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Validity of Lead Exposure Markers in Diagnosis and Surveillance

Joseph H. Graziano

Extensive research has been devoted to the development of biomarkers of environmental and occupational exposure to lead (Pb). This body of work can serve as a paradigm for biomarker development for other chemical exposures. Early efforts focused on indirect measurements of exposure by analyzing precursors and enzymes of a biosynthetic pathway (heme) in blood and urine. However, the direct measurement of Pb in blood has become increasingly simple and reliable and is now widely accepted for pediatric surveillance programs, in part because of known associations of Pb with adverse health outcomes. Other markers of exposure include measurements of Pb in important compartments: bone Pb, tooth Pb, and chelatable Pb. In addition, the technique of stable isotope dilution is available, since Pb exists in numerous nonradioactive isotopic forms. The strengths and weaknesses of all Pb biomarkers for confirming a diagnosis or for epidemiologic research vary widely depending upon the hypothesis under investigation.

Indexing Terms: *toxicology/x-ray fluorescence/stable isotope dilution spectrometry/atomic absorption spectrophotometry/enzyme inhibition*

During the past 50 years, efforts have been made to develop techniques to quantitatively evaluate environmental and occupational exposure to lead (Pb). Thus, although the word "biomarker" is relatively new, this area of research is not. Indeed, the body of work concerning methods to quantitate Pb exposure may well serve as a paradigm for investigations concerned with other chemical exposures.

In the 1930s, scientists measured Pb in biological fluids by crude and insensitive colorimetric chemical methods (*1*); by the late 1960s they used more sensitive "first generation" atomic absorption methods (*2*). The relatively poor precision of the early atomic absorption methods and the need for a large blood sample volume (7 mL) led to a successful search for indirect measures of Pb exposure, which focused on the toxic effects of Pb on the heme biosynthetic pathway. For 20 years these indirect methods proved to be highly effective, particularly for surveillance. Other more costly and elaborate technologies, such as x-ray fluorescence (XRF) (*3*) measurements of Pb in bone and of $^{206}Pb/^{207}Pb$ isotope ratios in blood (*4*), also evolved, although their utility was limited to research.[1]

Division of Environmental Sciences and Department of Pharmacology, Columbia University, College of Physicians & Surgeons, 630 West 168th Street, New York, NY 10032; fax 212-305-8780; E-mail jg24@cunixf.cc.columbia.edu.

[1] Nonstandard abbreviations: XRF, x-ray fluorescence; BPb, blood lead concentrations; UPb, urine lead; ALA-D, δ-aminolevulinic acid dehydratase; ALA, δ-aminolevulinic acid; and EP, erythrocyte protoporphyrin.

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Today, however, after improvements in the technology of atomic absorption spectrometry, the implementation of stringent quality-control programs, and decades of research on the health effects of Pb, the direct measurement of Pb in blood has returned as the most widely used and reliable method for detecting and diagnosing Pb poisoning (*5–7*).

Early Studies of Lead in Blood and Urine

Between 1937 and 1941, Kehoe et al. (*8*) conducted a classic series of Pb-balance studies in normal human volunteers. Sixteen laboratory assistants or graduate students were exposed to daily doses of Pb ranging from 0.3 to 3.0 mg/day for 16–208 weeks, by either ingestion or inhalation. At the time, blood lead concentrations (BPb) of <3.86 μmol/L (80 μg/dL) were believed to be inconsequential, and BPb concentrations of <0.97 μmol/L (20 μg/dL) were not detectable. Given our present knowledge of Pb toxicity, one shudders when reading Kehoe's work documenting increases in blood and urine Pb (UPb) concentrations, with BPb averaging >2.90 μmol/L (60 μg/dL) for years in some individuals. Those experiments did provide the first crude description of dose–response relationships between exposure and BPb, of Pb bioavailability, and of the kinetic behavior of Pb in humans. The extremely wide fluctuations over time in BPb and UPb within and among individuals with constant, controlled Pb exposure would have led one to believe that the direct measurement of Pb in biological fluids might never prove useful for medical surveillance and (or) diagnosis. The wide fluctuations, however, were probably due in large part to laboratory imprecision before the introduction of modern analytical technology, "clean" laboratories, and quality control.

The Heme Pathway as a Source of Biomarkers of Pb Exposure

Because of its affinity for several functional groups, particularly sulfhydryl groups, Pb inhibits enzymes in many biochemical pathways. Although several enzymes of the heme pathway are inhibited by Pb, two have proved to have the greatest diagnostic utility: δ-aminolevulinic acid dehydratase (ALA-D; EC 4.2.1.24) and heme synthetase (EC 4.99.1.1). Pb exposure produces an accumulation of their substrates, δ-aminolevulinic acid (ALA) and protoporphyrin, respectively. Because of the ease of sampling blood, measurement of these enzymes and substrates in red cells have been widely investigated as biomarkers of exposure; all of these are actually markers of the *effects* of Pb on heme biosynthesis.

Erythrocyte δ-aminolevulinic acid dehydratase. In 1970, Hernberg and coworkers (*9*) described a strong negative association between red cell ALA-D activity

and BPb, indicating that ALA-D measurement could serve as a biomarker of Pb exposure. ALA-D activity decreases logarithmically with BPb, such that 50% inhibition occurs at a BPb of only 0.72 μmol/L (15 μg/dL). In the ensuing decade, several studies revealed the potential clinical diagnostic utility of ALA-D measurements (*10*). However, the instability of the enzyme, which must be assayed within 24 h of blood sampling, limits its practical utility for surveillance. Moreover, the inhibition of the enzyme is so extensive at BPb ≥1.45 μmol/L (30 μg/dL) that the assay cannot readily distinguish between moderate and severe exposure.

δ-Aminolevulinic acid. Inhibition of ALA-D leads to an accumulation of ALA, first within cells, then in plasma, and finally in urine. Historically, the measurement of ALA in urine was an important tool in occupational medicine as a means of screening workers for objective evidence of Pb toxicity (*11*). By today's standards, however, urinary ALA is a relatively insensitive measure of Pb toxicity, because its level of excretion does not increase drastically until the BPb exceeds 1.93 μmol/L (40 μg/dL). Moreover, the need for quantitative 24-h urine collections can pose logistic limitations. Nevertheless, such measurement is simple and noninvasive, and it continues to be used in parts of the world where environmental Pb exposure is extensive and financial resources limited (*12*).

Erythrocyte protoporphyrin (EP). Heme synthetase, or ferrochelatase, is an enzyme located at the inner mitochondrial membrane, a site where Pb accumulates. The enzyme inserts an iron atom into protoporphyrin IX, forming heme. Like ALA-D, heme synthetase contains sulfhydryl groups that are sensitive to Pb. In 1973, Piomelli and coworkers developed a relatively simple fluorometric technique for measuring porphyrins in red blood cells (*13*). With that method they described the "hockey stick" relation between log EP and BPb in a group of 1038 New York City children, whereby EP begins to increase after BPb reaches ~0.82 μmol/L (17 μg/dL) (*14*). The further development of the hematofluorometer as a device that could quickly, reliably, and inexpensively analyze EP concentrations on fingerstick blood samples led to the widespread adaptation of the EP test as the method of choice for Pb surveillance (*15, 16*). Ironically, the mechanism(s) whereby Pb exposure leads to increased EP remains somewhat controversial. The observation that men with occupational Pb poisoning have normal levels of ferrochelatase activity in lymphocytes (*17*) has led some to conclude that in vivo enzyme inhibition does not account for the rise in EP (*18*). However, Pb accumulates in red cells (rather than lymphocytes), and inhibition of human reticulocyte ferrochelatase becomes more pronounced as iron supplies become rate-limiting (*19*).

Regardless of the mechanism, among individuals, wide variations in EP occur at any given BPb. Although there may be a genetic component to this variability, it is clear that iron deficiency also contributes to increased EP (*20*). During two decades as a screening test, the EP test was a wonderful method for detecting two prevalent conditions of early childhood: Pb exposure and iron deficiency. In the US, however, the 1991 Centers for Disease Control revision of "acceptable" BPb, from 1.21 to 0.48 μmol/L (25 to 10 μg/dL) (*21*), has led to the gradual abandonment of the EP test as a means of surveillance because it is incapable of identifying children with BPb of 0.48–1.21 μmol/L (10–25 μg/dL). The more costly measurement of BPb has quickly become the method of choice for surveillance and diagnosis of Pb exposure.

Measurements of Pb in Calcified Tissues

The skeleton is the primary long-term storage compartment for Pb. In children, 70%–80% of the body Pb burden is in bone, whereas in adults it is 90%–95% (*22, 23*). The earliest attempts to use skeletal Pb concentration as a biomarker of Pb exposure focused on the harvesting of deciduous teeth in school-age children. A classic study by Needleman et al. (*24*) reported associations between dentine Pb and classroom performance in children in the second grade. However, the limitations of tooth Pb as a biomarker are obvious, in that teeth are not readily available for analysis.

X-ray fluorescence (XRF) has slowly evolved as a method for the direct in vivo measurement of Pb in bone, thus providing an integrated estimate of Pb accumulation for epidemiologic studies. Several XRF technologies exist, some involving K x-rays and some L x-rays; the latter has theoretical limitations (*25*) and requires that the subjects be sedated, as slight movements greatly influence the measurements. K-XRF has become the more widely accepted technique, although a standardized protocol does not yet exist (*26*). The selection of the technique varies with many factors, including age, the outcome to be studied, the anticipated distribution of Pb in bone, and other technical factors (*3*).

Although proponents have sometimes exaggerated its virtues (*27*), XRF bone Pb measurements are certainly appealing for an integrated estimate of the largest Pb storage compartment. In some research settings, K-XRF measurements promise to resolve issues concerning relations between past heavy exposure and various health outcomes. The integration of past exposure, however, precludes the examination of temporal relations (or critical periods) with past exposure. Bone Pb K-XRF is also relatively costly, involves low-dose radiation exposure, requires considerable time, and is not widely available. Furthermore, the uncertainty of the measurement increases as bone Pb decreases (*26*), and many nonoccupationally exposed individuals have bone Pb concentrations undetectable by current methods. Given that environmental Pb exposure is generally decreasing over time in developed countries that have abandoned leaded gasoline additives and lead paint, that more and more interest and resources are devoted to the detection and management of very low-level exposure, and that even the EP test has proven to be insufficiently sensitive for their current needs, bone Pb measurements for diagnosis and surveillance will prove to be of limited use.

Stable Isotope Measurements

Another even more elaborate method for studying Pb exposure takes advantage of the fact that Pb exists in several experimentally useful, nonradioactive isotopic forms (i.e., ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb). Most Pb deposits vary in isotope ratio between the extremes of Broken Hill, Australia (e.g., $^{206}Pb/^{207}Pb$ approximates 1.04), the geologically oldest, and the younger deposits of the US Mississippi Valley ($^{206}Pb/^{207}Pb$ approximates 1.30). Manton (*4*) was the first to appreciate that, under certain conditions, the isotope ratios of Pb in human whole blood may be used experimentally to identify and quantify the various sources of environmental Pb exposure. More recently, two elegant studies have utilized the stable isotope technology to identify the predominant sources of childhood lead exposure in London and Glasgow, UK (*28*), and Omaha, NE (*29*).

Thus, mass spectrometry of stable Pb isotopes in whole blood allows one to utilize the stable isotope dilution technique as a forensic tool for environmental and (or) clinical studies. For example, Manton observed that the isotopic ratio of Pb in the air in Dallas fluctuated with time, depending on the source of Pb used in the manufacture of tetraethyl Pb. By taking advantage of the fact that, as a recent immigrant to the US, his blood isotopic ratio was distinctly different from that of the environment in Dallas, he was able to calculate the amount of Pb entering his own blood and that of his wife from environmental sources (*4, 30*).

In collaboration with Manton, we have recently initiated controlled clinical studies of the bioavailability of Pb in various matrices. For example, one study examines the bioavailability (i.e., fraction absorbed) of Pb in wine. By storing wine in a Pb-crystal decanter with a relatively low $^{206}Pb/^{207}Pb$ ratio, we have generated wine that has a distinctly different isotopic ratio than that observed in the blood of the typical American. The magnitude of the shift in blood $^{206}Pb/^{207}Pb$ after ingestion of a glass of wine allows us to precisely calculate the quantity of Pb absorbed from the wine. Obviously, however, the financial cost and high degree of technical proficiency required for stable isotopic Pb analyses limit the utility of this technology to research.

The $CaNa_2EDTA$ Mobilization Test

Under baseline conditions, the elimination of Pb in urine is generally not considered a sensitive indicator of Pb exposure. However, Pb elimination after a single injection of the chelating agent $CaNa_2EDTA$ is significantly associated with BPb as well as with various heme pathway biomarkers. Until recently, many pediatricians relied on the so-called "Pb mobilization test" to determine which children with BPb of 1.45–2.12 μmol/L (30–44 μg/dL) should receive a full course of chelation therapy (*31*). Those who eliminated >0.6 μg Pb/mg $CaNa_2EDTA$ were considered to have "positive" tests, while those excreting less Pb were considered negative.

The mobilization test is based on two assumptions that are not obvious: that "good responders" will continue to respond well and "poor responders" will continue to eliminate relatively little Pb. Based on statistical probability (i.e., regression to the mean), this assumption is probably false. In addition, a mobilization test with $CaNa_2EDTA$ or any other chelator assumes that children with a negative mobilization test cannot benefit from further treatment, an assumption for which there is no evidence. In addition, the test requires an 8–24 h urine collection in very small children (or occupationally exposed adults) and is therefore inherently error prone. Moreover, the physiological source of Pb mobilized into urine is not certain. Finally, it requires parenteral drug administration and nursing care, and is therefore relatively costly and not risk free. This $CaNa_2EDTA$ test has therefore fallen into disfavor.

Blood Lead as a Marker of Exposure

The isotopic dilution technique has also been used to determine the half-lives of Pb in blood (*32*); this has also been accomplished by more conventional atomic absorption BPb analyses (reviewed in *33*). Most investigators report at least two half-lives for Pb in blood, one of ~1 month and a second of ~4 years; the latter reflects the replenishment of the blood by the bone storage compartment. Thus, BPb is most useful as a marker of recent Pb exposure rather than cumulative lifetime exposure.

The complexity of the kinetics of Pb in blood has contributed to the reluctance by some to rely on it as a biomarker of Pb exposure. For several reasons, that reluctance has slowly disappeared. Reliable, sensitive methods for the detection of concentrations as low as 0.05 μmol/L (1 μg/dL) (*5–7, 34*) have become widely available; these now require smaller volumes of blood than in the past. All the alternative biomarkers described above have their own serious limitations due to lack of sensitivity, high technology, or kinetics. With regard to kinetics, EP and ALA-D, for example, are clearly influenced by red cell life span and turnover. However, the most important factor leading to the shift toward BPb as the biomarker of choice is that *all* of the major studies of pregnancy outcome (reviewed in *35*), cognitive function in children (e.g., *36–38*) or renal function in adults (*39*) have included BPb as the primary biomarker and all have reported adverse associations with BPb.

In summary, the direct measurement of Pb in blood has become the most widely used and informative biomarker of Pb exposure. Because of the well-defined associations between BPb and various adverse health outcomes, the measurement of BPb is the definitive means by which diagnoses are made. In the US, BPb has also become the method of choice for surveillance, because the EP test does not have the ability to detect children with BPb in the range of 0.48–0.97 μmol/L (10–20 μg/dL). However, in countries with higher environmental exposure, the measurement of EP (or even ALA in urine) allows for triage of cases so as to optimize the utilization of financial resources. Other more technologically sophisticated techniques for monitoring exposure

(e.g., XRF or stable isotope dilution) are available, but most of these have limitations that confine their use to research studies.

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