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ORIGINAL ARTICLE

Individual whole-body concentration of ^{137}Cesium is associated with decreased blood counts in children in the Chernobyl-contaminated areas, Ukraine, 2008–2010

Anna Lindgren[1], Eugenia Stepanova[2], Vitaliy Vdovenko[2], Daria McMahon[3], Oksana Litvinetz[2], Elena Leonovich[2] and Wilfried Karmaus[4]

The Narodichesky region, Zhitomir Oblast, Ukraine, is situated ~80 km from the Chernobyl Nuclear Power Plant, which exploded in 1986 and polluted the environment. A previous study found that children living in villages with high activity of ^{137}Cesium (Cs) in the soil had decreased levels of hemoglobin, erythrocytes and thrombocytes. These findings motivated the present study that used a more comprehensive exposure assessment, including individual whole-body concentrations (WBC) of ^{137}Cs (Bq/kg). This cross-sectional sample examined between 2008–2010, included 590 children in the age 0–18 years. Children with higher individual log(WBC) activity in the body had significantly decreased hemoglobin, erythrocyte and thrombocyte counts. The effect of log(WBC) on decreased thrombocyte count was only seen in children older than 12 years. The average village activity of ^{137}Cs (kBq/m^2) in soil was associated with decreased blood counts only indirectly, through ^{137}Cs in the body as an intermediate variable. Children in this study were born at least 4 years after the accident and thus exposed to low doses of ionizing radiation from ^{137}Cs. This cross-sectional study indicates that low levels may be associated with decreased blood counts, but we cannot exclude that these results are due to residual confounding factors.

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INTRODUCTION AND BACKGROUND

The Narodichesky region in Zhitomir Oblast, Ukraine, is one of the most contaminated populated areas around the Chernobyl Nuclear Power Plant, which exploded in 1986. The population in the contaminated areas is still exposed to ionizing radiation from the accident, mainly from the long-lived radioactive isotope ^{137}Cesium (Cs),[1] which has a physical half-life of 30 years. A previous longitudinal follow-up (1993–1998) found that children living in villages with high activity of ^{137}Cs in the soil had decreased levels of hemoglobin, erythrocytes and thrombocytes.[2] These findings motivated the present study, which used a more comprehensive exposure assessment, including individual whole-body concentrations (WBC) of ^{137}Cs (Bq/kg).

Radiation exposure in the years after the accident has been declining not only because of physical decay of the radionuclides but also because of vertical migration deeper into the soil, which shields off the exposure, and because of countermeasures to reduce the doses.[3] The average total (external and internal) radiation dose from Chernobyl for the population in the Narodichesky region has been estimated to be 17 mSv for the single year 1986, 13 mSv in cumulative dose for 1987–90, and only 3.2 mSv in cumulative dose 2001–05.[1] The average total dose from the accident for the single year 2008 in the Narodichesky region was estimated to be 0.13–2.2 mSv, depending on village.[4] The exposure is thus small in children born today compared with early after the accident.

External exposure from soil is only a part of the total dose, in the Narodichesky region it was ~70% of total exposure during 1986–2005.[1] In addition to external exposure radiating directly from radioactive deposition in the soil, people are internally exposed from ingestion of contaminated food; high activity of ^{137}Cs has been found in milk, meat and especially forest products such as mushrooms, wild berries, game meat and fish from local ponds.[3,5,6] Radioactivity from inhaled dust was an additional major source of internal exposure in the first years after the accident, but is an insignificant pathway today.[7] The levels of food contamination do not necessarily decrease as steadily as the soil radiation, as it's not affected by the vertical migration, but rather fluctuate from year to year depending on the harvest. The internal exposure from food makes up an increasing proportion of the total exposure in later years in many areas.[7,8]

Generally, a high level of ^{137}Cs deposition in soil can be expected to also contribute to a high level of ^{137}Cs in the body, and such a correlation has been reported,[9] but there are also studies that have found no correlation.[10,11] The lack of agreement depends on many factors such as the soil-transfer to vegetation, dietary habits and countermeasures applied to protect the population.

^{137}Cs is an alkali metal that has very similar characteristics to Potassium and is homogenously spread in the body. The average biological half-life in the body is 100 days before excretion. The uptake is rapid and complete both by inhalation and ingestion.[12]

[1]Department of Occupational and Environmental Medicine, Lund University, Lund, Sweden; [2]Department of Radiation Pediatrics, Congenital and Hereditory Pathology, Research Center for Radiation Medicine, Academy of Medical Sciences of Ukraine, Kyiv, Ukraine; [3]Department of Epidemiology and Biostatistics, Norman J. Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA and [4]Division of Epidemiology, Biostatistics and Environmental Health, School of Public Health, University of Memphis, Memphis, Tennessee, USA. Correspondence to: Dr Wilfried Karmaus, Division of Epidemiology, Biostatistics and Environmental Health, School of Public Health, University of Memphis, 301 Robison Hall, Memphis, TN 38152, USA.
Tel.: +1 901 678 2491, Fax: +1 901 678 0372,
E-mail: karmaus1@memphis.edu
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Considering the homogenous spread of moderate amounts of ^{137}Cs in the body, organ-specific doses resulting from ^{137}Cs in the body can in the Zhitomir region be expected to be low.

Although the acute detrimental effects of high-dose radiation on the hematopoietic system are well known, both from Chernobyl,[13] and other radiation accidents,[14] there is little information on long-term low-dose radiation effects on the hematopoietic system. The Techa river incident, a plutonium processing facility established in 1948 with radioactive waste leakage into the river, caused decreased levels of thrombocytes, erythrocytes and hemoglobin, in addition to a range of other symptoms, which together were labeled "chronic radiation syndrome".[15,16] However, in residents of the areas along the Techa river, the accumulated bone-marrow doses from internal ^{137}Cs and ^{90}Sr were up to 3–4 Sv, considerably higher than the total accumulated doses in the Narodichesky region.

Regarding health effects in the Chernobyl area, in addition to our previous study, we have identified six published articles on long-term effects on red blood cells, but these are in people born before the accident or irradiated in utero.[17–22] Stepanova et al.[22] found more transitory, prehemolytic and degenerative forms of erythrocytes in children irradiated in utero, in comparison with control children.

A study with cross-sectional comparisons in 1986, 1992 and 1998 found no difference in mean hemoglobin, erythrocyte or thrombocyte counts in children residing in settlements with ^{137}Cs soil activity of 37–555 kBq/m^2 compared with children living in settlements with less than 37 kBq/m^2.[19] However, this study only describe means in the two exposure groups without statistical adjustment for any factors, which is typical for many of the studies of health effects from Chernobyl.[23] In contrast, in a repeated measurement study (1993–1998), which included children born before and after the accident, we found decreased hemoglobin, erythrocyte and thrombocyte counts in children residing in villages with ^{137}Cs soil activity of 266–310 kBq/m^2 and 350–879 kBq/m^2 compared with 29–112 kBq/m^2.[2]

The purpose of the analysis at hand is to investigate whether our previous findings can be replicated and expanded upon by using more comprehensive exposure assessment, including not only average village soil activity of ^{137}Cs but also individual measurements of WBC of ^{137}Cs.

METHODS

Study Population

Settlements in the Narodichesky region (Zhitomir Oblast, Ukraine), are located ~80 km from the Chernobyl Nuclear Power Plant. In 2001, a population census determined that this region had ~11,400 inhabitants, including 2000 children. The study area has been extensively described in previous publications.[2,24] The lifestyle in the Narodichesky region is similar in all villages, with predominantly locally homegrown food supply and little migration. All children in the Narodichesky region are provided with free breakfast and lunch at school. The menu at school has to meet standards of sanitary hygiene and follow the provision of the Ukrainian government.[25] The meals served in school are prepared from products imported from areas free from contamination with radionuclides. Meals consumed at home are prepared from local produce (milk, meat, fish, vegetables, berries, mushrooms and fruit).

Study Selection and Missing Data

This cross-sectional study included 590 children in the age 0–18 years; 566 (age 2–18) had complete exposure measurements on ^{137}Cs WBC and residential ^{137}Cs soil estimates, and were used in the analyses.

We examined predominantly children of school age. In addition, some preschool children were assessed upon request of their parents. On the day of examination, all school children present in a class were transported to the Central Hospital of Narodichi by their teachers. Parents were notified of the forthcoming examinations in advance, and their verbal permission was requested for young children (0–6 years). Parents of all school children gave their written permission for the examination.

We excluded from the analysis 23 children with missing values of ^{137}Cs WBC and one child with missing information on ^{137}Cs in soil. Missing WBC was found exclusively in children 0–4 years of age, as these children had difficulties to comply with the exposure assessment.

Exposure Assessment

Village average soil activity of ^{137}Cs. The average ^{137}Cs soil activity per village for 2008 was obtained from a report published by the Ukrainian Ministry of Health.[4] The Ministry publishes yearly estimates for the purpose of monitoring the population exposure. The report stated that the estimates of ^{137}Cs in soil for 2008 were based on soil measurements conducted in 1992 and that a decay function was applied to obtain the estimates for 2008. The estimated average external effective dose for each village in 2008 was also obtained from this publication.

Individual measurements of WBC of ^{137}Cs. WBC of ^{137}Cs was measured for all children in 2008–2010 using a gamma-spectrometer (Whole-Body Counter SCRINNER-3 M, designed and produced by INECO (Ukrainian Institution of Human Ecology, Academy of Technological Sciences). "SCRINNER-3 M" is used in 12 regional medical centers of the Ukraine for mass screening of residents of contaminated areas,[26] and is also used in Belarus, at the Whole-Body Counter Laboratory at the Institute of Radiation Safety.[27]

The whole-body counter is designed as a standard chair (with lead (Pb) shielding in the back and seat of the chair for local protection from background radiation). The single detector of the "SCRINNER-3 M" is incorporated into the back of the chair and detects γ-rays from the top of the head to the knee level. The angle between the back of a chair and the seat is 100°, and the distance form the back and the base of the chair to detector is 40 cm. Children were sitting in upright position during the measurements.

The "SCRINNER-3 M" is equipped with Pb collimator: thickness 50 mm, coaxial scintillation detector NaI(Tl): Ø150×100 mm. Before the measurements, "SCRINNER-3 M" is calibrated using phantoms in six age groups, composed from taurpaline filled with dried peas with known radionuclide content. Additional information about the phantoms is presented in Supplementary File S1. IAEA has made intercomparisons of chair type whole-body counters with other whole-body counters, and also of "dried pea phantoms".[28] The general characteristics of the " SCRINNER-3 M" in calibrated state is given in Table 1. The minimum detection activity (MDA) for 3 min exposition on a 70-kg adult phantom (with a maximum error of 30%) was 340 Bq. The measurements were conducted according with the recommendations provided by the Research Center of Radiation Medicine Academy of Medical Sciences of Ukraine.[29,30] The protocol has been published.[31] Each measurement continued until its error dropped to the level of 30% or lower as required by the standards of the International Commission on Radiological Protection (ICRP). Under these measurement conditions, the required measurement error for most children was obtained within 3 min, and only a few children required up to 5 min. Calculations of measured activity (Bq) accounted for child's weight by including calibration coefficients. To further reduce residual confounding by weight, we took further account of child's weight by using WBC (Bq/kg) as the unit for our regression analyses, instead of the measured activity (Bq).

Every child had only one measurement of the WBC of ^{137}Cs. Almost all of the children, 561 out of 566, had this measurement in 2009. The measurements were predominately conducted in three months: April ($n=255$), September ($n=209$) and December ($n=55$). A few measurements were done in others months. As levels of ^{137}Cs are known to potentially vary by season due to different food consumption during different seasons (i.e., mushrooms in the autumn),[32,33] we adjusted for season in the final multivariable statistical analyses.

Calculations of Individual Internal Effective Dose From ^{137}Cs

The internal dose calculations were based on the age-dependent committed dose coefficients for ingestion e(nSv/Bq) from ICRP publication 72.[34] Based on the point-measurements of ^{137}Cs in the body, a constant steady state of the measured activity was assumed for the whole year. A biological half-life of 100 days was assumed, with an effective half-life of 99 days. The intake in Bq to remain in steady state was first calculated for 1 day: (ln 2/99) $\times$ ^{137}Cs (Bq). The doses were then calculated as: daily intake to remain in steady state (Bq) $\times$ e (nSv/Bq) $\times$ 365, and converted to mSv/year.

Table 1. General characteristics, WBC "SCRINNER-3 M".

Multichannel Pulse Analyzer (MCA)	ADC (1024 ch), interface serial RS-232
Detector	NaI(Tl) Ø150 × 100 mm
Collimator	Pb, thickness 50 mm
Measured radioisotope	^{137}Cs
Registration energy, kEV	662
MCA step, kEV/Ch	2
Energy resolution, %	10.2
Registration efficiency for 70 kg adult, Bq/cpm	6,5
Background attenuation factor for 70 kg adult	0.77
Background counts, cpm	2200
Minimum detection activity (MDA, sensitivity level for 3 min exposition of 70 kg adult), Bq	340

Abbreviation: WBC, whole-body counter.

Medical Assessment and Questionnaires

Medical assessments followed the same protocol as the previous studies.[2,23] Blood was collected in tubes containing EDTA. A blood count, including erythrocytes (red blood cell count; RBC), leukocytes, thrombocytes (PLT) and hemoglobin (Hb), was conducted using Sysmex model F-800 (TOA Medical Electronics Company, Kobe, Japan). Normal blood smears were stained using the standardized azure B-eosin GIEMSA Y Romanowsky procedure and the cells were counted. We calculated the color parameter (CP) to classify anemia. CP is an old parameter equal to mean corpuscular hemoglobin (MCH) $\times$ 0.03 with MCH = Hb (g/l) / number of erythrocytes (1×10^{12} cells/l).[35]

CP <0.8 (hypochrome) may be indicative of iron-deficiency anemia, CP = 0.8–1.0 (normochrome) is considered normal range, and CP >1.0 (hyperchrome) may be indicative of B12/folate-deficiency anemia. The reference values for hemoglobin, erythrocytes and thrombocytes are provided in (Table 2).[36,37]

More extensive confounder information was gathered in this study compared with our previous investigation. Information on potential confounders such as environmental tobacco smoke (ETS), active tobacco smoking and type of fuel used for residential heating or cooking was collected during interviews. Medical assessments, interviews and the measurements of WBC of ^{137}Cs were performed on the same day.

Statistical Methods

Multiple Linear Regression analyses were performed using Rx64 (R Development Core Team, 2012), version 2.15.0 to estimate the associations of ^{137}Cs in soil and WBC with blood counts. The outcome variables were hemoglobin, erythrocyte count and thrombocyte count. The exposure variables were log(WBC) and ^{137}Cs in soil (kBq/m^2). The log transformation of WBC (Bq/kg) was done to fulfill the model assumptions of linearity and constant variance. We adjusted all estimates for sex of the child, age (continuous), season (spring, summer, autumn and winter), ETS (yes/no), active tobacco smoking (0 cigarettes/day, 1–10 cigarettes/day, >10 cigarettes/day) and use of coal/wood for cooking (yes/no). These variables were deemed as potential confounders, and kept in the model regardless of their statistical significance, for model consistency. All 566 children had complete information on these variables. None of the 566 observed values were excluded from the analyses, despite a few extreme values on WBC. A few extreme values were expected since the ^{137}Cs intake may be highly variable.

Effects of interaction between ^{137}Cs in soil and age, and log (WBC) and age on blood counts, were tested by partial *t*-test with age as a continuous variable. If the interaction terms were significant we presented seperate effect estimates for different age groups. As a sensitivity analysis, we included CP as an additional categorical variable (hypochrome, normochrome and hyperchrome) in the model that estimated effects of ^{137}Cs in soil and log(WBC) on Hb. The purpose was to see if the effect estimates changed when adjusting for hypochrome and hyperchrome erythrocytes, which are often caused by nutritional deficiencies. The significance level for all analyses was $\alpha = 0.05$.

Path analysis, that is, covariance analysis of linear structural equations (28), was performed using SAS version 9.3. Path analysis is a method used to decompose sources of correlation to see how much of the total effects of a variable is due to its "direct effect" (partial correlation with the outcome after adjustment for other variables) and "indirect effect" (correlation with variables which have a partial correlation with the outcome). Path analysis can be used to test the assumption that a variable is an intermediate step in a chain of responses. If it is, the exposure variable should have a direct effect on the assumed intermediate variable, which should have a direct effect on the outcome.

Table 2. Ukrainian reference levels for Hb, RBC and PLT, and prevalence of children with current values below reference levels.

	Reference level	(*n* Below)/ (total *n*)	% Below
Hb[a] (g/l)	0–4 years ≥ 110	1/8	12.5%
	5–7 years ≥ 115	5/25	20%
	8 and older ≥ 120	117/533	22%
Total		123/566	21.7%
RBC[b] ($\times 10^{12}$ cells/l)	2–5 years ≥ 3.9	4/16	25%
	6–11 years ≥ 4.0	26/134	19.4%
	12 to 17 years ≥ 4.5	340/416	81.7%
Total		370/566	65.4%
PLT[c] (1×10^9 cells/l)	All ages ≥ 150	4/566	0.7%

Abbreviations: Hb, hemoglobin; RBC, red blood cells; PLT, platelet.
[a]Shabalov.[36]
[b]Nicholson and Pesce.[37]
[c]Stepanova *et al.*[2]

Based on results from our previous study, we hypothesized that ^{137}Cs in soil (1) has a direct effect on blood counts, and (2) also has an indirect effect on blood counts through WBC Bq/kg as an intermediate variable. The full model that we first specified is included in the Supplementary File S2. Blom transformation was used to standardize the variables. The model was modified to fulfill the criteria for model fit, with non-significant χ^2-test, root mean square residual close to zero, goodness of fit index (GFI) and adjusted goodness of fit index (AGFI) >0.98 indicating a good fit.[38] The final fitted path models supported by the data are presented in Supplementary File S2.

RESULTS

Population

Fifty percent of the children were recruited from the small town Narodichi, and the rest from 27 different rural villages (Table 3). Five children were living outside the Narodichesky region.

All children were born after the accident, the oldest child in 1990 (Table 4). The majority (73.5%) of the children were between the ages of 12–18 at the time of examination. Just under half of the sample (45%) was exposed to ETS, but only 5% of the children were active daily tobacco smokers (Table 4).

Exposure Description of WBC and ^{137}Cs kBq/m^2

The individual WBC Bq/kg had a right-skewed distribution with few observations above 300 Bq/kg (Figure 1a). The average activity of ^{137}Cs in soil of the village had a left-skewed distribution with approximately half of the population living in a village with >200 kBq/m^2 (Figure 1b, Table 3). Overall, the individual WBC had moderate positive correlation with the activity of ^{137}Cs in soil (Spearman's rank correlation, $r_s = 0.508$ $P < 0.001$; Figure 1c).

The WBC was highest for measurements performed in the spring (Figure 2). The mean internal effective dose was 0.14 mSv/year (Table 4). There were five children with an estimated effective internal dose >1 mSv/year.

Blood Markers

Overall, many children appeared to be low in hemoglobin concentrations and erythrocyte counts. In all, 21.7% of the children were below the reference values for Hb, and 65.4% were below the reference values for erythrocytes (RBC) for their age.

Table 3. Description of current ^{137}Cs exposure level for villages included in the study.

			External exposure (official estimates for year 2008)		Internal exposure (point-measures, 2008–2010)		
			Official average surface density of ^{137}Cs in soil[4]	Official average external effective dose[4]	^{137}Cs WBC, measured in this study (mean)	Internal effective dose calculated in this study	
						Mean	Range[a]
Village	N	%	(kBq/m^2)	(mSv/year)	^{137}Cs (Bq/kg)	(mSv/year)	
Jitomir[b]	1	0.2	—	—	18	0.03	—
Kiev[b]	1	0.2	—	—	17	0.02	—
Brodnik	16	2.8	41	0.11	27	0.03	(0.02–0.06)
Liplanchina	1	0.2	53	0.15	32	0.06	—
Vyasovka	8	1.4	54	0.15	31	0.04	(0.01–0.06)
Rubejevka	2	0.4	55	0.15	47	0.02	(0.02–0.03)
Radcha	3	0.5	57	0.16	33	0.04	(0.03–0.04)
Budo-Golubevi	2	0.4	58	0.16	131	0.15	(0.09–0.20)
N. Radcha	2	0.4	72	0.20	24	0.04	(0.04–0.05)
Klochki	2	0.4	73	0.20	16	0.04	(0.03–0.04)
Norinci	15	2.7	77	0.21	26	0.04	(0.02–0.09)
Guto-Mariatyn	6	1.1	80	0.22	61	0.07	(0.02–0.14)
Babinichi	10	1.8	87	0.24	68	0.06	(0.02–0.10)
Laski	19	3.4	88	0.24	15	0.03	(0.005–0.05)
Jerev	14	2.5	90	0.25	44	0.06	(0.03–0.08)
Bolotnica	17	3	91	0.25	30	0.05	(0.01–0.21)
Latashi	5	0.9	92	0.26	16	0.03	(0.02–0.05)
S. Dorogin	4	0.7	114	0.32	30	0.06	(0.02–0.15)
Zakusily	33	5.8	114	0.31	72	0.09	(0.03–0.22)
N. Dorogin	2	0.4	117	0.32	20	0.04	(0.03–0.04)
Zalesie	2	0.4	137	0.38	91	0.13	(0.07–0.18)
Suharevka	15	2.7	145	0.40	95	0.11	(0.04–0.21)
Moteyki	1	0.2	155	0.43	201	0.38	—
Megeliska	28	4.9	163	0.45	125	0.12	(0.03–0.25)
Selec	23	4.1	214	0.59	135	0.23	(0.03–1.36)
Narodichi	284	50.2	242	0.67	123	0.17	(0.009–1.41)
Basar	49	8.7	252	0.70	138	0.16	(0.01–0.34)
Lubarka	1	0.2	274	0.76	139	0.27	—

Abbreviations: Cs, cesium; WBC, whole-body concentration.
Official residential soil activity and external doses of ^{137}Cs for each village, and measured individual whole-body concentration and internal dose of ^{137}Cs ($n = 566$).
[a]Range (Min, Max) is not presented for the villages where only one child was measured.
[b]There are no official estimates of the external effective dose from ^{137}Cs in Jitomir and Kiev, as these areas are considered non-contaminated.

Hemoglobin values and erythrocyte counts below reference levels were more common in the older children (Table 2). For thrombocyte counts (PLT), only 0.7% of the children were below the references values for their age.

Plots of the bivariate relationships between individual WBC and log(WBC) with blood counts, suggest negative associations between WBC and Hb, RBCs and PLTs (Figure 3). Although less apparent, similar relationships were observed between ^{137}Cs activity in soil (kBq/m^2) and Hb, RBCs and PLTs (Figure 4). The log-transformed WBC (Bq/kg), log(WBC), had a more linear relationship with the outcomes, and was used for the multiple linear regression analyses.

In multiple linear regression analyses, log(WBC) was associated with the hemoglobin level (Table 5). One unit increase in log(WBC) decreased hemoglobin by -5.94 g/l, 95% CI (-6.75, -5.13). One unit increase in ^{137}Cs activity in residential soil was associated with decreased Hb, when adjusting for confounders other than individual log(WBC), but when adjusting for log(WBC) the effect was small and statistically insignificant (Table 5). There was no interaction between log(WBC) or ^{137}Cs in soil and age, regarding the effects on Hb.

The log(WBC) was also associated with erythrocyte count (RBC; Table 5). One unit increase in log(WBC) decreased the RBC by -0.238×10^{12}/l, 95% CI (-0.27, -0.21). One unit increase in ^{137}Cs activity in soil was also associated with decreased RBC when adjusting for confounders other than individual log (WBC), but when adjusting for log (WBC) the effect was very small and instead associated with increased RBC (Table 5). There was no interaction between log(WBC) or ^{137}Cs in soil and age, regarding the effects on RBC.

Regarding the effects on thrombocytes, there was a significant interaction between log(WBC) and age (P-value $= 0.004$), when using age as a continuous variable, but non-significant for ^{137}Cs in soil and age. We therefore show separate results for age <12 years and 12–18 years. In children 12 years and older, log(WBC) was associated with thrombocyte count (Table 5). One unit increase in log(WBC) decreased the count by -15.99×10^9/l, 95% CI (-21.37, -10.62). In children 12 years and older, one unit increase in ^{137}Cs activity in soil was also associated with decreased thrombocytes when adjusting for confounders other than individual log(WBC), but when adjusting for log(WBC) the effect was very small and statistically insignificant (Table 5). In children 2–11 years, there was no statistical association between log(WBC) or ^{137}Cs in soil with the thrombocyte count.

Sensitivity Analysis Using CP

There were 18 children with CP <0.8, indicating low levels of hemoglobin per erythrocyte; 527 children had a normal

Table 4. Characteristics of the study population ($n = 566$), and distribution exposure and outcome variables.

	N	%
Sex		
Female	266	47
Male	300	53
Age group (at time of examination), years		
<6	16	2.8
6–11	134	23.7
12–18	416	73.5
Birth year		
2002–2007	29	5
1996–2001	230	40.6
1990–1995	307	54.2
Environmental tobacco smoke (ETS)		
No	313	55
Yes	253	45
Active daily tobacco smoking, (cigarettes/day)		
No	538	95
1–10	23	4
>10	5	0.9
Use of coal/wood for cooking		
No	403	71
Yes	163	29
Activity of ^{137}Cs in residential soil (kBq/m^2)		
<116	161	28.4
116–164	48	8.4
>165	357	63.1

	Mean	Median	Range
Age (years)	12.9	13	2–18
Weight (kg)	47.4	48	12.5–99.7
Activity of ^{137}Cs in residential soil (kBq/m^2)	189.4	242	10–274
Activity of ^{137}Cs in the body (Bq)	4447	3404	185–42,480
WBC^{137}Cs (Bq/kg)	100.5	77.1	4–916
Log (WBC)	4.2	4.3	1.4–6.8
Effective internal dose (mSv/year)	0.14	0.10	0.005–1.41
Hemoglobin (g/l)	125.5	124	96–155
Erythrocyte cell count (RBC) 1×10^{12} cells/l	4.2	4.1	3.1–5.2
Thrombocyte count 1×10^{9} platelets/l	250.5	249	138–422

Abbreviations: Cs, cesium; WBC, whole-body concentration.

CP = 0.8–1.0, and 21 children had a CP >1.0 indicating high levels of hemoglobin per erythrocyte. Adjusting for CP as a categorical variable with three groups (CP <0.8, CP = 0.8–1.0, CP >1.0) did not change the effect estimate for log(WBC) on Hb, which changed by less than 2%. The effect of ^{137}Cs activity in soil remained small and statistically insignificant after adjustment for CP. The fact that adjustment for CP did not change the results, decreases the likelihood (but does not fully exclude) that iron-deficiency anemia or B_{12}/folate-deficiency anemia (which typically exhibits abnormal CP) are confounders in this study.

Path analysis Investigating ^{137}Cs/kg as an Intermediate Variable Between ^{137}Cs in Soil and Hb, RBC and PLT

The partial correlations supports our hypothesis that residential soil activity of ^{137}Cs had an indirect effect on blood counts through a direct effect of WBC (Bq/kg). There was no support for any direct effects from ^{137}Cs in soil. The graphs of the final fitted models supported by the path analysis can be seen in the Supplementary File S2.

DISCUSSION

The main strength of the study was, compared with our previous study, that the village levels of ^{137}Cs exposure were complemented by assessment of individual levels of ^{137}Cs WBC, which can be expected to decrease the amount of measurement error. We also gathered individual information on risk factors such as active tobacco smoking, ETS and household fuels such as coal and wood, which had only marginal influence on the risk estimates. As the residents in the investigated areas are poor, anemia due to poor nutrition is not unlikely, and we hypothesized this could confound or dilute the effects of radiation. Our results, however, show that most children had normal CP, which indicates that iron or B_{12}/folate deficiency due to poor nutrition is not a main cause of anemia in this area, and our sensitivity analysis shows that having low/high CP was not associated with ^{137}Cs exposure. These findings speak against that the low blood counts are due to nutritional deficiencies. However, we cannot exclude completely that a combination of iron-deficiency and B_{12}-may exist. The combination of both would result in a normal CP. Hence, the absence of other biochemical parameters such as ferritin and B_{12} is a limitation of our study. The expected effect of long-term exposure to ^{137}Cs on red blood cells is most likely related to activation of free radical oxidation in these cells. It is also possible, that long-term exposure ^{137}Cs may directly affect bone marrow and cause decreased production of blood cells and development of normochrome (normocytic) anemia, which was also mainly observed in this population.

Our results show that children of the Narodichesky region with high levels of log-transformed WBC of ^{137}Cs had decreased levels of hemoglobin, and erythrocyte and thrombocyte counts. The average village level of ^{137}Cs in soil was not associated with decreased blood counts after adjustment for individual body burden of ^{137}Cs. The effects of log(WBC) on decreased thrombocyte counts were only seen in children 12 years and older.

Our results, supported by a path analysis, suggest that internal exposure of ^{137}Cs affects blood counts, and that ^{137}Cs in soil has only indirect effects on the blood counts via internal exposure to ^{137}Cs as an intermediate variable, but no direct effect due to external exposure was detected. However, this finding should be interpreted with caution and cannot be fully attributed to internal dose being more important than external exposure, as it may also be influenced by measurement errors of the external exposure. The external exposure was assessed on average village level, while internal ^{137}Cs was measured on an individual level, that is, a larger measurement error can be expected to dilute the effects of external exposure. However, the strength was that there was a high variability in the level of ^{137}Cs soil contamination between the villages.

The level of external exposure has been decreasing for each year since the accident, but the ranking of which villages receives high or low external exposure is identical since 1992.[4] The average soil contamination for the village of a child can thus be used as a proxy of both cumulative and current exposure of that child. The population is stable and rarely relocates to places with different external exposure levels, which further strengthens the validity as a proxy for long-term exposure.

Internal exposure to ^{137}Cs measured by WBC is a point measurement primarily reflecting current intake, but it may also have long-term predictive value if dietary habits and levels of dust inhalation are stable. The very highest ^{137}Cs WBC are likely to result from very recent intakes of contaminated food. The fact that we adjusted for season and also took the logarithm of WBC, shrinks the values of the peak whole-body concentrations and probably makes the concentrations more representative of average long-term intake.

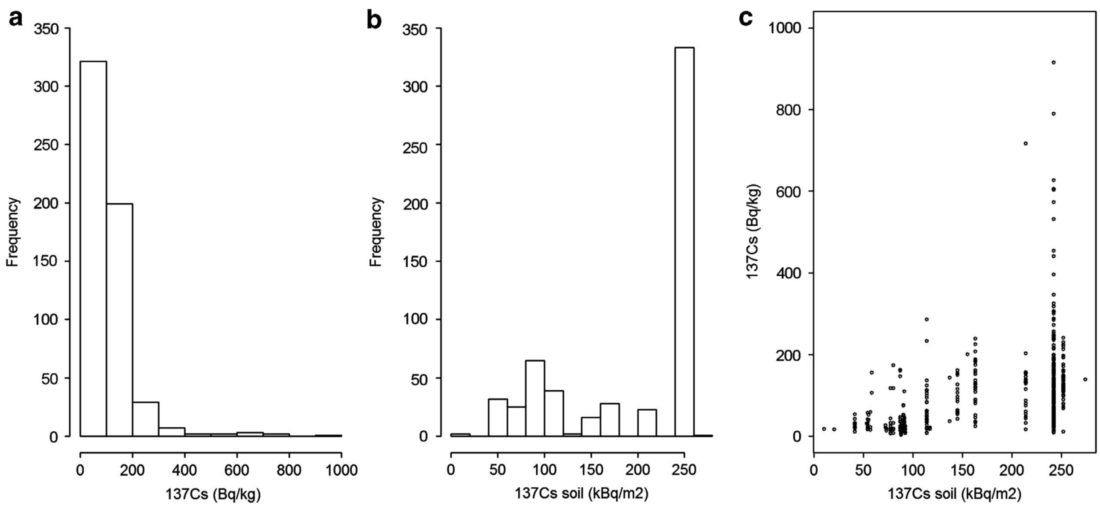

Figure 1. Exposure distributions of (a) ^{137}cesium (Cs) whole-body concentration (WBC, Bq/kg), (b) ^{137}Cs in Soil(kBq/m^2) and (c) the correlation between ^{137}Cs in soil (*x* axis) and ^{137}Cs WBC (*y* axis). ($n = 566$).

The cross-sectional nature of the study, however, was a limitation. To study dose–effect relationships, one needs to have information about the total cumulative dose for the entire period of residence in the Narodichesky region.

As WBC reflects dietary intake, the most likely potential confounding factors would be nutritional deficiencies or environmental contaminants correlated with ^{137}Cs. There may be a possibility of environmental confounding or partial effects due to other long-lived radionuclides released from the Chernobyl accident, such as ^{90}Strontium, ^{239}Plutonium and ^{241}Americium. These radionuclides were not as widely geographically distributed as ^{137}Cs, but mainly deposited within 100 km of the Chernobyl reactor due to dispersal with larger particle size.[3] However, these radionuclides have a relatively high deposition in Zhitomir Oblast,[1,39] but have not been measured to the same extent as ^{137}Cs. They are expected to make a small contribution to the total radiation dose in the population, but they are prone to bone deposition and are also accumulated in the bone due to very long physical and biological half-life.[12] Especially, Plutonium and Americium are concentrated in the endosteum,[12] which is also where stem cell production in the bone marrow is concentrated.[40] In addition, a large amount of Pb was dumped on the burning Chernobyl reactor to extinguish the fire, and asPb is a known cause of anemia the possibility of confounding due to Pb has to be considered. However, a study conducted in areas 30–100 km from Chernobyl found levels of Pb in soil to be low, between 2–27 mg/kg, which equals background values in these soil types.[41]

The present study supports the previous finding that children living in villages with high level of ^{137}Cs in soil had decreased levels of hemoglobin, red blood cells and thrombocytes.[2]The previous study showed that thrombocytes were less affected in later years, which is also in agreement with our current finding that thrombocytes are only affected by WBC in older children. A previous study by Babeshko et al.,[19] did not find any difference in blood cells count depending on village level of ^{137}Cs in soil, but this may depend on the simple comparison of means between the two groups of exposure, without adjustment for other factors. Previous studies in Zhitomir Oblast have found morphological changes in red blood cells in children irradiated in utero and living in the exposed areas.[22] This is not directly comparable as our children are born at least a few years after the accident, and thus been exposed to lower doses of radiation.

Studies in Kyrgyzstan have found decreased Hb and erythrocytes in children and adults living in close proximity to radioactive waste dumps, compared with control groups.[42,43] However, the exact levels of radioactive exposure for these individuals were not presented.

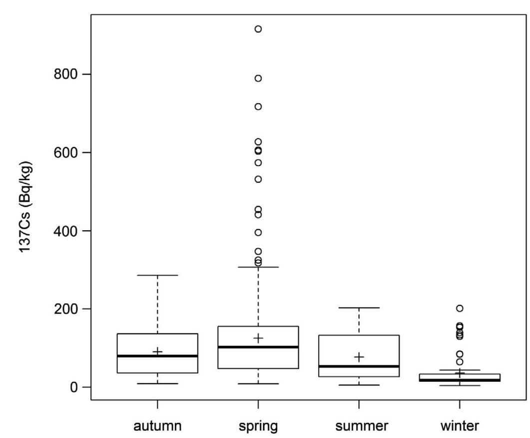

Figure 2. Whole-body concentration (WBC) by season. Boxplots displaying the exposure distributions of ^{137}cesium (Cs) whole-body concentration (Bq/kg), depending on season of the measurement ($n = 566$). The cross refers to the mean WBC.

The current total doses from the Chernobyl accident in Zhitomir Oblast have been estimated to be low, on average 0.13–2.2 mSv/year in 2008,[4] and this agreed well with the doses observed in our study. For some children, the levels were above the 1 mSv/year, which is the reference level as recommended by the ICRP for population exposure from a non-natural source,[44] but well below the 20 mSv/year which is the ICRP reference level for occupational exposure.

After the Techa river incident, in which people were exposed to accumulated bone marrow doses of up to 3–4 Sv, hematopoiesis recovery was seen after reduction in red bone marrow dose rates to 100 mSv/year or lower.[16] However, the dose reconstruction is retrospective in these studies, and the high uncertainty in the dose estimates can be expected to have diminished the ability to find effects from lower doses.

The ability to find effects of low-level exposure in epidemiological studies is highly dependent on the precision of the exposure assessment. WHO concluded that there is a complete lack of analytical studies of long-term effects from Chernobyl.[23] This is the only study on long-term effects from ^{137}Cs on blood cells, which

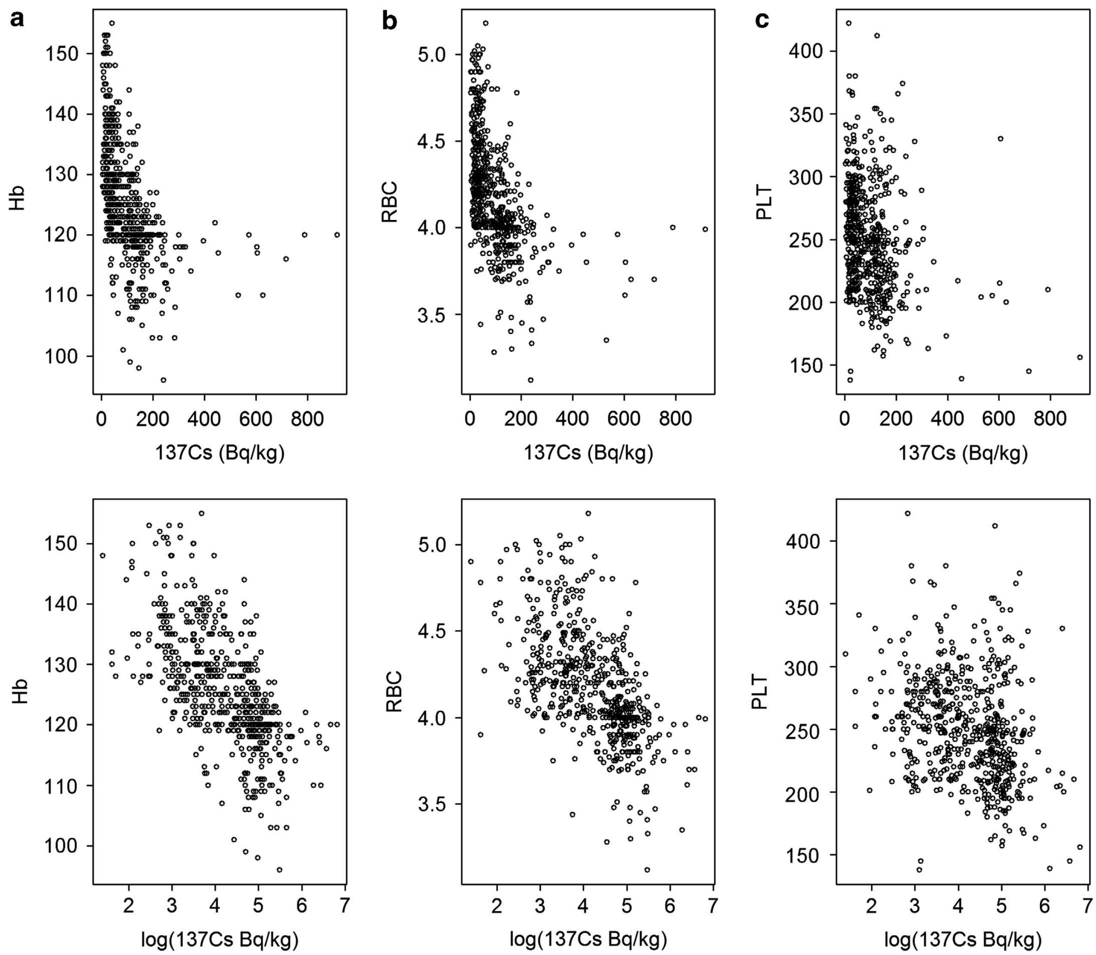

Figure 3. Individual whole-body concentration (WBC) of ^{137}cesium (Cs) (Bq/kg) and log(WBC), plotted against individual blood counts: hemoglobin (Hb) g/l, erythrocyte count (red blood cell; RBC) 1×10^{12} cells/l and thrombocyte count (platelet; PLT) $\times 10^9$ platelets/l, ($n = 566$).

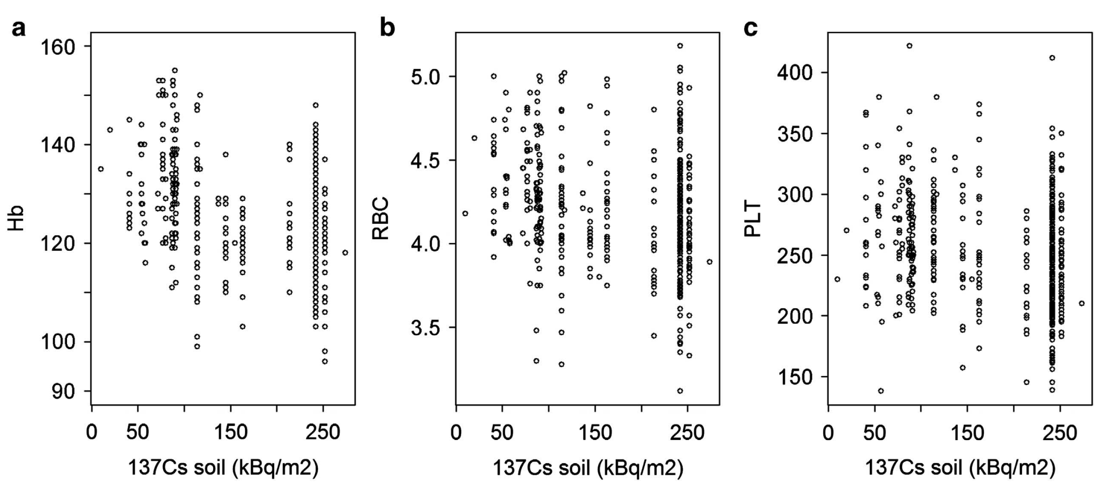

Figure 4. Residential activity of ^{137}Cesium (Cs) in soil (kBq/m^2), plotted against individual blood counts: (a) hemoglobin (Hb) g/l, (b) erythrocyte count (red blood cell; RBC) $\times 10^{12}$ cells/l and (c) thrombocyte count (platelet; PLT) $\times 10^9$ platelets/l ($n = 566$).

has used individual exposure assessment. Our findings suggest that the radiation exposure from fallout after the Chernobyl accident may still affect children in the contaminated areas. However, possible confounding factors from other radionuclides or heavy metals released from Chernobyl cannot be excluded and should be considered in future studies.

Although an association between low erythrocytes/Hb/thrombocytes and high ^{137}Cs was detected, the blood counts were only mildly decreased. No major symptoms should be expected of these subnormal blood counts observed in the children, except possible tiredness in the children with most apparent anemia. However, even if mildly decreased blood counts do not markedly

Table 5. Linear relationship between the whole-body concentration (WBC) of ^{137}Cs (Bq/kg) and the activity of ^{137}Cs in residential soil (kBq/m^2) and blood counts ($n = 566$).

	Estimate	SE	P-value
Hemoglobin (g/l)			
Log(WBC)[a]	−5.940	0.411	<0.001***
^{137}Cs in soil (kBq/m^2)[a]	−0.00241	0.00621	0.6978
Red blood cells (1×10^{12} cells/l)			
Log(WBC)[a]	−0.238	0.0148	<0.001***
^{137}Cs in soil (kBq/m^2)[a]	0.000548	0.000224	0.0149*
Platelets (1×10^9 plt/l)			
Age <12			
Log(WBC)[b]	−4.7479	5.348	0.3762
^{137}Cs in soil (kBq/m^2)[b]	−0.08901	0.06682	0.1850
Age ≥12			
Log(WBC)[a]	−15.99	2.735	<0.001***
^{137}Cs in soil (kBq/m^2)[a]	−0.052	0.0446	0.24387

Abbreviation: Cs, cesium.
[a]Adjusted for Cs in soil (kBq/m^2), log(^{137}Cs Bq/kg), age, sex, exam season, ETS, active tobacco smoking and use of coal/wood for cooking.
[b]Not adjusted for active smoking, as no children below 12 were smokers.

affect a healthy child under normal circumstances, the resources to cope with diseases or hard physical exercise may be diminished, especially if the subnormal blood counts reflect underlying disease with diminished cell production capacity.

Children in this study were born at least 4 years after the accident and thus exposed to low doses of ionizing radiation from ^{137}Cs. This cross-sectional study indicates that these levels may be associated with decreased blood counts, but we cannot exclude that our results are due to residual confounding factors. As WBC reflects dietary intake, the most likely potential confounding factors could be nutritional deficiencies or environmental factors causing anemia/decreased blood count. Future studies are needed to investigate the possible role of other radionuclides that may be correlated with ^{137}Cs, and possibly other heavy metals, in addition to ^{137}Cs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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