detected in 96 patients (54.5%). Additional descriptive data (Apgar, birth weight, silverman, size, and other clinical characteristics) can be found extensively reported in table 1. The difference in PABL and other numerical variables according to the presence or absence of complications is reported in table 2. Both milliliters and mL/kg of PABL were higher in the any complication than in the no complication group (mean 15.24 vs. 6.37 and 10.93 vs. 2.91, both p < 0.001). Basal hemoglobin and hematocrit did not significantly differ between both groups. However, the last hemoglobin and hematocrit were lower in the any complication compared to the no complications group (p < 0.001). When examining patients according to their term classification (Table 3) moderate pre-term, very pre-term and extreme PNs had higher mean PABL in both milliliters and mL/kg compared to term and late pre-term patients. Both of these differences were statistically significant (p < 0.001). The PABL examined through patients requiring and not requiring blood transfusions was also examined (Table 2) and those that did require them had higher milliliters and mL/kg PABL than those who did not (24.71 vs. 8.21 and 21.16 vs. 4.21, respectively, p < 0.001 in both cases). On the other hand, the association between the presence of any complication and term classification is reported in table 4. Here, a higher proportion of the presence of any complication was seen in the groups moderate pre-term, very pre-term and extremely pre-term (14 [73.7%], 24 [75.0%] and 11 [100.0%], respectively) compared with term and late pre-term (26 [44.8%] and 21 [37.5%], respectively). This difference was statistically significant (< 0.001). The association between the need for blood transfusions and term classification was also reviewed and is presented in table 4. The groups very pre-term and extremely pre-term had the highest proportion of need for blood transfusions (14 [43.8%] and 7 [63.6%], respectively), while term, late pre-term and moderate pre-term had lower proportions (7 [12.1, 2 [3.6%] and 2 [10.5%], respectively) with a p < 0.001. Finally in this section, additional results (descriptive statistics, associations between PABL and blood transfusions and other variables) are available in the supplementary material (Supplementary Tables S1-11).

Table 3. Difference in phlebotomy associated blood-loss in milliliters and milliliters/kilogram according to term classification

Variable	Term	Late preterm	Moderate preterm	Very preterm	Extreme preterm	p
Phlebotomy associated blood-loss in milliliters*	8.9509	7.2741	12.3132	20.3047	14.8818	< 0.001
Phlebotomy associated blood-loss in milliliters/kilogram*	2.9777	3.2653	8.6901	17.0702	19.7224	< 0.001

*Expressed as average.

Table 4. Proportion of any complication and need for blood transfusion according to term classification

Variable	Presence of any complication (%)	Absence of any complication (%)	p
Term/	26 (44.8)	32 (55.2)	< 0.001
Late preterm/	21 (37.5)	35 (62.5)	
Moderate preterm/	14 (73.7)	5 (26.3)	
Very preterm/	24 (75.0)	8 (25.0)	
Extremely preterm/	11 (100.0)	0 (0.0)	
Variable	**Need for blood transfusion (%)**	**No need for blood transfusion (%)**	**p**
Term/	7 (12.1)	51 (87.9)	< 0.001
Late preterm/	2 (3.6)	54 (96.4)	
Moderate preterm/	2 (10.5)	17 (89.5)	
Very preterm/	14 (43.8)	18 (56.3)	
Extremely preterm/	7 (63.6)	4 (36.4)	

/Reported as frequencies and percentages.

Discussion

The present study reports on the PABD and its associations in PN and TN from a cohort in northern Mexico. To the authors' best knowledge, no previous study regarding this matter has been published in this region. As previously stated, anemia is a highly relevant problem with an even higher morbidity and difficulty to resolve in PN[5]. Furthermore, those requiring blood transfusions as treatment face another set of possible risks[6-8]. Therefore, optimal control of all factors increasing the risk of anemia for PN and TN is of the utmost importance. Although not extensively studied, PABL has been reported by previous authors to contribute to the risk of anemia[1-4,15]. In PN and TN a single mL/kg of PABL can represent 1% or higher of their total blood volume[10]. Previous guidelines and recommendations regarding PABL vary greatly (0.8-4.0 mL/kg) and have been reported in general or by age specific groups[10]. Key points to minimize PABL include following the safe limits established by previous guidelines, lowering such limits in sick or hemoglobin-deficient patients, coordinating the clinical and research PABL needs to not cross the established limits and minimize the number and discomfort associated to PABL procedures[10]. In addition, specific subgroups of patients with certain ethnic backgrounds, diseases or other conditions may be underrepresented by actual guidelines and more research on their PABL limits is needed[10]. In the present study, mean PABL in mL/kg in the overall sample (7.3) and in the term groups moderate pre-term (8.69), very pre-term (17.07) and extreme pre-term (19.72) surpass these previously stated safe limits. However, previous reports on PABL in PN in other populations have also documented similar or higher averages[12,13]. Therefore, previously stated safe limits[10] could not be reflective of actual "real-world" clinical practices. In addition, in the aforementioned study by Madsen et al.[12] and in another study by Hack and Khodabux[16], a higher PABL was associated with the need for blood transfusions, which agrees with the findings in the present paper. Another relevant finding in the present paper was a higher PABL in patients experiencing any complications and in more pre-term patients. Previous reports have also documented that PABL is higher on more pre-term patients[15], but scarce information is available on the association between PABL and complications other than anemia in PN. It must be stated that the degree of pre-maturity, the need for blood transfusions and the presence of complications are also inter-associated variables as seen in the present paper. Therefore, it is hard to ascertain if PABL has any

causative effect on the other variables, or if it is only a marker of the severe diseases, high risks and laboratory-investigative needs seen in PN. On a final note, the authors would like to comment on the present study's weakness. First of all, it inherently possesses bias associated with retrospective studies, such as information bias, as clinical records were not originally written by the present authors. This retrospective bias extends to PABL, as it was quantified with a specific sheet in the clinical record and the study's investigators were not present for its quantifications. In addition, the temporal relationship between the PABL and other variables is uncertain. Larger, prospective studies controlling other variables would be of great importance to critically determine the real impact of PABL in the possible complications of PN.

Conclusions

PABL is associated with the presence of complications, more pre-term patients and the need for blood transfusions. Larger, prospective studies with an emphasis on other variables' control and temporality are needed to fully grasp the clinical implications of a high mL/kg PABL.

Funding

The authors declare that they have not received funding.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary. Relevant guidelines were followed.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

Supplementary data

Supplementary data are available at DOI: 10.24875/BMHIM.24000148. These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

References

1. Villeneuve A, Arsenault V, Lacroix J, Tucci M. Neonatal red blood cell transfusion. Vox Sang. 2021;116:366-78.
2. Von Lindern JS, Lopriore E. Management and prevention of neonatal anemia: current evidence and guidelines. Expert Rev Hematol. 2014;7:195-202.
3. Kilpatrick ES, Ginn EL, Lee BH. Reducing neonatal phlebotomy blood losses through the accurate calculation of minimum test volume requirements. Ann Clin Biochem. 2021;58:593-8.
4. Whitehead NS, Williams LO, Meleth S, Kennedy SM, Ubaka-Blackmoore N, Geaghan SM, et al. Interventions to prevent iatrogenic anemia: a laboratory medicine best practices systematic review. Crit Care. 2019;23:278.
5. Kirpalani H, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, et al. The premature infants in need of transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. J Pediatr. 2006;149:301-7.
6. Christensen RD. Associations between "early" red blood cell transfusion and severe intraventricular hemorrhage, and between "late" red blood cell transfusion and necrotizing enterocolitis. Semin Perinatol. 2012;36:283-9.
7. Crawford TM, Andersen CC, Hodyl NA, Robertson SA, Stark MJ. The contribution of red blood cell transfusion to neonatal morbidity and mortality. J Paediatr Child Health. 2019;55:387-92.
8. D'Amato G, Faienza MF, Palladino V, Bianchi FP, Natale MP, Christensen RD, et al. Red blood cell transfusions and potentially related morbidities in neonates under 32 weeks' gestation. Blood Transfus. 2020;19:113-9.
9. Gibson BE, Todd A, Roberts I, Pamphilon D, Rodeck C, Bolton-Maggs P, et al. Transfusion guidelines for neonates and older children. Br J Haematol. 2004;124:433-53.
10. Howie SR. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ. 2011;89:46-53.
11. Becquet O, Guyot D, Kuo P, Pawlotsky F, Besnard M, Papouin M, et al. Respective effects of phlebotomy losses and erythropoietin treatment on the need for blood transfusion in very premature infants. BMC Pediatr. 2013;13:176.
12. Madsen LP, Rasmussen MK, Bjerregaard LL, Nøhr SB, Ebbesen F. Impact of blood sampling in very preterm infants. Scand J Clin Lab Invest. 2000;60:125-32.
13. Ringer SA, Richardson DK, Sacher RA, Keszler M, Churchill WH. Variations in transfusion practice in neonatal intensive care. Pediatrics. 1998;101:194-200.
14. Karnati S, Kollikonda S, Abu-Shaweesh J. Late preterm infants - changing trends and continuing challenges. Int J Pediatr Adolesc Med. 2020;7:38-46.
15. Puia-Dumitrescu M, Tanaka DT, Spears TG, Daniel CJ, Kumar KR, Athavale K, et al. Patterns of phlebotomy blood loss and transfusions in extremely low birth weight infants. J Perinatol. 2019;39:1670-5.
16. Hack KE, Khodabux CM, Von Lindern JS, Brouwers HA, Scherjon SA, Van Rijn HJ, et al. Bloedtransfusiebehoefte bij prematuren in 2 nederlandse perinatologische centra sterk bepaald door bloedafname voor diagnostiek. Ned Tijdschr Geneeskd. 2008;152:1419-25.