Bone Lead Measured by X-ray Fluorescence: Epidemiologic Methods

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In vivo X-ray fluorescence (XRF) measurement of bone lead concentration (XRF) has emerged as an important technique for future epidemiological studies of long-term toxicity. Several issues germane to epidemiologic methodology need to be addressed, however. First, sources of variability in measurements of bone lead need to be quantified, including imprecision related to the physical measurement itself and the variability of lead deposition over the two main compartments of bones (cortical vs. trabecular) and within each compartment. Imprecision related to the physical measurement can be estimated for each individual measurement based on the variability of the signal and background. Second, approaches to low-level data need to be debated. We argue for using the minimal detection limit (MDL) to compare instruments and interpret individual measurements; however, with regard to epidemiologic studies, we would abandon the MDL in favor of using all point estimates. In analyses using bone lead as an independent variable, statistical techniques can be used to adjust regression estimates based on estimates of measurement uncertainty and bone lead variability. Third, factors that can be expected to modify the relationship between bone lead and toxicity such as gravidity history, endocrinological states, nutrition, and other important influences on bone metabolism, need to be identified and measured in epidemiologic studies. By addressing these issues, investigators will be able to maximize the utility of XRF measurements in environmental epidemiologic studies. — Environ Health Perspect 103(Suppl 1):105–110 (1999)

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Introduction

Lead is a familiar topic to all environmental scientists to some degree. Perhaps the most studied of all toxics and the subject of hundreds of scientific publications each year, attention to lead has recently increased yet again. Most responsible has been a spate of longitudinal epidemiologic studies indicating an adverse effect of lead on indices of neurobehavioral function and intelligence at low levels of exposure, a revelation that led to a recent report to Congress (1), a revised health advisory by the Centers for Disease Control (2), and a flurry of attention from the media.

Despite this activity, there remains a host of important unknowns surrounding lead toxicity, particularly regarding the long-term effects of chronic low-level exposure and methods for identifying individuals who are at risk. Central to these issues is the realization that lead accumulates in the skeleton and remains there, long after exposure may have decreased. Whereas levels of lead in blood, the most commonly used indicator of internal lead exposure, tend to decrease rapidly with an average half-life of about one month (3), levels of lead in bone have a half-life of years to decades (4,5). In children, some 70 to 80% of total body lead burden is contained in bone; the figure rises to 90 to 95% in adults (6,7). Among the considerations this phenomenon raises are: a) levels of lead in bone are a useful indicator of prior exposure to lead; and b) are bone lead stores themselves a risk factor for future toxicity?

These considerations would remain academic if it were not for the recent development of x-ray fluorescence (XRF) techniques for in vivo measurement of bone lead stores. The purpose of this article is to briefly review XRF and discuss considerations that are specific to environmental epidemiologic research.

In Vivo X-ray Fluorescence

Technical aspects of in vivo XRF, under steady development for over 15 years by investigators in the United Kingdom, Sweden, Australia, and the United States, have been reviewed in detail elsewhere (8–10). In short, XRF instruments utilize low-level gamma radiation to provoke the emission of fluorescent photons from the target area of a test subject’s anatomy. The photons are detected and counted over the wavelength spectrum (Figure 1), from which the characteristic emissions of lead are then carefully extracted. Measurements are noninvasive, painless, and require very little radiation exposure (11).

Two main XRF techniques exist: L-XRF, which uses weakly-penetrating radiation and concentrates on the emissions from L-shell electrons (12); and K-XRF, which uses radiation that penetrates bone more deeply and concentrates on K-shell electron emissions. The latter technique has a number of variations with respect to the radioactive isotopes used to generate the source radiation, the geometry of the source-detector arrangement, software, electronics hardware, and the anatomic target(s) chosen for the measurement. A comparison of L- and K-XRF can be found elsewhere (6).

Our group chose to use a K-XRF instrument with a 109Cd source in a backscatter geometry to measure bones of the lower leg (Figure 2). The number of lead fluorescent photons is compared with the
number of photons from the coherent scatter signal (which comes principally from calcium hydroxyapatite), resulting in µg of lead per g of bone mineral (µg/g) as the unit of measurement. This method of normalization renders the measurement insensitive to variations in bone shape, size, density, and histomorphometry, overlying tissue thickness, and movement (13). Validation studies comparing measurements from an instrument with this design against chemical analysis in cadaver studies have shown a high degree of accuracy (14–16).

Our instrument has two features that are adapted specifically to large epidemiologic studies. The software that runs the instrument and analyzes the resultant spectra is algorithm-driven, allowing the instrument to be operated by a trained technician rather than a physicist (17). In addition to measuring bone lead concentration, our instrument estimates each individual measurement’s uncertainty (equivalent to 1 standard deviation of replicate measurements) based on analyses of the characteristic X-rays (12), which creates the potential to adjust epidemiologic analyses for individual measurement error. Measurement uncertainty tends to increase with obesity (thicker overlying tissue) and lower bone density (18).

Existing Studies of Bone Lead Using in Vivo XRF

Most investigations have so far been conducted with K-XRF instruments in surveys of workers employed in factories with known lead exposure (2,19–21), in studies comparing lead burden estimated by XRF with lead burden estimated by chelation and cumulative indices of blood lead (22–24), and in pilot studies of control subjects with no known lead exposure (10,12,25). One group of investigators has been using an L-X-ray fluorescence technique to measure lead burden in children (26); the same investigators also conducted a survey of lead burden among factory workers (27).

These studies have confirmed that the kinetics of XRF-measured bone lead are quite unlike blood lead. Bone lead increases in proportion to the level and duration of exposure, and diminishes very slowly once exposure ceases. A high correlation is seen between XRF-measured tibia lead and a time-integrated blood lead index (16,21). Heterogeneity among bone sites and bone types (cortical vs trabecular) seems to exist, with cortical bones such as the tibia exhibiting very slow kinetics as opposed to the faster turnover of trabecular bones such as the patella and calcaneus (18,21). After treatment with the chelating agent calcium disodium edetate, bone lead levels drop slowly (28) if at all (21), as opposed to blood lead levels, which decrease more rapidly.

Investigations using XRF that attempt to correlate bone lead levels with indices of health are just beginning to appear in the literature. Two cross-sectional investigations did not find any relationship between XRF-measured bone lead and indicators of kidney function, including several sensitive markers of early tubular and glomerular damage such as urinary N-acetylglucoaminidase activity, retinol binding protein, urinary β2-microglobulin, and urinary albumin (29,30).

The Future of K-XRF and Epidemiology: Methodology

The development of in vivo XRF has in essence expanded the pool of biologic markers ("biomarkers") for lead. Others have traditionally included measurement of lead in blood, hair, urine, and teeth; chelatable lead; erythrocyte protoporphyrin in blood; and porphyrin cogeners and aminolevulinic acid dehydratase in blood and urine (Table 1). Under classification schemes designed to define biologic markers, the most apparent position of bone lead is as a biologic marker of internal dose (Table 1).

In addition, bone lead may also be able to serve as a biologically effective marker of dose (31) that is specific to skeletal tissue. Lead is toxic to osteoblasts in vitro (32) and lead exposure has been associated with decreased skeletal growth, as manifested by height and chest circumference (33). Lead exposure has also been associated with lower indices of red cell production, even at relatively low blood lead levels (34). Since hematopoiesis occurs in bone marrow, it is possible that bone lead would be a more accurate dose marker than blood lead for this form of toxicity.

More epidemiologic research that tests the ability of XRF-measured bone lead to serve as a biologic marker for both occupa-
Table 1. Examples of biologic markers for monitoring lead and their characteristics.

| Biologic markers                      | Half-life | Comments regarding use as indicator of accumulated lead exposure |
|---------------------------------------|-----------|---------------------------------------------------------------|
| Blood lead                            | Weeks to months | Dependent on urine flow                                        |
| Urine lead                            | Weeks     | Large intraindividual variability; prone to external contamination or leaching |
| Hair lead                             | Months    | Prone to external contamination; kinetics uncertain            |
| Nail lead                             | Months    | Prone to external contamination; kinetics uncertain            |
| Tooth lead                            | Decades to ∞ | Must await shedding of deciduous tooth, isolate circumpalatal dentine; in vivo measurement of whole tooth lead available, but of uncertain utility |
| Bone lead                             | Years to decades | Cortical bone has long half-life; trabecular bone has shorter half-life |
| Chelatable lead*                      | Years     | Involves injection and timed collection of urine; represents “chelatable” compartment of lead found mostly in soft tissue, partly in bone |
| Biologically effective dose markers   | Years to decades | May be related to abnormal skeletal development and/or hematopoiesis (untested) |
| Bone lead                             | Years     | Mostly trabecular bone; significant bone lead toxicity         |
| Erythrocytic protoporphyrin           | Weeks to months | Level increases due to lead’s inhibition of hematopoiesis; integrates exposure over several months; sensitivity poor for sustained mild-to-moderate elevations of blood lead (25–40 mcg/dl); increased levels also seen in iron deficiency |
| Urinary ALAD, coproporphyrins         | Weeks     | Activity inhibited by lead but also by other metals, e.g. methymercury, and by ethanol intoxication |
| Urinary pentaerythritol dehydrogenase  |           | Levels also increased in certain hepatic diseases |

ALAD, aminolevulinic acid dehydratase. *The amount of lead found in urine over a defined time period following injection of calcium disodium ethylenediamine–tetraacetate, a chelating agent.

significantly assist the epidemiologist (see next section).

Another source of variability is intra-individual variability in the distribution of lead in the skeleton. There is clear evidence from both autopsy studies and multiple-site XRF studies that bone lead kinetics dichotomize between a cortical bone compartment and a trabecular bone compartment (21,27,29,39). This can be taken into account by measuring both a predominantly cortical bone (i.e., the mid-tibia) and a predominantly trabecular bone (e.g., the calcaneus or patella) in future XRF studies. Our group has favored the patella over the calcaneus as a predominantly trabecular bone, since it seems to have more bone mass and therefore affords better measurement precision.

In addition, however, there is evidence that skeletal lead deposition has significant within-compartment variability. Wittmers et al. (39) found that the coefficient of variation for chemically measured bone lead concentrations of samples taken from multiple sites on the tibia from a single individual was 14%. In another study that examined six bone sites and conducted separate chemical analyses of cortical bone separated from trabecular bone, the bone lead concentration of the mid-tibia (a site frequently targeted in XRF studies) was found to have a correlation coefficient of 0.55 in comparison to the mean of the bone-lead concentrations from the cortical segments of the other five sites (40). A single XRF bone lead measurement is therefore likely to be only partly representative of skeletal lead burden, even within its own compartment.

Thus, in studies using XRF, the environmental epidemiologist will be confronted with XRF data with several sources of variability. One source, measurement uncertainty, can be individually quantified. The other, variability of skeletal lead distribution, can be reduced by measuring two or more bone sites; however, some residual variability will no doubt remain. These sources of variability will reduce both the power with which an association can be made between bone lead and a toxicological outcome, and the magnitude of the effect estimate once an association has been made (41).

Minimal Detectable Limits, Measurement Error, and Adjustment for Measurement Error

Classical laboratory practice calls for calculation of a minimum detectable limit (MDL). Measurements that fail to exceed
this value are put into a single category and are assumed to be indistinguishable from zero. With regard to in vivo XRF, physicists have defined the MDL as 2X standard deviation of net peak area counts (42), 3X standard deviation of background counts (43), or more recently, 2X the median of the uncertainty values (a function of both the peak and background count standard deviations) (44). Although analysis of in vivo XRF data is still in its infancy, several statistical methods could be borrowed from environmental pollution research for assigning a value to below-MDL measurements in order to retain them in continuous variable analyses (45,46).

In vivo XRF data are quite distinct from most laboratory data, however. Due to the random nature of radiation interaction and the calibration procedure, XRF instruments provide an unbiased point estimate of level concentration that may be zero or negative. In other words, if a bone with absolutely no lead is being measured multiple times by in vivo XRF, the measurements will oscillate around zero. Negative results obviously have no intrinsic meaning; they are negative only because the true bone lead concentration is close to zero and there is error associated with the measurement. In addition, as mentioned above, each in vivo XRF measurement is accompanied by an estimate of the uncertainty associated with that measurement.

Certainly, from a clinical perspective, i.e., the interpretation of any given individual's single K-XRF measurement, it is important to rely on the concept of an MDL; it defines a level below which most measurements of a true zero will fall. The MDL is also an important parameter for comparing the precision of various XRF instruments.

From the epidemiologist's perspective, however, useful information would be lost if, in the analysis of in vivo XRF data, an MDL were used to censor data or amalgamate low-level bone lead estimates into a single category. It is preferable to use all the point estimates, even if zero or below zero, in conjunction with the individual estimates of uncertainty. In essence, the individual estimates of measurement uncertainty carry information about the true bone lead concentrations. For example, for two identical and positive bone lead measurements falling below the MDL, the reading with the smaller estimate of measurement uncertainty will have a higher chance of corresponding to a true bone lead concentration greater than zero. We can use the estimate of measurement uncertainty together with the actual bone lead reading to modify the standard regression analyses when bone lead is used as a predictor variable.

Ignoring the error of the K-XRF measurement in ordinary least squares analyses produces estimates of the slope that are biased towards the null (47,48). The degree of attenuation in the slope depends on the magnitude of the uncertainty of the measurement error relative to the variability of the true bone lead values in the data. If $\lambda$ is the quotient between the variance of the predictor over the sum of the predictor variance and the average measurement error variance, an analysis using bone lead measured as a predictor would produce estimates of the true slope attenuated by a factor of $\lambda$. That is, the estimated slope is $\lambda$ times the true slope.

We can obtain an estimate of the coefficient of attenuation ($\lambda$) using the individual measurement error estimates produced by the K-XRF instrument, thus correcting the standard ordinary least squares estimates. Roughly speaking, the adjusted estimated slope is equal to the ordinary least squares slope divided by the coefficient of attenuation. We can also use the estimate of measurement uncertainty to estimate the standard error of the adjusted regression coefficients. Although the estimated slope is revised upward, the extra variability of the adjusted slope estimate generally results in reduced power for testing the null hypothesis that the slope is equal to zero.

Modifiers of Bone Lead and Toxicity

Finally, it is important for the epidemiologist to anticipate factors that may modify the relationship between bone lead and toxicity. As a biologic marker of internal dose, the bioavailability of bone lead for organ toxicity outside of bone is theoretically dependent on processes that affect bone metabolism, and, therefore, the liberation of lead from bone.

Of particular interest are pregnancy, lactation, and osteoporosis. For example, blood lead concentration has been found to be elevated in postmenopausal women in comparison to premenopausal women, even after controlling for age, calcium intake, and other variables potentially related to external lead exposure and mineral metabolism (49). The extent of the elevation among the postmenopausal women was also higher among those who were nulliparous, thus providing indirect evidence that increased mobilization of lead from bone can occur during the osteoporosis associated with menopause and the bone demineralization of pregnancy. Individual case reports have provided evidence that lead stored from exposures many years previously can be mobilized during pregnancy (50,51).

Attention must be paid to other influences of bone metabolism that have been shown to affect lead kinetics. A case was recently reported of a woman with elevated K-XRF measured bone stores who experienced marked elevations of blood lead when she developed hyperthyroidism, which is accompanied by greatly increased bone turnover (52). Experimental therapy to increase bone mass and surgical treatment of hyperparathyroidism were both found to be independently associated with reductions in blood lead (53). It is likely that other endocrinologic, nutritional, and miscellaneous factors that affect bone metabolism also affect lead mobilization. For example, hypercorticosism from endogenous overproduction states or the exogenous administration of steroid medications is known to enhance bone demineralization (54). Conversely, high levels of dietary calcium and regular exercise are known to inhibit bone demineralization (55).

These phenomena make it advisable for an environmental epidemiologist to collect data on parity, nutrition, and endocrinologic factors that might modify the relationship between XRF-measured bone lead and measures of outcome.

Conclusion

In vivo XRF promises to add significantly to our knowledge of lead toxicity by providing a biologic marker of lead accumulation. To optimize the use of this biologic marker in epidemiologic studies, it will be important to increase instrument precision, develop intercalibration protocols, estimate variability in skeletal lead distribution, validate the individual estimates of measurement uncertainty, apply techniques for adjusting analyses for measurement uncertainty, and measure factors that may modify bone lead-outcome relationships.

Even as this research progresses, however, it may be important to assess other potential biologic markers, particularly those that might serve as accurate biologic markers of effective lead dose. By so doing, we will be able to better bridge the domains of toxicology and epidemiology to shed more light on the ultimate effects of this remarkable toxin.
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