Predictive value of determinations of zinc protoporphyrin for increased blood lead concentrations

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Blood lead (PbB) and red cell zinc protoporphyrin (ZPP) concentrations are widely used biomarkers for lead toxicity. It is uncertain, however, whether either or both are needed for monitoring lead exposure and how discordant PbB and ZPP values should be interpreted. We reviewed the results of PbB and ZPP determinations in 94 workers in a lead-battery plant over a 13-year period and retrieved all 807 sets of tests in which both PbB and ZPP were available, with a follow-up PbB value 6 months later. PbB exceeded 1.93 μmol/L (40 μg/dL) in 414 (51%), and 2.90 μmol/L (60 μg/dL) in 105 (14%) of the blood samples. We derived the test properties of various ZPP concentrations for concurrent “toxic” PbB concentrations, defined as >1.93 and 2.90 μmol/L (40 and 60 μg/dL). The results indicated that, given a population of lead-exposed workers with a 10% prevalence of PbB of >2.90 μmol/L (60 μg/dL), a policy of testing PbB only in those with ZPP >0.71 μmol/L (40 μg/dL) would obviate 42% of the PbB tests, but would miss about three cases with toxic PbB concentrations in every 200 workers at risk. A finding of increased ZPP concentrations with a concurrent “nontoxic” PbB was associated with an increased risk of a toxic PbB concentration 6 months later. We conclude that (a) screening by testing only ZPP does not safeguard exposed persons against lead toxicity, and (b) the frequency of PbB monitoring should be guided by estimates of the risk of future lead toxicity in individual workers.

Lead poisoning is a preventable disease of environmental origin that is caused primarily by inhalation and gastrointestinal absorption in work settings (1–4). Its main manifestations are renal (5), nervous (6–8), systemic (9, 10), immunologic (11), and hematological. Lead inhibits heme biosynthesis and causes anemia, basophilic stippling, a decline in red cell δ-aminolevulinic acid dehydrogenase, and an increase in urinary δ-aminolevulinic acid, urinary coproporphyrin, red cell zinc protoporphyrin (ZPP), and pyrimidine 5′-nucleotidase (12). Subjective symptoms are useless as indicators of early lead poisoning (13). Therefore, monitoring of exposed workers is based on laboratory tests.

The biomarker of lead exposure is its concentration in the circulation (PbB). The threshold of PbB is 0.24 μmol/L (5 μg/dL) for increased red cell δ-aminolevulinic acid dehydrogenase, 0.48 μmol/L (10 μg/dL) for increased 5′-nucleotidase, 0.72–1.45 μmol/L (15–30 μg/dL) for increased urinary δ-aminolevulinic acid and ZPP; and 1.93 μmol/L (40 μg/dL) for increased urinary coproporphyrin (12). The threshold of PbB has been also reported to be 1.45 μmol/L (30 μg/dL) for fatigue (9), 1.93 μmol/L (40 μg/dL) for deficits on performance of cognitive tasks (7), and 2.90 μmol/L (60 μg/dL) for impaired renal tubular functions (5). Biochemical and clinical harmful effects may, therefore, be produced by PbB concentrations as low as 0.24 and 1.45 μmol/L (5 and 30 μg/dL), respectively. What then should be considered as a “safe” exposure to lead in occupational workers, and what is the threshold PbB concentration that should preclude additional exposure?

This threshold is a compromise between safety and cost. The World Health Organization study group recommended in 1980 that this threshold be set at 1.93 μmol/L (40 μg/dL) (14). The Occupational Safety and Health

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Administration (OSHA) requires that employees be removed from additional exposure when their PbB concentrations exceed 2.90 μmol/L (60 μg/dL) [or averages ≥ 2.41 μmol/L (50 μg/dL)], until the PbB concentration declines below 1.93 μmol/L (40 μg/dL) (15). Israeli law similarly sets the PbB threshold at 2.90 μmol/L (60 μg/dL) but permits employees to return to work if repeated PbB concentrations are less than this limit (16).

A second widely used biomarker of lead exposure is red cell ZPP. ZPP has been claimed to be better correlated than PbB with fatigue, sleep disturbances, and arthralgia (10), and with CNS and gastrointestinal symptoms (17). On the other hand, PbB was reported to correlate better with sensory-motor slowing and memory impairment (7) and ZPP is insensitive for the detection of increased PbB at the critical thresholds of 0.24–0.58 μmol/L (5–12 μg/dL) in children and adult women (18). Correlation coefficients between logZPP and PbB have varied between 0.72 and 0.90 for men (18–22) and between 0.53 and 0.56 for women (19, 21). Although statistically significant, these correlations are too low to warrant a prediction of PbB from ZPP concentrations in individual cases (20, 23, 24). Similarly, various threshold ZPP concentrations have been found to have a limited sensitivity for toxic PbB concentrations (18, 20).

The discrepancies between ZPP and PbB concentrations could have several causes. ZPP may be a better measure of chronic lead exposure because of its longer half-life (63 days), whereas PbB could be a superior indicator of short exposure (25). In chronic exposure, PbB peaks at 3–6 months, whereas ZPP peaks at 6–9 months. After the elimination of exposure, PbB concentrations remain above healthy reference values for up to 2 years, whereas PbB concentrations decrease more rapidly (23, 25, 26). Another explanation could be the lower specificity of ZPP, which increases not only in lead poisoning but also in iron deficiency and in anemia of chronic disease (27). Lastly, the discrepancies may be products of methodological difficulties: ZPP values vary between different brands of fluorometers (5), may be affected by plasma (27), and decline in the first 6–8 min before stabilizing (28).

It is uncertain, therefore, which test is best suited to safeguard exposed workers against unacceptable risks. On one hand, most workers are intermittently rather than constantly exposed to lead; consequently, some authors have recommended the use of both exposure (PbB) and biological (ZPP) response tests (14). On the other hand, PbB determinations require a relatively complicated analysis, whereas ZPP determinations are inexpensive and easy to perform; consequently, other authors have advocated PbB determinations only for subjects with an increased ZPP (18–20, 29, 30).

Restricting PbB determinations to only those workers with ZPP concentrations exceeding a certain threshold would avoid PbB testing in a proportion of the monitored population, but it may miss cases with toxic PbB concentrations. We know of no previous attempts to explore this trade-off. The objective of this study is to report PbB and ZPP concentrations in workers in a lead-battery plant. This study attempts to estimate (a) the proportion of PbB tests that would be avoided by a policy of restricting PbB testing to only subjects with ZPP concentrations above a certain threshold, and the cost of such a policy in terms of undiagnosed cases with toxic PbB concentrations because of false-negative ZPP results; and (b) the importance of a finding of high ZPP and concurrent “nontoxic” PbB. “Toxic” PbB concentrations are defined as those exceeding either 1.93 or 2.90 μmol/L (40 or 60 μg/dL).

**Materials and Methods**

**STUDY POPULATION**

In Israel, testing for ZPP and PbB is required by law once every 6 months. Workers found to have a PbB concentration ≥ 2.90 μmol/L (60 μg/dL) are considered unfit for work until their blood lead concentrations decline below this concentration. Red cell ZPP concentrations do not affect decisions regarding future employment. We reviewed the results of PbB and ZPP determinations in 94 workers in a lead battery plant from 1980 to 1993 and retrieved all 807 combinations of tests in which both PbB and ZPP were available, with a follow-up PbB value 6 months later.

**PbB DETERMINATION**

PbB was measured using a modification of the method described by Fernandez (31) in a 1:10 dilution, by volume, of whole blood in 0.35 mol/L ammonium nitrate that contained 10 mL/L Triton X-100. The determinations were performed by electrothermal atomization atomic absorption spectroscopy, using a Perkin–Elmer 5000 equipped with a Zeeman background corrector. The standards were taken as absolute values, and quality was controlled using samples from an interlaboratory comparison program run by the Center of Toxicology, Quebec, Canada. The lead concentration was calculated by the method of standard addition, in which known amounts of lead are added to blood samples. In our laboratory, 0.24 μmol/L (5 μg/dL) is the lower limit of detection, linearity is from 0.24 to 2.90 μmol/L (5 to 60 μg/dL), and the coefficient of variation is 5%.

**ZPP DETERMINATION**

ZPP was determined using the ProtoFluor-Z Hematofluorometer (Helena Laboratories) that was calibrated daily, using high- and low- concentration calibrator solutions. The instrument excites the blood sample, pretreated by ProtoFluor Z reagent containing potassium cyanide, at 450 nm and measures the emitted fluorescence at 595 nm. This fluorescence is proportional to the ZPP/heme ratio. The ProtoFluor-Z Hematofluorometer expresses the results in terms of either μmol/mol heme, or μmol/L whole blood for a standardized hematocrit of 42%. In our laboratory, the correlation between the two was 0.981.
Similar to other reports (19, 22, 25, 26), we chose to present ZPP concentrations in \( \mu \text{mol/L} \) (\( \mu \text{g/dL} \)) whole blood.

**ANALYSIS**

ZPP concentrations were divided into low, \(<0.73 \mu \text{mol/L} \) (\(<41 \mu \text{g/dL} \)); intermediate, \(0.73–1.75 \mu \text{mol/L} \) (\(41–99 \mu \text{g/dL} \)); and high, \(\geq1.77 \mu \text{mol/L} \) (\(100 \mu \text{g/dL} \)). The correlation between concurrent PbB and ZPP values, as well as the predictive value of ZPP, PbB, and several other variables for a PbB concentration of 1.93 and 2.90 \( \mu \text{mol/L} \) (\(40 \) and \(60 \mu \text{g/dL} \)) or more after 6 months was explored by univariate and logistic regression analysis.

**RESULTS**

**RELATIONSHIP BETWEEN PbB AND ZPP CONCENTRATIONS**

The correlation between PbB and logZPP in a total of 807 blood samples was 0.583. PbB exceeded 1.93 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) in 414 (51%), and 2.90 \( \mu \text{mol/L} \) (\(60 \mu \text{g/dL} \)) in 105 (14%) of the blood samples.

Receiver-operator curve estimates of the test properties of various ZPP thresholds for concurrent toxic PbB concentrations (Fig. 1) indicated that a ZPP threshold of 0.71 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) had a sensitivity and specificity of 84.5% and 63.6%, respectively, for a PbB concentration above 1.93 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) and 96.2% and 44.2%, respectively, for a PbB concentration of 2.90 \( \mu \text{mol/L} \) (\(60 \mu \text{g/dL} \)). A ZPP threshold of 0.35 \( \mu \text{mol/L} \) (\(20 \mu \text{g/dL} \)) had a sensitivity and specificity of 95.7% and 19.1%, respectively, for a PbB above 1.93 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) and 99.0% and 13.1%, respectively, for a PbB of 2.90 \( \mu \text{mol/L} \) (\(60 \mu \text{g/dL} \)).

These test properties indicate that, in a hypothetical population with a 1% prevalence of PbB concentrations exceeding 1.93 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) or more, a policy of PbB-testing of only those with ZPP above 0.71 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) would obviate 35% of the PbB tests and miss about 3 cases with this PbB concentration per 200 persons at risk. However, given a 50% prevalence of PbB concentrations exceeding this concentration, such as that in our study population, such a policy would obviate 26% of the PbB tests but would miss about 8% of the cases with this PbB concentration among the workers at risk (Fig. 2A). Given a 10% prevalence of PbB concentrations exceeding 2.90 \( \mu \text{mol/L} \) (\(60 \mu \text{g/dL} \)), similar to that in our exposed workers, a policy of PbB testing only of those with ZPP above 0.71 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) would obviate 42% of the PbB tests but would miss about 3 cases with this PbB concentration in every 200 workers at risk (Fig. 2B).

**PREDICTIVE VALUE OF PbB AND ZPP CONCENTRATIONS FOR PbB CONCENTRATIONS 6 MONTHS LATER**

**Univariate analysis.** Breakdown of PbB, ZPP, age, systolic blood pressure, diastolic blood pressure, hemoglobin concentration, white blood cell count, and body mass index (body weight divided by square of height) by PbB concentration [less or more than 2.90 \( \mu \text{mol/L} \) (\(60 \mu \text{g/dL} \))] 6 months later, revealed that PbB, ZPP, age, and body mass index had a predictive value for PbB (Table 1).

The predictive value of PbB and ZPP for blood lead concentrations exceeding 1.93 \( \mu \text{mol/L} \) (\(40 \mu \text{g/dL} \)) 6 months later are presented in Table 2. Of a total of 129 pairs of test results with PbB <1.45 \( \mu \text{mol/L} \) (\(30 \mu \text{g/dL} \)) and low ZPP <0.73 \( \mu \text{mol/L} \) (\(41 \mu \text{g/dL} \)), only 8 (6.2%) had toxic PbB concentrations 6 months later. Intermediate [1.44–1.88 \( \mu \text{mol/L} \) (\(30–39 \mu \text{g/dL} \))] PbB and intermediate [0.73–1.75 \( \mu \text{mol/L} \) (\(41–99 \mu \text{g/dL} \))] ZPP concentrations were associated with a sevenfold increase in the risk for a toxic PbB 6 months later, relative to those with low PbB and ZPP concentrations. A ZPP concentration exceeding 1.75 \( \mu \text{mol/L} \) (\(99 \mu \text{g/dL} \)) with concurrent nontoxic PbB concentrations was associated with a 12-fold increase in the risk of toxic PbB concentrations 6 months later, whereas high ZPP and high PbB concentrations were associated with a 15-fold increase in this risk.

Table 3 presents the predictive values of PbB and ZPP concentrations, taking into account the ZPP thresholds and PbB concentrations.
for toxic blood lead concentrations, defined as those exceeding 2.90 μmol/L (60 μg/dL). Of a total of 250 pairs of test results with PbB 1.93 μmol/L (40 μg/dL) and low ZPP, 0.73 μmol/L (41 μg/dL), only 3 (1.2%) had toxic PbB concentrations 6 months later. Intermediate [1.93–2.85 μmol/L (40–59 μg/dL)] PbB and intermediate [0.73–1.75 μmol/L (41–99 μg/dL)] ZPP concentrations were associated with a 14-fold increase in risk for a toxic PbB 6 months later, relative to those with low PbB and ZPP concentrations. A ZPP concentration exceeding 1.75 μmol/L (99 μg/dL), even with concurrent nontoxic PbB concentrations, was associated with a 28.7-fold increase in the risk of toxic PbB concentrations 6 months later. High ZPP and high PbB concentrations were associated with a 44-fold increase in this risk.

**Multivariate analysis.** Logistic regression revealed predictive values (odds ratios with 95% confidence limits) for PbB on follow-up of 2.13 (1.34–3.38) for PbB, 4.05 (2.44–6.73) for ZPP, 1.47 (0.73–2.94) for age, and 1.81 (1.03–3.25) for body mass index.

**Discussion**

The presented findings were derived from a selected population of exposed workers and should not be generalized over other populations. Still, we believe that they warrant two conclusions. The first conclusion is that the sensitivity and specificity of ZPP for a concurrent toxic PbB concentration, whether defined as 1.93 or 2.90 μmol/L (40 or 60 μg/dL) or more, are not high enough to justify a policy of PbB-testing in only exposed workers with ZPP exceeding a predetermined threshold concentration. Such a policy may or may not be useful in populations with extremely low prevalence of lead toxicity but not in lead-exposed workers similar to those in our study population, with a 14% prevalence of PbB concentrations above 2.90 μmol/L (60 μg/dL) and a 51% prevalence of PbB concentrations above 1.93 μmol/L (40 μg/dL). In such cases, we cannot afford the false-negative rate of ZPP determinations. False-negative rates may be reduced, of course, by using lower ZPP thresholds with higher sensitivity for toxic PbB concentrations; however the higher false-positive rate reduces the savings incurred by obviating PbB testing.

**Table 1. Values for biological variables obtained at initial physical examination for 94 workers in a lead battery factory (1980–1993), separated according to PbB values obtained at 6-month follow-up.**

<table>
<thead>
<tr>
<th>Variables on index examination</th>
<th>&gt;2.90 μmol/L (60 μg/dL)</th>
<th>&lt;2.90 μmol/L (60 μg/dL)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>40.2 ± 9.3</td>
<td>37.6 ± 9.5</td>
<td>.003</td>
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<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79.5 ± 8.1</td>
<td>78.5 ± 5.9</td>
<td>.17</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>113.9 ± 15.2</td>
<td>112.0 ± 12.6</td>
<td>.19</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>69.1 ± 3.8</td>
<td>68.6 ± 4.1</td>
<td>.19</td>
</tr>
<tr>
<td>White blood count/cmm</td>
<td>7688 ± 2543</td>
<td>7938 ± 2226</td>
<td>.21</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>157 ± 10</td>
<td>158 ± 11</td>
<td>.33</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.6 ± 3.8</td>
<td>25.2 ± 3.3</td>
<td>.001</td>
</tr>
<tr>
<td>Body mass index (% ≥ 27.8 kg/m²)</td>
<td>46.9</td>
<td>27.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PbB values [% ≥2.90 μmol/L (60 μg/dL)]</td>
<td>36.9</td>
<td>8.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ZPP values [% ≥1.77 μmol/L (100 μg/dL)]</td>
<td>45.1</td>
<td>10.4</td>
<td>&lt;.001</td>
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</table>

* Mean ± SD.
The second conclusion relates to the optimal frequency of PbB monitoring. Frequent monitoring of PbB certainly confers a higher safety; however, it costs more. Our analysis suggests that this frequency should be adjusted according to the risk of future toxic PbB concentrations in individual workers. This risk may be estimated by present PbB and ZPP concentrations. For example, a ZPP of 1.77 μmol/L (100 μg/dL) or more, with a concurrent nontoxic PbB of 2.85 μmol/L (59 μg/dL) or less was associated with an ~30-fold increase in relative risk for a PbB above 2.90 μmol/L (60 μg/dL) 6 months later. Although increased ZPP concentrations in and of themselves are not considered to warrant decisions regarding future employment, they do identify those workers who should be more frequently monitored for lead toxicity.

We recommend including both PbB and ZPP determinations during monitoring of lead-exposed workers and adapting the frequency of these determinations according to the estimated risk of individual workers. PbB determinations remain the most suitable method for monitoring current lead toxicity, whereas increased ZPP concentrations, even with concurrent nontoxic PbB, have a predictive value for incipient lead toxicity. According to existing regulations, workers whose PbB exceeds a predefined toxic concentration, whether 1.93 or 2.90 μmol/L (40 or 60 μg/dL), should be withheld from additional exposure. ZPP concentrations exceeding 0.71 μmol/L (40 μg/dL), with concurrent nontoxic PbB concentrations, warrant "closer follow-up". The frequency of examinations during this closer follow-up should be a compromise between the estimated individual risk and the costs incurred by laboratory expenditures and loss of work time.

References

6. Singer R, Valciukas JA, Lilis R. Lead exposure and nerve conduc-