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Zinc-Protoporphyrin Determination as a Screening Test for Lead-Exposure in Childhood

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Lead is an ubiquitous, toxic metal to which we are exposed in normal life since its principal source is the combustion of leaded petrol. Although the clinical picture of lead intoxication is well established, it is known that exposure to low doses of lead may produce toxic effects, particularly in the developing nervous system of children, which will be persistent and irreversible (Finkelstein et al. 1998). Lead exposure has therefore been considered to be the most severe environmental disease in American children (Needleman 1993).

Lead levels considered to be toxic have progressively decreased as noxious effects of the metal have been discovered at increasingly lower exposure concentrations. As in the 1970s blood lead levels (BPb) of 30 μg/dL were considered safe, present knowledge indicates that toxic manifestations begin with exposures above 10 μg/dL, without assuring the innocuity of exposure at lower lead doses; the only really safe level is 0 μg/dL (American Academy of Pediatrics1987). Since the effects of low-dose lead exposure are clinically inapparent, the only efficacious means of control will be exposure prevention acting on sources of the metal. In 1991, the Centers for Disease Control recommended massive screening of children to detect exposure and influence control measures prior to the onset of early harmful effects (Pantell et al. 1993).

The appropriate screening technique had to be easy to perform, rapid, reliable and economical, with zinc protoporphyrin (ZPP) determination being recommended as a screening method for lead intoxication, given the good correlation between high blood lead levels and increases in ZPP (Blumberg et al. 1987, Kammholz et al. 1972, Peter et al. 1978). This relationship is based on the fact that one of the main toxic effects of lead is produced on heme biosynthesis. Lead provokes ferrochelatase (heme synthetase) inhibition, which impedes binding of iron to protoporphyrin IX, thus leading to protoporphyrin accumulation. This increase in protoporphyrins would serve as a toxic-exposure marker (Lichtman and Feldman 1963, Takebayashi et al. 1993).

This increase in protoporphyrins following Pb exposure is produced only at high exposure levels (over 20 μg/dL), showing good correlation with

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blood lead levels above 40 μg/dL (Froom et al. 1998, Leung et al. 1993,Piomelli et al. 1973, Yip et al. 1983). This rise, at high BPb, limits the usefulness of ZPP determination in the screening of individuals exposed to toxic lead levels, since it would fail to detect those presenting BPb below 20 μg/dL.

The aims of this study were to assess the usefulness of ZPP determination for screening lead exposure in children, bearing in mind the gradual drop in the lead toxicity threshold in the last few years, and detect the optimum ZPP cut-off point for screening at the currently considered toxic levels.

MATERIALS AND METHODS

The study was conducted between January 1 and December 31, 1993, at the Hospital Materno-Infantil Vall d'Hebron, Barcelona (Spain); blood samples came from children of the Metropolitan Area of Barcelona. From routine extractions performed at the hospital laboratory, a convenience sampling was made, and all samples drawn on the days when BPb and ZPP determination could be performed.

Blood was obtained by venopuncture using stainless steel needles and disposable plastic syringes and placed in tubes with EDTA as anticoagulant (Microtainer, Becton Dickinson, USA). Samples were stored at 4°C until processing.

An atomic absorption spectrophotometer with a graphite furnace (Perkin-Elmer, USA) was used for BPb determination following the method previously optimised in our laboratory (Sole et al. 1998). Under the described analytical conditions, the limit of Pb detection (signal equivalent to three times the background noise) was approximately 0.009 μmol l^{-1}. All samples were analysed in triplicate and results referred to a standard curve performed previously at each session. Standards with a certified amount of Pb (CRDL Std 2, Radian Corp. USA) were intercalated among samples to ensure accuracy and reliability of each analysis. Samples which did not differ by more than 2% of the standard value were considered acceptable.

ZPP was determined by hematofluorimetry (Helena Laboratories, USA) in the same samples using the usual method for this technique. Following calibration with known protoporphyrin values, and with the same precision criteria as above, the instrument shows protoporphyrin concentration in μg/dL. The results obtained were corrected by the individual's haemoglobin value and converted into μmol ZPP/mol heme. All samples were analysed in triplicate and controls with a known amount of ZPP intercalated among them for accuracy verification. Haemoglobin determination was also performed using a Coulter automatic counter.

To assess ZPP as a screening method for lead exposure and to detect its optimum cut-off point, sensitivity, specificity and predictive values were calculated for different ZPP cut-off values. These results were plotted in Receiver Operator Characteristic (ROC) curves, which graphically

represent the relationship between sensitivity and specificity for each of the cut-off points (Burgueño et al. 1995). The best cut-off point would be that which presents high values for both sensitivity and specificity and would therefore be near the upper left corner of the graph. Overall precision of the test for all cut-off points was assessed by calculation of the area below the curve (ABC), which is defined as the probability of correctly classifying a pair of individuals (one healthy and one sick) randomly chosen from the population, using the results obtained when applying to them the diagnostic test. Values above 0.7 indicate a good discriminative capacity of the test. Calculation of the confidence interval also aids utility assessment of the test, if the interval does not include the null value (0.5) it may be concluded that the test is useful for detecting intoxicated subjects. The method of Garrido et al. (Garrido and Madero, 1996) was used for calculation of the area below the ROC curve and its confidence interval. Linear regression was calculated to assess the correlation between BPb and ZPP values.

A total of 1158 blood samples from the same number of children was analysed. Ages ranged from neonates to 15 years, with a mean of 6.32 years and a standard deviation of 4.63 years. The sample presented a slight predominance of males (n=637; 55%) to females (n=521; 45%).

RESULTS AND DISCUSSION

Lead poisoning prevalence: According to current recommendations considering an individual to have lead poisoning when blood lead levels exceed 10 μg/dL, 49 of the 1158 children analysed presented levels within the toxic range, which represents an intoxication prevalence of 4.2%. Sixteen children presented BPb values above 15 μg/dL (1.4%), and 6 (0.5%) above 20 μg/dL.

Zinc protoporphyrin: ZPP values of the children studied were 55.2 ± 0.72 μmol ZPP/mol heme (mean ± SEM). The distribution histogram of ZPP values is shown in figure1. Increases in ZPP values are only seen in children with blood lead values over 20 μg/dL, much higher than the limit of toxicity (figure 2).

Assessment of ZPP as a marker for Pb exposure: Figure 3 shows the ROC curves for different BPb decision thresholds (10, 15 and 20 μg/dL). No ZPP cut-off point presents high sensitivity and specificity values (near the upper left corner of the graph) in any of the curves, although values are better at the higher BPb decision threshold.

Sensitivity and specificity values for different ZPP cut-off points in each of the lead exposure thresholds considered are presented in Table 1. Values of areas below the curve for each BPb threshold considered and their standard errors and 95% confidence intervals are shown in Table 2.

In the present study we attempted to assess the usefulness of ZPP determination for the detection of lead poisoning in our geographic area for which high levels of lead pollution have not been described.

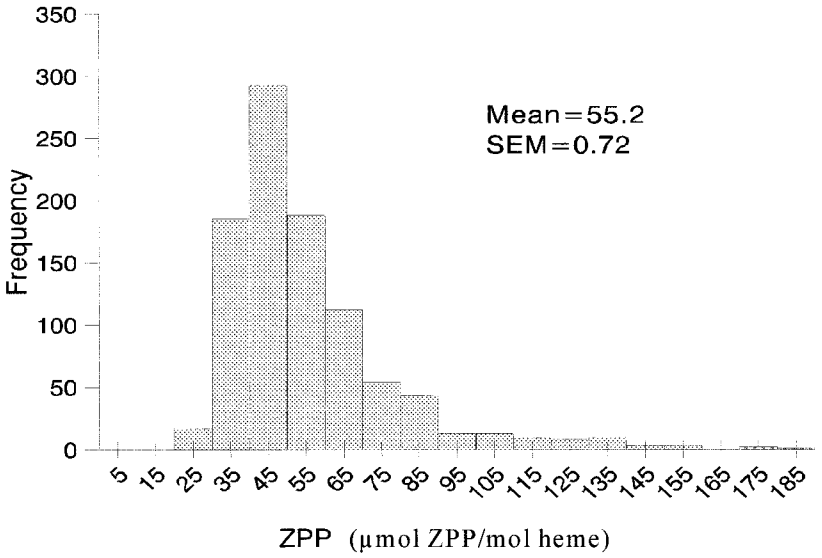

Figure 1. Distribution histogram of ZPP values in the children of our series. Each bar represents an interval of 10 µmol ZPP/mol heme.

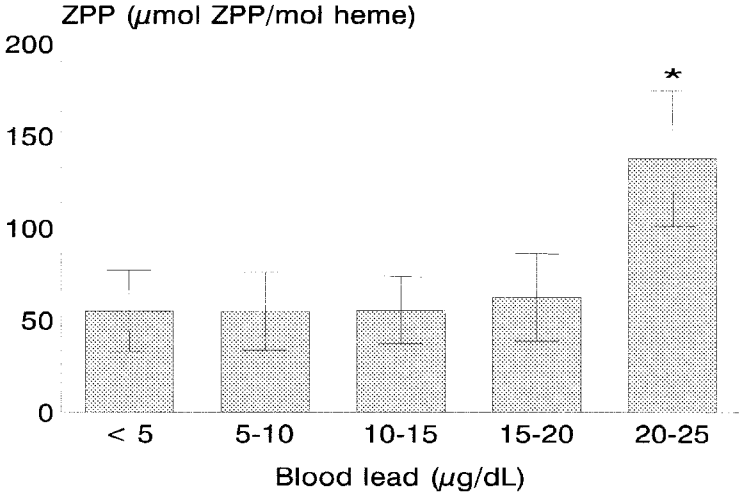

Figure 2. ZPP values related to plumbemia. Children were grouped in 5 mg/dL of BPb intervals. * Difference statistically significant ($p<0.05$).

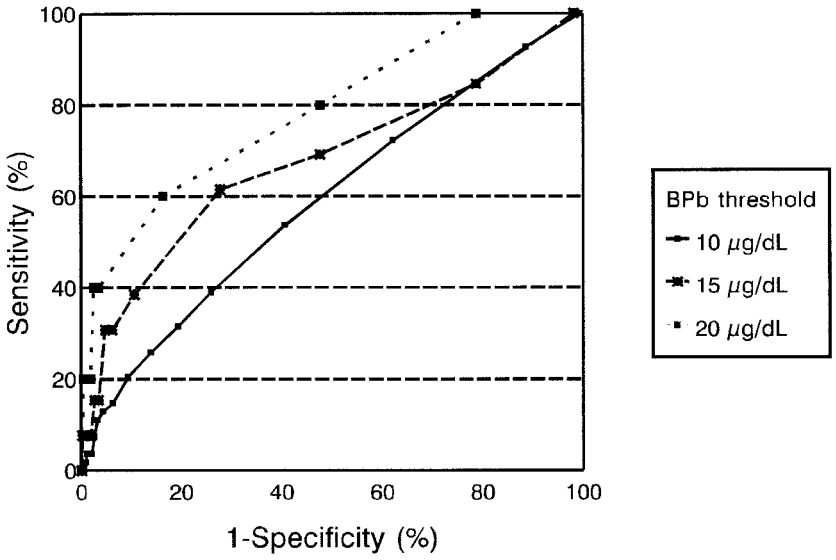

Figure 3. Receiver Operator Characteristic (ROC) curves for each of the blood lead thresholds evaluated: 10, 15 and 20 µg/dL. Plots represent different ZPP cut-off points.

Table 1. Sensitivity and specificity values for different ZPP cut-off points considering different BPb thresholds.

	BPb 10 µg/dL		BPb 15 µg/dL		BPb 20 µg/dL	
ZPP cut-off	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
30	100	0.66	100	1.9	100	1.9
60	53.7	59.3	61.5	72.3	60	72
90	25.9	86	30.8	93.8	40	93.8
120	13	95.6	15.4	97.3	20	97.4
150	3.7	97.9	7.7	99.5	15	99.5
180	1.85	99.1	0	100	0	100

Table 2. Area below the ROC curves for different BPb threshold levels.

BPb threshold	**Area Below Curve**	**SEM**	**95% Confidence Interval**
10 µg/dL	0.52	0.01	0.49-0.55
15 µg/dL	0.575	0.03	0.51-0.64
20 µg/dL	0.59	0.06	0.47-0.72

BPb= Blood lead level. SEM= Standard error for the mean

Although the use of ZPP has been recommended for population screening of lead exposure owing to the advantages presented by the method over BPb determination (economy, ease of use and transport), recent studies (Leung et al. 1993) have shown that these ZPP elevations are produced only at BPb levels of 20-40 μg/dL, much higher than the toxicity limit currently considered.

The usefulness of the test as a screening method is determined by its capacity to detect children who present blood lead values above 10 μg/dL. Thus, for different ZPP cut-off points, sensitivity, specificity and predictive values were calculated.
Sensitivity of the test (capacity to detect intoxicated individuals) was high for low cut-off points (30 - 40 μmol ZPP/mol heme). If these cut-off points were used, all intoxicated subjects would be detected, but on the basis of considering as exposed many individuals who are really not, since at these cut-off levels specificity (capacity to detect a non-intoxicated individual) is very low (from 0.66 to 1.9%).

Furthermore, it should be taken into account that these ZPP levels lie within the normal range (prevalence of ZPP > 40 in our series was 88%) and therefore the majority of the population would be above the cut-off point. If much higher cut-off points, around 120, were used, the situation would be inverted. At these ZPP levels, the test proves highly specific, i.e. those who presented a negative test would in all likelihood not be intoxicated, but sensitivity would be extremely low (between 13 and 20% depending on the BPb threshold considered).

The optimal cut-off point will be that which presents high values of both sensitivity and specificity and will therefore be capable of differentiating intoxicated individuals with a high probability. The ZPP value that presents better results of sensitivity and specificity is 60 μmol ZPP/ mol heme. At this cut-off point, 53.7% sensitivity is obtained, with 59.3% specificity, although these values remain very low since almost half of the individuals would be incorrectly classified. For screening purposes, a test which is only capable of detecting 53% of exposed subjects and which presents a negative result in only 59% of those not exposed would not suffice.

Considering the results obtained when Positive and Negative Predictive values of the test are calculated for the different cut-off points, we reach the same conclusions. For all cut-off values of ZPP, Positive Predictive Values (PPV) are around 95%, and Negative Predictive Values are under 20%.

The prevalence of ZPP values above the chosen cut-off points should also be taken into account. At low cut-off points, the majority of the population will present values higher than that chosen, presenting positive values in the test without being intoxicated, while with high cut-off points most of the population will be below.

Other published studies reach similar conclusions. High specificity with low sensitivity is achieved with high ZPP cut-off points, and, inversely, lower cut-off points show low specificity with high sensitivity, so no cut-off point

that may be useful to detect lead exposure can be established (Leung et al. 1993, Turk et al. 1992). Studies of Leung (Leung et al. 1993) and McElvaine (McElvaine et al. 1991) found sensitivity of 27% and specificity of 85% using ZPP cut-off points of 35 μmol ZPP/mol heme. Using the same cut-off points, DeBaun found true positive and false positive result rates of 23% and 4%, respectively (DeBaun and Sox 1991). Similar results were reported by Turk (Turk et al. 1982) who found that 65% of individuals with BPb values over 25 μg/dL presented ZPP values lower than 35 and would therefore be undetected.

This relationship between sensitivity and specificity to determine the usefulness of a diagnostic test can be represented on the ROC curve, which constitutes the most adequate method for evaluating the usefulness of a test when it presents several possible cut-off points and which represents graphically the relationship between sensitivity and specificity.

In our sample, the curves obtained are better the higher the BPb threshold considered. The area below the ROC curve (an overall measure of the test's value) in our series was 0.521 for the currently accepted toxicity threshold (0.48 μmol/L), with a 95% confidence interval from 0.49 to 0.55. This result indicates that it is only capable of correctly classifying 52.1% of cases, whereas at random 50% would be classified correctly. The confidence interval, which includes the 0.5 value, supports the lack of ability of the test to distinguish between sick and healthy individuals. Our results are similar to those described by Leung (Leung et al. 1993) and Rolfe (Rolfe et al. 1993), who also found an area below the ROC curve lower than 0.6.

A greater usefulness of the test is observed when higher BPb thresholds are used: the area below the curve increases with the increase in the threshold considered (Table 1), approaching an acceptable value for decision limits of 20 μg/dL of blood lead (ABC = 0.59).

Our data confirm the scant utility of ZPP for lead poisoning screening, and the absence of optimal cut-off values. The lack of utility of this test signifies that at present the only recommended method for lead intoxication screening is determination of blood lead levels.

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REFERENCES

American Academy of Pediatrics (1987) Committee on environmental hazards. Committee on accident and poison prevention. Statement on childhood lead poisoning. Pediatrics 79: 457-465.

Blumberg WE, Eisinger J, Lamola AA, Zuckerman DM (1977) Zinc protoporphyrin level in blood determined by a portable hematofluorometer: a screening device for lead poisoning. J Lab Clin Med 89: 712-723.

Burgueño MJ, García-Bastos JL, González Buitrago JM (1995) Las curvas

ROC en la evaluación de las pruebas diagnósticas. Med Clin (Barc) 104: 661-670.
DeBaun MR, Sox HC (1991) Setting the optimal erythrocyte protoporphyrin screening decision threshold for lead poisoning: a decision analytic approach. Pediatrics 88: 121-131.
Finkelstein Y, Markowitz ME, Rosen JF (1998) Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. Brain Res Rev 27: 168-176.
Froom P, Kristal-Boneh E, Benbassat J, Ashkanazi R, Ribak J (1998) Predictive value of determinations of zinc protoporphyrin for increased blood lead concentrations. Clin Chem 44: 1283-1288.
Garrido G, Madero R (1996) Area bajo la curva ROC. Med Clin (Barc) 106: 355-356.
Kammholz LP, Thatcher LG, Blodgett FM, Good TA (1972) Rapid protoporphyrin quantitation for detection of lead poisoning. Pediatrics 50: 625-631.
Leung FY, Bradley C, Pellar TG (1993) Reference intervals for blood lead and evaluation of zinc protoporphyrin as a screening test for lead toxicity. Clin Biochem 26: 491-496.
Lichtman HC, Feldman F (1963) In vitro pyrrole and porphyrin synthesis in lead poisoning and iron deficiency. J Clin Invest 42: 830-839.
McElvaine MD, Orbach HG, Binder S, Blanksma LA, Maes EF, Krieg RM (1991) Evaluation of the erythrocyte protoporphyrin test as a screen for elevated blood lead levels. J Pediatr 119: 548-550.
Needleman HL (1993) The current status of childhood lead toxicity. Advances in Pediatrics vol 40. Mosby-Year Book Inc. St Louis, pp 125-139.
Pantell RH, Takayama JI, Newman TB (1993) Costs and benefits of lead screening. JAMA 270: 2054.
Peter F, Growcock G, Strunc G (1978) Fluorometric determination of erythrocyte protoporphyrin in blood: a comparison between direct (hematofluorometric) and indirect (extraction) methods. Clin Chem 24: 1515-1517.
Piomelli S, Davidow B, Guinee VF, Young P, Gay G (1973) The FEP (free erythrocyte porphyrins) test: a screening micromethod for lead poisoning. Pediatrics 50: 254-259.
Rolfe PB, Marcinak JF, Nice AJ, Williams RH (1993) Use of zinc protoporphyrin measured by the Protofluor-Z hematofluorometer in screening children for elevated blood lead levels. Amer J Dis Child 147: 66-68.
Sole E, Ballabriga A, Dominguez C (1998) Lead exposure in children: levels in blood, prevalence of intoxication and related factors. Biometals 11: 189-197.
Takebayashi T, Omae K, Hosoda K, Satoh T, Hamaguchi T, Sakurai H (1993) Evaluation of d-aminolevulinic acid in blood of workers exposed to lead. British J Ind Med 50: 49-54.
Turk DS, Schonfeld DJ, Cullen M, Rainey P (1992) Sensitivity of erythrocyte protoporphyrin as a screening test for lead poisoning. N Engl J Med 326: 137-138.
Yip R, Schwartz S, Deinard AS (1983) Screening for iron deficiency with the erythrocyte protoporphyrin test. Pediatrics 72: 214-219.