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Health investigations of depleted uranium clean-up workers

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Key words
Depleted uranium; decontamination; clean-up workers

Summary
Background: *The soil contaminated by depleted uranium (^{238}U) ammunition during the NATO bombing of Serbia and Montenegro was cleaned-up for four months in 2002. A team of 11 clean-up workers (expert members) were medically examined three times: before decontamination as a preliminary medical check-up, immediately after decontamination, and four years after cleaning up contaminated ground.* **Objectives:** *This short report presents investigations and health risk assessments of clean-up workers in radioactive decontamination operations and an assessment of the environmental health perspectives for citizens living in surrounding areas.* **Method:** *The method of initial health disorders was used, analyzing the most sensitive biological materials, such as blood cells or chromosome damage, DNA strand breaks, radio-toxicological examination of urine.* **Results:** *The total number of blood cells did not change, but variations of the relative number (percentage) of cells in the leukocyte formula were observed. The total number of DNA alterations was higher immediately after decontamination than before decontamination. Four years after decontamination the number of DNA alterations had decreased. However, the number of damaged cells (lymphocytes containing chromosome lesions) was higher in both medical examinations, immediately after and four years after decontamination.* **Conclusions:** *Disease or tumours due to ^{238}U did not develop in the group of depleted uranium clean-up workers during the investigation period of four years. Further monitoring of haematological and chromosomal effects and the health condition of workers is necessary.*

Riassunto
Indagini sulla salute di addetti alla decontaminazione di uranio impoverito». *Il terreno contaminato da munizioni contenenti uranio impoverito (^{238}U), durante il bombardamento della Serbia e del Montenegro da parte della NATO, è stato bonificato e decontaminato nel corso di un periodo di 4 mesi nel 2002. Una squadra di 11 operatori esperti della decontaminazione sono stati sottoposti a visita medica in 3 occasioni: prima delle operazioni di decontaminazione, come controllo medico preliminare, subito dopo la fine delle operazioni di decontaminazione, e a 4 anni dalla fine delle operazioni. Nel contributo qui presente vengono descritte le indagini e valutazioni del rischio per la salute di operatori di decontaminazione radioattiva, e una valutazione delle prospettive per la salute per le popolazioni residenti nelle aree circostanti. È stato utilizzato il metodo dell'indagine delle iniziali condizioni di salute, mediante la ricerca nei liquidi biologici di indicatori tra i più sensibili, come la conta delle cellule ematiche circolanti, la misura del danno cromosomico e della rottura dei filamenti del DNA, analisi radiotossicologica delle urine. I risultati hanno dimostrato che il numero totale delle cellule ematiche non è variato, tuttavia sono state osservate*

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variazioni del numero relativo (percentuale) delle cellule nella formula leucocitaria. Il numero totale di alterazioni del DNA è risultato più alto subito dopo le operazioni di decontaminazione che prima della decontaminazione. A quattro anni dalla fine delle operazioni di decontaminazione il numero di alterazioni del DNA è risultato aumentato. Tuttavia il numero di cellule danneggiate (linfociti con lesioni cromosomiche) è risultato aumentato in occasione di entrambi i controlli clinici, cioè subito dopo decontaminazione e a quattro anni dalla fine della decontaminazione. Si conclude che non si sono sviluppati malattie o tumori dovuti a ^{238}U nel gruppo di operatori di decontaminazione dell'uranio impoverito durante il periodo di studio di 4 anni. Si raccomanda di eseguire ulteriori studi di monitoraggio ematologico, degli effetti cromosomici nonché delle condizioni di salute dei lavoratori interessati.

Introduction

This short report presents an effort to utilize the method for investigation of health consequences due to radiation originating from depleted uranium decontamination operations by means of analysis of initial health disorders carried out on the most sensitive biological materials such as blood cells or chromosomes.

The method was used on an expert team of 11 clean-up workers who worked for four months on the decontamination of soil contaminated by depleted uranium (^{238}U) ammunition used during the NATO bombing of Serbia and Montenegro in 1999 (10).

All workers participating in decontamination operations were subjected to medical check-ups in accordance with the programme for investigation of individuals in areas of ionizing radiations, as prescribed by national regulations (6). During their work, the team members wore protective clothing and used protective equipment and personal thermo-luminescent dosimeters (TLD). During decontamination procedures, the clean-up workers lived near the contaminated area.

Methods

The group of 11 clean-up workers were medically examined three times: before decontamination as a preliminary medical check-up (January 2002), immediately after decontamination (June 2002) and four years after cleanup of contaminated ground as a control medical check-up (2006). The programme of targeted examination was carried out with the purpose of examining the effects and consequences of ^{238}U contamination. The programme consisted of: general clinical check-ups, general haematological tests and cytogenetic analysis, measurement of radioactivity of urine and renal function tests. For morphological analysis and detection of immature forms, the blood cells were investigated in stained smears of capillary blood. Smears of capillary blood were coloured using the conventional May Grünvald-Giemsa technique and the Kaplan method. Blood smears were observed by light microscopy under immersion. The number of lymphocytes and granulocytes were counted automatically from venous blood (2). Lymphocytes were isolated and prepared using the standard methods for analysis of chromosome aberrations. Giemsa-stained preparations were scored in immersion by light microscopy and 200 metaphases were analyzed (3, 8).

The gamma-spectrometric methods were used to measure radioactivity of 24-hour urine samples (5, 7). One sample of urine was tested by alpha spectrometry. For renal function tests an analysis was performed to quantify: creatinine, urea, and microglobulin-β2 in urine (4, 9).

All 11 clean-up workers were equipped for four months with personal thermo-luminescent dosimeters (TLD), which were read by a thermoluminescence scanner. Dosimeters (Lif, type 100) were checked after decontamination procedures by Harshaw TLD Reader Model 6600. The effective dose was estimated by processing the values obtained (6, 8, 10).

Statistical analysis

For statistical analysis Student's t-test was used and Student's paired t-tests for processing the clinical/laboratory results and dosimeter data. Pearson's

χ^2 tests, in the form of contingency tables, were used for the analysis of two attributive characteristics (to analyze chromosome aberrations). Also, Mc Nemar's paired test was used to analyze chromosome aberrations.

Results

The preliminary medical investigations showed that the general health conditions of the individuals were normal, and the same was true for investigations immediately after and four years after decontamination operations. Neither variations nor significant changes in blood cell count before or after decontamination operations were observed. The number of erythrocytes, reticulocytes, leucocytes, and platelets did not significantly change. However, the percentage of lymphocytes increased in the leukocyte formula in 8 out of 11 cases, (from 35% up to 43%, on average), while the percentage of monocytes increased in 7 cases. After 4 years, the number of lymphocytes decreased ($1,5x10^9$/l, on average), as a percentage in the leukocyte formula (27%). The percentage of eosinophiles was higher after decontamination (2.27%) than before decontamination (0.82%). Basophiles were not detected. During the investigation, immediately after and four years after decontamination, neither young, premature cells nor leukaemic blasts were detected in the 100 cells counted in the smears (0%).

Chromosomal aberrations appeared in five cases after decontamination, compared to two cases in the preliminary check-ups. In two cases chromosomal changes were found before decontamination (subject numbers 8 and 11; table 1). Subject number 11 had one double chromosome break (acentric fragment). Subject number 8 had two chromatid breaks in one cell.

Preliminary checkups identified 3 chromosomal lesions in 2 cells – lymphocytes, but checkups immediately after decontamination showed 12 chromosomal lesions in 7 lymphocytes (table 1). This is a higher number of chromosomal lesions, ($p<0.05$), but 3 of them (in 2 cases: subject numbers 2 and 10; table 1) were identified as complete aberrations (double breaks and specific figures; table 1), 2 acentric and dicentric chromosomes. No specific figures, identified as not complete aberrations, such

Table 1 - Cytogenetic analysis of team members before and after decontamination work

N.	Chromosome aberrations and lesions															Damaged Cells		
	Acentric Chromosome			Breaks Chromatid			Dicentrics Chromosome			Exchanges Chromatid			Total					
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1					2	1					1		0	3	1	0	2	1
2		1			1	1		1					0	3	1	0	2	1
3					1						1		0	2	0	0	1	0
4						1							0	0	1	0	0	1
5						1							0	0	1	0	0	1
6													0	0	0	0	0	0
7													0	0	0	0	0	0
8				2									2	0	0	1	0	0
9													0	0	0	0	0	0
10		1			1	1							0	2	1	0	1	1
11	1		1		1						1		1	2	1	1	1	1
All	1	2	1	2	6	4	0	1	0	0	3	0	3	12	6	2	7	6

N. numerical code of subject investigated
I. before decontamination – preliminary check-ups
II. immediately after decontamination
III. 4 years after decontamination – control check-ups

as a chromatid breaks and chromatid exchanges, appeared in other cells. There were no significant changes in the number of aberrations, specific for last irradiation as dicentric (or acentric), compared with preliminary or control check-ups (after 4 years). In control check-ups 6 chromosomal lesions in 6 lymphocytes (table 1), appeared in 6 subjects: (subject numbers 1, 2, 4, 5, 10 and 11; table 1). Subject number 11 had 1 acentric chromosome, but he already had a similar aberration before decontamination. Another 5 lesions in chromosomes in lymphocytes were observed as chromatid breaks. Damaged cells (lymphocytes containing chromosome aberrations and/or chromatid lesions) were higher four years after decontamination.

Radioactivity of the urine, measured by gamma spectrometric, did not increase (table 2). The measured activity was due to radioactive cesium, ^{137}Cs, and was registered only as an unnatural radionuclide in urine. It was under 1Bq/1 litre of urine. Only one sample of urine (target members with dicentric chromosome) was tested by alpha spectrometry. Measurement of isotopic ratios $^{235}U/^{238}U$ allowed us to exclude urine contamination with ^{238}U. Creatinine, urea, and microglobulin-β2 in the urine and blood did not significantly change.

Table 2 - Dosimeters results of team members before and after decontamination work

N.	Effective dose TLD (mSv)	Urine radioactivity (Bq/l)	
		I	II
1	0.48	0.12	0.18
2	0.28	0.22	0.22
3	0.25	0.47	0.41
4	0.55	0.36	0.23
5	0.37	0.50	0.39
6	0.40	0.21	0.35
7	0.48	0.44	0.26
8	0.26	0.31	0.18
9	0.31	0.34	0.18
10	0.38	0.39	0.14
11	0.40	0.25	0.30
Dose and radioactivity	<1mSv	<1Bq/l	<1Bq/l

N. numerical code of subject investigated
I. before decontamination – preliminary check-ups
II. immediately after decontamination

The thermo-luminescence dosimeters that were worn for four months over the protective clothing recorded doses between 0.25 mSv and 0.55 mSv (table 2) and were always below 1 mSv.

Discussion

The total number of blood cells did not change, but variations in the relative number (percentage) of cells in the leukocyte formula were observed. The percentage of eosinophyles increased, while the percentage of lymphocytes decreased. This may be due to the high number of damaged lymphocytes containing DNA lesions.

The total number of DNA lesions was high. The chromatid break was a onefold chain break and cannot be considered as specifically due to ionizing radiations (8). Depleted uranium (^{238}U), although radioactive, is a predominantly toxic rather than a radiation health risk (2, 11). Double break DNA (dicentric and acentric chromosomes), were observed immediately after clean-up activity in only one of the 11 subjects. The frequency of characteristic chromosome aberrations was identified as 1%, which is similar to that for general populations exposed to natural uranium (7). The chromosome changes were reversible and after four years the total number of DNA lesions decreased. The number of damaged cells did not change, but was still increased. No diseases were reported and no impact on the health conditions of workers were observed. However a role of ^{238}U cannot be excluded, especially bearing in mind that the source of depleted uranium did not exist in the past. For this reason, investigations should be continued.

Young cells, leukocyte precursors or blasts were not found in the blood over the four years. This means that no disorders were observed in the bone marrow; and consequently that the risk of malignant haematological diseases was very low, but cannot be excluded in the future, after many years (9). The study of cohort personnel involved in missions in the Balkans, found no correlation between the tumours observed and presence of depleted uranium (1). However, Belorussian decontamination operators who cleaned up contaminated ground after

the Chernobyl accident, developed solitary tumours up to 15 years later (9). During our investigations, neither systemic nor solitary tumours were observed in any of the 11 team members. Further monitoring should be carried out on long-term effects.

Low concentrations of uranium in the urine may occur even 5,000 days after contamination. Uranium can cause renal damage; microglobulinuria and aminoaciduria (4, 9). Neither gamma nor alpha emissions were detected in urine. Significant contamination did not occur. Renal function was normal.

The thermo-luminescence dosimeters did not record doses of ionizing radiation exceeding those of the natural background activity. This can be explained by the type of radioactive emissions to which clean-up workers were exposed during decontamination operations as well as by the distance and site of dosimeter application (2).

NO POTENTIAL CONFLICT OF INTEREST RELEVANT TO THIS ARTICLE WAS REPORTED

REFERENCES

1. GUSTAVSSON P, TALBACK M, LUNDIN A: Incidence of cancer among Swedish military and civil personnel involved in UN missions in the Balkans 1989-99. Occupational Environmental Medicine 2004; *61*: 171-173
2. ICRP: Uranium. In *Individual Monitoring for Intakes of Radio-nuclides by Workers: Design and Interpretation. International Commission on Radiological Protection*. Annals of the ICRP 1996; *19*: 141-154
3. JOVICIC D, MILACIC S, KOVACEVIC R: Cytogenetic analysis of chromosomal status of subjects from the regions in the vicinity of uranium-contaminated areas. Abstracts of *ICC XV, 15th International Chromosome Conference* 5-10 September 2004. London, UK: Brunel University, Chromosome Research 2004; *12:* s156
4. KLASSEN CD: *Casarett and Doull's toxicology: the basic science of poisons*. McGraw-Hill, Medical Publishing Division. Sixth Edition 2001: 857-859
5. KNEZEVIC I, MILACIC S, NOVAK LI, NESIC V: The Effect of $CaNa_2EDTA$ on Excretion of ^{210}Pb, ^{210}Po and Stable Lead in Cases of Chronic Lead Intoxication. Radiation Protection Dosimetry 1998; *79*: s471-s472
6. Law on Ionizing Radiation Protection, Official Gazette of the Federal Republic of Yugoslavia 1996; N. 46/96
7. MILACIC S, PETROVIC D, JOVICIC D, et al: Examination of health status of populations from Uranium-contaminated Regions. Environmental Research 2004; *95*: 2-10
8. MILACIC S: The frequency of chromosomal lesions and damaged lymphocytes of workers occupationally exposed to x-rays. Health Physics 2005; *88*: 334-339
9. OKEANOV AE, SOSNOVSKAYA EY, PRIATKINA OP: A national cancer registry to assess trends after the Chernobyl accident. Swiss Med Wkly 2004; *134*: 645-649
10. UNEP: *Depleted uranium in Serbia and Montenegro – Post-conflict Environmental Assessment*. United Nations Environment Program - UNEP. Geneva, Switzerland: 2002. Available on www.unep.org
11. WHO: *Depleted uranium: Sources, Exposure and Health Effects*. Department of Protection of the Human Environment. Geneva: World Health Organization, 2001