Use of Zinc Protoporphyrin in Screening Individuals for Exposure to Lead

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We studied the relation between the concentrations of lead in blood (PbB) and zinc protoporphyrin in blood (ZPP) in a group of 801 men occupationally exposed for more than one year to lead or inorganic lead compounds. Linear regression of PbB on log ZPP provided 95% tolerance intervals for PbB values for a given ZPP value. The intervals we found are too large to warrant the estimation of PbB on the basis of ZPP measurements in health surveillance of lead workers. Instead we propose a procedure in which ZPP can be used as an indicator to decide which individuals exposed to lead need further investigation of PbB in light of existing limit values for PbB. The procedure is applicable only for PbB values of 2.4 μmol/L or more but may reduce considerably the costs for screening individuals or groups of people exposed to lead.

Additional Keyphrases: reference values • receiver-operating characteristic curves

Exposure to lead and its inorganic compounds leads to an increased concentration of lead in blood (PbB). It has now generally been accepted that, under conditions of more or less constant and prolonged exposure, PbB reflects the quantity of "biologically active" lead in the body (1–4). Analysis of PbB is, therefore, the first choice for the assessment of internal exposure of lead. Moreover, assessment of health risks due to exposure to lead is generally based on the concentration of lead in blood. However, determination of PbB is expensive and time-consuming, especially if large groups of people have to be screened.

As early as the 19th century, patients suffering from lead intoxication were recognized as showing symptoms of effects by lead on the formation of heme (5). Thus, these effects have been investigated extensively (6). Lead inhibits at least two enzymes that are essential for the formation of heme, namely, δ-aminolevulinate dehydratase (ALA-D; EC 2.6.1.43) and ferrochelatase (EC 4.99.1.1).1 Both the inhibition of ALA-D in erythrocytes and the resulting increase in excretion of the substrate δ-aminolevulinic acid in urine are used for early detection of the biological responses to lead, and indirectly as a measure of increased internal exposure of lead. Because of the interaction of lead with ferrochelatase in bone marrow, no iron is inserted into the substrate protoporphyrin IX, so that the concentration of the latter is increased in erythrocytes. Lamola and Yamane (7) showed that instead of iron, zinc is incorporated into this free erythrocyte protoporphyrin to form zinc protoporphyrin (ZPP). ZPP thus reflects an effect of lead on the hemopoietic system, mainly resulting from the deposits of lead in the bone marrow. PbB, on the other hand, reflects both recent and earlier exposure. Numerous authors [see review by Wildt et al. (8)] have shown that under steady-state conditions of exposure the logarithm of ZPP is linearly related to PbB. Iron-deficiency anemia may also result in an accumulation of ZPP, whereas in erythropoietic protoporphyrina, a rare inborn error of metabolism, the increased protoporphyrin in erythrocytes remains mainly unchelated.

The concentration of ZPP can be measured directly in a smear of blood, which can be drawn from an ear lobe or obtained by a finger prick. Because of the simplicity of the measurement, which does not require specially qualified people, determination of ZPP has been applied as another method to screen lead workers for a biological response to an increased exposure to lead (1, 8–10). Here we further contribute to the discussion on the validity of using the concentration of ZPP to estimate PbB. We propose using the ZPP value and the statistical correlation between PbB and ZPP to decide, on an individual basis, whether additional analysis of PbB is necessary to assess if someone has an increased health risk due to lead exposure.

Subjects and Methods

We studied 801 men (ages 16–64 years) employed in lead-processing industries and exposed to lead or inorganic lead compounds for at least one year. Of this group, 55% were Dutch by birth, the others were of Mediterranean origin. The men were involved in the production of batteries, pigments, or soldered cans or were employees of metal recycling industries. Most of them have been checked for lead in blood several times over the last few years; for those cases, we used the first measurement in this study. In general, the individual PbB values showed relatively little variation over time. In view of the nature and time of exposure (more than a year), steady-state concentrations of PbB in the bone marrow are likely.

Venous blood was collected in 5-mL lead-free tube containing 7.5 mg of disodium EDTA (Venoeject, Terumo, Italy). We mixed 100 μL of blood with 900 μL of 1 mol/L nitric acid solution with swirling on a shaker. The precipitate was centrifuged and the lead in the clear supernatant was determined by atomic absorption spectrophotometry (Model 4000, equipped with a Model 500 graphite atomizer Perkin-Elmer, Haarzode, Belgium) (11). The quality of the analysis was checked by participation in the U.K. External Quality Assurance Scheme of the Queen Elizabeth Hospital, Birmingham, U.K. At three-week intervals, we analyzed lead-supplemented human blood samples. On the average, our results differed by 7.7% (range 0–22.6%) from the average results of 80 participants, but there was no systematic deviation. We determined the concentration of ZPP with a direct-reading hematofluorometer (Model 4000 Environmental Sciences Associates, Bedford, MA) after oxygenation of a 100-μL blood sample by swirling on a shaker. The apparatus, which measures the ratio of the fluorescence of ZPP and the absorbance of light by oxyhemoglobin in the sample, was calibrated at a fixed concentration of hemoglobin (Hb), 8.4 mmol/L (for men). The Ct for 15 analyses of the same sample carried out on five successive days was 2.6% at a ZPP content of 95 μmol/mL.

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1 Nonstandard abbreviations: ALA-D, aminolevulinate dehydratase; ZPP, zinc protoporphyrin; and Hb, hemoglobin.

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HB and about 1.5% at concentrations of ZPP between 150 and 625 μmol/mol HB.

The 95% tolerance intervals for PbB as calculated for log ZPP (Figure 1) are the simultaneous tolerance intervals of Lieberman Miller (cf. 12), based on the regression of PbB on log ZPP. For PbB, 1 μmol/L corresponds to 207 μg/L; for ZPP, 1 μmol/mol HB corresponds to 25 μg/g HB.

Results and Discussion

Figure 1 shows the relation between PbB and log ZPP. The highest concentration of PbB was 6.2 μmol/L and of ZPP 1253 μmol/mol HB. Assuming a linear relation, the regression of log ZPP on PbB gives the following equation:

\[
\text{log ZPP} = 0.56 \text{ PbB} + 0.86 \ (r = 0.83, P < 10^{-4})
\]

This relation is in good agreement with results of other studies 8).

Table 1 lists 95% tolerance intervals for PbB at four different concentrations of ZPP, obtained by regressing PbB on log ZPP separately for the Dutch workers, the Mediterranean workers, and the whole group. Given the considerable overlap of the intervals of the Dutch and the Mediterranean workers, we decided to treat the Dutch and Mediterranean workers as a single population. Considering the four occupations studied—production of batteries, of pigments, or of soldered cans, and metal recycling—separately only slightly narrows the PbB tolerance intervals. The upper values remain more than twice the lower values.

The large ZPP-related intervals for PbB preclude an acceptable estimation of the concentration of PbB on the basis of ZPP. The width of the intervals is partly explained by the absence of a strong biological relation between PbB and ZPP. Close correlation between PbB and ZPP exists only under steady-state conditions of exposure because of the time lag in the formation of ZPP (13). Although the exposure of most of the present lead workers meets this requirement, as shown by analysis of blood samples of the same workers over several years, other factors probably also influence the width of the intervals, for example:

- The kinetics of lead absorption and formation of ZPP may differ among people.

### Table 1. 95% Tolerance Intervals for PbB at Different Concentrations of ZPP

<table>
<thead>
<tr>
<th>ZPP, μmol/mol HB</th>
<th>Dutch workers (n = 556)</th>
<th>Mediterranean workers (n = 246)</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>1.48–4.05</td>
<td>1.44–4.09</td>
<td>1.52–4.02</td>
</tr>
<tr>
<td>500</td>
<td>2.00–4.84</td>
<td>1.86–4.63</td>
<td>2.03–4.58</td>
</tr>
<tr>
<td>750</td>
<td>2.31–4.99</td>
<td>2.13–4.95</td>
<td>2.33–4.92</td>
</tr>
<tr>
<td>1000</td>
<td>2.52–5.23</td>
<td>2.31–5.17</td>
<td>2.54–5.16</td>
</tr>
</tbody>
</table>

- Iron deficiency contributes to an increase in ZPP (7).
- Interindividual variability affects the kinetics of formation of ZPP.
- Increased concentrations of bilirubin in serum have a small, positive effect on the ZPP reading (10).
- Analytical variation in the analysis of both analytes, especially PbB, may affect the results. (The orders of magnitude of these variations have already been given.)

In 1982 the Council of Ministers of the European Community (14) issued a Directive on the protection of workers against health risks due to exposure to lead. For PbB, three so-called action values (1.45, 2.4, and 2.9 μmol/L) and a limit value (3.4 μmol/L) have been defined. Concentrations of PbB between 3.4 and 3.9 μmol/L are considered acceptable if ZPP is <500 μmol/mol HB.

Because for many ZPP values the resulting PbB intervals include the action or limit values as given in the European Community Directive, giving rise to indecisive worker policy, we conclude that an acceptable estimate of PbB by measuring ZPP is not possible in health surveillance of individual lead workers. Individual ZPP values may be used only as a very rough indicator of individual PbB, and the average ZPP of a group of workers only approximates the average PbB. This conclusion is supported by the work of Herber (9) and Grandjean and Lintrup (10). Accepting PbB as the best marker to estimate health risk implies that every worker who is possibly exposed to lead should be monitored for PbB at regular intervals. For this reason, in the U.S. as well as in the European Community, assessment of PbB is required in workplace monitoring. However, detection of individuals with high PbB concentrations by screening for ZPP would be desirable because the latter is considerably less costly. Therefore, we further analyzed the relationship between PbB and ZPP.

Using the PbB and ZPP data of the 801 lead workers, we calculated the sensitivity and specificity of different ZPP cutoff points at the five standard values of PbB mentioned in the Directive of the European Community. (Within this context, sensitivity and specificity refer to the epidemiological characteristics of the relationship between PbB and ZPP. That is, the sensitivity is defined as the percentage of the cases with ZPP values above a chosen cutoff point, given a PbB value above a chosen standard value. The specificity is defined as the percentage of the cases with ZPP values below a chosen cutoff point, given a PbB value below a chosen standard value. These percentages depend on the characteristics of the population under study.)

Appropriate cutoff points for ZPP at the four PbB standard values can be derived from plots of sensitivity against (1 − specificity), the so-called receiver-operating character-
istic (ROC) curves (15) (Figure 2). The optimum cutoff point is determined as the point the farthest from the diagonal; the cutoff point maximizes the discriminative power of the test by minimizing the fractions of false-positive and false-negative results. Table 2 gives the optimum ZPP cutoff points at the five PbB standard values as derived from Figure 2.

Screening with the aim of meeting the above Directive demands a sensitivity of 100% because every person with a PbB concentration exceeding an action value or the limit value should be identified. To have a sensitivity of 100%, one must use a lower cutoff point of ZPP and thus a lower specificity, leading to suboptimal discriminative power (Table 2). Table 2 shows that ZPP cannot be used as a discriminative indicator for the lowest PbB standard value (specificity = 0%). In that case all samples would have to be analyzed for PbB. This parallels the report of Meredith et al. (16).

The positive predictive value of a screening result (i.e. the fraction of the samples for which PbB exceeds the standard PbB value of all samples for which ZPP exceeds the chosen cutoff point) depends not only on sensitivity and specificity, but also on the prevalence in the study population of values of PbB that exceed the standard value under consideration: the lower the prevalence, the greater the number of false-positive test results. Table 3 presents positive predictive values of ZPP cutoff points that yield a sensitivity of 100% at different prevalences.

ZPP can be used satisfactorily for pre-screening only if one can estimate reasonably accurately the prevalence of the standard PbB values in the population under study. Recently, Health Hazard Surveys carried out in several lead-processing industries in The Netherlands (17) have reported prevalences of 60%, 33%, 19%, and 10% for PbB >2.4, >2.9, >3.4, and >3.9 μmol/L, respectively, in battery works (n = 200); 25%, 14%, 7%, and 3%, respectively, in pigment production (n = 556); and 100%, 87%, 87%, and 87%, respectively, in flame cutting of lead-painted con-

![Table 2. ZPP Cutoff Points for Optimum and Required Sensitivity, and Related Specificity, at Five Values for PbB](image)

<table>
<thead>
<tr>
<th>PbB, μmol/L</th>
<th>ZPP, μmol/L</th>
<th>Optimus, %</th>
<th>ZPP, μmol/L</th>
<th>Required, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.45</td>
<td>55</td>
<td>80</td>
<td>87</td>
<td>0</td>
</tr>
<tr>
<td>2.4</td>
<td>90</td>
<td>95</td>
<td>84</td>
<td>40</td>
</tr>
<tr>
<td>2.9</td>
<td>170</td>
<td>90</td>
<td>90</td>
<td>45</td>
</tr>
<tr>
<td>3.4</td>
<td>250</td>
<td>94</td>
<td>88</td>
<td>120</td>
</tr>
<tr>
<td>3.9</td>
<td>250</td>
<td>100</td>
<td>86</td>
<td>250</td>
</tr>
</tbody>
</table>

* Action and limit values specified in European Community Directive (see text).

b ZPP cutoff points as derived from Fig. 2.

![Table 3. Positive Predictive Value (PPV) of ZPP Cutoff Points at Different Prevalences of Four Values for PbB](image)

<table>
<thead>
<tr>
<th>PbB, μmol/L</th>
<th>ZPP</th>
<th>Sens., %</th>
<th>Spec., %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>40</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2.9</td>
<td>45</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3.4</td>
<td>120</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3.9</td>
<td>250</td>
<td>100</td>
<td>86</td>
</tr>
</tbody>
</table>

* Sensitivity as required by the European Community Directive.

b Specificity as derived from Fig. 2.
structions (n = 8) (18). Rifle instructors showed no values for PbB > 2.4 μmol/L (n = 125). Hernberg and Tola (19) reported decile distributions and ranges of PbB values found in different types of work in Finland. These results allow an estimation of the prevalences in groups of lead workers.

The ultimate goal of the use of ZPP as a screening method is an economical one. Does the method save labor and time over screening via PbB? Potential savings can be computed with the formula S = nP - (nZ + fP), where S = savings, n = the number of samples, P = the cost of a PbB analysis, Z = the cost of a ZPP analysis, and f = the number of samples with true- and false-positive ZPP values that require additional PbB analysis. Figure 3 gives the relation between savings and percentage of positive ZPP values, assuming that the cost of a ZPP analysis is about 10% of the cost of a PbB analysis, which is the case with our laboratory. Savings vary between 10% “negative savings” (when all samples have positive ZPP values) and 90% of the costs of PbB analysis (when no samples have positive ZPP values).

In summary, measuring ZPP to screen lead workers for a PbB of 1.45 μmol/L has no economical value. All such samples must also be analyzed for PbB. However, screening for ZPP to detect the higher “action values” and the “limit value” for PbB may save considerable costs.

The results in this study were obtained from a Health Hazard survey in lead-processing industries, financed by the Directorate-General of Labour of the Ministry of Social Affairs and Employment in The Netherlands. We thank Mr. F. J. A. Krouwenberg and Mrs. M. E. van Pijkeren for their skillful assistance in carrying out the analyses of lead and zinc protoporphyrin, and Mr. J. F. Bleichrodt, Dr. J. J. van Hemmen, and Prof. Dr. R. L. Zielhuis for advice in preparing this manuscript.

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