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Association of Blood Lead Level with Neurological Features in 972 Children Affected by an Acute Severe Lead Poisoning Outbreak in Zamfara State, Northern Nigeria

Jane Greig¹, Natalie Thurtle², Lauren Cooney², Cono Ariti³, Abdulkadir Ola Ahmed⁴, Teshome Ashagre⁴, Anthony Ayela⁴, Kingsley Chukwumalú⁴, Alison Criado-Perez⁴, Camilo Gómez-Restrepo⁴, Caitlin Meredith⁴, Antonio Neri⁵, Darryl Stellmach⁴,⁶, Nasir Sani-Gwarzo⁷, Abdulsalami Nasidi⁸, Leslie Shanks², Paul I. Dargan²,⁹

¹ Manson Unit, Médecins Sans Frontières, London, United Kingdom, ² Public Health Department, Médecins Sans Frontières, Amsterdam, The Netherlands, ³ Medical Statistics Department, London School of Hygiene and Tropical Medicine, London, United Kingdom, ⁴ Nigeria Mission, Médecins Sans Frontières, Sokoto, Nigeria, ⁵ Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, Georgia, United States of America, ⁶ Institute of Social and Cultural Anthropology, University of Oxford, Oxford, United Kingdom, ⁷ Department of Public Health, Federal Ministry of Health, Abuja, Nigeria, ⁸ Nigeria Centre for Disease Control, Abuja, Nigeria, ⁹ Guys and St. Thomas’ NHS Foundation Trust and King’s College London, London, United Kingdom

Abstract

Background: In 2010, Médecins Sans Frontières (MSF) investigated reports of high mortality in young children in Zamfara State, Nigeria, leading to confirmation of villages with widespread acute severe lead poisoning. In a retrospective analysis, we aimed to determine venous blood lead level (VBLL) thresholds and risk factors for encephalopathy using MSF programmatic data from the first year of the outbreak response.

Methods and Findings: We included children aged ≤5 years with VBLL ≥45 μg/dL before any chelation and recorded neurological status. Odds ratios (OR) for neurological features were estimated; the final model was adjusted for age and baseline VBLL, using random effects for village of residence. 972 children met inclusion criteria: 885 (91%) had no neurological features; 37 (4%) had severe features; 47 (5%) had reported recent seizures; and six (1%) had other neurological abnormalities. The geometric mean VBLLs for all groups with neurological features were >100 μg/dL vs 65.9 μg/dL for those without neurological features. The adjusted OR for neurological features increased with increasing VBLL: from 2.75, 95%CI 1.27–5.98 (80–99.9 μg/dL) to 22.95, 95%CI 10.54–49.96 (≥120 μg/dL). Neurological features were associated with younger age (OR 4.77 [95% CI 2.50–9.11] for 1–3 years, 2.69 [95%CI 1.15–6.26] for 2–<3 years, both vs 3–5 years). Severe neurological features were seen at VBLL <105 μg/dL only in those with malaria.

Interpretation: Increasing VBLL (from ≥80 μg/dL) and age 1–<3 years were strongly associated with neurological features; in those tested for malaria, a positive test was also strongly associated. These factors will help clinicians managing children with lead poisoning in prioritising therapy and developing chelation protocols.

Introduction

Lead poisoning is not a new phenomenon. Though debate continues as to whether it was described by Hippocrates [1], and the hypothesis that it contributed to the fall of the Roman Empire remains in dispute [2,3], humans have been exposed to lead as a toxicant since at least the start of industrialisation. It continues to be a significant cause of morbidity. In 2004, lead poisoning accounted for about 0.6% of the global burden of disease and 9 million disability-adjusted-life-years [4]. Lead affects mechanisms as diverse as energy metabolism, apoptosis, cell adhesion, intercellular and intracellular signalling, protein maturation, and genetic regulation [4]. As a result, lead poisoning causes a continuum of sub-clinical and clinical features including hypertension [5], nephropathy [6], infertility [7], anaemia, behavioural changes including violence [8], and decreased IQ (intelligence quotient) [9]; as well as severe manifestations such as acute encephalopathy and death [10,11,12].

In resource-rich nations, deaths from lead encephalopathy are a largely historical phenomenon. Hundreds of children died from lead poisoning in the USA in the first half of the 20th Century when lead use was widespread [13]. Lead-related deaths in US
cities in the 1950s and 1960s were primarily related to lead paint
ingestion [11,12]. One Baltimore hospital reported 36 cases of
severe lead encephalopathy between 1954 and 1956 [14], while 38
cases were reported in Chicago between 1959 and 1963 [13] -
examples of a broader problem that reported figures likely grossly
underestimate. The last recorded death from lead encephalopathy
in the USA was in 2006 [16], preceded by one in 2000 that was
itself the first since 1990 [17]. Reports of lead-related deaths are
uncommon in resource-poor settings, and mostly detected though
outbreaks, such as the 18 deaths in Senegal linked to lead acid
battery recycling in 2007 [18].

In March 2010, a Médecins Sans Frontières (MSF) disease
surveillance team in Zamfara State, northern Nigeria, was
contacted by leaders and health staff of a local village with reports
of high mortality in young children following an unknown illness.
MSF was invited to assist in investigating these reports by the state
Ministry of Health. MSF carried out an initial assessment and
rapidly assembled a dedicated response team and 24-hour care in
the village clinic. Children presented with sudden onset of
abdominal pain and/or vomiting, intractable seizures with or
without fever, then sometimes rapid progression to death.
Symptoms were unresponsive to initial treatment by the MSF
team for common endemic diseases such as malaria and
meningitis, and anti-convulsants had little effect. Over 2 months
until 17 May 2010, nearly 300 children aged ≤5 years presented
in four villages with these symptoms with a mortality of 48%.
There were anecdotal reports of a recent increase in small-scale
ore processing with dry-milling to extract gold. An outbreak of
severe lead poisoning was confirmed [19,20,21]. Initially, seven
rural villages were identified as extremely lead contaminated due
to lead exposure harming children by relocating ore processing
and discharged to an uncontaminated location when possible.

Treatment in the absence of environmental remediation has
as undertaken [20]. Health promotion has focused on removing the
lead exposure harming children by relocating ore processing
activities and minimising further lead contamination of the
villages.

The focus of the MSF emergency medical response was clinical
lead surveillance and chelation therapy. All children aged ≤5
years from the seven villages where remediation was taking place
were offered screening by MSF as their villages were remediated.
Treatment in the absence of environmental remediation has
limited impact, so screening in unremediated villages was
considered futile. Children with venous BLL (VBLL) ≥45 µg/
dl. [the MSF protocol and CDC recommended chelation
threshold] from remediated villages [24] were offered chelation
therapy, the outcomes of which will be reported separately.
Children from unremediated villages presenting to an MSF
facility with signs of encephalopathy and therefore at immediate risk
of death were tested and treated with chelation therapy if required,
and discharged to an uncontaminated location when possible.

Although there has been some characterisation of the clinical
pattern of lead toxicity [4,10,25] and the VBLL threshold for
encephalopathy is often stated to be in the range of 70–100 µg/dL
[4,26], there are limited data on the VBLL threshold above which
life-threatening effects including encephalopathy are likely to
occur in children [10,25,27,28]. In this paper we describe nearly
1000 lead exposed children with VBLL ≥45 µg/dL, and the
clinical and demographic characteristics associated with neuro-
ological features using basic clinical examination. This is the largest
reported retrospective analysis of data from children ≤5 years with
VBLL in this range.

Methods

For the period June 2010 to the end of June 2011, we included all
children aged ≤5 years with a first-ever VBLL ≥45 µg/dL
recorded before chelation therapy and whose neurological status
was recorded within 7 days of this VBLL. Screening and treatment
were provided by MSF. Children were identified via an MSF
doctor-to-door census that detailed children aged ≤5 years living in
each residential compound. Screening for enrolment to the MSF
celation programme included a brief clinical history and
examination, with particular attention to neurological status.
Neurological assessment included history of seizures, change in
behaviour, delay or loss of developmental milestones, peripheral
neuropathies, gait, assessment of reflexes, and level of conscious-
ness (alert/voice/pain/unresponsive [AVPU] assessment scale
[29]). Detailed demographic and clinical data were recorded on
standardised forms by medical staff. Only key data were entered
into an electronic database, including enrolment data: AVPU,
neurological manifestations (summarised as none, present [not
severe], severe), recent seizure history (yes/no). In addition, key
impatient data were entered: seizures during hospitalisation (yes/
no; if yes, change during admission) and other neurological
symptoms during hospitalisation (none, present [not severe], severe;
if present, change during admission). Neurological status
for this analysis was described by a composite measure of
neurological signs or symptoms and AVPU as severe neurological
features (1), presumptive seizures (2), mild neurological features (3),
or no neurological features identified (4) (Table 1), with categories
1–3 combined as “any neurological features”.

VBLL was measured using the Lead Care II point-of-care
analyzer (Magellan Biosciences, Chelmsford, Massachusetts), using
manufacturer-recommended protocols, with samples testing above
the upper limit of 65 µg/dL rested using a dilution method
developed with CDC and described elsewhere [30]. Regular
quality control for VBLL was provided by CDC, USA, using
inductively-coupled plasma mass spectrometry (ICPMS): point-of-
care values of 120 clinical samples (13% diluted in project from
>65 µg/dL) were on average 4.0 µg/dL lower than ICPMS
limits of agreement [31] −19.7 µg/dL to +11.7 µg/dL. VBLL
results were categorised: 45–64, 65–79, 80–99, 100–119, 120–199,
≥200 µg/dL. Haemoglobin was measured on all venous samples
by the HemoCue Hb 301 point-of-care testing system (HemoCue,
Angelholm, Sweden) prior to 22nd November 2010, and by the
Sysmex Automated Hematology Analyzer, KX-21N (Sysmex,
Hyogo, Japan) after this date. Venous samples were also used to
assess alanine transaminase (ALT) (HumaLyser 2000 [Human,
Wiesbaden, Germany]). Where symptoms such as fever were
suggestive of malaria, a malaria rapid diagnostic test (RDT) was
performed (HRP-2 tests as the endemic cases and seasonal
outbreaks are almost exclusively due to Plasmodium falciparum).
Prevalence levels in children between the ages of 6 months to 59
months in the area is 48.2% [32]. Children with positive test
results were immediately treated with artemisinin-based combina-
tion therapy (ACT) for uncomplicated malaria and artemether
for severe malaria.

Haematological and biochemical parameters were categorised
[33]: haemoglobin (g/dL) low (<10 if <2 years old, <11 if 2–5
years), high (>13 if <2 years, >14 if 2–5 years), or normal; ALT
(U/L) normal (0–42), mildly elevated (>42–100), moderately
Neurological status categories and definitions.

| Neurological status category | Definition |
|-----------------------------|------------|
| 1 Severe neurological features: | Seizures witnessed by medical staff, and/or altered consciousness (an AVPU of V or P or U). |
| 2 Presumptive seizures: | A guardian’s report of recent (within the past few days) seizure activity and an AVPU of A on presentation; but no seizures witnessed by medical staff. |
| 3 Mild neurological features: | Any neurological signs or symptoms noted by medical staff but without reported history or witnessed seizure; and an AVPU of A on presentation.* |
| 4 No neurological features identified: | No significant neurological signs or symptoms identified on brief clinical examination by the initial treating doctor; and no history of recent seizures. |

*Noted abnormalities were hypo-reflexia, inconsolable crying, agitation, and decreased mobility.

"Any neurological features" = categories 1+2+3.

January 2010 and 30 June 2011 approximately 95% of children in the seven remediated villages were screened; 972 children met the inclusion criteria (Figure 1). Among another 160 children meeting age, VBLL and date criteria, but with inadequate record of neurological status in their clinical file, 24 (15%) had VBLL more (≥100–1000) or severely elevated (≥1000). MUAC (mid-upper arm circumference) for nutritional status was recorded as green (≥135 mm), yellow (≥125 to <135 mm), orange (≥110 to <125 mm; moderately malnourished) or red (<110 mm; severely malnourished) for children aged ≥6 months, or not applicable for infants <6 months. Age at time of VBLL was grouped as 0–<6 months, 6–<12 months, 1–<2 years, 2–<3 years, 3–5 years, based on USA Environmental Protection Agency guidance regarding behavioural and physiological development stages for environmental assessment [34].

Selected clinical and laboratory data were routinely entered into an electronic database specifically designed (by JG) to support patient care and programme management. Data were analysed using STATA 10.1 (StataCorp, Texas, USA). Baseline characteristics were described as counts and percentages of patients in each category and compared with chi-squared or Fisher’s exact tests unless a high proportion of data was missing. VBLL by neurological status was calculated as geometric means (95% CI) due to non-normally distributed data, accommodating clustering by village of residence by using robust standard errors. Log VBLL values by neurological status categories were compared by Analysis of Variance with Schefe test between groups. Odds ratios (OR) for any neurological features (compared to none) were estimated for the following variables based on review of descriptive data, completeness and plausibility, with random effects (for village of residence as a variable potentially incorporating various unmeasured factors such as level of environmental contamination): gender; age at time of VBLL; baseline VBLL category; nutritional status (MUAC); and haemoglobin. A multivariable multi-level logistic regression random effects model to estimate adjusted OR of the outcome of any neurological symptoms included factors significant at p<0.10 in the unadjusted analysis or with plausible epidemiological or biological association. Backward selection was used to choose prognostic variables for the final model, discarding those no longer associated (p>0.10) with the outcome after adjustment for other variables. P-values were calculated for the strength of association of each variable with the outcome using Wald tests, and the VBLL categorical variable assessed as continuous to test for trend. Interaction was not plausible with the retained variables. The sensitivity of the model was assessed for severity of neurological features. The role of malaria was assessed in patients who had been tested for malaria due to symptoms (33%), adjusting for age and VBLL.

Ethics statement

This study met the standards set by the independent MSF Ethics Review Board for retrospective analyses of routinely collected programmatic data [35], here being data collected to facilitate life-saving clinical care. These standards include, but are not limited to, assurances of confidentiality, involvement of local partners and minimal harm to patients. Coded identification numbers were used and personal identifiers and all unnecessary data were removed from the dataset. Review of anonymous routinely collected programmatic data does not constitute research under the National Health Research Ethics Committee of Nigeria guidelines. CDC USA staff involvement did not require CDC Human Subject Review as CDC personnel were not involved in treating patients.

Results

Patient characteristics

Between 1 June 2010 and 30 June 2011 approximately 95% of children in the seven remediated villages were screened; 972 children met the inclusion criteria (Figure 1). Among another 160 children meeting age, VBLL and date criteria, but with inadequate record of neurological status in their clinical file, geometric mean VBLL was lower than for those with recorded neurological status (65.3 µg/dL [95% CI 61.5–69.4] vs 79.4 [95% CI 62.6–100.7]; p = 0.010), however there was no difference in the proportion female (46.9% vs 49.2%; p = 0.59), or the age distribution (p = 0.87). Among the 972 children included, 340 (35%) had a VBLL ≥80 µg/dL. The maximum VBLL recorded was 708 µg/dL. 73 (8%) had a VBLL 120–199 µg/dL and 27 (3%) had a VBLL ≥200 µg/dL (Table 2). Proportionally more children aged 1–<2 years had VBLL ≥120 µg/dL than in the other age groups (25% [39/159] vs 7% for all other ages combined [61/183]; all other age groups were each separately <13%; p<0.001) (Table 2). 14 children had died by 30 June 2011; lead poisoning was likely a primary cause in five of these deaths as these children had recent high VBLL (104–460 µg/dL) and symptoms consistent with lead encephalopathy, with no other obvious cause of death.

Most (383 [91%]) children had no neurological features, 34 (4%) had seizures witnessed by medical staff and/or reduced level of consciousness, 47 (5%) had a guardian-reported history of recent seizures but no altered consciousness on presentation, and six (1%) displayed some other signs of neurological abnormality (Table 3). The geometric mean VBLL for all groups with neurological features was >100 µg/dL (157.6 µg/dL for mild neurological features, 106.9 µg/dL for presumptive seizures and 170.1 µg/dL for severe neurological features) compared with 65.9 µg/dL for those without neurological features (Table 3; Figure 2). The range of VBLL in each group was wide, such that children with no neurological features at first presentation had elevated (≥100–1000), or severely elevated (≥1000).
VBLLs up to 345 μg/dL, and children with neurological features had VBLL as low as 47 μg/dL (Table 3); only children with concurrent malaria had severe neurological features below VBLL 105 μg/dL.

Within age groups, neurological features were most common in 1–2 year olds (25% vs 6% for other ages; p<0.001). 233 (72%) of 325 children tested for malaria had a positive RDT result. A positive malaria test was more common in children with neurological features than in those without (82% [56/68] vs 69% [177/257], respectively; p = 0.034) (Table 3); 19 children with neurological features did not have a malaria test recorded. A positive malaria test was more common in children with VBLL >80 μg/dL (n = 194 tested for malaria) who had any neurological features (24% vs 18%; p = 0.23). A high proportion (24%) of children had no ALT results recorded; a low proportion of children had elevated ALT levels (9%), none were severely elevated (Table 3).

Factors associated with neurological features

In logistic regression (unadjusted OR, random effects for village of residence), presentation with any neurological features was not associated with gender (OR = 1.08, 95%CI 0.68–1.72; p = 0.73), or low haemoglobin (OR = 1.39, 95%CI 0.77–2.50; p = 0.27), but was associated with age (highest for 1–2 years [OR = 9.42, 95%CI 5.23–16.96; p<0.001] compared with 3–5 years), increasing VBLL (test for trend p<0.001, starting from 80.0–99.9 μg/dL with OR = 4.76, 95%CI 2.02–11.31; p<0.001). The final multivariate analysis retained only age and VBLL as strongly associated with any neurological features after adjustment for the other factors and random effects for village (both p<0.001). Children aged 1–2 years had the highest odds of neurological features compared with children age 3–5 years (adjusted OR 4.77, 95%CI 2.50–9.11); age 2–3 years was also associated with neurological features (adjusted OR 2.69, 95%CI 1.15–6.26) compared with children age 3–5 years. Increasing VBLL

Table 2. First-ever pre-chelation VBLL test (in categories, μg/dL) by age group (number and % of row).

|        | 45–64.9 | 65–79.9 | 80–99.9 | 100–119.9 | 120–199.9 | 200+ | Total |
|--------|---------|---------|---------|-----------|-----------|------|-------|
| 0–6 months | 33 (65%) | 5 (10%) | 3 (6%)  | 5 (10%)  | 4 (8%)  | 1 (2%) | 51    | 5%    |
| 6–12 months| 52 (51%) | 5 (5%)  | 20 (20%)| 14 (14%) | 7 (7%)  | 4 (4%) | 102   | 10%   |
| 1–2 years  | 75 (47%) | 4 (3%)  | 23 (14%)| 18 (11%) | 22 (14%)| 17 (11%)| 159   | 16%   |
| 2–3 years  | 53 (54%) | 8 (8%)  | 14 (14%)| 11 (11%) | 11 (11%)| 1 (1%) | 98    | 10%   |
| 3–5 years  | 363 (65%)| 34 (6%) | 88 (16%)| 44 (8%)  | 29 (5%) | 4 (1%) | 562   | 58%   |
| Total      | 576 (59%)| 56 (6%) | 148 (15%)| 92 (9%)  | 73 (8%) | 27 (3%)| 972   |       |

VBLL = venous blood lead level.

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compared with the reference category of 45–64.9 μg/dL) was strongly associated with increased odds of neurological features (test for trend p<0.001): adjusted OR 2.75, 95%CI 1.27–5.98 (VBLL 80–99.9 μg/dL); 3.84, 95%CI 1.62–9.09 (100–119.9 μg/dL); and 22.95, 95%CI 10.54–49.96 (≥120 μg/dL) (Table 4).

A sensitivity analysis using only the outcome of severe neurological features compared with all other patients gave similar overall results. The association with VBLL was stronger and the trend robust (test for trend p<0.001), but significant only from 100–119.9 μg/dL (adjusted OR 7.35, 95%CI 2.02–26.79; for VBLL ≥120 μg/dL adjusted OR 23.96, 95%CI 7.33–78.31). The adjusted OR were also higher for all ages with the largest associations for children aged 1–3 years (OR=6 for both 1–2 y and 1–3 y) compared with children aged 3–5 years.

Severe neurological features in children with a positive malaria RDT result were seen at VBLL as low as 50 μg/dL, while in those with a negative RDT the lowest VBLL with severe neurological features was 105 μg/dL, and in the one child with severe neurological features and no malaria test result the VBLL was 293 μg/dL. In the 325 children with a malaria RDT result, the unadjusted OR of a positive RDT with any neurological features was 2.04 (95% CI 0.96–4.35; p = 0.065). After adjustment for age and VBLL there was evidence of a strong association of malaria with neurological features (adjusted OR = 3.60, 95% CI 1.42–9.09; p = 0.007); after adjustment for malaria, age and VBLL showed a similar pattern of association with neurological features as in the entire cohort, that is, highest adjusted OR in 1–2 year olds and increasing adjusted OR with increasing VBLL.

Discussion

The MSF programmatic data collected during the response to the Zamfara lead poisoning disaster presents a cohort size and severity that is unprecedented. While lead poisoning was endemic in resource-rich countries in the early to mid-20th Century, an acute outbreak of this magnitude has not been reported in the literature. Chisolm et al. reported 197 children exposed to lead including 41 cases of severe encephalopathy in 1952–1954 [12] and Greengard et al. reported 38 severe cases in 1962 and 1963 with lead blood levels in 20 fatalities of 90–825 μg/dL [15]. More recently, the Treatment of Lead-exposed Children (TLC) trial group reported a cohort of 780 patients, although all had VBLL <45 μg/dL [36] and thus are not comparable with the patients in Zamfara. Reports in recent decades with similar numbers of children screened for lead poisoning have been based on general population datasets, with much lower geometric mean VBLLs, generally <10 μg/dL [37,38]. Media reports of lead poisoning of hundreds of children in China in 2009 [39,40] indicated comparatively low lead levels, with no detailed reports published of acutely severely intoxicated children. Other recent studies have concerned low-level endemic lead poisoning [41,42]. In the 972 children included in this analysis, the geometric mean VBLL was 79.4 μg/dL, 35% had VBLL ≥80 μg/dL, and 9% had some clinically identified neurological features consistent with lead poisoning. An additional 696 children (35% of those screened) had first-ever VBLLs of 10–44.9 μg/dL and thus did not require chelation (and are not reported in this analysis), such that the range of exposure of this cohort of children is consistent with the entire clinical and sub-clinical spectrum of lead pathophysiology.

Children with severe neurological features of altered consciousness or seizures witnessed by clinical staff had significantly higher VBLL than those with only a guardian-reported recent history of seizures. Having any neurological feature was strongly associated with increasing initial VBLL, with evidence of an increased OR at a VBLL of 80–99.9 μg/dL, becoming stronger at 100–119.9 μg/dL and larger still from 120 μg/dL. This is consistent with previous ranges given for the threshold above which encephalopathy risk is increased [4,10,26], strengthened by the robust...
evidence from this large cohort of poisoned children. Clinicians managing patients with lead poisoning should take into consideration the presence of neurological features and, particularly, the potential risk of life-threatening encephalopathy at VBLL above 80 \( \mu g/dL \), particularly (as discussed in more detail below) in those with concurrent malaria.

Severe neurological features were not seen below VBLL 50 \( \mu g/dL \) and in the absence of a positive malaria test, not below 105 \( \mu g/dL \). Most children with neurological features at a lower VBLL (<80 \( \mu g/dL \)) also had a positive malaria test. In 325 children with symptoms suggestive of malaria who were tested by RDT, a positive result was independently associated with neurological features in addition to the trend associating increasing VBLL with neurological features. It is challenging to distinguish between features of cerebral malaria and lead encephalopathy clinically, and the presence of both lead and malaria were strongly associated with neurological features. We postulate that it is also possible that haemolysis associated with malaria increases the relative proportion of free plasma lead at a given (whole blood) VBLL, increasing the lead available to cause encephalopathy. The interaction between malaria infection and lead toxicity is still uncertain and is an area for future research.

Of children with no neurological features at the time of initial examination, 31% had VBLL \( \geq 80 \mu g/dL \), comparable to the United States National Academy of Sciences cohort [10]. Clinical signs and symptoms varied among children with similar blood lead levels, confirming that clinical features alone are poor predictors of the severity of lead poisoning and the risk of long-term

### Table 3. Characteristics by neurological status in children ≤5 years, with first-ever pre-chelation VBLL \( \geq 45 \mu g/dL \) from 1 June 2010 to 30 June 2011.

|                      | No neurological features (\( n = 885 \)) | Mild neurological features (\( n = 6 \)) | Presumptive seizures (\( n = 47 \)) | Severe neurological features (\( n = 34 \)) | p-value* | All (\( n = 972 \)) |
|----------------------|----------------------------------------|----------------------------------------|------------------------------------|---------------------------------------------|----------|---------------------|
| Geometric mean VBLL (95% CI) (\( \mu g/dL \)) | 65.9 (59.7–72.7) | 157.6 (52.1–476.3) | 106.9 (76.1–150.3) | 170.1 (112.0–258.2) | <0.001 | 79.4 (62.6–100.7) |
| VBLL range (\( \mu g/dL \)) | 45.0–345.1 | 49.2–708.0 | 46.9–531.0 | 50.6–459.9 | 45.0–708.0 |
| VBLL ≥80 \( \mu g/dL \), n (%) | 276 (31%) | 5 (83%) | 32 (68%) | 27 (79%) | <0.001 | 340 (35%) |
| Sex male, n (%) | 450 (51%) | 2 (33%) | 27 (57%) | 15 (44%) | 0.56 | 494 (51%) |
| Age category, n (%) | | | | | | |
| 0–<6 months | 48 (5%) | 0 | 2 (4%) | 1 (3%) | 51 (5%) |
| 6–<12 months | 92 (10%) | 1 (17%) | 5 (11%) | 4 (12%) | 102 (10%) |
| 1–<2 years | 119 (13%) | 3 (50%) | 17 (37%) | 20 (59%) | 159 (16%) |
| 2–<3 years | 87 (10%) | 1 (17%) | 5 (11%) | 5 (15%) | 98 (10%) |
| 3–5 years | 539 (61%) | 1 (17%) | 18 (38%) | 4 (12%) | 562 (58%) |
| Malaria RDT, n (%) | | | | | | |
| Symptomatic; RDT negative | 80 (9%) | 1 (17%) | 4 (9%) | 7 (9%) | 92 (9%) |
| Symptomatic; RDT positive | 177 (20%) | 4 (67%) | 26 (55%) | 26 (76%) | 233 (24%) |
| Asymptomatic/missing* | 628 (71%) | 1 (17%) | 17 (36%) | 1 (3%) | 647 (67%) |
| Nutritional status (MUAC), n (%) | | | | | 0.045 | |
| Red (<110 mm) | 8 (1%) | 0 | 2 (4%) | 1 (3%) | 11 (1%) |
| Orange (≥110 <125 mm) | 23 (3%) | 0 | 5 (10%) | 1 (3%) | 29 (3%) |
| Yellow (≥125 <135 mm) | 56 (6%) | 1 (17%) | 3 (6%) | 5 (15%) | 65 (7%) |
| Green (≥135 mm) | 613 (69%) | 3 (05%) | 27 (57%) | 18 (53%) | 661 (68%) |
| Not applicable (<6 m old) | 48 (5%) | 0 | 2 (4%) | 1 (3%) | 51 (5%) |
| Missing (≥6 m old) | 137 (15%) | 2 (33%) | 8 (17%) | 8 (24%) | 155 (16%) |
| Laboratory tests at time of VBLL, n (%) | | | | | | |
| Normal haemoglobin (g/dL) | 213 (24%) | 0 | 13 (28%) | 3 (9%) | 229 (24%) |
| Low (<10 if <2 y; <11 if 2–5 y) | 664 (75%) | 6 (100%) | 33 (70%) | 30 (88%) | 733 (75%) |
| High (>13 if <2 y; >14 if 2–5 y) | 2 (0%) | 0 | 1 (2%) | 1 (3%) | 4 (0%) |
| Missing | 6 (1%) | 0 | 0 | 0 | 6 (1%) |
| Normal ALT, n (%) | 604 (68%) | 1 (17%) | 26 (55%) | 23 (68%) | 654 (67%) |
| Mildly elevated (>42–100 U/L) | 73 (8%) | 0 | 2 (4%) | 2 (6%) | 77 (8%) |
| Mod. elevated (>100–1000 U/L) | 11 (1%) | 0 | 0 | 1 (3%) | 12 (1%) |
| Severely elevated (>1000 U/L) | 0 | 0 | 0 | 0 | 0 |
| Missing | 197 (22%) | 5 (83%) | 19 (8%) | 8 (24%) | 229 (24%) |

*Malaria test performed only on symptomatic children, but no result in database not confirmation that asymptomatic.

1 p-values only given when <20% data missing. VBLL = venous blood lead level. RDT = rapid diagnostic test. MUAC = mid upper-arm circumference. Mod = moderately.

m = months. y = years. ALT = alanine transaminase.
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neurological damage [21]. This variation may be due to a number of factors including duration of exposure and other factors which may influence absorption, storage, and effects of lead in the body such as genetic polymorphisms [43], co-morbidities, and essential trace element deficiency. The limited sensitivity and specificity of such as genetic polymorphisms [43], co-morbidities, and essential trace element deficiency. The limited sensitivity and specificity of neurological assessments and categorisation may also have contributed to the recorded variation.

A larger proportion of children with neurological features were aged 1–3 years compared with those without neurological features. The greatest proportion of high VBLLs were seen in children aged 1–<2 years, and the strongest association with neurological features was in this age group after adjusting for VBLL. During the initial outbreak investigation (before lead poisoning was confirmed and before chelation therapy began) proportionally more children aged 1–<2 years died amongst children displaying probable or suspected neurological features. We hypothesise that this risk pattern for both high VBLL and stronger association with neurological features may reflect a number of factors: variation in bioavailability of ingested lead; variation in exposure due to behavioural (i.e., hand-mouth) and developmental factors; immaturity of the blood/brain barrier compared with older children and possibly other age-related influences on activities and movement around the contaminated villages and ore processing areas. Conversely, there are several reasons why children younger than 1 year may have been less at risk of raised VBLL and neurological effects: shorter duration of potential exposure by virtue of younger age; poorer mobility and less marked hand-mouth behaviour; and physiological variation in absorption. However, the neurological damage done by lead, beyond fatal lead encephalopathy, is long-term [4,26] and only continued monitoring will allow us to fully document and understand the long-term sequelae of lead toxicity in this cohort.

Lead poisoning related to industrial activity is an ongoing problem, with a recent outbreak in Senegal secondary to used lead acid battery recycling linked to 18 child deaths and 81 additional cases of poisoning [18]. In the United Nations-Administered Province of Kosovo, camps for internally displaced people were contaminated by industrial lead production, and children born there between 1999 and 2007 had lead levels up to 74 µg/dL [44]; children near a recently closed auto-battery recycling plant in the Dominican Republic had venous blood lead levels up to 130 µg/dL [45]; and concerns continue about widespread exposure of children to lead from industries in China [36,46]. Other heavy metals such as mercury and cadmium also continue to cause contamination [47] and poisoning associated with industrial development [48,49]. With increased industrialisation in resource-poor countries and dumping of toxic waste, we expect greater frequency of undetected lower level poisoning and an increased risk of severe environmental toxicological emergencies. Whilst the Zamfara outbreak was at the most severe end of the spectrum, the true extent of lower-level poisoning and the implications for those exposed are unclear.

Limitations to our data arise from the circumstance in which they were collected, namely an emergency humanitarian response to mass mortality and morbidity from lead poisoning. While screening was offered to all children in the seven villages remediated in the first year, tragically, hundreds of children had died before the excess mortality was notified and cause identified in these villages; these children were not included in this analysis. In the first 2 months of the response, 42 children did not have VBLL recorded immediately prior to starting chelation therapy (with an earlier screening BLL ≥65 µg/dL) due to logistical challenges, including three who had had witnessed seizures and three with presumptive seizures; these children were not included in this analysis. The neurological data collected may have missed subtle neurological abnormalities that would not have been identified with the simple tools used that focused on detecting life-threatening encephalopathy. The VBLLs in the first months with the simple tools used that focused on detecting life-threatening encephalopathy. The VBLLs in the first months and possibly other age-related influences on activities and movement around the contaminated villages and ore processing areas.

### Table 4. Final multi-level logistic regression model to assess factors associated with having any neurological features at time of first-ever pre-chelation VBLL.

| Age at time of VBLL | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | p-value* |
|---------------------|------------------------|----------------------|----------|
| 0–<6 months         | 1.35 (0.36–5.04)        | 1.66 (0.44–6.31)     | <0.001   |
| 6–<12 months        | 2.07 (0.93–4.63)        | 1.46 (0.61–3.51)     |          |
| 1–<2 years          | 9.42 (5.23–16.96)       | 4.77 (2.50–9.11)     |          |
| 2–<3 years          | 3.54 (1.59–7.88)        | 2.69 (1.15–6.26)     |          |
| 3–5 years           | 1.00 (1.00–1.00)        | 1.00 (1.00–1.00)     |          |
| VBLL                |                        |                      | <0.001   |
| 45–64.9             | 1.00 (1.00–1.00)        | 1.00 (1.00–1.00)     |          |
| 65–79.9             | 1.21 (0.26–5.62)        | 1.02 (0.21–4.87)     |          |
| 80–99.9             | 3.06 (1.43–6.53)        | 2.75 (1.27–5.98)     |          |
| 100–119.9           | 4.87 (2.11–11.22)       | 3.84 (1.62–9.09)     |          |
| 120+                | 38.77 (18.14–82.86)     | 22.95 (10.54–49.96)  |          |

*P-value from the Wald test of no association of the attribute with the outcome adjusted for the other variables in the model. VBLL = venous blood lead level. OR = odds ratio. (n = 972). 
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associations were not substantially altered (data not shown). Children with inadequately recorded neurological status at time of VBLL had a lower geometric mean VBLL, which may indicate that these children were less likely to have exhibited neurological features. A sensitivity analysis of the model including all these patients as having had no neurological features did not alter the patterns of association (data not shown). The date of birth is often inaccurate or unknown in this remote, rural region, therefore most ages in this analysis are approximations, and thus age was categorised. In addition there was a substantial amount of missing data for some important variables such as the malaria RDT result. The fact that only symptomatic children were tested suggests that clinical malaria was present; however, as the RDT may be positive for weeks after treatment, microscopy would be needed to confirm acute infection. There is the potential that the neurological features seen may be related to comorbidities other than malaria or to permanent damage from previous high VBLLs. As with any large dataset, despite standardised forms, continuous use of data for clinical purposes, and regular data cleaning, it is possible that there was some misclassification, but this is considered unlikely to be differential. The strength of this dataset is its unequalled size, severity of lead exposure, and scope of routinely collected clinical data.

Conclusion

In this large cohort of 972 children exposed to environmental lead contamination, 55% of children ≥5 years with a VBLL requiring chelation therapy (≥45 μg/dL) had VBLLs ≥80 μg/dL. There was evidence that a VBLL ≥80 μg/dL was associated with neurological features and strengthened as VBLL increased, and neurological features were also more likely in children aged 1–3 years compared to those 3–5 years. Severe neurological features seen may be related to comorbidities other than malaria to permanent damage from previous high VBLLs. As with any large dataset, despite standardised forms, continuous use of data for clinical purposes, and regular data cleaning, it is possible that there was some misclassification, but this is considered unlikely to be differential. The strength of this dataset is its unequalled size, severity of lead exposure, and scope of routinely collected clinical data.

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