Phlebotomy Overdraw in the Neonatal Intensive Care Nursery

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Abstract. *Objective.* **Because blood loss attributable to laboratory testing is the primary cause of anemia among preterm infants during the first weeks of life, we quantified blood lost attributable to phlebotomy overdraw, ie, excess that might be avoided. We hypothesized that phlebotomy overdraw in excess of that requested by the hospital laboratory was a common occurrence, that clinical factors associated with excessive phlebotomy loss would be identified, and that some of these factors are potentially correctable.**

Design, Outcome Measures, and Analysis. **Blood samples drawn for clinical purposes from neonates cared for in our 2 neonatal special care units were weighed, and selected clinical data were recorded. The latter included the test performed; the blood collection container used; the infant's location (ie, neonatal intensive care unit [NICU] and intermediate intensive care unit); the infant's weight at sampling; and the phlebotomist's level of experience, work shift, and clinical role. Data were analyzed by univariate and multivariate procedures. Phlebotomists included laboratory technicians stationed in the neonatal satellite laboratory, phlebotomists assigned to the hospital's central laboratory, and neonatal staff nurses. Phlebotomists were considered experienced if they had worked in the nursery setting for >1 year. Blood was sampled from a venous or arterial catheter or by capillary stick from a finger or heel. Blood collection containers were classified as tubes with marked fill-lines imprinted on the outside wall, tubes without fill-lines, and syringes. Infants were classified by weight into 3 groups: <1 kg, 1 to 2 kg, and >2 kg. The volume of blood removed was calculated by subtracting the weight of the empty collection container from that of the container filled with blood and dividing by the specific gravity of blood, ie, 1.050 g/mL. The volume of blood withdrawn for individual laboratory tests was expressed as a percentage of the volume requested by the hospital laboratory.**

Results. **The mean (± standard error of the mean) volume of blood drawn for the 578 tests drawn exceeded that requested by the hospital laboratory by 19.0% ± 1.8% per test. The clinical factors identified as being significantly associated with greater phlebotomy overdraw in the multiple regression model included: 1) collection in blood containers without fill-lines; 2) lighter weight infants; and 3) critically ill infants being cared for in the NICU. Because the overall R^2 of the multiple regression for these 3 clinical factors was only .24, the random factor of individual phlebotomist was added to the model. This model showed that there was a significant variation in blood overdraw among individual phlebotomists, and as a result, the overall R^2 increased to .52. An additional subset analysis involving 2 of the 3 groups of blood drawers (ie, hospital and neonatal laboratory phlebotomists) examining the effect of work shift, demonstrated that there was significantly greater overdraw for blood samples obtained during the evening shift, compared with the day shift when drawn using unmarked tubes for the group of heavier infants cared for in the NICU.**

Conclusion. **Significant volumes of blood loss are attributable to overdraw for laboratory testing. This occurrence likely exacerbates the anemia of prematurity and may increase the need for transfusions in some infants. Attempts should be made to correct the factors involved. Common sense suggests that blood samples drawn in tubes with fill-lines marked on the outside would more closely approximate the volumes requested than those without. Conversely, the use of unmarked tubes could lead to phlebotomy overdraw because phlebotomists may overcompensate to avoid having to redraw the sample because of an insufficient volume for analysis. We were surprised to observe that the lightest and most critically ill infants experienced the greatest blood overdraw. Because the volume indicators on the outside of syringe barrels are seemingly analogous to the blood collection tubes with fill-lines, it was also unexpected to observe that blood overdraw was greater with syringes than with either marked or unmarked tubes. It is likely that this is attributable in part to the unavoidable presence of the air bubble inevitably originating in the syringe tip. Educating individual phlebotomists, nurses, and other members of the care team on reducing unnecessary blood loss, eg, ordering only essential blood tests, exercising the greatest care in the smallest infants, practice in drawing blood samples into syringes, etc, may also help. Other promising means for reducing laboratory blood loss include technologic improvements to further reduce laboratory sample volume required, more reproducible and better capillary blood sampling containers, and use of point-of-care laboratory testing in which little to no blood loss results.** *Pediatrics* **2000;106(2). URL: http://www.pediatrics.org/cgi/content/full/106/2/e19;** ***anemia, phlebotomy, infants, intensive care, blood transfusions.***

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ABBREVIATIONS. VLBW, very low birth weight; RBC, red blood cell; NICU, neonatal intensive care unit; IICU, intermediate intensive care unit.

During the first weeks of life, blood loss attributable to laboratory testing is acknowledged as the primary factor leading to anemia in critically ill infants.[1] This is particularly true for very low birth weight (VLBW) preterm infants (ie, those with birth weights <1500 g) whose total blood volumes are small but whose need for blood testing is great. The latter is attributable to cardiorespiratory illness that these infants frequently manifest.[2–5] Because clinically significant anemia is treated with red blood cell (RBC) transfusions, reducing laboratory phlebotomy loss in VLBW infants would likely reduce their need for RBC transfusions.

Our observation of the laboratory phlebotomy practices in our nurseries led us to hypothesize that phlebotomy overdraw in excess of that requested by the laboratory was a common occurrence. Because we could find little quantitative data in the neonatal literature to support this, we sought to test this hypothesis in the clinical setting. Methodologies used in previous studies to assess neonatal phlebotomy loss have been imprecise, ie, based on volumes recorded in the hospital chart or the volume requested by the laboratory or unspecified.[2,6–9] Moreover, none of these studies have validated the accuracy of their assessment of blood loss. In the present study, we determined if the volume of blood drawn for laboratory testing from neonates is representative of that requested by the hospital laboratory. We hypothesized that a clinically significant volume of excessive blood would be drawn (ie, laboratory overdraw), that clinical factors associated with excessive phlebotomy loss would be identified, and that some of these factors are potentially correctable.

METHODS

The study received approval from our local human subjects review committee. Patient confidentiality was maintained with subject identification used only for gathering missing information from the subject's hospital record.

The study design was one in which laboratory samples drawn for clinical purposes were weighed to determine whether the blood volume drawn was equivalent to that requested by laboratory personnel. To test for a possible change in phlebotomy overdraw over time, the study was conducted during 2 distinct periods—August 6, 1997 to October 3, 1997 and December 9, 1997 to February 3, 1998. No change in laboratory instrumentation, blood volume requirement, or phlebotomy methodology took place between or during the 2 periods. The phlebotomists included laboratory technicians stationed in the neonatal intensive care unit (NICU) satellite laboratory, phlebotomists assigned to the hospital's central laboratory, and neonatal staff nurses. During the study, these individuals were not informed about the goals or findings of the study.

Blood samples were taken only from infants admitted to our 2 special care nurseries, ie, the NICU and the intermediate intensive care unit (IICU). Because of the study's focus on clinical factors typically contributing to anemia, blood samples were excluded if drawn for research purposes or if drawn from infants being treated with extracorporeal membrane oxygenation. Because phlebotomist participation was voluntary, samples included were those in which the NICU laboratory technician had sufficient time to weigh and to record information regarding associated factors. This included >90% of the NICU phlebotomists, 80% of the hospital phlebotomists drawing blood on NICU infants, and 55% of the NICU nurses.

Blood was sampled from a venous or arterial catheter or by capillary stick from a finger or heel. Before analysis, each sample was weighed and relevant clinical information was recorded. The latter included: 1) the laboratory test ordered; 2) date and time of phlebotomy; 3) type of collection container; 4) whether one or more collection containers were filled at the time of phlebotomy; 5) patient's weight and location (ie, NICU or IICU); 6) and the phlebotomist's name and clinical designation (ie, NICU laboratory technician, hospital phlebotomist, or neonatal staff nurse). Phlebotomists were considered experienced if they had worked in the nursery setting for >1 year. Infants were classified by weight into 3 groups: <1 kg, 1 to 2 kg, and >2 kg.

The volume of blood removed was calculated by subtracting the weight of the empty collection container from that of the container filled with blood and dividing by the specific gravity of blood, ie, 1.050 g/mL.[10] The weights of empty blood collection containers were determined by weighing 10 empty collection containers using a scale measuring in .001-g increments (Mettler Instruments AE240 Dual Range Balance, Mettler Instrument Corporation, Hightstown, NJ). Blood collection containers included Microtainer tubes (Becton-Dickinson and Company, Franklin Lakes, NJ), Sarstedt CB 300 Microvette tubes (Aktiengesellschaft and Company, Numbrecht, Germany), 1-mL blood gas syringes (Radiometer America Inc, Westlake, OH), and Microhematocrit Capillary Tubes (Fisher Scientific, Chicago, IL). The coefficients of variation for the weights of individual empty collection containers ranged between 1% and 2%. Blood collection containers were classified as tubes with marked fill-lines imprinted on the outside wall, tubes without fill-lines, and syringes. Only the Microcontainer tubes and syringes had fill-lines or volume markings on the outside. For some data items, not all of the information requested was recorded or legible. Most commonly, this was the name of the phlebotomist, the experience of the hospital phlebotomist, and the time of sampling.

The volume of blood withdrawn for individual laboratory tests was expressed as a percentage of the volume requested by the hospital laboratory. For example, a value of 120% indicates that 20% more blood was drawn than was requested by the laboratory (ie, 20% overdraw). For simplicity, blood lost on gauze pads, syringes, bedding, and intravenous tubing was not included. A previous study has estimated this to be 10% of the total volume withdrawn.[11]

Data Analysis

Univariate analysis was used to test for significant associations of the study variables with the relative volume drawn, ie, the percentage of blood overdraw. This involved the comparison of means by *t* test or analysis of variance as appropriate based on the number of groups being compared. To identify study variables and interactions among study variables that were significantly associated with blood overdraw, multiple linear regression—using backward and forward stepwise selection procedures—was also performed. The latter procedures were performed using the SAS/STAT REG procedure (SAS, Cary, NC).[12] The effect of work shift and phlebotomist experience on blood overdraw was evaluated using the mixed-effects model, which included the effect of the individual phlebotomist on the amount of blood overdraw as a factor in the model. This analysis was performed using the SAS/STAT MIXED procedure. Differences for the univariate comparisons were considered significant if the P value was <.05. For the multiple regression analysis, a P value <.10 was used for entry and a P value >.10 for removal from the model. Except where otherwise specified, data are presented as the mean ± standard error of the mean.

RESULTS

The total number of samples was 578, with 309 obtained from infants located in the NICU and 269 samples from IICU infants. The mean volume of blood overdraw for all blood tests was 19.0% ± 1.8% ($P < .0001$). The relative volume of blood drawn differed markedly based on individual laboratory tests (Fig 1). For 4 of the 11 blood tests, significantly ($P < .05$) more blood was drawn than was requested by the hospital laboratory. The mean volume of blood drawn for 2 of the 11 tests (electrolytes with bilirubin and drug levels) was significantly less ($P < .05$) than the volume of blood requested by the lab-

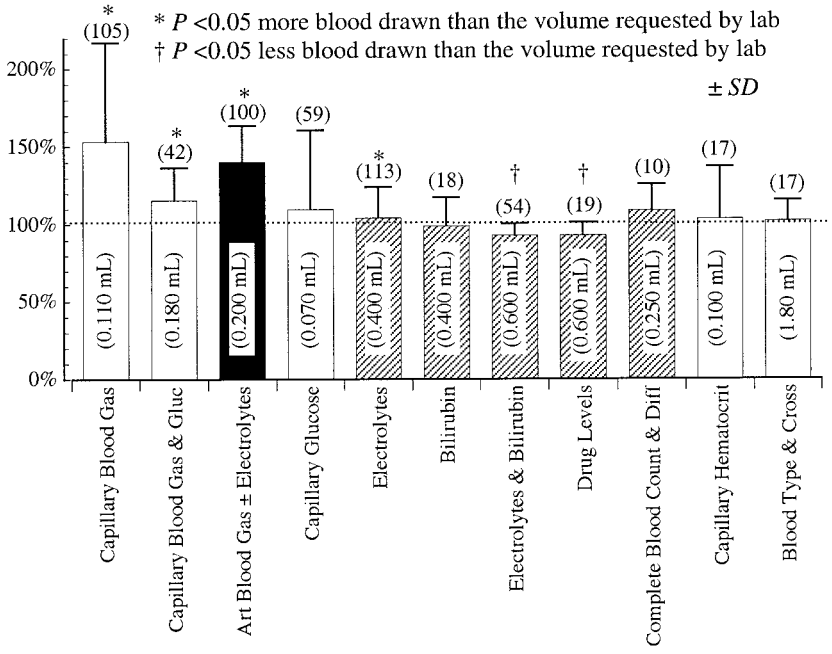

Fig 1. Phlebotomy loss by test requested. Open bars indicate samples drawn in containers without fill-lines; diagonally cross-hatched bars, samples drawn into containers with fill-lines; filled bar, samples drawn into syringes. Numbers above the bars within parentheses indicate the number of samples; numbers within the bars indicate the milliliters of blood requested by the laboratory. The dotted line at 100% indicates that the volume of blood drawn and the volume requested were identical.

oratory. For the remaining 5 tests, there was no statistical difference in the volume drawn and volume requested.

As suggested by the data shown in Fig 1, when analyzed by univariate analysis (Table 1), the greatest blood overdraws were observed among tests drawn into collection containers not having fill-lines. Additional univariate comparisons were conducted to identify factors other than tube fill-lines that were associated with phlebotomy overdraw. The clinical factors identified included infants of lowest weight at the time of phlebotomy, infants receiving care in the NICU versus IICU, drawing single tests compared with drawing multiple tests at one time, phlebotomist's designation, and the August to October study period versus the December to February study period (Table 1).

TABLE 1. Univariate Comparison of Clinical Study Parameters for Entire Database

	Number of Blood Samples	Phlebotomy Overdraw*	*P* Value†
Type of collection tube			
Marked tube	238	99 ± 1.2	<.0001
Unmarked tube	226	125 ± 2.4	
Syringe	114	148 ± 6.0	
Weight at sampling			
<1 kg	103	129 ± 4.3	.0205
1–2 kg	244	117 ± 3.0	
>2 kg	231	116 ± 2.4	
Infant's location			
NICU	309	130 ± 2.8	<.0001
IICU	269	106 ± 1.8	
Number of tubes sampled			
Single tube drawn at a time	503	121 ± 2.0	<.0001‡
Multiple tubes drawn at a time	74	104 ± 3.2	
Phlebotomist's designation			
Hospital phlebotomist	346	110 ± 1.7	<.0001‡
Nurse	114	148 ± 6.0	
NICU laboratory technician	98	122 ± 3.1	
Study period			
August to October	259	126 ± 3.3	.0013‡
December to February	319	114 ± 1.7	

* Percentage of volume drawn relative to that requested by the hospital laboratory.
† *P* values indicate comparison of clinical study parameters for the conditions shown.
‡ Study variables not found to be significant when analyzed using the forward and backward multiple regression methods.

To more definitively identify individual factors affecting phlebotomy overdraw from those that were significant in the univariate analyses, 2 separate multiple regression selection methods were applied. These were the forward stepwise and backward elimination selection methods. Both selection methods identified the same 3 factors as being statistically associated with the greatest percent overdraw. Factors identified as being significantly associated with greater phlebotomy overdraw with the multiple regression methods included: 1) the patient's location in the NICU ($P = .012$; Fig 2); 2) blood collection with a container without fill-lines ($P < .0001$); and 3) lighter weight infants ($P = .008$).

When these 3 dominant variables were tested for interaction with one another using all possible combinations of variables, the only significant interaction identified was between collection container type and the infant's weight at sampling (Fig 3). The interaction observed indicated that collecting blood samples in unmarked tubes was associated with significantly higher phlebotomy overdraw in the 2 groups of infants weighing <2 kg, compared with those weighing >2 kg. Furthermore, across all weight classes, blood samples drawn in syringes had greater phlebotomy overdraw relative to those drawn in either marked or unmarked tubes.

The R^2 for the regression model with the 3 factors

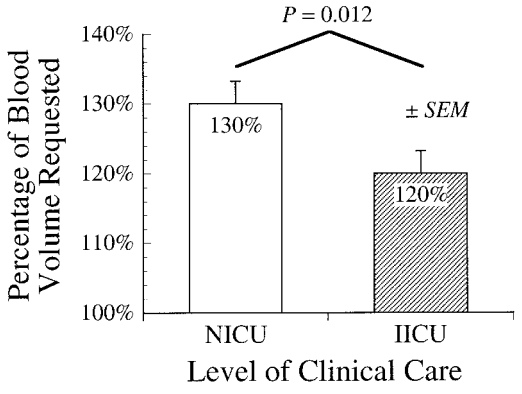

Fig 2. Comparison of least square means from the multiple regression model of phlebotomy overdraw between patients being cared for in the NICU versus those cared for in the IICU. (The overall of least square means overdraw using the multiple regression model was 125% ± 2.0%, compared with 119% ± 1.8% as determined without using the model.)

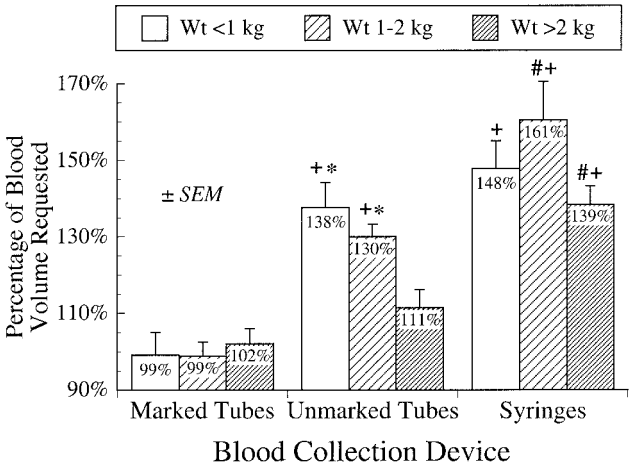

Fig 3. Comparison of least square means from the multiple regression model of phlebotomy overdraw using marked collection tubes, unmarked collection tubes, and syringes in infants grouped by body weight. Bars with the following symbols indicate a significantly greater excess phlebotomy: (*) compared with infants >2 kg with the same tube type; (+) compared with marked collection tubes with same weight group; and (#) compared with unmarked collection tubes with same weight group.

and 1 interaction effect was .24. It was then considered whether more of the variation in blood overdraw might be explained by including the random factor of individual phlebotomists in the model. Using only the blood sample data for which the name of the phlebotomist was known (n = 509), a mixed model was fitted to include the effect of the individual phlebotomist. This model had an R^2 of .52 and showed a significant variation in blood overdraw among individual phlebotomists ($P < .0001$).

To determine whether either the phlebotomist's experience or the work shift during which the blood was sampled was associated with phlebotomy overdraw, each phlebotomist group was examined separately in a subgroup analysis. Phlebotomist experience in both groups showed no significant effect on blood overdrawn. There were too few inexperienced nurses (n = 3) to determine whether nursing experience was a significant factor in phlebotomy overdraw. Among hospital phlebotomists not permanently stationed in the NICU, phlebotomies performed in the evening hours had significantly greater excess volumes of blood when analyzed by the mixed model analyses (34.1% ± 6.9% vs 9.8% ± 4.8%; $P < .004$). This was particularly true for the group of evening phlebotomists when unmarked collection tubes were used or when blood was drawn from the groups of the heavier or the more severely ill infants receiving treatment in the NICU. The blood overdraw when using unmarked collection tubes for severely ill infants was 65.7% ± 10.0% for the evening shift, compared with 22.7% ± 7.1% for the day shift (P = .01). When using unmarked collection tubes for infants >2 kg, the blood overdraw was 46.2% ± 13.7% for the evening shift, compared with −8.7% ± 7.9% for the day shift (P = .01). For the NICU laboratory technicians, there was no significant effect of work shifts on blood overdraw (P = .17).

DISCUSSION

With increasing survival of critically ill VLBW infants has come greater awareness that laboratory phlebotomy loss in the weeks immediately after birth is the primary contributor to the early anemia—and the RBC transfusion needs—that these infants universally experienced. Our finding that phlebotomy overdraw averaged 19% ± 1.8% per test among infants cared for in an intensive care nursery provides evidence that laboratory phlebotomy overdraw can be substantial. Clinical factors associated with increased phlebotomy overdraw in this setting included collection containers without a fill-line (including syringes), low infant body weight, and the severity of illness (as suggested by the subject's nursery location). Because the overall R^2 in the multiple regression model was only .24 for these 3 factors, the effect of individual phlebotomists on the variation of blood overdraw was examined and found to be highly significant, with the R^2 value increasing to .52. The R^2 value indicates that the results of our regression model account for only 52% of the total variation in blood overdraw. This implies that the other 48% of the total variation not accounted for is attributable to factors not included in our analysis. Although it is uncertain whether phlebotomy overdraw experienced by our neonates can be extrapolated to NICUs elsewhere, we speculate that our experience is not unique.

Previous evidence supporting laboratory phlebotomy loss as the primary contributor to anemia in the weeks immediately after birth is the close relationship reported between the volume of blood removed for laboratory testing and the volume of blood transfused[2,6–9] (Table 2). These previous studies have reported phlebotomy loss among predominantly preterm infants during the first 6 weeks of life of 11.0 mL/kg to 21.7 mL/kg—with the volume removed approximating that transfused. Thus, the mean phlebotomy overdraw of 19% identified in the present study is equivalent to 2.1 to 4.1 mL/kg/week of blood. In none of the studies listed in Table 2 were phlebotomy attributable to blood drawn for research

TABLE 2. Studies in Neonates Reporting Both Phlebotomy and RBC Transfusion Data

Reference	Group Studied	Postnatal Age Included (Weeks)	*n*	Mean Birth Weight (Grams)	Weekly Phlebotomy (mL/kg*)	Weekly Transfusion (mL/kg*)	Phlebotomy Versus RBC Transfusions Volumes	
							r	R^2
9	NICU infants	Birth to 1 wk	18	1822	21.7	12.3	Not reported	Not reported
2	<1500 g	Birth to 6 wk	57	Not reported	11.1	6.7	.82	.67
6	<1500 g	Birth to 4 wk	60	1161	12.7	10.6	.91	.83
7	Hospital A <1500 g	Birth to 2 wk	270	1073	8.2	15.7	Not reported	Not reported
7	Hospital B <1500 g	Birth to 2 wk	978	978	21.4	16.8	Not reported	Not reported
8	<1250 g	Birth to 2 wk	80	948	20.7	21.7	Not reported	Not reported
			Mean	1196	15.9	14.0	.87	.75
			Standard deviation	360	6.0	5.2	.06	.11
			n	5	6	6	2	2

* Based on birth weight.

purposes; and with the exception of birth weight, all were derived from unselected patient groups cared for in NICU settings. Except for the study by Obladen et al,[6] who visually estimated laboratory phlebotomy loss in blood collection tubes with graduated fill-lines on the outside, these previous studies are all silent on how phlebotomy loss was determined. Based on the finding of the present study of significant phlebotomy overdraw—even without including hidden blood loss (ie, that on cotton swabs and bedding, in the dead space of syringes, and intravenous tubing)—the data in Table 2 likely represent an underestimate of phlebotomy loss among infants cared for in NICU settings.

Common sense suggests that blood samples drawn in tubes with fill-lines marked on the outside would more closely approximate the volumes requested than those without. Conversely, the use of unmarked tubes could lead to phlebotomy overdraw as the phlebotomist overcompensates to avoid having to redraw the sample because of an insufficient volume for analysis. Although the frequency of blood tests having to be redrawn as a result of an insufficient test volume was not specifically recorded, the experience of our NICU is that at most this occurs only 5 to 10 times a week.

Several factors were unexpectedly identified as being associated with laboratory overdraw. We were surprised that the lightest and the most critically ill infants experienced the greatest blood overdraw. We had anticipated that the lighter weight, sicker infants being cared for in the NICU would have had less blood overdraw as a result of recognition by the medical care team that this patient group—with their high frequency of RBC transfusions—is the one that can least afford to have phlebotomy overdraws.

Because the volume indicators on the outside of syringe barrels are seemingly analogous to the blood collection tubes with fill-lines, it was also unexpected to observe that blood overdraw was greater with syringes than with either marked or unmarked tubes. It is likely that that is attributable in part to the unavoidable presence of the air bubble inevitably originating in the syringe tip.

Interpretation of data in the present study has several limitations based on the study design. First, for practical reasons, only data recorded by NICU phlebotomists volunteering to do so were included. Thus, although sample selection was arbitrary, it was not random. Second, we made no attempt to quantitate hidden blood loss.[11] Moreover, hidden blood loss itself might differ depending on the type of tube used in the collection or the site of blood sampling, ie, capillary or from an indwelling catheter. Although the rationale for the study was not publicized, participating phlebotomists may have been more careful than normal in drawing only the volume requested since they observed blood samples being weighed and clinical data being recorded. Voluntary participation might also have resulted in a greater proportion of blood samples being drawn when the workload of the phlebotomists was less intense. We speculate that these latter 2 considerations could have resulted in an underestimate of the true magnitude of phlebotomy overdraw.

Our results strongly indicate that phlebotomy overdraw is a significant contributor to the development of anemia among critically ill infants. The R^2 value of .52 in the 2 multiple regression methods indicates that other important factors not included in the present analysis were also operative. We are uncertain what these factors may have been.

Substantial data exist to support laboratory phlebotomy loss as an important contributor to neonatal anemia and the need for RBC transfusion. Estimates of laboratory phlebotomy loss among VLBW infants in the early weeks of life, when cardiorespiratory illness is typically most severe, have been shown to approximate the volume of packed RBCs transfused.[6–8,13] This, along with highly significant direct correlation reported between the volume of blood removed and that transfused during this period (ie, correlation coefficients of .8 to .9),[2,13] strongly suggest that the nearly 20% phlebotomy overdraw is a significant predisposing factor to the RBC transfusions that these infants commonly receive. Based on these data, we estimate that phlebotomy overdraw is responsible for 5% to 15% of the RBC transfusions received by VLBW infants.

The present data suggest practical ways that laboratory phlebotomy loss might be further reduced. Use of blood collection tubes with fill-lines is a sim-

ple and obvious means for reducing blood loss. To realize this in practice, manufacturers will need to provide blood-sampling containers with calibrated fill-lines. Educating individual phlebotomists, nurses, and other members of the care team on reducing unnecessary blood loss, eg, ordering only essential blood tests, exercising the greatest care in the smallest infants, practice in drawing blood samples into syringes, etc, may also help.[14] Although not investigated in the present study, other promising means for reducing laboratory blood loss include technologic improvements to further reduce laboratory sample volume required, more reproducible and better capillary blood-sampling containers, and use of in-line point-of-care monitors in which little to no blood is required.[15,16]

Continued systematic studies of phlebotomy loss will be necessary to establish the efficacy of these and other modalities. Likewise, future studies should assess the application of one—or more likely several—of these modalities to document whether reduction of phlebotomy loss diminishes clinically significant anemia, thereby reducing the need of these infants for RBC transfusions.

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