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Primary prevention of pediatric lead exposure requires new approaches to transfusion screening

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Abstract

Objective—Over the past several decades there have been numerous initiatives to limit environmental lead exposure, especially among children. Recently, the CDC accepted a series of recommendations to further expand prevention of childhood lead exposure, but these recommendations do not address lead exposure occurring via blood transfusion. We sought to facilitate further assessment of transfusion-associated lead exposure by designing a procedure to test packed red blood cells (pRBCs) prepared for transfusion.

Study Design—The relationship between pRBC and whole blood lead concentration was investigated in 27 samples using a modified clinical assay. Subsequently, lead concentrations of 100 pRBC units were determined.

Results—Our sample preparation method demonstrated correlation between whole blood lead and pRBC lead concentration ($R^2 = 0.82$). In addition, all 100 pRBC units tested had detectable lead. The median pRBC lead concentration was 0.8 $\mu\text{g/dL}$, with a standard deviation of 0.8 $\mu\text{g/dL}$ and a range of 0.2 $\mu\text{g/dL}$ to 4.1 $\mu\text{g/dL}$. In addition, after only a few days of storage, approximately 25% of whole blood lead was found in the supernatant plasma.

Conclusions—Transfusion of pRBCs is a source of lead exposure. Here we report the quantification of lead concentration in pRBCs. Among the samples that we tested, there was a greater than 20-fold range in lead concentration. Pre-transfusion testing of pRBC units according to the method we describe or donor screening of whole blood lead and selection of below-average units for transfusion to children would diminish an easily overlooked source of pediatric lead exposure.

Introduction

Lead is recognized as a health hazard to people of all ages. In developing children, lead is especially hazardous and is associated with poor academic performance, reduced verbal development, and long-term, irreversible neurologic and cognitive impairment.¹⁻⁴ Over the past decade, several studies have demonstrated impaired intellectual development among children with blood lead levels below 10 µg/dL, which had previously been below the United States Centers for Disease Control and Prevention (CDC) “level of concern” for children.⁵⁻⁶ In response to these studies, the CDC recently adopted a series of recommendations indicating that there is no safe level of blood lead in children.⁷ However, these recommendations do not address strategies to avoid lead exposures occurring via blood transfusions, even though transfused lead is substantially more bioavailable than oral lead⁸⁻⁹ and a dose-response relationship between the lead concentration of transfused pRBCs and post-transfusion blood lead concentration is known to exist among very premature infants.¹

Because blood lead is confined almost exclusively within erythrocytes, pRBCs are the primary blood product capable of transmitting lead via transfusion.¹⁰ Although there are several tests available to measure lead in whole blood, there is not a widely-available laboratory test to measure the lead content of pRBCs prepared for transfusion. The purpose of this paper is to investigate a methodology to measure lead in pRBC units which, if employed, could prevent children from receiving pRBC transfusions from donors with above-average lead content.

Materials and Methods

The study protocol was approved by the Institutional Review Board at Vanderbilt University Medical Center (VUMC).

Assay Modification for Measurement of pRBC Lead

Twenty whole blood specimens with known lead concentrations ranging from 1.0 to 22.7 µg/dL were randomly selected from clinical runs at ARUP laboratories in Salt Lake City, UT. All 20 specimens were between 1 and 5 days old at the time of analysis. These samples were collected in trace element free K₂EDTA or Na₂EDTA tubes, converted to pRBC specimens consistent with a clinically validated method for Mg-RBC measurements, and then analyzed using a clinically validated method for whole blood lead measurements. Specifically, approximately 1 mL of well-mixed blood was removed by pipetting and added to a 10 mL trace-element free polypropylene tube. The tube was spun in a Jouan model C4i centrifuge (Thermo Scientific, Asheville, NC) at 3,000 rpm for five minutes. The plasma was removed and 3 mL of wash solution was added (0.003 M EDTA, di-potassium salt; 0.010 M MOPS; 0.080 M Trizma base; 0.149 M choline chloride). The wash solution and pRBCs were mixed by gentle inversion. The resulting suspension was spun for five minutes at 3,000 rpm. The majority of the wash solution was removed, being careful not to disturb the RBCs. The cells were washed, mixed, centrifuged, and separated again. Then, 50 µL of packed RBCs were pipetted, lysed, diluted and aspirated into an inductively coupled plasma-mass spectrometer (ICP-MS; Perkin-Elmer SCIEX ELAN 9000 and DRCII) which had been

calibrated for whole blood lead testing. Counts per second of the natural isotopes of lead (^{204}Pb , ^{206}Pb , ^{207}Pb , ^{208}Pb) were obtained and summed. Controls carried through the standard preparation for whole blood lead were used to validate the calibration and the run consistent with routine clinical practice.

Determination of Lead Content in Supernatant Plasma

Seven whole blood samples that had been tested by the whole blood method at ARUP laboratories approximately 3 days prior on average were centrifuged and prepared by the pRBC method as described above. Prior to washing the cells, the plasma was collected and lead concentration was determined on a 50 μL aliquot by ICP-MS.

pRBC Unit Testing

Residual tubing segments from 100 units of pRBCs of blood group A (n=25), B (n=17), AB (n=7) and O (n=51) donors were randomly selected from the VUMC blood bank inventory. These sealed tubing segments were sent in a 4°C shipment to the Trace and Toxic Elements laboratory at ARUP Laboratories. The tubing segments were stored at 4°C until they were analyzed. At the time of analysis, the packed red blood cells were washed to remove extracellular contaminants and storage solution. Then, 50 μL of packed RBCs were pipetted, lysed, diluted and aspirated into an inductively coupled plasma-mass spectrometer as described above.

Results

Comparison of pRBC and Whole Blood Lead Measurements

Whole blood and pRBC data were compared based on two criteria: percent recovery and relative difference Bland-Altman comparison. The average percent recovery of lead from pRBCs was 77% of the whole blood lead content. Linear regression analysis between methods indicated a proportional bias of 68%, constant bias of 0.86 and a correlation coefficient of 0.825 (Figure 1). The relative difference Bland-Altman plot shows almost an equal number of points above and below the line of identity (Figure 2) and a statistically insignificant slope (F -statistic = 0.36, p = 0.56).

Lead concentration of supernatant plasma

The whole blood lead concentration in 100 μL of whole blood was comparable to the sum of the lead concentration in 50 μL of pRBCs plus the lead concentration in 50 μL of supernatant plasma (see Figure 3). The removed plasma contained, on average, 25% of the original whole blood concentration (range: 12% – 43%) while the pRBCs contained, on average, 90% of the original whole blood lead concentration (range: 55% to 119%). The summed total of pRBCs plus supernatant plasma averaged 115% of the whole blood lead concentration, with a range from 83% to 143%.

Lead concentrations of pRBC specimens

Units of pRBCs obtained from the VUMC Blood Bank had a mean lead concentration of 1.1 $\mu\text{g/dL}$ and a median concentration of 0.8 $\mu\text{g/dL}$, with a standard deviation of 0.80 $\mu\text{g/dL}$.

The minimum lead concentration was 0.2 µg/dL, while the maximum was 4.1 µg/dL (Figure 4). We did not identify any statistically significant associations between donor blood group and blood lead concentration.

Discussion

The positions of the World Health Organization (WHO) and the CDC on the topic of pediatric lead exposure have evolved over time. Until recently, the CDC described pediatric blood lead levels (BLLs) below 10 µg/dL as below the “level of concern”.⁷ Similarly, the WHO had previously set a tolerable weekly oral intake of 25 µg/kg of lead.¹¹ However, more recent studies have shown that there is no level of lead exposure that is harmless to children.^{5–6} Accordingly, the WHO no longer publishes a tolerable weekly intake level for lead and the CDC no longer considers BLL<10 µg/dL below the “level of concern” in children.^{7,12–13} These recent changes in position by the CDC and the WHO reflect a consensus that even low-level lead exposure must be avoided in children, and that mechanisms of exposure that previously were discounted¹⁴ – such as blood transfusion – deserve renewed scrutiny.

It has been more than 10 years since a dose-response relationship between the lead concentration of transfused RBCs and the post-transfusion blood lead concentration was established in very premature infants.¹ Our results show a wide range in pRBC lead concentration among donors, with the lowest-testing unit in our cohort containing less than one twentieth of the lead concentration of the highest-testing unit (Figure 4). We feel that it would be feasible to meet most if not all of the need of pediatric patients at our hospital with pRBCs containing <1 µg/dL of lead. However, such an initiative would require institutional support and/or regulatory requirements mandating such testing.

Because blood lead is almost exclusively stored in erythrocytes¹⁰, we initially expected that our pRBC lead concentration measurements would be approximately equal to our whole blood lead concentration measurements. We suspected that our 77% recovery rate may be explained by differences in specimen processing between whole blood and pRBC samples. To determine whether our underestimation of lead concentration in pRBC specimens was due to the presence of a substantial concentration of lead in the supernatant plasma, we measured the lead concentration of pRBCs and separated plasma in samples over a range of whole blood lead concentrations. These experiments showed that even after only a few days of storage, lead was released from erythrocytes into the supernatant plasma. Interestingly, the oldest sample of the group (tested 6 days post-collection) had a nearly equal distribution of lead between the plasma and pRBC compartments (43% and 55%, respectively), indicating that lead equilibrates between cells and supernatant plasma during storage, and is no longer contained primarily in the RBCs after a few days.

Currently, there are several practices in place that are intended to protect neonates and small children from the risks of transfusion related disease transmission and antibody formation.^{15–18} In addition, neonates typically receive aliquots from the same donor unit until it expires or is depleted, thereby reducing potential infectious and non-infectious disease risk by reducing donor exposures. Given that the effects of lead are greatest in the

developing brain^{2,19}, that the symptoms of low-level lead exposure are difficult to detect¹⁹, and that lead exposure has repeatedly been linked to decreased IQ^{5–6,19}, it seems rational to limit lead exposure to premature infants and children with chronic, transfusion-dependent anemia.

We further note that selecting an appropriate testing method to be used in lead determination is extremely important. Previous studies that measured lead in blood donors have not clearly stated whether their methods were standardized for whole blood specimens, pRBC specimens, or both.^{1, 14, 20} The assay that we describe in this study allows for a simple, predictable estimation from donor whole blood lead concentration to expected pRBC lead concentration, and vice versa. However, our pRBC assay is not intended to supplant whole blood lead testing as a method of donor blood screening. We imagine that donor whole blood could be assayed for lead at the same facility where infectious disease testing occurs and lead concentrations could be communicated to the hospital blood bank prior to transfusion.

We are aware that many hospital laboratories do not have ICP-MS equipment, on-site clean room facilities or comparable assays that would allow for the direct implementation of the procedure we have described here for measuring lead in pRBCs. However, the implementation of screening for whole blood lead at the time of collection could be conducted at any facility offering whole blood lead testing using a more widely available technique. Both graphite furnace atomic absorption spectrometry (GF-AAS) and ICP-MS demonstrate adequate detection limits for whole blood lead testing (< 1 to $2 \mu\text{g/dL}$ and $< 0.1 \mu\text{g/dL}$, respectively). Nevertheless, as we demonstrate here, ICP-MS is a very sensitive assay that is capable of detecting low levels of lead that, according to the new position outlined by the CDC, may pose a health risk. Because ICP-MS is available at reference laboratories, implementation of our procedure would not require a large capital outlay by hospitals to purchase new instrumentation, but rather the addition of lead screening to the list of tests performed by reference laboratories prior to release of a blood product.

Infectious disease screening for blood donations, which includes assaying for Hepatitis B, Hepatitis C, HIV 1 & 2, Human T-Lymphotropic Virus Types I & II, *Trypanosoma cruzi*, syphilis, and West Nile Virus costs approximately \$50 total.²¹ This is a substantial discount compared to individual patient testing for infectious diseases, due to the high volume of blood donors. Therefore, even though blood lead testing currently costs approximately \$50–\$80 per patient tested, we believe that performing lead testing at a high volume as a part of the donor screening process would substantially reduce the cost per test, ultimately raising the cost of screening only by a nominal amount.

Our study is limited by several factors. First, the lead concentrations that we report in pRBC units at our institution may not be generalizable to other regions. Urban areas with extensive older housing stock and/or numerous immigrants from areas with high levels of environmental lead may result in a blood donor pool with higher average blood lead levels than we report here. In addition, blood lead concentrations are known to be seasonal, so the mean pRBC lead concentration may vary based on the time of year.²² Furthermore, due to our relatively small sample size, there may be outliers at the high end of the lead

concentration spectrum that were not included in our cohort. Finally, when we converted our whole blood specimens to pRBC specimens, we assumed a hematocrit of 50% for each sample. We chose this approach because, unfortunately, it was not possible for us to know the hematocrit of each of our donors. While this approach did allow us to ensure that we would be able to accurately measure lead and to standardize each analysis for a defined volume of pRBCs, it would be necessary to know the precise hematocrit of a donor in order to more accurately correlate pRBC lead concentration to whole blood concentration. Finally, there was one sample with particularly poor agreement between whole blood and pRBC method (see Figure 1 and Figure 2). We speculate that these erythrocytes may have been particularly fragile, or could have been substantially more hemolyzed than other samples in the cohort. This contention is supported by our experiments demonstrating that a substantial portion of blood lead can be found in the supernatant plasma after several days of storage (Figure 3).

Conclusion

Due to recent changes in policy by the CDC and WHO, minimization of lead exposure in children is a subject that is now being addressed with renewed urgency. Atypical sources of lead exposure, such as blood transfusion, deserve new attention. Results from the assay used to test pRBC units for lead in this study correlate with whole blood lead levels across the measured range. In addition, all tested pRBC specimens contained detectable concentrations of lead, indicating that all pRBC transfusion recipients would theoretically be exposed to at least some lead. Ultimately, a prospective clinical trial comparing the incidence of intellectual disability to degree of lead exposure among a cohort of infants receiving blood transfusions is needed to establish a guideline for acceptable levels of lead in pRBC units. At present, we believe that it would be prudent to minimize transfusion-associated lead exposure by reserving units of pRBCs with the lowest lead concentrations for pediatric patients, especially premature infants and children with chronic, transfusion-dependent anemias.

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Abbreviations

ARUP	ARUP Laboratories
CDC	The United States Centers for Disease Control and Prevention
ICP-MS	Inductively coupled plasma-mass spectrometer
RBC	Red Blood Cells
pRBCs	Packed Red Blood Cells
VUMC	Vanderbilt University Medical Center

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Figure 1.

Comparison of whole blood lead to pRBC lead as determined in 20 samples. Dashed line is the line of identity.

Figure 2.

Relative difference Bland-Altman plot comparing whole blood and pRBC lead concentrations. Bold dashed line is the mean relative difference with upper and lower dashed lines + and - 2 standard deviations, respectively. Solid black line is the linear regression line. Units of lead concentration: $\mu\text{g/dL}$.

Figure 3.
Supernatant plasma contains measurable lead after a few days of storage.

Figure 4. Histogram of pRBC lead concentration in 100 specimens obtained from the VUMC blood bank.