Primary Prevention of Pediatric Lead Exposure Requires New Approaches to Transfusion Screening

Eric Gehrie, MD[1], Amaris Keiser, MD[2], Sheila Dawling, PhD[1], James Travis, BS[3], Frederick G. Strathmann, PhD[3,4], and Garrett S. Booth, MD[1]

Objective 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 pRBCs and whole blood lead concentration was investigated in 27 samples using a modified clinical assay. Lead concentrations were measured in 100 pRBC units.

Results Our sample preparation method demonstrated a correlation between whole blood lead and pRBC lead concentrations (R^2 = 0.82). In addition, all 100 pRBC units tested had detectable lead levels. The median pRBC lead concentration was 0.8 μg/dL, with an SD of 0.8 μg/dL and a range of 0.2-4.1 μg/dL. In addition, after only a few days of storage, approximately 25% of whole blood lead was found in the supernatant plasma.

Conclusion Transfusion of pRBCs is a source of lead exposure. Here we report the quantification of lead concentration in pRBCs. We found a >20-fold range of lead concentrations in the samples tested. Pretransfusion testing of pRBC units according to our proposed approach 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. *(J Pediatr 2013;163:855-9)*.

Lead is a recognized health hazard to people of all ages. Lead exposure is especially hazardous to developing children, 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 in children with blood lead levels <10 μg/dL, which had previously been below the US Centers for Disease Control and Prevention (CDC)-specified "level of concern" for children.[5,6] In response to these studies, the CDC recently adopted a series of recommendations that state that there is no safe blood lead level in children.[7] However, these recommendations do not address strategies to avoid lead exposure occurring via blood transfusions, even though transfused lead is substantially more bioavailable than oral lead[8,9] and a dose–response relationship between the lead concentration of transfused packed red blood cells (pRBCs) and posttransfusion blood lead concentration has been demonstrated in very preterm 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 several tests are available to measure lead in whole blood, there is no widely available laboratory test for measuring the lead content of pRBCs prepared for transfusion. The purpose of the present study was to investigate a methodology to measure lead in pRBC units to prevent children from receiving pRBC transfusions from donors with above-average blood lead content.

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 selected at random from clinical runs at ARUP Laboratories in Salt Lake City, Utah. All 20 specimens were 1-5 days old at the time of analysis. The samples were collected in trace element-free K_2EDTA or Na_2EDTA tubes, converted to pRBC specimens consistent with a clinically validated method for red blood cell magnesium 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

CDC	Centers for Disease Control and Prevention
ICP-MS	Inductively coupled plasma mass spectrometry
pRBC	Packed red blood cell
VUMC	Vanderbilt University Medical Center
WHO	World Health Organization

From the [1]Departments of Pathology, Microbiology, and Immunology, [2]Pediatrics, Vanderbilt University Medical Center, Nashville, TN; [3]ARUP Institute for Clinical and Experimental Pathology, and [4]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT

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a 10-mL trace element–free polypropylene tube. The tube was spun in a centrifuge (Jouan model C4i; Thermo Scientific, Asheville, North Carolina) at 3000 rpm for 5 minutes. Then the plasma was removed, and 3 mL of wash solution (0.003 M EDTA, dipotassium salt, 0.010 M 3-(N-Morpholino)propanesulfonic acid, 0.080 M Trizma base, and 0.149 M choline chloride) was added. The wash solution and pRBCs were mixed by gentle inversion, and the resulting suspension was spun at 3000 rpm for 5 minutes. The majority of the wash solution was removed, with care taken to not disturb the RBCs. The cells were washed, mixed, centrifuged, and then separated again. Then 50 μL of pRBCs were pipetted, lysed, diluted, and aspirated into an inductively coupled plasma mass spectrometer (SCIEX ELAN 9000 and DRCII; PerkinElmer, Waltham, Massachusetts) that 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 earlier on average were centrifuged and prepared by the pRBC method as described above. Before cell washing, plasma was collected and lead concentration determined in a 50-μL aliquot by inductively coupled plasma mass spectrometry (ICP-MS).

pRBC Unit Testing

Residual tubing segments for 100 pRBC units from blood group A (n = 25), B (n = 17), AB (n = 7), and O (n = 51) donors were selected at random from the VUMC blood bank inventory. The sealed tubing segments were maintained at 4°C and shipped to the Trace and Toxic Elements Laboratory at ARUP Laboratories, where they were stored at 4°C until analysis. At the time of analysis, the pRBCs were washed to remove extracellular contaminants and storage solution. Then 50 μL of pRBCs 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 2 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 correlation coefficient of 0.825 (Figure 1). The relative difference Bland-Altman plot showed an almost equal number of points above and below the line of identity (Figure 2) and a statistically insignificant slope ($F = 0.36$; $P = .56$).

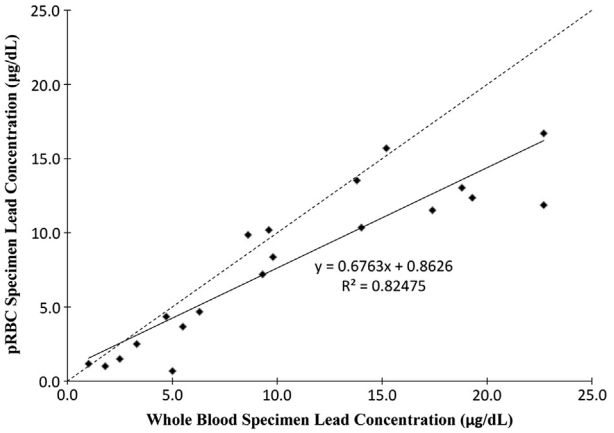

Figure 1. Comparison of whole blood lead and pRBC lead as measured in 20 samples. The *dashed line* is the line of identity.

Lead Concentration of Supernatant Plasma

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

Lead Concentrations of pRBC Specimens

Units of pRBCs obtained from the VUMC Blood Bank had a mean lead concentration of 1.1 μg/dL and a median concentration of 0.8 μg/dL, with an SD of 0.80 μg/dL. The minimum lead concentration was 0.2 μg/dL, and the maximum was 4.1 μg/dL (Figure 4). There were no 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 considered pediatric blood lead levels <10 μg/dL as below the "level of concern."[7] Similarly, the WHO had previously set a tolerable weekly oral lead intake of 25 μg/kg.[11] However, more recent studies have demonstrated 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 a blood lead level <10 μg/dL below the "level of concern" in