CME Article #3

A Prospective Study of Prenatal and Childhood Lead Exposure and Erythropoietin Production

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Learning Objectives

- Summarize what previous studies have shown about the association of blood lead (BPb) concentration, hemoglobin (Hgb), and erythropoietin (EPO) production.
- Compare levels of Pb in blood and tibial bone, hemoglobin levels, and EPO production in lead-exposed and unexposed children 4.5 to 12 years of age.
- Identify the likely underlying cause of these findings and their implications for lead-exposed children.

Abstract

We test the hypothesis that chronic lead (Pb) exposure may be associated with an inability to maintain an adequate serum erythropoietin (EPO) concentration. From a longitudinal study of Pb exposure and infant and childhood development, we measured blood Pb (BPb) and serum EPO concentrations serially at ages 4.5, 6.5, 9.5, and 12 and tibia (cortical) Pb concentration at age 12. Pb-exposed children aged 4.5 and 6.5 produced increased concentrations of EPO to maintain normal Hgb concentrations. EPO production declined between ages 4.5 and 6.5. At ages 9.5 and 12, further diminution of the association was found. No association was found between tibia Pb and EPO. The continued decline in the slope of the relationship between EPO and BPb with age, after adjustment for hemoglobin, implies a gradually decreasing capacity to produce EPO (J Occup Environ Med. *2004;46:924–929*)

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High dose-environmental lead (Pb) exposure is associated with anemia,[1–3] the mechanisms of which are not totally clear. It is known that Pb exposure is associated with impaired heme synthesis,[4–6] but even very high blood lead concentrations (BPb) cannot account for the full decrement in hemoglobin (Hgb) synthesis.[5] Pb has other effects on erythrocytes, including inhibition of pyrimidine-5′-nucleotidase activity,[7] ineffective erythropoiesis,[8] and shortened red cell survival,[9–10] but taken together, these still do not account for the effects on Hgb concentration.

Our interest in the mechanism of Pb-induced anemia was sparked by a report by Grandjean et al,[11] who described delayed blood regeneration capacity in Pb-exposed workers who had normal Hgb concentrations prior to donation of a unit of blood. We hypothesized that Pb may inhibit the production of erythropoietin (EPO), a glycoprotein hormone that regulates both steady-state and accelerated erythrocyte production. More than 90% of EPO is produced in the proximal tubule of the kidney, the site of substantial Pb accumulation.

Using data from a long-term prospective study of environmental Pb exposure in pregnant women and their offspring,[12–14] we previously examined the relationships between BPb and EPO during pregnancy and in children at ages 4.5, 6.5, and 9.5 years. In pregnant women, EPO was significantly depressed among those with moderately elevated BPbs.[15] Further, we found that children with

elevated BPb maintained normal Hgb values, but required hyperproduction of EPO to do so.[16] We hypothesized that children with moderately elevated BPb have shortened red cell survival, a phenomenon previously described in children with Pb encephalopathy[9] and in Pb workers.[10] Here, we extend our observation of the same cohort of children and examine the associations between Pb exposure during childhood and EPO concentrations up to age 12. We previously reported relationships between BPb and EPO through age 9.5. The skeleton is the largest reservoir of Pb in the body, and the half-life of Pb in bone is of the order of years. Thus, bone Pb is believed to be the best surrogate of lifetime exposure.[17–19] The current analyses are unique in that cumulative Pb exposure is assessed using both average BPb from birth through age 12, and tibial bone lead concentrations measured at age 12.

Methods

This study was conducted in two towns in Kosovo, Yugoslavia: Kosovska Mitrovica (K. Mitrovica), the site of a Pb mine, smelter, refinery, and battery plant; and Pristina, a relatively unexposed town 25 miles to the south. Children were selected for follow-up from a previous prospective study of 1502 pregnant women residing in these towns. Pregnancy outcomes[20] and childhood developmental outcomes have been previously described.[13–15] Field work was completed in December 1998, months before the outbreak of war in Kosovo.

Subjects

In brief, 706 mother-infant pairs from the pregnancy study were invited to participate in a follow-up study involving repeated visits at 6-month intervals. Of these, the parents of 541 consented and brought their children to at least one visit. Of those who consented, 311 (53.5%), 267 (49.4%), 260 (48.1%), and 280 (51.8%) participated in the visits at ages 4.5, 6.5, 9.5, and 12 years, respectively. For the present study, whole blood for BPb and Hgb were available for 272, 201, 234, and 280 children at each of these ages, respectively; sera for EPO analyses were available for 211, 178, 234, and 280 children, respectively.

In December, 1998, we measured tibia lead (Tib-Pb) in cohort participants between the ages of 11 and 13. Logistical and cost constraints limited the number of children in whom we could measure bone Pb. We therefore selected a subsample of the cohort with a broad range of Pb exposures from among those with eight or more serial BPb measurements. This subsample included 209 children: 111 in K. Mitrovica and 98 in Pristina. Blood samples were obtained on 280 children: 49 children at age 11 (17.5%), 91 children at age 11.5 (32.5%), 107 children at age 12 (38.2%), 32 children at age 12.5 (11.4%), and 1 child at age 13.5 (0.3%). The mean (± standard deviation, SD) age of the children was 11.72 (± 0.47) years.

Laboratory Analyses

At mid-pregnancy, delivery and at each 6-month follow-up visit, venous blood samples were taken for the measurement of BPb,[21] erythrocyte protoporphyrin (EP),[22] and Hgb. At each visit, additional sera were obtained and frozen immediately to create a serum bank, from which the current study is derived. Whole blood and serum samples were stored at 4°C and −20°C, respectively, and appropriately transported to Columbia University, where all assays were performed. The laboratory participates in the BPb and EP quality control program of the Centers for Disease Control and Prevention. During the study period relevant to this analysis, agreements with the quality control values for BPb and EP, measured by intraclass correlation coefficients, were both 0.99.

All available sera from the visits at ages 4.5, 6.5, 9.5, and 12 years were analyzed in duplicate for EPO using a commercially available enzyme immunoassay.[23] In our laboratory, the limit of detection of the assay was 0.6 mIU/mL, and the coefficients of variation for duplicate measures was 9.8%, 8.3%, 7.9%, and 3.3%, respectively, at ages 4.5, 6.5, 9.5, and 12 years.

Bone Lead

Tibia bone lead (Tib-Pb) was measured using ^{109}Cd-based K-shell-x-ray fluorescence;[24] details of the technology used in the current study have been previously described.[25] Measurement uncertainty with this procedure, which uses previous-generation spectroscopy electronics, in children is similar to that in adults. With our instrumentation, the minimum-detectable Tib-Pb was 4 μg Pb/g bone mineral (ie, three times the standard deviation of repeated measures of a blank sample). For Tib-Pb that measured close to or less than this limit, the instrumentation occasionally recorded negative values. This results from production of an unbiased point estimate of Tib-Pb that oscillates around the true Tib-Pb value. In the present sample, Tib-Pb measurements ranged between −14.4 and +193.5 μg Pb/g bone mineral. Previous investigations of the use of negative values of Tib-Pb[26,27] suggest that their retention in analyses is useful because alternative procedures, such as recoding them to "0" or to a multiple of the standard deviation of the background noise, introduce bias. We performed our analysis twice, once retaining the negative values and once adding a constant of 15 to eliminate negative values when the logarithmic transformation was used; this transformation was undertaken to make the distributions approximately symmetrical. Results from both analyses were comparable, and we report analyses using the latter algorithm.

Statistical Analysis

The association between BPb and EPO was examined using regression methods. Because the distributions of EPO and BPb are skewed, loga-

rithmic transformations were used for these variables. All analyses were performed using SAS version 6.12 or 8.1 (SAS Institute, Cary, NC). First, we evaluated the association between BPb and EPO at each age using linear regression analysis, controlling for concurrent Hgb concentration. Concurrent Hgb was always controlled because it is the most important predictor of EPO.[15,16] Second, we combined data from all ages and used repeated measures analysis to determine whether the associations between BPb and EPO, controlling for Hgb, changed over time. Third, we estimated cumulative BPb using the algorithm described below. Finally, we repeated the analyses using Tib-Pb as the cumulative exposure variable.

The repeated measures models were fitted using the marginal generalized estimating equations approach,[28] which allows for independent effects of age and BPb at each age on EPO at each age while controlling for the within-subject correlations. In all, 875 observations from 373 subjects with EPO, BPb and Hgb measures were available for this analysis. The 875 observations included 101 (27.1%) subjects who had one measure, 111 (29.8%) who had two, 92 (24.7%) who had three, and 69 (18.5%) who had all four EPO measures.

Cumulative lifetime exposure to Pb was first estimated using the average of BPb measurements obtained at 6-month intervals from birth until age 12. The distribution of BPb is skewed; we therefore used a base 10 logarithmic transformation. Because BPbs measured serially in this population are highly correlated,[13] for children missing measurements we estimated the cumulative lifetime average BPb using all available data.

For subjects with missing values for BPb or Hgb at a specific time point, we used the following algorithm to substitute values. If BPb (or Hgb) measurements were available for both the 6 months prior to and the 6 months after the missing values, we substituted the mean of these measures. If BPb (or Hgb) was available for only one of these time points, that value was substituted.

Results

Mean BPb, Hgb, and EPO data are presented in Table 1 as functions of age and town of residence. As expected, BPbs were substantially higher in K. Mitrovica than Pristina, and BPbs declined somewhat with age. Mean Hgb concentrations remained constant and within the normal range. Mean EPOs were significantly different between towns at ages 4.5 and 6.5 but not at ages 9.5 or 12.

Hgb concentration is the strongest determinant of EPO. We therefore examined EPO concentrations within strata of Hgb (Fig. 1). As expected, children with higher Hgb had lower EPO concentrations. Relatively few children were anemic. For example, at age 4.5 years, 3.3% had Hgb $<$11.0 g/dL at ages; at ages 6.5, 9.5, and 12, less than 0.5% of the children had Hgb $<$11.0 g/dL. Within each Hgb stratum, EPO was significantly higher in K. Mitrovica than in Pristina at ages 4.5 and 6.5, and similar to that in Pristina for ages 9.5 and 12.

As expected, Hgb was always inversely and significantly associated with EPO (data not shown). Associations between BPb and EPO, adjusted for Hgb and specific to each age, are shown in Table 2. Statistically significant associations were found between BPb and EPO at ages 4.5 and 6.5, although the magnitude of the association at the latter age was greatly reduced with age. At ages 9.5 and 12, BPb was no longer significantly associated with EPO and the magnitudes of the associations continued to decline.

TABLE 1
Hematological Parameters for 280 Children in Kosovska Mitrovica and Pristina, Yugoslavia, at Ages 4.5, 6.5, 9.5, and 12 Years

		Town			
		K. Mitrovica		Pristina	
Parameter	Age	Mean	SE*	Mean	SE*
Blood lead concentration (μg/dL)	4.5	39.3	1.45	9.0	0.25
	6.5	36.2	1.15	8.1	0.35
	9.5	28.1	0.92	6.6	0.22
	12	30.6	0.84	6.1	0.17
Hemoglobin concentration (g/dL)	4.5	12.4	0.081	12.3	0.065
	6.5	13.0	0.074	12.7	0.086
	9.5	12.9	0.063	13.0	0.075
	12	12.9	0.071	12.9	0.072
Erythropoietin concentration (mIU/mL)	4.5	7.8	0.73	5.6	0.32
	6.5	9.7	0.43	8.2	0.46
	9.5	8.8	0.35	8.8	0.59
	12	9.6	0.40	9.2	0.35

* Standard error of the mean.

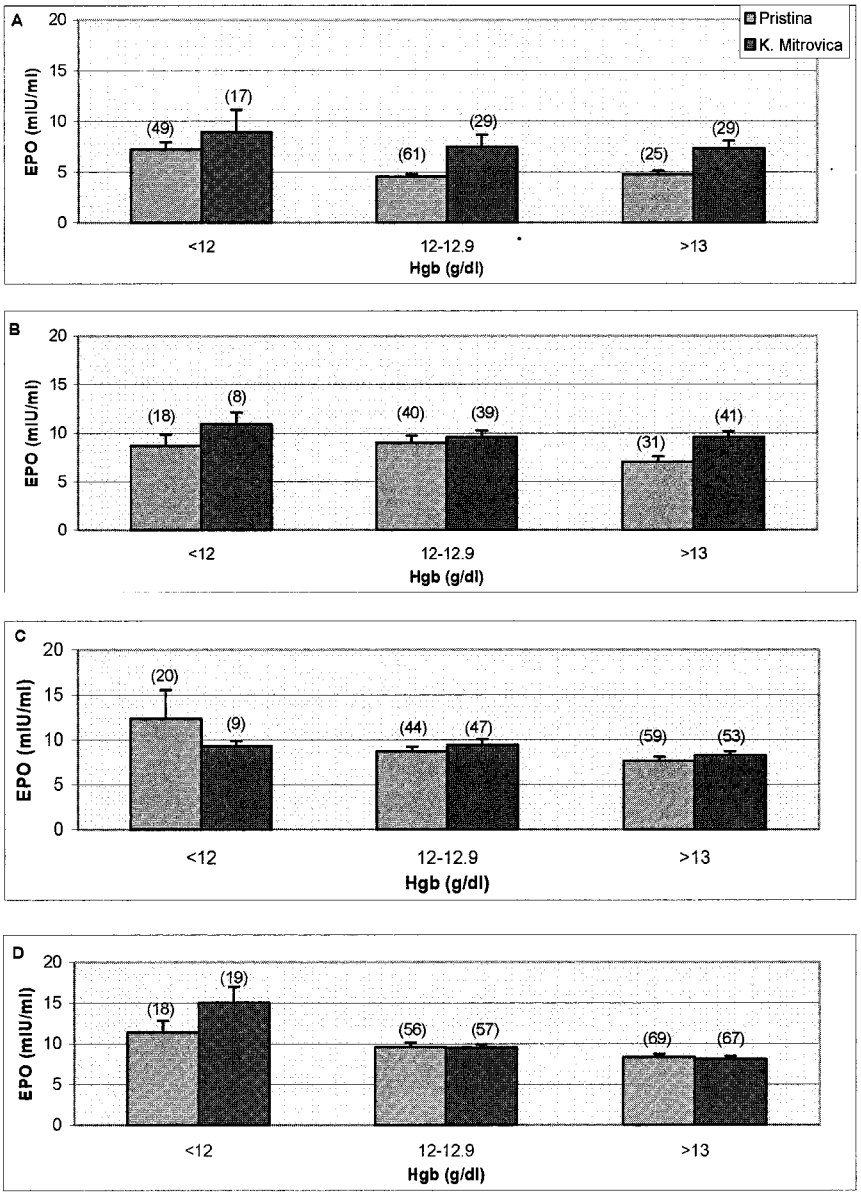

Fig. 1. Mean serum EPO concentrations in children in Kosovska Mitrovica and Pristina, Yugoslavia, stratified by hemoglobin concentration. At ages 4.5 (A) and 6.5 (B) children in K. Mitrovica had significantly higher serum EPO. At ages 9.5 (C) and 12 (D), serum EPO concentrations were comparable. Error bars indicate standard errors. Numbers in parentheses indicate number of children each bar represents.

Results of the repeated measures analysis confirmed these findings. At ages 4.5 and 6.5, after adjustment for Hgb, BPb was positively and significantly associated with EPO ($P < 0.0001$ and $P = 0.0007$, respectively). This association diminished at ages 9.5 and 12 ($P > 0.10$). To test whether the association between BPb and EPO varied by age, we repeated all analyses, including the appropriate interaction terms. Based on this model, a significant interaction between BPb and age was found, indicating that the association changed with age. As shown in Fig. 2, the slopes of the regression lines declined in a clockwise rotation with age. We found no significant interactions between Hgb and age. Neither Tib-Pb nor average cumulative lifetime BPb was associated with EPO measured at age 12. Furthermore, there was no evidence of nonlinear associations between these variables and EPO.

TABLE 2
Regression Coefficients Relating Blood Lead Concentration to Serum Erythropoietin Concentration for All Children 4.5, 6.5, 9.5, and 12 Years of Age

Age (Years)	B*	SE†	*P* Value
4.5	0.21	0.043	0.0001
6.5	0.11	0.041	0.0103
9.5	0.029	0.033	0.39
12	0.016	0.031	0.60
Average to age 12‡	0.022	0.034	0.51
Bone Pb	0.0089	0.033	0.79

* Estimated regression coefficient.
† Standard error of estimated regression coefficient.
‡ Average of all blood lead concentrations through age 12 years.

Discussion

This prospective analysis investigating one mechanism that contributes to Pb-induced anemia builds on our previous work with this cohort. We previously reported that Pb-exposed children aged 4.5 and 6.5 produced increased concentrations of EPO to maintain normal Hgb concentrations.[16] However, the degree of EPO hyperproduction declined between ages 4.5 and 6.5. In addition, by age 9.5, a further diminution of the association was found. Extending these analyses to age 12, we find even less of an association between BPb and EPO. Taken with our observations on the pregnant mothers of these children, our current data provides further confidence in the hypothesis that in nonanemic Pb-exposed young children, increased erythrocyte production is required to maintain normal Hgb concentrations. The continued decline in the slope of the relationship between EPO and BPb with age, after adjustment for Hgb, implies that in children with chronically elevated BPb, the capacity to produce EPO declines over the course of time. This decline is likely the result of cell damage in the proximal renal tubule, the site of EPO production and Pb toxicity.

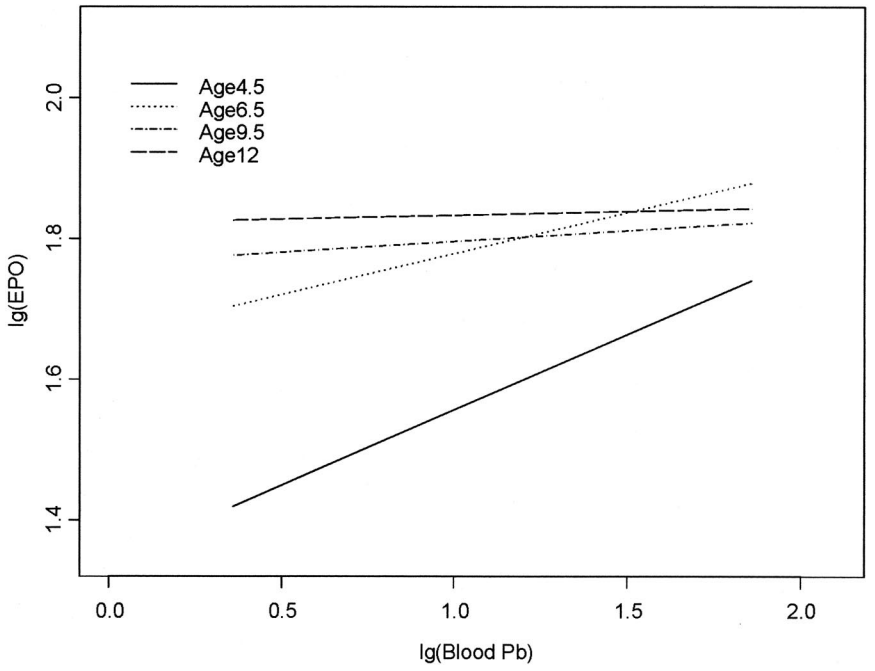

Fig. 2. Relationships between log (BPb) and log (EPO), adjusted for Hgb, at ages 4.5, 6.5, 9.5, and 12 years in children in Kosovska Mitrovica and Pristina, Yugoslavia.

To our knowledge, this is the first study to measure serum EPO longitudinally in children between the ages of 4.5 and 12 years. In Pristina, ie, the relatively low-exposure town, we observed a gradual increase in serum EPO, from 5.6 mU/mL at age 4.5 to 9.2 mU/mL at age 12. This trend was not as clear in K. Mitrovica. We note that, in our laboratory, nonanemic pregnant women from Pristina (mean BPb 3.6 μg/dL) had a mean serum EPO of 9.8 mU/mL,[15] roughly comparable with the children at age 12. Thus, it appears that serum EPO undergoes a gradual increase during childhood in the face of unchanging Hgb concentrations (Table 1). The reason for this rise is not apparent.

There is substantial evidence to suggest that the hyperproduction of EPO in Pb exposed young children is a response to Pb-induced shortened red cell survival. As early as 1925, a review by Aub et al[1] described the anemia of Pb poisoning as one that occurs in two phases. Initially, accelerated erythrocyte destruction is responsible for a fall in Hgb concentration. Animal studies revealed that during this phase, splenectomy could reverse the anemia, presumably by lengthening red cell survival. In rabbits with acute Pb poisoning, peripheral red cell destruction was followed by bone marrow hyperplasia, undoubtedly caused (in hindsight) by increased EPO production. Over the course of time, however, anemia and bone marrow hypoplasia ensued, suggesting either failure of the erythropoietic stem cells, a decline in EPO production, or both. Leikin and Eng[9] found a similar progression in children with BPbs ranging from 60 to 238 μg/dL.

More recent data find stronger associations between cumulative Pb exposure and Hgb. Hu et al[29] found a significant association between tibia (and patella) bone Pb and Hgb among nonanemic male carpenters but not association between BPb and Hgb. The authors suggest that these observations reflect the inhibition of erythropoiesis through reduced EPO synthesis, with bone Pb serving as a proxy for renal Pb accumulation. Among long-term Pb workers, EPO concentrations were significantly lower among those with BPbs $>$ 30 μg/dL than among those with BPbs less than 20 μg/dL.[30,31] We do not find an association between Tib-Pb and Hgb (data not shown), nor do we find an association between Tib-Pb and EPO in our children. This may be the result of a gradual decline in the ability to produce EPO, which is not clinically significant at age 12. In an analysis of the pregnant mothers of these children,[15] we found a significant inverse association between BPb and EPO. We note that the mothers were exposed to high airborne Pb for most, if not all, of their lives.

Somewhat contrary to our findings, Liebelt et al[32] found decreased EPO concentrations with increasing BPb among 86 children between ages 1 and 6 years (mean age 34 months). These children were recruited from either a university based Pb clinic or a primary care clinic. Mean BPb in this population was 18 μg/dL (range 2 to 84 μg/dL) and mean EPO was 5.9 mIU/mL (range 2.3 to 11.5 mIU/mL). The difference in results, in part, may be the result of confounding by age in their study.

In summary, the Yugoslavia Prospective Cohort Study of Pb has provided a unique opportunity to assess the relationships between Pb exposure and EPO in a population well characterized from birth onward. Collectively, our studies of EPO in this cohort indicate that the spectrum of nephrotoxicity of Pb includes a gradual decline in EPO production.

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