

Roberta Liberatori<sup>1</sup>, Riccardo Romeo<sup>1</sup>, Brunetta Porcelli<sup>2</sup>, Lucio Barabesi<sup>3</sup>, Pietro Sartorelli<sup>1</sup>

## Erythropoiesis, erythropoietin and blood lead levels

<sup>1</sup> Section of Occupational Medicine and Toxicology, University of Siena, Italy

<sup>2</sup> Department of Internal Medicine, Endocrine-Metabolic Sciences and Biochemistry, University of Siena, Italy

<sup>3</sup> Department of Quantitative Methods, University of Siena, Italy

**ABSTRACT.** In a previous study EPO values were significantly lower in exposed subjects (PbB  $\geq$  30 mcg/dl) than in controls (PbB  $\leq$  20 mcg/dl).

The aim of the study was to verify if high PbB cause the reduction of EPO in connection with the serum concentration of this hormone expected considering the Hct and hemoglobin (Hb) levels.

Both in Pb exposed workers and controls blood levels of EPO, PbB, Hb, Hct were measured.

It was not observed any line relation between Hct e log EPO ( $p=0.01$ ) and any multiple regression relation between log EPO, Hct and PbB into the total studied population.

In our subjects PbB does not change physiologic relation between EPO and Hct and between EPO and Hb. Maybe because normal levels of Hct and Hb reduce EPO production in the healthy population. In physiologic condition of erythropoiesis, EPO does not seem influenced by PbB.

**Key words:** erythropoietin (EPO), lead levels (PbB), haematocrit (Hct).

**RIASSUNTO.** Alcuni Autori hanno riscontrato concentrazioni più basse di eritropoietina (EPO) in soggetti con più elevati valori di piombemia (PbB).

Scopo del nostro studio è stato quello di verificare se alti livelli di PbB causino una diminuzione dell'EPO in rapporto a quanto atteso in base ai livelli di ematocrito (Hct) ed emoglobina (Hb) e la presenza di una eventuale correlazione tra le due sostanze.

Sono state analizzate le cartelle cliniche di 35 pazienti con esposizione occupazionale a Pb e di 154 pazienti non professionalmente esposti a Pb appartenenti a un gruppo di controllo. A ciascuno sono stati dosati EPO, PbB e Hct. Il valore medio di PbB negli esposti era di 42,03  $\mu$ g/dl e nei non esposti di 10,06  $\mu$ g/dl con una differenza statisticamente significativa ( $p=0,0001$ ).

La differenza dei valori medi di Hct e logEPO tra i due gruppi studiati non è statisticamente significativa ( $p=0,5$  e  $p=0,7$ ).

Tra le due variabili Hct e log EPO non esiste nessuna correlazione lineare (coefficiente di Pearson = 0,19) e si può escludere l'ipotesi di dipendenza ( $p=0,01$ ).

Si è quindi considerato un modello di regressione multipla lineare in cui la variabile di risposta era log EPO e le variabili esplicative erano Hct e PbB. Dall'analisi è risultato che tale modello non spiega adeguatamente la relazione tra le variabili ( $F=2,63$ ,  $p=0,07$ ).

I risultati ottenuti confermano l'assenza di una *relationship* tra PbB ed EPO. Tuttavia in letteratura alcuni studi evidenziano come, pur in assenza di una dipendenza diretta, i valori di EPO risultino influenzati dai livelli di PbB. Nella popolazione studiata la PbB non sembra variare la fisiologica relazione EPO/Hct ed EPO/Hb. Ciò può essere attribuito al fatto che

### Introduction

The proliferation and maturation of bone marrow cells engaged in erythropoiesis is regulated by erythropoietin (EPO) produced in the renal tubule (1, 2). Several studies confirmed that tissues hypoxia, in case of anaemia and hypoxemia, is the most powerful stimulus to the EPO's secretion (3, 4, 5).

Lower EPO levels were correlated with higher blood lead levels (PbB) in 1500 pregnant women, probably through the toxic effect of lead (Pb) on the renal tubule, that represents the EPO production site (6). It was also supposed that reduced serum levels of EPO are partly responsible for saturnine anaemia. In a previous study (7) EPO values were significantly lower in exposed subjects (PbB  $\geq$  30 mcg/dl) than in controls (PbB  $\leq$  20 mcg/dl). However, a correlation was not demonstrated between blood lead concentrations and erythropoietin in any group.

Osterode *et al.* (8) found that in the subjects exposed to Pb, EPO was in the normal range and did not increase in the presence of anemia induced by Pb. Consequently they suggested two new mechanisms of Pb induced anaemia: the reduction of erythroid progenitor (burst-forming units-erythrocytes, BFU-E cells), and the inappropriate renal EPO production in the presence of severe exposure to Pb, which would lead to an inadequate maturation of BFU-E cells.

Moreover, there is a relationship between EPO and haematocrit (Hct) that was found to change at the cut point of Hct = 38% with two different curves, one for Hct < 38% and another for Hct > 38% (9, 10). The EPO response to anemia in the single subject can be evaluated by the observed/predicted (O/P) ratio. In this way EPO/Hct relationship changes were demonstrated in the different kind of anaemia allowing a functional classification of anaemia (10).

### Aim of the study

The aim of the study was to assess if the increase of PbB levels can cause the reduction of EPO value demonstrating a toxic effect of Pb in the EPO production site.

la produzione di eritropoietina è depressa quando i valori di Hct e Hb risultano relativamente elevati, cioè in pratica nel range di normalità come avveniva nella popolazione studiata. Non si può escludere che l'assenza dell'anemia possa in qualche modo nascondere l'effetto del metallo sull'EPO. Fortunatamente grazie alle migliorate condizioni lavorative ed all'utilizzo delle nuove tecnologie industriali, l'abnorme assorbimento di Pb è oramai limitato ad eventi accidentali e comunque non raggiunge quasi mai livelli tali da causare intossicazioni acute caratterizzate tra l'altro dall'insorgenza di anemia. Queste mutate condizioni ostacolano la verifica delle ipotesi sopra formulate. In base ai risultati ottenuti si può comunque concludere che in condizioni di sanguificazione normali l'EPO non è influenzata dalla PbB.

**Parole chiave:** eritropoietina (EPO), piombemia (PbB), ematocrito (Hct).

**Subjects and methods**

A random population of 35 occupationally lead exposed workers (30 males and 5 females) and 154 non-professionally exposed volunteers (125 males and 29 females) was examined. The main professional involved sectors of exposed workers were glass, ceramics and foundries.

All subjects had normal renal function and did not suffer from diseases than affect EPO production.

PbB, hemoglobyn (Hb) and Hct were measured. EPO was analyzed by commercial radioimmunoassay kits (Medical System S.p.A., Genoa Italy). Blood samples were taken in the morning; the serum obtained was frozen at -20 °C until assay.

The mean homogeneity in the groups for each considered variable (i.e. PbB, EPO and Hct) was assessed by the two-sample *t*-Student test. In order to avoid non-nor-

mality, a logarithmic transformation was performed on the variable EPO. Subsequently, considering log(EPO) and Hct as response variable and regressor respectively, a linear regression analysis and a nonparametric regression analysis were carried out on controls. In addition, a linear multiple regression was also implemented by considering log(EPO) as response variable and Hct and PbB as regressors. The data were analyzed using the SAS 9.1 software.

**Results**

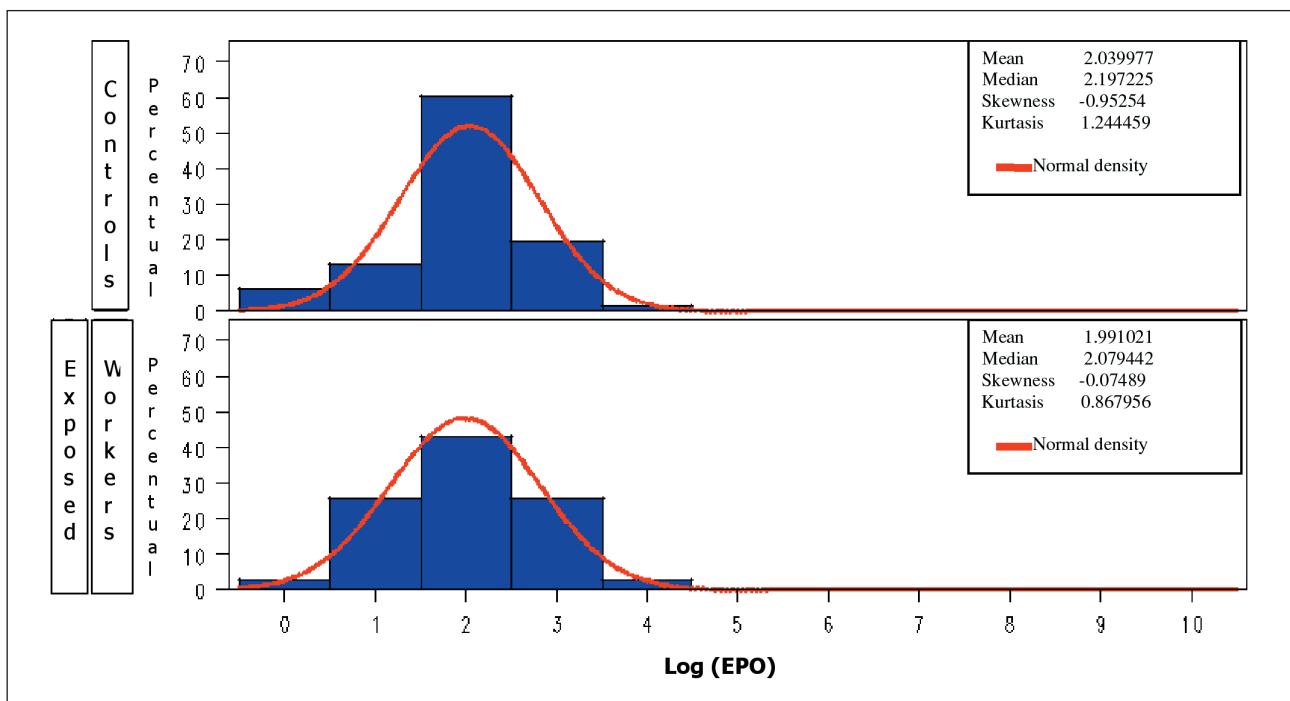
Details of the studied population are summarized in table I.

PbB mean value was significantly higher in exposed workers than in non-professionally exposed subjects (p=0.0001). The mean values of log(EPO), Hb and Hct did not show any significant differences between the two groups on the basis of the two-sample *t*-test (p=0.7, p=0.6 and p=0.5, respectively).

Figure 1 shows the sampling distribution of log(EPO) in exposed and unexposed subjects: it is apparent that the two sampling distributions are very similar.

**Table I. Details of the studied population**

	Exposed workers (n. 35)	Non-professionally exposed subjects (n. 154)
Male	30	125
Female	5	29
Mean age (years)	50±13	50±13
PbB (µg/dl)	42.03	10.06
EPO (mU/ml)	10.28	9.71
Hct (%)	44.96	45.57



**Figure 1. Sampling distribution of log(EPO) in exposed workers and controls**

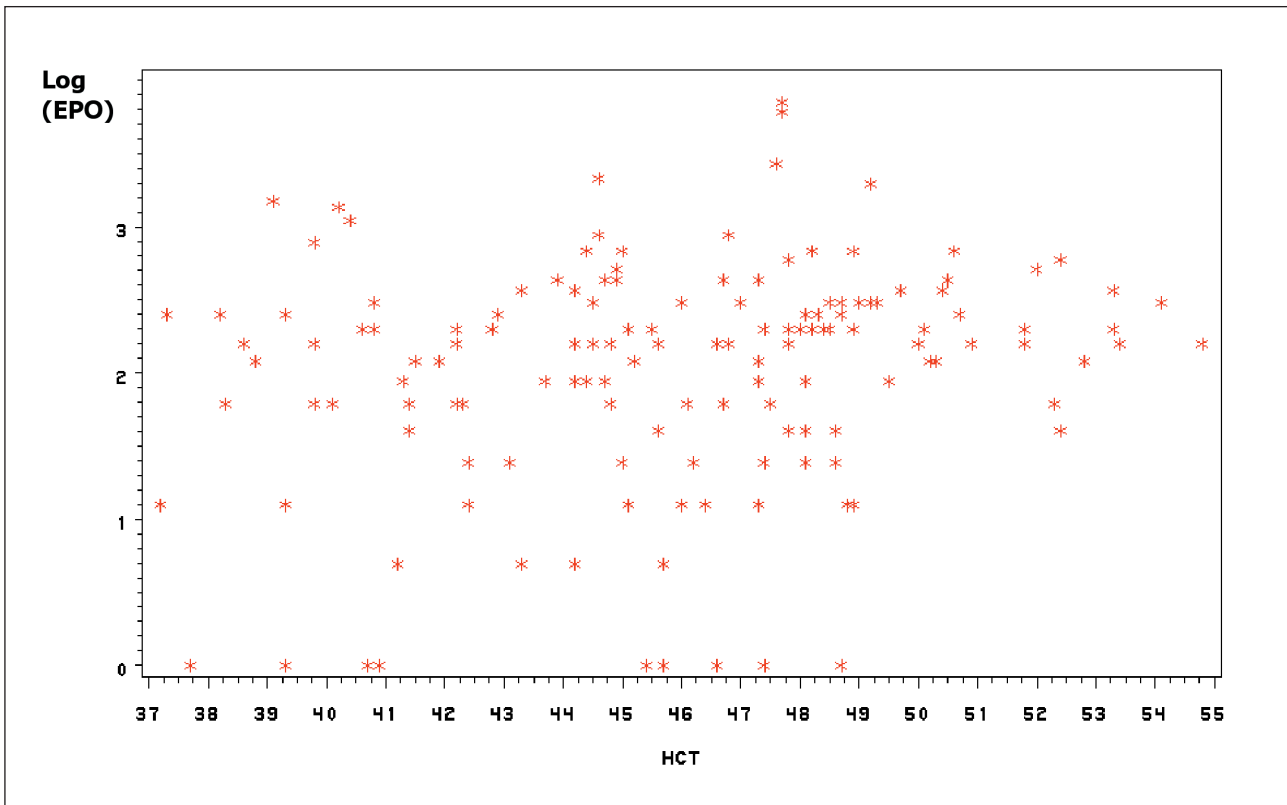


Figure 2. Scatter-plot of Hct and log(EPO) data

In order to consider the joint distribution of Hct and log(EPO) the scatter-plot of the corresponding observations was considered in figure 2.

No linear dependence exists since the Pearson's coefficient turns out to be 0.19 while the two variables turn out

to be independent by assuming the bivariate normality.

The dependence analysis for the variables log(EPO) and Hct was solely considered for the exposed subjects. In turn no dependence exists in this group (Pearson coefficient = 0.1) (figure 3).

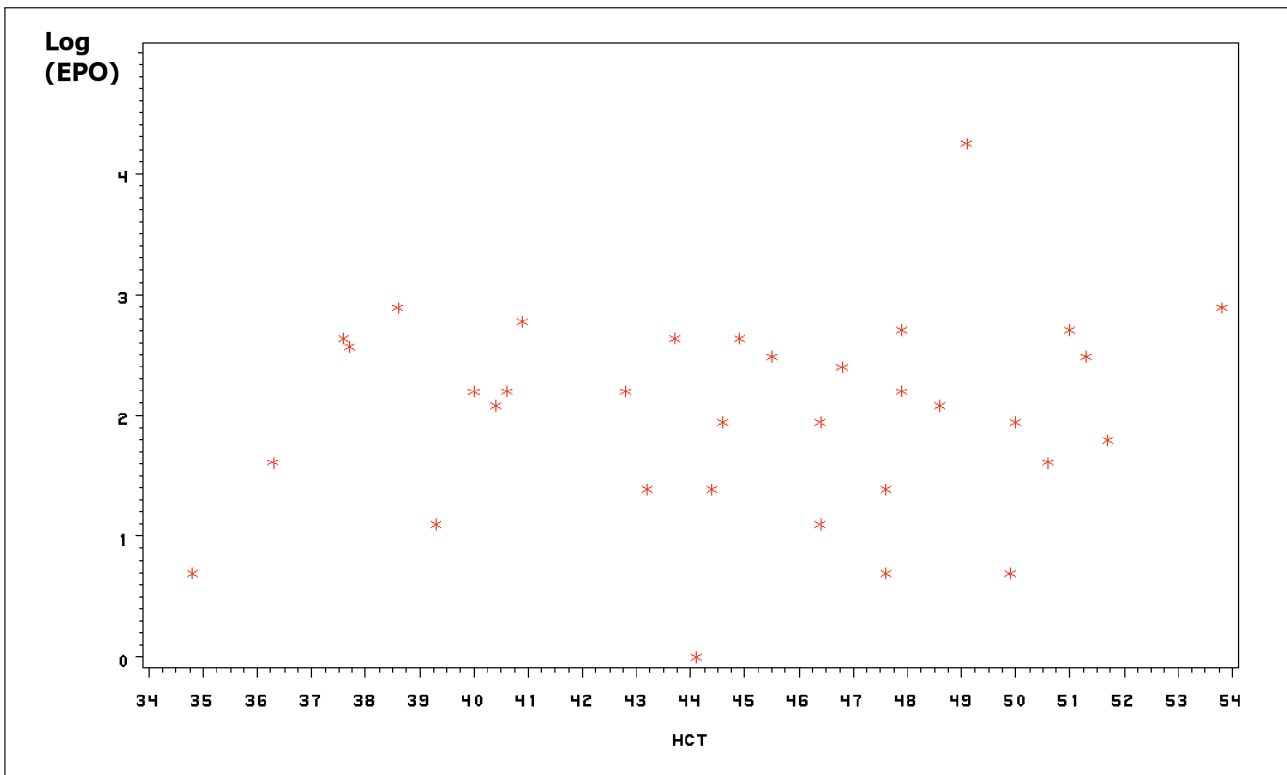


Figure 3. Scatter-plot of Hct and log(EPO) data in Pb exposed subjects

The multiple regression linear model was considered by assuming log(EPO) as response variable, while the regressors were Hct and PbB. Since the two groups do not seem to statistically differ, they were considered as a single population. Unfortunately, the model is not useful in practice since the existence of a linear relationship was rejected on the basis of the *F*-test ( $F= 2.63$ ,  $p=0.07$ ).

## Discussion

Results confirm the absence of relationship between PbB and EPO. Nevertheless, some studies in literature show that EPO values are influenced by PbB levels too without direct dependence. Since EPO values depend on hypoxia derived from anaemia, they were assessed in the function of Hct and Hb levels.

In the studied population PbB does not change physiologic relation between EPO and Hct and between EPO and Hb maybe because in a healthy population Hct > 38% and normal Hb levels reduce EPO production. But the absence of anaemia could hide the PbB effect on EPO. On the other hand at higher PbB levels the Pb capability to cause anemia through the inhibition of various enzymatic activities, could be increased by a contemporary EPO reduction. This would explain results obtained in pregnant women by Graziano *et al.*

However in physiologic conditions of erythropoiesis EPO does not seem influenced by Pb.

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**Reprint request:** Roberta Liberatori - Section of Occupational Medicine and Toxicology, University of Siena, Viale Bracci 16, 53100 Siena, Italy - Tel. 0577586750, Fax 0577586759, E-mail: robertaliberatori@inwind.it